

https://dspace.org/

Factors during pregnancy affecting the susceptibility of offspring to Type 2 diabetes

Toman, Marketa

2012-01-10
http://hdl.handle.net/10539/10940

Downloaded from a DSpace repository. https://dspace.org/

FACTORS DURING PREGNANCY AFFECTING THE SUSCEPTIBILITY OF OFFSPRING TO TYPE 2 DIABETES

Marketa Toman

A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg,
in fulfilment of the requirements for the degree

of

Doctor of Philosophy

Johannesburg, South Africa, 2011

DECLARATION

I, Marketa Toman declare that this thesis is my own work. It is being submitted for the degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

…….

……day of ……., 2011.

The study reported in this thesis was performed in the Department of Chemical Pathology of the National Health Laboratory Service at the Faculty of Health Science of the University of the Witwatersrand, Johannesburg, South Africa.

DEDICATION

To my parents, my husband Zdenek

and to the memory

of my dear friend Elizabeth Campbell.

PRESENTATIONS DERIVED FROM THIS STUDY

CONGRESS POSTER PRESENTATIONS

- ***Factors During Pregnancy Associated with Fetal Growth***. The 37th SEMDSA (Society for Endocrinology, Metabolism and Diabetes of South Africa) Workshop, 2-6 April 2001, Johannesburg. Abstract published in the JEMDSA (The Journal of Endocrinology, Metabolism and Diabetes of South Africa), April 2001, Vol.6, No. 1, Pg 46.

- ***Maternal Factors Involved in the Control of Fetal Growth.*** EUROMEDLAB, 14th IFCC European Congress of Clinical Chemistry and Laboratory Medicine, 26–31 May 2001, Prague, Czech Republic, Europe. Abstract published in the Clin Chem Lab Med 2001; 39, Special Supplement pp S1 – S448, May 2001.

- ***Maternal Nutrition During and After Pregnancy and its Influence on Hormone Levels***. The 38th SEMDSA (Society for Endocrinology, Metabolism and Diabetes of South Africa), Sommerset West, April 2002. Also presented at the Faculty Research Day in the WITS University Medical School in August 2002. Abstract published in the JEMDSA (The Journal of Endocrinology, Metabolism and Diabetes of South Africa), April 2002, Vol.7, No. 1, Pg 35.

- ***Fetal Growth and Maternal Amino Acids***. EUROMEDLAB, 15th IFCC European Congress of Clinical Chemistry and Laboratory Medicine, 1– 5 June 2003, Barcelona, Spain, Europe.

- ***Maternal Amino Acids and Fetal Development***. Presented at the Faculty of Health Sciences Research Day, 4th August 2004.

- ***Leptin and adiponectin serum levels in pregnant African women and their offspring.*** Presented at the IDF (International Diabetes Federation) 2006 - 19th World Diabetes Congress. 3.12. -7.12. 2006, Cape Town, South Africa. Abstract published in the Special Supplement to Diabetic Medicine, Volume 23, December 2006 (P1427).

ORAL PRESENTATION

- ***Leptin and adiponectin serum levels in pregnant African women and their offspring***. Presented at the Health Science Research Day, 23.8.2006. University of the Witwatersrand, Faculty of Health Sciences.

ACKNOWLEDGEMENTS

This study would not have been possible without the contribution and support of many people.

My grateful thanks to my supervisor, Associate Professor Nigel Crowther, who provided me with his expert knowledge, advice and support throughout the period of this study.

I would also like to express my thanks to my co-supervisor, Professor IP Gray, previous Head of the Department of Chemical Pathology, who introduced me to this research field.

I thank Professor F Guidozzi, the Academic Head, Dept. of Obstetrics and Gynaecology, my co-supervisor, for his professional advice and guidance and allowing me to conduct part of my project in the Antenatal Clinic of the Charlotte Maxeke (Johannesburg General) Hospital.

Thank you to Dr. J. MacKeown, a dietitian, who kindly supplied me with her food frequency questionnaire and who coded all the dietary intake information for this study. I would also like to thank her for her valuable professional advice and support.

Sincere thanks to Professor P. Rheeder for his consultations and guidance during the statistical analyses and for his assistance with the linear mixed models.

Many thanks to Dr. I. Erasmus and Mrs. Z. Holland for performing the ultrasound scans of pregnant women participating in this study for their expert knowledge in this field. Additional thanks to Mrs. Holland for providing me with the ultrasound images used in my Thesis.

I thank Sr. B. Mabilane (Gaofetoge), research assistant, for her help with the enrolment and follow-up of participants. In addition, I would like to thank Sr. Mabilane together with Sr. N. Holden and Sr. N. Ramela for collecting blood samples from mothers and infants and for their assistance during the anthropometrical measurements.

I would like to thank Sr. E. Hennessy, Sr. J. Meredi, Mrs. A. Polchet and the staff of the Antenatal Clinic, the Labour Ward and the Postnatal Wards for their tolerance, understanding and professional help and advice.

Many thanks to Mrs. L. Battaglia and the staff of the NHLS library for their kind assistance in obtaining numerous research publications for me. And a big thank you to my colleagues for their encouragement and interest in my work, especially Carolyn, Delia and Rita for their support and help.

I would like to thank everybody who contributed to this study in any way, including my family, friends and also all the participating women and babies.

Finally, I wish to thank Professor J. Paiker, the current Head of the Department of Chemical Pathology, for allowing me to carry out my study in parallel with my employment.

This study would not be possible without the financial support from:

Medical Research Council, MRC

National Health Laboratory Service, NHLS

University of the Witwatersrand, WITS

South African Sugar Association, SASA

ABSTRACT

Objectives. The Pregnancy, Nutrition & Diabetes (PND) study aimed to evaluate the dietary status of urban Black pregnant women attending the Antenatal Clinic at the Charlotte Maxeke (Johannesburg General) Hospital. In addition, the study investigated the effects of maternal dietary intake and hormonal levels during pregnancy on fetal growth, birth size and the early postnatal development of risk factors for future Type 2 diabetes. The study analysis precedes a detailed description of the study population, including its comparison with other populations.

Methods. 126 women were enrolled in the study before 24 weeks of gestation. Twice during pregnancy (weeks 20-24 and 30-36, visits V1 and V2) and approximately six months after the delivery (visit V3), volunteers participated in a standard 75 g oral glucose tolerance test (OGTT). Blood pressure, anthropometric and socio-demographic data were taken and a food frequency questionnaire was administered at each visit. The daily maternal intakes of total energy and macronutrients expressed as a % of total energy intake (%E) were calculated and further evaluated. A comparison with the crude nutrients intakes was also performed. During both pregnancy visits, an ultrasound examination was carried out to obtain estimates of fetal biometry and fetal well-being and the postnatal anthropometric parameters were also measured. Blood samples were collected at fasting, 30min, 60min and 120min of the OGTT for the measurements of maternal glucose (GLC), insulin (INS), C-peptide (C-PEP) and proinsulin (PI) and fasting samples for the determination of placental lactogen (HPL), insulin-like growth factor 1 (IGF-1), insulin-like growth factor binding protein 1 (IGF-BP1), free

thyroxin (FT4), cortisol (CORT) and leptin (LEPT). Infant fasting blood samples were used for the analysis of the GLC, INS, PI, IGF-BP3 and LEPT. The plasma glucose samples were analysed on the Beckman Glucose Analyser 2, whilst all the other analytes were measured by an immunoassay method on 96-well plates.

Results – DESCRIPTIVE DATA.

Study participants. The majority of the study participants had completed high school education, however were unemployed and of low and very poor socio-economic levels. In comparison with another study from the developing world, the Pune study in India, the women from the current study were older, heavier with greater body mass index (BMI) and they were also taller. They had higher head and arm circumference.

Birth outcomes. The birth size of African babies in the current study, although smaller in all parameters, was still relatively closer to the size of the Caucasian babies seen within the Southampton (*Godfrey et al. 1996*) or Helsinki (*Forsén et al. 1997)* studies than to the birth size of the babies from Trivandrum (*Jaya et al. 1995)* or Pune (*Rao et al. 2001*) in India. These relatively smaller African babies have comparable (or even larger) maternal placental sizes in relation to the mothers of both European studies. However, in comparison with the small Indian babies, the major difference noted was a substantially smaller placental size of the Pune mothers.

Compared to z-scores from the World Health Organization (WHO) child growth standards (*WHO Anthropo 2010*), the PND study babies were born lighter and shorter with a larger head circumference (HC). A growth delay observed near the neonatal visit was followed

by catch up growth in weight for age, however no catch-up growth was observed for length for age by the second postnatal visit.

Dietary intakes. The PND study women had pregnancy energy intakes slightly below the national level intakes *(Steyn et al. 2006*), except for the energy intake at V3, which exceeded the national level. The lower energy intake during pregnancy is attributable to a lower intake of dietary protein and total carbohydrate. The fat intake was substantially higher in the PND study, with levels almost double at the second postnatal visit. This discrepancy may be due to differences between investigated populations or due to increased requirements of energy during lactation.

In comparison with the Recommended Dietary Allowances (RDA), during the pregnancy period women in the current study had lesser crude protein and higher carbohydrate intake. Intake of the crude protein after delivery was low in comparison to the RDA for lactating women. Total carbohydrate intake exceeded the RDA. The relation between the intake of macronutrients expressed as a percentage of total energy (%E) and RDA was similar.

The current study participants had a lower intake of energy, especially during the pregnancy period, when compared to populations in the developed world. They had a lower intake of protein at all visits in comparison with the national average for women or with that found in the Southampton study or when compared to the usual American diet. The energy-adjusted intake of fat and carbohydrate were comparable with the Southampton and American data. This population in our study was therefore more comparable to that of Pune than to American or Southampton populations. However, despite similar total energy intakes as the

Pune Study, total protein and fat intakes were higher in the African mothers, which may explain their higher weights and birthweights of their babies.

Results – ANALYTICAL DATA

Dietary intakes. Maternal dietary intakes showed significant effects on the fetal biometrical measurements, mainly involving the fetal head, femur length and the size of abdomen. The outcomes also show a significant relationship between maternal protein intake and baby's birth weight. Associations were also found for maternal dietary intakes and the neonatal length and BMI and the markers of the infant β-cell function. Intakes of the plant protein and polyunsaturated fat supported the linear growth. There was no correlation between maternal dietary intake and the fetal growth rate. Effects of the total energy intake and carbohydrate seemed to be direct, while the effect of protein and fat may be delayed, possibly involving metabolic adaptation of the mother and the partitioning of nutrients between the mother, placenta and fetus. (See Table: Associations between the maternal dietary intake and the neonatal INSF1 levels, Pg. xiv).

Maternal hormones. The data of the current study show significant relationships between maternal pregnancy hormones and fetal growth rate and the postnatal growth rate, although maternal anthropometry and fetal gender and BMI are also significantly involved. The maternal thyroid hormones seem to play an important role in fetal and postnatal growth and insulin metabolism (N=76, p=0.002, ß=-0.343; AR^2=0.106) in the association with the neonatal fasting insulin.

Discussion. The outcomes of the current study show that the African pregnant women in the study had lower energy intakes attributable to a lower intake of dietary protein and total carbohydrate.

Maternal dietary intakes showed significant effects on the fetal biometrical parameters. Protein dietary intake was positively associated with baby's birth weight and was shown to be statistically significant. Associations were also found for maternal dietary intakes and the infant postnatal BMI and the markers of the infant β-cell function. Quality of the protein and fat has different effects on fetal/infant growth.

Maternal hormones showed correlations with fetal growth rate and the outcomes of the current study also show that maternal hormones can affect neonatal and early postnatal infant glucose levels and β-cell function. They are also linked to the programming of early obesity. The maternal thyroid hormones seem to play an important role.

The maternal low energy, protein (especially plant protein) and PUFA intakes during gestation may be the reasons for the lower z-score birth parameters of the infants in comparison with the World Health Organization (WHO) child growth standards (*WHO Anthro 2010*). The disproportionally larger head of the newborn may be an outcome of the brain sparing effect. A suggestion for an increased maternal dietary intake of energy, protein and PUFA has been made.

Table: Associations between the maternal dietary intake and the neonatal INSF1 levels.

NUTRIENT	Intake V1 (N=76)					Intake V2 (N=75)			
Variable	p	β	95%CI (b)	AR^2	**Variable**	p	β	95%CI (b)	AR^2
TOT PROT1[%E]	0.002	0.231	0.01; 0.06	0.108	PL PROT2[%E]	0.010	-0.298	0.00; 0.00	0.076
Crude amount	0.045	0.231	0.00; 0.03	0.041	*Crude amount*	NSF			
AN PROT1[%E]	0.007	0.307	0.00; 0.01	0.082	TOT FAT2[%E]	0.004	0.327	0.00; 0.00	0.095
Crude amount	0.020	0.267	0.00; 0.02	0.059	*Crude amount*	0.004	0.330	0.00; 0.01	0.096
TOT FAT1[%E]	0.022	-0.263	0.00; 0.00	0.057	MUFA2[%E]	0.007	0.312	0.00; 0.00	0.085
Crude amount	NSF				*Crude amount*	0.008	0.304	0.00; 0.00	0.080
PUFA1[%E]	0.012	-0.287	0.00; -0.02	0.051	PUFA2[%E]	0.003	0.335	0.01; 0.03	0.100
Crude amount	NSF				*Crude amount*	0.001	0.389	0.01; 0.02	0.140
					TOT CHO2[%E]	0.005	-0.321	0.00; 0.00	0.091
					Crude amount	NSF			
					TOT ENERGY2	0.029	0.253	0.00; 0.03	0.051

* The units for the confidence interval (CI) of the slope (b) are expressed in the units of transformation as stated in Appendix G.

p (probability), ß (Standardised regression coefficient), N (number of subject); CI (confidence interval); b(slope); NSF (non significant association)

INS F1 (neonatal fasting insulin); TOT (total); AN (animal); PL (plant); PROT (protein); CHO (carbohydrate);

PUFA (polyunsaturated fatty acid); MUFA (monounsaturated fatty acid);

TABLE OF CONTENTS

Pg

DECLARATION ii

DEDICATION iii

PRESENTATIONS DERIVED FROM THIS STUDY iv

ACKNOWLEDGEMENTS vi

ABSTRACT ix

TABLE OF CONTENTS xv

LIST OF FIGURES xxvi

LIST OF TABLES xxix

NOMENCLATURE xxxii

CHAPTER 1

Pg

LITERATURE REVIEW

Conceptual Framework …… **1**

1.1 INTRODUCTION ……..…… **2**

1.1.1 Fetal origins of adult disease (FOAD) – historical background 4

1.1.2 World epidemiological studies in developed and developing countries 10

1.1.3 “Thrifty genotype” and “Thrifty phenotype” 22

1.1.3.1 "Thrifty genotype" hypothesis 22

1.1.3.2 "Thrifty phenotype" hypothesis 24

1.1.4 "Developmental origins" hypothesis of future health and disease 28

1.2 DIABETES MELLITUS …… 32

1.2.1 Aetiology, pathophysiology, complications and diagnostic criteria for Type 2 diabetes mellitus 32

1.2.1.1 Aetiology and pathophysiology of Type 2 diabetes 32

1.2.1.2 Assessment of insulin resistance 36

1.2.1.3 Long-term complications of Type 2 diabetes mellitus 38

1.2.1.4 The diagnosis of diabetes mellitus 38

1.2.2 Epidemiology of diabetes mellitus and costs to society 39

1.2.3 Diabetes in the South Africa 41

1.2.4 Gestational diabetes mellitus 42

1.2.5 Endocrine pancreas and pancreatic β-cells 44

1.2.5.1 Development of the pancreatic endocrine cells 44

1.2.5.2 Regulation of β-cell mass 45

1.2.5.3 Maternal islet adaptation during pregnancy 46

1.2.5.4 Effect of nutrition 47

1.2.5.5 Effect of glucose 48

1.2.5.6 Effect of hormones 48

1.2.5.7 Fetal insulin 49

1.3 THE STAGES AND THE "CRITICAL PERIODS" IN THE HUMAN GROWTH AND DEVELOPMENT ……..… 50

1.3.1 Maternal adaptations to pregnancy 51

1.3.2 Embryonic and Fetal development 51

1.3.2.1 Introduction 51

1.3.2.2 Early development 54

1.3.2.3 Trophoblast invasion and establishment of the utero-placental circulation 58

1.3.2.4 Early nutrition 60

1.3.2.5 Growth factors and blastocyst development 60

1.4 DETERMINANTS OF FETAL GROWTH AND FETAL PROGRAMMING …… 61

1.4.1 Assessment of the birth outcome 61

1.4.2 Determinants of fetal growth 64

1.4.2.1 Fetal genome 69

1.4.2.1.1 Epigenetic regulation 69

1.4.2.1.2 Imprinted genes 69

1.4.2.2 Maternal factors influencing fetal growth 70

1.4.2.2.1 Maternal age and body composition 71

1.4.2.2.2 Pregnancy spacing 73

1.4.2.2.3 Maternal race, ethnicity, socio-economic status 73

1.4.2.2.4 Maternal health 73

1.4.2.3 Effects of maternal nutrition 74

1.4.2.3.1 Nutritional deprivation 74

1.4.2.3.2 Overnutrition 75

1.4.2.3.3 Macronutrients in fetal growth 75

1.4.2.3.4 Micronutrients in fetal growth 80

1.4.2.4 Effects of placenta 80

1.4.2.4.1 Placental volume 81

1.4.2.4.2 Utero-placental blood flow 81

1.4.2.4.3 Placental transport 82

1.4.2.4.4 Placental endocrine function 86

1.4.2.4.5 Intrauterine hypoxia 89

1.5 AIMS OF THE STUDY …….. 92

1.5.1 **INTRODUCTION TO THE RESEARCH STUDY**……. 92

1.5.2 **AIMS AND OBJECTIVES OF THE PRESENT STUDY**…….. 93

CHAPTER 2 **Pg**

MATERIALS AND METHODS

2. 1 STUDY POPULATION AND STUDY DESIGN …….. 96

2. 2 ENROLMENT AND FOLLOW-UP ……. 98

2.2.1 Sampling and Enrolment 98

2.2.2 Organisation of the study 101

2.2.3 Feasibility study 102

2. 3 PROCEDURES PERFORMED IN DAY WARD

(PRE- AND POSTNATAL) ……..…. 103

2.3.1 Anthropometric measurements of women and babies 103

2.3.1.1 Weight and height 103

2.3.1.2 Head, arm and abdominal circumference measurements 104

2.3.2 Blood pressure measurement 104

2.3.3 Oral Glucose Tolerance Test (OGTT) 104

2.3.3.1 Procedure of the OGTT 104

2.3.3.2 Blood collection procedure during the OGTT 105

2.3.4 Postnatal visits of babies 106

2.3.4.1 Preparation for the blood collection 106

2.3.4.2 Heel-prick blood collection method 106

2.4 QUESTIONNAIRES AND FORMS ……..… 107

2.4.1 Inclusion criteria questionnaire 107

2.4.2 Food frequency questionnaire 108

2.4.2.1 Questionnaire procedure 109

2.4.2.2 Questionnaire coding and evaluation 110

2.5 ULTRASOUND SCAN …….. 111

2.5.1 Technical equipment 111

2.5.2 Ultrasound examinations 112

2.5.2.1 Ultrasound examination at the first visit 112

2.5.2.2 Ultrasound examination at the second visit 115

2.6 DELIVERY AND DELIVERY RECORDS ……. 120

2.7 BIOCHEMICAL ANALYSIS ……..….. 121

2.7.1 Specimen collection and storage 121

2.7.2 Determination of glucose 123

2.7.3 Immunoassays 124

2.7.3.1 Instruments 124

2.7.3.2 Non-competitive immunoassay 125

2.7.3.3 Non-competitive assays used in the present study 125

2.7.3.4 Competitive immunoassay with enzymatic label 134

2.7.3.5 Competitive immunoassay in the present study 135

2.8 STATISTICAL ANALYSIS ……..….. 140

2.8.1 Preparation of the database 140

2.8.1.1 Dietary intake data 140

2.8.1.2 Issue of the breast-feeding and formula feeding 142

2.8.1.3 Calculation and Indices 142

2.8.1.4 Variables in the database 143

2.8.1.5 Missing values 143

2.8.2 Descriptive statistics. 144

2.8.2.1 The z-scores for neonates and infants 145

2.8.3 Analytical statistics 145

2.8.3.1 Analysis of dietary intake 146

2.8.3.2 Analysis of maternal pregnancy parameters vs fetal, birth and infant parameters 146

2.8.3.3 Confounders 147

2.8.3.4 Data analysis framework 149

CHAPTER 3 **Pg**

RESULTS - DESCRIPTIVE DATA

3.1 CHARACTERISTICS OF STUDY PARTICIPANTS …… 152

3.1.1 Ethnic groups 152

3.1.2 Age distribution 152

3.1.3 Socio-economic background 153

3.1.3.1 Level of education 153

3.1.3.2 Employment status 155

3.1.3.3 Family income 155

3.1.3.4 Number of household occupants 155

3.1.3.5 Number of mothers' siblings 157

3.1.4 Family history of diabetes and hypertension 157

3.2 STUDY ATTENDANCE …… 159

3.2.1 Follow-up profile 159

3.2.2 Reasons for the loss to follow-up 160

3.3 ANTHROPOMETRY …… 163

3.4 MEASURED METABOLIC PROFILE ……..... 165

3.4.1 OGTT levels of glucose and hormones involved in glucose metabolism 165

3.4.1.1 Glucose levels during the two-hour OGTT 165

3.4.1.2 Insulin levels during the two-hour OGTT 167

3.4.1.3 C-peptide levels during the 2-h OGTT 167

3.4.1.4 Proinsulin levels during the 2-h OGTT 170

3.4.2 Area under the curve for glucose, insulin, proinsulin and C-peptide during the 2-h OGTT 172

3.4.3 Measurement of other metabolic products 174

3.5 NUTRIENT INTAKES ……..….. 176

3.6 ULTRASOUND EVALUATION ……..….. 178

3.7 DELIVERY OUTCOMES …….. 180

3.8 EVALUATION OF NEONATES AND INFANTS …….. 181

3.8.1 Infants' age, weight and anthropometry 181

3.8.2 Infants' glucose and hormonal levels 183

RESULTS - ANALYTICAL DATA

3.9 SUMMARY TABLES ON MATERNAL DIETARY EFFECTS……. 185

3.9.1 Maternal dietary intake and associations with fetal weight and biometry 188

3.9.2 Maternal dietary intake in association with the birth parameters 191

3.9.3 Maternal dietary intake and the infants' anthropometric parameters 191

3.9.4 Maternal dietary intake and the infants' metabolic parameters 194

3.10 MULTIPLE LINEAR REGRESSION INVOLVING MATERNAL AND FETAL – INFANT PARAMETERS …… 200

3.10.1 Associations of the maternal anthropometry and blood pressure with the fetal-birth-postnatal size and growth rate parameters 201

3.10.2 Associations of the maternal glucose and hormones with the infant birth-postnatal size and growth rate parameters 203

3.10.3 Associations of the maternal glucose and hormones with the infant postnatal glucose and metabolic parameters 205

3.11 ASSOCIATIONS WITH THE SOCIO-ECONOMIC STATUS (SES) OF THE PARTICIPANTS …… 209

3.12 FURTHER ASSOCIATIONS…… 215

3.13 SAMPLE BIAS ……... 216

CHAPTER 4

Pg

DISCUSSION – DESCRIPTIVE DATA

4.1 ENROLMENT AND CHARACTERISTICS OF STUDY PARTICIPANTS ……. 217

4.1.1 Ethnic composition of the study population 217

4.1.2 Socio-economic background 218

4.1.2.1 Employment status 219

4.1.2.2 Family income 219

4.1.3 Family history of diabetes and hypertension 220

4.2 ANTHROPOMETRICAL DATA AND BIRTH OUTCOME …….. 221

4.2.1 Maternal anthropometry 221

4.2.2 Birth outcome 223

4.3 MATERNAL DIETARY INTAKES IN COMPARISON WITH RDA AND OTHER STUDIES …… 226

4.3.1 PND study and national South African data on women 15-64 years old 228

4.3.2 Comparison with the RDA 231

4.3.3 Comparison with other studies 232

4.3.4 Low energy reporting 234

4.4 SELECTION OF THE METHOD FOR DIETARY INTAKE ASSESSMENT IN THE CURRENT STUDY ……. 235

4.5 DISCUSSION OF THE ANALYTICAL PART OF THE DATA ANALYSIS ……. 239

4.5.1 Discussion on the maternal nutrient intake effects 239

4.5.1.1 Maternal nutrients and fetal biometry at US1 and US2 239

4.5.1.2 Maternal nutrients and birth weight 243

4.5.1.3 Maternal nutrient intake and postnatal anthropometry 245

4.5.1.4 Maternal nutrients and the postnatal metabolic parameters 251

4.5.2 Discussion on fetal metabolic parameters 255

4.5.2.1 Fetal and postnatal growth rates 256

4.5.2.2 Birth size 260

4.5.2.3 Postnatal infant anthropometry 261

4.5.2.4 Postnatal infant metabolic variables 263

4.5.3 Discussion on the correlation with Socio-economic Status (SES) 266

CHAPTER 5 Pg

SUMMARY AND CONCLUSIONS …… 268

LIMITATIONS OF THE CURRENT STUDY …….. 277

IMPLICATIONS OF THE STUDY ……. 278

FOLLOW-ON RESEARCH FROM THIS PROJECT…… 280

APPENDICES

			Pg
			282
A	Enrolment diagram		283
B	Inclusion criteria questionnaire		284
C	Food frequency questionnaire		287
D, E, F	Maternal energy and macronutrient dietary intake regression analyses and the list of used confounders and their significance		291- 293
	D	Maternal protein intake analysis	294
	E	Maternal fat intake analysis	305
	F	Maternal energy, carbohydrate and added sugar intake analysis	317
G	G1	List of variables and their transformations for data on mothers	330
	G2	List of variables and their transformations for data on ultrasound, Delivery and infants	331
H	Maternal dietary intakes summary tables		332
I	Correlations used in the regression models listed in Appendix J		337
J	Linear regression models		344
K	Tables of outcomes from the linear mixed model analyses		349
L	Permission to reprint the RDA/DRI values		355
M	Ethics Clearance		356

REFERENCES **357**

LIST OF FIGURES

Pg

CHAPTER 1.

Figure:

1.1 Ischaemic heart disease (IHD) in men in Hertfordshire at age of 59-70. (Source of the data: *Barker et al. 1992*) …….. 6

1.2 Impaired glucose tolerance (IGT) and Type 2 diabetes prevalence expressed as odds ratio in men in Hertfordshire at age of 59-70. (Source of the data: *Barker et al. 1992.)* ……. 6

1.3 Age at adiposity rebound in relation to BMI at 1 y and 12 years of age for both genders combined (Helsinki study; *Eriksson et al. 2003a*) ……. 13

1.4 Age at adiposity rebound and accumulative incidence of diabetes (Helsinki study; *Eriksson et al. 2003a*) ……. 13

1.5 Glucose and insulin levels of offspring born before famine, exposed to famine during different times of prenatal life or conceived after famine (Dutch Hunger Winter study; *Ravelli et al. 1998*) …….. 15

1.6 Systolic blood pressure (Figure A), LDL cholesterol (Figure B) as cardiovascular risk markers and the HOMA-IR index (Figure C) at 8-year old children of Pune study plotted according to their birth weight (*Bavdekar et al 1999)* ……. 19

1.7 "Thrifty genotype" and "Previously neutral polymorphism" hypotheses (Adapted from *Turner et al. 1993*, Fig. 1) …….. 23

1.8 "Thrifty phenotype" hypothesis (*Hales et al.1992)* …… 26

1.9 Scheme of time periods and events during gestation with incorporated timing for the PND study visits (drawing based on *O'Rahilly et al.2001a*) …….. 53

1.10 Embryonic period and the Carnegie developmental stages. (Diagram based on that of *Moore 1993d and O'Rahilly et al. 2001a)* ……. 55

1.11 The structure of mature placenta. (Source: *Sood et al. 2006; freely obtainable via PNAS open access)* …… 58

1.12 Table A. Potential determinants of fetal growth and birth weight (Based on *Kramer 1987*)…….…… 66

Table B. Regulation of fetal development and programming. (Based on *Fall 2003a, Fig 6* and *Godfrey 2006, Fig 2.4*) ……. 68

CHAPTER 2.

Figure:

2.1 Study flow diagram ……..….. 97

2.2 Ultrasound images of fetal biometry ……..…… 113

2.3 Four quadrant method for AFI estimation …….. 116

2.4 Spectral Doppler ultrasound waveform analysis performed on the maximum velocity of red blood cells from umbilical artery ……..…. 118

2.5 Data analysis framework: Effects of maternal nutrition …… 150

2.6 Data analysis framework: Effects of maternal glucose and hormones …….. 151

CHAPTER 3.

Figure:

3.1 Language-cultural background of women enrolled into the study …… 153

3.2 Distribution of the mothers' age at enrolment …… 154

3.3 Education level of baby's parents at the time of enrolment …… 154

3.4 Employment status of baby's parents at the time of enrolment …….. 156

3.5 Family monthly income ……..…. 156

3.6 Number of adults and children over 3 years of age sharing the household ….…. 157

3.7 Number of mother's siblings …… 158

3.8 Family history of diabetes and hypertension for mothers and fathers …… 158

3.9 Frequency of the loss to follow-up at different stages of the study …… 159

3.10 Glucose levels during the two-hour OGTT for mothers at all visits …… 166

3.11 Insulin levels during the OGTT for mothers at all visits …… 168

3.12 C-peptide levels during OGTT for mothers at all visits ……..…. 169

3.13 Proinsulin levels during OGTT for mothers at all visits …….. 171

3.14 Associations between maternal protein intakes and the neonatal fasting insulin and suggested mechanism …….. 199

LIST OF TABLES

Pg

CHAPTER 1.

Table:

1.1 Epidemiological studies on nutrition ……..76 - 79

CHAPTER 3.

Table:

3.1 Reasons for excluding the woman/baby from a visit or from further follow-up (Tables A, B and C)……..161-162

3.2 Anthropometric measurements and BP of mothers at the antenatal (V1 and V2) and postnatal visit (V3) …… 164

3.3 Areas under curve of glucose, insulin, proinsulin and C-peptide during the two hour-glucose tolerance test (Tables A and B) ……. 173

3.4 Levels of other metabolites measured at both pregnancy visits (V1, V2) and the visit (V3) of mothers …… 175

3.5 Average daily nutrient intake per person during the antenatal (V1 and V2) and postnatal (V3) visits …….. 177

3.6 Ultrasound outcome: Fetal biometry, estimations of fetal weight, gestational age and growth rate, calculated indices and ratios …….. 179

3.7 Delivery outcomes for 50 female and 55 male newborns and Ponderal index (A). Birth weight by baby's gender is shown in section B…… 180

3.8 Table A: Babies' age, weight and anthropometry at both postnatal visits (Ba1, Ba2)…….. 182
Table B: Babies' anthropometry at both postnatal visits expressed as z-scores …….. 183
3.9 Babies' glucose, hormones, insulin resistance assessment (HOMA-IR) and postnatal growth at both visits (Ba1, Ba2)……. 184
3.10 Table A: Associations between maternal dietary intakes and fetal weight and biometry ……..…190
Table B: Associations between maternal dietary intakes and infants' anthropometry……..…..192
Table C: Associations between maternal dietary intakes and infants' metabolic parameters……..……195
3.11 Major outcomes for maternal dietary intakes and the neonatal INS F1 levels……..198
3.12 Table A: Associations of maternal anthropometry and her blood pressure with the fetal-birth- postnatal size and growth rate parameters …… 202
Table B: Associations of maternal glucose and hormones with infants' birth-postnatal size and the growth rates ……. 204
Table C: Associations between maternal glucose and hormones and infants' postnatal metabolic parameters…… 206
3.13 Summary of major outcomes for maternal glucose and hormones and infants' parameters and growth rates……...208
3.14 Table A: Relationship between mother's employment status and maternal, birth and infant postnatal parameters ……210

Table B: Associations of father's employment status with birth and infant postnatal parameters. ……. 210

3.15 Associations between parental education and maternal cosrtisol ……..211

3.16 Relationship between maternal nutrient intakes and SES …… 213

3.17 Gestational age association with fetal biometrical parameters …….. 215

CHAPTER 4.

Table:

4.1 Comparison of maternal characteristics ……. 222

4.2 Comparison of the birth characteristics between different studies…….. 225

4.3 Table A: Comparison of the maternal daily dietary intake of macronutrients with DRIs, overall intake in South African women, the Pune Maternal Nutrition Study, the Southampton study and the usual American diet …… 227

Table B: Comparison of the protein and fat categories and the added sugar Maternal daily dietary intake with the data on South African women …… 230

NOMENCLATURE

%E	percent of energy
11ß-HSD (-1, -2)	11ß-hydroxysteroid dehydrogenase (-1, -2)
AC	abdominal circumference
ADA	American Diabetes Association
AFI	amniotic fluid index
AGA	appropriate for gestational age
AIDS	Acquired immunodeficiency syndrome
AN PROT	animal protein
ANC	Antenatal Clinic
AR^2	adjusted multiple regression coefficient
AUC	area under the curve
B1	vitamin B1, thiamin
B12	vitamin B12, cobalamin
B2	vitamin B2, riboflavin
B6	vitamin B6, pyridoxin
Ba1, Ba2	first postnatal baby's visit, second postnatal baby's visit
BD	Becton Dickinson
BHC	birth head circumference
BL	birth length
BMI	body mass index
BP	blood pressure
BPD	biparietal diameter
BTT	Birth-to-ten Study (Birth-to-twenty Study)
BW	birth weight
Ca	calcium
CHD	coronary heart disease
CHO	carbohydrate
coefficient B	non- standardized regression coefficients
coefficient β	standardized regression coefficients

CORT	cortisol
C-PEP	C-peptide
CV	coefficients of variation
CVS	cardiovascular system
DIA BP	diastolic blood pressure
DM	Diabetes Mellitus
DNA	Deoxyribonucleic acid
DOH	“Developmental origins” hypothesis
DOPA (L-DOPA)	L-3,4-dihydroxyphenylalanine
DRI	Dietary Reference Intakes
EASIA	Enzyme Amplified Sensitivity Immunoassay
EDD	estimated date of delivery
EFW	estimated fetal weight
EIA	enzyme immunoassay
ELISA	enzyme-linked immunoassay
EU	United Europe (European Union)
FA	fatty acids
FFA	free fatty acids
FFQ	food frequency questionnaire
FL	femur length
FOAD	“Fetal origins of adult disease”
FT3	free triiodothyronine
FT4	free thyroxine
g	gram (unit of mass); also gravity (see centrifuge)
	(derived units: kg, mg, μg, ng)
GDM	gestational diabetes mellitus
GH	growth hormone
GLC	glucose
GLUT	glucose transporter
GRRT	growth rate
$H_2 O_2$	hydrogen peroxide

H_2O	water
HB	haemoglobin
HC	head circumference
HCG	human chorionic gonadotrophin
HDL	high density lipoprotein
HIV	Human immunodeficiency virus
HOMA-ß	Homeostasis model assessment of pancreatic ß-cell function
HOMA-IR	Homeostasis model assessment of insulin resistance
HPA axis	hypothalamic-pituitary-adrenal axis
HPL	human placental lactogen
HRP	horseradish peroxidase
ID	identity document
IGF (-1, -2)	insulin-like growth factor (-1, -2)
IGF BP	insulin-like growth factor binding protein
IGF-BP (-1, -2, -3..)	insulin-like growth factor binding protein (-1,-2,-3..)
IGT	impaired glucose tolerance
IHD	ischaemic heart disease
INS	insulin
INS RES	insulin resistance
IQR	interquartile range
IUGR	intrauterine growth retardation/restriction
kcal	kilo calorie (unit of energy)
kJ	kilo Joule (unit of energy)
l	liter (unit of volume; derived units: dl, ml, µl)
LBW	low birth weight
LC PUFA	long-chain polyunsaturated fatty acids
LDL	low density lipoprotein
LGA	large for gestational age
LMP	last menstrual period
m	meter (unit of length; derived units: cm, mm, µm)
Mab	monoclonal antibodies

Mg	magnesium
MHz	megahertz, unit of the sound frequency
MmHg	millimeters of mercury (unit of pressure)
MODY	Maturity-onset diabetes of the young
mol	unit of amount of substance; derived units: mmol, μmol,nmol,pmol
MRC	Medical Research Council
MUFA	monounsaturated fatty acids
MWG	maternal weight gain
NADPH	nicotinamide adenine dinucleotide phosphate
NRPNI	National Research Programme for Nutrition Intervention
O_2	oxygen
^oC	Degree Celsia
OGTT	oral glucose tolerance test
p	statistical value of significance
p.f.	post fertilisation
PAR	Predictive adaptive response
PEPCK	phosphoenolpyruvate carboxykinase
PI	proinsulin, also pulsatility index (see context)
PIGF	placental growth factor (also PLGF)
PL PROT	plant protein
PLW	placental weight
PND	Pregnancy, Nutrition & Diabetes Study
PONDIX	ponderal index
PP cells	pancreatic polypeptide producing cells
PUFA	polyunsaturated fatty acids
R	correlation coefficient, also Rand (SA currency; see context)
R^2	multiple regression coefficient
RCT	randomised control trial
RDA	Recommended Dietary Allowances
Rh	Rhesus factor
RI	resistance index

SA	South Africa
SAFA	saturated fatty acids
SYS BP	systolic blood pressure
SD	standard deviation
SEM	standard error of the mean
SES	socio – economic status
SGA	small for gestational age
SR	standard residuals
SST	Serum Separator Tube
T3	triiodothyronine
T4	thyroxine
TMB	tetramethylbenzidine
TOT CHO (TCHO)	total carbohydrate
TOT ENERGY	total energy
TOT PROT	total protein
TSH	thyroid stimulating hormone
UBCoS	Uppsala Birth Cohort Study
UK	United Kingdom
ULSAM	Uppsala Longitudinal Study of Adult Men
US	ultrasound
USA	United States of America
V1, V2, V3	visit 1, visit 2, visit 3
VLBW	very low birth weight
WHO	World Health Organization
WITS	Witwatersrand
Zn	zinc

CHAPTER 1

LITERATURE REVIEW

Conceptual Framework

In this presented Report the “Pregnancy, Nutrition & Diabetes (PND)” Study is being introduced. The research of the PND study overlaps different research fields, namely nutrition, endocrinology and obstetrics. Due to the study complexity, a rather broad literature review was produced, enabling the reader to gain a better understanding of the current research field and the placement of the PND study within the context of this research.

The Literature Review begins with a historical background of the fetal programming hypothesis, introducing the first studies investigating associations between fetal environment, birth size, postnatal growth and later offspring health. Complementary studies on animals and new epidemiologic studies on humans are also discussed. The knowledge gained from these studies gave rise to a new hypothesis, the “Thrifty phenotype hypothesis”, which introduced the idea of metabolic “programming”. Opposing this theory is the “Thrifty genotype hypothesis”, which explains chronic diseases on the bases of thrifty genes. The “Thrifty phenotype hypothesis” was subsequently revised and broadened into the “Developmental origins” hypothesis.

The PND study investigated the relationship of maternal nutrition and hormones with respect to increased risk for Type 2 diabetes later in life. As this is a prospective study, only known markers for this disease were explored. However, the second section of Chapter 1 provides information on the pathophysiology and epidemiology of Type 2 diabetes with a special focus on gestational diabetes and the development of the pancreatic ß-cells.

The third section of Chapter1 reviews stages of human development, concentrating on stages of embryonic and fetal development and the infant birth size. A comprehensive understanding of fetal development and maternal adaptations during pregnancy is important in a search for their determinants, which are crucial for the interpretation of the outcomes observed in the current study.

The last section of the Literature Review Chapter explores in greater detail the determinants of fetal growth and birth weight, as many of them are closely linked to the current study.

1.1 INTRODUCTION

Epidemiological studies carried out worldwide during the last 20 years have shown that chronic diseases may have their origin in fetal and early postnatal life by a process of “programming”. Programming was defined as a ”permanent or long-term change in the structure or function of an organism resulting from a stimulus or insult acting at a critical period of early life” (*Lucas 1998*).

The original observation that initiated the fetal programming hypothesis was that low birth weight and poor fetal growth were associated with coronary heart disease in adulthood (*Barker et al.1986*). Further studies observed similar associations between different patterns of early growth and increased risk of future metabolic and cardiovascular diseases (*Barker et al. 1993a; Barker et al.1993b; Eriksson 2001; Hales et al. 2001; Barker et al. 2002)*. Maternal health and nutritional status were implicated in programming the development and function of pancreatic β-cells, kidney nephrons, or other organs, leading to future health problems (*Hales et al.1992; Barker et al. 1993b; Yajnik et al. 1995; Ravelli et al. 1998; Robinson 2001*; *Fall 2003a; De Boo et al. 2006*).

The first studies were carried out on populations of older people, retrospectively. The existing birth records were compared with current occurrence of illness or death and risk factors were thus identified. A few studies investigated children and young adults. In parallel, a growing number of animal research studies and in vitro experiments investigated how nutritional manipulation in early stages of growth and development could program birth and adult size and their relation to future disease (*Barker et al. 1992; Lucas 1998; Ozane 2001*).

The outcomes from animal studies, in vitro experimentation and epidemiological surveys were the basis on which the present "Developmental origins" of health and disease hypothesis was developed. Thus Barker's "Fetal origins" theory gave rise to a whole new discipline in biomedical science.

The research activities and directions since the very first pioneering studies can be chronologically illustrated in following categories:

1) Establishment of the relationship between birth parameters and later disease
2) Investigation of patterns of early postnatal growth and later disease
3) Focusing on fetal growth in relation to the birth outcomes (with known links to future disease)
4) Nutrition and other determinants of fetal growth
5) Molecular mechanism behind the programming effect (mainly animal and in vitro experiments)
6) Early detection, treatment and prevention

Although the knowledge in each category progressed, the research is not closed, as new links may be discovered and different conditions and different populations can still be compared. Early disease detection and the possibility of early treatment in combination could prevent many debilitating, life shortening and costly chronic diseases.

1.1.1 Fetal origins of adult disease (FOAD) – historical background

Observations that adverse events in childhood may have long term effects on the individual's health existed already early in the 20th century (*Barker 1998; Barker 2002; Fall 2003a*). Explanations were attributed to different living conditions during childhood. It was the study of DJP Barker and his colleagues (*Barker et al.1986*), who retrospectively investigated neonatal and post-neonatal death rates among babies in **England and Wales**,

from which a novel concept of “Fetal origins of adult chronic diseases” evolved. This hypothesis states that poor nutrition during fetal life causes disproportionate fetal growth and results in coronary heart disease (CHD) in adulthood (*Barker1995*). Results of this study showed that the geographical pattern in rates of neonatal mortality early in the 1900s coincided with the adult mortality rate distribution of coronary heart disease in the 1960s and 1970s. As the most commonly reported reason for the infant’s death at that time was low birth weight, a suggestion was made that low growth rate before birth may be linked to coronary heart disease later in life. A similar pattern was discovered between mortality rates among mothers with a poor maternal health and later death rates from stroke among their offspring (*Barker et al. 1987*).

Barker’s original observation was followed by a series of epidemiological studies carried out on groups of men and women from Hertfordshire, Preston and Sheffield in the UK, for whom birth and early infancy growth records were available.

The **Hertfordshire** study (*Hales et al. 1991; Barker et al 1993a; Barker 1995*) investigated present cardiovascular mortality of men currently in their 60s’ in connection with birth weight and measurements of growth in infancy. Those who were born small and who were small at one year of age showed the highest risk of death from ischaemic heart disease (IHD) and stroke as adults (Figure 1.1).

Also in Hertfordshire, babies with slowest prenatal and early postnatal growth had the highest incidence of impaired glucose tolerance (IGT) and Type 2 diabetes in later life (Figure 1.2). This was the first indication of a link between Type 2 diabetes, pregnancy outcome and infant development. Low birthweight was also associated with an increased risk for the

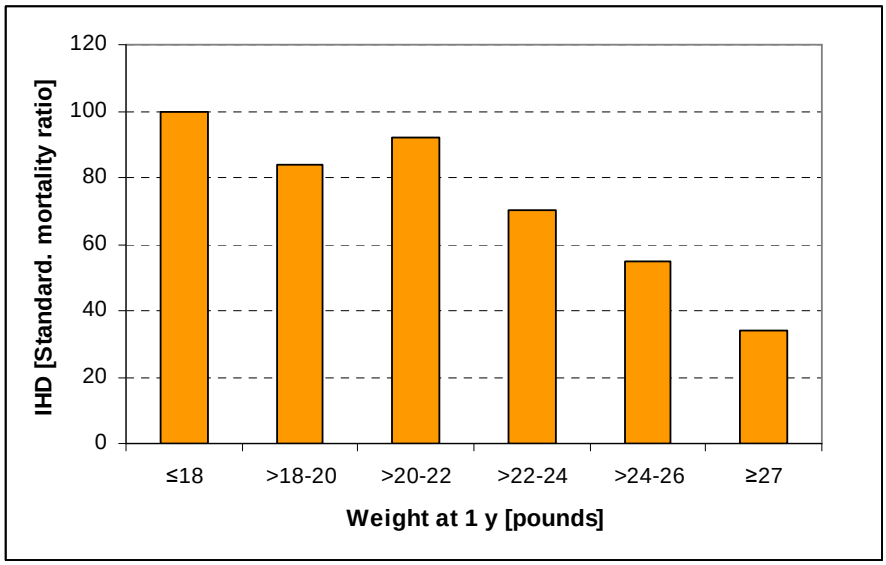


Figure 1.1 Ischaemic heart disease (IHD) in men in Hertfordshire at age of 59-70. (Weight at 1 year in kg: 8.2, 9.1, 10.0, 10.9, 11.8 and 12.3) (Source of the data: *Barker et al. 1992*)

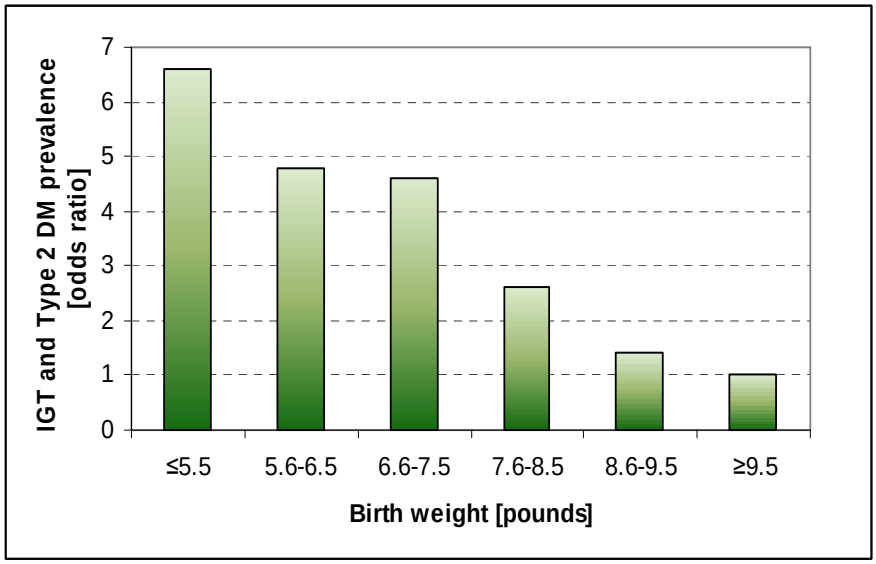


Figure 1.2 Impaired glucose tolerance (IGT) and Type 2 diabetes prevalence expressed as odds ratio in men in Hertfordshire at age of 59-70. (BW in kg: 2.5, 2.54-2.95, 2.99-3.41, 3.45-3.86, 3.90-4.31) (Source of the data: *Barker et al. 1992.)*

metabolic syndrome[1], elevated concentrations of fibrinogen and markers of ß-cell function - proinsulin, 32 – 33 split proinsulin and insulin. It was suggested that metabolic syndrome and insulin resistance might all be linked and determined by factors reducing fetal and infant growth. A possible influence of maternal nutrition on pancreatic ß-cell development was considered as an alternative to the "thrifty genotype" theory (see Chapter 1.1.3.2) (*Hales at al. 1991; Barker 1992; Barker et al 1992; Barker et al 1993a; Barker 1995*).

In the Preston study two high-risk categories of babies leading to hypertension later in life were identified according to their birthweight and placental weight (*Barker et al. 1990*; *Hales et al. 1992*; *Barker et al.1993a).* The first group of babies, who also developed insulin resistance and/or impaired glucose tolerance in adulthood, were babies born small at term with low ponderal index, small head circumference (symmetrically growth restricted) and with disproportionately large placentas.

The second group, who developed high blood pressure in adulthood, were babies with above-average birthweight, large placentas and head circumference but of a short birth length (asymmetrically growth retarded). These babies did not develop metabolic syndrome but they

[1] The Metabolic Syndrome (originally named Syndrome X or Insulin Resistance Syndrome) was proposed by GM Reaven (*1988*). It referred to metabolic and vascular abnormalities i.e. hypertension, glucose intolerance and dyslipidaemia (elevated plasma triglycerides and low high-density lipoprotein cholesterol levels) occurring together in individuals more commonly than could be explained by pure chance. The original factors were extended to visceral obesity, hypercoagulability and microalbuminuria (*WHO 1999; Zimmet et al. 2001)*. These abnormalities represent a high risk of progressing to Type 2 diabetes, coronary heart disease and other diseases associated with insulin resistance (*Turner et al.1993; Reaven 1995*; *Bogardus 1995; Johnson et al. 2006*). The above metabolic and physiological disorders may have a common aetiology in insulin resistance (*WHO 1999*).

were predisposed to development of other abnormalities such as raised plasma fibrinogen levels later in life *(Barker et al. 1990; Barker 1992; Barker et al. 1992; Barker et al.1993a; Phillips et al. 1994; Barker 1995).*

Individuals who were found with the lowest blood pressures as adults were born large with small placentas. These effects were irrespective of the social class at the time of birth or social class in adult life *(Barker et al. 1990)*. Similarly, as in the Hertfordshire study, it has been postulated that the blood pressure in adulthood is related to the intrauterine environment and factors constraining fetal growth. As the systolic BP in adults rose with the increasing placental size at birth, a suggestion was made that the large placenta might be an adaptive response to a poor maternal nutrition during pregnancy (*Leon 1999*).

A study in Sheffield (*Barker et al. 1993b; Barker 1995; Barker1998*) investigated how fetal growth and size at birth were related to later disorders in cholesterol metabolism, blood coagulation and subsequent death rates from cardiovascular disease. Research was based on detailed obstetric and delivery records of only men born in one maternity hospital between the years 1907 and 1924. Findings were consistent with the earlier Hertfordshire and Preston studies (see above; *Barker et al. 1993b*). Reduced fetal growth associated with changes in blood vessels and pancreatic β-cells was implicated in programming of blood pressure, glucose and insulin metabolism and cardiovascular disease. Again, as in the previous studies of Hertfordshire and Preston, the maternal undernutrition during mid- or late gestation was suggested as the factor causing disproportionate fetal growth. Changes in length of gestation or social class had no influence on cardiovascular mortality.

As the hospital records on the birth, placental and infant size showed consistent trends with development of later disease among the older population, the interest of some studies focussed on younger population groups. Two studies in Salisbury and Southampton investigated a population of 4-year old children and 20-year old men respectively. Also outcomes for these age groups were found in agreement with the above studies on older people.

The **Salisbury study** (*Law et al. 1991; Barker 1992; Barker et al. 1993b)* found relationships between fetal growth, birth weight, birth size, placental weight and blood pressure and insulin response similar to those described previously.

The **Southampton** prospective observational study (*Godfrey et al. 1996*) investigated the effect of maternal nutrition on placental and fetal growth. The results showed that placental and birth size correlated with maternal intake of carbohydrate and protein. High carbohydrate intake in early pregnancy suppressed placental growth, having a greater effect in combination with low dairy protein intake in late pregnancy, whilst the birth weight was found inversely related to the meat protein intake also in late pregnancy. Not only the protein source, but also protein density appeared to be important for placental and fetal growth.

1.1.2 Worldwide epidemiological studies in developed and developing countries.

Similar trends have been reported from epidemiological studies carried out in different populations and ethnic groups in Europe (Uppsala study; Helsinki study; Dutch famine study), United States (New York Nurses' Health Study; Pima Indians in Arizona), in China, India (Pune study) and others (*Hales et al. 2001*). The only exception to this is the Leningrad Siege Study. Some of the most important studies will be discussed below.

The Uppsala studies (Sweden). Two cohorts were retrospectively investigated for relation between the birth size and a future disease outcome. The Uppsala Longitudinal Study of Adult Men (ULSAM) found an inverse correlation between birth weight and hypertension, and with some other components of the metabolic syndrome (insulin resistance, truncal obesity and high plasminogen activator inhibitor-1 levels) in later life *(Byberg, et al. 2000*). However, contrary to the Preston study, where the risk for future hypertension rose with increasing placental weight, in the ULSAM cohort this trend was opposite. The men with lowest blood pressures had higher placental weights at delivery, irrespective of their birth weight (*Leon 1999*).

In the ULSAM cohort, it also has been established that the indirect relationship between size at birth (as low ponderal index) and later development of Type 2 diabetes was mediated through insulin resistance rather than through impaired β-cell function. Obesity in adulthood added to the risk (*Lithell et al. 1996*).

The second cohort (Uppsala Birth Cohort Study – UBCoS) study based on detailed obstetric records of Swedish men and women retrospectively studied a relation between size at birth and mortality from cardiovascular diseases. There was found an inverse correlation between fetal growth rate and current mortality from IHD (*Leon et al.1998; Leon 1999*). This finding shows that the fetal growth rate may be even more important predictor of the future disease risk than are the birth parameters.

In the Helsinki study (Finland) first a cohort of men born in Helsinki University Hospital between 1924-33 was investigated. Later, this cohort was extended by a group of men and women born between 1934-1944.

The first study aimed to evaluate the relation between birth size and death rates from coronary heart disease (CHD). Further follow-up tried to establish if the childhood growth could alter links between impaired fetal growth and the later death from CHD (*Forsén et al.1997; Eriksson et al. 1999*).

Low birth weight and body size at birth (i.e. low ponderal index), as well as low height, weight and BMI at 1 year of age were associated with increased risk of later CHD. Rapid catch-up growth after 1 year of age in men born with ponderal index below 26 kg/m^3 contributed to a further risk increase (*Forsén et al.1997*; *Eriksson 2001; Eriksson et al. 2001*). However, gaining weight during the first year of life was associated with decreased risk for developing CHD later in life, especially for those who were born with an above average ponderal index and had an above average height and BMI at 1year of age *(Eriksson et al. 2001)*.

Further investigation showed that the early adiposity rebound[2] was connected to the development of both obesity and Type 2 diabetes (Figure 1.3, 1.4). Two different pathways leading to these diseases were identified. Low birth weight, thinness at birth and infancy followed by an accelerated growth during early childhood were associated with type 2 diabetes, whilst high birth weight and high neonatal ponderal index were linked to obesity later in life. However, some people born with a higher birthweight also developed Type 2 diabetes, if they experienced slow linear growth during early infancy and had a high childhood BMI. Therefore the association between the birthweight and Type 2 diabetes was actually U-shaped (*Eriksson 2001; Eriksson et al. 2002; 2003a; 2003b; Barker et al. 2002*). This pattern has previously been reported in other population groups, e.g. Pima Indians (see further; *McCance et al. 1994*).

[2] During period of childhood, a child passes through a stage, when its body mass index (BMI) decreases to a minimum (after age of 2 years) before increasing again after 6 years of age, the so called **adiposity rebound period.** Age at adiposity rebound corresponds to the age of the lowest BMI between one and 12 years (*Eriksson et al. 2003a*).

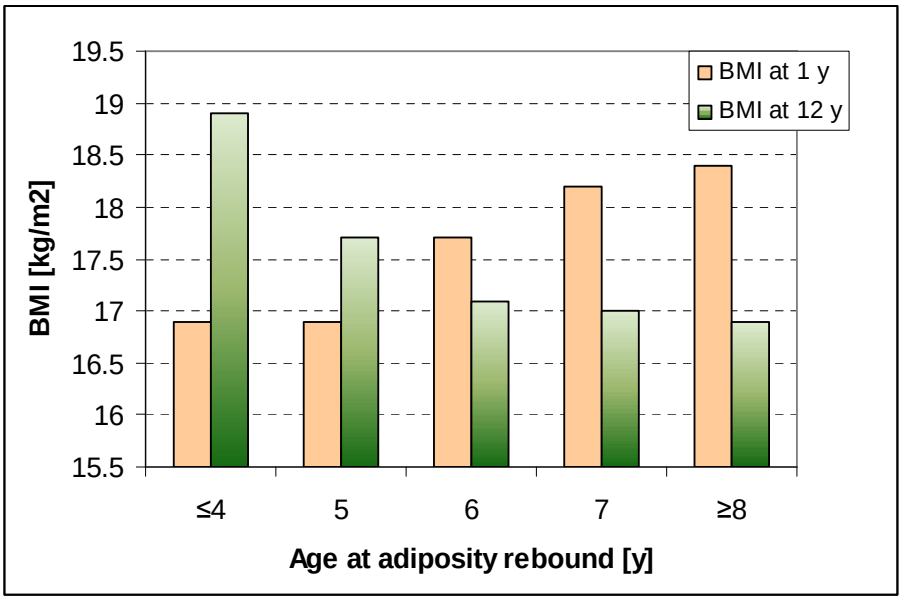


Figure 1.3 Age at adiposity rebound in relation to BMI at 1 y and 12 years of age for both genders combined (Helsinki study; *Eriksson et al. 2003a*).

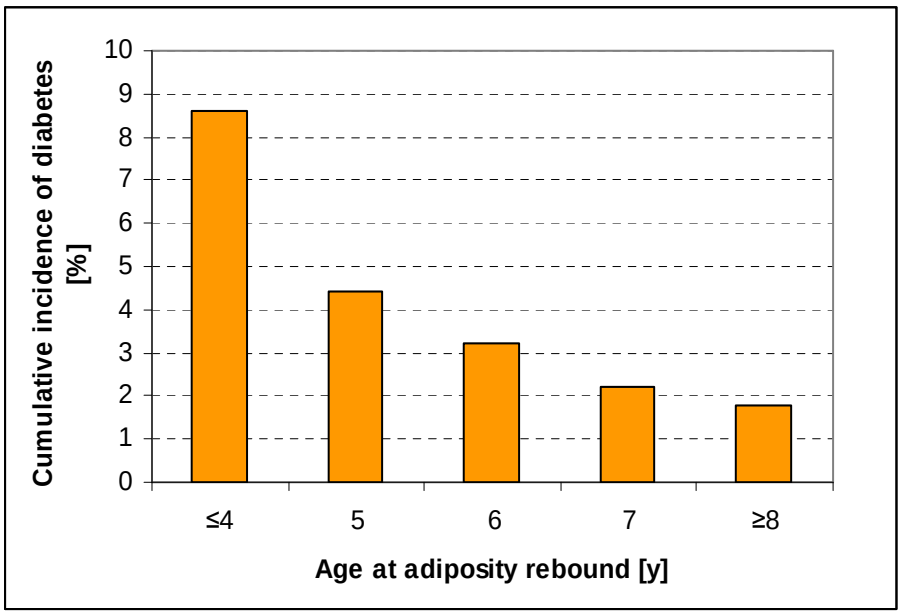


Figure 1.4 Age at adiposity rebound and accumulative incidence of diabetes (Helsinki study; *Eriksson et al. 2003a*).

Dutch Hunger Winter study. This study analysed the period of famine in Holland during November 1944 to May 1945. During this time, the daily energy intakes in this otherwise adequately nourished population decreased below 1000 calories per day (*Ravelli et al. 1998).* Due to the relatively short duration of the famine, this study enabled a comparison between different pregnancy exposures.

Those people who were exposed to the famine *in utero* early in gestation, showed higher rates of subsequent obesity, measured by BMI and waist circumference (*Ravelli et al. 1999*). They also had higher prevalence of CHD later in life than non-exposed people, independently of their birth weight (*Rosenboom et al 2000*).

Individuals exposed to the famine in mid and late gestation had the highest blood glucose levels at 2 hours of their oral glucose tolerance test (OGTT) as adults, especially if born thin and becoming obese as adults (Figure 1.5; *Ravelli et al. 1998).*

Undernutrition at this time of gestation led to impaired glucose tolerance and Type 2 diabetes, while exposure to famine in early gestation was associated with abnormalities in lipid metabolism. Outcomes also showed that maternal diet during pregnancy could programme metabolism without altering size at birth. Only late pregnancy exposure to famine caused variations in birth size and body proportions (*Ravelli et al. 1998; Rosenboom et al 2000; Hales et al. 2001; Stein et al. 2004*).

This study has not found any relation between the low birthweight or small birth size and later development of hypertension, as did other previously reported studies.

Recent findings for this cohort are also discussed in Chapter 1.4.2.1.2.

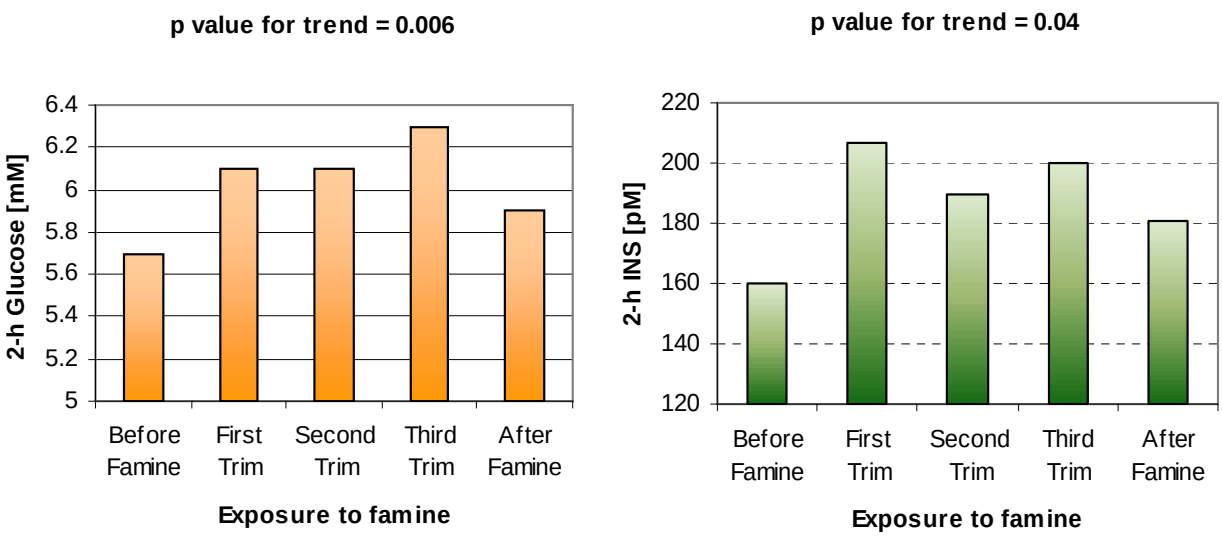


Figure 1.5 Glucose and insulin levels of offspring born before famine, exposed to famine during different times of prenatal life or conceived after famine (Dutch Hunger Winter study). Data expressed as geometric means; n=702 (*Ravelli et al. 1998*).

The Leningrad Siege study (Russia; siege of Leningrad during period of 1941 to 1944; *Stanner et al. 2001*). This study investigated three cohorts. The first cohort included people exposed to starvation *in utero*, with continuation in infancy. The second cohort involved those who were exposed to the siege in early infancy only (at least 10 weeks old and adequately nourished during pregnancy). In the third cohort there were people born out of the city, therefore not exposed to starvation. No association was found between intrauterine starvation and glucose intolerance, dyslipidaemia, hypertension or cardiovascular disease later in life.

This outcome differed from the Dutch Hunger Winter study, however, the conditions of both studies were also different. As discussed above, the famine in the otherwise adequately nourished Dutch population was of a relatively short duration and soon after the liberation of Netherlands in May 1945 the daily caloric intake more than doubled (*Ravelli et al 1998*). In contrast, the children born during or after the siege of Leningrad, continued to be exposed to poor nutrition and had no catch-up growth (*Hales et al. 2001; Stanner et al. 2001*).

The American Nurses' Health Study. A cohort of registered nurses with mean age of 42 years was followed between 1976 and 1992. An association was reported between low birth weight and the later increased risk of non-fatal coronary artery disease and stroke (*Rich-Edwards et al. 1997*).

A study in **Beijing**, **China,** followed records of people in their 40s' born during time of chronic malnutrition in China. The mothers of studied offspring were thin and short (155.6 cm) in average. Low maternal BMI (20.9 ± 6.2 kg/m^2) at 15 and (24.8 ± 2.4 kg/m^2) at 38 gestational weeks, as well as the baby's birth weight, were significantly correlated with increased levels of plasma glucose, insulin and triglycerides in adult offspring (*Mi et al. 2000*). The birth weight, but not maternal BMI, was also inversely associated with the offspring's blood pressure later in life.

Pima Indians in Arizona are a population with the highest reported prevalence of Type 2 diabetes, following their transition from farming to sedentary lifestyle (*Turner et al. 1993)*. Birth weight was found to be associated with the prevalence of diabetes in a U-shape manner (*McCance et al. 1994*). Thus, low birth weight was associated with a high risk of adult

diabetes, as was a high birth weight. A high prevalence of parental diabetes in the low birth weight group of infants, suggested possible genetic predisposition of these babies to insulin resistance and Type 2 diabetes. Due to a relatively high mortality among low birth weight infants, the authors proposed, that those who lived and later became diabetic, might represent a selective survival mechanism. The association of the high birth weight babies with later diabetes may be due to gestational diabetes.

The study in Mysore, an urban area in **South India,** investigated the relationship between data from birth records and current glucose and insulin metabolism of the 39-60 years old adults (*Fall et al. 1998*). The high prevalence of Type 2 diabetes (15%) correlated with short stature at birth and high ponderal index (obesity). The high ponderal index was associated with reduced β-cell function.

Pune Maternal Nutrition and Fetal Growth Study in India is a large community-based observational study involving women from rural areas near the city of Pune, Maharashtra and their offspring. This was the first study to follow maternal pre-pregnancy anthropometric measurements in relation to the offspring's birth weight and size. Maternal nutrient intake during pregnancy was also evaluated (*Yajnik et al. 2003a*). The birth parameters of Pune babies and maternal size and nutrition were compared with the Southampton babies (*Godfrey et al. 1996*) of a relatively well-nourished population in the UK (see the Southampton study).

The mothers in Pune study were lighter (42.0 kg), shorter (1.52 m) and had lower BMI (18.0 kg/m^2) than mothers in the Southampton study (63.0 kg, 1.62 m, 23.5 kg/m^2 respectively; *Yajnik 2002: Fall 2003b*).

The full term babies in Pune had lower birth weight than the Caucasian babies (2.7kg vs. 3.5kg), they were small and thin with low muscle mass but centrally obese ("thin-fat phenotype"), preserving fat during their intrauterine development (*Yajnik et al. 2003a; Fall 2003b; Yajnik 2004*). Nearly 30 % of the Pune babies were born with the weight below 2.5 kg (*Rao et al.* 2001; *Yajnik 2002*), which was in accordance with the national data. Indian babies are known to be among the smallest in the world. This is associated with chronic undernutrition in the Indian population (*Yajnik 2004*). The size of Pune babies reflected parental size. The nutritional history of the mother was reflected in her anthropometric measurements, especially in her head circumference, which in turn was strongly correlated with the overall fetal growth of her offspring (*Yajnik et al. 2003a, b*).

In Pune, the links between offspring's birth weight and future insulin resistance and Type 2 diabetes were also studied in 8-year old children (*Yajnik 2002).* The systolic blood pressure, plasma LDL cholesterol, fasting and 30-min plasma insulin, 30-min plasma glucose and HOMA-IR index of these 8-year old children were all inversely associated with the children's birth weight (see Figure 1.6 A, B, C; *Bavdekar et al. 1999*).

Taller children of shorter parents were found to be at higher cardiovascular risk. The strongest predictor was the growth velocity between 4 and 8 years of age (*Bavdekar et al. 1999; Yajnik 2004*). Together with rapid childhood growth the "thin-fat" phenotype represented increased risk for future obesity-associated diseases (insulin resistance and hypertension) and Type 2 diabetes. Risk was higher than in Caucasian population (*Yajnik et al. 2003; Fall 2003a; Yajnik 2004).*

Figure 1.6 A

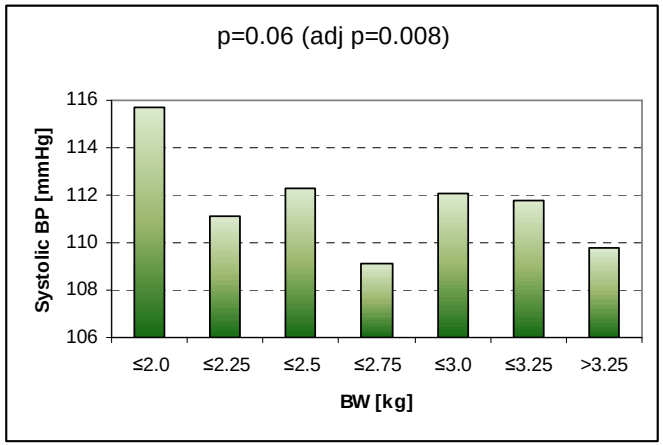


Figure 1.6 B

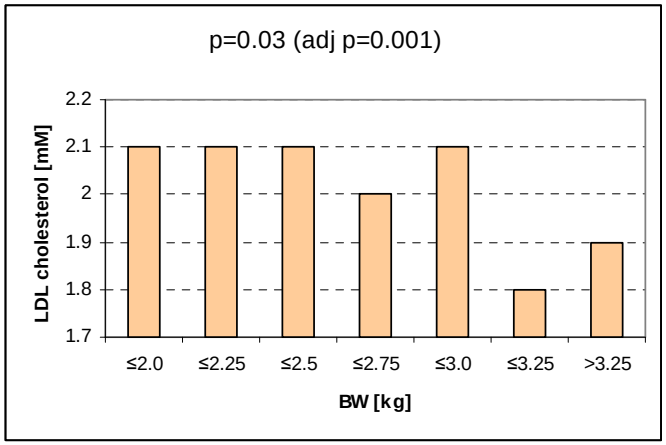


Figure 1.6 C

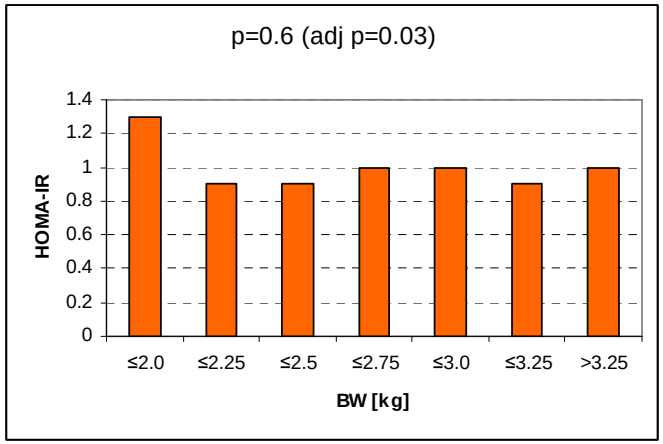


Figure 1.6 Systolic blood pressure (Figure A), LDL cholesterol (Figure B) as cardiovascular risk markers and the HOMA-IR index (Figure C) in 8-year old children of Pune study plotted according to their birth weight. The differences across the birthweight categories became stronger after adjustment for age, sex and current body weight (adjusted p value).

(According to: *Bavdekar et al. 1999)*

The South African Studies.

There are a limited number of studies investigating the relationship between birth and postnatal characteristics in respect to the health - disease risk within South Africa. Fields of interest, which have been partly assessed, include obesity, metabolic syndrome, bone metabolism and osteoporosis (*Levitt et al. 2005a*). Some examples of these studies include the following.

The Birth-To-Ten (BTT) study is a longitudinal, prospective study of urban children and their families. It included all births during a 7-week period from April to June 1990 in Soweto-Johannesburg, enrolling over 4000 cases (*Richter et al. 1995)*. The original 10-year follow-up period was extended by further 10 years into the Birth-To-Twenty study. Systolic blood pressure in 5-year-old children of this follow-up inversely correlated with their birth weight. This observation was independent of confounding variables such as current weight, height, gestational age or socio-economic status (*Levitt, et al. 1999b*).

Low birth weight and rapid weight gain in childhood led to poor glucose tolerance in 7-year-old BTT children, using the oral glucose tolerance test for assessment (*Crowther et al. 1998*). Low birth weight children without catch-up growth had diminished glucose tolerance. Catch-up growth between birth and 7 years in the absence of catch-up growth between birth and 1 year was characterised by elevated proinsulin and insulin levels. The catch-up growth within the first year of life as well as between birth and 7 was associated with lower proinsulin and insulin levels (*Crowther et al. 2008*).

Other South African cohort studies:

Levitt et al analysed the birth records and traced individuals born in the Groote Schuur Hospital Maternity Center in Cape Town 20 years ago. Those born with low birth weight had elevated systolic and diastolic blood pressures and elevated fasting plasma glucose levels at 20 years of age. Low birth weight individuals had high blood pressure and glucose levels without being obese as adults. The hypothalamic-pituitary-adrenal (HPA) axis has been implicated in early life programming of future cardiovascular and metabolic disease in humans (*Levitt et al. 2000b*).

In a cohort of 100 prematurely born or SGA infants in the Johannesburg General Hospital, glucose tolerance was associated with baby's birth weight irrespective of gestational age (*Gray et al. 2002*). Mothers treated for hypertension had babies of lower birthweight. Neonatal catch-up growth rate was negatively correlated with birth weight, and positively correlated with insulin sensitivity.

In summary, these epidemiological studies, with the exception of the Dutch Hunger Winter study and the Leningrad study, all demonstrate the negative effect of low birth weight. Low birth weight, thinness at birth (measured by low ponderal index) and a short stature reflect reduced intrauterine growth. The published data show that these parameters are associated with a high blood pressure, coronary heart disease and metabolic disease later in adulthood. Large placental weight (in the Preston study), failure of catch-up growth in infancy and a rapid growth in childhood with subsequent obesity (Helsinki study) further increase the disease risk. The relation between birth weight and future disease has a U-shape character, therefore also babies of above-average birth weigh and high ponderal index at birth may

experience increased risk of future disease. Poor maternal nutrition has been implicated in programming effects leading to impaired fetal and infant growth.

1.1.3 "Thrifty genotype" and "Thrifty phenotype"

Two possible hypotheses have been formulated to explain the mechanism by which poor fetal growth is linked to adult chronic health status. Both hypotheses will be discussed in the following sections.

1.1.3.1 "Thrifty genotype" hypothesis (JV Neel, 1962).

This hypothesis proposes that the genes selected during human evolution, adjusted the carbohydrate and lipid metabolism to an optimal use of energy resources between times of abundancy and times of shortage (*Turner et al.1993; Coughlan et al. 1997; Zimmet et al. 2003*). These "thrifty" genes represented a survival mechanism in the time of irregular nutrient resources. However, in the environment of continuous high-energy food availability combined with a sedentary life-style, these genes became diabetogenic (*Neel 1962; Cassell et al. 2003*). Pancreatic β-cell overstimulation leads to hyperinsulinaemia, insulin resistance and exhaustion of pancreatic islets, ultimately resulting in diabetes (*Neel 1962, Turner et al. 1993; Mackenzie et al. 1996; Coughlan et al. 1997).*

The application of the "Thrifty genotype" hypothesis was then extended to fetal life. The relationship between adult diseases and in utero environment was explained by suggesting that in times of poor maternal nutrition the babies that survived were those that carry genetic

polymorphisms (i.e. the “thrifty genes”) that make them less insulin sensitive. This insulin resistance ensures that glucose levels are maintained for brain consumption.

The “Thrifty gene” hypothesis also suggests that some of the mutations neither presented any survival advantage at the time of the unstable food supplies (hunter-gatherer), nor were pathogenic. However, they possibly accumulated (silent polymorphism), and in an altered environment became detrimental (*Turner et al. 1993;* Figure 1.7).

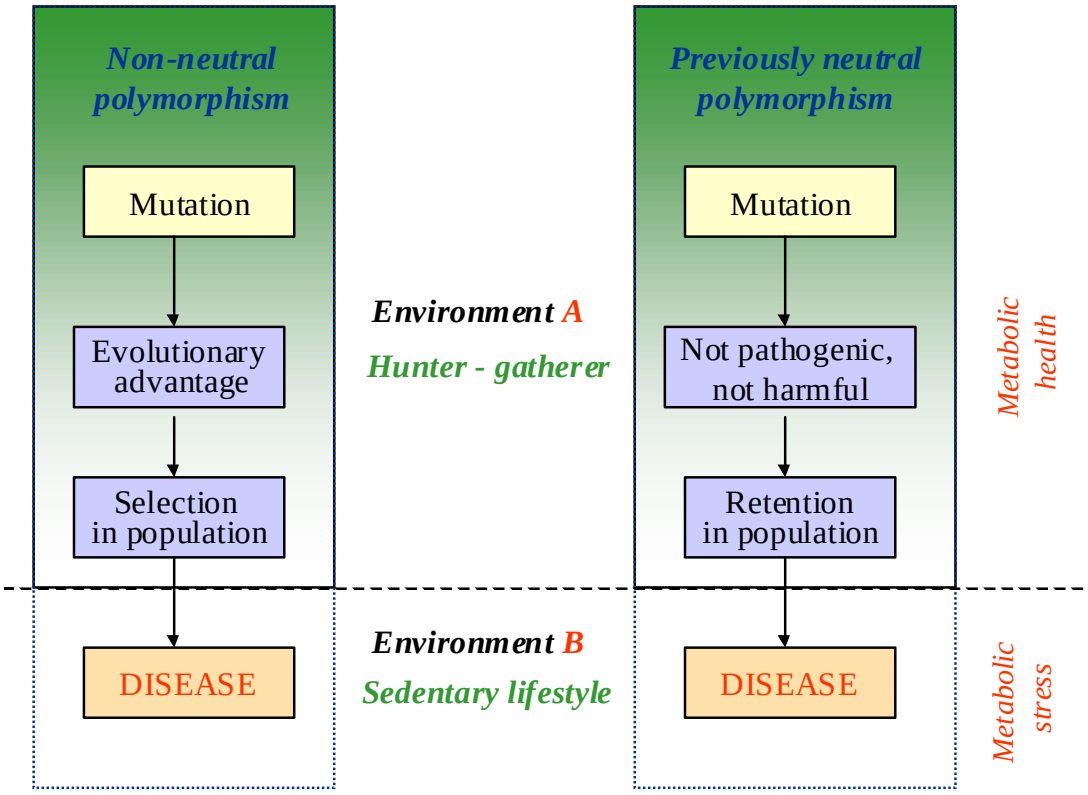


Figure 1.7 “Thrifty genotype” and “Previously neutral polymorphism” hypotheses (Adapted from *Turner et al. 1993*, Fig. 1)

1.1.3.2 “Thrifty phenotype” hypothesis.

The “Thrifty phenotype” hypothesis (*Hales et al. 1992)* proposes that Type 2 diabetes is possibly an outcome of environmental factors, namely maternal undernutrition, which act *in utero* in the process of metabolic “programming”. The metabolic programming is an essential tool for survival under conditions of inadequate food supply. The postnatal environment (nutrient availability) will decide if the predisposed individual will eventually progress to the disease in later life (Figure 1.8). The genetic factors are not considered in this theory or they may have a minimal effect.

During the periods of rapid cell replication or growth, the organs and other developing systems become sensitive to environmental effects (*Barker 1997*). Inadequate maternal nutrition during these critical phases of early life can permanently alter the structure or function of different organs and tissues, including blood vessels, during their development (*McCance 1962; Ozane et al. 1999*). The timing of the external insult could determine the kind of future metabolic problem. This process is known as the “programming effect” (*Lucas 1991*; *Barker 1997; Hales et al.2001*). In an optimal *in utero* environment, the programming process is part of natural development.

A fetus exposed to low oxygen or low nutrient environment has insufficient energy resources for normal growth. For immediate survival, it adapts to the situation by a selective down-regulation of the growth and metabolic rates of different tissues. The oxygenated blood flow is diverted to vital organs, such as brain, heart, lung and adrenals (*Campbell 1967*; *Barker 1997; Barker 1998; Ozanne et al. 1999*) at the expense of the liver, pancreas, spleen,

kidneys, muscle and gut (*Desai et al.1996*; *Haugen et al.2005*). This mechanism is also known as the "Brain sparing effect*" (Barker 1997; Barker 1998).*

Therefore, temporarily, the fetus will benefit. In the long term, if the low nutritional environment remains constant, programming would be appropriate and aid survival. However, if there is a poor intrauterine nutritional environment followed by abundant nutrition, these changes could have harmful consequences (Figure 1.8; *Ozanne et al.1999*).

Pancreatic islets of Langerhans are highly vascularised *(see Chapter 1.2.5.1; Bonner-Weir 1990*). Therefore, changes to the islet vasculature induced by poor fetal nutrition will affect the pancreatic β-cells and limit their insulin production (*see Chapter 1.2.5.4)*. In combination with insulin resistance caused by poor fetal nutrition and enhanced by adult obesity this would lead to impaired glucose tolerance, and Type 2 diabetes later in life (*Hales et al. 1992*; *Barker et al. 1993a; Ozanne et al. 1999*; *Hales et al. 2001*).

In a similar way, a low number or size of nephrons in the kidney could lead to increased susceptibility to hypertension later in life. Alternatively, changes in hepatic development could cause an increased risk of cardiovascular and metabolic disease in adulthood (*Haugen et al. 2005*). Experiments with rats (*Desai et al. 1996; Lucas 1998*), evaluating a low protein diet to mothers during gestation, showed an alteration in hepatic carbohydrate metabolism in these offspring. The metabolic shift was associated with increased liver PEPCK (phosphoenolpyruvate carboxykinase) enzymatic activity and a decrease in the glucokinase enzymatic activity, stimulating glucose production rather than utilization.

Each of these enzymes originates from the hepatocytes of different liver zones (catabolic periportal vs anabolic perivenous –linked to glucokinase), therefore it has been suggested, that programming affects these zones differently. Similarities with this outcome were observed also with activities of other liver enzymes of carbohydrate metabolism (*Desai et al. 1995; Byrne et al. 2000).*

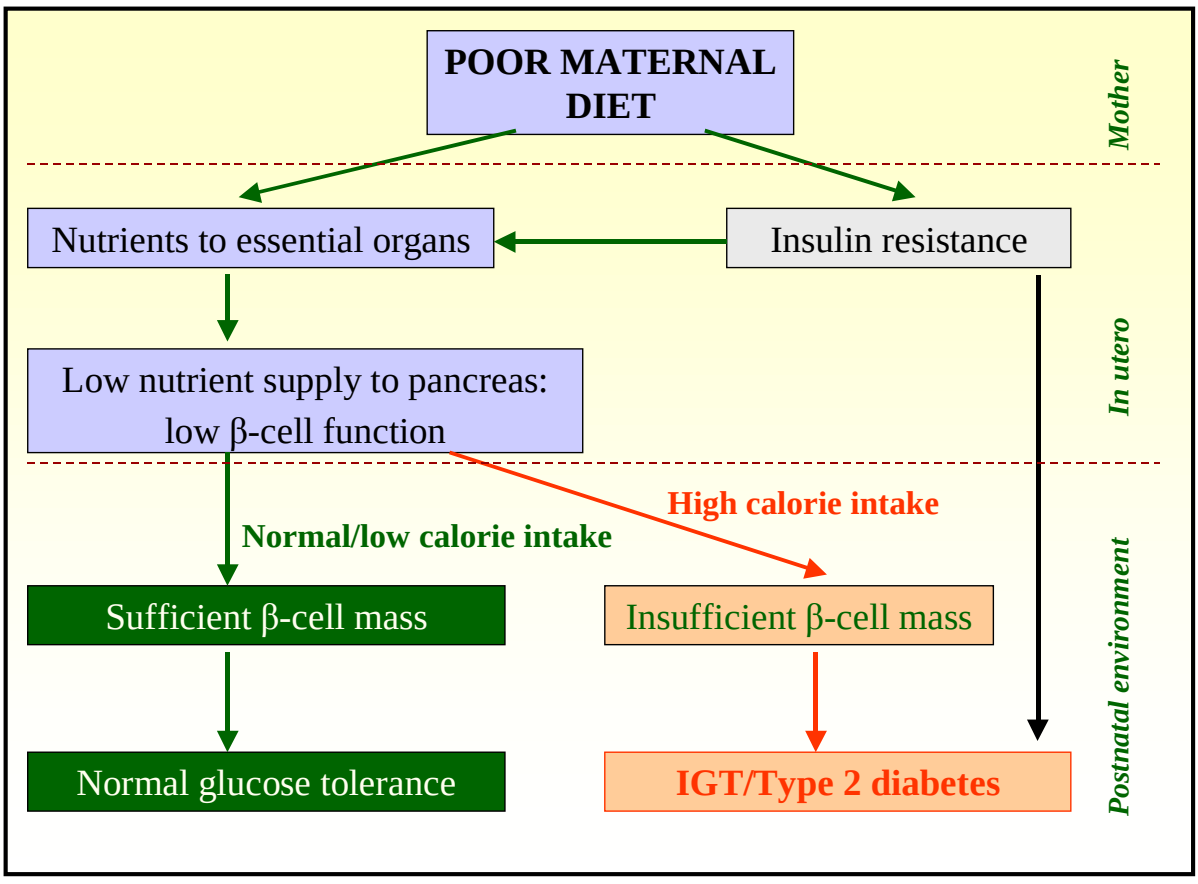


Figure 1.8 "Thrifty phenotype" hypothesis. Effect of maternal undernutrition on the aetiology of Type 2 diabetes (*adapted from Hales et al.1992*).

The risk of future chronic disease can be modified by additional (lifestyle) postnatal factors such as catch-up growth and adult obesity (*Eriksson et al. 2001; 2003a,b*). Thus insulin resistance or metabolic syndrome are more common in subjects with low birth weight who became obese as adults (*Phillips 1996*). Low body mass index in adulthood has a protective role (*Hales et al. 1992; Wilkin 1993*; *Barker et al. 1993a).*

The Thrifty Phenotype Hypothesis may be further supported by the follow-up studies of Danish diabetic twins (*Poulsen et al. 1999a,b; Poulsen et al. 2006, Poulsen et al. 2009*) or of a large cohort of Swedish twins (*Iliadou et al. 2004*). In the former study the Type-2 diabetic monozygotic co-sibling had lower birthweight than the unaffected twin and the non-diabetic monozygotic twins (usually sharing one placenta) were more insulin resistant than dizygotic twins (developing in separate placentas). From these outcomes the authors suggested that the intrauterine environment is an important determinant of future disease development, where low birthweight or monozygosity negatively influence glucose homeostasis, irrespective of genetic cause. There are, however, other studies on twin gestations that have been published, which were inconclusive and had variable outcomes. For example, in the New Zealand twin study (*Williams et al 1999),* mothers who smoked during pregnancy when compared to non-smokers had babies of a lower birth weight and of a higher blood pressure at ages 9 and 18 years, supporting the "Fetal origins hypothesis". However, opposing this hypothesis, the twin vs. singleton babies had a lower birth weight associated with a lower blood pressure at age 9 years. De Geus et al (*2001*) investigated blood pressure among participants of the Netherlands Twin Registry in their adulthood, comparing them with their singleton siblings. No significant difference in blood pressure was found, despite a significantly lower birth weight among twins. Similarly, comparing the differences in birth

weight within twin (monozygotic and dizygotic same sex) pairs with their blood pressure at age of 7 years, the authors (*Zhang et al 2001a*) failed to find any significant outcomes linking the *in-utero* environment with the blood pressure in the child. Contrary to this, a similar study in the UK (*Poulter et al. 1999*) investigating female twin pairs, found an inverse association between the birth weight of a twin and her blood pressure later in adulthood. Therefore, more studies on twins or multiple pregnancies, where the fetuses develop in a shared environment, may be needed to clarify possible *in utero* effects linked to future adult diseases.

With additional knowledge emerging, the "Thrifty phenotype" hypothesis has further been revised by Barker and Hales (*Barker 1995, 1998; Hales et al. 2001)* and quite recently broadened into the "**Developmental origins" hypothesis** (DOH) by Gluckman and Hanson (*Sharp 2005; Gluckman et al. 2006*). The Developmental Origins Hypothesis accepts the concept of gene-environment interaction and explains the origins of human disease from the aspect of human evolution. This hypothesis will be discussed in the following section.

1.1.4 "Developmental origins" hypothesis of future health and disease.

As the early life events involved in programming are not restricted to the prenatal period only, but also include the neonatal period, infancy and childhood (*Forsén et al. 1997*; *Eriksson et al. 1999b, 2001, 2002, 2003a,b; Eriksson 2001; Barker et al. 2002),* a broader terminology of "developmental" instead of "fetal origins" has been used.

An important natural feature (in many plants, animals and humans; *Crespi et al. 2005*) is a "phenotypic plasticity". In this process, a single genotype can lead to a range of phenotypes suitable for different environments *(Barker 2004; Gluckman et al. 2005b; Denver et al. 2006; Gluckman. et al. 2006).* While one phenotype may represent an advantage in one environment (selects survival/fitness in early life), others may be disadvantageous and carry a disease risk (*Gluckman et al. 2005a*). These developmental trajectories may be programmed by an insult acting during "critical windows" of early development through the mechanism of "developmental plasticity" or they can be a consequence of the circumstances to which the parents or grandparents were exposed (*Bateson et al. 2004*). Once the organ development is completed, the plasticity cannot apply.

During fetal or postnatal development an environmental insult may lead to a so called biological "trade off"*(Kuzawa 2005; Crespi et al. 2005),* which produces an immediate short-term advantage (survival, growth) at the cost of a future long-term disadvantage (e.g. in reproductive health, longevity, disease).

Contrary to the biological trade-off, the "Predictive adaptive response" (PAR; term was introduced by Gluckman and Hanson; *Gluckman et al. 2005a*) does not have any immediate effect. It is believed that maternal information (metabolic and endocrine signals) allows the developing fetus to sense and evaluate its environment during the plastic period and to "predict" and adapt its development to the expected conditions in postnatal life (*Gluck et al. 2005b*). This is a natural process, in which phenotypic changes may be mediated via epigenetics (changes in gene expression; see Chapter 1.4.2.1; *Cassell et al. 2003*).

The correct assessment of the future environment ("Appropriate PAR") brings benefit to the individual in respect of maximal fitness in adulthood (*Gluckman et al. 2005a*). However, if the selected trajectory is incorrect (failure in maternal signaling or sudden changes in future environment; termed "Inappropriate PARs"), the individual may be exposed to an increased risk of future disease (*Gluckman et al. 2005a; 2007*). According to this hypothesis, the fetus is able to sense the availability of nutrients *in utero* and infer the postnatal nutrient environment. The fetus adapts (during critical windows of its development) to the expected future nutrient supply in a manner that brings it maximal survival and reproductive fitness benefit. The programmed changes are irreversible and result with permanently altered responses to the nutrient or other stimuli during future life (*Srinivasan et al. 2003*). Therefore, if a mismatch between prediction and reality occurs, the selected phenotype will lead to a disease.

An example of a mismatch between prenatal and postnatal environmental conditions may be seen in offspring of the Dutch Hunger Winter study (See Chapter 1.1.2), when compared to offspring of the Leningrad Siege study, where the selected developmental trajectories led to a "survival phenotype", as the prediction was correct (*Rickard et al. 2000).*

Mismatches between pre- and postnatal environments are common, particularly in the developing world where the environment is changing grossly with industrialisation, urbanisation, nutrient availability and climate deviations. Human life span extends far beyond the reproductive years, as many previously deadly diseases are currently treatable. As a consequence, the manifestation of chronic diseases is on the increase. The mismatch in prediction is of a little significance when life span was short, as the disease manifestation could not possibly occur (*Gluckman et al. 2006*).

Intergenerational effect.

Interaction of genetic and environmental components in the "Predictive adaptive response" system includes the intergenerational effect, namely the adaptive response of the fetus will reflect experiences (nutrition, life *in utero*, birthweight, health…) of recent ancestors (*Cassell, et al.; 2003 Kuzawa 2005*). The birthweight of the baby was found related to the birthweight of both parents, however only to the height associated with the maternal female line (height of the maternal grandmother) and to the maternal grandfather's social class (*Emanuel et al. 1992*). Thus the growth and development *in utero* of female ancestors may affect the birthweight of the offspring three or more generations later.

On the time scale, the intergenerational effect might be seen as an intermediate kind of human adaptive mechanisms similar to plasticity (*Kuzawa 2005*). However, as it responds gradually to environmental changes experienced across generations (carry-over effect), it acts more slowly than plasticity (real time response to environment), but much faster than the genetic response (non-inducible, driven by the natural selection process; *Jablonka et al. 1995*).

The intergenerational effect was seen also in the Dutch Hunger Winter study offspring. Mothers exposed to famine during their first trimester had offspring with normal birthweight. The birthweights of the first-born child correlated with mother's birthweight. According to the research data, babies born in consecutive pregnancies are usually heavier than the first-born children (*Billewicz et al. 2005*). However, in this study, the birthweight of all further offspring and the second-generation offspring were decreased (*Lumey 1998b*).

The possible mechanistic links between poor fetal development and adult diseases, particularly Type 2 diabetes, have now been described. The following section will provide information on the pathophysiology of Type 2 diabetes in order to bring it into context with the fetal programming hypothesis.

1. 2. DIABETES MELLITUS

1.2.1 Aetiology, pathophysiology, complications and diagnostic criteria for Type 2 diabetes mellitus.

1.2.1.1 Aetiology and pathophysiology of Type 2 diabetes

Type 2 diabetes mellitus is a metabolic disorder characterised by chronic hyperglycaemia. It occurs as a result of a combination of insulin deficiency (secretory failure of pancreatic β-cells) and insulin resistance (decreased sensitivity or responsiveness) of target tissues (*WHO 1999*). Type 2 diabetes is the predominant form, accounting for 90-95% of all diabetics (*ADA 2008).*

The aetiology of Type 2 diabetes is still unknown, although it is believed, that both environmental and genetic factors contribute to the disease.

Genetic factors.

There are genetic differences in susceptibility for Type 2 diabetes (*Zimmet et al. 2003*; see also Chapter 1.1.4 Intergenerational effect). Several studies investigated the first-degree relatives of patients with Type 2 DM. Despite the normal glucose results during an oral-glucose tolerance test, they had several metabolic abnormalities (*Eriksson et al. 1989*), such as hyperinsulinaemia, decreased C-peptide levels and C-peptide to insulin ratios (*Humphriss et al. 1997).* Insulin resistance and impaired insulin secretion were both necessary for the development of impaired glucose tolerance in these subjects.

There may be genetic defects of β-cell function (such defects are known in types of maturity onset - MODY diabetes (*Polonsky 1995; Byrne et al. 1996)* or mitochondrial DNA defects (*ADA 2008))* or genetic defects in insulin action, e.g. mutations of the insulin receptor (*WHO 1999*). However, these mutations are rare and explain only a small percentage of the genetic input into Type 2 diabetes.

Recently new loci containing relatively common genetic variants, which are significantly associated with Type 2 diabetes risk, were identified by Sladek et al (*2007*). The genes at these loci have a role in the development of the pancreas; they possibly regulate insulin secretion and may be involved in many other functions (insulin sensitivity, cell proliferation and specialisation).

Age.

Type 2 diabetes mellitus used to be commonly associated with elderly people. However, during the last two decades there has been a decline in age of onset, now being seen in

adolescents and more commonly in children (*Arslanian et al. 1998*; *Rosenbloom et al. 1999)*. An association with childhood obesity has been made. For example, in Japanese children Type 2 diabetes is seven times more common than Type 1 diabetes (*Rosenbloom et al.1999)* and a high prevalence of Type 2 diabetes in children is also found in the United States, Pacific Islands, Hong Kong, Australia and the United Kingdom (*Zimmet et al. 2001*). This creates a serious problem with respect to earlier onset of diabetic complications and reduced quality of life.

Diseases.

Diseases of the exocrine pancreas (e.g. pancreatitis, trauma, infection) or different endocrinopathies (e.g. acromegaly, hyperthyroidism, Cushing syndrome) may also cause Type 2 diabetes (*ADA 2008*).

Lifestyle.

The prevalence of diabetes increases in parallel with obesity, hypertension and cardiovascular disease, the products of the so-called "epidemiological transition" - rapid cultural and social development in a form of "fat-rich" or "energy-dense" diet, urbanisation, migration, unhealthy sedentary lifestyle and changes in behavioral patterns (*Coughlan et al. 1997*). The lowest prevalence of diabetes is found in developing non-industrialised countries, where the traditional lifestyle of hunter-gatherers or farmers still exists (*Fall 2001*). Thus sedentary lifestyle, overeating and obesity are high risk factors for Type 2 diabetes.

According to the National Center for health Statistics in the USA and based on The National Health and Nutrition Examination Survey (NCHS), one third of the American adults were obese in the year 2005 - 2006 (http://www.cdc.gov/nchs/data/databriefs/db01.pdf).

This means that one in three American adults were obese, reaching the prevalence of approximately 40% among people 40-59 years old, with similar rates for men and women. The highest prevalence of obesity (61%) in the USA was seen among non-Hispanic black women aged 60 years and above, compared to 32 % of non-Hispanic white women. The lowest contribution towards the national average was found among men aged 20-39 years, from whom only 28% were found obese (http://www.cdc.gov/nchs/data/databriefs/db01.pdf).

Obesity represents a high risk for Type 2 diabetes (*WHO 1999*). From research observations it is known, that the abdominal visceral adipose tissue correlates with insulin resistance (*Bergman et al. 2001*) and decreased insulin secretion (*Cruz et al. 2002*). The β-cell of a person who is obese, insulin resistant and chronically overnurished, is trying to compensate for the high glucose levels by hypersecretion of insulin to maintain euglycaemia. Therefore, these subjects' insulin levels are elevated, while the glucose levels may appear normal and person is metabolically healthy. The compensation mechanism involves an expansion of the β-cell mass (by proliferation and/or neogenesis) and enhanced insulin secretion to various stimuli. However, once the ability to compensate fails, a person progresses to Type 2 diabetes via progressive impairment of β-cell function. The changes in the β-cell most probably occur in very early stages before the disease manifestation. It has been suggested by Prentki et al (*2006*), that this situation occurs in people with "susceptible" islets (e.g. due to programming *in utero*) while people with "robust" β-cells can maintain the high insulin output.

A number of epidemiological studies investigating the role of low birth weight in aetiology of Type 2 diabetes have been performed. **Insulin resistance**, especially if accompanied with obesity in adulthood, was suggested as the mediator of Type 2 diabetes

rather than impaired β-cell function e.g. in Uppsala study, (Chapter 1.1.2; *Lithell et al. 1996*), Preston study (*Phillips et al. 1994*) and Haguenau in France (*Jaquet et al. 2000*).

1.2.1.2 Assessment of insulin resistance

Insulin resistance and impaired insulin secretion serve as indicators of the development of Type 2 diabetes (*DeFronzo et al. 1979*). The euglycaemic-hyperinsulinemic clamp is considered the gold standard technique for the assessment of insulin sensitivity (*DeFronzo et al. 1979*; *Bergman et al 1985*). However, this technique is invasive, and involves study conditions, which are not completely physiological. This also applies to the frequently sampled intravenous glucose tolerance test (FS IVGTT) with minimal model analysis (*Bergman et al. 1979*), although this method is less invasive than the clamp method.

Frequently, only the fasting glucose, insulin or C-peptide samples are measured or an oral glucose tolerance test (OGTT) is performed. The application of these methods is simpler, less invasive, and less costly, and therefore more practical especially for larger research studies, when compared to the clamp and FS IVGTT methods. From the plasma glucose levels and the insulin responses measured during the OGTT insulin resistance (or sensitivity) and β-cell secretion can be evaluated by means of various indices *(Radziuk 2000; Soonthornpun et al 2003; Muniyappa et al 2008*) such as the Insulinogenic index *(Seltzer et al. 1967*), Insulin sensitivity index (ISI; *Matsuda et al 1999);* AUC Insulin/AUC Glucose *(Strumvoll et al. 2000b),* QUCKI *(Katz et al. 2000),* HOMA index *(Matthews et al 1985).*

The standard OGTT, originally introduced for the diagnosis of impaired glucose tolerance (IGT) and diabetes mellitus by the World Health Organization (WHO), uses 75g of

glucose load and the sampling times of fasting (0 minutes) and 120 minutes (*WHO Criteria 2006*). In many clinical and research studies, the duration of the OGTT is extended beyond 2 hours and/or additional sampling times are included, according to the purpose of the test. Commonly, the blood samples are collected at fasting (0 min), 30, 60, (90) and 120 minutes (*Matsuda et al 1999; Strumvoll et al 2000a; Yasuhara et al 2003; Pontiroli et al 2004; Keskin et al 2005*).

HOMA INDEX:

The Homeostasis model assessment (HOMA) is a computer-generated model for the assessment of insulin resistance (HOMA-IR) or of pancreatic ß-cell function (HOMA-ß; *Matthews et al. 1985*).

The HOMA method has shown that both increased insulin resistance and decreased ß-cell secretory function are associated with Type 2 diabetes (*Haffner et al 1997*). However, as the HOMA-ß model extrapolates from fasting steady state data to the assessment of dynamic ß-cell secretary function, the evaluation of insulin resistance from HOMA-IR is preferred by the majority of studies (*Muniyappa et al. 2008*).

The HOMA-IR index (and its logarithmic and reciprocal forms) correlate with the clamp technique in many studies (*Bonora et al 2000*; *Yokoyama et al 2003*; *Wallace et al 2004*; *Chen et al 2005* and others) and in combination with its simple and less invasive application and lower costs it is considered a valid and effective method for the assessment of insulin resistance/sensitivity in both Type 2 diabetic and non-diabetic subjects. The HOMA-IR index was also previously applied for the assessment of insulin resistance in pregnant women (*Moses et al 2006*; *Parretti et al 2006; Ozcimen et al. 2008; Retnakaran et al 2008*) and children (*Arslanian 1998; Arslanian et al 1998*). An agreement between HOMA-IR and

glucose clamp derived values was demonstrated in a South African Black urban population (*Ntyintyane et al. 2010*) and the HOMA-IR index was also used in other South African studies involving urban Black men and women (*Ferris et al 2005; Crowther et al 2006)* and Black pregnant rural women *(Mamabolo et al 2007).*

1.2.1.3. Long-term complications of Type 2 diabetes

Long-term complications of diabetes include microvascular damage (neuropathy, nephropathy, retinopathy) and macrovascular complications (coronary artery and peripheral vascular disease, stroke), leading to renal failure, blindness, foot ulcers and amputations associated with diasability and reduced life expectancy (*ADA 2008).*

1.2.1.4 The diagnosis of diabetes mellitus

In respect to the fundamental feature being hyperglycaemia, the diagnosis of diabetes mellitus is based on blood glucose levels.

According to the World Health Organization (*WHO 2006*) Criteria the diagnosis of diabetes depends on fasting and postprandial glucose levels as follows:

Fasting level of plasma venous glucose ≥7.0 mmol/l

or 2h post glucose load* ≥ 11.1 mmol/l or both.

*A glucose load represents 75 g of anhydrous glucose dissolved in water, which is applied in a standard oral glucose tolerance test, OGTT, after an 8 hour fast.

The American Diabetes Association (*ADA 2009*) uses three ways to diagnose diabetes mellitus: fasting **or** 2-h plasma post glucose load levels as described by the WHO criteria **or** symptoms of hyperglycaemia and the causal plasma glucose >11.1mmol/l.

1.2.2 Epidemiology of diabetes mellitus and costs to society.

Diabetes is a chronic disease and one of the most common non-communicable diseases and is a leading cause of morbidity and premature mortality. It has been declared as a world pandemic, affecting 246 million (6%) people in 2007 in the developed and developing countries. The numbers are expected to rise to 380 million (7.3%) in 2025, which means that 1 out of 14 adults will be affected with diabetes. Diabetes is among the first five leading causes of death in most developed countries (3.8 million deaths per year world-wide), which makes it comparable to the magnitude of death due to HIV/AIDS (http://www.diabetesatlas.org; 3rd edition). It has been observed that 80% of deaths in Type 2 diabetic subjects are due to cardiovascular complications (*Zimmet et al. 2003)*.

The estimated prevalence rates of diabetes among adult people by region predicted for the year 2030 (http://www.diabetesatlas.org, 4th edition) are as follows:

1. North America and Caribbean (12.1%)
2. Middle East and North Africa (10.8%)
3. South-East Asia (9.1%)
4. Europe (8.1%)
5. South and Central America (7. 8%)

6. Western Pacific (5.7%)

7. Africa (with the lowest prediction of nearly 4.7%)

(Presented with the permission of the International Diabetes Federation.)

Although at present the non-industrial African Region accounts for the lowest prevalence of diabetes (3.8% assessed for the year 2010), the highest increase in the number of people with diabetes in this Region within the next 20 years (98.1%) is expected (http://www.diabetesatlas.org, *4th edition, Table No 3.1*). Also for other countries undergoing rapid industrialisation and urbanisation, such as the Middle East and South America, the predicted rise in prevalence of Type 2 diabetes is high. On the other hand, the lowest increase of 20% for the same time period is forecast for the European Region, where programmes for the prevention, early identification and treatment of diabetes were formulated and implemented on national and international levels. These strategies are summarised in the Saint Vincent Declaration, a document formulated by the European health experts together with the WHO and the IDF organisations in Italy, 1989 (www.ncbi.nlm.nih.gov/pubmed/11918624; www.idf.org;)

Costs to society

Chronic diseases mean a lifetime cost to the individual and to the health care system. More than 80% of present diabetics live in low- and middle- income countries and the treatment is either not affordable to many of them or they receive care below optimal level (*Fall 2001*). As a consequence, these people have a higher prevalence of diabetic complications and face a more rapid progression to end-stage renal disease than diabetics in developed countries. In the US the total estimated costs of diabetes were $ 174 billion in the

year 2007, which includes medical expenditures and reduced national productivity *(ADA 2008*).

1.2.3 Diabetes in South Africa.

Nearly 80 % of the 40.5-million South African residents are represented by Black South Africans (*Motala et al. 2008*). The majority of this population lives in rural areas, although increasing numbers are migrating to cities seeking a better quality of life and job opportunity (*Levitt 1996*). Historically, in rural areas where the traditional lifestyle still prevails, diabetes mellitus is almost non-existant (*Bourne et al. 2002*).

Significant disparity still exists in South Africa, with the result that there is an obvious unequal distribution of wealth and opportunities between developed and developing areas. The lifestyle in the urban areas has changed dramatically and primarily, because of increasing obesity, the urban areas have higher prevalence rates for diabetes mellitus than rural areas (*Bradshaw et al. 2007; Motala et al. 2008).* The highest prevalence of diabetes was found in Asian Indians, followed by Coloured, urban Black African and White groups (*Mollentze et al. 2005; Bradshaw et al. 2007*). Among the Black African population, the highest prevalence for diabetes of 8% was found in an urban area near Cape Town. Obesity and the pattern of fat distribution were identified as independent risk factors (*Levitt et al. 1993*). In Durban (*Omar et al. 1993*) the diabetes prevalence rate in the Zulu ethnic group was shown to be 5.3 % (age and sex adjusted), being twice as more common in women than men (*Bradshaw et al. 2007*). A

cross-sectional survey (*Motala et al. 2008)* of a rural northern KwaZulu-Natal revealed a crude overall prevalence of diabetes of 4.6%, which was similar for both men and women.

Diabetes mellitus was responsible on average for 4.3% (22 412 cases) of all deaths in South Africa in year 2000, which represented the 7th commonest cause of death in the country (*Bradshaw et al. 2007*). Obesity and the pattern of fat distribution were the highest risk factors for Type 2 diabetes, next to age and urbanization (*Levitt 1996; Motala et al. 2008*).

1.2.4 Gestational diabetes mellitus.

Gestational diabetes mellitus (GDM) is a common metabolic disorder during pregnancy. The GDM prevalence varies among different populations (in a range of 1-14%) and it is in direct proportion to the prevalence of Type 2 diabetes in particular population/ethnic groups (*Perucchini et al. 1999*). GDM is defined as carbohydrate intolerance causing hyperglycaemia in pregnant women with onset during pregnancy (*WHO 1999; Ben-Haroush et al. 2004*).

Risk factors that may warn to the development or presence of GDM within the current pregnancy include history of glucose intolerance or large-for-gestational age babies within a previous pregnancy, an obviously large baby at current pregnancy, high-risk ethnic groups or high glucose levels (*WHO 1999*) and obesity (*Ben-Haroush et al. 2004*).

GDM diagnosed in early pregnancy is associated with increased number of obstetric complications, recurrent GDM in next pregnancy and future development of metabolic

syndrome and Type 2 diabetes (*Ben-Haroush et al. 2004; Vohr et al. 2008*). The offspring of women with GDM are more likely to develop metabolic syndrome later in life. This seems to be related to maternal glycaemia in the third trimester, maternal obesity, neonatal macrosomia and childhood obesity (*Vohr et al. 2008*). Pregnant women diagnosed with GDM should be re-tested 6 weeks after delivery and re-classified as being diabetic, having impaired glucose tolerance (IGT) or normal glucose levels (*Peruccini et al. 1999*).

Normal pregnancy is associated with progressive insulin resistance that may reach levels of Type 2 non-pregnant diabetics during the last trimester *(Dunne 1999; Buchanan 2001*). Increasing maternal weight, increased secretion of steroids, secretion of insulin and increased secretion in growth hormone all make pregnancy "diabetogenic". Insulin resistance may vary significantly among pregnant women and the reduction in insulin sensitivity is linked to increasing requirements for placental glucose transport and supply to the fetus (*Dunne 1999*; *Jansson et al. 2001*). Women with a history of GDM showed a higher degree of insulin resistance in comparison to non-pregnant women without a history of GDM (*Buchanan 2001*).

Gestational diabetes mellitus can lead to fetal overgrowth and large-for-gestational-age newborns, more commonly known as macrosomia. This condition may be associated with complications during delivery and with neonatal hypoglycaemia. In addition, the mother has an elevated risk for pregnancy-induced hypertension (*Jansson et al. 2001).*

It can therefore be seen that the aetiology of Type 2 diabetes involves both insulin resistance and ß-cell secretory failure. Many studies have now shown that low birth weight is

associated with insulin resistance in adulthood but some studies have also shown reduced islet cell mass and poor ß-cell function in low-birthweight subjects (*Crowther et al. 2000*).

The next section of this report will therefore describe the developmental pathways for islet ß-cells in the fetal pancreas.

1.2.5 Endocrine pancreas and pancreatic β-cells.

Poor early development of the islets of Langerhans and β-cells has been suggested as a major factor in the aetiology of Type 2 diabetes mellitus *(Hales et al. 1992, Swenne 1992).*

1.2.5.1 Development of the pancreatic endocrine cells.

Human pancreatic endocrine cells can be detected already at about 8 gestational weeks (*Clark et al. 1983; Piper et al. 2004).* Mature islets of Langerhans consist of four distinct cell types with exact topography within the islet and within the pancreas. The β-cells (producing insulin) are found in the central core of the islet surrounded by δ-cells (secreting somatostatin) and α- or PP cells in the periphery (*Vinik et al. 1997*). Islets with a high population of α-cells (secreting glucagon) are found in the body and tail of pancreas, while islets with a high population of PP cells (producing pancreatic polypeptide) are located in the head of the pancreas (*Bonner-Weir 1990*). Recently discovered glucagon secreting endocrine ε- cells are still poorly understood (*Prado et al. 2004; Andralojc et al. 2009*). The islets of Langerhans represent 2-4% of the total pancreatic mass at birth, decreasing to 1% in adulthood. The β-cells account for the largest proportion (60-80%) of all islet cells. The development and

function of islet cells is regulated by expressions of specific genes further controlled by transcription factors (*Reusens et al. 2006*).

Each islet is highly vascularised *(Bonner-Weir 1990*) starting from 14 weeks post-conception (*Piper et al. 2004*) with greater blood flow directed to the islets (10-15 %) than to the pancreas as a whole (*Hales et al. 1992).* The human β-cell can produce insulin from between seven and eight weeks *in utero* (*Scharfmann et al. 2008*), however, its secretion in response to glucose is absent until about 28 gestational weeks (*Nicolaides et al. 1989; see Chapter 1.2.5.7*).

1.2.5.2 Regulation of β-cell mass

The β-cell mass can change by increasing or decreasing cell volume and/or cell number in order to maintain euglycaemia (*Bonner-Weir 1994*). The islet mass increases by neogenesis, enlargement and replication of existing cells (*Bonner-Weir 1990)* with cell atrophy and apoptosis contributing to the islet mass decrease (*Vinik et al. 1997).*

The pancreatic β-cell mass increases rapidly from about week 20 (*Bouwens et al. 2005)* of fetal life until about the fifth postnatal month (*Wilkin 1993; Khan et al. 1994).* It approximately doubles from the neonatal to the infant period *(Rahier et al. 1981*) reaching at least half the adult complement at the age of 1 year (*Hales et al. 1992).* The β-cell proliferation during the fetal period correlates with gestational age and from weeks 17-32 decreases until about 6 month postnatally.

Apoptosis shows a different pattern from proliferation; in the early fetal period and after 6 months of age it is rare. However, there is a "wave" of β-cell apoptosis in the human fetus occurring during the perinatal period (2 months before and after delivery; *Kassem et al. 2000*). In the rat fetus, the wave of islet-cell "developmental" apoptosis occurs between day 12 – 17 after birth (*Petrik et al. 1999*), during which many original fetal phenotypes of β-cells are replaced in the following "wave" of neogenesis (*Hill et al. 2000*). The mechanism regulating the apoptosis is still unclear.

In human adults and other mammals, the β-cell mass has some but limited flexibility, provided by the balance between cell regeneration and cell apoptosis or decreased replication rate. This ensures an appropriate production of insulin for maintaining euglycaemia in conditions such as pregnancy (see below) or obesity. If this balance becomes disturbed, impairment in glucose homeostasis may follow (*Bernard et al 1999).*

1.2.5.3 Maternal islet adaptation during pregnancy

During pregnancy, the maternal β-cells undergo changes in order to cope with the increased metabolic demands of the fetus (*Swenne 1992*). These changes include increased β-cell proliferation, increased insulin synthesis and glucose-stimulated insulin release at a lower threshold. Lowering the threshold for insulin secretion allows the release of larger amounts of insulin at normal blood glucose concentrations (*Sorenson et al. 1997*). Inadequate compensation may result in gestational diabetes (*Bouwens et al. 2005*).

1.2.5.4 Effect of nutrition.

Nutrition plays the major role in the growth of pancreatic β-cells (*Wilkin 1993*). Prenatal undernutrition causes impairment of fetal β-cell growth and results in glucose intolerance (*Seckl 2004*). Infants who are small for gestational age show selectively reduced β-cell mass in comparison with other types of islet cells (*Petrik et al. 1999; Hay Jr 2006a)*. Amino acids are the major factors controlling β-cell growth and development and promote insulin secretion in fetal pancreas until late fetal life (*De Gasparo et al. 1978; Cetin et al. 1988*).

A low protein diet changes the balance between β-cell replication and apoptosis (*Petrik et al 1999*). Rats fed a low protein diet during gestation had growth retarded offspring with reduced β-cell mass (volume) at birth and permanently impaired insulin response to glucose. Reduced pancreatic expression of IGF-2 (islet survival factor; see above) was associated with the lower β-cell proliferation rate and increased apoptosis in the fetus and neonate (*Petrik et al. 1999*). Contrary to this, maternal total caloric restriction during pregnancy affected neogenesis but preserved β-cell proliferation rate (*Bertin et al. 1999*). Again in adult rat, pre- and postnatal low protein diet decreased pancreatic islet blood flow and insulin release. Low blood flow values may reflect an adaptive physiological response, an impaired pancreatic vasculogenesis or dysfunction of endothelial cells (*Iglesias-Barreira et al. 1996*).

Overnutrition during pregnancy, and also maternal diabetes, leads to β-cell hyperplasia in the rat fetus (*Armitage et al. 2005*). An increased exposure of pregnant rats to dietary fat, irrespective of total calorie intake, resulted in hyperglycaemia and hyperinsulinaemia in the

adult offspring (*Martin-Gronert et al. 2006*). This condition led to β-cell exhaustion, associated with decreased insulin sensitivity and decreased insulin secretory capacity (*Armitage et al. 2005*).

1.2.5.5 Effect of glucose.

In experiments with adult non-pregnant rats, glucose infusion for 24 h nearly doubled the β-cell numbers. This effect was reversed later after glucose withdrawal and was associated with increased beta cell apoptosis (*Bouwens et al. 2005*). Also in rats, high maternal blood glucose levels (as in maternal diabetes) in pregnancy cause hyperplasia of beta cells in the neonate (*Wilkin 1993; Bonner-Weir et al. 1994)* and glucose intolerance in the offspring (*Srinivasan et al. 2003*). Further, a high carbohydrate milk formula administered to rat pups during suckling period, which is the critical period of pancreatic development in rats, leads to adaptive changes in islet function and results in a life long hyperinsulinaemia in these offspring (*Srinivasan et al. 2003*).

1.2.5.6 Effect of hormones.

The pregnancy upregulation of β-cell mass is possibly directly mediated by the placental hormones lactogen and prolactin (*Brelje et al. 1993; Sorenson et al. 1997*). Placental lactogen, prolactin, growth hormone and various growth factors (see below) stimulate directly the β-cell proliferation (*Bouwens et al. 2005*) in islet cultures in a dose dependent manner (*Billestrup et al. 1991*). This includes insulin-like growth factor 1 (IGF-1), platelet-derived growth factor, epidermal growth factor and others. Placental lactogen seems to be the principal hormone responsible for this regulation (*Brelje et al. 1993; Bonner-Weir et al. 1994)*.

The timing of apoptosis is regulated by insulin-like growth factor 2 (IGF-2), which acts as a pancreatic islet survival factor by reducing the apoptosis, as was demonstrated on isolated islets from 5-day old rats (*Petrik et al 1998; Petrik et al. 1999; Bouwens et al.2005*). On the other hand, hyperinsulinism during infancy is associated with constantly elevated β-cell proliferation and apoptosis (*Kassem et al. 2000*). Maternal gestational food restriction caused fetal growth retardation and a long-lasting decrease in rat fetal β-cell mass, associated with development of glucose intolerance in adult rats. The reduction in fetal β-cell mass was caused by an elevation in glucocorticoid concentration as a response to maternal undernutrition (*Gesina et al. 2006*). Glucocorticoids can inhibit insulin release and β-cell replication, acting through the glucocorticoid receptors in pancreatic islets. Normally, glycaemic stimulation supersedes this effect. However, steroids in an excessive amount may override the limited compensatory potential (growth and insulin production) of the β-cells, causing development of diabetes (*Swenne1992, Dodic et al.1999*).

1.2.5.7 Fetal insulin.

Fetal insulin production starts early in fetal life (*Scharfmann et al. 2008*), reaching a plateau in mid gestation (*Fowden et al. 2004*). Insulin has major fetal growth-promoting effects, which may be mediated in many ways. It can increase the rate of transfer of nutrients including amino acids into cells, promote protein synthesis and inhibit the effects of catabolic hormones (*Milner1989*) and any abnormalities in fetal insulin secretion disturb fetal growth (*Fowden 1992*).

Fetal plasma insulin concentrations correlate with maternal hormone level (*Milner1989*), although maternal insulin does not cross the placental membrane (*Kalhan et al. 1975*). Fetus increases insulin production in response to maternal hyperglycaemia, stimulating its growth (*Murphy et al. 2006*). Insulin effects on fetal growth were confirmed in gene knock-out animal models and also in humans with mutation in genes related to insulin action, such as the insulin receptor and glucokinase, resulting in lower birth weight and size (*Ong et al. 2004*).

Studies have shown that β-cell function in adults is related to birthweight and other birth parameters. Therefore it is important to understand the basis of human fetal development. The following section of this report will cover this field.

1.3 THE STAGES AND THE "CRITICAL PERIODS" IN THE HUMAN GROWTH AND DEVELOPMENT.

As described in Chapter 1.1, the process of programming occurs at critical periods of prenatal and early postnatal development. However, as various organs and structures develop during different periods, the timing of programming extends over several stages of human development. In this Chapter, some of the human developmental stages with respect to their critical periods will be reviewed, with major focus on the embryonic and fetal periods.

1.3.1 Maternal adaptations to pregnancy

Pregnancy is associated with adaptive changes in maternal organ systems and functions in order to accommodate the increasing demands of developing fetus, placenta and uterus. The metabolic changes are mainly related to the production of polypeptide and steroid hormones by the feto-placental unit (*Picciano 2003*) and begin early in pregnancy.

From about week 6 of gestation there is an expansion in plasma volume reaching a maximum around 32-34 weeks (*Ciliberto et al. 1998*). The plasma volume expansion has an influence on uterine blood flow (*Fall 2003a*). An insufficient volume expansion may lead to pre-eclampsia, IUGR, premature labour or oligohydramnios (*El-Haddad et al. 2006*).

Cardiac output increases due to increases in stroke volume and heart rate. The red blood cell mass rises, but to a lesser extent than the plasma volume. This leads to haemodilution and a relative physiological anaemia with normal haemoglobin levels at term *(Ciliberto et al. 1998; Picciano 2003*). Haemoglobin concentration below 10g/dl is usually associated with iron deficiency (*Hoque et al. 2009*), although definition of anaemia in pregnancy is said to be present when haemoglobin level is below 11g/dl (*Rastogi et al 2002*; *Milman 2008*).

1.3.2 Embryonic and fetal development

1.3.2.1 Introduction.

The first eight weeks (56 days) of human life after fertilisation are known as the **embryonic period** (See Figure 1.9). The conceptus, placenta and the majority of organs and

body structures undergo a rapid cell growth, cell divisions and differentiations during their establishment *(Moore 1993a;* See Figure1.10). During this period the embryo is highly vulnerable. The term embryo is used from the second gestational week, when the bilaminar embryonic disc is formed (*Moore 1993a*).

The fetal period extends from the ninth gestational week post fertilisation until birth (See Figure 1.9). Organs and structures established during the embryonic life develop further, increase in size and mature. The periods of rapid growth are also critical for further development, as the organs and tissues are sensitive to external insults.

During the third to fifth month post fertilization, the fetus is characterized by growth in length and increasing calcification. During the last two months of pregnancy fetus gains weight rapidly due to fat deposition (*Luke 1994; Sadler 2000b).* At 36 weeks the head circumference (HC) and the abdominal circumference (AC) are approximately equal. After this, the AC may be greater than that the HC. The male fetus is known to grow faster than the female and weighs more at birth *(Moore 1993b).*

Early fetal growth is rather slow, followed by accelerated growth between 16-17 to 26-27 weeks (about 85g per week). It reaches maximum growth between 26-27 to 37-38 weeks (about 200g/week) and finally, at the end of the gestation it decreases, with fetus gaining about 70g per week (*Neumann et al. 1984*). The median growth rate of a singleton pregnancy is about 250g/week at 33 gestational weeks (*Luke 1994*).

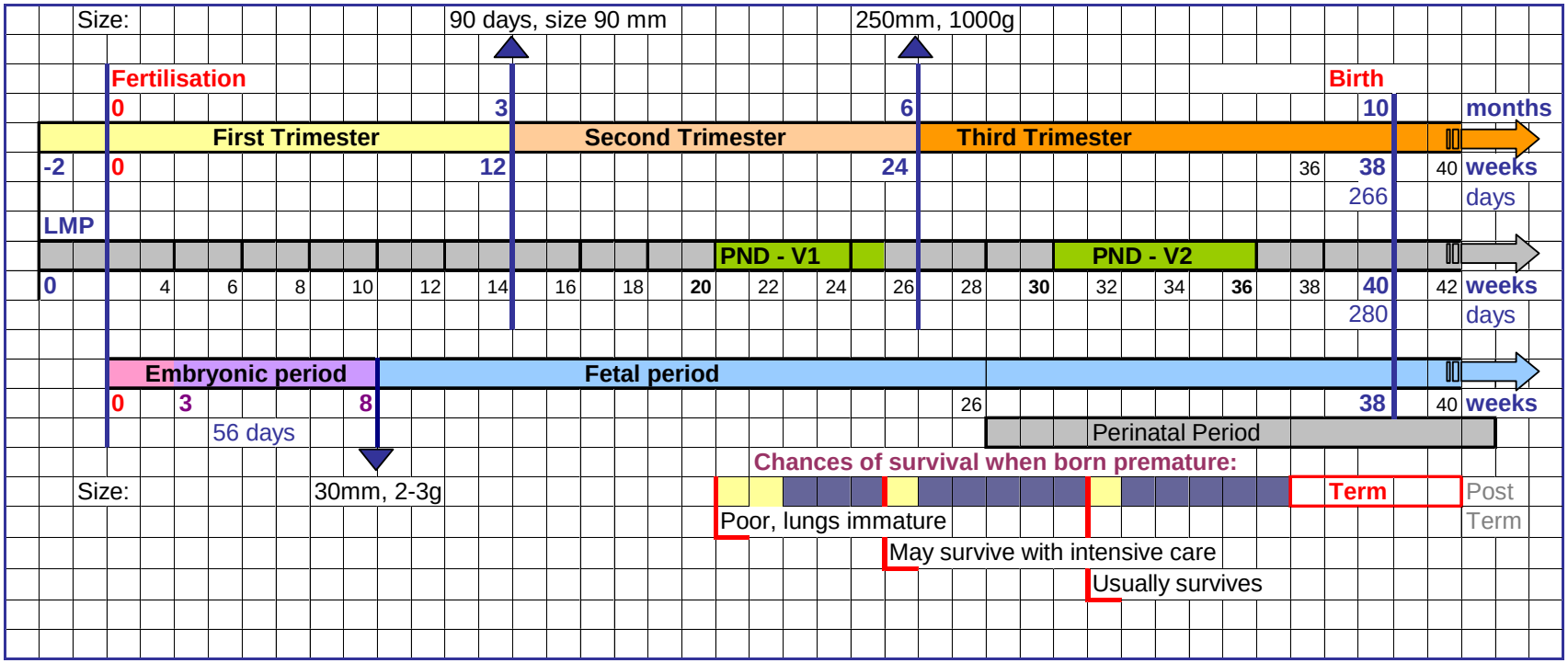


Fig. 1.9 Scheme of time periods and events during gestation with incorporated timing for the "Pregnancy, Nutrition & Diabetes" (PND) study visits. (Drawing based on *O'Rahilly et al. 2001a*, Figure 8.1.)

1.3.2.2 Early development

After fertilisation of the ovum, a rapid cell division gives rise to the zygote, then the morula and then the blastocyst. This period is often referred as to the pre-implantation stages of the embryo. Already at the 2-8 -cell stage, human embryonic gene transcription is activated (*O'Rahilly 2001b*).

The blastocyst stage represents a spherical structure of approximately 16-32 cells (day 4 post fertilisation) with a cavity inside (*O'Rahilly 2001b)* and differentiated outer and inner cell layers *(Lewis et al. 1990d;* http://embryology.med.unsw.edu.au). The inner cell mass consists of two layers forming together the embryonic disc – bilaminar embryo (Week2). The placenta and the placental membranes originate from the outer layer, termed the trophoblast (*Kliman 1998;* http://embryology.med.unsw.edu.au). The cavity within the blastocyst later develops into the extraembryonic cavities – chorionic, amniotic and yolk sac (*Lewis et al. 1990d*).

At approximately 3 to 4 weeks (p.f.) the bilaminar embryonic disc becomes trilaminar. From the three germ layers, ectoderm, mesoderm and endoderm, organs begin to form (See Fig. 1.10; *Sadler 2000a).*

The development of placenta.

The early placentation is stimulated by the blastocyst implantation process. The blastocyst attaches through the outer trophopoblastic cells to the uterine lining **(**uterine endometrial epithelium) at approximately day 6 p.f. (*Moore 1993a).*

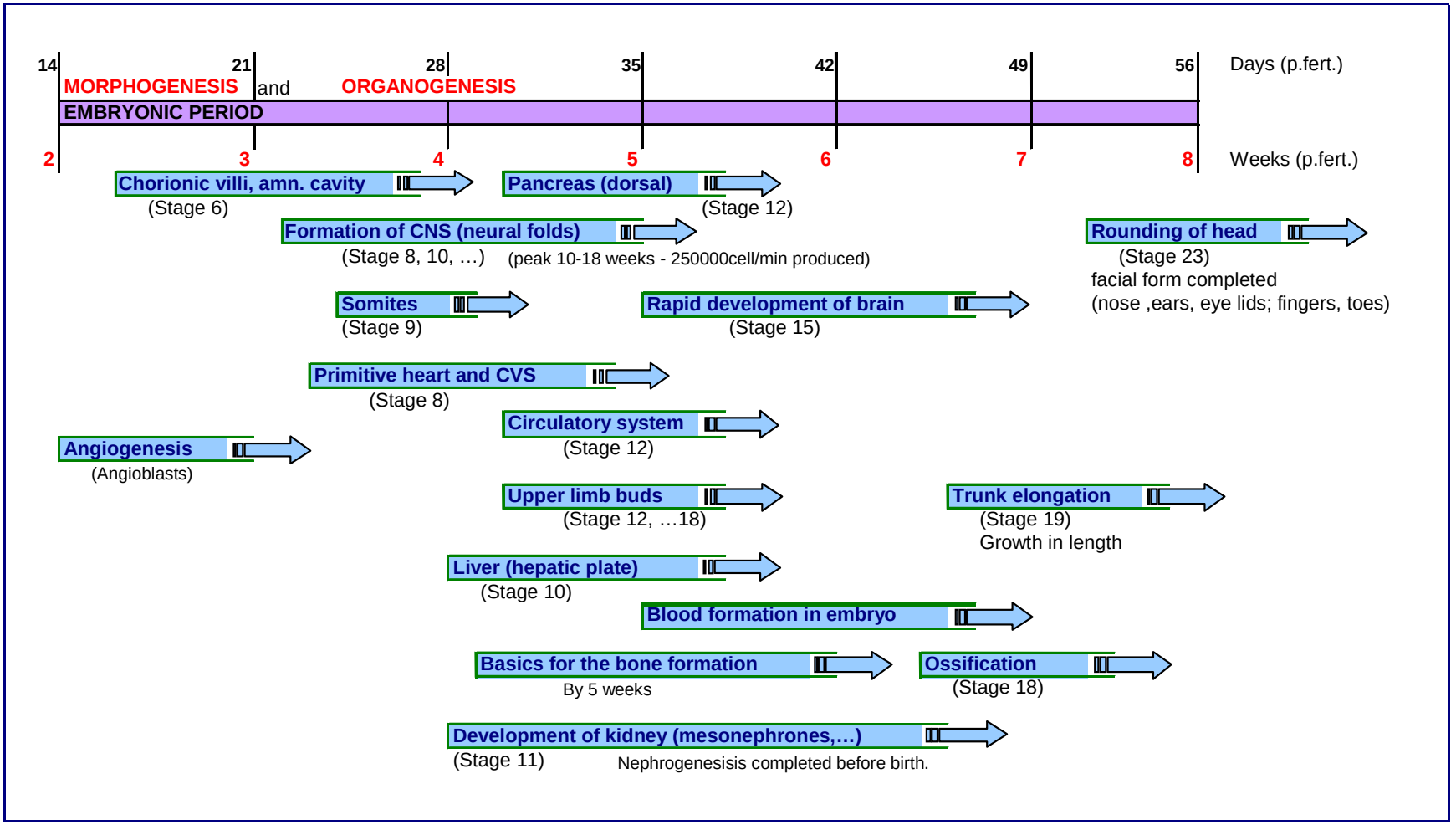


Fig. 1.10 Embryonic period and the Carnegie developmental stages.

(Diagram based on that of *Moore 1993d and O'Rahilly et al. 2001a)*

The trophoblast rapidly differentiatiates into two cell layers – the syncitiotrophoblast (outer layer) and the cytotrophoblast (inner layer), surrounding the implanted blastocyst (9-10 days p.f.) *(O'Rahilly et al. 2001c*).

The maternal endometrium contains active glands and spiral arteries (*Sadler 2000a*; see Figure 1.11). The endometrium is transformed into decidua. Maternal decidual cells contain nutrients (glycogen and lipids**;** see Chapter 1.3.2.4) and start secreting various hormones and factors, such as prolactin, insulin-like growth factor binding protein-1, tissue factor, interleukin-15, and vascular endothelial growth factor (*Sadler 2000a; Gude et al. 2004*).

The process of decidualisation is regulated by the hormones progesterone (secreted by the corpus luteum), human chorionic gonadotrophin (HCG; secreted by the syncitiotrophoblast), human chorionic somatomammotrophin, human placental lactogen (HPL; promoting growth) and steroid hormones. These hormones are involved in the nourishment of endometrium and preservation of the corpus luteum, until the placenta is ready to maintain the secretory function (*O'Rahilly et al. 2001c;* http://www.embryology.ch/indexen.html;). Within the syncytiotrophoblast layer special cavities known as lacunae are created, which later fuse into intervillous spaces and fill with maternal blood (http://www.embryology.ch/indexen.html). During the first trimester, before the blood flow is established, the intervillous spaces most likely contain acellular fluid *(O'Rahilly 2001d; Burton et al. 2002*).

The chorionic villi develop from the trophoblastic sprouts ("finger-like" projections), which are created when the trophoblast invades the maternal decidua. There are three basic

stages of villi development, being repeated in cycles until delivery. The primary villi are the trophoblastic sprouts composed of the sincitiotrophoblast in the outer and cytotrophoblast in the inner layer. In the next development, the primary villi are invaded by the extra-embryonic mesenchyme (as the villous core), and further by the fetal capillaries (vascularisation of the villi). These developmental stages are known as the secondary and tertiary villi (*Castelluci et al. 2000).* The villi further branch, increasing thus the mass and the surface of the placenta, and specialise *(Castelluci et al. 1990*).

In mature placenta, the chorionic villi represent the essential functional unit, where an exchange of nutrients, gases and waste products between the mother and the fetus takes place (see Figure 1.11). The layers of sincitiotrophoblast, cytotrophoblast, connective tissue and the fetal capillary endothelium create the placental "membrane", also known as the placental "barrier", preventing maternal and fetal blood from mixing. The sincitiotrophoblast (outer) layer is directly in contact with maternal blood in intervillous spaces *(Castelluci et al. 2000*). Poor vascularisation and other abnormalities in villi formation or function are associated with reversed end-of diastolic flow in umbilical artery velocity waveform *(Divon et al. 2002*; see Chapter 2.5.2.2).

The composition of the placental membrane changes over time. At the end of the first trimester the villous cytotrophoblastic layer slowly disappears and the membrane becomes thinner, providing a shorter diffusional distance (*Lewis et al. 1990d*).

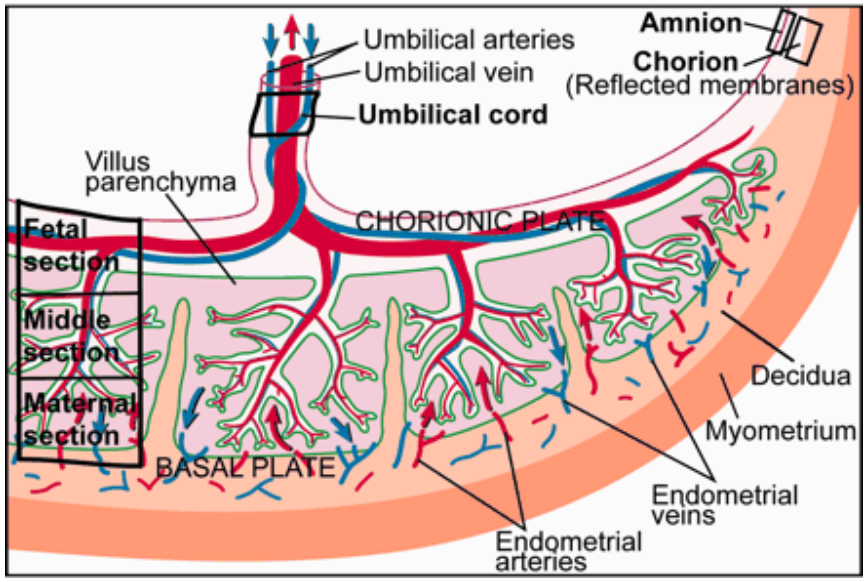


Fig. 1.11 The structure of mature placenta.

(Source: *Sood et al. 2006; freely obtainable via PNAS open access)*

1.3.2.3 Trophoblast invasion and establishment of the utero-placental circulation.

At the beginning (5-10 gestational weeks), the extravillous trophoblast invades into the decidua and forms temporary plugs in the maternal arteries. This causes an obstruction to the blood flow into the intervillous space, creating a low-oxygen environment (*Kingdom et al. 1999; James et al 2006*). The trophoblast plugs are released at about 12 weeks of gestation, when narrow uteroplacental spiral arteries are transformed into "funnel-shaped deltas" (*Lewis et al. 1990d; Jaffe et al. 1997)*. As a consequence, the continuous utero- placental circulation is established and expands to the rest of the placenta (*Jauniaux et al. 2003*).

Oxygenated maternal blood carrying nutrients passes from the endometrial spiral arteries into intervillous spaces, where it partially mixes with deoxygenated (venous) blood.

Nutrients, oxygen and other substances cross the placental membrane into the chorionic villi capillaries and through the umbilical vein are delivered to the fetus (*Gude et al. 2004*).

Deoxygenated fetal blood is carried from the fetus through umbilical arteries into capillaries of the chorionic villi branches where it reaches placental membrane and fetal waste products are passed to the maternal side. Maternal blood leaves the intervillous spaces by the uterine (endometrial) veins (*Lewis et al. 1990d;* http://www.embryology.ch/indexen.html*).*

The trophoblast invasion is regulated by specific factors. These include the vascular endothelial growth factor (*potent stimulator of sprouting angiogenesis*) and its receptors and insulin-like growth-factor 2 (IGF-2; *Myatt et al. 2006*). Also the insulin-like growth factor binding protein 1 (IGF-BP1), which is produced by maternal decidua, may inhibit the trophoblast invasion, if present in excessive concentration (*Krampl et al 2002*).

As mentioned earlier, the first trimester development occurs in a low oxygen environment. This arrangement is important for organogenesis (*Myatt et al. 2006*), as it protects embryo/fetus from oxygen free radicals and also stimulates the extravillous trophoblast outgrowth (*Burton et al. 2002*; *James et al. 2006*). Hypoxia also promotes angiogenesis and up-regulates vascular endothelial growth factor expression and downregulates placental growth factor (PIGF) (*Khaliq et al. 1999; Kingdom et al. 1999 and 2000*). The release of trophoblastic plugs is associated with a sudden increase in oxygen tension, meaning an oxidative stress (*Jauniaux et al. 2000 and 2002; Burton et al 2001*). It may have a role in the stimulation of normal placental differentiation. However, where the antioxidant defence is poor, it may represent a pathological condition (*Jauniaux et al. 2000*).

1.3.2.4 Early nutriton.

During the first trimester, due to a low oxygen environment, the early metabolism is anaerobic and histotrophic (*Burton et al. 2001; Jauniaux et al. 2003)*. It is based on uterine glandular secretions containing maternal plasma proteins, glycogen and other molecules that are delivered to the intervillous space (*Burton et al. 2002*). These nutrients (proteins in the form of amino acids) are transported by diffusion across the placental membrane to the exocoelomic cavity and the yolk sac (*Wild et al. 1999; Jauniaux et al. 2003*). Some proteins are synthesized by the yolk sac (*Wild et al. 1999).*

The embryo prefers pyruvate and lactate during the cleavage stage, and glucose during blastocyst formation and the early post-implantation stages for energy substrates (*Leese et al. 1999; Burton et al. 2002*). Amino acids are also utilised for energy production, and they stimulate blastocyst formation and are involved in a variety of other functions, such as control of pH and signal transduction, besides their main role in protein synthesis (*Fleming 2006*).

With establishment of the placental circulation (9-12 weeks p.f.), the nutrition becomes haemotrophic, delivered from the mother across the placental barrier (*Burton et al. 2001*).

1.3.2.5 Growth factors and blastocyst development.

Based on animal models and *in vitro* experiments, it was concluded that the pre-implantation embryo may be regulated by insulin and IGF-1 supplied from the mother and IGF-2 originating from the embryo itself. IGF-1 is known to have a stimulatory effect on development of human pre-implantation embryos *in vitro* (*Wild et al. 1999*).

1.4 DETERMINANTS OF FETAL GROWTH AND FETAL PROGRAMMING.

With the establishment of the relationship between birth outcomes and diseases in adulthood (See Chapter 1.1), the research interest concentrated on fetal development/growth and factors leading to programming effects. However, especially in human trials, it may be complicated or even impossible to directly investigate such factors and their role in fetal programming. Therefore many pioneering studies used animal or *in vitro* experiments. The simplest indirect approach to evaluate effects *in utero* was to monitor birth outcomes.

1.4.1 Assessment of birth outcomes.

The size at birth reflects the length of gestation and the fetal growth rate (*Thame et al. 2004; Kramer 2003)*, and they serve as indicators of future disease risk. In epidemiological studies of the assessment of pregnancy outcome, birth weight was the commonest measure used, as it was routinely recorded in many hospitals and health centers (see Chapter 1.1). It is considered to be the single most important determinant of neonatal morbidity and mortality (*Kramer 1987*). However, the birth weight only approximately reflects the environment and growth *in utero,* due to a number of other factors influencing fetal development (*Ounsted et al. 1985*). A better assessment of fetal growth is provided by the ultrasound technique, which enables a direct evaluation of the size from fetal biometric measurements (see Chapter 2.5.2.2). Growth charts have also been constructed to assess and monitor fetal growth (*Reece 1993; Maršál et al. 1996; van Bogaert 1999; Mamelle et al. 2001*).

According to birth weight neonates can be allocated into following categories:

LBW (low birth weight) – weight below 2500g irrespective of gestational age (term or pre-term babies) (*WHO Criteria;* http://www.who.int/whosis/indicators/2007LBW/en/print.html)

VLBW (very low birth weight) – below 1500g

Extremely low BW – below 1000g.

Low birth weight is a consequence of a shorter duration of gestation or it is due to a slow rate of fetal growth (*Kramer 2003*), therefore such babies can be both, born preterm or at term. The preterm (premature) babies are born before 37 completed gestational weeks (see Fig. 1.9). About one third of premature babies with a birth weight of 2500 g or less are in fact small for gestational age babies (*Neumann et al. 1984*).

According to the birth size, neonates can be appropriate (AGA), large (LGA) or small (SGA) for gestational age:

AGA is a baby of a size within 2 standard deviations (SD) of the mean populational weight for a particular gestational age (*Persson 1989*). In practice a range between 10th and 90th percentiles is used instead.

LGA indicates a baby of a birthweight exceeding the upper border of the range for the AGA infant. Conventionally, for obstetric reasons, LGA baby is one whose birthweight is equal or greater than 4000g. High maternal BMI, excessive pregnancy weight gain and maternal diabetes are the main reasons for fetal macrosomia (*Brudenell 1989).*

SGA indicates size below a defined reference range for the AGA infant. Commonly babies born below 2 500 g are considered as SGA (*Wigglesworth 1989*). An expert committee in Manchester 2006 (*Clayton et al 2007*) set up recommendations for definition of SGA neonates, that uses a cut-off level at 2 standard deviations (SD) below the mean for the weight and/or length. Identification of the SGA baby can be done only after excluding the involvement of any pathological process (see IUGR; *Peleg et al. 1998*). SGA neonates have low lean mass and often central adiposity. Rapid weight gain in infancy leads to later obesity. According to research studies, the infants without catch-up growth in height or HC show the worst outcome (*Clayton et al. 2007*).

IUGR (intrauterine growth retardation/restriction). Such a baby is small due to pathological restriction of its existing growth potential (*Campbell 1989)*. Growth retardation can be classified as symmetrical or asymmetrical retardation. The **symmetrically** retarded fetus/neonate is proportionally small (*Peleg et al. 1998).* This retardation originates in early pregnancy, before the 14th week, and reasons are mainly infections (e.g. cytomegalovirus, rubella, toxoplasmosis), or genetic malformations (as Trisomies 13, 18, 21; *Mitchell 2001*) or severe malnutrition. Because the insult occurs early in development, the "brain-sparing effect" (see Chapter 1.1.3.2) cannot apply and number of brain cells is reduced with possible impact on intellect (see Fig. 1.10; *Hinchliffe et al. 1992*). These infants will stay small, as there is a poor chance for catch-up growth (*Neumann et al. 1984*).

Assymetrical growth retardation is more common. The fetus is small in a disproportionate way (small abdomen, lack of subcutaneous fat, normal body length and head), due to the "brain sparing effect" (see Chapter 1.1.3.2; *Neumann et al. 1984; Hinchliffe*

et al. 1992; Peleg et al. 1998). Placental abnormalities and insufficiency, poor utero-placental blood flow, general malnutrition, maternal smoking or multiple pregnancies may be the reasons (*Neumann et al. 1984; Hinchliffe* et al. *1992; Mitchell 2001*). This type of growth retardation is related to late pregnancy (after 28 to 32 weeks), when growth proceeds mainly via cellular hypertrophy (*Neumann et al. 1984; Hinchliffe et al. 1992*).

As the genetic growth potential is not known and the cut-off limit for IUGR fetuses may still include some normally nourished babies, the definition of growth restriction is rather complicated. IUGR babies have significantly increased perinatal and infant morbidity and mortality, as well as a higher prevalence of developmental and physical growth handicaps (*Villar 1986; Hinchliffe et al.1992; Peleg et al. 1998*). IUGR accounts for about 10% of all pregnancies, ranging from 3% to 25% according to the maternal health and also varies among populations (*Dogra et al. 2006*). Frequently, but not always, the IUGR fetuses are SGA (*Campbell 1989*). In developed countries IUGR is strongly related to maternal smoking, while in developing countries the major cause seems to be maternal malnutrition (*Bergmann et al. 2008*).

1.4.2 Determinants of fetal growth.

As it was explained in Chapters 1.1.3 and 1.1.4, the fetus responds to the adverse effects of the environment during the critical periods of development by downregulating its growth rate, in order to preserve its energy for the brain and key organs. Depending on the magnitude and timing of the adverse effects, a low birth weight and disproportionate size are the likely birth outcome. For this reason, the fetal growth rate and also the early postnatal

growth patterns became important indicators of the future disease risk. A great research interest has thus been directed towards the identification and monitoring of the potential determinants of the fetal and postnatal growth and the mechanisms of their involvement in fetal programming.

The control of fetal growth is quite complex, as it involves gene-environment interaction (see Chapter 1.1.4). One has to realise, that in fact it is the combination of various effects that leads to the fetal adaptation, although, one of these effects may dominate. If this dominant effect is also modifiable, an opportunity for a timely intervention in preventing the adverse programming outcome is present. Thus, better understanding of the growth determinants and their effects is important for early detection, treatment or prevention of chronic diseases of adult life.

Already two decades ago, Kramer (*1987*) reviewed 895 publications and found 43 potential determinants of low birth weight (See Figure 1.12A). As birth weight is an outcome of fetal development, the determinants of birth weight have to be common for fetal growth as well. The differences in outcome are then attributable to the different timing of the exposure ("critical window" periods) of the fetus to these determinants. Some of them may also be pre-determined (as genetic background) or act indirectly via maternal exposure during her childhood.

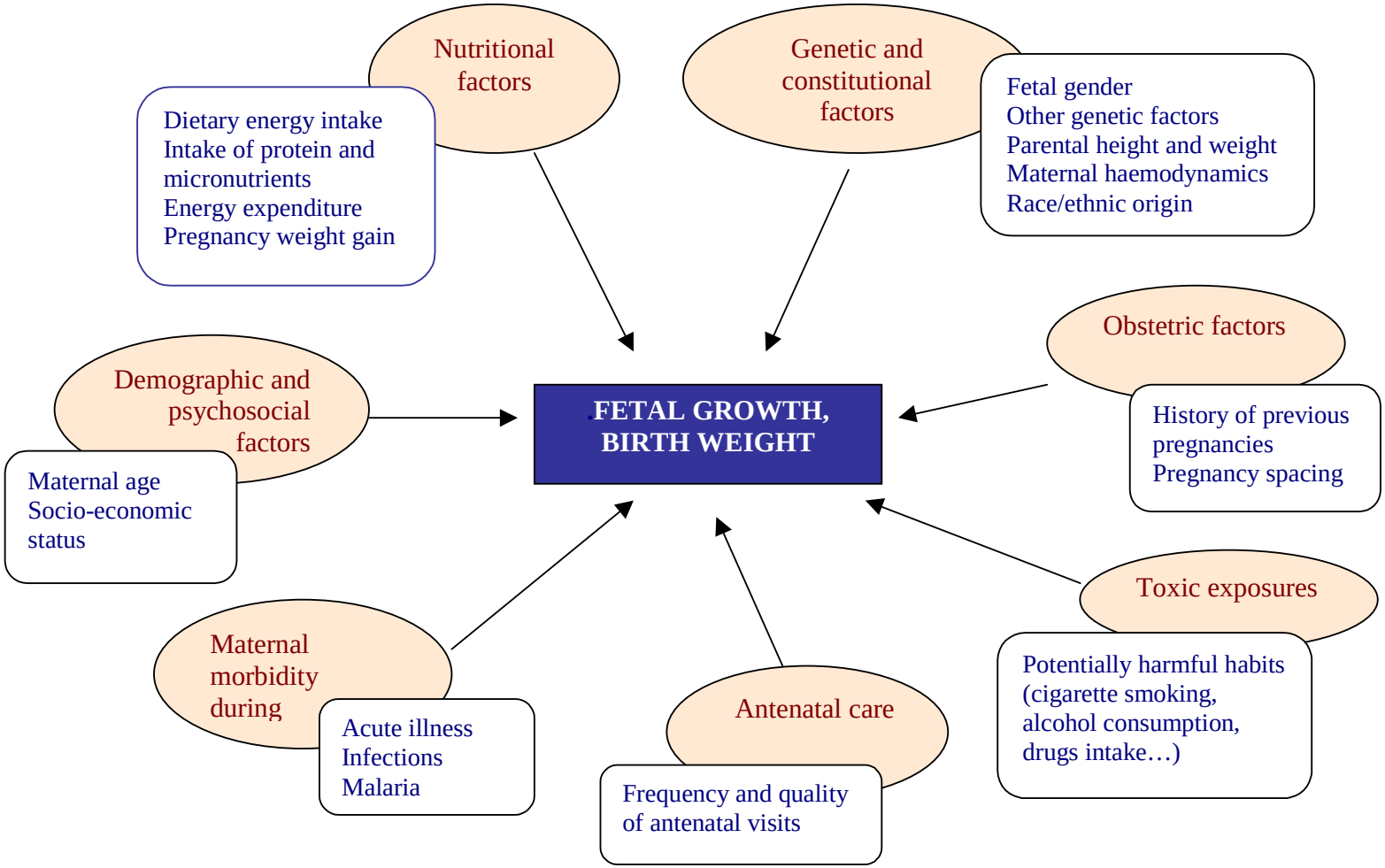


Figure 1.12A. Potential determinants of fetal growth and birth weight (Based on *Kramer 1987*).

The following factors were found to have a relatively well-established independent causal effect on fetal growth in populations worldwide. However, the extent of this causality for each of these factors may differ with respect to the developmental status of the country (*Kramer 1987*; See further Chapters 1.4).

Fetal gender

Racial/ethnic origin

Paternal height and weight

Maternal height

Maternal weight (her own birth weight, pre-pregnancy weight and pregnancy weight gain)

Maternal nutrition (calorie intake)

Maternal health (malaria, general morbidity and pregnancy-related illness)

Maternal habits (alcohol drinking, cigarette smoking or tobacco chewing) Pregnancy history (parity, previous low birth weight infants)

Figure 1.12B suggests a possible simplified mechanism for fetal growth and development in the programming of future risk for Type 2 diabetes. Well-known categories of determinants of fetal growth/birth weight are included and the effects of individual determinants will be discussed in the following chapters.

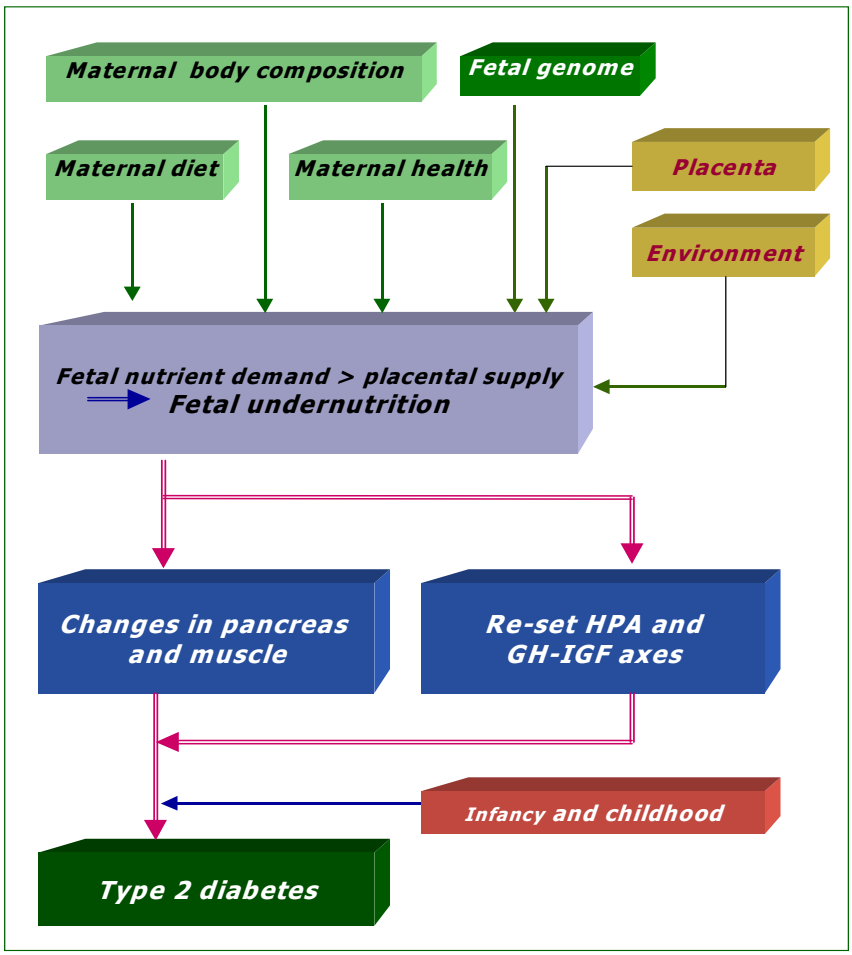


Figure 1.12B Regulation of fetal development and programming.

(Based on *Fall 2003a*, Fig 6 and *Godfrey 2006*, Fig 2.4)

1.4.2.1 Fetal genome.

1.4.2.1.1 Epigenetic regulation.

The term epigenetics refers to changes in gene expression, which do not result from the DNA sequence (*Whitelaw et al. 2006*). These changes include DNA methylation (enzymatic attachment of methyl groups at certain positions in genomic DNA) and histone modifications (histone proteins are responsible for packaging DNA into chromosomes) (*Whitelaw et al. 2006)*. These epigenetic changes are responsible for maintaining correct gene expression for any particular cell. They are inherited and they can be influenced by environment (*Reik et al 2001a; Whitelaw et al. 2006)* such as maternal nutrition and health status (*Wu et al. 2004*; *Junien et al. 2007).*

1.4.2.1.2 Imprinted genes.

The majority of genes in the fetal genome are expressed from both maternal and paternal alleles, although some genes are inherited exclusively from one allele only. These are known as imprinted genes (*Steinhoff et al. 2009*) and can be influenced by epigenetic changes (*Fall 2003a).*

Imprinted genes control normal fetal development, placental growth and structure and possibly influence the expression of placental nutrient transporters. In this way they are involved in the control of fetal nutrient demand and supply (*Murphy et al. 2006*). There are about 60 imprinted genes known in humans. Imprinted genes of paternal origin were found to

enhance fetal growth, while maternally-imprinted genes are associated with reduced fetal growth (*Reik et al.2001b).*

A recent study by Heijmans et al (*2008*) provided the first evidence that environmental events occurring during early human development may produce permanent epigenetic changes with health consequences in future life. People who were conceived during the famine in Holland were investigated (see Chapter 1.1.2). The offspring exposed to famine during the first 10 postconceptional weeks showed less DNA methylation of the imprinted IGF2 gene than their non-exposed siblings of the same sex. Those who were exposed to famine during late pregnancy did not differ in methylation pattern.

Epigenetic changes may not alter DNA sequence, but they can select which genes are expressed by silencing otherwise activated genes and vice versa. This may predispose the individual to future disease (*Heijmans et al. 2008*).

1.4.2.2 **Maternal factors influencing fetal growth.**

Besides the genetic component, the maternal factors that may affect the fetal development include the age of the mother, her body composition, health, number of children she delivered before and the spacing between subsequent pregnancies, her social class and her habits such as smoking or drinking alcohol, and most importantly, the quantity and quality of nutrients she ate before and during pregnancy. The majority of these factors will be discussed in the following sections. However, as already stated, although a direct effect of a single factor may have been studied and identified, in reality their effects add up or combine. Also it is

important to realise, that the final outcome depends on the equilibrium between fetal demand and maternal supply.

1.4.2.2.1 Maternal age and body composition

Maternal age.

Adolescent pregnancies and higher maternal age are associated with **a** high risk for adverse pregnancy outcomes (low birth weight, growth retardation, stillbirth, premature birth, congenital anomalies, pregnancy induced hypertension, anaemia; *Luke 1994; King 2003*).
An inadequate supply of nutrients in young mothers, who are still experiencing their own growth will lead to competition for nutrients with the fetus (*King 2003*). Pregnancy in older mothers may be complicated by pre-existing metabolic health problems, such as chronic hypertension, diabetes, hypothyroidism and during gestation by fetal karyotype abnormalities, gestational diabetes, pregnancy induced hypertension and pre-eclampsia, which may affect fetal development and delivery (*Dildy et al 1996*).

Maternal birth size.

Although infant's birth weight shows correlation with both parental birth weights, the maternal effects are stronger (*Fall 2003a*). Mother's own birthweight strongly correlates with her infant's birth weight and placental weight, irrespective of parity and dietary intake (*Godfrey et al. 1996*).

Maternal weight.

Maternal pre-pregnancy weight (adjusted for height as BMI) and pregnancy weight gain reflect maternal nutritional status (*Neggers et al. 2003; Thame et al. 2004*). Apart from the gestational age these are the major determinants of fetal weight and birthweight (*Luke 1994*), which may be further modified by environmental factors (*Neggers et al 2003*). Low weight gain is a strong predictor of preterm delivery and LBW babies, especially in women who are underweight before pregnancy (*Schieve et al. 2000*; *Neggers et al. 2003*). Overweight women and women over 35 years of age experience rather low pregnancy weight gain (*Strauss et al. 1999*).

High maternal body mass in pregnancy leads to childhood obesity and development of insulin resistance and CHD in her offspring later in life, irrespective of its birthweight (*Godfrey et al. 2000; Eriksson et al. 2003b*).

Maternal height.

Short stature appears to predispose to lower size at birth. In the Pune Maternal Nutrition Study, the Indian mothers were short, of a low weight and BMI and also their babies were short and underweight, although centrally obese (see Ch 1.1.2). Placental and birth weights do not correlate with maternal age and social class, if corrected for maternal height (*Godfrey et al. 1996*).

Maternal constraint.

The uterus itself has growth limiting capacity for the fetus, which is important in controlling fetal overgrowth (*Murphy et al. 2006*). From animal experiments (embryo transfer/

manipulation studies) it is known that the intrauterine environment (uterine size) can override the growth potential set up by the fetal genome (*Godfrey et al. 1998*). A larger placenta enables the fetus to reach larger size (*Godfrey et al. 2000*).

1.4.2.2.2 Pregnancy spacing

Some studies investigated the effect of the time span between subsequent pregnancies on the pregnancy outcome and concluded that short intervals between subsequent pregnancies may result in maternal undernutrition and thus increase the likelihood of premature birth and low birth weight (*Rawlings et al. 1995; Zhu et al. 1999; King 2003*).

1.4.2.2.3 Maternal race, ethnicity, socio-economic status.

Maternal race and ethnicity may significantly influence the baby's birthweight, which may reflect social class, development status of the country and is gender specific. (*Kramer 1987; Shiono et al. 1986; Scholl et al. 2002*).

1.4.2.2.4 Maternal health

Maternal endocrine status, infections and diseases may influence fetal growth and development. Maternal hypertension (*Law et al. 1991*), renal disease and heart and respiratory disease (*Murphy et al. 2006*) are connected to an increased risk of growth retardation or preterm delivery.

Fetuses of diabetic mothers have higher glucose, insulin and leptin levels. They are born with greater birth weight and carry an increased risk of future obesity and glucose intolerance (*McMillen et al. 2006*).

1.4.2.3 Effects of maternal nutrition.

The importance of maternal nutrition in fetal programming has been emphasized by Hales and Barker (*1992)* in their Thrifty Phenotype Theory (see Chapter 1.1.3.2). However, nutrient effects on growth and development had already been shown in research studies, especially in animals. McCance (*1962*) performed experiments with dietary intervention on rats in different litter sizes and discovered that there was a critical period after birth during which the lack of nutrients resulted in smaller animal size and a permanent growth programming effect. Lucas (*1991*) described the effect of nutrition (human milk vs. standard or enriched pre-term infant formula) given to preterm infants during the first postnatal month on their further growth and development, and marked the neonatal period as critical for nutritional manipulation after preterm birth. Based on this finding and summarizing outcomes of animal studies, he established a concept of biological programming. He concluded that an inadequate nutrition during critical periods of early development might result in pathological processes later in life. Further research showed that inadequate maternal nutrition is one of the factors in utero, resulting in adverse changes in fetal growth and development (*Campbell et al. 1996; Cox 1999*).

In the following sections the effects of energy, macronutrient and micronutrient intake on fetal growth or birth outcome will be discussed.

1.4.2.3.1 Nutritional deprivation.

This effect was seen in babies born to mothers affected by famine during the Dutch Hunger Winter and during the Siege of Leningrad (see Chapter 1.1.2). The birth outcomes

differed according to the timing of the famine exposure. Only the third trimester exposure to famine caused differences in birth size and body proportions. Changes induced by inadequate maternal nutrition in early pregnancy may persist into adulthood without affecting birth weight (*Lumey1998a*; *Stein et al. 2004*).

1.4.2.3.2 Overnutrition.

Pregnancy overnutrition in humans is associated with development of gestational diabetes and pre-eclampsia and may lead to development of cardiovascular problems (*Martin-Gronert et al. 2006*) and/or metabolic syndrome phenotype in the offspring later in life (*Armitage et al 2005*).

1.4.2.3.3 Macronutrients in fetal growth.

In animal experiments, low protein diet has been studied quite extensively (see Chapter 1.1.3.2). Also the study of *Bieswal et al. (2006*) showed that low protein and low calorie intake in rats during pregnancy had resulted in growth retardation and low birth weight. The extension of the restricted diet after birth into the lactation period led to a life-long growth restriction. However, overfeeding of the growth-retarded offspring during the suckling period resulted in catch-up growth with the weight exceeding normally fed controls.

Numerous epidemiological studies, mainly involving populations of adults with available birth records or of pregnant women, have investigated the effects of macronutrients on pregnancy outcome or their relationship with the development of chronic diseases later in life. Some examples are listed in Table 1.1.

Table 1.1 **Epidemiological studies on nutrition**. Dietary intakes of protein (milk protein), carbohydrate and fat (fish ω-3 fatty acids) and their association with birth parameters and/or chronic disease later in life.

Authors	Population	Number	Study topic	Outcome
Campbell et al 1996	Follow-up study of men and women born in years 1948-1954 in Aberdeen, Scotland	253 men and women	Investigating how maternal pregnancy diet is associated with blood pressure of the offspring in adulthood	The offspring of women with **low animal protein** intake (<50g/d) and **high carbohydrate** intakes in late pregnancy had high blood pressure later in life. The same outcome was also associated with intake of **high animal protein** (>50g/d) and **low carbohydrate**. Elevated blood pressure was associated with lower placental size and low BW, irrespective of maternal nutrition.
Godfrey et al. 1996	The Southampton Study including pregnant women attending the Princess Ann Maternity Hospital, who completed gestation with a term delivery	538 women	Maternal dietary intake during early and late pregnancy and its relationship with the placental and fetal weight.	**High carbohydrate** intake in early pregnancy and **low dairy and meat protein** intake in late pregnancy suppressed placental growth and reduced birth weight

Authors	Population	Number	Study topic	Outcome
Sloan et al 2001	The New York Trial, including low income urban pregnant women	2163 women	Dietary protein intake (evaluated by two 24-h dietary recall) during pregnancy (months 4-6 and month 8) and its association with pregnancy outcome.	A negative effect of **high protein** diet was observed. Mean protein intake ≥85g/d resulted in decreased BW by 71g in comparison with a mean intake of protein 50-84.9g/d
Moore et al. 2004	Pregnant women 18-41 y old living in Adelaide, Australia	557 women	Maternal dietary intake during pregnancy and the birth size of the offspring. A dietary intake was assessed by means of a FFQ, administered during early and late pregnancy.	A direct association between the percentage of energy derived from **protein** taken in early pregnancy and the birth weight and placental weight was observed. The energy derived from **carbohydrate** intake at the same time of gestation was inversely related with neonatal ponderal index.
Rao et al. 2007	A community-based study in a rural area of Haryana in India	140 pregnant women (3rd pregn. trimester)	Maternal dietary intake of protein and total energy in association with BW	Low BW was linked to the third trimester mean **protein** intake of less than 40 g/d (average was 50.8g/d) and mean energy intake below 1500kcal
Andreasyan et al. 2007	The Tasmanian Infant Health Survey (1988-1989)	1040 mother-infant pairs	Maternal dietary intake during pregnancy and birth size	**High protein intake** was associated with lower BW and PONDIX. High protein diet taken at the 3rd trim. led to decreased PONDIX in large BW babies.

Authors	Population	Number	Study topic	Outcome
Milk (protein) and fish (fat) consumption:				
Rao et al. 2001	The Pune Maternal Nutrition Study including pregnant rural Indian women near Pune City.	797 women	Maternal dietary intake during pregnancy and birth size	Maternal consumption of **milk, fruits and green leafy vegetables** at 28 gest. weeks correlated with the birth size. No such correlation was observed for maternal energy or protein intakes. The **dietary fat** consumption at 18 weeks of gestation was associated with neonatal length. An important role of **micronutrients** in fetal growth was suggested.
Ludvigsson et al. 2004	Swedish pregnant women	12880 women	Milk consumption during pregnancy and the relationship with birth weight, IUGR and preterm delivery.(Questionnaire and birth registry)	Low **milk** dietary intake was associated with IUGR of the newborn
Olsen et al. 2007	The Danish National Birth Cohort (1996-2002)	50 117 mother-infant pairs	Milk consumption during pregnancy and the relationship with the birth size	Milk consumption during pregnancy increased babies BW for age and decreased rates of SGA fetuses.

Authors	Population	Number	Study topic	Outcome
Olsen et al. 2002	Swedish pregnant women	8729 women	Consumption of seafood and birth weight.	Low consumption of **fish** was linked to preterm deliveries, intrauterine growth restriction and low neonatal weight. Beneficial effect of **ω-3 fatty acids** derived from fish or fish oil supplements on birth outcomes were observed.
Mitchell et al. 2004	Auckland babies born between October 1995 and August 1996 and their mothers.	844 cases, 870 controls	Effect of maternal diet during pregnancy with respect to size at birth (AGA vs SGA)	A reduced risk of small for gestational age babies (SGA) was connected to increased fish consumption in early pregnancy.
Inconclusive:				
Mathews et al. 1999	The Portsmouth study, including healthy white nulliparous women attending the local antenatal clinics during 2-year period (May 1994-Feb1996).	693 women	Maternal dietary intake and smoking during pregnancy and their association with plac. and birth weights at term deliveries.	Only a weak nutritional effect on placental and birth weights was found. The maternal nutrition in developed countries might only have a small effect on placental and birth weights.

The epidemiological studies listed in Table 1.1 investigated separate nutrients or nutrients in combination, intakes during early or late pregnancy and also separate food items such as milk, fruits and vegetables and fish. In summary, low protein intakes and high protein densities were associated with decreased birth weight. The consumption of milk, fruits, vegetables and fish was linked to increases in birth weight and a beneficial effect of n-3 PUFA on birth size was reported.

1.4.2.3.4 Micronutrients in fetal growth.

At the time of organ and tissue development, during stages of cell proliferation and differentiation, micronutrients play essential roles in signaling (e.g. retinoic acid), enzymatic catalysis (e.g. copper) or as structural elements (e.g. zinc in transcription factors). Vitamins are important in cellular metabolism, maintenance and growth (*McArdle et al. 1999*) as antioxidants. Lack of vitamin B6 may impair pancreatic insulin production.

1.4.2.4 Effects of placenta

The intrauterine environment is believed to be a major determinant of fetal growth and development (*Godfrey et al. 1988*), where the placenta, a highly specialised organ supporting the fetus with nutrients and oxygen, represents the only communication pathway between the mother and the fetus. The complexity of this role involves the placenta in a variety of functions, such as transport, respiratory, metabolic and endocrine functions. The placenta also mediates heat exchange between the fetal and maternal circulations and serves as an immunological barrier with mother (*Lewis et al. 1990d*; *Gude et al. 2004; Myatt 2006*).

The placenta constantly develops and matures during pregnancy. Therefore, any disruption during placental development will result in a modified function and adverse effects on the fetus. The timing of an insult will determine the deviation in placental function (*Myatt 2006*). It is known, for example, that placentas of pregnancies complicated with preeclampsia and IUGR are associated with defects in transition of maternal placental arteries into low resistance vessels, which limits the oxygen supply to the fetus (*Macara et al. 1996; Dugoff et al. 2005*).

1.4.2.4.1 Placental volume.

Placental volume and the rate of placental growth are correlated with fetal size. These effects seem to be regulated by maternal weight and weight gain and her nutritional status (*Neumann et al. 1984; Thame et al. 2000 and 2004*).

1.4.2.4.2 Utero-placental blood flow

The Doppler ultrasound blood flow waveform analysis helps to identify fetal hypoxaemia, growth restricted fetuses (*Maršál 1989, Dugoff et al. 2005*) and utero-placental complications (*Bower et al. 1993*). Increased vascular resistance of uterine arteries due to inadequate trophoblast invasion (Chapter 1.3.2.2; *Divon 2002; Murphy 2006)* or umbilical cord abnormalities (*Somerset et al. 1993*) will show decreased end-diastolic flow velocity resulting in high values of placental indices - the resistance index (RI) and the S/D ratio (See Chapter 2.5.2.2).

IUGR fetuses have reduced perfusion of fetal tissues, and the umbilical vein blood flow is reduced in relation to fetal size (*Reece et al.1993; Murphy 2006*).

1.4.2.4.3 Placental transport

Depending on the nature of transported substance/solute and placental integrity, a substance may cross placenta intact, it may be metabolised, consumed or altered by placenta, or it may not be transported at all (*Hay 1994*). Transport can be mediated by diffusion, via transporter proteins or by endocytosis/ exocytosis processes utilising concentration gradients or transmembrane potentials as moving forces (*Moritz et al. 2006*).

Transport of gases (oxygen, carbon dioxide) is regulated by placental blood flow and simple diffusion. The high permeability of the placental membrane to oxygen and carbon dioxide enables rapid exchange of these gases and placental blood flow is thus the rate-limiting step for oxygen delivery to the fetus (*Sadler 2000c; Gude et al. 2004*).

Transport of glucose and amino acids depends on the availability from the maternal supply and on specific placental transport systems (*Bell et al. 1999; Jansson et al. 2003*). Their activity and expression is essential for delivery of nutrients to the fetus, and thus for the regulation of fetal growth.

Glucose transport.

Glucose is the most frequently transported carbohydrate and serves as the primary substrate for fetal oxidative metabolism (*Gude et al. 2004; Myatt 2006*). The placenta regulates the rate of glucose transfer and also consumes glucose in variable amounts. This regulation is linked to the magnitude of the concentration gradient between fetal and maternal

glucose levels, which are always relatively lower on the fetal side *(Jansson et al. 2001*). The fetus produces minimal amounts of glucose and only with advancing gestation, as pancreas develops, does the endogenous production of insulin becomes stimulated (see Chapter 1.2.5; *Gude et al 2004; Hay 2006b*). As maternal insulin does not cross the placental membrane, the correlation seen between maternal and fetal insulin levels may be due to a significant correlation between maternal and fetal glucose concentrations (*Nicolaides et al. 1989*).

The fetus requires an increase in the glucose supply with maturation and growth of different organs and therefore, in order to maintain the maternal glucose equilibrium, the rate of maternal glucose production increases. This is accompanied by development of gestational glucose intolerance and a state of insulin resistance within the mother (*Hay 2006b*).

Placental transport of glucose is mediated by facilitated diffusion, involving glucose transporters (*Illsley 2000*), which increase or decrease their expression in response to changes in glucose gradient (*Leonce et al. 2006).* In humans there are three transporters located on placental membranes: GLUT 1, the major and also rate-limiting glucose transporter present in placental membranes, and further GLUT 3 and GLUT 4 (*Illsley 2000*; *Murphy et al. 2006*).

Long-term changes in fetal glucose concentrations may lead to programming effects with adverse outcomes in the developing fetus. Maternal diabetic pregnancy leads to fetal hyperglycaemia that is associated with elevation of the growth-stimulating factor: insulin, insulin-like growth factor 1 (IGF1) and leptin (*Jansson et al. 2002; Myatt et al. 2006*). This may result in changes to the glucose transporter *(Hay Jr. 2006a*). Indeed, an increase of the basal membrane GLUT 1 expression (whilst activities of GLUT 3 and GLUT 4 remained

unaffected) was found by Illsley et al *(2000*) and also by other researchers (*Jansson et al. 1999*). Authors believed that these changes led to increased glucose transport to the fetus of diabetic mothers, in spite of her adequate control of the disease. No changes to glucose transport have been associated in women with gestational diabetes mellitus (*Jansson et al. 2001*).

The hypoglycaemia present in SGA fetuses may be a consequence of reduced supply/transfer of maternal glucose to the fetus, which may occur, for example, with increased placental glucose consumption. From experimental studies in sheep it is known that placentae of preterm IUGR pregnancies show increased glucose consumption in comparison with placentae of normal pregnancies (*Leonce et al. 2006; Murphy et al. 2006).*

In addition, in pregnancy, glucocorticoids can interfere with the expression of placental glucose GLUT 1 and GLUT 3 transporters (*Hahn et al. 1999*), adding further to the likelihood of adverse effects, including restriction of growth of the developing fetus.

Amino acid transport.

During the early preimplantation stages of development in the mouse embryo or in cultures of human fertilised eggs a preference for amino acids was noted, changing from non-essential (during cleavage) to essential amino acids (8-cell stage and later; *Van Winkle 2001*), with both contributing to an improvement in the conceptus/embryo development.

The fetus utilises amino acids mainly for protein synthesis, or they also can be metabolised by the fetus. The placental transport of amino acids from mother to fetus is

mediated by transporter proteins, found in both microvillous and basal plasma membranes (*Gude et al. 2004; Murphy et al. 2006)*. There are about 15 different amino acid transporter systems found in the human placenta (*Myatt et al. 2006*). Amino acids are more effective in insulin stimulation than glucose (see Chapter 1.2.5.4) therefore changes in their transport systems will reflect in fetal growth (*Jansson et al. 2002*). Reduction in the system A placental transporter expression and activity has been observed in hypoxia and IUGR pregnancies (*Ayuk et al. 2002; Nelson et al. 2003*), whilst in pregnancies complicated by diabetes mellitus or associated with accelerated fetal growth an enhancement of transporter activity was observed (*Jansson et al. 2002*).

The amino acid transporter system A appears to be hormonally regulated by human growth hormone in early pregnancy, and by insulin and leptin in late pregnancy (*Jansson et al. 2003; Ericsson et al. 2004*).

Transport of fatty acids.

Essential fatty acids and long-chain polyunsaturated fatty acids (LC PUFA) are important for fetal growth and development, namely during brain neurogenesis and adipose tissue changes in late pregnancy. The fetus depends on maternal supplies of LC PUFA (*Duttaroy 2009*). LC PUFA can cross the placental membrane by means of passive diffusion, however, it seems that their transport is mediated by several transport/binding proteins present on the membrane. These proteins are regulated by fatty acid activated transcription factors (*Duttaroy 2009)*. Free fatty acids (FFA) from maternal blood can cross the placenta by passive diffusion or their transport is mediated by the fatty acid binding and transfer proteins located in placental membranes (*Murphy et al. 2006*), which control the direction and the amount of transported fatty acid (*Gude et al. 2004*). Elevated maternal pregnancy levels of triacylglycerol

ensure availability of an FFA source (*Duttaroy 2009*). The triacylglycerol cannot cross the placental membrane, therefore it is metabolized by placental lipoprotein lipase to FFAs and glycerol. FFAs are transported to the fetal side and re-esterified with intracellularly generated glycerol phosphate (*Myatt et al. 2006*; *Duttaroy 2009*).

1.4.2.4.4 Placental endocrine function

The placenta produces numerous hormones in order to adjust maternal metabolism, enable "communication" with the fetus and regulate fetal growth (*Murphy et al. 2006*). Placental hormones are secreted into both maternal and fetal circulation and vary with development. The majority of receptors for these hormones are located on the placental syncytiotrophoblast, supporting the endocrine and paracrine function of the placenta. There are indications, that the placenta may regulate its own function in response to outside stimuli such as nutrition. (*Jansson et al.2003*).

The effect of hormones on fetal growth may be direct or indirect, mediated by changes in placental growth, fetal metabolism, including production of growth factors and other hormones by feto-placental tissues (*Fowden et al. 2004*).

Thyroid hormones.

The placenta is largely impermeable to maternal peptides. Hormones important in postnatal growth, such as growth hormone and thyroxine, are present in the human fetus from early gestation but their influence on fetal growth is less than in postnatal life (*Girling 2006)*. Iodine crosses the placenta, thyroxine (T4) is transferred only during the first trimester and triiodothyronine (T3) and thyroid stimulating hormone (TSH) do not cross at all (*Girling*

2006). Fetal thyroid hormone concentration is reduced in hypoxaemic conditions, leading to assymetrical IUGR with reduced muscle mass (*Fowden et al. 2004*).

During late pregnancy, the placenta supports fetal growth via hormones that affect the transport and/or utilisation of maternal nutrients. This includes growth hormone and insulin-like growth factors (*Gluckman 1995*). With low nutrient income, concentration of anabolic hormones like insulin and IGF-1 is diminished, and that of catabolic hormones (glucocorticoids) is elevated (*Barker 1998*).

Insulin-like growth factors (IGFs) and their binding proteins (IGF-BPs) play a major role in maternal tissue growth and development and in embryonic and fetal growth (*Westwood 1999*). Production of IGF-1 is regulated by placental growth hormone (GH), both secreted by the placental trophoblast (*Westwood 1999; Verhaeghe et al. 2002*). IGF receptors are controlled by six IGF-BPs. IGF-BP3 is the main IGF-1 binding protein, however the biologically active free fraction of IGF-1 is controlled by IGF-BP1 (*Qiu et al. 2005*). IGF-BP1 is produced by the liver and abundantly by maternal decidua, which points to its important role during the implantation process *(Westwood 1999*). The concentration of IGF-BP1 increases from early pregnancy (*Krampl et al. 2002; Verhaeghe et al. 2002*) and reaches steady maximum at mid-gestation. Insulin may be involved in this regulation by acting on hepatic GH receptor or IGF-BP1 liver production (*Qiu et al. 2005).* There is a structural similarity between insulin and IGF-1 and IGF-BPs regulate IGF-1's hypoglycaemic activity. Therefore IGF-1 may have insulin independent function in glucose metabolism and homeostasis. It can increase glucose disposal in skeletal muscle (*Qiu et al. 2005*). From in vitro studies it seems

that IGF-BP1 may have a role in endometrial development and elevated levels of IGF-BP1 can inhibit trophoblast invasion. Increased maternal concentrations of IGF-BP1 limit IGF-1 mediated growth, resulting in IUGR (*Krampl et al. 2002; see also Chapter 1.3.2.3).*

IGF-1 does not cross the placenta, but maternal levels may influence substrate transfer across the placenta and in this way regulate fetal growth (*Lind 1989*).

Glucocorticoids.

Glucocorticoids have a critical role in tissue development and maturation. An excess of glucocorticoids leads to growth restriction, hypertension, hyperglycaemia and upregulation of the hypothalamic-pituitary adrenal axis (HPA) (*Myatt 2006*).

Glucocorticoid levels are low in the fetus, with an increase in levels before the end of gestation to promote organ maturation (*McNeil 2007).* Glucocorticoids are supplied to the fetus by the mother across the placenta. This transport, and therefore the fetal exposure to glucocorticoids, is controlled by placental 11ß-hydroxysteroid dehydrogenase2 (HSD2), which converts cortisol into inactive corticosterone (*Fowden et al. 2004*). The second source of fetal glucocorticoids is derived from fetal adrenal gland, regulated by the HPA axis (*McNeil 2007*).

Deficiency (mostly due to mutation of the 11ß-HSD2 gene caused by chemicals) or alterations of the 11ß-HSD2 activity lead to overexposure of the fetus to glucocorticoids. This is the case in maternal undernutrition, protein deficiency, maternal stress, placental hypoxia (due to inadequate trophoblast invasion), and in maternal treatment with synthetic

glucocorticoids to reduce the risk of preterm labour (*Seckl 2004; McNeil 2007)*. Reduced placental expression of the 11ß-HSD2 is associated with IUGR.

11ß-HSD1 isoform, expressed also in placental tissues (maternal and fetal), can act in both directions, to deactivate and to regenerate cortisol from cortisone. Increased cortisol production was found during the third trimester (*Chan et al. 2007*).

Leptin.

Leptin receptors are present on pancreatic β-cells. This enables leptin to directly inhibit insulin secretion. On the other hand, insulin stimulates adipogenesis and leptin synthesis, creating a negative feedback loop ("adipoinsular axis").

There is a significant placental transfer of maternal leptin to the fetus, increasing with advancing gestation (*McMillen et al. 2006*). Leptin is also produced by placental trophoblast and is secreted to both maternal and fetal circulations. Leptin may have a significant role during the to gestational diabetes and IUGR (*Sagawa et al. 2002*). The study of Ong et al (*1999*) showed a correlation between cord blood leptin levels and the pattern of weight gain in early postnatal life. Leptin has a stimulating effect on fetal weight gain (*Harigaya et al. 1997*) and in angiogenesis (*Cao et al 2001*).

1.4.2.4.5 Intrauterine hypoxia

Low oxygen levels (hypoxia) can have harmful effects on placental tissues and placental function. Clinical conditions commonly associated with intrauterine hypoxia include maternal anaemia, pregnancy at high altitude and maternal pre-eclampsia (*Burton et al. 1999)*.

Maternal anaemia

In maternal anemia there is reduced capacity of the maternal blood to carry oxygen *(Hoque et al. 2009)*. This condition is defined as haemoglobin levels below 11 g/dl in the first and third trimester and below 10.5 during the second trimester (*Law et al. 1991; Milman 2008*). In South Africa the level of 10.0 g/dl is used (See Chapter 1.3.1.1). Nutritional derived iron deficiency is the most frequent type of anaemia in Sub-Saharan Africa however parasitic infections and HIV are strong contributing factors (*Hoque et al. 2009*).

Low maternal haemoglobin and iron levels during pregnancy were associated with large placental weights, placental to birth weight ratio (*Godfrey et al. 1991; Law et al. 1991*) and proportionally low cord blood haemoglobin, serum iron, transferrin saturation and ferritin. The latter suggests reduced iron supply to the fetus in maternal anaemia, as fetus extracts iron in proportional amounts with the maternal levels (*Singla et al. 1996*). An increase in placental weight is seen as a compensatory mechanism (*Burton et al.1999)*.

Iron deficiency anaemia is associated with low birth weight, premature birth and SGA infants (*Allen 2001; Milman 2008*), while the anaemia caused by pregnancy volume expansion is not related to adverse birth outcomes (*Rasmussen 2001;*). However, a steep decline in the iron stores was detected in pregnant urban White and Black South African women between the first and third trimester of gestation (*Guidozzi et al. 1995*), pointing to the importance of iron supplements in these population groups.

High altitude hypoxia

Pregnancies at high altitude (>2700m) are associated with hypoxia due to low atmospheric oxygen concentration (*Burton et al. 1999)*. This is connected with 2-5 fold-increased incidence of low birth weight, growth restricted fetuses, high risk of preeclampsia (*Zamudio et al. 2006*).

Oxygen level regulates development, proliferation and vascularisation of the villous trophoblast (*Myatt 2006)*.

Chronic hypoxia alters nutrient supply to the fetus; the concentration of placental GLUT1 is reduced and markers of hypoxia, erythropoietin receptor and transferrin, are increased (*Zamudio et al. 2006*). Also, amino acid system-A transporter activity and expression is downregulated in cultured term human trophoblasts (*Nelson et al. 2003*). Maternal placental hypoxia stimulates IGF-BP1 production, which is one of the mechanisms leading to IUGR (*Krampl et al. 2002*).

Adaptation to altering oxygen levels is mediated by the hypoxia-inducible transcription factors 1 and 2 (*Schäffer et al. 2005*). They activate corresponding target gene transcription according to the level of oxygenation (*Schäffer et al. 2005; Myatt 2006*).

Disturbance of physiological oxygen concentrations may lead to abnormal placental development (*Schäffer et al. 2005*). The Doppler blood flow velocity waveform analysis helps to detect fetal hypoxemia (See Chapters 1.4.2.4.2 and 2.5.2.2).

1.5 AIMS OF THE STUDY

1.5.1 Introduction to the research study.

Chapter 1 provides a detailed overview of the published literature that is relevant to the PND study research field, but, in some instances, also includes information that exceeds the research aims of the present study.

The major research focus of the current study concerns the determinants of fetal growth and birth weight (excluding the genetic factors). Two of these determinants, maternal nutrition and maternal pregnancy hormones, were investigated and their relationship with maternal anthropometry, fetal biometry, birth outcomes, postnatal infant growth and metabolic parameters was evaluated in the study.

Closely related to the current study research were also chapters including the stages of fetal development, placental transport of nutrients and endocrine pancreas as these were related to the study results interpretation.

It is important to realize the magnitude of the global prevalence of diabetes and the necessity of its early prevention. The latter does have a bearing on the study aims and naturally outcomes will have important implication. Also the studies from both the developed and developing countries were relevant to the PND study aims, as the nutrient intakes, anthropometry and birth parameters of infants in this study population were compared with those from countries in a similar transient state and from countries at a more advanced level of economic development.

1.5.2 Aims and objectives of the present study

In humans, various epidemiological trials have investigated the effects of maternal nutrition on birth outcomes and future disease risk. However, very few such studies have been conducted in Sub-Saharan Africa and South Africa itself.

In South Africa, the national prevalence of low birth weight is 8.9 %, displaying a wide range from 0.6% in Gauteng Province to 24.5% in the Northern Cape Province (SADHS survey, 2009; http://www.childrencount.ci.org.za). The targeted World prevalence, established at The World Summit for Children (1990) is below 10% (*Ramakrishnan 2004*). As was shown in the Dutch Hunger Winter study (see Chapter 1.1.2), the programming effect, which will lead to disease later in life, manifests as low weight at birth. However, the majority of epidemiological studies (see Chapter 1.1.2) found low birth weight to be associated with an increased risk of future disease, and this was confirmed in experimental studies on animal models with dietary restriction during pregnancy. As maternal health and nutrition are factors influencing fetal growth and development, more research in this field in African population is required.

South Africa is undergoing a rapid migration of people from rural to urban areas, which is associated with a transition in lifestyle and dietary patterns. Low energy intakes were seen in rural areas, while high energy intakes were recorded in urban areas (*MacIntyre 1998*). Urbanisation leads to lower physical activity and increased obesity (*Steyn 2005*). Although, as already mention in Chapter 1.2.2, the prevalence of diabetes is currently lowest in the African Region. At the same time the highest increase in prevalence of diabetes is expected in this

Region for the year 2030 as a consequence of rapid industrialisation and urbanisation. The prevalence of diabetes in South Africa (Chapter 1.2.3) is relatively high, ranging from 4.6 % in rural to 8% in urban areas.

South Africa has one of the highest HIV infection rates in the world. The national prevalence is 10.8% (www.hsrc.ac.za/Factsheet-40.phtml). This has put a great burden on the national health services and thus the resources and facilities for monitoring and treatment of non-communicable diseases, such as diabetes, are limited, and in many rural settlements are non-existent. Less than 20 % of the global costs of diabetes are associated with the Africa region (http://www.diabetesatlas.org; 4th ed.).

In summary, with increasing urbanization, the prevalence of Type 2 diabetes is expected to rise. Resources for the treatment of Type 2 diabetes however are limited. Therefore preventive measures need to be taken urgently. This should involve more research on nutritional status of mothers during pregnancy and what influence it may have on the developing fetus. Early detection of the problem and possible dietary intervention could influence the later outcome of the disease.

The Hertfordshire study was the first to show that slow prenatal and rapid postnatal growth was associated with impaired glucose tolerance and Type 2 diabetes. Also, a low birth weight, elevated markers of β-cell function (proinsulin, 32-33 split proinsulin and insulin) and a high BMI in adulthood were linked to the metabolic syndrome (Chapter 1.1.1). Further studies extended these risk indicators with thinness at birth (measured by ponderal index), small head circumference, high placental weight, short stature and disproportionate growth

assessed by the high placental weight to BW ratio all being linked to poor glucose tolerance and/or increased insulin resistance. Maternal nutrition was one of the factors implicated in fetal programming and increased risk of future metabolic disease.

The purpose of the present study was therefore to evaluate the dietary status of urban Black pregnant women attending the Johannesburg General Hospital. A further aim was to investigate the effects of maternal diet and hormonal levels during pregnancy on fetal and infant growth and other risk indicators associated with future Type 2 diabetes.

This is an exploratory study attempting to identify effects of individual nutrients and hormones on the prenatal and postnatal infant variables in order to identify potential factors and areas for possible future intervention. Therefore it does not include a predictive, complex model building design.

The aims of this study involve the following research questions:

- Does maternal pregnancy dietary intake of macronutrients affect fetal, birth or postnatal size or growth?
- Does maternal pregnancy dietary intake of macronutrients affect infant glucose and markers of infant β-cell function i.e. INS, PI, HOMA-IR?
- Does maternal pregnancy hormonal levels affect fetal, birth or postnatal size or growth?
- Does maternal pregnancy hormonal levels affect infant glucose and markers of infant β-cell function i.e. INS, PI, HOMA-IR?

CHAPTER 2

MATERIALS AND METHODS

2.1 STUDY POPULATION AND STUDY DESIGN.

This prospective longitudinal study took place at the Johannesburg General Hospital and the Faculty of Health Sciences of the WITS (Witwatersrand) University in the Gauteng province of South Africa.

The study population were pregnant women of a Black ethnic origin attending the Johannesburg General Hospital Antenatal Clinic. The study took place from March 2000 to September 2005 and consisted of two major parts in which pregnant women and then the same women and their offspring were followed.

Study design.

Twice during pregnancy (visits V1, V2) and approximately half a year after the delivery (V3), participating women had a standard oral glucose tolerance test (OGTT), blood pressure and anthropometric measurements taken (see Figure 2.1). Health status and socio-demographic data were recorded and nutritional status was assessed by means of a food frequency questionnaire (FFQ). During both pregnancy visits an ultrasound examination was carried out.

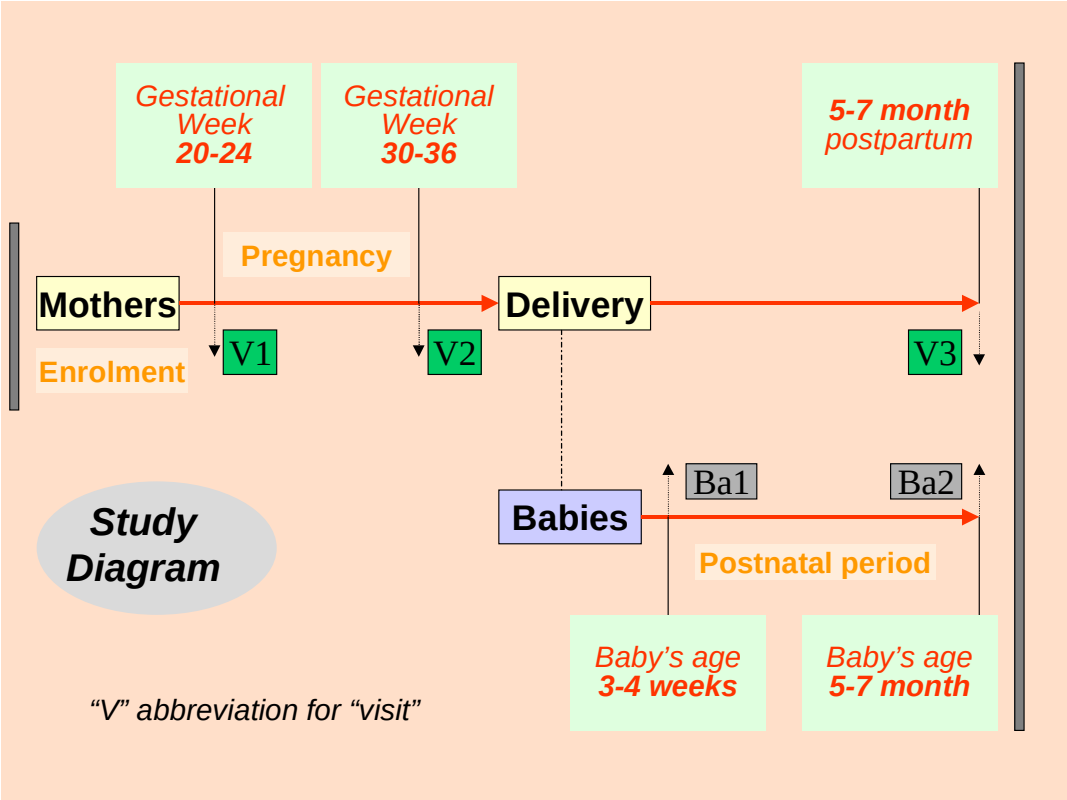


Figure 2.1 Study flow diagram.

The offspring of these mothers were followed. Three to four weeks after birth and half a year postnatally fasting blood samples and anthropometric data were collected (Figure 2.1).

Measurements and data collection methods in this study included:

a) Interview - consultation, structured questionnaires

b) Documentary source - patient's file and hospital records

c) Non-invasive measurements and methods - blood pressure, anthropometric measurements, ultrasound scan, biochemical analysis of blood samples

d) Invasive methods - oral glucose tolerance test, blood collections

Enrolment and ultrasound measurements took place in the Antenatal Clinic, while the anthropometric measurements, oral glucose tolerance test and questionnaires were carried out in the Day Ward of the Department of Chemical Pathology at the WITS Faculty of Health Sciences.

2. 2 ENROLMENT AND FOLLOW-UP

2. 2. 1 Sampling and enrolment

The present study participants were enrolled from the Antenatal Clinic in the Johannesburg General Hospital. The average age of women attending the Antenatal Clinic was approximately 30 years (see Chapter 3.1, Fig 3.2), although the age range of volunteers at the study enrolment extended from 18 - 40 years. The average gestational age at the first visit to the clinic was around 30 weeks of gestation. There were some women who presented for the first visit as early as in the first trimester, but also individuals who checked in just a few weeks or days before delivery. From all the pregnant women registered in the Antenatal Clinic, about 60-70% attended further visits and delivered in the same hospital (*information obtained from the Antenatal Clinic records*). Midwives and doctors closely followed all the patients at regular intervals for the duration of their pregnancy and at delivery.

The present study benefited from the routine organisation of the clinic. The Antenatal Clinic utilised a Voluntary Counselling and Testing program initiated by the Department of Health (*Gauteng Department of Health*). All expectant mothers underwent counselling for routine blood tests, which included syphilis, Rhesus (Rh) factor, haemoglobin (HB) levels, and

HIV. The HIV test was offered to each woman registered to the ANC. Women who were HIV positive were allocated for antiretroviral treatment with Nevirapine during the labour. According to the clinic records 25 - 30% of attendants were HIV positive.

The sampling and enrolment methods for the current study were designed to be in accordance with the routine of the Antenatal Clinic. The sampling was done in a number of sampling periods. These were of varying time intervals (from one week to one month), determined by the numbers of women successfully enrolled into the study (Appendix A). This procedure was regulated by the capacity of the Day Ward and ultrasound unit for study participants. Therefore, the convenient sampling method was used. Selection bias was avoided as all the women during the sampling periods had an equal chance of being selected (all patients' files were investigated for a match with the study enrolment criteria on each sampling day). The subject characteristics measured in the current study did not differ across groups of women sampled of different time periods.

Estimation of the sample size.

As this study involved pregnant women and neonates, the intention was to keep the number of subjects as low as possible, while still obtaining a sufficient power for data analysis. Because of the complexity of the current study, the sample size was estimated using the general rule for the relationship between the sample size and the number of variables in the multiple regression model (*Petrie et al. 2000a*). According to this rule a minimum of 10 subjects are needed for each explanatory variable entering the regression model. Therefore, at the lower range, between 70 – 150 cases were assumed to be sufficient for the analysis in the current study.

In total, 126 pregnant women were enrolled and attended the first study visit (V1). The loss to follow-up is reviewed in Chapter 3.2.

The selection criteria were as follows:

1) Pregnant woman of a Black ethnic origin
2) Age between 18 – 40 years
3) Gestation below 24 weeks
4) Non-diabetic (WHO 1999 criteria)
5) Women with a chronic disease requiring treatment were excluded from enrolment
6) Singleton pregnancy only
7) Haemoglobin levels above 10g/dl [3], syphilis and HIV screening negative
8) Blood pressure below 140/90 mmHg

The patient's file and the antenatal card information included personal details (name, ID, date of birth, address, hospital number, next of kin, demographic data), past medical history, history of previous pregnancies, family medical history and social status. During a patient's visit the file and the card were updated with routine examination outcomes, test results related to the clinic practice, with clinical or ultrasound assessment of the gestational length, fetal growth and well-being.

[3] concenration of haemoglobin defining pregnancy anaemia in South Africa (*Hoque et al.2007*)

The present study was described to volunteers using pamphlets and in a personal discussion with pre-selected individuals. The pamphlets contained information on the study, contact details of study investigators and they were printed in the most common languages spoken by Antenatal Clinic visitors. A nurse, who spoke a majority of these languages, helped with sampling and enrolment.

2.2.2 Organisation of the study

All participants had their Antenatal Clinic files and cards labelled with study stickers. Nurses from the Antenatal Clinic were familiar with the study requirements. Pamphlets with study details and study requirements were handed to the staff and displayed on information boards of the Labour and Postnatal Wards. The nurses were asked to report any delivery or admission for delivery of study participants to the study investigator by phone.

Mothers with their newborns were seen first in the Postnatal Wards. There were two Postnatal Wards in the hospital; one for mothers who underwent a normal vaginal delivery (complicated or uncomplicated), and the second for mothers who delivered by caesarean section.

2. 2. 3 Feasibility study

The first fifty participants were included into a feasibility study. This was used to set up and optimise the enrolment and study routine.

The routine procedure used at the Antenatal Clinic helped to exclude women unsuitable for the study at a very early stage and enabled close monitoring of participants during pregnancy follow-up.

Further adjustments were made to the study protocol at the end of the feasibility study:

a) The inclusion criteria questionnaire was reviewed and adjusted. Information available in the patient's antenatal card or file was removed from the questionnaire, and a photocopy of the hospital document was kept instead. Evaluation of the socio-economic status was extended by information referring to the number of household occupants and family monthly income.

b) Measurements of baby's abdominal circumference (AC) during postnatal visits and of maternal head and mid-upper arm circumference, as well as the postprandial C-peptide concentrations and the leptin assay were added into the study (see also Chapter 2.8.1.5).

The feasibility study and the main study data were combined together for analysis.

2.3 PROCEDURES PERFORMED IN DAY WARD (PRE- AND POSTNATAL)

2. 3. 1 Anthropometric measurements of women and babies

2. 3. 1. 1 Weight and height

Measurements of participants' weight and height were taken after removal of shoes and any heavy clothing. An accurate electronic weighing scale with an attached manually operated measuring rod was used (Modern scale Co. (Pty) Ltd, Johannesburg, South Africa). Measurements were taken to the nearest 0.1 kg, and the nearest 0.1 cm respectively. Babies were weighed and measured on a special weighing and measuring station. This included a digital baby scale Seca 736 equipped with the Seca 231 measuring rod, both distributed by Delta Surgical, cc, Johannesburg, South Africa. Babies were weighed undressed.

Body mass index (BMI) in women and babies was calculated according to the formula: BMI = weight [kg]/ height ^{2} [m^2].

Ponderal index (PONDIX), measure of adiposity for newborns, was calculated according to the formula:

PONDIX = birth weight [kg] / birth length^3 [m^3] *100 (*Leon 1999*)

2.3.1.2 Head, arm and abdominal circumference measurements

The head circumference of the mother (at occipitofrontal level) was measured by an ordinary non-elastic tape measure calibrated in centimetres and millimetres. Measurements were taken to the nearest 0.1 cm. Mother's mid-arm circumference was measured using an ordinary non-elastic tape measure. Measurements were taken to the nearest 0.1 cm. The abdominal circumference of the baby was measured using an ordinary non-elastic tape measure. The tape was placed around baby's abdomen at the umbilical level (measured on expiration).

2. 3. 2 Blood pressure measurement

The participant's blood pressure (BP) was measured in the supine position after resting. A mercury sphygmomanometer (Baumanometer, kompax desk model; supplied by Surgical Instruments (PTY), LTD, Johannesburg, SA) was used and BP was estimated to the nearest 2 mmHg by auscultation. An adult standard cuff size was used for all the women, except one case, when a larger cuff size had to be used.

2. 3. 3 Oral glucose tolerance test (OGTT)

2. 3. 3. 1 Procedure of the OGTT

The same oral glucose tolerance test procedure was carried out on pregnant and non-pregnant women (WHO Criteria 1999). The participant was instructed to abstain from any food and beverages from 10.00 p.m. the night before. Limited water intake was allowed. The

first (“fasting”) blood sample was collected between 8.00 and 8.30 a.m. (see blood collection procedure). An immediate blood glucose level was estimated by means of a Precision MediSense Blood Glucose Testing System utilising Precision Plus Electrodes (Abbot Laboratories; Abbot Park, Illinois, USA). This was a simple test carried out before the glucose load to confirm if the woman arrived for the visit “fasting”, or to determine if she was a diabetic. In the former case the OGTT was performed on another day, and in the latter case the woman was referred to the Antenatal Clinic and was excluded from the study.

The participant was given 75 grams of pure glucose (D glucose anhydrous; Saarchem-Merck, South Africa) in 200 millilitres of water to drink over a 5-minute period. Further blood samples were taken at 30, 60 and 120 minutes after taking the oral glucose load.

2. 3. 3. 2 Blood collection procedure during the OGTT

An intra-venous catheter was inserted by needle into the forearm vein (*antecubital fossa*) of the participant whilst in a supine position. A baseline fasting blood sample was collected into a plain 10 ml collection tube to obtain serum and one 5 ml potassium oxalate/sodium fluoride tube to obtain plasma sample (Vacutainer blood collection tubes; BD Diagnostics; Becton Dickinson Vacutainer Systems; Franklin Lakes, New Jersey, USA). The arm was kept warm with an electric heating pad to encourage circulation for ease of blood collection. Blood samples were stored at 4°C until the OGTT was completed.

2. 3. 4 Postnatal visits of babies

2. 3. 4. 1. Preparation for the blood collection

Mothers of neonates were instructed to feed the baby before 6.00 am, so the babies would have fasted for three and half to four hours before the test which was commenced between 8.30 and 9.00 am. The older babies (5-7 months) fasted for 6 hours. Both fasting periods were selected in accordance with an average interval between normal feedings at the particular age category (*Lewis et al.1990a*).

Babies were weighed and measured and their well-being was discussed with the mother. A trained nurse collected a blood sample by a heel-prick method (see below). After blood collection, mother was encouraged to breastfeed her baby. Baby's formula for newborns (Nestlé NAN Formula Number 1; *Nestlé, Vevey, Switzerland)* was also made available.

2. 3. 4. 2 Heel-prick blood collection method

The fasted baby was placed in the supine position and kept warm under a blanket. A heating pad placed around the baby's calf was occasionally used to encourage circulation to the heel area. The puncture site on the baby's heel was cleaned with an antiseptic swab. After the site had dried, the skin was punctured on the side of the heel with a sterile lancet (size 1.00mm x 2.5 mm). The first blood drop was wiped away. The Microtainer tube was held right under the blood collection site and blood drops were collected on the gel separator at the bottom of the tube *(Meites 1988).* Two small-volume pediatric Microtainer tubes were used. (BD Microtainer Brand Serum Separator Tube (SST); Catalogue No 365960; Becton Dickinson Vacutainer Systems; Franklin Lakes, New Jersey, USA). Between 0.8 -1.0 ml of

blood in total was collected. After collection, the tubes were sealed with a stopper and left standing for five minutes at room temperature to clot. Then they were kept at 4°C before the separation step (see Section 2.7.1 Sample preparation).

2. 4 QUESTIONNAIRES AND FORMS

2. 4. 1 Inclusion criteria questionnaire

At the volunteer's first visit to the Day Ward two questionnaires were completed: the inclusion criteria questionnaire and the food frequency questionnaire, which was re-administered at each visit**.**

Inclusion criteria questionnaire was a structured questionnaire collecting information on personal data, contact details, socio-economic status, education, occupation, medical history (see Appendix B).

Assessment of Socio – Economic Status (SES):

Education level of mother/father was classified into following categories:

	Age
1) Illiterate	N/A
2) Primary school level	<12 years
3) Standard 6 – 7	12-14 years
4) Standard 8 – 12, matriculation	14-18 years
5) Post matriculation, tertiary education	> 18 years

Employment status of the mother/father at the time of enrolment:

1) Student
2) Employed
3) Unemployed

Family monthly income categories:

1) Below or equal to R 1000
2) Over R 1000 but below or equal to R 2000
3) Over R 2000 but below or equal to R 3000
4) Over R 3000 but below or equal to R 4000
5) Over R 4000

Physical acitivity: A participant was asked if she was most of the time active (walking, standing, physically working, excercising) or resting a lot (sitting, lying).

2. 4. 2 Food frequency questionnaire

Nutritional intake during pregnancy (V1 and V2) and postpartum (V3) was assessed by means of a semi-quantitative food frequency questionnaire (FFQ) (Appendix C) supplied by a registered dietician (J MacKeown, Dental Research Institute, MRC) collaborating with the study. This questionnaire was adapted from the original used at Rowett Research Institute, Aberdeen, Scotland, for South African populations (*MacKeown 1999*). It has been validated previously by Margetts et al. *(1989*). A quantitative FFQ has been proven as a reproducible,

relatively valid and culturally sensitive method for assessing the dietary intake of Black South African in the North-West province (*MacIntyre1998, 2001a, 2001b).* The repeatability and validity of the FFQ have not been re-evaluated in the current study.

2. 4. 2. 1 Questionnaire procedure

Participants were asked to indicate how frequently the listed food items were consumed per week, with an extension to a frequency per month for the rarely eaten foods (Appendix C). The questionnaire was separated into 8 major food groups, listing over 50 main food items. The participants also indicated how many main meals and how many snacks they ate per day. Data on portion sizes was collected. Examples of serving sizes were available for display to each person undergoing the interview. Where there was a difference in volume between the raw and the cooked food item (e.g. rice or pasta), the cooked nutrient was considered. No correction was made for seasonal food, like fruits and vegetables. At the end of the questionnaire, participants were asked about the number of portions of certain food groups they ate the day before the interview and if this was a typical intake or different from their normal daily intake. This 24-hour recall was not included in the coding for nutritional intake, but served as a cross-check of the information reported in the FFQ to minimise possible over or underestimation of food consumption. In the case of disagreement between the two methods, the respondent would have to repeat the procedure.

The questionnaire was administered to each participating woman by one of the three trained interviewers (study coordinator, 2 nurses). Participants could choose to be interviewed also in their native languages Zulu (isiZulu), Xhosa (isiXhosa), Sotho (seSotho), or Tswana

(seTswana; http://countrystudies.us/south-africa/45.htm), although the majority selected English.

2. 4. 2. 2 Questionnaire coding and evaluation

The food information collected by the questionnaire was reviewed and coded onto computer coding sheets by the dietician, using the 1991 South African Medical Research Council (MRC) Food Composition Tables and codes (*Langenhoven 1991a*). Either the household measure recorded or standard portion sizes were used according to the National Research Programme for Nutrition Intervention (NRPNI) Food Quantities Manual (*Langenhoven 1991b*). A specific computer program written by the University of the Witwatersrand Computer and Networking Centre was used to determine the mean daily intake for each participant (MacKeown). Although only total energy and macronutrient intakes have been reported in this study, the micronutrient intakes were also recorded.

The macro and micronutrients assessed using the FFQ were as follows:

Macronutrients: protein (plant, animal, total), fat (saturated fatty acids - SAFA, monounsaturated fatty acids - MUFA, polyunsaturated fatty acids - PUFA, total) cholesterol, total available carbohydrate, dietary fibre, added sugar.

Micronutrients: calcium, vitamin B12, iron, magnesium, phosphorus, potassium, sodium, zinc, copper, vitamin A (RE), thiamin (vit B1), riboflavin (vit B2), nicotinic acid, pyridoxin (vit B6), folic acid, ascorbic acid, panthotenic acid, biotin, vitamin D, vitamin E, manganese.

2. 5 ULTRASOUND SCAN

2. 5. 1 Technical equipment

The feasibility study was performed on a Toshiba ultrasound machine (Tecmed). The instrument employed in further scans was the SONOLINE Sienna Ultrasound Imaging System from Siemens Medical Systems, Inc., Ultrasound Group, USA. *(Supplier: Authorized EC Representative Siemens Aktiengesellschaft Bereich Medizinische Technik in Erlagen, Germany).*

There were no significant differences found between the variances of fetal biometry measurements obtained from the Toshiba vs. the SONOLINE machine, therefore the Feasibility study results were merged with the Main study data. (Analysed by one-way ANOVA with the Levene's test for homogeneity of variances. *Petrie et al 2000c.*)

The gestational age (US MA) and the fetal weight estimates on both instruments were derived from different software pre-programmed formulas (Campbell and Shepard formulas respectively for Toshiba and the Hadlock formulas for SONOLINE). Therefore the estimates derived from the Toshiba instrument were recalculated using the Hadlock formulas only (*Hadlock 1984;* see Chapter 2.5.2.1) to enable comparisons between the data.

Fetal biometry and amniotic fluid index were evaluated by the real time B-mode ultrasound. Fetal well being and utero-placental blood flow in the third trimester were assessed by the Doppler ultrasound techniques (http://www.ob-ultrasound). Only trans-abdominal scans using a 3.5 MHz curvilinear transducer probe were performed in this study.

2. 5. 2 Ultrasound examinations

Ultrasound examination was carried out twice on all study participants. Routinely the scan followed directly after the OGTT procedure in the Day Ward or it was performed as close to this visit timing as possible. A requirement of the study was to carry out all scans on one ultrasound machine and by one operator.

2. 5. 2. 1 Ultrasound examination at the first visit (V1; 20-24 gestational weeks)

Fetal weight and pregnancy dating were assessed using following fetal biometry ultrasound measurements (see Figure 2.2):

1. Biparietal diameter (BPD)
2. Head circumference (HC)
3. Abdominal circumference (AC)
4. Femur length (FL)

These parameters are commonly measured at ultrasound examination during the **second trimester** (18 - 24th week of gestation) and further to estimate fetal growth and look for possible presence of fetal anomalies. At this time the fetal anatomy is well developed and structures of interest like face, spine, fingers are large enough to be visible for accurate evaluation (*Reece 1993).*

Head circumference **Abdominal circumference**



Biparietal diameter **Femur length**

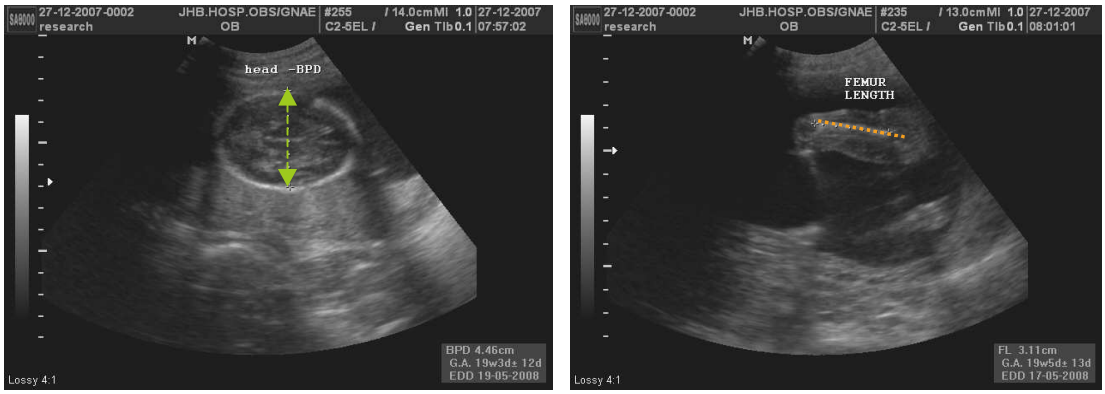


Figure 2.2 Ultrasound images of fetal biometry

Gestational age estimate (Menstrual age by ultrasound; USMA) was derived from all four biometric measurements and the regression equation of Hadlock (*1984):*

US MA [weeks] based on all four parameters (BPDcm, HCcm, ACcm, FLcm).

US MA = 10.85 + 0.060 * HC * FL + 0.6700 * BPD + 0.1680 * AC

In some instances the HC measurement was not recorded. In these cases calculation of US MA was based on the regression equation by Hadlock using three measurements only (BPDcm, ACcm, FLcm; *Sonoline Sienna User Reference 2000; Hadlock 1984).*

US MA [weeks] = 10.61 + 0.175 * BPD * FL + 0.2970 * AC + 0.7100 * FL

Estimated date of delivery (EDD) (*Sonoline Sienna User Reference 2000*)

US EDD = 280 days – US MA (in days) at "today's" ultrasound scan, added to "today's" date (where 280 days is an average length of human gestation).

Estimated fetal weight (EFW) (*Sonoline Sienna User Reference2000; Hadlock 1985*) was calculated according to the regression equation by Hadlock employing all four parameters: (BPDcm, HCcm, ACcm, FLcm)

EFW [g] =

10^(1.3596 – 0.00386*AC*FL+0.0064*HC+0.00061*BPD*AC+0.0424*AC+0.174*FL)

1SD = 7.4 %, 2 SD = 14.8 %

(where 10^(x) means exponent for a base-10)

For three measurements only:

EFW [g] with exclusion of HC based on (BPDcm, ACcm, FLcm).

EFW = 10^(1.335-0.0034*AC*FL+0.0316*BPD+0.0457*AC+0.1623*FL)

2 SD = 15.0 %

(where 10^(x) means exponent for a base-10)

2. 5. 2. 2 Ultrasound examination at the second visit (V2; 30-36 gest. weeks).

The same fetal biometry measurements were carried out at ultrasound visit 2 in order to assess the fetal size and weight. As single parameters in later pregnancy, the fetal length and the abdominal circumference can give the best estimate of fetal age and fetal weight respectively (*Reece 1993*).

The gestational age was calculated by the ultrasound software. However, the estimate from the first ultrasound scan was used for all pregnancy dating, as due to normal fetal biologic variations the third trimester ultrasound dating is not as reliable (*Reece 1993*).

Amniotic fluid volume is an important indicator of fetal well-being. It is commonly estimated by the ultrasound technique and expressed as an amniotic fluid index (AFI; *Phelan et al. 1987; Nwosu et al. 1993*; *Schrimmer et al. 2002*). At term the index reaches level of 11-16 cm (*Durbin et al. 2005*). Too high or too low AFI (*Gramellini et al. 2004*) is found with pathologic conditions of polyhydramnios and oligohydramnios respectively (*Lewis et al. 1990b*). The former may be related to congenital malformations, or maternal diabetes, the later may be a consequence of placental insufficiency with decreased placental blood flow and growth restriction (*Sadler 2000c, Lewis 1990b*).

Amniotic fluid index (AFI) was determined by the "four quadrant method" (*Moore 1990, Nwosu et al. 1993, Schrimmer et al. 2002).* The AFI represents a sum of the largest vertical depth of fluid in each of four arbitrarily divided uterine quadrants (pockets; D1 – D4). See Figure 2.3.

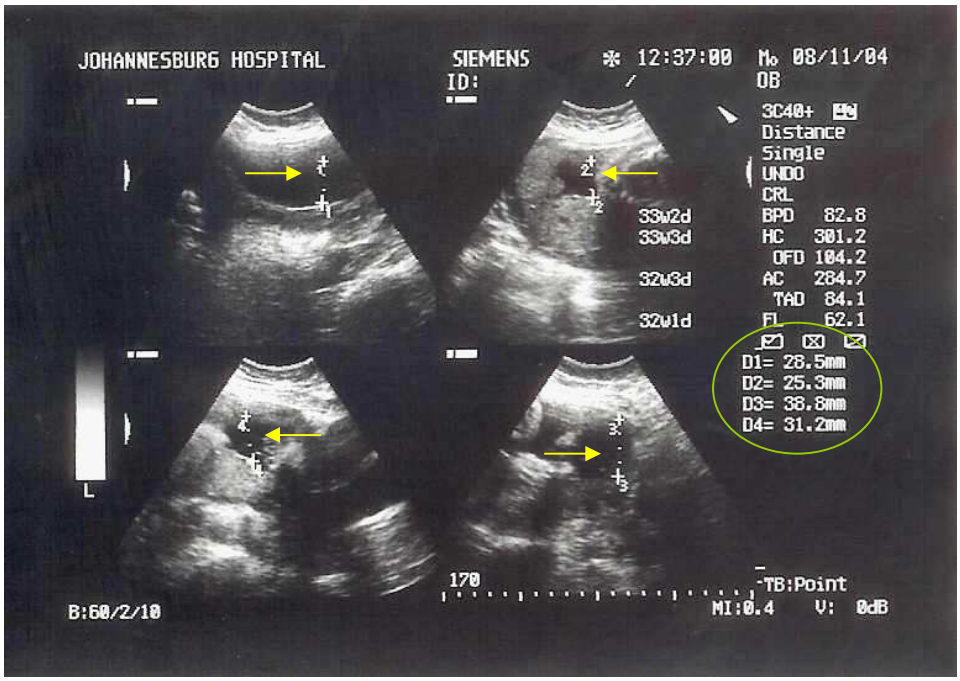


Fig. 2.3 Four quadrant method for AFI estimation.

Doppler measurements.

To assess the uteroplacental blood flow, fetal well-being and predicting fetal distress, hypoxaemia or growth-retarded fetuses, the spectral Doppler ultrasound waveform analyses was carried out (see Chapter 1.4.2.4.2). The Doppler ultrasound is a non-invasive real time imagining technique commonly used in obstetrics to evaluate maternal and fetal haemodynamics, e.g. to study pattern of uteroplacental and umbilical blood flows (*Maršál 1989*). The Doppler waveform analysis follows the maximum velocity of red blood cells from large arteries during the cardiac cycle (*Maršál 1989; Maršál et al.1999*). A forward flow is seen during the systole and the diastole in normal pregnancies. Low, absent or even reversed

diastolic flow indicates various degrees of increased down-stream resistance (*Lewis 1990c).* The velocity of blood flow can be calculated.

The umbilical cord contains two arteries (carrying deoxygenated blood and waste products from fetus towards placenta) and one umbilical vein (carrying oxygenated blood and nutrients from placenta to fetus) branching into chorionic plate vessels. The cord on average is 55 cm long and about 1-2 cm in diameter. The length may vary between 30 and 90 cm (*Moore 1993c).* All Doppler scans were done on an umbilical artery.

The spectral Doppler ultrasound waveform analysis uses three major indices to evaluate blood flow (*Thompson et al. 1988*; see Figure 2.4):

1. The Resistance index (RI; also known as Pourcelot ratio) represents a measure of resistance to arterial flow (*US-TIP*):

RI = (systole – diastole) / systole. With diastole missing (in severe cases of fetal distress) the ratio is equal to 1, which presents the index maximum.

2. Pulsatility index (PI**;** also known as Gosling and King's index; PI = (S – D) / A) can also reflect reverse flow (S states for a systole, D for a diastole and A is a mean height over one cardiac cycle).

3. Systole to diastole (S/D) ratio introduced by Stuart et al in 1975 as simplified Pulsatility index (also known as A/B ratio) (*Divon 2002*).

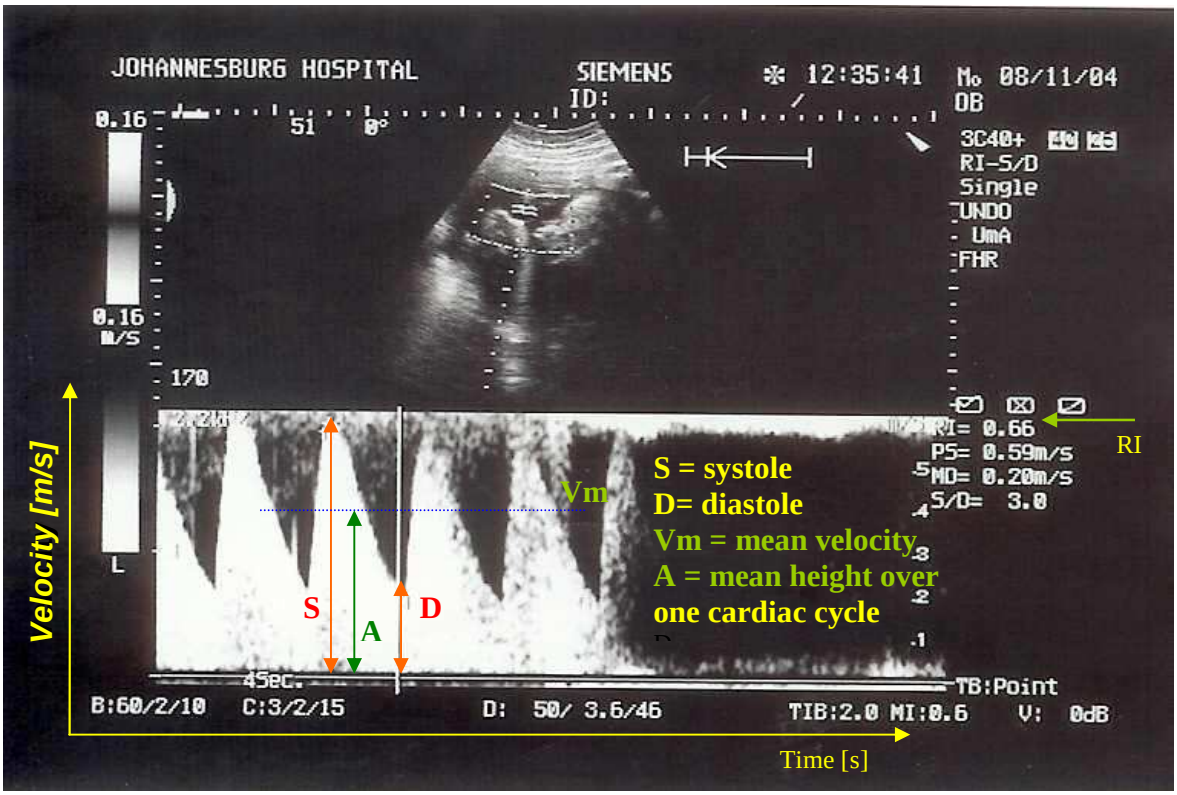


Figure 2.4: Spectral Doppler ultrasound waveform analysis[4] performed on the maximum velocity of red blood cells from umbilical artery.

The fetal well-being was assessed from the global ultrasound outcome.

[4] All ultrasound images (Figures 2.2, 2.3, 2.4) were taken in the Antenatal Clinic by study sonographer Mrs. Z. Holland, for the purpose of this study.

Assessment of intrauterine growth retardation (IUGR)**.**

In addition to the RI, tabulated ratios of **HC to AC** (by *Campbell et al.1977*) and of **FL to AC** (by *Hadlock et al. 1983*) can be used in assessment of fetal growth retardation.

The fetal liver is the most affected fetal organ in cases of abnormal fetal development like IUGR or macrosomia (see Chapter 1.1.3.2. Brain sparing effect). The AC measurement reflects the liver size and it is considered as the most sensitive single parameter for growth retardation detection with sensitivity of 83% and false positive rate of 10% (*Reece 1993)* Head measurements and femur length are usually unaffected (or head is larger) in asymmetrical IUGR, while AC is disproportionately reduced (*Hadlock et al. 1983; Campbell 1989;* see Chapter 1.4.1). Therefore, the HC/AC ratio in serial measurements is able to distinguish normal growth from symmetric and asymmetric IUGR. Until 36 gestational weeks the HC is larger than the AC giving a ratio >1 (*Moore et al. 1993b*).

In the present study the ratio of Hadlock (FL/AC *100) was used. Between 21 to 42 gestational weeks this ratio stays practically constant (22 ± 2) with upper limit (90th percentile) of 23.5. This limit corresponds to a sensitivity of 63.3% and specificity of 90% for detecting IUGR (*Hadlock 1983).* Values exceeding 23.5 after 21 are considered to be indicative of IUGR.

Neonatal **Ponderal index** is also used as relatively reliable method for recognising asymmetric IUGR (*Reece 1993.)*

2.6 DELIVERY AND DELIVERY RECORDS.

Nurses from the Labour Ward were asked to report to study investigators the admission or delivery of any woman volunteering in the study. Delivery information was available in the Labour Ward birth register. Detailed notes on labour proceedings and the well-being of the mother and her infant were recorded in patient's hospital file and kept in the Postnatal Ward where mother was admitted after delivery.

The following information was captured from Delivery records:

Date of delivery

Time of delivery

Type of delivery:

(Normal vaginal delivery;

Caesarean section)

Delivery complications

Placental weight

Cord length

Blood loss

Newborn's details:

Birth weight

Birth length

Head circumference

Apgar score

Sex

Assessment of age at delivery

Apgar score (introduced by Dr. Virginia Apgar in 1952, Columbia University, USA; *Casey et al 2001*) is a quick and simple method to assess newborns health status. It is performed at 1, 5 and 10 minutes after birth. Baby is evaluated on five criteria: skin colour,

heart rate, reflex irritability, muscle tone and breathing on a scale of zero to two for each criterion. Total Apgar score is a sum of these scores. Scores over 7 are considered as generally normal (http://embryology.med.unsw.edu.au/Child/apgar.htm).

The babies with an abnormal Apgar score (below 7 at 10 minutes) were not selected for further study. One neonate was excluded for this reason.

2.7 BIOCHEMICAL ANALYSIS

2.7.1 Specimen collection and storage.

Pregnant women and mothers.

Serum: blood was collected by venepuncture into plain blood collection tubes (Vacutainer blood collection tubes; BD Diagnostics; Becton Dickinson Vacutainer Systems; Franklin Lakes, New Jersey, USA), allowed to clot, and kept at 4°C until all the OGTT samples were collected. The chilled blood samples were processed further; serum was separated from cells by centrifugation (Beckman, TJ-6, table top horizontal rotor/swing-out head centrifuge) at speed of approximately 1300 - 1400 x g for 15 to 20 minutes at approximately 4°C. Samples were stored at -20°C until assayed. For longer storage a set of samples was kept at -75°C, as well as samples for IGF-1 estimation.

Serum from fasting samples collected at each of the three maternal visits was used for measurement of insulin, proinsulin, leptin, IGF-1, IGF BP-1, IGF BP-3, thyroid hormones, HPL, C-Peptide and cortisol. In addition, the serum samples collected during postprandial time points (30', 60' and 120') of the OGTT were used for insulin, proinsulin and C-peptide assays. The HPL was measured only during pregnancy (visits V1 and V2) and cortisol only at V2 and V3 from fasting blood samples collected between 8.00 and 8.30 a.m (see Chapter 2.3.3.1). Haemoglobin concentration was obtained from the Antenatal Clinic records at the time of enrollment.

Plasma: blood was collected into Vacutainer tubes containing potassium oxalate and sodium fluoride as an anticoagulant. Collected samples were thoroughly mixed with the anticoagulant. Tubes were kept at 4°C until all the OGTT samples were collected and the chilled samples were processed further. Plasma fraction was separated by centrifugation (Beckman, TJ-6, table top swing head centrifuge) at speed of approximately 1300 - 1400 x g for 15 to 20 minutes at 4°C. Samples were stored at -20°C until assayed. For longer storage a set of samples was kept at -75°C. Plasma samples (F, 30', 60' and 120' of the OGTT) were used for measurement of glucose levels.

Baby samples.

For babies, only blood for obtaining serum samples was collected to reduce volume of blood withdrawn. Therefore, the glucose measurement was performed on serum samples. The small-volume paediatric Microtainer tubes were used (BD Microtainer Brand Serum Separator

Tube (SST); Becton Dickinson Vacutainer Systems; Franklin Lakes, New Jersey, USA). Fasting blood samples were kept at 4°C until completion of baby's visit (see the heel prick sampling procedure above). Afterwards, samples were spun down in centrifuge and stored at –20°C. For longer storage one sample was kept at -75°C. Serum samples were used for the measurement of glucose, insulin, proinsulin, leptin and IGF BP-3.

2.7.2 Determination of Glucose.

Glucose in maternal plasma or babies' serum samples was analysed using a Beckman Glucose Analyser 2 (Beckman Instruments, Fullerton, California, USA).

Principle: The system employed an oxygen consumption rate method developed by Beckman Instruments and utilised specific Beckman reagents. The method is based on the enzymatic reaction of β-D-glucose from a standard or patient's sample with dissolved oxygen present in the reagent solution as follows:.

$$\beta\text{-D-glucose} + O_2 \xrightarrow[H_2O]{\text{glucose oxidase}} \text{Gluconic acid} + H_2O_2$$

An electronic system equipped with a Beckman oxygen electrode detects a rate of oxygen consumption, which is directly proportional to the concentration of glucose in the sample. The technique was selected for its very low sample consumption of 10 microlitres.

Sensitivity: Lowest detectable value 0.6 mmol/l (10 mg/dl; *Beckman -User Manual*)

Cross reactivity: No interference found with pathologic levels of bilirubin, uric acid, creatinine, lipids or haemolysis up to 1 g of haemoglobin per 100 millilitres. No effect of drugs (DOPA, salicylates) or agents preventing glycolysis or coagulation.

Precision.

Intra assay variation – Standard deviation (SD) was less than 0.14 mmol/l for twenty replicates of the Beckman 8.3 mmol/l aqueous standard.

Inter assay variation - was less than 0.17 mmol/l

2.7.3 Immunoassays.

The majority of analytical tests in this study utilised an enzyme immunoassay carried out on 96-well microplates to determine an antigen of interest from collected serum or plasma samples. Two basic approaches were used: Assays with competitive binding and assays with non-competitive binding.

2.7. 3. 1 Instruments

Instruments employed in present study were the following:

Microplate shaker/incubator: Amesham UK, Model ZLE.164.

Microplate washer: Labsystems Multiwash, Model 8070-06; Labsystems Ltd., Helsinki, Finland; manufactured in USA.

Microplate reader: Labsystem Multiscan Biochromatic, Type 348, Labsystems Ltd., Helsinki, Finland.

2.7. 3. 2 Non-competitive immunoassay.

Antigen present in the sample, standard or control is bound between two monoclonal antibodies with specific binding sites. The first antibody is attached to a solid phase (capture antibody) and the second antibody carries a label for detection:

- wall – capture antibody – antigen – detection antibody - label

The unbound fraction is separated from immobilised "sandwich" complex and the amount of labelled antibody is detected by a spectrophotometer. The amount of analyte is **directly proportional** to the concentration of the detection antibody, bound to the well of the microplate.

2.7. 3. 3 Non-competitive assays used in the present study

Insulin (INS)

INS- EASIA (Enzyme Amplified Sensitivity Immunoassay) kit; BioSource Europe S.A., Nivelles, Belgium;

Catalogue Number: KAP 1251

Distributed by the Laboratory Specialities, cc, South Africa.

Principle and procedure of the test:

1) Standards, quality controls and samples were incubated with the capture monoclonal antibodies (Mab1) attached to the walls of a microtitre plate and with the detection antibody-horseradish peroxidase conjugate (Mab2-HRP). A sandwich complex was achieved in one incubation step:
    - microtitre well wall – Mab 1 – human insulin – Mab 2-HRP.
2) Unbound Mab2-HRP conjugate was washed off.
3) Tetramethylbenzidine (TMB) – H_2O_2 was added as a substrate to produce a chromogenic reaction catalysed by the remaining bound Mab2-HRP conjugate.
4) After a short incubation the reaction was stopped by an acid solution resulting in yellow reaction product.
5) The absorbance values were measured on 96 well-plate reader with colorimetric detection at a wavelength of 450 nm.
6) The concentration of unknown samples was obtained by interpolation from a standard curve plot of absorbance values against the known concentration values, of the standard samples.

Analytical sensitivity.

The assay detection limit (defined as A_{blank} + 2*SD_{blank} above the mean of zero calibrator) was 0.15 µIU/ml (1.04 pmol/l)

Specificity.

Cross-reactivity: was measured on the high value calibrator. Human proinsulin cross reactivity was negligible.

Precision.

Intra-assay and inter-assay coefficients of variation (CVs) were 5.3 % and 5.6 % for low, and 3.0 % and 9.8 % respectively for low to medium concentrations of the analyte.

Upper detection limit: 500 µIU/ml (3450 pmol/l)

Proinsulin (PI)

INTACT PROINSULIN ELISA kit; DakoCytomation Ltd, UK;

Catalogue Number: K 6242

Distributed by DakoCytomation, South Africa

Principle of the test:

The assay is based on the same principle as the insulin assay.

Procedure:

A sandwich complex was achieved in two incubation steps. Each step involved a washing procedure removing unbound antigen after the first incubation period and unbound Mab2-enzyme following the second incubation period. Also the chromogenic reaction was achieved in two steps. After incubating the sandwich complex with an enzyme substrate, an amplifier was added into wells. Nicotinamide adenine dinucleotide phosphate (reduced form; NADPH)

was the substrate for the chromogenic reaction. The enzyme amplification system served as a highly sensitive detector of alkaline phosphatase molecules, which was the enzymatic label used in this assay. The absorbance values were measured at the wavelength of 490 nm.

Analytical sensitivity.

The assay detection limit was below 0.5 pmol/l.

Specificity.

No *cross-reactivity* was detected with human insulin, C-peptide or 32-33 split proinsulin; 100% cross-reactivity found for 64-65 split proinsulin. No interference from heterophilic (e.g. human anti-mouse) antibodies or rheumatoid factor was seen.

Precision.

Intra-assay and inter-assay CVs were 4.0 % and 5.5 % respectively for low and

2.4 % and 3.3 % respectively for medium concentrations of the analyte.

Upper detection limit: 100 pmol/l

Leptin

HUMAN LEPTIN ELISA, Clinical Range kit; Supplied directly from: BioVendor Laboratory Medicine, Inc., Czech Republic, EU.

Cat. No: RD191001100

Principle of the test:

The analysis is based on the same principle as in the insulin assay.

Procedure:

A sandwich complex was achieved in a two-step procedure as in the proinsulin assay. Horseradish peroxidase was used as an enzymatic label for Mab2. The chromogenic reaction was achieved in one step only using TMB as a substrate. The absorbance of the product was read at 450 nm.

Analytical sensitivity.

The assay detection limit (A_{blank} + 3*SD_{blank})) was 0.5 ng/ml.

Specificity.

No *cross-reactivity* was reported in the kit insert.

Precision.

Intra-assay and inter-assay CVs were 7.5 % and 9.2 % respectively for low and

6.7 % and 3.2 % respectively for medium concentrations of the analyte.

Upper detection limit: 50 ng/ml

Insulin-Like Growth Factor 1 (IGF-1)

OCTEIA IGF-1 kit; IDS (Immunodiagnostic Systems Limited), UK.

Catalogue Number: AC-27F1

Distributed by the AEC-Amersham (PTY), LTD, South Africa

Principle of the test:

The assay is based on the same principle as the insulin assay.

Procedure:

Prior to the analysis, samples were pre-incubated with a proprietary reagent to dissociate IGF-1 from binding proteins. The walls of microplate were pre-coated with purified sheep polyclonal anti-IGF-1 antibodies. Detection monoclonal antibodies were linked to HRP. A sandwich complex was achieved in a one-step procedure. The chromogenic reaction was accomplished in the same manner as in the insulin assay. The absorbance values were measured at a wavelength of 450 nm.

Analytical sensitivity.

The assay detection limit was 1.9µg/l.

Specificity.

Cross-reactivity: no interference was observed with IGF-2, insulin or proinsulin.

Precision.

Intra-assay and inter-assay CVs were 3.5 % and 7.0 % respectively for low and

2.3 % and 7.1 % respectively for low to middle concentrations of the analyte.

Upper detection limit: 1070 μg/l (1 μg/l = 0.131 nmol/l)

Insulin-Like Growth-Factor-Binding Protein 1 (IGF BP-1)

IGFBP-1 IEMA (former PP12 IEMA) kit; Oy Medix Biochemica Ab, Finland. Catalogue Number: 108 51 ETMB

Distributed by the AEC-Amersham (PTY), LTD, South Africa

Principle of the test:

The assay is based on the same principle as the insulin assay.

Procedure:

A sandwich complex was achieved in a two-step procedure. Further reaction steps and reagents used were as in the insulin assay. The absorbance values were measured at a wavelength of 414 nm.

Analytical sensitivity.

The assay detection limit was 0.4 μg/l.

Specificity.

No *cross-reactivity* was observed with human IGFBP-2, IGFBP-4, IGFBP-5 or IGFBP-6 up to concentrations of 5000 μg/l or purified human IGF BP-3 up to hundred thousand micrograms per litre.

Precision.

Intra-assay and inter-assay CVs were 5.4 % and 5.8 % respectively for low and

5.4 % and 5.9 % respectively for higher concentrations of the analyte.

Upper detection limit: 180 μg/l

Insulin-Like Growth-Factor-Binding Protein 3 (IGF BP-3)

IGFBP-3 ELISA kit; BIOCODE S.A., Belgium.

Catalogue Number: EL 2014

Distributed by the AEC-Amesham (PTY) LTD, South Africa

Principle of the test:

The assay is based on the same principle as the insulin assay.

Procedure:

The assay procedure was as in the insulin assay. The enzyme label linked to Mab2 was alkaline phosphatase. Para-Nitro Phenyl Phosphate was used as a substrate for chromogenic reaction. The absorbance values were measured at a wavelength of 405 nm.

Analytical sensitivity.

The assay detection limit was 10.5 ng/ml.

Specificity.

No *cross-reactivity* was found with IGF-1, IGFBP-1 or IGFBP-2.

Precision.

Intra-assay and inter-assay CVs were 4.3 % and 13.7 % respectively for low and

8.3 % and 8.3 % respectively for medium concentrations of the analyte.

Upper detection limit: 6500 ng/ml

Human Placental Lactogen (HPL)

HPL ELISA kit; DRG Diagnostics, Germany.

Catalogue Number: EIA-1283

Distributed by AEC-Amersham (PTY) LTD, South Africa.

Principle of the test:

The assay is based on the same principle as the insulin assay.

Procedure:

The assay procedure, label, chromogenic reagent and wavelength at which absorbance readings were taken were as in the insulin assay.

Analytical sensitivity.

The assay lower detection limit was 0.3mg/l.

Specificity.

No *cross-reaction* with human chorionic gonadotropin (HCG), alpha fetoprotein, human growth hormone or prolactin has been detected.

Precision.

Intra-assay and inter-assay CVs were 6.1 % and 8.8 % respectively for low and 6.7 % and 5.7 % respectively for medium concentrations of the analyte.

Upper detection limit: 20 mg/l

2. 7. 3. 4 Competitive immunoassay with enzymatic label.

A fixed quantity of labelled antigen and unknown amount of antigen present in the sample, standard or control (analyte) compete for a limited number of specific antibody binding sites. At equilibrium two types of immobilised complexes coexist:

- wall – capture antibody – antigen – label
- wall – capture antibody – analyte

Unbound reagents are separated. Bound antigen-label complex is detected by a spectrophotometer. The amount of analyte is inversely proportional to the amount of labelled antigen.

2. 7. 3. 5 Competitive assays in the present study

They were all solid phase immunoassays.

Cortisol

CORTISOL ELISA kit; DRG Diagnostics, Germany.

Catalogue Number: EIA-1887

Distributed by the AEC-Amersham (PTY) LTD, South Africa.

Principle of the test and procedure:

1. Cortisol-HRP conjugate and unknown amount of cortisol present in the sample, standard or control competed for a limited number of specific antibody binding sites coated on the wells of the microtitre plate. Two immobilized fractions were created:
    - wall – antibody – cortisol – HRP
    - wall – antibody – cortisol from sample
2. Washing step removed unbound reagents.
3. Tetramethylbenzidine (TMB – H_2O_2) was added as a substrate to produce a chromogenic reaction catalysed by the remaining bound cortisol-HRP conjugate.
4. After a short incubation period the reaction was stopped by an acid solution resulting in a coloured reaction product.
5. The absorbance values were measured on a 96 well-plate reader with colorimetric detection at a wavelength of 450 nm.
6. The concentration of unknown samples was obtained by interpolation from a standard curve plot of absorbance values against known standard sample concentration values.
7. The unknown amount of cortisol in the sample was inversely proportional to the reaction product, and so to the amount of labelled cortisol.

Analytical sensitivity.

The assay detection limit was 2.5 ng/ml (6.9 nmol/l).

Specificity.

Cross-reactivity was reported with cortisol (100%), corticosterone (31 %), progesterone, deoxycortisol and dexamethazone <2%.

Precision:

Intra-assay and inter-assay CVs were 5.0 % and 4.4 % respectively for low and

9.6 % and 4.0 % respectively for low to medium concentrations of the analyte.

Upper detection limit: 800 ng/ml

During the study, the manufacturer two times modified the test procedure. In the washing step (No 2) the volume of washing buffer was increased from 250 µl to 350 µl and eventually to 400 µl.

Cross-reactivity in the latest modified kit was reported higher for corticosterone (45 %) and for progesterone <9%.

Precision of the latest modified kit:

Intra-assay and Inter-assay CVs were 8.1 % and 6.6 % respective for low and 3.2 % and 7.7 % respective for low to medium concentration of the analyte.

A comparison was done between both assays and the results obtained from the new assay corresponded well (CV <10%) with the results obtained using the original assay.

C-Peptide (C-PEP)

C-PEP-EASIA kit; BioSource Europe S.A., Belgium.

Catalogue Number: KAP 0401

Distributed by the Laboratory Specialities cc, South Africa.

Principle of the test and procedure:

Principle and the procedure for the test were the same as for the cortisol assay, except that an extra incubation step was included to enhance the sensitivity of the assay. This was accomplished by carrying out the competitive incubation in two separate steps; first pre-incubating samples in antibody-coated wells followed by later addition of the antigen-HRP conjugate. Afterwards the assay proceeded according to the routine as described for the cortisol assay.

Analytical sensitivity.

The assay lower detection limit was 0.01 nmol/l.

Specificity.

Cross-reactivity: 30% cross-reactivity found with monkey C-peptide, and low cross-reactivity with biosynthetic human proinsulin. No interference was detected with purified porcine proinsulin, C-peptide, insulin or glucagon or beef glucagon.

Precision.

Intra-assay and inter-assay CVs were 6.2 % and 5.7 % respectively for low and 6.7 % and 3.7 % respectively for low to medium concentrations of the analyte.

Upper detection limit: 7.5 nmol/l

(Conversion factor: 1 pmol = 3 ng)

Second C-peptide EASIA kit.

During the course of the study some changes were made to the original C-peptide kit by the manufacturer. The "increased sensitivity" option was removed, and the standard curve dilutions and conjugate preparation were changed. The volumes of calibrators, samples, standards, enzyme-labelled antigen and stop solution were also modified. The principle of the assay stayed identical.

Analytical sensitivity:

The assay lower detection limit was 0.01 pmol/ml (0.01 nmol/l) as in the previous assay.

Precision:

Intra-assay and inter-assay CVs were 5.8 % and 9.2 % respectively for low and

8.4 % and 7.2 % respectively for low to medium concentrations of the analyte.

Upper detection limit: 5.0 pmol/ml

A comparison was done between both assays and the results obtained from the new assay corresponded well (CV <10%) with the results obtained using the original assay.

Free Thyroxine (FT4)

ACTIVE ®Free T4 EIA kit; DSL (Diagnostic System Laboratories, Inc.), USA. Catalogue Number: DSL-10-40100.

Distributed by the Laboratory Specialities, cc, South Africa.

Principle of the test and procedure:

Principle and the procedure of the test were the same as in the cortisol assay.

Analytical sensitivity.

The assay lower detection limit was 0.05 ng/dl.

Specificity.

Cross-reactivity of the thyroxine antibody was with l-thyroxin 100%, d- thyroxine 98%, d-triiodothyronine 1.5% and l-tri-iodothyronine 3.0%. No further cross-reactivity or interference was found. The enzyme-T4 conjugate did not show any measurable binding to serum proteins especially thyroid hormone binding globulin and albumin.

Precision.

Intra-assay and inter-assay CVs were 7.8 % and 10.8 % respectively for low and 6.5% and 7.9 % respectively for low to medium concentrations of the analyte.

Upper detection limit: 7.0 ng/dl

Conversion factor: 1 ng/dl = 12.9 pmol/l

2.8 STATISTICAL ANALYSIS

Statistical analysis was conducted using descriptive and analytical techniques.

Software used: Statistica version 7, StatSoft, USA.

2.8.1 Preparation of the database

2.8.1.1 Dietary intake data.

Dietary intakes of macronutrients were expressed as a percentage of the total energy intake. Total energy was reported in kilojoules (kJ; 1kJ=0.239kcal).

Energy available to the body, metabolisable energy, was calculated using Atwater's general factors: 16 kJ/g of carbohydrate, 17 kJ/g of protein and 37 kJ/g of fat based on the MRC Food Composition tables and codes (*McKeown 1999; Langenhoven 1991a*). The Atwater system enables one to estimate metabolisable energy of the food from the sum of its major components: carbohydrate, protein, fat and alcohol. The Atwater factors express the number of kilocalories (kJ) per gram of these nutrients (*Mc Neill 2000*). As there was no alcohol intake among the study participants recorded, this factor was excluded from the contribution towards the metabolisable energy.

Thus the equation used was as flows:

Tot Energy (kJ) = (17kJ/g x g tot protein) + (16 kJ/g x g tot carbohydrate) + (37 kJ/g x g tot fat)

Protein component.

Total protein is made up of animal protein and plant protein.

Fat component.

The total fat includes mainly saturated fats (SAFA), monounsaturated fats (MUFA) and polyunsaturated fats (PUFA). The non-fatty acid components are phospholipids, sterols and glycerol from triacylglycerols. The composition tables adjust for the non-fatty acid components by means of conversion factors (see Chapter 2.4.2.2; *McKeown 1999; Langenhoven 1991a, 1991b*).

Carbohydrate.

Total carbohydrate is a sum of the total available carbohydrate and dietary fibre.

Total "available carbohydrate" is composed of free sugars, dextrins, starch and glycogen.

It represents the part of carbohydrate that is digested and absorbed and produces a glycogenic effect. The dietary fibre is a sum of cellulose, soluble and insoluble polysaccharides (other than cellulose) and lignin. The fibre is not digestible, therefore it is not absorbed in the human small intestine *(Englyst et al. 2000; Mc Neill 2000*)

For the analysis of the relationship between nutrient intakes and fetal, maternal and postnatal parameters the fat, protein and carbohydrate intakes were expressed as a percentage of the total energy intake [%E]. For macronutrient subtypes (animal protein, plant protein etc.) their percentage contribution to the total amount of the particular macronutrient was calculated.

2.8.1.2 Issue of the breast-feeding and formula feeding.

At the baby's first visit the majority of mothers were breast-feeding their babies and occasionally using infant formula. There were very few babies strictly breast-fed or babies fed with the infant formula only.

At the infants' second visit the majority of mothers used combination of infant formula and solids, sometimes babies were still breast-fed. Cases, where the baby was only breast-fed were very rare.

Although some records were not available, it can be concluded, that the analysed population at both visits was quite homogenic and the categories of infants either breast-fed or fed with the infant formula only would have been too few for statistical analysis.

2.8.1.3 Calculations and Indices

The **BMI** for the mother and her infant and the **PONDIX** for the newborn were calculated from the weight and length measurements as stated in Chapter 2.3.1.1.

Area under curves (AUC) of glucose, insulin, proinsulin and C-peptide was calculated using trapezoidal integration (*Le Floch et al. 1990*).

Insulin resistance as HOMA-IR index – see also Chapter 1.2.1.2.

In the current study, the insulin resistance in mothers and infants was evaluated by means of the HOMA-IR; where:

HOMA-IR = (Glucose F [mM] * Ins F [uIU/ml])/ 22.5 (*Matthews et al. 1985)*

For the analysis, the data was log-transformed (log HOMA-IR; see Appendix G).

The HOMA- ß index was not used in the current analysis, although it may be considered in the future follow-up analysis, where it will be compared with some other indices.

2.8.1.4 Variables in the database

All variables were tested for normality using the Shapiro-Wilk's test. Skewed variables were transformed (see Appendix G) before further analysis.

2.8.1.5 Missing values

The "missing data" in the database were due to the following reasons:

- some variables were included into the study at later stages (see the Feasibility study). This applies to the C-peptide concentrations 30min-120min at V1, neonatal abdominal circumference, income index and the number of household occupants.

  Originally, the participant was asked about the family monthly income, however, in the majority of cases the mother did not know the father's income. Therefore indicators such as the number of mother's siblings, education and employment were used instead. Later in the study, the income index was introduced, splitting monthly income into divisions of R 1000 difference. The women were asked how much money (as per income division) do they have each month to buy food and to run the household.

- loss to follow-up between pregnancy period and delivery (see Chapter 3.2)
- loss to follow-up between delivery and postnatal period (see Chapter 3.2)
- missing records in the patient hospital file (birth records, haemoglobin data)
- some measurements of the fetal head circumference were not available

- in the biochemical analysis a few results were excluded as the samples did not produce reproducible results (although re-tested in two separate assays from different triplicates)
- other variables derived from variables with missing data

2.8.2 Descriptive statistics.

Data was expressed as the mean or median with standard deviation (SD) or interquartile range (25th and 75th centiles) in tables and with standard error of the mean (SEM) in graphs.

Changes in mean values between visits were evaluated according to the number of visits being compared. For the data collected at two visits only, the variables were analysed by means of the Student's paired t-test. Other variables such as the maternal anthropometry, maternal hormones and nutrient intakes collected at each of the three study visits were analysed by menas of a linear mixed model, accounting thus for multiple testing. Similarly, the OGTT results presented on glucose, insulin, proinsulin and C-peptide (in section 3.4.1) were analysed using the linear mixed model (xtreg in STATA statistical software) to evaluate the mean effect over time. The outcomes were adjusted (for 3 evaluations) at each visit using Bonferroni correction. In cases where the unadjusted p values were 0.000, the adjusted value was interpreted as $p<0.01$. (For details see Appendix I; Analysis framework see Figures 2.5 and 2.6 – the fat arrows indicate comparison steps taken in the descriptive analysis).

To evaluate the normality of the distribution, the Shapiro-Wilk's W test was used.

Where necessary, the variables were transformed in order to normalise the distribution. Some Associations between some variables were evaluated by the Pearson correlation (Chapter 3.10; selection of variables).

2.8.2.1 The z-scores for neonates and infants

The birth and the postnatal infant anthropometry was also expressed as weight for length, weight for age, length for age, BMI for age and HC for age by means of the z-scores using the WHO child growth standards (*WHO Anthro, version 3.1.0, June 2010*).

The z-score expresses how many standard deviations above or below the mean the certain value lies. Using a defined international standard, the z-scores enable to compare the outcomes with other international studies.

2.8.3 Analytical statistics

The data analysis framework is presented in the Figures 2.5 and 2.6.

The first Figure involves analysis of maternal nutrient intakes, whilst the seconf Figure involves analysis of maternal glucose and hormones. For further details see Chapters 3.9 and 3.10.

2.8.3.1 Analysis of dietary intake.

The major part of the analysis was carried out by multiple linear regression analysis. Each independent variable was assessed with particular dependent variable for a linear relationship and outliers using Residual Analysis (Predicted vs. residuals plot and Normal Probability plot; for transformations applied see Appendix G). Outliers reaching or exceeding 3 standard residuals (SR) were excluded (see Appendix D,E,F and).

The effect of maternal nutrient intake on fetal, birth, and postnatal parameters was assessed in regression models without and in the presence of confounders (See Appendix D,E,F).

The regression coefficients β (standardized) and B (non standardized) and p-value were recorded, as well as the squared multiple correlation coefficient (R^2) and adjusted R^2 to assess the predictability of the particular model.

All outcomes from the multiple regression analysis involving nutrient intake and fetal, birth and postnatal parameters are entered in tables in Appendices D, E, F. The significant outcomes adjusted for confounders and outliers are summarised in the analytical section of the Results Chapter.

2.8.3.2 Analysis of maternal pregnancy parameters vs fetal, birth and infant parameters.

To evaluate possible effects of maternal anthropometry and metabolic profile on fetal growth, birth parameters and the postnatal factors indicating risk associated with future Type 2 diabetes development, correlation analysis was performed. All maternal variables of the V1

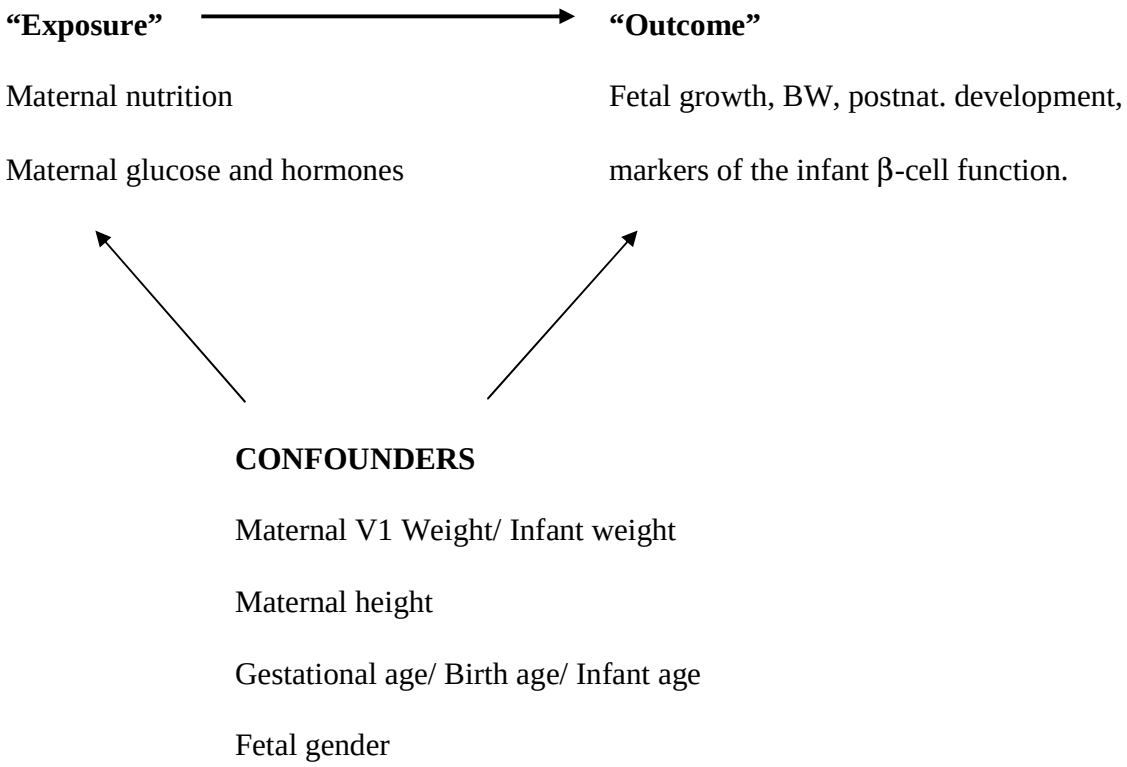
were entered into parametrical analysis with fetal/birth and infant postnatal parameters. The same analysis was repeated for the maternal parameters of V2. All outcomes with $p < 0.1$ were selected for a subsequent analysis with a backward stepwise multiple linear regression to evaluate the strongest effects (see Appendix H and Chapter 3.10).

2.8.3.3 Confounders.

Selection of confounder for the current study.

The choice of confounders used in the current study was based entirely on the literature.

As birth weight represents a proxy outcome of fetal development, it was assumed, that the confounders for fetal development and birth did not differ. However, their effects may differ according to the timing of the exposure. As there was not much information on confounders and the significance of different exposure times during fetal development, a set of four possible confounders with well-established effects on birth weight were selected (*Kramer 1987, Kramer 2003*; See Chapter 1.4.2). Their number was limited with respect to the rule on the number of explanatory variables used in a regression analysis (*Petrie et al 2000a)*. This set of confounders was applied in all the regression models for both pregnancy visits and for birth (See the diagram below).



Some of the tested confounders may have actually acted as modifiers (*see Kramer 1987*), however, the effect of modification was not assessed in the current study. Effects of these confounders can be followed in Chapter 3.9 and 3.10 in Tables 3.10A-C and 3.12 A-C and in Appendix D,E,F **Pg. 292.**

The effects of other potential confounders (such as ethnic origin, maternal age, maternal health during pregnancy, maternal habits, maternal and family health history; *See Chapter 1.4.2*) were not investigated in the current study, however they were dealt with in following ways:

In the study design:

- Investigating only South African urban pregnant women of a Black ethnic origin, although ethnic language subgroups were not investigated.
- By setting up study enrolment criteria (such as maternal age restriction by excluding teenage pregnancies and pregnancies of older mothers; non diabetic participants with no other chronic disease requiring treatment, normal blood pressure and a haemoglobin level above 10g/dl[3]; singleton pregnancy)

In the analysis:

- Confounders such as socio-economic status (education, employment, income, number of siblings, number of household occupants), mother's physical activity or stress were investigated in separate statistical analyses.
- Information was collected and filed for the further analysis (e.g. family health history; previous pregnancies, cultural-language groups; health problems during pregnancy; previous pregnancies…)

Effect absent (smoking, drinking alcohol, malaria)

2.8.3.4 Data analysis framework.

Figures 2.5 and 2.8 represent the study data analysis framework for maternal dietary intake and maternal hormones respectively. Broad arrows indicate comparisons in descriptive analysis. Changes between maternal paramenters comparing all visits were evaluated by the linear mixed model. Changes between the fetal or the infant postnatal parameters were analysed by the paired t-test. Thin arrows indicate regression analysis between maternal and fetal/birth/infant parameters, including the growth rates.

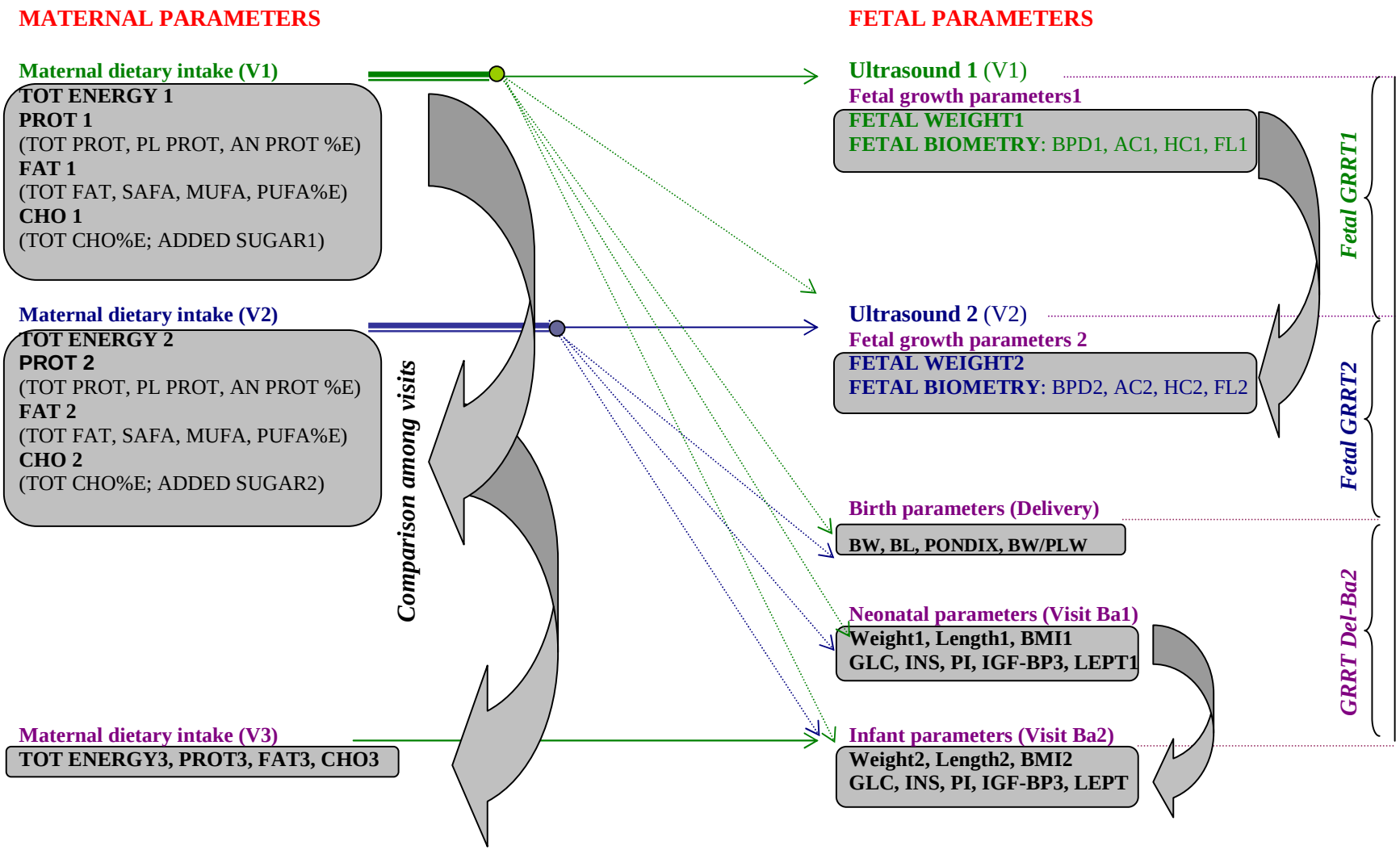


Figure 2.5. Data analysis framework: Effects of maternal nutrition on fetal, birth and postnatal parameters. Thin arrows indicate regression analysis between maternal and fetal/birth/infant parameters. Broad arrows indicate comparisons in descriptive analysis.

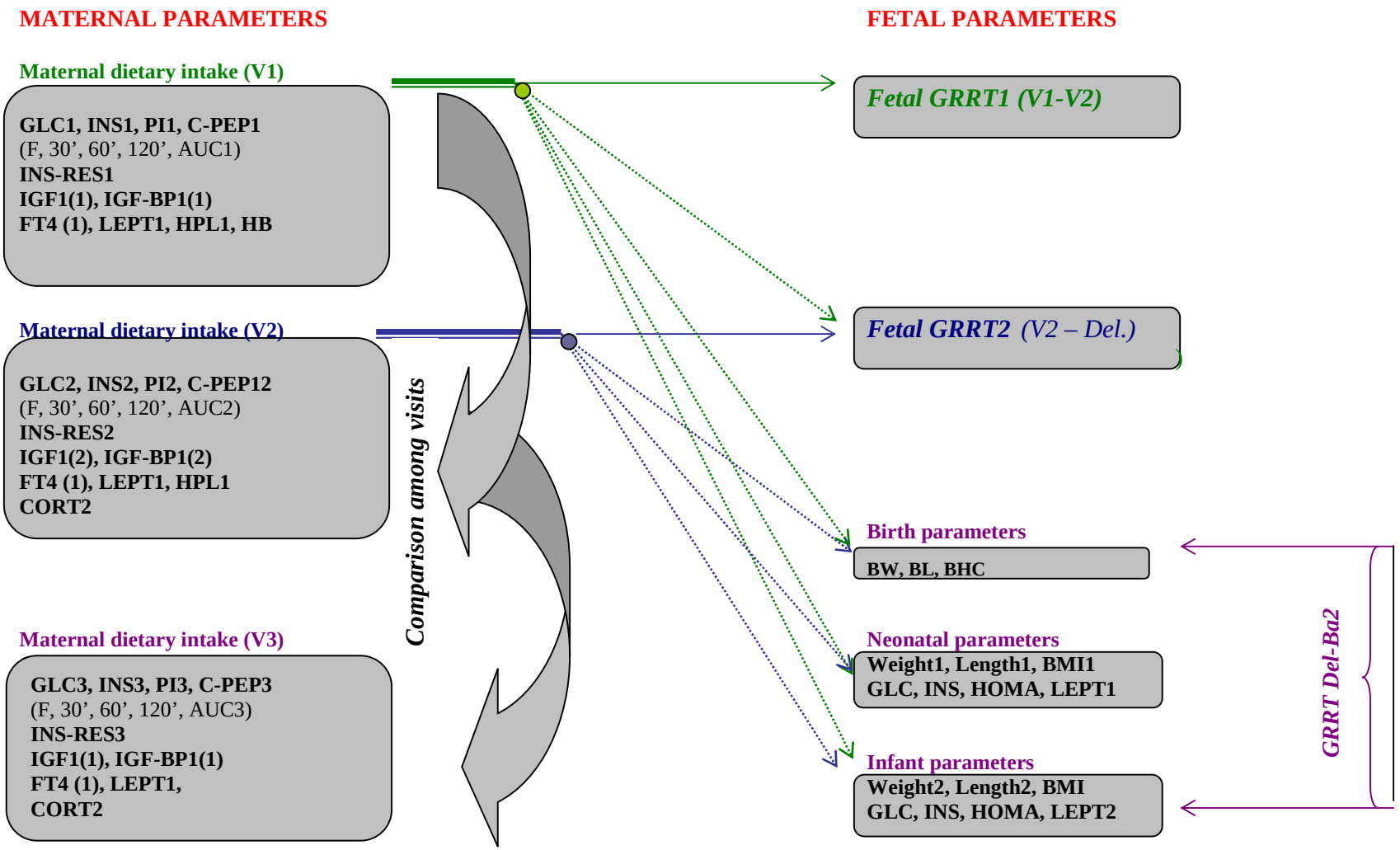


Figure 2.6. Data analysis framework: Effects of maternal glucose and hormones on fetal, birth and postnatal parameters. Thin arrows indicate regression analysis between maternal and fetal/birth/infant parameters. Broad arrows indicate comparisons in descriptive analysis.

CHAPTER 3

RESULTS - DESCRIPTIVE DATA

In this Chapter the results of the study are presented. The section of Descriptive data shows the characteristics of the study participants, the study follow-up and the descriptive data obtained from the mothers, fetuses, neonates and infants.

3.1 CHARACTERISTICS OF STUDY PARTICIPANTS

3.1.1 Ethnic groups

The majority of the study participants were Zulu speakers (Figure 3.1), exceeding the other most frequent language groups, Xhosa and Sotho, by 3.4 and 4.8 fold respectively.

3.1.2 Age distribution

The age at enrolment was restricted by study selection criteria (Chapter 2.2.1) to 18-40 years. According to Figure 3.2 the majority of women enrolled into the present study were 25-30 years of age. The remainder of women was predominantly in the older rather than the younger age groups.

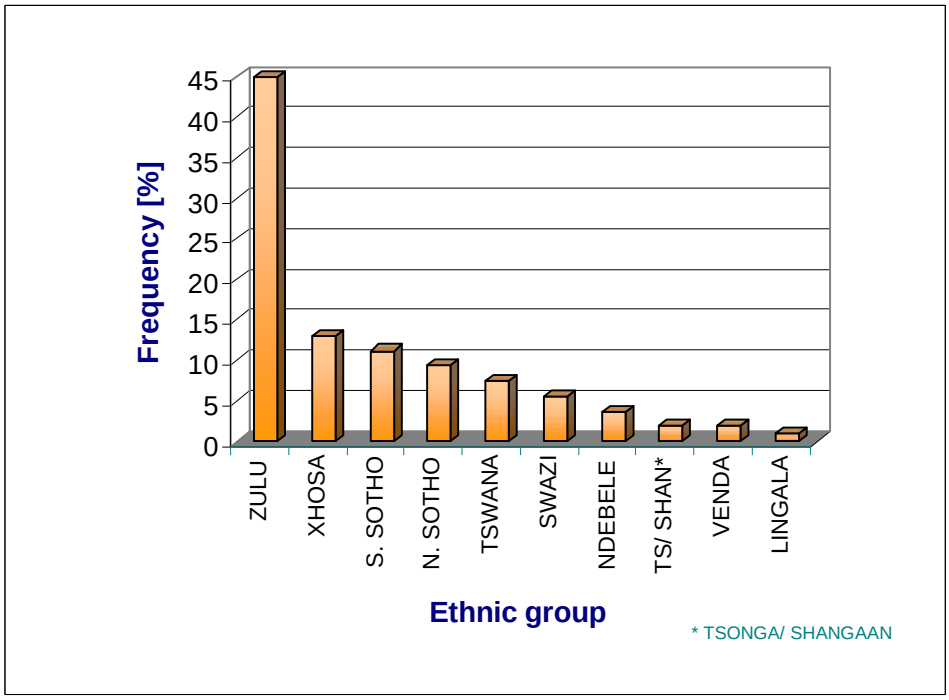


Figure 3.1 Language-cultural background of women enrolled into the study.

3.1.3 Socio-economic background

3.1.3.1 Level of education (Figure 3.3)

The majority of women participating in the study completed their education between 14-18 years of age (standard 8 and matriculation category of the education level; see Chapter 2.4.1). This category exceeded the other categories of education by at least 3.5 fold.

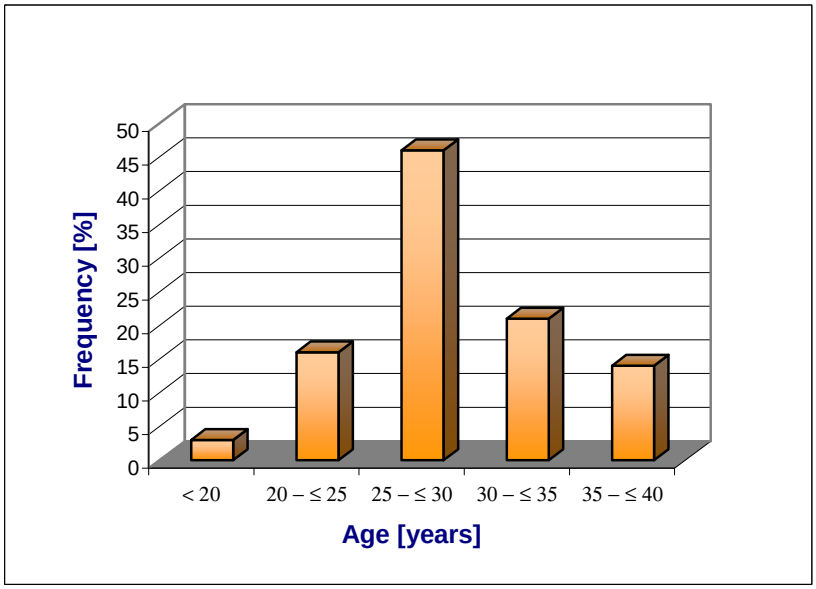


Figure 3.2 Distribution of the mothers' age at enrolment.

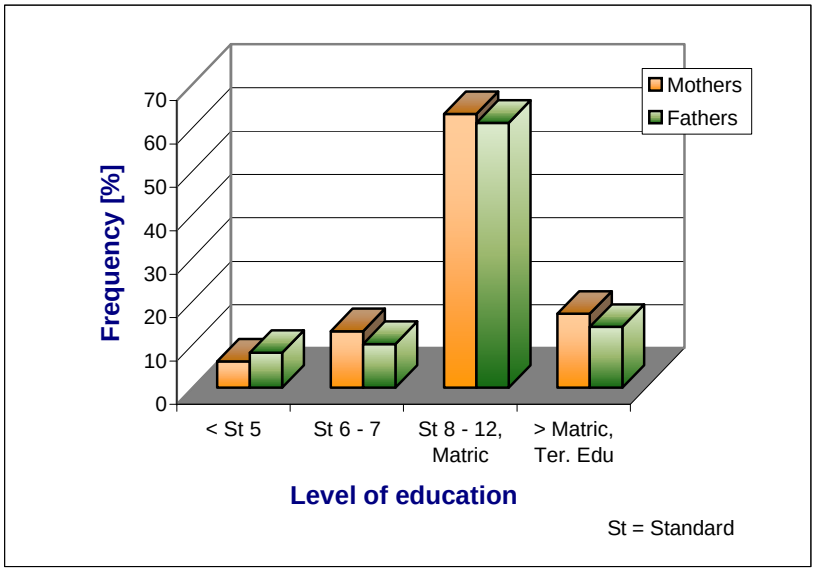


Figure 3.3 Education level of baby's parents at the time of enrolment. (See Chapter 2.4.1) Levels of education correspond to the <12 years, 12-14 Years, 14-18 years and >18 years of age categories respectively.

The education of the mothers was comparable with the education level of the infants' fathers, although more men contributed to the lowest education category than women did. Educational status was known in 93.5% of the fathers.

3.1.3.2 Employment status (Fig 3.4)

Although similarly educated, women in their early pregnancy were twice as likely to be unemployed as were their partners. A small fraction of participating women and men were unemployed and studying, being supported by their parents or partner. Employment status of all fathers was known.

3.1.3.3 Family income (Figure 3.5)

Family monthly income in the majority of participants was in the lowest category (below R 1000).

3.1.3.4 Number of household occupants (Figure 3.6).

This figure demonstrates the crowding in the household. The most common number of household occupants was between 2-4 people, although larger "families" of more than 5 individuals were not exceptional. (However, the exact information on the number of rooms in each household was not available. Two families sharing a household or one family sharing a single room with relatives or non-family members was not a rare situation).

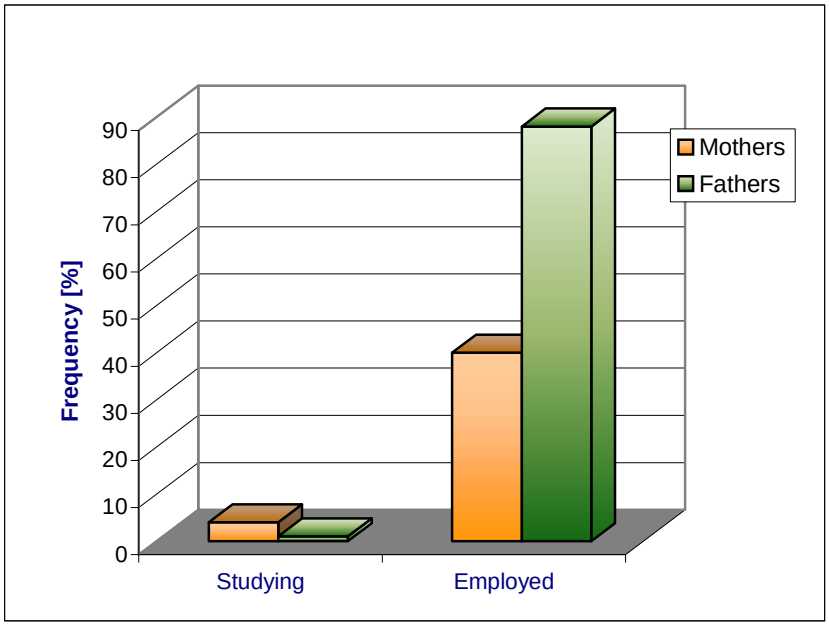


Figure 3.4 Employment status of baby's parents at the time of enrolment. Studying parent was economically supported by the partner or parents.

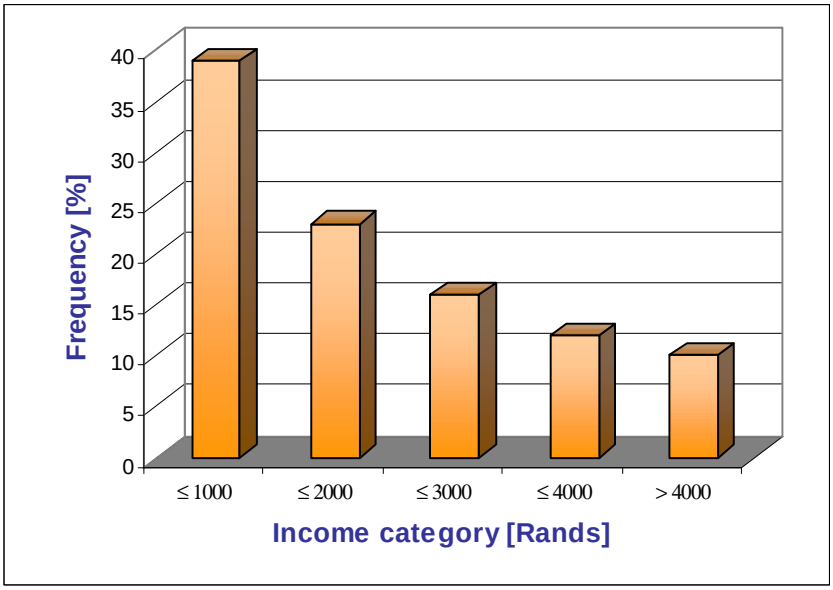


Figure 3.5 Family monthly income in categories (ranges) of Rands with minimum of R 400 and maximum of R 9 000.

3.1.3.5 Number of mothers' siblings (Figure 3.7)

The data in Figure 3.7 reflects the number of siblings of the mother. Between 1 to 6 brothers or sisters was commonly reported, followed by a steep decline towards higher numbers. A single child in a family was rare.

3.1.4 Family history of diabetes and hypertension (Figure 3.8)

Although the study participants were pre-selected as non-hypertensive and non-diabetic, there was a small fraction of those who had a first-degree relative (mother, father, brother or sister) who had been treated for one or both of these conditions. Among these groups, the maternal family members showed a higher frequency of treated individuals for any of the above conditions. The family medical history of the infant's father was not known by about 14% of the mothers.

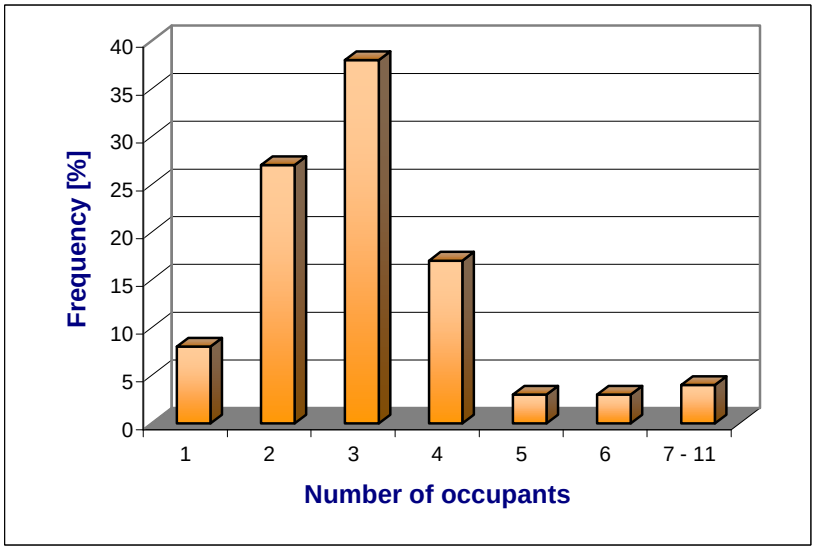


Figure 3.6 Number of adults and children over 3 years of age sharing the household.

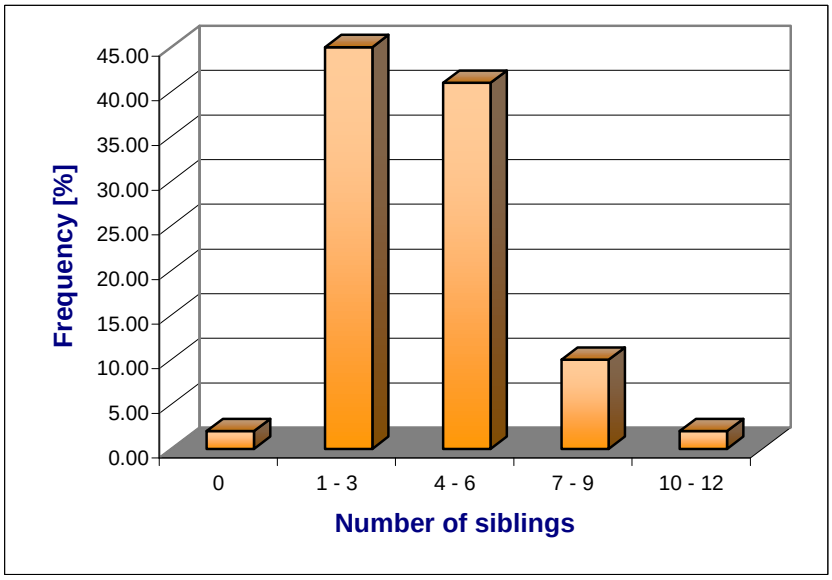


Figure 3.7 Number of mother's siblings as an estimation of the size of the family the mother came from.

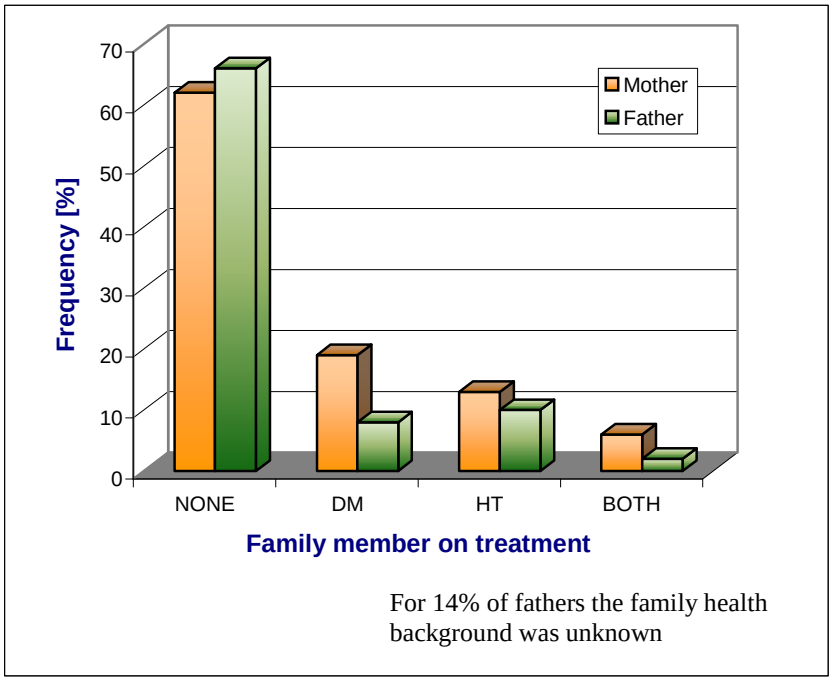


Figure 3.8 Family history of diabetes and hypertension for mothers and fathers.

3.2 STUDY ATTENDANCE

3.2.1 Follow-up profile

Figure 3.9 demonstrates loss to follow-up in different stages of the study. From the original 126 enrolled mothers at the first visit, 108 (85.7%) completed both pregnancy visits and were included in the final pregnancy database. A small decline in these numbers occurred at the stage of delivery, when only 105 (83.3%) of the delivery records of enrolled participants became available. Considering all stages of the study, the major drop out in the participating volunteers was experienced during the postnatal period, as only 77 (61.1%) babies continued in the study with the first postnatal visit and 74 (58.7%) babies completed whole study by the second postnatal visit. Therefore 41.3 % of enrolled participants in total did not complete the study. Reasons are summarized in Table 3.1 (See also Discussion).

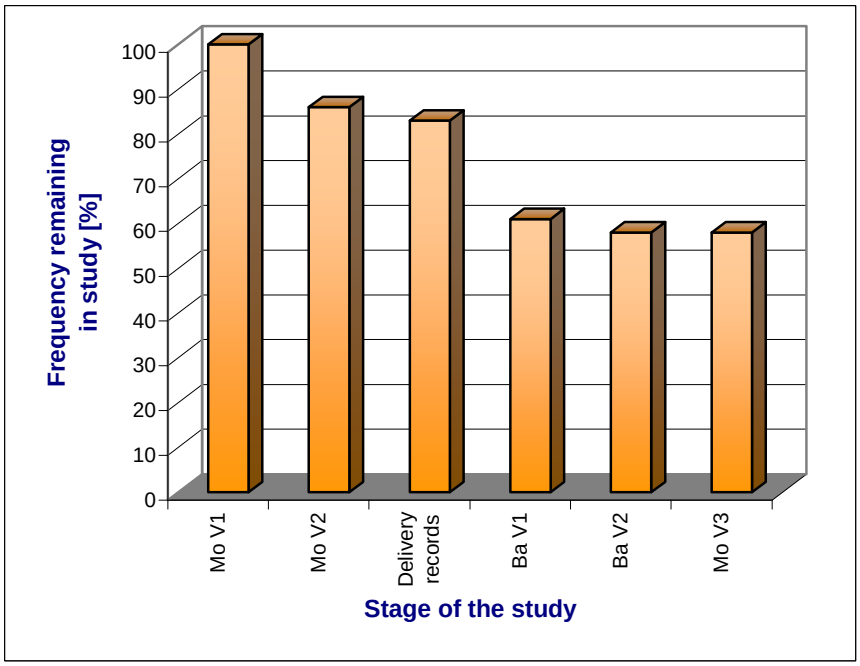


Figure 3.9 Frequency of the loss to follow-up at different stages of the study.

3.2.2 Reasons for the loss to follow-up (Table 3.1 A, B, C)

The following tables summarise the reasons for exclusion of participants from a particular visit or from further follow-up. In a few cases, a participating woman was allowed to continue in the study if she missed one visit, as she could still significantly contribute to the study outcomes. The reasons for exclusion from the second follow-up (Table 3.1A) included a variety of causes, although premature delivery accounted for 7 of these. Two other reasons were miscarriage and a missed appointment. During the enrolment of the study a number of volunteering women were lost due to limited time given by the woman's employer for these pregnancy visits. Cases like twin pregnancy, hypertension and pregnancy anomalies were excluded early in the study. In one case a women attending the antenatal clinic and enrolled into the study was found not to be pregnant and one woman with premature delivery still attended V2.

In conclusion, from 126 women who were enrolled into the study 18 were excluded from the second pregnancy visit V2.

Reasons for the loss to follow-up during delivery (Table 3.1 B) included deliveries in different hospitals. Some women who resided temporarily in Johannesburg for the period of gestation decided to deliver in their home area. Exceptions were two women who delivered out of the Johannesburg General Hospital (one in neighboring Botswana), obtained their birth records for the study purpose and returned with their infants for the study follow-up. In one incident a women delivered her baby in her car on the way to Durban. For obvious reasons the complete birth records were not available, except the birth weight and the length taken by

paramedics. In total, 105 birth records were available, most of them complete with some exceptions when a particular measurement was not taken or was not recorded in the woman's hospital file.

Table 3.1 Reasons for excluding the woman/baby from a visit or from further follow-up (Tables A, B and C).

A. Pregnancy period.	**Number**
Twin pregnancy	1
Pregnancy anomalies	1
Hypertension	1
Unwell during OGTT	1
Not pregnant (negative hPL confirmed by ultrasound)	1
Not able to attend visits/ refused to participate	3*
Lost contact	1
Miscarriage	3
Premature birth	7

* One woman missed the second visit, however she attended other visits and completed the study.

B. Delivery.	**Number**
Stillbirth	1
Delivered elsewhere and discontinued participation	4

After delivery many mothers discontinued their participation (Table 3.1 C) for various reasons (e.g. did not want to expose their neonate to heel pricks or baby's father did not wish the mother to continue).

From the total number of babies attending the study postnatally, some of the visits had to be cancelled. Two babies were suffering from recurrent episodes of upper respiratory tract infections during the winter season and were on antibiotic treatment. One baby was breast-fed before blood collection and therefore had to be excluded from the visit, and one mother became pregnant prior to the conclusion of the study.

C. Postnatal period.	**Number**
Newborn with health problem	2
Mother discontinued participation (unable to attend or refused to continue)	11
Mother and baby left Johannesburg	3
Subsequent pregnancy (before V2)	1
Lost contact	9
Mother and baby attended only one postnatal visit (stayed in follow-up)*	11

* Includes also cases when baby was unwell or breast-fed before the blood collection.

3.3 **ANTHROPOMETRY** (Table 3.2)

As expected, the mean maternal weight during pregnancy increased significantly (n=107; p<0.01). There were also significant differences between V1 and the postpartum visit V3 (n= 73; p=0.018) and V2 and V3 (n=72; p<0.01). The differences in pattern and significance of outcomes for maternal BMI were as for the maternal weight. The linear mixed model and the subsequent Bonferroni correction for the p value were applied to evaluate differences between the repeated maternal visits (See Methods Chapter 2.8.2 and Appendix K). Maternal weight gain was calculated from the difference between two pregnancy visits, as no maternal weight was taken at delivery or before the V1.

Mean systolic blood pressure at the first pregnancy visit (V1) was 108.1 mmHg (ranging from 80 – 130 mmHg) and a very similar value was recorded at the second pregnancy visit (V2; 109.2 mmHg ranging from 90-130 mmHg). Half a year postpartum (V3) a rise in the systolic blood pressure was noticed, reaching 115.9mmHg and ranging from 80 – 140 mmHg. This systolic BP was significantly different in comparison with the V1 (n=71; p=<0.01) or the V2 (n=70; p<0.01) level, using the linear mixed model analysis. The very same pattern and significance of outcomes were obtained for the diastolic BP with means and ranges 68.2 mmHg (50-90mmHg), 69.9 mmHg (50-90mmHg) and 77.4 mmHg (60-100 mmHg) for the V1, V2 and V3 respectively (See Appendix K, Table K1 for more details).

Table 3.2 Anthropometric measurements and BP of mothers at the antenatal (V1 and V2) and the postnatal visit (V3).

	V1	(n=108)	V2	(n=107)	V3	(n=73)
Variable	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Weight [kg]	74.1	13.3	78.6[b]	12.9	71.6[e,g]	15.5
Height [m]	1.59	0.06	-	-	-	-
BMI [kgm^{-2}]	29.3	4.8	31.1[b]	4.6	28.5[e,g]	5.6
MWG (V1-V2) [g/d]	-	-	59.6[#1]	33.1	-	-
Systolic BP [mmHg]	108.1	10.1	109.2	10.7	115.9[#2;e,h]	13.2
Diastolic BP [mmHg]	68.2	8.2	69.9	8.4	77.4[#2;e,h]	9.4

MWG (maternal weight gain); [#1](n=106), [#2](n=71);

b: $p<0.010$, V1-V2
e: $p<0.010$, V2-V3
g: $p<0.050$, h: $p<0.010$, V1-V3

3.4 MEASURED METABOLIC PROFILE

The OGTT results presented on glucose, insulin, proinsulin and C-peptide below (in section 3.4.1) were analysed by a linear mixed model. The comparisons were adjusted (for 3 evaluations) at each visit using Bonferroni correction. (See Chapter 2.8.2 and Appendix K).

3.4.1. OGTT levels of glucose and hormones involved in glucose metabolism.

3.4.1.1 Glucose levels during the two-hour OGTT (Fig 3.10).

Maternal mean plasma glucose levels were higher at all time points during the two-hour glucose tolerance test at V2 compared to V1. Except for the fasting levels (3.8 ± 0.05 mmol/l at V1 and 3.9 ± 0.04 mmol/l at V2), significance was observed for differences at the time points of the glucose curve (5.7 ± 0.1 vs 6.2 ± 0.1 mmol/l at 30 min, 5.7 ± 0.1 vs 6.5 ± 0.1 mmol/l at 60 min and 5.4 ± 0.1 vs 5.9 ± 0.1 mmol/l at 120 min with $p<0.01$ at all instances). Values are expressed as mean ± SEM.

When V1 data was compared with V3, the V3 glucose levels were higher at all time points but only reached significance at fasting and 30 minutes ($p<0.01$ at both instances). The actual values were 3.8 ± 0.1 and 4.4 ± 0.1mmol/l for fasting glucose and 5.7 ± 0.1 and 6.3 ± 0.2 mmol/l for 30-minute glucose levels at V1 and V3 respectively. When comparing V2 and V3 glucose concentrations, the fasting value was higher for V3 (3.9 ± 0.1 vs 4.4 ± 0.1 mmol/l at V3; $p< 0.01$), but at 30 min the levels were very similar (6.3 mmol/l for V3). Afterwards, at 60 min and 120 min, the V3 levels fell below those for V2 reaching a significant difference ($p<0.01$) only at 1 hour (6.4 ± 1.4 and 6.0 ± 1.8 mmol/l for V2 and V3 respectively).

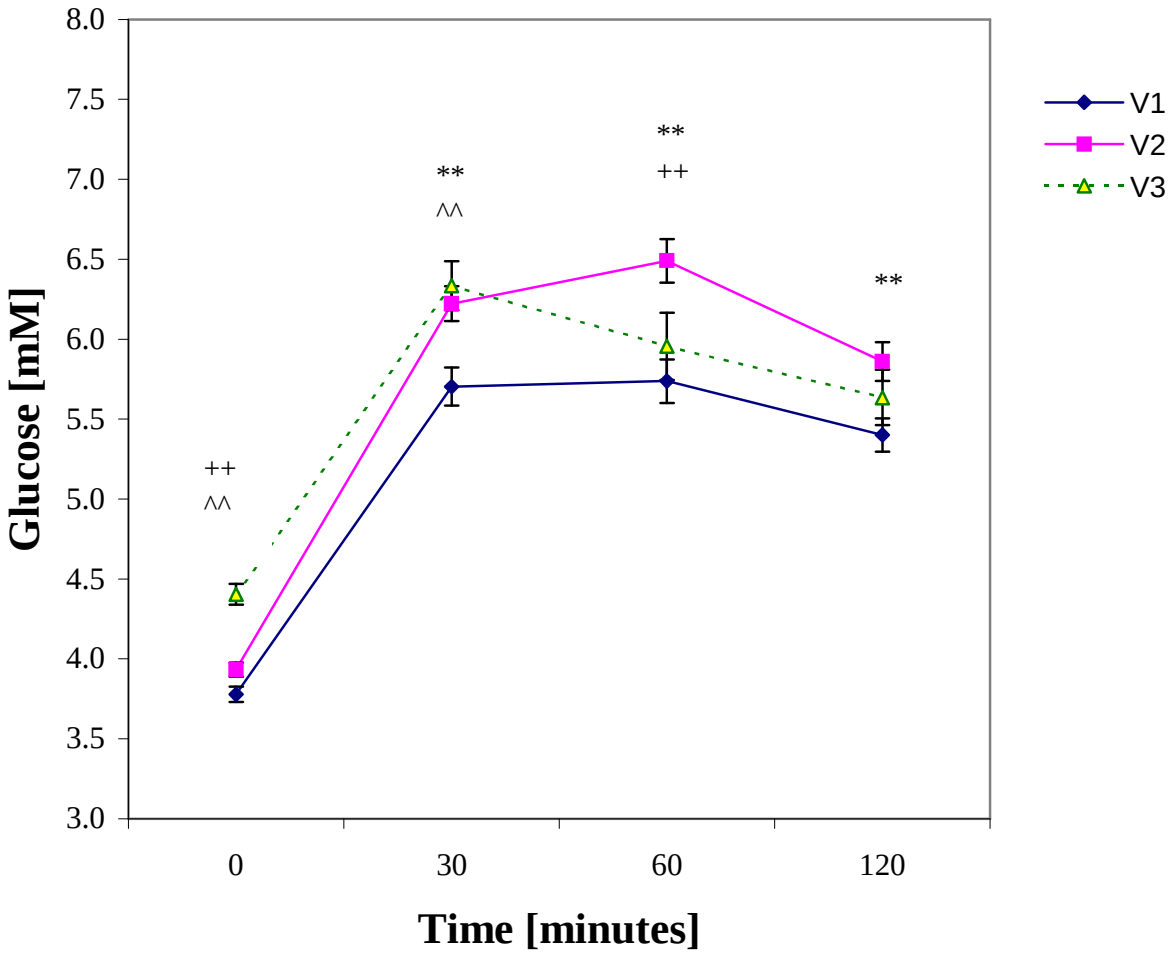


Figure 3.10 Glucose levels during the two-hour OGTT for mothers at all visits. Levels are presented as the mean value with a standard error of the mean (SEM).

**p<0.01 V1 vs V2

++p<0.01 V2 vs V3

^^ p<0.01 V1 vs V3

3.4.1.2 Insulin levels during the two-hour OGTT (Figure 3.11).

There was no significant difference between the mean serum insulin levels during the first pregnancy visit (V1) and levels at V3 at fasting (64.2 ± 2.5 vs 74.6 ± 4.7 pmol/l) or any further time point (504.1± 28.0 vs 502.9 ± 42.7 pmol/l respectively for 30 min, 399.5 ± 22.7 vs 348.5 ± 24.7pmol/l respectively for 60 min and 335.7 ± 20 vs 318.5 ± 30.8 pmol/l respectively for 120 min). All insulin values for the third trimester pregnancy visit (V2; 645.1 ± 44.9 pM for 30 min, 653.2 ± 47.1 pM for 60 min and 461 ± 29.6 pM for 120 min) were significantly higher than the levels at V1 and V3 ($p<0.01$ at all instances), except for the fasting concentration (72.9 pM at V2).

3.4.1.3 C-peptide levels during the 2-h OGTT (Figure 3.12).

Mean serum C-peptide concentrations increased significantly from the first to the second pregnancy visit at the last two time points of the OGTT (from 2.9 ± 0.1 to 3,4 ± 0.1 nmol/l at 60 min and from 2.8 ± 0.1 to 3.1 ± 0.1 nmol/l at 120 min with $p<0.01$ at both instances). Thus fasting levels (0.5 ± 0.02 vs. 0.6 ± 0.02 nmol/l) and 30 min levels (2.7 ± 0.1 vs. 2.8 ± 0.1 nmol/l) did not significantly differ. C-peptide concentrations at the postpartum visit (V3) were significantly lower than the levels at the second pregnancy visit (2.5 ± 0.1, 2.5 ± 0.1 and 2.3 ± 0.1 nmol/l for 30, 60 and 120 min respectively, where $p<0.05$ at all instances), except for the fasting levels (0.5 ± 0.03 nmol/l). Mean C-peptide levels between the V1 and V3 differed significantly only at 1-h and 2-h concentrations (2.9 ± 0.1 vs 2.5 ± 0.1 at 60 min and 2.8 ± 0.1 vs 2.3 ± 0.1 nmol/l at 120min with $p<0.01$ at both instances).

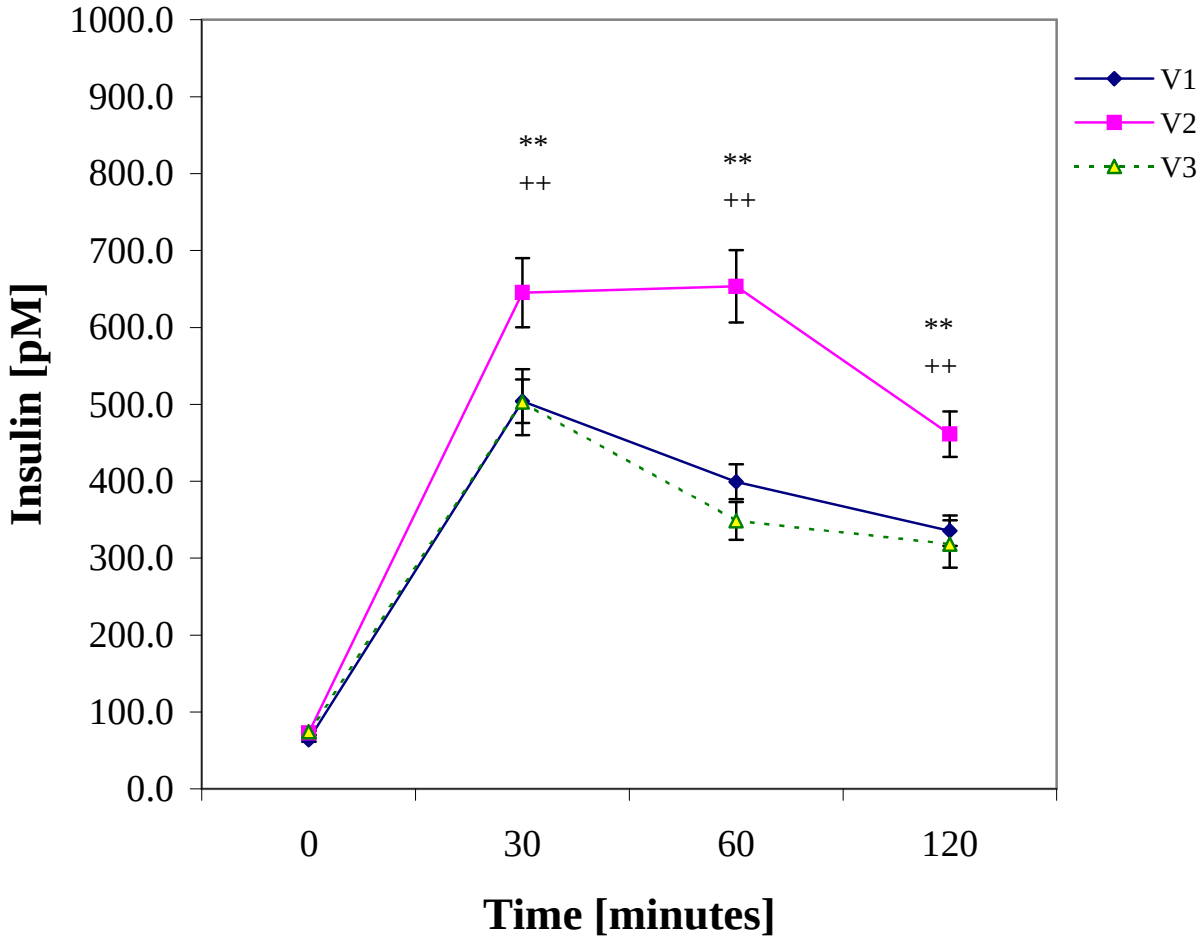


Figure 3.11 Insulin levels during the OGTT for mothers at all visits. Levels are presented as the mean value with a SEM.

**p<0.01 V1 vs V2

++p<0.01 V2 vs V3

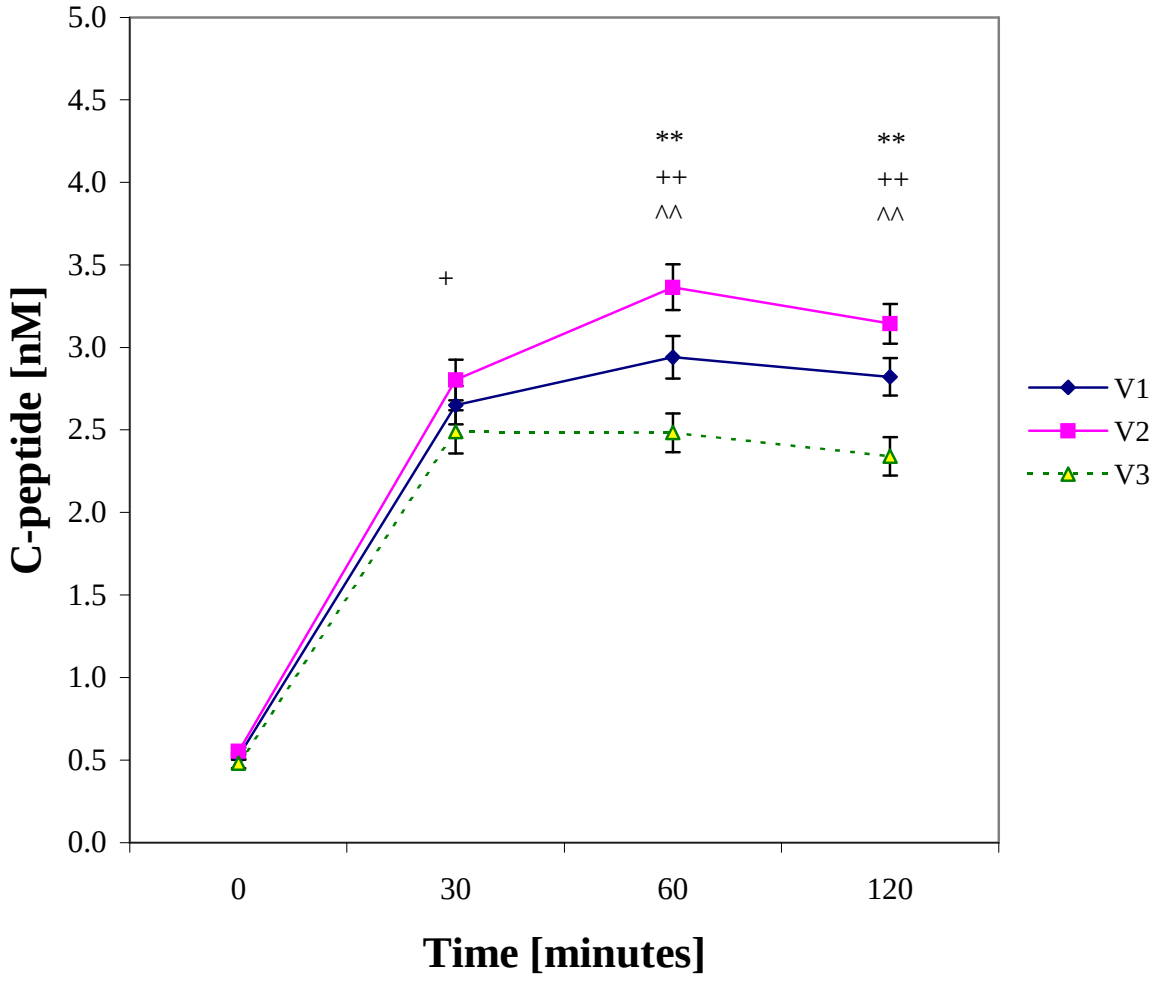


Figure 3.12 C-peptide levels during OGTT for mothers at all visits. Levels are presented as the mean value with SEM.

**p<0.01 V1 vs V2

+p<0.05, ++p<0.01 V2 vs V3

^^p<0.01 V1 vs V3

3.4.1.4 Proinsulin levels during the 2-h OGTT (Figure 3.13).

The mean serum proinsulin pregnancy levels and levels half a year after delivery were different between all the three curves at each time point, except for all the visits' fasting levels and the 30 min and 60 min concentrations during gestation. At all instances, concentrations rose from the fasting level until the two-hour collection time. The lowest proinsulin concentrations were found at the first pregnancy visit. The proinsulin levels continued to increase at the second pregnancy visit, and were the highest half a year after delivery.

Measured proinsulin concentrations in the sequence of V1, V2 and V3 were as follows: 4.3 ± 0.2, 4.9 ± 0.3 and 7.1 ± 0.5 pmol/l for 30 min, 5.9 ± 0.4, 7.0 ± 0.4 and 8.8 ± 0.6 pmol/l for 60 min and 8.5 ± 0.6, 9.9 ± 0.7 and 12.0 ± 0.8 pmol/l for 120 min with $p<0.01$ for all comparisons except for the non-significant outcomes for the pregnancy 30min and 60 min levels. Fasting proinsulin levels were 1.7 ± 0.1, 2.1 ± 0.1 and 2.6 ± 0.2 pmol/l, showing also no significant differences between these levels.

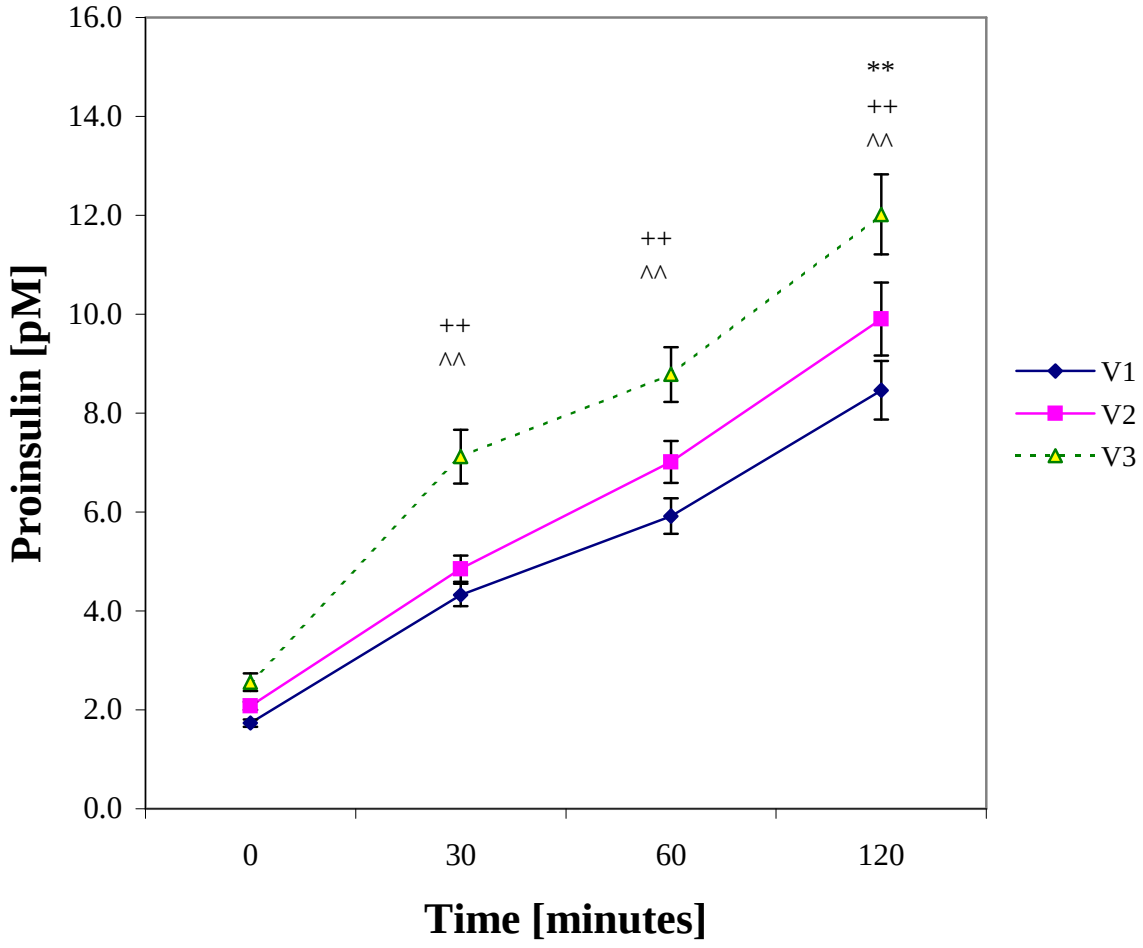


Figure 3.13 Proinsulin levels during OGTT for mothers at all visits. Levels are presented as the mean value with a SEM. `

**p<0.01 V1 vs V2

++p<0.01 V2 vs V3

^^p<0.01 V1 vs V3

3.4.2 Area under the curve for glucose, insulin, proinsulin and C-peptide during the 2-h OGTT (Table 3.3 A)

During pregnancy, the area under the curve for glucose, insulin, proinsulin and C-peptide increased significantly ($p < 0.001$ for all). The decline in the area under the glucose curve between V2 and V3 was not significant. However, in the case of the insulin curve, the area under the curve at V3 decreased significantly from V2 and it reached an even lower value than the one at the first pregnancy visit ($p<0.001$). The change between V1 and V3 was not significant. Similar changes in the area under the curve for C-peptide was discovered with a $p<0.001$ for V2 vs V3.

The area under the proinsulin curve differed in its character between visits, as it was constantly increasing from V1 towards V3. All increases were significant ($p<0.001$ at all instances).

Derived variables (Table 3.3 B).

HOMA-IR index reflecting the mother's insulin resistance increased significantly between both pregnancy visits ($p<0.05$) and also increased further after pregnancy ($p < 0.05$ for V2 vs V3 and $p<0.01$ for the difference between V1 and V3).

Table 3.3 Areas under curves of glucose, insulin, proinsulin and C-peptide during the two-hour glucose tolerance test (Table A) and HOMA-IR index (Table B).

	V1	(n=108)	V2	(n=107)	V3	(n=73)
Table A	**Mean/ Median**	**SD/ IQR**	**Mean/ Median**	**SD/ IQR**	**Mean/ Median**	**SD/ IQR**
GLC AUC [mmol.l^{-1}. min]	638	564-706	695[c]	633-785	693[i]	154
INS AUC [pmol·l^{-1}.min]	38994	27940-54136	50446[c]	35366-79302	34931[f]	24571-52847
C-PEP AUC [pmol· ml^{-1}. min]	306[#2]	104	322[#3 c]	239-424	264[#1; f]	96
PI AUC [pmol·l^{-1}.min]	588[#3]	401-783	665[b]	489-975	1007[f,i]	524

[#1](n=73), [#2](n=79), [#3](n=106); IQR [interquartile range]. For the comparison of AUC the paired t-test with Bonferroni correction were applied.

Table B	V1	(n=108)	V2	(n=107)	V3	(n=74)
Variable	**Median**	**IQR**	**Median**	**IQR**	**Median**	**IQR**
HOMA-IR [index]	1.42	1.07-1.83	1.73[a]	1.23-2.26	1.87[d,h]	1.22-2.77

IQR (interquartile range; 25th and 75th centiles)

a: p<0.050; b: p<0.010; c: p<0.001, V1-V2
d: p<0.050; f: p<0.001, V2-V3
h: p<0.010; i: p<0.001, V1-V3

3.4.3. **Measurement of other metabolic products** (Table 3.4 A)

Mean haemoglobin level at the women's registration at the antenatal clinic was 12.4 g/dl. The HPL and IGF-1 concentrations increased from V1 to V2 ($p<0.001$ HPL and $p<0.01$ for IGF-1), whilst the IGF-BP1 and FT4 concentrations decreased ($p<0.05$ for IGF-BP1 and $p<0.01$ for FT4).

The IGF-1, IGF-BP1, FT4 and cortisol levels differed between the last pregnancy visit and the visit half a year following delivery ($p<0.01$ for each comparison). The mean IGF-1 concentration peaked at V2, whilst the IGF-BP1 declined from V1 to V3. The FT4 reached a nadir at V2. Cortisol declined between V2 and V3.

The difference in IGF-1 and IGF-BP1 levels was found to be significant between the early pregnancy visit and half a year after delivery ($p<0.01$), while no significant difference between these two visits was observed for FT4 levels. Maternal serum leptin showed no significant differences for any pair visit comparison, just missing the 5% level significance for pregnancy fasting concentrations after the p value adjustment with Bonferroni correction.

The linear mixed model analysis was applied for IGF-1, IGF-BP1, FT4 and LEPT between visits comparison, while the Student's t-test was applied for the variables with the data measured at two occassions only.

Table 3.4 Levels of other metabolites measured at both pregnancy visits (V1, V2) and the visit (V3) of mothers.

A.	V1	(n=108)	V2	(n=107)	V3	(n=74)
Variable	**Mean/ Median**	**SD/ IQR**	**Mean/ Median**	**SD/ IQR**	**Mean/ Median**	**SD/ IQR**
HB [g dl^{-1}]	12.4[#1]	1.1	-	-	-	-
HPL [mg l^{-1}]	2.9	2.4-3.6	5.6[c]	4.5-7.1	-	-
IGF 1 [µg l^{-1}]	182.3	148.5-235.1	324.1[b]	115.4	113.4[e,h]	31.2
IGF BP1 [µg l^{-1}]	68.0	47.1-88.8	61.4[a]	39.3-80.5	3.7[e,h]	2.3-7.5
FT4 [pmol l^{-1}]	12.5	11.2-14.1	11.5[b]	10.7-13.0	12.6[e]	1.6
CORT [ng ml^{-1}]	-	-	298.5	251.9-354.2	110.1[f]	44.6
LEPT [ng ml^{-1}]	24.4	15.3-40.1	28.2	14.3-42.4	24.3	12.5-40.5

[#1](n=105); HB (haemoglobin); IQR (interquartile range; 25th and 75th centiles)

a: $p<0.050$; b: $p<0.010$; c: $p<0.001$, V1-V2
e: $p<0.010$; f: $p<0.001$, V2-V3
h: $p<0.010$, V1-V3

3.5 NUTRITIENT INTAKES

The average daily nutrient intake for the study participants is summarised in Table 3.5. No differences were found between the daily maternal energy or macronutrient intakes (in absolute or relative amounts) during pregnancy (i.e. between V1 and V2 visits). Between V2 and V3 maternal intake of total fat increased ($p<0.05$), as did monounsaturated (MUFA; $p<0.05$) and polyunsaturated fatty acid (PUFA; $p<0.01$) intakes. The total energy intake, plant protein, total carbohydrate, total available carbohydrate and added sugar levels produced non-significant outcomes only after the adjustment with the Bonferoni correction.

The total energy intake differed between the first pregnancy visit and V3 ($p<0.05$). An increase was seen in plant protein intake, total fat intake, MUFA and PUFA intakes with $p<0.01$ at all instances. Total carbohydrate and total available carbohydrate intakes also showed increases at V3 ($p<0.05$ at both instances).

Total protein expressed as a percentage of total energy intake significantly decreased after pregnancy ($p<0.01$ for both comparisons between V1 and V3 and V2 and V3). Increase in total fat expressed as a percentage of energy intake between V1 and V3 was nearly significant ($p=0.069$), reaching significance for the comparison between V2 and V3 ($p<0.05$).

Table 3. 5 Average daily nutrient intake per person during the antenatal (V1 and V2) and postnatal (V3) visits.

	V1	(n=107)	V2	(n=107)	V3	(n=74)
Nutritional factor	**Median**	**IQR**	**Median**	**IQR**	**Median**	**IQR**
ENERGY [kJ]	7002	5573-9346	7059	5619-9146	7890[g]	6487-10274
TOT PROT [g]	54.0	44.0-68.3	53.8	44.0-72.6	55.3	42.1-71.0
PL PROT [g]	21.7	14.8-28.0	22.1	16.2-28.1	22.1[h]	18.5-32.4
AN PROT [g]	31.8	23.6-42.0	31.4	23.5-45.3	30.6	19.9-38.8
TOT FAT [g]	64.7	47.0-94.8	69.0	47.0-93.2	86.7[d,h]	64.7-104.7
SAFA [g]	22.5	16.7-30.5	23.4	16.0-30.4	24.9	17.6-32.2
MUFA [g]	20.6	13.8-28.2	21.1	14.6-29.0	28.2[d,h]	19.9-33.3
PUFA [g]	16.7	11.0-30.1	15.7	11.6-27.6	25.5[e,h]	18.9-33.5
Cholesterol [mg]	212.8	116.9-298.0	187.1	122.6-301.4	205.6	130.9-322.5
Tot avail CHO [g]	215.4	167.0-280.9	218.7	167.5-273.4	235.1[g]	184.7-321.9
TOT CHO [g]	234.5	183.5-300.7	234.8	179.1-288.4	249.9[g]	201.1-338.8
Added sugar [g]	51.9	28.8-74.8	50.1	33.4-74.6	59.8	40.2-77.4
Dietary fibre [g]	14.6	10.5-20.3	15.4	11.7-21.3	15.0	11.5-21.3
TOT PROT [% E]	12.9	11.0-15.4	13.0	11.2-14.8	11.7[e,h]	10.3-12.8
TOT FAT [% E]	35.3	28.4-41.8	35.0	30.0-40.5	37.5[d]	31.4-44.6
TOT CHO [% E]	51.9	45.9-59.4	53.3	46.5-59.7	51.2	45.3-58.0

SAFA (saturated fatty acids), PUFA (polyunsaturated fatty acid); MUFA (monounsaturated fatty acid); TOT (total); AN (animal); PL (plant); PROT (protein); CHO (carbohydrate); SD (standard deviation), IQR (interquartile range; 25th and 75th centiles).

d: $p<0.050$, e: $p<0.010$, V2-V3; g: $p<0.050$, h: $p<0.010$, V1-V3

3.6 ULTRASOUND EVALUATION (See Table 3.6)

One hundred and seven ultrasound scans were performed at 22.7 ± 2.1 weeks (i.e. V1) and the mean estimate of fetal weight was 539.5 ± 192.4 g. The biparietal diameter (BPD) measurement was 5.6 ± 0.7 cm, the abdominal circumference (AC) of 17.7 ± 2.5 cm was still smaller than the fetal head circumference (HC), 20.3 ± 2.4 cm, and femur length (FL) was 3.9 ± 0.6 cm. As expected, the fetal weight and all the ultrasound biometric parameters increased significantly ($p<0.001$) between the two antenatal ultrasound evaluations. The second evaluation was carried out at 33.1± 2.1 weeks of gestation and included 105 mothers. The fetal weight estimate was 1968.3 ± 408.6 g, the BPD was 8.3 ± 0.4 cm, the size of the fetal abdomen was 28.1 ± 2.2 cm, slowly approaching the size of fetal head (HC of 29.8 ± 1.5 cm). The fetal femur was approximately 1.5 fold longer (6.2 ± 0.4cm) than that of the previous scan. Resistance index was measured at the second scan only and gave an average result of 0.7 ± 0.1. The mean amniotic fluid index at 33.1 weeks was 12.3 ± 4.4 cm.

The fetal growth rate calculated from the difference in weights between ultrasound 1 and 2 showed an increase of 20.0 ± 4.2 g per day on average. During the time period between ultrasound 2 and the birth, the mean fetal growth rate increased to 29.8 ± 13.3 g per day ($p<0.001$).

Hadlock's ratio [FL/AC] for prediction of IUGR (Chapter 2.5.3.2) was evaluated from the second ultrasound scan and the proportion of IUGR cases was calculated. There were 8.6 % of fetuses with the ratio exceeding the cut-off value of 23.5. Among them, 2.9% were borderline cases (with the ratio 23.6, the 90th percentile in the current study) and 1.9% of fetuses had both elevated FL/AC ratio and BW below or equal to 2500g.

Table 3.6 Ultrasound outcome: Fetal biometry, estimations of fetal weight, gestational age and growth rate, calculated indices and ratios.

	US 1	(n=107)	US 2	(n=105)
Variable	**Mean/ Median**	**SD/ IQR**	**Mean/ Median**	**SD/ IQR**
Gestational age [weeks]	22.7	2.1	32.9[c]	31.7-34.4
EFW [g]	511.0	388-660	1926[c]	1710-2155
BPD [cm]	5.6	0.7	8.3[c]	0.4
AC [cm]	17.7	2.5	27.9[c]	26.6-29.4
HC [cm]	20.3[#4]	2.4	29.8[#2,c]	1.5
FL [cm]	3.9	0.6	6.2[c]	0.4
RI [index]	-	-	0.7[#1]	0.1
AFI [cm]	-	-	11.8[#3]	9.1-14.9
GRRT [g day^{-1}]	19.7[#6]	17.4-22.2	27.4[#5,c]	23.4-33.2
FL/AC ratio (n=85)§	22.1	1.4	22.0	1.2

[#1](n=94), [#2](n=95), [#3](n=96), [#4](n=98), [#5](n=102), [#6](n=105);
c: $p<0.001$

GRRT-US1 is the growth rate between US1 and US2. GRRT-US2 is the growth rate between US2 and birth.

§ In respect of the validity criteria for the use of the Hadlock ratio, only 85 values qualified for this analysis (See Chapter 2.5.2.2).

EFW (estimated fetal weight), BPD (biparietal diameter), AC (abdominal circumference), HC (head circumference)
FL(femur length), RI (resistance index), AFI (amniotic fluid index).

3.7 DELIVERY OUTCOMES

Fifty female and fifty five male infants were born in the study for whom the birth records were available. The mean birth age was 39.5 ± 1.4 gestational weeks based on the fetal age estimate from the first ultrasound scan. The mean birth weight (BW) was recorded as 3218.3 ± 440.1 g, birth length (BL) was 49.4 ± 2.4 cm, head circumference at birth (BHC) was 34.6 ± 1.5 cm and placenta weighed 625.2 ± 121.1g (Table 3.7A). Ponderal index was 26.7 kg/m^3 in average and the BW/PLW ratio 5.31 ± 1.2.

Table 3.7 Delivery outcomes for 50 female and 55 male newborns and Ponderal index (A). Birth weight by baby's gender is shown in section B.

A. Variable	N	Mean/Median	SD/IQR
Age at birth [weeks]	105	39.5	1.4
Birth weight [g]	105	3170	2920 - 3400
Birth length [cm]	101	49.4	2.4
Head circumference [cm]	102	34.6	1.5
Placental weight [g]	88	600.0	550.0 -700.0
BW/PLW	88	5.1	4.5-6.1
Ponderal Index [$kg.m^{-3}$]	101	26.2	24.8 - 28.3

B. Variable	N	Median	IQR
Birth weight (girls) [g]	50	3105.0	2880-3386
Birth weight (boys) [g]	55	3170.0	2996-3540

$p < 0.05$ girls vs boys

There was a significant difference in the birth weight between boys and girls (p=0.036; Table 3.7 B), which was lost if corrected for gestational age, maternal height or BMI. No further significant differences between genders were observed for the remaining birth parameters or the age at birth. The mean birth weight correlated significantly positively with all measured birth parameters. The birth length and the birth head circumference were the most strongly associated parameters (p<0.001; R=0.668 and R= 0.612 for BL and HC respectively). The ponderal index showed also relatively strong relationship (p=0.001; R=0.421), followed by the placental weight (p <0.05; R=0.238). Analyses were performed by the one way ANOVA and the parametrical correlation with Pearson p value.

From 105 available birth weight records only 1.9 % (2 infants) of these full term babies had a birth weight below 2 500g, another 1.9% (2 infants) were of 2500g and 6.7% (7 babies) exceeded the weight of 4000g at birth.

3.8 EVALUATION OF NEONATES AND INFANTS

3.8.1 Infants' age, weight and anthropometry (Table 3.8)

At the first postnatal visit infants were 3.7 ± 1.3 weeks of age calculated from birth. As expected, the weight, length, BMI and abdominal circumference significantly increased between visits (n=74; p< 0.001 for all comparisons).

Table 3.8a Babies' age, weight and anthropometry at both postnatal visits (Ba1, Ba2).

	Ba 1	(n=77)	Ba 2	(n=74)
Variable	**Mean/ Median**	**SD/ IQR**	**Mean/ Median**	**SD/ IQR**
Age [weeks from deliv.]	3.7	1.3	22.7[c]	21.9-24.4
Weight [g]	3925	595	7532[#4,c]	1003
Length [cm]	52.2	2.9	64.8[#4,c]	3.0
BMI [kgm^{-2}]	14.6	13.3-15.4	17.5[#3,c]	16.5-18.9
AC [cm]	35.5[#1]	33.0-37.4	43.0[#2,c]	40.5-45.0

[#1](n=65), [#2](n=71), [#3](n=72), [#4](n=73);

c: $p<0.001$

The birth and the postnatal anthropometrical parameters were expressed as z-scores (WHO Anthro 2010; see Chapter 2.8.2.1) in order to compare the size of babies with adjustment for age or length (Table 3.8b). From the table it is apparent that on average the babies in the current study are born slightly shorter and of a lower birth weight for either length or age (all the birth z-scores have a negative value), and with a relatively larger head circumference. With increasing baby's age, the weight z-scores become positive, whilst the baby's length for age scores decreased. Both the weight for age and the length for age pass through a nadir at approximately 3-4 weeks of baby's postnatal age, "catching-up" later around the second postnatal visit. However, the z scores for length remain behind the international standard at half a year after birth. The increases in BMI for age correspond to the increases observed in weight z-scores.

Table 3.8b. Babies' anthropometry at birth and at both postnatal visits expressed as z-scores.

z-scores	Birth	(n=101)	Ba 1	(n=77)	Ba 2	(n=74)
Variable	**Mean**	**95% CI**	**Mean**	**95% CI**	**Mean**	**95% CI**
Weight/Length	-0.22	-0.47; 0.03	0.13	-0.25; 0.51	0.58	0.32; 0.84
Weight/Age	-0.20	-0.38; -0.02	-0.36	-0.55;-0.17	0.14	-0.10; 0.38
Length/Age	-0.05	-0.30; 0.20	-0.63	-0.94;-0.33	-0.34	-0.64;-0.04
BMI/Age	-0.26	-0.47; -0.05	-0.01	-0.29; 0.26	0.46	0.21; 0.71
BHC/ Age	0.38	0.15; 0.62	-	-	-	-

No gender difference in z scores for birth parameters was found.

3.8.2 Infants' glucose and hormonal levels (Table 3.9)

Average fasting glucose and leptin concentrations between the two postnatal visits increased from 4.0 ± 0.5 mmol/l to 4.3 ± 0.6 mmol/l (n=70, $p < 0.001$) for glucose and from 3.9 ± 2.9 ng/ml to 4.1 ± 2.5 ng/ml (n=69, $p< 0.001$) for leptin. Insulin concentrations increased from 37.4 ± 17.0 pmol/l to 42.5 ± 15.9 pmol/l, however this change was insignificant (n=68, p=0.065). Mean fasting proinsulin (3.7 ± 1.5 pmol/l) concentration declined by the second postnatal visit to levels of 3.1 ± 1.3 pmol/l (n=70; $p< 0.001$). Insulin-like growth factor binding protein 3 (IFG BP3) increased between visits (from 1 535 ± 324 μg/l to 2 122 ± 536 μg/l; n = 70; $p< 0.001$). Neonatal insulin resistance, measured by the homeostasis model analysis (HOMA-IR), increased significantly during the early postnatal life from 0.98 ± 0.41 to 1.05 ± 0.47 (n=68, $p< 0.001$). There was no significant change in the postnatal growth rate.

Table 3.9 Babies' glucose, hormones, insulin resistance assessment (HOMA-IR) and postnatal growth at both visits (Ba1, Ba2).

	Ba 1	(n=76)	Ba 2	(n=74)
Variable	**Mean/ Median**	**SD/ IQR**	**Mean/ Median**	**SD/ IQR**
GLC F [mmol.l^{-1}]	4.0[#7]	0.5	4.3[c]	0.6
INS F [pmol.l^{-1}]	34.2	28.0-41.1	38.0[#4]	32.1-50.0
PI F [pmol.l^{-1}]	3.4[#7]	2.8-4.6	2.8[c]	2.2-3.5
IGF BP3 [µg. l^{-1}]	1535[#7]	324	2122[c]	536
LEPT [ng ml^{-1}]	3.0	1.9-5.2	3.3[c]	2.5-5.2
HOMA-IR [mmol.mU.l^{-2}]	0.88[#6]	0.69-1.11	1.05[#4,c]	0.91-1.37
GR RT (Del – Ba1,Ba2) [g day^{-1}]	29.1	10.7	26.9[#4]	24.0-30.1
GR RT (Ba 1 – Ba 2) [g day^{-1}]	-	-	26.5[#1]	23.3-29.4

[#1](n=69), [#2](n=70), [#3](n=71), [#4](n=72), [#5](n=74), [#6](n=75), [#7](n=77)

c:$p<0.001$

Normally distributed variables and skewed variables transformed into the logarithmic scale were analysed by means of the Student's paired t-test. For very skewed variables, or in instances where the paired distributions could not be transformed to normality in the same way, the non-parametrical Wilcoxon matched pairs test was applied.

RESULTS - ANALYTICAL DATA

The aim of the analytical part of the data analysis was to identify significant relationships between maternal dietary intakes, maternal glucose and hormonal levels and fetal, birth and infant parameters. The dietary intakes are relatively modifiable factors and they can influence maternal glucose and hormonal levels, and thus the maternal health during the childbearing age. Therefore great attention was given to this first-step analysis in order to identify macronutrients with possible impact on fetal/infant growth and the markers of infant pancreatic ß-cell function. The analysis of nutrient-hormone interactions and the building of a complex statistical model will be the next step in the data analysis of this study, however, it was not one of aims of the current report.

3.9 SUMMARY TABLES ON MATERNAL DIETARY EFFECTS

The maternal macronutrient intakes were analysed in an energy-adjusted form expressed as a percentage of energy intake. As all the macronutrients contribute to the total energy intake, they are also highly intercorrelated. Thus, in a regression model, it is not possible to distinguish between their individual effects on the response variable (*Willet et al.1986; Dallal 2007*). For this reason, and to investigate the isolated effects of each macronutrient, the analysis was carried out in individual linear regression models. The

response variables were regressed on each of macronutrients with and without adjustment for the selected confounders (See Chapter 2.8.3.3)

For the data analysis framework diagrams see Chapter 2.8.3.4, Figure 2.5.

In this section of the Results Chapter the significant outcomes for energy and macronutrients presented in the Appendix D, E and F tables are summarised.

Due to a large number of analyses and results it was not possible to display single models with all the covariates and also it was difficult to summarise such outcomes. Therefore, a graphical illustration was selected for this purpose, which also enables the effects of the selected confounders on the analysed variable to be followed (Tables 3.10 A-C). All significant outcomes from the tables in Appendix D, E, F were entered in Tables 3.10 A-C with significant p values indicated using astrices. The top (blue) astrices represent associations unadjusted for confounders, whilst the bottom (red) astrices represent the model adjusted for the confounders listed below the table.

Again, due to a large number of analyses and data, only significant results of the regression models adjusted for confounders (see Appendix D, E, F) are presented in a summary form in the tables.

Variables of interest:

- Fetal weight (FW), biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC) and femur length (FL) from fetal biometry,
- Birth weight (BW), birth length (BL), ponderal index (PONDIX) and birth weight to placental weight ratio (BW/PLAC W) from birth parameters,
- Infant weight (WEIG), length (LENG) and body mass index (BMI) from the postnatal anthropometrical parameters
- Fasting glucose (GLC F), insulin (INS F), proinsulin (PI F), leptin (LEPT F) and insulin-like growth factor binding protein 3 (IGF BP3) as markers of infant β-cell function and obesity.

As no significant relationships between maternal dietary intake and fetal growth rate were found, these results are not presented.

Maternal intakes of macronutrients (except added sugar) were expressed as percentage of the total energy intake (%E). Unless stated otherwise, all further analyses used macronutrient intake expressed in this way.

3.9.1 Maternal dietary intake and associations with fetal weight and biometry (Table 3.10A).

In all the significant associations between maternal dietary intake and the fetal biometry parameters, and where the regression model included adjustment for confounding variables, gestational age always dominated. This led to the loss of many significant correlations after the adjustment for age, and those that remained, were accompanied by a strong adjusted regression coefficient (AR^2). In some instances maternal weight (associations involving **BPD2, AC2** and **HC2**) and fetal sex (for **AC1** associations) also showed significant relationships.

Among unadjusted models, only the early pregnancy dietary intakes (V1) showed significant associations with concurrent (US1) and later (US2) fetal ultrasound parameters. These included maternal **AN PROT1, PL PROT1** and **SAFA1** intakes correlating with fetal weight and the biometrical parameters of the concurrent ultrasound visit. These associations were negative, except for the **PL PROT1** intake. Similarly, the dietary intake of **PUFA1** supported fetal growth at the later pregnancy visit and the intake of **MUFA1** was associated with the length of the fetal femur bone (**FL2**), also only at V2. These two positive relationships reflected in the **TOT FAT1** intake, which also positively associated with **FL2**.

In adjusted models, the early macronutrient intakes of the mother (V1) produced a lesser number of significant associations with the concurrent fetal ultrasound parameters

(US1). The strongest association observed was the positive correlation of the **TOT ENERGY1** intake with the fetal **AC1,** possibly including a contribution from **ADDED SUGAR1.** The **TOT PROT1** intake was directly related to the fetal **BPD1** measurement, whereas the **TOT ENERGY1** intake correlated negatively with **BPD1**. However, this latter association was very weak and the significance of the outcome depended on exclusion of an outlier value. The scatter plot confirmed a poor linear relationship between both variables (not presented), therefore this outcome may have been attributable to a Type I error. Similarly, this applied to the association between maternal **TOT ENERGY2** intake and the concurrent size of fetal abdomen (**AC2**) and **SAFA2** intake and **HC2.** The fetal abdomen, in turn, was significantly and positively related to the intake of maternal protein (**AN PROT2** and **TOT PROT2**). There were two significant positive associations for **TOT ENERGY2** with the concurrent fetal weight **(FW2)** and length (**FL2)**. The relationship between **TOT CHO1** intake and the fetal head (**BPD2**) was very weak and may also indicate a chance outcome.

The strongest relationship between the maternal dietary macronutrient intake and fetal biometry was found for the **PROT1** intake and the fetal head size (**HC2**) at the later ultrasound scan, adjusted or unadjusted for confounders. The association was negative.

Table 3.10 A **Associations between maternal dietary intakes and fetal weight and biometry.**

	US V1 parameters				US V2 parameters				
Fetal biometry	**FW1**	**BPD1**	**AC1**	**HC1**	**FW2**	**BPD2**	**AC2**	**HC2**	**FL2**
TOT PROT1		**			*-			***- ***-	*-
PL PROT1		*	**	*					
AN PROT1	*-		**-	*-	*-				
TOT PROT2							*		
AN PROT2		NOT APPLICABLE					**		
TOT FAT1									***
SAFA1	**-	*-	**-	*-					
MUFA1									***
PUFA1					***		***	*	***
SAFA2		NOT APPLICABLE						*-	
TOT CHO1			*			*			
ADDED SUGGAR1			*						
TOT ENERGY1		*	***						
TOT ENERGY2		NOT APPLICABLE			**		*		**

+/- direction of the association (no mark indicates a positive association)

Unadjusted	Adjusted for gestational age, MW1, M height, fetal sex	
***	***	p≤ 0.010
**	**	p≤ 0.025
*	*	p< 0.050

3.9.2 Maternal dietary intake and association with the birth parameters.

The total protein intake of the mother near her last pregnancy visit (**TOT PROT2**) correlated ($p<0.05$) with baby's **BW** after adjustment for maternal height, weight, birth age and the baby's gender. This was the only association found with any of the birth parameters (See Appendix H).

3.9.3 Maternal dietary intake and the infants' anthropometric parameters (Table 3.10 B).

Neonatal visit Ba1:

The babies' neonatal length and BMI showed many strong correlations with all the maternal macronutrients intakes during the pregnancy period, except for the intake of carbohydrates and added sugar and the total energy. There was a reciprocal relationship between outcomes for the length and BMI variables. There was also a discrepancy between the direction (positive or negative) of the effects of the macronutrients taken during the second vs. the third pregnancy trimester. The **PUFA1** and the **TOT PROT2** (specifically **PL PROT2**) showed beneficial effects on the neonatal length (**Length1**), whilst the **TOT PROT1** (namely **AN PROT1**) and the total fat (**TOT FAT2** with a major contribution of the **PUFA2)** were found positively related to the neonatal **BMI1.**

Table 3.10 B Association between maternal dietary intakes and the infants' anthropometry

Postnatal Ba1 Postnatal Ba2

Postnatal Anthropom.	Length1	BMI1	Weight2	Length2	BMI2
TOT PROT1	**_	*** ***	*		*** ***
AN PROT1	***_	 ***			 *
TOT PROT2	*** ***	 **_			
PL PROT2	*** *	 ***_	*_		 **_
TOT FAT1		*_ *_			
SAFA1					** *
MUFA1				*_ **_	
PUFA1	** ***	*_ **_			
TOT FAT2	***_ ***_	** ***			** ***
SAFA2					* **
PUFA2	*_ **_	*** ***	**		* ***
ADDED SUGGAR1					*_ *_
TOT CHO2					 *_
TOT ENERGY3			 *		

+/- direction of the association (no mark indicates a positive association)

Unadjusted	Adjusted for babies' age and sex	
***	***	p≤ 0.010
**	**	p≤ 0.025
*	*	p< 0.050

Postnatal visit Ba2:

There were no effects of maternal nutrient intake on the babies' neonatal weight (Weight1) and only a few correlations with the babies' postnatal weight at the second visit (**Weight 2**). These associations of the infant weight with **TOTPROT1** (positive), **PL PROT2** (negative), **PUFA2** (positive) and with the concurrent mother's intake of **TOT ENERGY3 (**positive**)** were all weak. Also, there was only one significant (negative) outcome seen for the babies' postnatal length (**Lenght2),** and it was with the maternal intake of **MUFA1**.

As with the first postnatal visit, there were also many correlations of macronutrients with the postnatal babies' **BMI2.** These were for the protein intake **TOT PROT 1**, specifically **AN PROT1** and the fat intake of **SAFA1** and **SAFA2**, which all correlated positively. There were also negative, weak correlations of **BMI2** with the maternal intakes of **added sugar** earlier in pregnancy and of the total carbohydrate (**TOT CHO2**) later in pregnancy. Further, there was a weak positive association between the concurrent maternal intake of total energy (**TOT ENERGY3**) and the babies' postnatal weight (**Weight2**).

In the majority of associations with the neonatal anthropometry, the adjustment for confounders strengthened the relationship between variables.

3.9.4 Maternal dietary intake and the infants' metabolic parameters (Table 3.10 C).

There were highly significant correlations found between maternal early pregnancy intakes of protein (**TOT PROT1, AN PROT1**) and fat (**TOT FAT1, PUFA1**) and the infant neonatal fasting insulin (**INSF1**) and the **HOMA-IR1** levels. Both metabolic variables showed similar associations and directions with the maternal protein and fat intakes as those found for the postnatal infant BMI (i.e. positive for the protein and negative for the maternal fat intakes). However, the later pregnancy intakes of maternal **PL PROT2** and fat (**TOT FAT2**, **MUFA2** and **PUFA2**) were correlated negatively with **INS F1** and **HOMA-IR1** for the protein and positively for the fat intakes. The p values were strong and were unaltered by adjustment for the infant age, sex and weight. The maternal intake of carbohydrate during the last pregnancy trimester (**TOT CHO2**) correlated negatively with the neonatal **INSF1** and the **HOMA-IR1** levels.

With a similar pattern, associations of maternal pregnancy macronutrient intakes with the babies' later postnatal **INSF2** and **HOMA-IR2** levels were observed, although with a weaker significance compared to the neonatal levels. In addition, the **AN PROT2** intakes correlated positively with the **INSF2** concentrations, whilst the relationships of the INSF2 and **HOMA-IR2** with the **TOT FAT1** and **PUFA1** and **2** were non-significant or appeared very weak.

Table 3.10 C Association between maternal dietary intakes and the infants' metabolic parameters.

	Ba1parameters				Ba2 parameters					
Postnatal Metabol. p.	**GLC F1**	**INS F1**	**HOMA1**	**IGF- BP3 (1)**	**GLC F2**	**INS F2**	**PI F2**	**HOMA2**	**LEPT2**	**IGF- BP3 (2)**
TOT PROT1		*** ***	*** ***	*		*		***		
PL PROT1							*- **-			
AN PROT1		*** ***	*** ***	* *		*		***		
TOT PROT2										*
PL PROT2		***- ***-	*- *-			***- ***-	**-	*- **-		
AN PROT2						* **				** **
TOT FAT1		*- *-	**- *-							
PUFA1		**- **-	***- ***-			*-				
TOT FAT2		*** ***	** **			**				
MUFA2		*** ***	*	** **		*** **		***		
PUFA2		*** ***	*** ***						*** **	
TOT ENERGY2	**- *-	** **			*- *-					
TOT CHO2		***- ***-	*- **-			***- ***-		**- ***-		
ADDED SUGGAR2							*			
TOT ENERGY3						***-		*-	*** *	
ADDED SUGGAR3									**	

+/- direction of the association
(no mark indicates a positive association)

Unadjusted
***
**
*

Adjusted for baby's age, weight, sex
*** p≤ 0.010
** p≤ 0.025
* p< 0.050

The only associations with the infant fasting glucose levels (neonatal and later postnatal **GLC F1 and 2**) were observed with the **TOT ENERGY2** maternal dietary intakes, in both instances negative and weak. A relatively stronger and positive association was observed for maternal **TOT ENERGY2** intake and the neonatal **INS F1** concentrations and for the maternal **TOT ENERGY3** dietary intake and the concurrent **INS F2** levels. The later relationship was negative.

Whilst there was no association between any maternal dietary intake variables and the neonatal proinsulin levels, the later postnatal concentrations (**PI F2**) correlated significantly and negatively with the maternal intake of plant protein at both pregnancy visits (**PL PROT 1 and 2**). However, the later correlation with the **PL PROT2** lost significance after correcting for baby's age, sex and weight. A positive association of **ADDED SUGAR2** with **PIF2** was observed.

The babies' fasting **IGF BP 1** (of visit 1 and 2) concentrations showed direct relationships with the mothers' concurrent intakes of **TOT PROT**, specifically with the **AN PROT**.

The positive associations found between the late pregnancy maternal intake of fat **(PUFA2**) and the concurrent intakes of **ADDED SUGAR3** and **TOT ENERGY3** with the babies' postnatal leptin levels (**LEPT2**) decreasead in their significance after adjustment for the babies' age, sex and weight.

The main focus of the nutritional analysis was to identify possible effects of maternal dietary intakes that could influence postnatal β-cell function of this infant. From Table 3.10C it is apparent, that the majority of associations include the neonatal **INSF1** and **HOMA-IR1** variables, and thus these variables may present the greatest opportunity for a possible nutritional intervention. As the neonatal **INSF1** is the major component of the **HOMA-IR1** index, and the **GLCF1** was related only to the **TOT ENERGY2** intake, a furthter detailed focus was placed on the associations of maternal dietary intake with **INSF1.**

Table 3.11 includes associations of the **INSF1** with macronutrient intakes adjusted and unadjusted for total energy intake. The intakes of the crude **TOT FAT1** and **PUFA1** earlier in pregnancy and of the **PL PROT2** and **TOT CHO2** during the second pregnancy visit did not produce any significant outcomes.

Table 3.11 Major outcomes for maternal dietary intakes and neonatal INSF1 levels

NUTRIENT	Intake V1 (n=76)					Intake V2 (n=75)			
Variable [g]	p	β	95%CI(b)	AR^2	**Variable [g]**	p	β	95%CI(b)	AR^2
TOT PROT1[%E]	0.002	0.231	-0.93; 3.10	0.108	PL PROT2[%E]	0.010	-0.298	0.00; 0.00	0.076
Crude amount	0.045	0.231	-0.96; 3.02	0.041	*Crude amount*	NSF			
AN PROT1[%E]	0.007	0.307	-0.01; 0.17	0.082	TOT FAT2[%E]	0.004	0.327	0.00; 0.00	0.095
Crude amount	0.020	0.267	-0.96; 3.01	0.059	*Crude amount*	0.004	0.330	0.00; 0.01	0.096
TOT FAT1[%E]	0.022	-0.263	0.00; 0.00	0.057	MUFA2[%E]	0.007	0.312	0.00; 0.00	0.085
Crude amount	NSF				*Crude amount*	0.008	0.304	0.00; 0.00	0.080
PUFA1[%E]	0.012	-0.287	0.00; -0.02	0.051	PUFA2[%E]	0.003	0.335	0.01; 0.03	0.100
Crude amount	NSF				*Crude amount*	0.001	0.389	0.01; 0.02	0.140
					TOT CHO2[%E]	0.005	-0.321	0.00; 0.00	0.091
					Crude amount	NSF			
					TOT ENERGY2	0.029	0.253	0.00; 0.03	0.051

* The units for the confidence interval (CI) of the slope (b) are expressed in the units of transformation as stated in Appendix G.

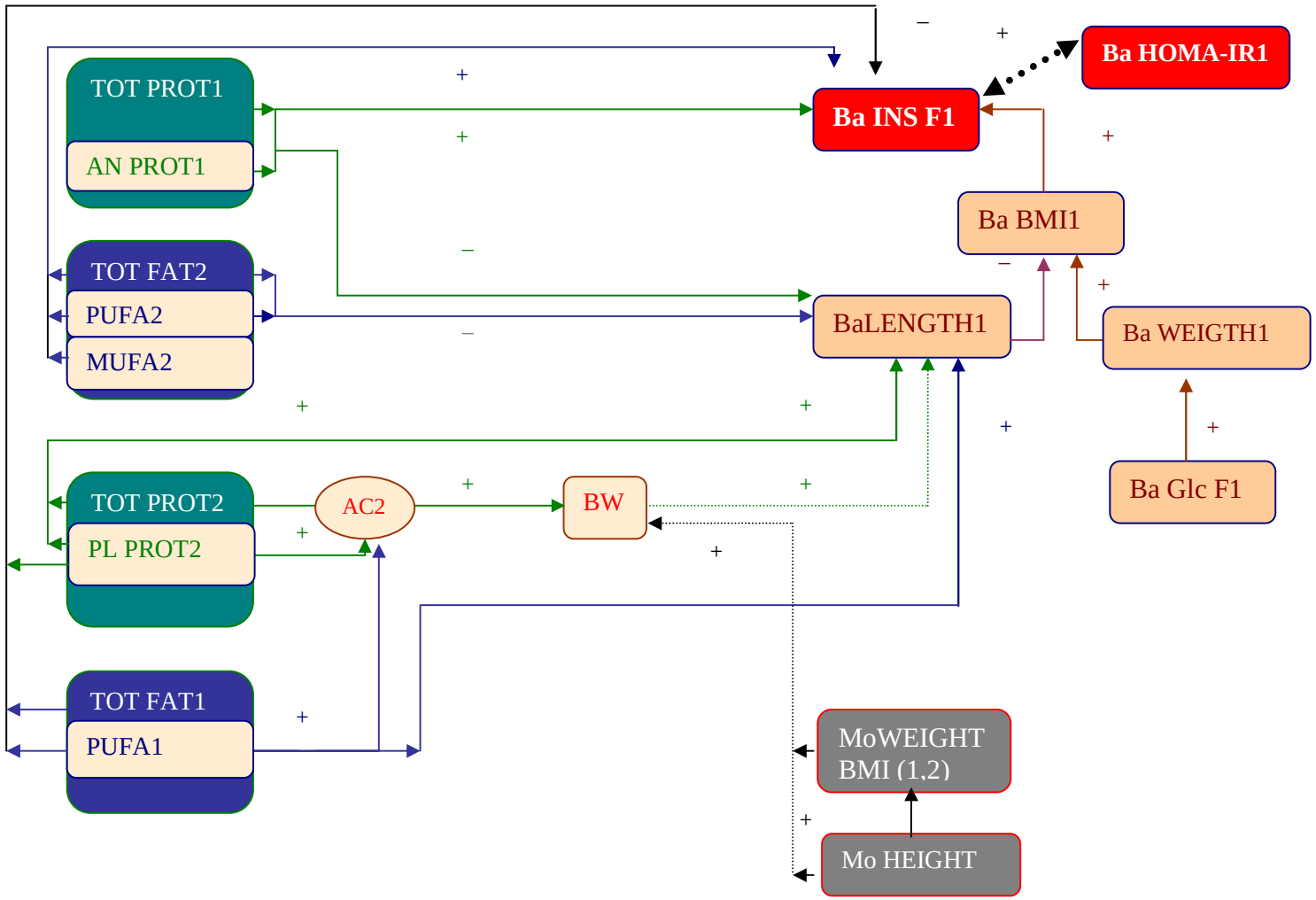


Figure 3.14 Association between maternal protein and fat intakes and the neonatal fasting insulin and suggested mechanism.

3.10 MULTIPLE LINEAR REGRESSION INVOLVING MATERNAL AND FETAL - INFANT PARAMETERS

Effects of maternal anthropometric and metabolic parameters on fetal growth rate, birth and infant postnatal parameters were investigated and results of this analysis are presented in this section (See Figure 2.6 for the analysis framework).

A. Detailed parametrical correlation analysis was performed between maternal anthropometric and metabolic parameters and fetal, birth and infant variables of interest in order to select variables significantly associated for further analysis. For this purpose a cut-off level of $p<0.1$ was used. These pre-selected variables are listed in detail in Appendix I.

B. The pre-selected variables were tested in the regression analysis as explannatory variables for their effects on the response variable using a "backwards selection" (*Petrie et al. 2000b*). Each regression analysis was performed with and without adjustment for confounders (see Chapter 2.8.3.3 and in Appendix D, E, F). In some instances both outcomes differed according to these approaches, therefore both final models were presented in the tables (Appendix J). Each of the presented models includes all final covariates significantly related to the fetal-infant parameter of interest. The summarised outcome can be followed below in Table 3.12 (A-C).

Separate analyses were done for maternal parameters at visit 1 (V1) and visit 2 (V2). Where necessary, the correction for an outlier was carried out. In such cases, when

differences in corrected/uncorrected B value-outcomes exceeded 10%, both outcomes were presented. Transformations and units of the variables are listed in Appendix G.

3.10.1 Associations of the maternal anthropometry and her blood pressure with the fetal – birth – postnatal size and growth rate parameters (Table 3.12 A).

As expected, the maternal pregnancy weight correlated strongly with the infants' birth parameters and also with the neonatal **Ba BMI1**. Adjusting for the birth age, the associations with the **BL** and **BHC** lost signficance. The relationship of **MW1** with the fetal growth rate between both pregnancy visits (**GRRT1**) was possibly mediated via fetal weight (Chapter 3.12; see confounders). The maternal height was positively associated with infant **BW** (Chapter 3.12; see confounders) and negatively with the late pregnancy growth rate.

The table also displays correlations between the postnatal confounders (infant age and sex) and the infant anthropometry. Whilst the birth size and the neonatal **BMI1**were strongly influenced by the birth age, during the later stages (BaV2) the effect of the infant sex was more dominant (with girls having greater **BMI2** than boys, and boys were growing faster than girls between both postnatal visits).

A strong direct association between maternal **SYS BP2** and the fetal **GRRT1** between both pregnancy visits was noted, adjusted or unadjusted for potential confounders.

Table 3.12 A Associations of maternal anthropometry and her blood pressure with fetal – birth – postnatal size and growth rate parameters.

Babies' parameters: ⇒ Maternal parameters: ⇓	BW	BL	BHC	BMI Ba1	BMI Ba2	GRRT1	GRRT2	GRRT D-Ba2
M Height	** 						* 	
MW1	*** ***	*** 	*** 	*** ***		*** ***	 	
MW2	*** ***	*** 	** 	** **		 	 	
SYS BP2	 	 	 	 		*** ***	 	

Babies' parameters: ⇒ Babies' parameters: ⇓	BW	BL	BHC	BMI Ba1	BMI Ba2	GRRT1	GRRT2	GRRT D-Ba2
Age	*** ***	*** ***	*** ***	*** ***	 	 	 	
Sex	* *	 	 	 	**_ **_	 	 	*** ***

+/- direction of the association (+ is not entered in the table)

Unadjusted	Adjusted for babies'age, weight, sex	
***	***	p≤ 0.010
**	**	p≤ 0.025
*	*	p< 0.050

3.10.2 Associations of the maternal glucose and hormones with the infant birth – postnatal size and the growth rate parameters. (Table 3.12B)

The maternal concentrations of **HPL at V1 and V2** and maternal **LEPT2** were directly associated with the growth rate between these two visits (**GRRT1**).

The binding proteins **IGF BP1 and 2** were significantly associated with the size of newborn's head (**BHC)**, maternal **proinsulins** were associated with the neonatal **BMIBa1** and maternal fasting glucose (**GLCF1**) and **IGF1 BP 2** concentration with the second-visit postnatal **BMI Ba2**. In all instances the relationships were negative.

The infant **BMIBa2** was also positively and strongly associated with the early pregnancy maternal levels of **LEPT 1** and her insulin resistance at the end of pregnancy (**INS RES2**). The **INS RES2** was also positively related to the infant postnatal growth rate (**GRRT D-Ba2**).

The maternal **FT4** of the **V1** showed a weak stimulatory effect on the late pregnancy **(GRRT2)** and the postnatal growth rates **(GRRT D-Ba2).** Some weak inverse associations were also seen for maternal **PIF2** with **GRRT2** and for **IGF BP2** with the infant **BL** and the postnatal **BMI Ba2.**

TAB 3.12B Associations between maternal glucose and hormones and Infants' birth – postnatal size and the growth rates.

Babies' parameters: ⇒ Maternal Hormones: ⇓	GRRT1	GRRT2	GRRT D-Ba2	BL	BHC	BMI Ba1	BMI Ba2
GLCF1							***_ ***_
PIF1						***_	
PI-30' (1)						***_	
IGF-BP1 (1)					**_ *_		
HPL1	*** ***						
FT4 (1)		*					
LEPT1				*	* *		*** ***
PIF2		*				***_	
PI-60' (2)						***_	
IGF-BP1 (2)				**_	***_ ***_		*_
HPL2	*** ***						
LEPT2	*** ***						
INS RES2			*** ***				*** ***

+/- direction of the association (+ is not entered in the table)

Unadjusted	Adjusted for babies'age, weight, sex	
***	***	p≤ 0.010
**	**	p≤ 0.025
*	*	p< 0.050

3.10.3 Associations of the maternal glucose and hormones with the infant postnatal glucose and metabolic parameters. (Table 3.12C)

The babies' fasting glucose levels at both postnatal visits showed a number of associations with maternal hormones. The **GLC F Ba1** was positively but weakly related to maternal **GLCF1** and **HPL2** if unadjusted for babies' age, weight and gender. The neonatal GLC F Ba1 was also in a positvie and strong relationship with the maternal levels of **CORT2** and **babies' concurent age,** and inversely was associated with the maternal **FT4 V2** (see model in Appendix J, Table J 2.6). The concentrations of maternal **HPL 1 and 2** were also positively associated with the infant postnatal glucose **GLC F Ba2.**

The early pregnancy maternal concentrations of **FT4 (V1)** were inversely related to fasting neonatal insulin **INS F Ba1**, **HOMA-IR Ba1** and also to the later postnatal leptin **LEPT F Ba2** levels.

Both postnatal leptin concentrations were related to the infant sex, each time being higher for the female offspring. However, the male offspring was heavier than the female offspring of the same age (N=77, R=0.231, F=4.23, p= 0.041; means: 3783g vs. 4056g for female vs male Ba1; N=73, R=0.363, F=10.80, p=0.002; means: 7111 vs 7813g for female vs male Ba2; Analysed by ANOVA).

TAB 3.12C Associations between maternal glucose and hormones and infants' postnatal metabolic parameters

Babies' parameters: ⇒ Maternal Hormones: ⇓	GLC F Ba1	INS F Ba1	LEPT Ba1	HOMA Ba1	GLC F Ba2	INS F Ba2	LEPT Ba2
GLCF1	*						
HPL1					*** ***		
FT4 (1)		***_ ***_		***_ ***_			**_
INS-30' (2)							**_
PIF2				*_ *_			
PIF-30' (2)					*_		
HPL2	*				** *		
FT4 (2)	***_						
CORT2	** ***						

Babies' parameters: ⇒ Babies' parameters ⇓	GLC F Ba1	INS F Ba1	LEPT Ba1	HOMA Ba1	GLC F Ba2	INS F Ba2	LEPT Ba2
Weight Ba1			***	***			
Age Ba1	***		**				
Weight Ba2						*	***
Sex			***_			*_	***_

+/- direction of the association (+ is not entered in the table)

Unadjusted	Adjusted for babies'age, weight, sex	
***	***	p≤ 0.010
**	**	p≤ 0.025
*	*	p< 0.050

A similar but weaker relationship with the infant sex was also observed for **INS F Ba2.** The infant weight showed a positive association with **HOMA-IR Ba1.**

Some weak associations were also seen for maternal proinsulins of the later pregnancy visit (**PIF2 and PIF-30' V2**) with **HOMA-IR Ba1** and for maternal **INS-30' V2** and babies' later postnatal **LEPT Ba2**. All associations were negative.

The major outcomes obtained for maternal glucose and hormones and infants' parameters and growth rates are summarized in Table 3.13 below.

Table 3.13 Summary of major outcomes for maternal glucose and hormones and infants' parameters and growth rates

ANTHROP.			(n=76)		**METABOL.**			(n=76)	
Variable	p	β	95%CI(b)*	AR^2	**Variable**	p	β	95%CI(b)*	AR^2
GRRT1					**GLC F Ba1**				
HPL1	<0.001	0.390		0.243	GLCF1	0.047	0.228	0.03; 1.69	0.039
HPL2	0.028	0.217	-0.17; -2.60	0.038	FT4 (2)	0.003	-0.347	-4.46; -20.16	0.156
LEPT2	<0.010	0.252	0.02; 0.12	0.055	HPL2	0.044	0.224	0.07; 3.15	0.156
GRRT2					CORT2	0.012	0.293	0.30; 2.19	0.156
FT4 (1)	0.046	0.197	0.02; 0.90	0.029	**INS F Ba1**				
PIF2					FT4 (1)	0.003	-0.343	-0.13; -0.58	0.106
GRRT D-Ba2					**HOMA Ba1**				
INS RES2	0.006	0.325	0.05; 0.28	0.093	FT4 (1)	<0.001	-0.417	-3.12; -9.10	0.227
					GLC F Ba2				
					HPL1	<0.001	0.532	1.19; 2.92	0.270
					HPL2	0.034	0.116	0.19; 3.99	0.049
					LEPTF Ba2				
					FT4(1)	0.012	0.291	1.48;10.85	0.072

* The units for the confidence interval (CI) of the slope (b) are expressed in the units of transformation as stated in Appendix G.

3.11 ASSOCIATIONS WITH THE SOCIO-ECONOMIC STATUS (SES) OF THE PARTICIPANTS.

Both the mother's and the father's education showed significant relationships with the family income ($p = 0.042$ and $p=0.017$ respectively). Also, there was a strong direct relationship between mother's and father's educational status ($P<0.001$). Mothers who were employed were more physically active ($p<0.001$) and their education was inversely and strongly ($p = 0.006$) associated with the number of siblings, as mothers from larger families had fewer opportunities to study. Due to an occurrence of low frequencies in the contingency tables, the Fisher exact test was applied.

Mothers who were employed were inclined to deliver taller babies (see Table 3.14A). The employed status of the female also positively correlated with her pregnancy weight, BMI and leptin. Furthermore, employed women showed elevated fasting insulin, and insulin resistance index at V1 and **GLC F**, **area under GLC curve** and **PI** levels at V2. The neonates of employed women had lower fasting glucose levels than neonates of unemployed women (Table 3.14A). Baby's **BL** also positively correlated with father's employment, whilst neonatal **PONDIX** was negatively associated with father's employment status (Table 3.14B).

(In all folowing tables, the 95% CI of the regression slope b is expressed in the transformation units of the particular variable. See Appendix G.)

Table 3.14 A Relationship between mother's employment status and maternal, birth and infant postnatal parameters.

Mo employment vs. Dependent variable:	N	p	β	95% CI (b)	AR^2
BL	99	0.034	0.212	1.96; 0.09	0.035
MW1	106	0.002	0.294	0.07; 0.02	0.111
MW2	105	0.004	0.280	0.06; 0.01	0.068
BMI1	106	0.004	0.272	0.06; 0.01	0.093
BMI2	105	0.010	0.252	0.05; 0.00	0.051
GLC F2	107	0.038	0.201	0.00; 0.02	0.031
Area GLC 2	107	0.009	0.252	0.06; 0.01	0.054
INS F1	108	0.016	0.231	0.14; 0.02	0.044
INS RES 1*	108	0.014	0.237	0.17; 0.02	0.049
PI F2	105	0.010	0.247	0.15; 0.02	0.118
LEPT F1;	106	0.007	0.180	0.18; 0.03	0.569
LEPT F2	105	0.008	0.184	0.98; 0.16	0.538
GLC F Ba1	75	0.032	-0.235	-0.02; -0.42	0.153

Mo (mother); *Maternal insulin resistance was calculated as HOMA-IR; Analysed by the regression analysis. All the associations were significant either adjusted or unadjusted for the gestational/postnatal age (where appropriate). An exception was the birth length correlation, which, due to a strong relationship with the birth age lost the significance in adjustment. For all other variables, the table shows associations corrected for the age.

Table 3.14 B Association of father's employment status with birth and infant postnatal parameters.

Father employment vs. Dependent variable:	N	p	β	95% CI (b)	AR^2
BL	100	0.001	0.306	0.74; 3.42	0.204
PONDIX	101	0.006	-0.271	-0.01; 0.07	0.064

Analysed by the regression analysis.

The associations including maternal weight, BMI, proinsulin, maternal leptin and infant glucose were adjusted for the gestational/baby's age (as appropriate). In addition, associations with maternal leptin were adjusted for maternal weight. All the other associations, where the age adjustment had no effect, are presented without adjustment. Maternal glucose, insulin and insulin resistance values lost their significance after adjusting for maternal BMI. An adjustment for the total energy intake did not change the above associations and was insignificant in the regression output. The strongest association observed was between the fathers' employment status and the infant birth length (adjusted for the birth age).

Interestingly, a correlation was found between parental education and maternal cortisol, where lower educational level of baby's mother or father was associated with higher maternal cortisol levels at V2 (Table 3.15).

Table 3.15 Association between parental education and maternal cortisol.

Independent variable	Dependent variable	N	p	β	95% CI (b)	AR^2
Mo education	Mo CORT V2	106	<0.001	-0.329	-0.08; -0.03	0.213
Fa education	Mo CORT V2	99	<0.001	-0.360	-0.07; -0.02	0.233

Analysed by the regression analysis. Adjusted for maternal BMI and gestational age. Mo (mother), Fa (father).

No correlation was observed between birth weight and any socio-economic indicators selected in this study.

Association between SES and nutrient intake.

Following variables were tested:

Nutrient intakes at V1 and V2:

Total Energy

Total Protein

Total Fat

Total carbohydrate

SES variables:

Income index (4 categories)

Household occupants (3 categories)

Number of mother's siblings (3 categories)

Mother's and father's education (each 4 categories)

Mother's and father's employment (2 categories; students were allocated to the non-employed category, as their number was low)

The nutrient intakes were normalized and the associations between variables were tested by means of one-way ANOVA with “unequal HSD” post-hoc test (due to different numbers in each category). The Levene’s test was used to test for the variances in the groups. In the case of positive outcome of the Levene’s test, the Kruskal Wallis ANOVA was applied. (See Table 3.16).

Table 3.16. Relationship between maternal nutrient intakes and SES.

Compared Variables	N	F	p	Post hoc p value
Mother education:				
TOT ENERGY1	107	4.1	0.009	0.028
TOT CHO1		3.9	0.011	0.037
Father education:				
TOT ENERGY1	101	5.3	0.002	0.002
TOT PROT1		3.6	0.017	0.019
TOT FAT1		4.7	0.004	0.005
TOT CHO1		4.2	0.008	0.006
Mother employment:				
TOT ENERGY1	107	9.4	0.003	0.006
TOT PROT1		5.8	0.018	0.030
TOT FAT1		5.9	0.017	0.029
TOT CHO1		8.9	0.004	0.008
Father employment:				
TOT ENERGY2	107	14.2	<0.001	0.006
TOT PROT2		12.0	0.001	0.011
TOT FAT2		10.9	0.001	0.015
TOT CHO2		13.9	<0.001	0.006

No significant association was found for the number of household occupants, the income index or the number of mother's siblings. Although for the income index and the mother's protein intake at V1 and also for mother's number of siblings and her total energy intake at V1 the ANOVA p value was significant, after application of the post hoc test the significance was lost.

Mother's education, and even more strongly father's education and mother's employment were associated positively with the nutrient intake at the first pregnancy visit. The father's employment status was associated positively with the mother's nutrient intake at the second pregnancy visit.

No significant association with maternal dietary intake was recorded for the family monthly income index.

3.12 FURTHER ASSOCIATIONS

The following associations are discussed in Chapters 4.5.1 and 4.5.2.

The strongest determinant of the fetal biometrical parameters was the gestational age at both pregnancy ultrasound visits (Table 3.17).

Table 3.17 Gestational age association with fetal biometrical parameters

	USMA1		(N=106)	**USMA2**		(N=105)
Parameter	β	95%CI (b)	AR^2	β	95%CI (b)	AR^2
FW	0.992	1.84;1.93	0.985	0.796	2.17;2.92	0.814
BPD	0.973	0.29;0.32	0.973	0.680	8.99;13.72	0.700
AC	0.952	0.01;0.01	0.947	0.677	0.31;0.47	0.718
HC	0.983	1.05;1.14	0.961	0.690	30.36;46.81	0.690
FL	0.971	0.25;0.27	0.966	0.784	9.61;13.08	0.817

All p values are <0.001.

95%CI of the slope – units are corresponding to the transformed form of the variable (see Appendix G2).

In addition, a significant positive correlation was found for the birth weight and maternal height adjusted for the birth age ($p=0.013$, $\beta=0.219$ for height and $p<0.001$, $\beta=0.423$ for birth age, $AR^2= 0.220$) and the maternal weight **MW1** correlated with the **FW1** (N=107; R=0.207; p=0.033; see confounders).

The male infants were growing faster than the female infants between birth and the last postnatal visit ($p <0.001$, $AR^2=0.210$, F=20.1 by ANOVA). Also, a significant gender difference in the infant postnatal leptin concentrations (**LEPT1 and 2**) with higher levels in females (N=76; R=0.286, p=0.012; analysed by ANOVA) was found. The male offspring

was heavier than the female offspring of the same age (N=77, R=0.231, F=4.23, p= 0.041; means: 3783g vs. 4056g for female vs male Ba1; N=73, R=0.363, F=10.80, p=0.002; means: 7111 vs 7813g for female vs male Ba2; Analysed by ANOVA).

A significant inverse relationship between both pregnancy visit maternal IGF BP1 was found with the birthweight (p=0.002, Spear R=-0.306, N=105).

These correlations are discussed in the Chapter 4.5.2.1.

3.13 SAMPLE BIAS

A possible sample bias due to attrition between the women who completed the study and the women who left the study after delivery was investigated. The data on the major variables (maternal age, nutrient intake, anthropometry and hormones) were compared for V1 and V2 with one-way ANOVA. No significant differences were found.

CHAPTER 4

DISCUSSION - DESCRIPTIVE DATA

This section of Chapter 4 includes discussion on the descriptive data. In the first part, the characteristics of the women enrolled into the study will be discussed. The next part will focus on the data obtained from anthropometric measurements and will compare the birth outcome with other studies. A discussion of the maternal dietary intakes and the selection of the food frequency questionnaire for this study will conclude this section.

4.1 ENROLMENT AND CHARACTERISTICS OF STUDY PARTICIPANTS

4.1.1 Ethnic composition of the study population

The majority of the study participants were Zulu speaking. This reflects the ethnic/language composition of the Black population of Gauteng Province, which has the highest urban concentration of Zulu people, while Kwa-Zulu Natal is the most Zulu-populated rural area in South Africa (www.sahistory.org.za). [3]

[3] The language groups among Black South Africans generally overlap with ethnic population groups. In South Africa there are four major ethnic divisions corresponding to four major subgroups of original Bantu languages: Nguni, Sotho, Tsonga-Shangaan and Venda (http://countrystudies.us/south-africa/45/htm).
Nguni includes the Zulu, Xhosa, Swazi and Ndebele speaking groups, accounting for two thirds of the South African Black population (www.sahistory.org.za)
Sotho includes the Northern Sotho (Pedi group), Southern Sotho and Tswana (Ba Tswana).

The women attending the 159ANC clinic came from all areas of Johannesburg and its townships, where they permanently resided or had moved to temporarily from other parts of the country for the duration of their pregnancy.

Only South African residents were included in the study. However, six women originating in Swaziland two women from Venda and one woman born in Zaire were also accepted. Swaziland and Lesotho are two small independent countries surrounded by South Africa. All these women were married to South Africans and resided in Gauteng for a minimum of 5 years. Thus, the study participants represented quite a variety of ethnic/language groups (Figure 3.1). However, due to small numbers in each of these groups, except the Zulu speakers, no further analysis in this respect has been carried out.
The comparison of the Zulu vs. combined non-Zulu ethnic groups was not carried out, as the non-Zulu combined population would not represent a homogenic population. The current study did not aim to investigate ethnic differences and such a study would require a different study design.

4.1.2 Soci-economic background.

The socio-economic background of participants was assessed from parental educational level, employment status, family monthly income, number of household occupants and number of mother's siblings. The household crowding factor was used in the Helsinki study, which reported an association between less crowded homes and higher social class (*Eriksson et al 1999).*

The women came from different socio-economic strata ranging from middle class communities to those of a very poor socio-economic status. In this study there were no women from the higher socio-economic background as these women are not likely to seek medical attention within the public service and they invariably/preferably attend private clinics. Likewise, very few of the women in the study had a tertiary education.

In South Africa, 80% of all pregnant women, of whom 85.2% are African, attend public sector antenatal clinics (*SADHS, 1998*).

4.1.2.1 Employment status.

Although similarly educated (Figure 3.3), women in their early pregnancy were twice as likely to be unemployed compared to their partners (Figure 3.4). This may be partially explained by the pregnancy, but more likely it reflects the pattern within the society. Richter et al *(2006*) followed immigrant groups from the Greater Johannesburg area and also found that unemployment of mothers was higher than that of fathers among migrants and original residents.

4.1.2.2 Family income

Family monthly income was difficult to evaluate. Although an attempt was made to obtain direct values in Rands, many women were unsure about the exact amount or hesitated to reveal their true income. Therefore, it was more practical to assess the family monthly income using categories with the range of R 1000 (Figure 3.5). The majority of participants fell in the lowest category (below R 1000).

Comparing this classification with the national estimate of average monthly earnings for the year 2005 (see below) (*Statistics South Africa 2006*), it confirms that the vast majority of study participants was coming from a poor socio-economic background. Only one family had a monthly income that exceeded the national average. The monthly income estimates in the formal non-agricultural industry, including bonuses and overtime for 2005 were R 7006 with large variance between industries from R 4000 (construction) to over R 14 000 (electricity, gas and water supply) per month.

4.1.3 Family history of diabetes and hypertension

In the present study, 35.7% of mothers of the Xhosa speaking group reported a first-degree relative having treatment for diabetes or hypertension or both, in comparison with the Zulu group, where 47.9% of the mothers' close relatives had been treated for the above conditions (Figure 3.8). However, these data may be biased, as mothers were not sure about their family members medical background, and it cannot be excluded, that in many cases the health status was unknown to members themselves. This situation was even more unclear in the case of the partner's family members. Therefore 14% of the men had to be excluded from the data set. As the majority of mothers reported no family history of diabetes in the first-degree relatives, the data was not used in further analysis.

In conclusion, the Black pregnant women enrolled into the study were women living, or temporarily residing, in the Gauteng area during their pregnancy. The majority were Zulu speakers, although a variety of ethnic/language groups was present and the

distribution of the major groups reflected the ethnic composition of the Gauteng Province (www.sahistory.org.za). A majority of women in the study had completed high school education, but few had any tertiary education. Despite similar education level with men, the unemployment rate among these women was almost twice as high.

The study participants were from low and very poor socio-economic levels. This was confirmed by the family income figures, which showed that the majority of families had income below the national average.

4.2 ANTHROPOMETRICAL DATA AND BIRTH OUTCOME

Maternal anthropometry, birth data and maternal dietary intakes were compared with available data from similar studies carried out in developing and developed countries.

4.2.1 Maternal anthropometry.

Maternal anthropometry was compared with the data from mothers at 28 weeks of gestation from the Pune Maternal Nutrition Study (Table 4.1; *Rao et al. 2001*). This pregnancy timing fell between the PND study V1 (20-24 gest. weeks) and V2 (30-36 gest weeks). At both visits, the PND study women were older, heavier with greater BMI and they were also taller. They had higher HC and ArC. However, the numbers of women in the PND study are much less than in the Pune study, which also is reflected in larger standard deviations. The measurements of the mother's head and arm circumference were added only later during the feasibility study, therefore the numbers are low and the data were not considered in the analytical part of the study.

The records of mothers in the current study were also compared with the European mothers in the Helsinki study (*Forsén et al. 1997)*. The records in the Helsinki study were taken at the onset of labour, whilst the records of the women in the current study were from 30-36 weeks of gestation. However, despite similar heights, the weights and BMIs of the European mothers were much lower than those of the women in the current study. One also has to consider, that the European mothers were two years younger.

TABLE 4.1: Comparison of maternal characteristics

	PND V1 (n=108)	**PND V2** (n=107)	**PUNE 28 g.w.** (n=633)	**HELSINKI*** (n=3076)
Variable	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Age [y]	29.0±4.6		21.4	27.4±5.7
Weight [kg]	74.1±13.3	78.6±12.9	47.3±5.2	67.3±9.1
Height [m]	1.59±0.06	-	1.51.9±0.05	1.58±0.06
BMI [kgm^{-2}]	29.3±4.8	31.1±4.6	20.5±1.7	26.9±3.2
MHC [cm] [#1]	-	57.0±2.3	52.3±1.5	-
MArC [cm] [#2]	-	28.2±3.5	22.7±1.7	-

[#1](Mother's head circumference; n=71); [#2](Mother's mid-upper arm circumference; n=68);

* (*Forsén et al. 1997*; maternal weight taken on admission to labour)

4.2.2 Birth outcome.

In comparison with the fullterm babies of the India study in Trivandrum - Kerala (Table 4.2; *Jaya et al. 1995*), the Johannesburg babies were heavier (female babies 3123.8g ± 334.0 vs 2819.7 g ± 410.9 and male babies 3304.1g ± 506.1 vs 2905.2 g ± 431.4), longer (49.4 cm ± 2.4 vs 47.7 cm ± 2.2) and had bigger head circumference (34.6cm ± 1.5 vs 32.4cm ± 1.7). The placental weight (625.5 g ± 121.1 vs 502.4 g ± 43.3) and ponderal index (26 $kg.m^{-3}$ ± 3.0 vs 23 $kg.m^{-3}$ ± 1.1) were higher. Despite an increasing average BW going from low to high socio-economic status in the Trivandrum-Kerala study (with a significant difference of 89.6g between the two extremes), the PND study babies' BW was higher at all instances and did not differe with the socio-economic status.

Table 4.2 shows the available data on birth outcomes of the Pune Maternal Nutrition Study (rural area; *Rao et al. 2001*), Southampton study (*Godfrey et al. 1996*) and of the Helsinki study (*Forsén et al. 1997;* see Chapter 1.1.2).

The African babies had much higher BW than the Indian babies, but lower than the European babies, irrespective of gender. A similar relationship was also observed for babies' BL and ponderal index. The HC of the PND study babies was very close to the Helsinki babies, with comparable SD, whilst the Indian babies were lower by more than 1 cm when compared to the PND study. Also the placental weight in the current study was very close to the results of the Helsinki study and even larger than the Southampton study. However, the placental weight of the Pune mothers was 43% less than that of mothers participating in the PND study, despite very similar birth age.

The larger size of the head of babies in the current study was confirmed by the z-scores. Similarly, from comparison with the z-scores, it was obvious that these babies were born shorter and lighter. The fast improvement in the weight for length ratio, exceeding the mean value for the standard population (WHO), and the parallel delayed linear growth indicated fat accumulation in these relatively stunted infants. This again was in agreement with the elevated BMI for age score (See Table 3.8b).

In conclusion, the birth size of these African babies, although smaller in all parameters, is still closer to the size of the Caucasian babies than to the birth size of the babies from Trivandrum or Pune of India. These relatively smaller African babies have comparable (or even larger) maternal placental sizes in relation to the mothers of both European studies. However, in comparison with the small Indian babies, the major noted difference was a substantially lower placental size of the Pune mothers.

TABLE 4.2: Comparison of the birth characteristics between different studies.

N	105	3230	633	538	3302
Variable	PND Study	Trivandrum, India	Pune, India	Southampton, UK	Helsinki, Finland
Birth weight ave [g]	3213.7 ± 439.7	2961 ± 348.8	2665 ± 358	3436±480	-
Birth weight F [g]	3123.8 ± 334.0	2819.7 ± 410.9		3344±463	-
Birth weight M [g]	3304.1 ± 506.1	2905.2 ± 431.4		3527±496	3440 ± 510
Birth length [cm]	49.4 ± 2.4	47.7 ± 2.2	47.8 ± 2.0	-	50.2 ± 1.9
Head circumference [cm]	34.6 ± 1.5	32.4 ± 1.7	33.1 ± 1.2	-	34.9 ± 1.4
Placental weight	625.2 ± 121.1	502.4 ± 43.3	360 ± 76.4	533.0±120	634 ± 127
Ponderal Index [$kg.m^{-3}$]	26.6 ± 3.0	23 ± 11	25.0 ± 5.0	-	27.0 ± 2.5
Age at birth [weeks]	39.5 ± 1.4	37-42 full term	39.4 ± 1.2	40.2±1.3	39.3 ± 2

4.3 MATERNAL DIETARY INTAKES IN COMPARISON WITH RDA AND OTHER STUDIES.

The maternal dietary intakes were compared with the Pune Maternal Nutrition Study (intake at 28 gest. weeks) and the Southampton study (intake at 32.7 gest. weeks) due to their ethnic and socio-economic similarities or differences. In addition, the maternal dietary intake during pregnancy and at V3 was compared with the Dietary Reference Intakes (DRIs)[4], with the overall dietary intakes of South African adult females (*Steyn et al 2006)* and with an analysis of the American diet (*Schaefer et al, 2000;* Table 4.3A). The American diet data was selected for comparison due to the known high caloric intake and prevalence of obesity among the American population (See Chapter 1.2.1.1).

Under the category of the DRIs, the Recommended Dietary Allowences (RDAs) were used for comparison. The RDA values differ for pregnant and lactating and non-pregnant women. The ranges of protein, fat and carbohydrate expressed as a percentage of total energy intakes are available only for the general adult population. Due to the half-year

[4] DRIs (2005) include reference values for specific nutrients. They replaced previous Recommended Dietary Allowances (RDAs), published from 1941 to 1989 by the National Academy of Sciences in USA, and Recommended Nutrient Intakes, published by the Canadian government. DRIs were developed by the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes of the Food and Nutrition Board, Institute of Medicine, the National Academies, in collaboration with Health Canada.

Table 4.3 A: Comparison of the maternal daily dietary intake of macronutrients with DRIs, overall intake in South African women, the Pune Maternal Nutrition Study, the Southampton study and the usual American diet.

	PND	**PND**	RDA	Pune St*[1]	South St*[2]	**PND**	RDA	SA women*[3]	American diet*[4]
Nutritional factor	**V1**	**V2**	Pregn.	28w	32.7w	**V3**	**Lactat.**	**Non-pregn.**	**Actual**
Energy [kJ]	**7002**	**7059**	-	7000	9682	**7890**	-	7239	9916 ± 1565
TOT PROT [g]	**54**	**53.8**	71	43.5	85.7	**55.3**	71/46	61.6	88.9 ± 14.0
TOT FAT [g]	**64.7**	**69.0**	-	32.4	93.2	**86.7**	-	46.6	92.2 ± 14.6
TOT CHO [g]	**234.5**	**234.8**	175	-	301.9	**249.9**	210/130	244.5	290.3 ± 45.9
Fiber [g]	**14.6**	**15.4**	-	-	-	**15.0**	-	18.7	28.4 ± 4.5
Cholesterol [mg]	**212.8**	**187.1**	-	-	-	**205.6**	-	197.9	348.4 ± 55.0
TOT PROT [%E]	**12.9**	**13.0**	10-35§	10.4	14.7	**11.7**	10-35§	14.1	15.0 ± 0
TOT FAT [% E]	**35.3**	**35.0**	20-35§	17.2	36.4	**37.5**	20-35§	22.9	35.0 ± 0
TOT CHO [% E]	**51.9**	**53.3**	45-65§	72.3	49.0	**51.2**	45-65§	62.1	49.0 ± 0

PND (Pregnancy, Nutrition & Diabetes – current study); *[1] (*Rao et al 2001*); *[2](Southampton study; *Godfreyet al. 1996*);

*[3] (*Steyn et al 2006*); *[4](*Schaefer et al, 2000*; N=1726, age=15-60y)

DRI (Dietary Reference Intakes); RDA (Recommended Dietary Allowance); § Acceptable Macronutrient Distribution Ranges for general adult population (reprinted with permission of the National Academy of Sciences. See Appendix I)

time span between the delivery and the last maternal visit V3, there are two values entered in Table 4.3 for the postpartum period; the first one is the RDA for the lactation period and the second is for non-pregnant women.

4.3.1 PND study and national South African data on women 15-64 years old (Table 4.3 A and B; *Steyn et al 2006).*

The PND Study pregnancy total energy intakes were slightly lower than the national level for non-pregnant women (7002 and 7059 vs 7239 kJ), however the energy intake at V3 exceeded the national level. The difference between the V2 and V3 intakes was 838kJ/d. There are increased requirements for energy intake during pregnancy at approximately 800 – 1200 kJ/day extra and for the first six month of exclusive breast-feeding of 2100kJ/d extra in well-nourished population (*McNeill 2000)*. Considering these amounts, the total energy intake of the current study participants appears to be low. This was largely due to a low intake of protein (54 and 53.8 g/day vs 61.6g/day) and total carbohydrate. Comparing the quality of the crude protein with the intakes in similar age groups of South African non-pregnant women (Table 4.3B), the consumption of animal protein was similar, however, the intake of the plant protein was substantially lower. As the dietary intake of plant protein in this study was positively associated with linear growth (See Chapters 3.9 and 4.5.1.3), this could contribute to the relatively stunted growth of infants during the early postnatal period (see Chapter 4.2.2). In turn, there was a substantially higher intake of fat in the PND study (64.7 and 69.0 g/day vs 46.6 g/day). Fat

intake increased at V3 to almost double the level of the South African average. The crude amount of the dietary fat intake was compared with the similar age categories of South African non-pregnant women (Table 4.3.B). The PND study women ate between 1.5-1.9 fold more of **SAFA**, 1.2–1.8 fold more of the **MUFA** and 1.2–2.5 fold more of **PUFA.** Thus, the most frequently consumed fat during the pregnancy period was SAFA, whilst the **PUFA's** intake increased dramatically after delivery. Looking at the changes in fat consumption between visits, the **MUFA** and **PUFA** intakes increased significantly, whilst the intake of **SAFA** remained constant.

The above findings may help explain the z-score data. The **PUFA** requirements during pregnancy are high (*Al et al 1995 and 2000*). The **LC-PUFA** is utilised by the fetus for brain and retina development, and for bone metabolism (see Chapter 4.5.1.1 and 4.5.1.3) and during the first two trimesters it is also required by the mother to build her pregnancy fat reserves. The **SAFA** intake in the current study (and in the literature) was negatively associated with bone growth. This combined with the low levels of maternal **PL PROT** intake could eventually lead to a shorter, lighter baby with a relatively larger head (see Chapter 5 "Summary and Conclusions"). The decline in z-scores for weight/age and especially for length/age at the Ba1 visit could correspond to the switch from the maternal placental **PUFA** supply to the breast/formula feeding, which would not fully support growth of the baby. An improvement in these scores at the Ba 2 visit may then reflect a mobilisation of the infant fat reserves and its own production of **PUFA**s.

It would be expected that these effects were not isolated, but they were complemented by effects of other factors, such as vitamins, minerals and maternal hormones. However, these were not components of the current study analysis.

Table 4.3 B: Comparison of the protein and fat categories and the added sugar maternal daily dietary intake with the data on South African women

	PND Study (N=107)	**PND** Study (N=107)	**PND** Study (N=74)	SAurban women* (n=1196)	SA women 15-29 years* (n=1726)	SA women 30-44 years* (n=1726)
Nutritional factor	V1	V2	V3	Non-preg.	Non-preg.	Non-preg.
PL PROT [g]	**21.7**	**22.1**	**22.1**	22.5	28.2	29.8
AN PROT [g]	**31.8**	**31.4**	**30.6**	40.1	34.8	31.8
SAFA [g]	**22.5**	**23.4**	**24.9**	17.6	15.1	13.1
MUFA [g]	**20.6**	**21.1**	**28.2**	20.8	17.7	15.9
PUFA [g]	**16.7**	**15.7**	**25.5**	13.1	13.7	10.2
Added sugar [g]	**51.9**	**50.1**	**59.8**	53.8	48.2	39.0
PL PROT [%E]	**5.1**	**5.3**	**5.2**	5.3	6.3	6.9
AN PROT [%E]	**8.0**	**8.0**	**6.5**	9.2	7.2	7.2
SAFA [%E]	**11.8**	**11.7**	**11.1**	8.6	6.7	6.3
MUFA [%E]	**10.7**	**10.9**	**12.1**	10.1	7.9	7.6
PUFA [%E]	**10.4**	**10.2**	**12.4**	6.5	6.5	5.1
Added sugar [%E]	**11.8**	**11.5**	**12.4**	12.6	10.4	10.4

*(*Steyn et al 2006*);

However, there were differences between both investigated populations. The current study investigated only urban (and pregnant) women 18-40 years old, while the overall national data include rural populations of a wide age range. Also, there is a large samples size difference.

MacIntyre (*1998*) reported differences in fat intake among 25-34.9 year old females in rural (50.6g/d), urban middle class (67.6 g/d) and urban upper class (73.0 g/d)

populations in the North West Province of South Africa. The pregnancy fat intake in the PND study was thus in accordance with these levels, considering that the women in the current study were of a low to middle class background.

The large increase in fat intake after delivery may be related to the higher energy requirements for breast-feeding (breast milk has high content of fat; *Gurr 2000*).

Bourne et al (*2002*) reviewed data from existing studies on the nutritional intake of the black population in South Africa and concluded, that within a 50-y period (1940-1990) there was a major shift occurring in the macronutrient intake in the urban Black South African population. Fat and animal protein intakes increased, while carbohydrate consumption and plant protein substantially decreased. Fat consumption increased from 16.4% to 26.2%, and carbohydrate intake decreased from 69.3% to 61.7% expressed as % of total energy (%E), producing a relative increase of 59.7% for fat and relative decrease of 10.9% for carbohydrate over the 50-y period. Women in the PND study seem to follow this same trend when considering the maternal V1 intake, fat intake (35.3 %E) would represent a relative increase of 34.7% between years 1990-2005 whilst the carbohydrate intake of 51.9%E would correspond to the relative decrease of 15.9% over the 15-y period.

4.3.2 Comparison with the RDA.

During the pregnancy period, women in the current study had lesser crude protein and higher carbohydrate intake than was the RDA. Expressing the macronutrients as a %E, the protein intake was on the low side of the recommended range, whilst total CHO was higher and the fat intake was equal or exceeded this range. Intake of crude protein after

delivery was low for the lactating women, but sufficiently high for the non-pregnant women. Total carbohydrate intake exceeded both ranges. The relationship between the intake of macronutrients expressed as a %E and RDA was similar as during the gestational period.

In conclusion, based on the comparison with the RDA, it was the protein intake adjusted or unadjusted for energy, which appeared to be low and possibly insufficient for the requirements of pregnancy and lactation in the current study group. Although the fat intake appeared to be sufficient, this was largely due to the high consumption of **SAFA**. This reflects an unbalanced diet composition, amplified by the low energy intakes, and supports the above theory for the possible mechanism explaining the lower weight and length z-scores.

4.3.3 Comparison with other studies.

The energy intake in South African women was much lower than in the Southampton study or in comparison with the American diet. Also, total fat intake during pregnancy was lower than in the Southampton or American populations and corresponded with the value found in the MacIntyre study for middle class women in urban areas of South Africa (see above). Total carbohydrate during all visits was below the levels of the Southampton and American data. The American population consumed more fibre than did the pregnant women in the current study. However, they also consumed more cholesterol.

When comparing protein, fat and carbohydrate adjusted for energy intake, the Southampton study and American data were similar, whilst the protein intake of South African women was lower. The energy-adjusted protein consumption in women in the PND study sits at the lower limit of the acceptable range of DRIs, below the levels found in the Southampton and

American diets. Energy-adjusted amounts of fat and carbohydrate are comparable or slightly higher than in the American population.

In comparison with the Pune Maternal Nutrition Study, the pregnancy intake of energy was very similar, although the protein intake (crude or energy adjusted) was higher and the fat intake (crude or energy adjusted) was twice as high in African women in the current study. However, in the Indian women the intake of carbohydrate was the major source of the total energy intake, and was found the highest among all the compared studies.

The Pune women were shorter and lighter than the women in the current study, even though the energy intakes of both study groups (PND and Pune) were similar. Indian rural women are involved in hard physical labour, associated with high energy expenditures (*NNMB 1991; http://www.unsystem.org/scn/archives/india/ch07.htm*). In the PND Study the energy expenditure has not been evaluated. However, low levels of physical activity in South African urban women have been reported by other authors (*Kruger et al 2003*) and it is possible that this may explain differences in anthropometry between the Pune and the PND women. Also low energy reporting by study participants cannot be excluded, although the 24-hour recall was used as a crosscheck in the study.

In conclusion, the study participants had a lower intake of energy, especially during the pregnancy period, when compared to populations in the developed world. This could have been due to a generally lower physical activity in the urban South African women and/or due to the low to middle social class of the current study participants. The energy intake was also positively influenced by the parental level of education and by their

employment status (see Chapter 3.11). Low energy reporting by study participants also cannot be excluded, although the 24-hour recall was used as a crosscheck in the study to minimize under- or over-reporting.

The total protein intake was low at all visits in comparison with the national average for non-pregnant women or with the Southampton study or for the usual American diet. The energy-adjusted intake of fat and carbohydrate were comparable with the Southampton and American data. This study population was therefore more comparable to that of Pune than to American or Southampton populations. However, despite similar total energy intakes compared to the Pune Study, the total protein and fat intakes were higher in the African mothers, which may explain their relatively higher weights and larger birthweights of their babies.

4.3.4 LOW ENERGY REPORTING

The actual level of the energy expenditure and the basal metabolic rate were not measured in the current study. Although the age- and sex- specific tables for energy expenditure are available (*Black 2000*), they are not optimal for an assessment of pregnant women, as they do not account for the energy supplied for the growth of the fetus, placenta, uterus, adipose tissue and breasts (*Kramer 1987, McNeill 2000*).

However, underreporting of calorie intake in the South African Black and Indian women was evaluated by Waisberg (*2009a,b*). The assessment was based on the calculation of the ratio for the reported energy intake vs. the basic metabolic rate (assessed by means of

the electrical impedance measurement) with the cut-off level of ≤ 1.14 for the low energy reporters. The study also utilized the same food frequency questionnaire as the current study. According to this evaluation, there were 10% among lean, 0% among overweight and 45% among obese African women who underreported energy intakes.

4.4. SELECTION OF THE METHOD FOR DIETARY INTAKE ASSESSMENT IN THE CURRENT STUDY

At present there is no dietary assessment method that could accurately measure the individual's true nutrient intake. Any method evaluating a dietary intake has its benefits and limitations (*Schaefer et al. 2000*) and has to be considered as an estimate (*MacIntyre 1998; MacKeown 1999; Sempos et al. 1999*).

A FFQ administered interview was selected for the present study as the most suitable way for the dietary intake assessment of the study volunteers. Selection of the method depends on the study design, the target population and aims of the study. For example, the diet records should not be used in populations where literacy levels are low, unless a trained observer is present. Although this method allows quite accurate dietary intake, it is labour-intensive and many subjects misreport, especially over longer periods of recording, resign or refuse to participate (*MacKeown 1999, Nelson 2000, Schaefer et al. 2000, Erkkola et al. 2001*).

Food frequency questionnaires are not designed to collect detailed data. The intakes of certain food categories or nutrients may be underestimated or overestimated and they depend on the respondent's memory *(Sempos et al. 1999; Schaefer et al. 2000; MacIntyre 2001a,b; Molag et al. 2007*). Therefore they are considered as a less accurate measure of the absolute levels of nutrient intakes than recalls or diet records (*MacKeown 1999; Schaefer et al. 2000*). However, the dietary recalls and diet records, if applied over a short period of time, do not accurately represent the habitual dietary intake as they may miss variations in the intake of less commonly eaten foods (*Willet et al.1986; Basch et al 1994; Nelson 2000*). Therefore 3-7 day long weighed-food record or collection of several dietary recalls is recommended (*Sempos et al. 1999; Schaefer et al. 2000; Bloemberg et al. 1989*). Average daily intake is then based on a combination of these records (*Bloemberg et al. 1989).* This would increase the precision of estimate, but also the costs and inconvenience of repeated interviews (*Nelson 2000, MacIntyre2001a,b*).

Epidemiological studies often use FFQs for an assessment of an individual's nutrient intake (*MacKeown 1999, Sempos et al. 1999*), because the FFQs are generally easier to administer, quicker to complete, cheaper than diet records or 24h dietary recall, and require minimal coding (*Margetts et al. 1989, Sempos et al. 1999*). This allows their application to a larger number of subjects, which in turn increases statistical power of the study. Due to the longer time period they cover, for the assessment of usual dietary intake they are considered to be better by some authors than the 24-h dietary recalls (*Margetts et al. 1989*). The semi-quantitative questionnaires are also a useful tool for ranking individual intakes.

The FFQ employed in this study was easy to complete and its length of administration complied with the available time during the study visit. The food list composition was based on many years of experience in dietary assessment (*MacKeown 2007*) and included food items commonly consumed among the African population group. As a part of this questionnaire, a 24-h dietary recall section was inserted as a cross-check (see Chapter 2.4.2.1).

For a comparison, the dietary intake assessment methods used in the studies of Pune, Southampton and South Africa are summarised below.

The assessment of dietary intakes of energy and macronutrients in the Pune Maternal Nutrition Study was carried out twice during the pregnancy period (weeks 18 and 28) and it was based on a modified 24-h dietary recall. The authors also assessed the dietary intakes in the form of food groups and this evaluation was performed by means of a FFQ (*Rao et al 2001*).

In the Southampton study, a FFQ was administered to mothers twice during gestation (weeks 15.3 and 32.7) for an assessment of the average daily nutrient intake. The study used a dietary history crosscheck in the form of a 4-day record during early pregnancy (*Godfrey et al 1996*).

The data on South African women (*Steyn et al. 2006*) were part of a study comparing nutrient intake between South Africa and Kenya and they are based on a

secondary analysis of previous dietary studies and of the National Food Consumption Survey and the South African Demographic and Health Study. Dietary intake was assessed by means of a 24-h dietary recall questionnaire.

All these studies employed trained field workers (nurses) who visited participating mothers at home to take anthropometrical measurements, to evaluate the socio-economic status and complete the dietary intake assessment.

American data are based on triplicate samples of a 3-day menu cycle, which was evaluated by a chemical analysis.

4.5 DISCUSSION OF THE ANALYTICAL PART OF THE DATA ANALYSIS.

4.5.1 Discussion of the maternal nutrient intake effects.

Tables 3.10 A-C and Appendices D,E,F and H show the associations between maternal dietary intake and the fetal – birth – infant postnatal parameters. Table 3.17 includes the relationship between fetal weight and biometry and the gestational age.

4.5.1.1 Maternal nutrients and fetal biometry at US1 and US2.

The gestational age adjusted models "explained" over 90% of variation in the response variable and the effect of maternal dietary intake in these models has only a weak contribution. This shows that the effect of maternal nutrient intake on fetal biometry is not so apparent as it becomes in the postnatal period. However, there were some significant relationships and these will be discussed below.

Amino acids are the essential building material for fetal growth. The timing of the first pregnancy visit fell into the period of major brain development, which requires protein and polyunsaturated fatty acids (*Westermarck 2000*). This may explain the positive correlation observed between the fetal biparietal diameter of the first visit **(BPD1)** and maternal total protein intake analysed for the same visit (**TOT PROT1;** see Table 3.10 A).

The maternal plant protein intake near the early pregnancy visit showed a supportive effect on the concurrent size of fetal head and abdomen, whilst the intake of animal protein showed a negative relatioship. Similarly, the early intake of **SAFA** revealed a negative association with these fetal biometrical parameters. On the other hand, the early dietary consumption of **PUFA** showed supportive effects on fetal growth later in gestation. The association with fetal head and length may involve other factors such as effects of micronutrients (e.g. calcium; rapid bone mineralisation occurs during the third trimester; *Lucas 1991*), or growth factors and other hormones, which stimulate the bone growth or mediate the transfer and availability of nutrients (Chapter 4.5.1.3). However, the strong positive relationships of **PUFA1** maternal intake with **FW2, AC2, HC2 and FL2** measurements seemed to be in agreement with the timing of the early pregnancy maternal adaptations. The **PUFA1**, next to its utilisations in fetus for the brain, retina and bone development, is contributing towards the building of maternal pregnancy fat stores, which in turn serve as the major energy source for the fetus especially during the last pregnancy trimester (see discussion Chapter 4.5.1.3). All these parameters were taken during the same ultrasound scan, therefore adjustment for the gestational age may bias these associations by "overcorrecting" in the regression model, resulting in loss of significance.

The strong association of the HC2 with maternal **TOT PROT** intake at **V1** was negative. However, the results do not indicate, which source of protein contributed towards this association the most. From the descriptive part of the study it is known that maternal intake of animal protein was the primary source of protein during pregnancy (Chapter 4.3.1). Therefore, as both proteins are expressed as % energy, it may be possible, that the negative direction reflects decreasing supplies of maternal **PL PROT** as fetal growth

accelerates in late pregnancy. However, it also may be possible that these negative effects of maternal dietary intake on skull growth represent some kind of a negative feedback system that will ensure that excessive skull growth does not occur at high levels of energy intake. However, we cannot currently prove this hypothesis. A rather complex relationship between maternal protein intake and the size of a baby was addressed by other authors before (*Duggleby et al 2002*), involving protein conservation and metabolic adaptation of the mother. Also the partitioning of nutrients between the mother, placenta and fetus needs to be considered.

The maternal total energy intake at V2 (**TOT ENERGY2**) positively correlated with the fetal abdominal circumference of the same visit (**AC2**; Table 3.12 A), and this may be related to **TOT PROT2** (possibly derived from **AN PROT1**) as this variable also correlated with **AC2** (Table 3.10 A). The fetal abdomen reflects the size of fetal liver (*Godfrey et al 1996;* see Chapter 2.5.3.2), especially during the third trimester, when the liver undergoes rapid growth (*Reece et al. 1993*).

Protein is important in liver development; the content of the liver protein reflects the quantity of dietary protein (*Kosterlitz 1947*). Maternal protein restriction during gestation led to significant growth retardation of neonatal rats *(Desai et al 1996*), involving decreased body weight and weight of liver and brain and changes in liver and serum IGF-I levels. Therefore a positive association between the **TOT PROT2** intake and fetal **AC** at V2 may be expected (Table 3.10 A). Similarly it would explain the correlation between the **TOT ENERGY1** and **AC1** during the second trimester (Table 3.12 C), however, the positive association of **AC1** with maternal protein intake just "missed" significance (at p=0.050; see Appendix D) at this visit. High maternal protein density intakes in some studies, however,

were reported to restrict fetal growth and resulted in low **BW** (*Sloan 2001; Andreasyan et al. 2007;* see Chapter 1.4.2.3.3). On the other hand, animals fed a low protein diet during pregnancy are known to have growth-retarded offspring with a reduced β-cell mass. Earlier in pregnancy another energy source related to **AC** may be derived from carbohydrate (data of the current study shows an association with **added sugar**). Parretti et al (*2001*) showed a significant positive correlation between maternal 60-minute glucose levels of the OGTT (at 28 gestational weeks) with fetal abdominal circumference. Similar correlations were reported also for 32 and 36 gestational weeks for maternal glucose levels at different times of the OGTT, whilst a negative association was found for the **HC/AC** ratio with the maternal glucose levels. During the third trimester maternal **PUFA1** may be the further contributor, as the accelerated fat accumulation by fetus during the third trimester would reflect in the **AC** measurement.

The maternal energy intake during the third trimester (**TOT ENERGY2**) correlated positively with the concurrent fetal weight (**EFW2**) and femur length (**FL2**). The positive correlations between fetal size and weight during the V2 and maternal intake of total energy was expected, as during the third trimester the fetus increases its size quite rapidly and requires therefore increased energy supplies (at least 800 extra kJ/d for mother; *McNeill 2000*). The contribution of **PUFA (**also **MUFA**) towards the **TOT ENERGY2** to support the bone growth of femur would also be expected (see Chapter 4.5.1.3).

4.5.1.2 Maternal nutrients and birth weight.

While many studies have reported no association between BW and maternal macronutrient intake, in the current study maternal protein intake (**TOT PROT2**) at the end of pregnancy showed a positive correlation with baby's **BW**.

A prospective study in Adelaide in Australia (*Moore et al 2004*) including over 500 Caucasian women 18-41 y old, found also a significant positive correlation between the 16w-gestation maternal protein intake and the baby's BW and placental weight. The protein intake in the Adelaide women was much higher than in the African women of the current study (89g/d = 16.6%E at 16 and 86g/d=16.2%E at 30-34 weeks of gestation).

The Aberdeen study in Scotland (*Campbell et al 1996*), which carried out a retrospective analysis of dietary and birth records of 253 mothers from 1948-1954, found, in turn, an inverse association between the maternal protein intake (as %E) at 30 weeks of gestation and the birthweight. Although the protein contribution to the total energy intake in the African population was slightly higher in comparison with the protein intake of mothers in Aberdeen (13% vs 12.2% in Aberdeen), the amount of crude protein intake in Johannesburg mothers was much lower (53.8 g vs. 73 g).

High protein supplementary intakes (≥85g/d) lead to a decreased birth weight by 71g in the New-York trial (*Sloan et al 2001*, See Chapter 1.4.2). This was in comparison with a mean intake of protein 50-84.9g/d.

The study from the rural area of Haryana in India (*Rao et al. 2007*) detected an association between a very low maternal daily caloric intake (below 6276 kJ) and low protein intake (below 40g) during the third trimester with the baby's low birth weight.

The Pune study in India (see Chapter 1.1.2) did not find any association between protein intake or energy intake and BW, however the authors found a strong association for micronutrients derived from green leafy vegetables and fruits and the fat derived from milk (*Rao et al 2001)*. The amount of protein present in consumed milk was associated with increased birth size and placental weight in the study from Denmark (*Olsen et al 2007;* see Chapter 1.4.2.3.3).

Therefore, the association between maternal protein intake and baby's BW may be an inverted U-shape, where not only low but also high protein intakes may impact negatively on baby's birth weight. However, since the nutrient intakes with respect to the total energy intake are intrrelated, it is likely that the low/high protein intake effect will be related to the ratio of other nutrient intakes, the total energy intake or to the quality of the protein itself. Unfortunately, the current study results did not show any further significant associations with BW and the effect of dietary intake with different ratios of macronutrinets was not evaluated.

4.5.1.3 Maternal nutrient intake and postnatal anthropometry.

The maternal total energy intake during pregnancy in the current study did not show any associations with the postnatal infant anthropometry, however the concurrent energy intake (**TOT ENERGY3**) was positively related to infant's weight (**WEIG2**) at the last postnatal visit. The majority of mothers breastfed their infants for a few months after delivery and many of them were continuing with breastfeeding in addition to using infant feeds near V3. Many studies analysed the relationship of postnatal nutrition to infant size (*Lucas 1991*), and found similar associations.

As stated already in the Results Chapter (3.10), in comparison with the fetal biometrical parameters, there were numerous associations between maternal macronutrient intakes and the infant neonatal length and both postnatal **BMI** measurements. As length is inversely related to **BMI**, these associations with **BMI** were in the reverse direction for length.

The total protein dietary intake at V1 (**TOT PROT1**) showed an association with baby's postnatal weight (**WEIG2**) and **BMI 1 and 2** (adjusted or unadjusted for confounders), which is most probably related to the animal protein intake (**AN PROT1**) also at V1. This would emphasise the importance of maternal protein intake in early gestation influencing the infant's **BMI** at 6 months after birth. Similarly, there was an effect of maternal protein intake later in pregnancy on baby's neonatal and later postnatal anthropometry. Also, this supported the association seen already between the fetal **AC2** and **TOT PROT2** intake (Chapter 4.5.1.1).

It was noted that the intakes of animal and plant proteins produced different effects on infant anthropometry. Whilst the animal protein contributed towards the infant's **BMI** (and the fetal **AC2** adjusted for age druing V2), the plant protein (or minerals associated with its intake) seemed to be supportive of linear growth. Therefore the association of **plant protein** with **linear growth** was studied using the available literature.

Duggleby et al (*2002*) studied protein metabolism in pregnant women and found a strong association between maternal protein synthesis and fetal growth during the second trimester. Thus, the mothers who had larger protein turnover also had babies who were longer at birth. However, this relationship appeared to be independent of the mother's height and protein dietary intake.

Muaku et al (*1996)* showed in pregnant, dietary-protein restricted Wistar rats that reduction in serum IGF-I may contribute to the reduced somatic and organ growth associated with low protein diet. Also, feeding a high protein diet to malnourished children due to diarrheal disease in Bangladesh (*Kabir et al. 1998*) led to an increase in their linear growth during the 6-month follow-up period, suggesting a key role for protein in the children's growth. An increased dietary protein intake in adolescents was associated with bone growth independently of calcium intake and mediated by IGF-1 (*Bonjour et al 2001*).

Inadequate protein intake seems to be associated with delayed skeletal growth and reduced bone mass, whilst sufficient intake of protein has to be accompanied with a sufficient intake of calcium (*Vatanparast et al. 2007*) to produce a beneficial effect on bone growth.

Different types of protein show different effects. The animal protein is high in sulphur-containing amino-acids, generating an acidic cellular environment. The plant protein (fruits and vegetables) is less acidic, or more basic. If the renal buffering capacity is overwhelmed, the bone reacts by releasing the carbonate, citrate and sodium and calcium to neutralize this acid (*Barzel et al. 1998*). However, the plant proteins found in soy, corn, wheat and rice (purified plant proteins) have similar sulphur content as some animal proteins in eggs, milk and muscle from meat, poultry and fish and increase urinary calcium *(Massey 2003*). On the other hand, the calcium in milk, potassium and magnesium in fruits and vegetables (e.g. legumes, grains) can decrease the urinary calcium losses (*Massey 2003; Vatanparast et al. 2007).* (In addition, there are also effects of other nutrients, such as vitamin D or caffeine on the bone health.)

Therefore, it may be possible that the plant protein intake recorded for the participants in the current study could contribute to the linear growth of the offspring measured during the neonatal period. The negative association of the animal protein intake with neonatal length could indicate either the increased intake of this protein or insufficient intake of the plant protein to stimulate the linear growth. The ratio between these two protein intakes may then be important.

Thus, during the early antenatal period, maternal protein intake (possibly the animal protein) may support general growth, i.e. increasing fetal size (as a “fuel to the growth process”; *Lucas 1991*) stimulating various growth factors. The plant protein, in turn, might

stimulate the growth in length, which association was then apparent in the postnatal infant parameters.

The maternal dietary intake of saturated fat during both pregnancy visits (**SAFA1** and **SAFA2**) was positively associated with the later postnatal infant **BMI**. According to Mensink et al (*2003),* dietary fats produce different effects on the blood lipid levels. The SAFA acts in the opposite direction to the PUFA and MUFA, which both lower the blood levels of triglycerides and LDL cholesterol. The SAFA-mediated increase in levels of maternal lipids during pregnancy may thus contribute to the programming of increased BMI of the child.

The demand for the long chain PUFA (LC-PUFA) by the fetus is well documented, as it is required for brain and retina development and for their future function (*Burdge et al.2006*). As maternal intakes of **PUFA and MUFA** showed associations with the postnatal anthropometry, the mechanism of their involvement in the **bone growth** was further investigated.

There is limited data on the effects of PUFA on human and especially infant skeletal metabolism, however, the involvement of fatty acids in bone modeling and remodeling was demonstrated in a number of animal studies. Some of these studies are discussed below.

The effect of PUFA on bone metabolism is produced via changes in prostaglanding synthesis (*Watkins et al. 2000a, 2001*). Arachidonic acid (AA, 20:4 n-6 – a long chain PUFA) is a precursor of prostaglandin (2-eicosanoids). Prostaglandin E2 (PGE2) produced

in bone tissue (*Sardesai 1992*) can stimulate bone formation as well as bone resorption. This action depends on its concentrations. Whilst a low concentration of PGE2 stimulates bone formation, a high concentration decreases this effect, and can further lead to bone resorption (*Watkins 2001, Mollard et al 2005*).

The stimulatory effect of PGE2 is mediated via increased osteoblastic production of the insulin-like growth factors and their binding proteins (*McCarthy et al 1991*; *Baylink et al 1993)*.

Studies with chickens and rats showed that increasing n-3 PUFA by dietary intake decreased the n-6 to n-3 ratio and reduced PGE2 and increased the serum levels of alkaline phosphatase (*Li et al 2003*; *Watkins et al 2003 and 2006*), leading to a preservation of femur bone.

In addition, studies on rats and men (*Kruger et al 2005; Watkins et al 2006; Högström et al 2007; Li et al 2010*) confirmed the beneficial effect of the docosahexaenoic acid (DHA; 22:6n-3 PUFA) and also of the conjugated linoleic acid (CLA; butter and milk fat; *Li et al 1999; Watkins et al 2000b)* on bone metabolism. Downregulation of PGE2 stimulated IGF/IGF-BP mediated bone formation.

Another mechanism by which n-3 PUFAs regulate birth weight involves their effect on gestational length. Increased intakes of n-3 PUFA decrease the production of uterine

prostaglandins, which initiate uterine contractions at the beginning of labour (*Olsen et al 1986*).

There is not much information on the effects of dietary **MUFA** on bone metabolism. Authors of a study on healthy men and women in Athens, Greece, reported a direct relationship between dietary intake of MUFA (from olive oil) and bone mineral density *(Trichopoulou et al.1997).* However, the association with the infant postnatal length in the current study was negative.

Dietary intake of **SAFA** was found to be inversely associated with bone density in animals and men (*Wohl et al 1998; Corwin et al 2005*).

Therefore, the effect of **PUFA1** on neonatal length most likely represents a real association. Unfortunately, the ratio between n-3 and n-6 PUFA was not investigated in this study. The positive relationship of **PUFA1** with neonatal length may indicate sufficient supply of n-3 PUFA during early pregnancy stages for the establishment of fetal long bones (gest. month 3-5; See Chapter 1.3.2.1).

The weaker negative association of **PUFA2** with neonatal length is more difficult to explain, as it is not supported by data in the literature. The associtions is weak and it is possible that the result occurred by chance.

As the effects of nutrients are not isolated, it is possible that, while the maternal fat intake (together with maternal fat stores) supports the accumulation of the fetal fat deposits,

the **PL PROT2** intake may support bone metabolism. However, further research is required to confirm this.

In combination this data shows differential effects of macronutrients on postnatal growth that may act during different stages of pregnancy.

4.5.1.4 Maternal nutrients and postnatal metabolic parameters

In this section the postnatal metabolic parameters associated with glucose metabolism (glucose and markers of the infant β-cell function **INS, PI, HOMA-IR**), obesity (**LEPT**) or nutrient status (**IGF-BP3**) were evaluated.

As described in Chapter 3.9, there were many associations between maternal dietary intake and markers of infant postnatal β-cell function. These mainly involved the neonatal **INS** and **HOMA-IR** levels. There were similar associations for the neonatal and later postnatal **INS** and **HOMA-IR** concentrations, therefore a similar mechanism is expected for these relationships. Also, both sets of metabolic variables showed similar associations with the maternal protein and fat intakes as were found for the postnatal infant **BMI** (i.e. positive for the protein and negative for the maternal fat intakes). No effects for **SAFA** consumption during any pregnancy visit were found.

Maternal total energy intake in the second trimester (**TOT ENERGY1**) did not show any significant correlations with postnatal glucose, or other examined variables. For the third trimester **TOT ENERGY2** intake, there were significant relationships found with

neonatal glucose (**GLC F1;** inverse correlation) and fasting insulin (**INS F1;** direct correlation) and with the 6-months postnatal fasting glucose (**GLC F2**; also inverse correlation). These outcomes are the result of the combined effects of protein, fat and carbohydrate energy sources.

An interesting observation was the concurrent negative association between maternal total energy intake at V3 (**TOT ENERGY3**) and infant insulin (**INS F2**) and **HOMA-IR2** and a positive correlation with infant leptin (**LEPT2)**. Leptin involvement could be related to infant weight, as serum/plasma leptin is known to correlate with body mass (*Helland et al 1998*) and also the **TOT ENERGY3** showed a significant positive relationship with the concurrent infant weight.

Similarly this applied to the correlation between **LEPT2** and **PUFA2**. The study of Ogueh et al (*1999*) found that human fetal blood levels of leptin increased with gestational age in agreeement with fetal adipose tissue development and accretion of fat. The authors also suggested that leptin had a role in fetal bone development, as it was assocaited with decreses in bone resorption, with a resulting increase in bone mass.

For the relatively increased insulin secretion (**INS F 1 and 2**) and elevated **HOMA-IR1** index it seems that the animal protein intake is responsible, while the plant protein intake (**PL PROT2**) has rather a protective role showing negative relationships with **INS F1 and 2** and **HOMA IR 1 and 2**. The **PL PROT1** intake also showed a negative relationship with infant proinsulin (**PI F2**) levels.

The effect of the plant protein may be mediated via its effect on the growth in length and decreased BMI. In addition, there may be a further protective effect on infant β-cell function (see negative correlations with **INS F1 and 2** and **HOMA-IR1**) of maternal **PUFA1** intake, which also shows a stimulatory effect on infant length. The animal protein intake is reflected in the total protein intake (**TOT PROT1**) where both have positive effects on **INS F1** and **HOMA-IR1.** Protective effect of PUFA was shown in the study of Salmerón et al (*2001*) in decreasing the risk of Type 2 diabetes in women.

A study in India found that a partial substitution of dietary linoleic acid (18:2 n-6 **PUFA**) with alpha- linolenic acid (18:3 n-3 PUFA) was associated with decreased blood lipid levels and improved peripheral **insulin sensitivity** in sucrose fed rats (*Ghafoorunissa et al. 2005*). An epidemiologic study of over 3000 people in Japan 35 –66 years old, assessed nutrient intake based on a diet history questionnaire and observed that increased intakes of alpha-linolenic acid lowered the prevalence of insulin resistance among normal weight individuals (*Muramatsu et al. 2010*).

Low carbohydrate intake (**TOT CHO2**) was related to elevated levels of infant **INS F1 and 2** and **HOMA-IR1 and 2** possibly due to their relationship with the infant **BMI2**.

The reasons for the positive correlations of maternal dietary intake of **MUFA2** and **PUFA2** (third trimester) and negative correlations of the total carbohydrate intake (**TOT CHO2**) with infant **INS F1 and 2** and **HOMA-IR1 and 2** are unclear. As explained in Chapter 4.5.1.3, the maternal intake of **PUFA2** may have improved fetal weight/fat mass during the accelerated growth phase of the third pregnancy trimester, which may have effects on infant insulin levels.

To further evaluate the association of the maternal dietary intake with neonatal **INS F1,** an analysis employing crude intakes of macronutrients was carried out.
All these associations also revealed significant outcomes, except for the **TOT FAT1** and **PUFA1** intakes for early pregnancy and the **PL PROT2** and **TOT CHO2** for late pregnancy.

The levels of the baby's **IGF-BP3** correlated with maternal **AN PROT** and **MUFA2** intakes, always showing a positive relationship. Therefore, maternal protein, specifically animal protein (**AN PROT 1 and 2**) seemed to be involved in the regulation of the infant postnatal concentrations of **IGF-BP3**. IGF BP3 concentrations are known to decrease, whilst IGF BP1 concentrations increase with maternal food restriction (*Gluckman 1995*). This was seen in studies with experimental animals, where reduced levels of IGF-BP3 in fetal sheep correlated with maternal preconceptional malnutrition (*Gallaher et al. 1998*). Qiu et al (*2004*) investigated early nutrition intervention in IUGR neonatal rat pups and discovered that pups on a high protein diet presented with the fastest catch-up growth. They had highest IGF-1 and IGF BP3 serum levels, while the low protein diet intervention group pups were small and IGF-1 and IGF-BP3 were significantly lower. IGF-BP3, due to its binding with IGFs, serves as a pool for the growth factors in the circulation (*Murphy 2006*). Therefore significantly increased levels of **IGF BP3** between the neonatal and the second postnatal visit in the current study may reflect a good nutritional status of the child.

The nutrient intake associations with infant postnatal metabolic parameters were adjusted for infant **BMI**, to exclude possible interference of **BMI** in their relationship. In the case of the protein results, the correction for **BMI** in the majority of outcomes

weakened the effect of protein. However, these associations still remained significant or near significance. Therefore, there is some small involvement of infant **BMI** in the outcomes. However, the direct influence of protein still remains (Appendix H).

After adjusting for fat intake and infant **BMI**, a number of significant outcomes between the maternal fat intake and the fetal metabolic parameters, weakened. The correlation between **PUFA2** and **LEPT2** however lost significance completely, therefore this association is driven mainly by infant **BMI** and the contribution of **PUFA2** is minimal (Table H2).

A few weakened correlations were seen after adjustment for **BMI** for **TOT CHO**, **added sugar** and **TOT ENERGY** intakes (Table H3), however most of the relationships maintained significance indicating that maternal total carbohydrate and total energy intakes do have direct effects on neonatal and infant metabolic parameters.

4.5.2 Discussion on maternal vs. fetal - infant metabolic parameters.

Maternal hormones in fetal growth, birth parameters and infant growth with respect to the risk assessment for the development of future Type 2 diabetes.

See Chapter 3.10 and Tables 3.12 A-C. The separate regression models are presented in Appendix J. Each of these models includes only the covariates that were significantly

related to the fetal-infant parameter of interest. All other covariates, which were included in these regression models, are presented in Appendix I.

4.5.2.1. Fetal and postnatal growth rates

GRRT1: Fetal growth rate between the two pregnancy visits (**V1 and V2**) was positively related to the maternal weight (**MW1**) and the placental lactogen (**HPL1 and 2**) at these visits (see Chapter 3.10). The concentration of maternal HPL, secreted by the placenta, reflects the placental function *(Gluckman 1995; Picciano 2003)*. A well functioning placenta will stimulate fetal growth, as HPL directly stimulates insulin and IGF-1 secretion and possibly regulates the supply of glucose and amino acids to the fetus (*Brelje et al. 1993; Handwerger et al. 2000; Picciano 2003*). The above finding supports the role of HPL in fetal growth. A similar outcome was reported by *Sørensen et al (2000*), who found a significant positive correlation between the HPL slope and the fetal growth rate.

The maternal size influences the neonatal size, partially due to the genetic component and partially because the maternal size reflects her own nutrient status (see Chapter 1.4). Thus, mothers of a larger weight (BMI) will tend to have babies of a large BW (univariate correlation in this study: p=0.002, R=0.306, N=104) and the BW is an outcome of the fetal growth (*Forsén et al. 1997; Romundstad et al. 2007*). Low pregnancy weight gain was associated with underweight mothers, leading to growth retarded fetus (*Strauss et al. 1999*; *Schieve et al. 2000*). There was a direct correlation between maternal weight and the placental volume and then between the placental volume and the fetal growth rate shown in the study of *Thame et al.* (*2004*). Obese mothers, however, also tend

to have smaller babies, therefore the relationship between maternal weight and birth weight is an inverted U-shape with a consequence of impaired glucose tolerance in the offspring of the thin and obese mothers (*McMillen et al. 2005*)

The regression analysis of the V2 parameters showed also a significant positive contribution of the maternal systolic blood pressure of V2 (**BP SYS2**) to the fetal **GRRT1.** It should be reminded, that hypertensive patients were not enrolled into the study. Results of various studies showed, that there was an increased incidence of preterm birth, low birth weight and also fetal death with low systolic and diastolic blood pressures (*Zhang et al.2001b*). This was possibly due to a low placental blood flow (*Jones et al. 1983*), resulting in a low nutrient supply to the fetus and in low oxygenation. However, high blood pressure present in mother before pregnancy was also linked to the small size of the newborn (*Romundstad et al. 2007*). Therefore the relation between the maternal blood pressure and fetal **GRRT** may also be an inverted U-shape.

Finally, the regression model adjusted for confounders (gest. age, fetal sex, maternal height and maternal weight), showed a significant influence of maternal leptin (**LEPT2**; removing significance of maternal weight). The major reason for this is likely to be the known positive effect of leptin on fetal growth (*Harigaya et al.1997; Helland et al. 1998*).

GRRT2: The fetal growth rate between V2 and delivery was significantly positively related to **maternal height** and maternal **FT4 at V1** or maternal fasting **PI at V2**. Maternal height adjusted for the birth age, was significantly associated with baby's

BW ($p=0.013$, $\beta=0.219$ for height and $p<0.001$, $\beta=0.423$ for birth age, $AR^2= 0.220$; see Appendix H.1.2). As with the maternal weight, there is an effect of maternal height on baby's size (*Kramer 2003*). Thus, taller mothers will have bigger babies than shorter mothers delivered at the same gestational age, which reflects the larger placental size of taller mothers (see maternal constraint, Chapter 1.4.2.2.1).

T4 is an anabolic hormone and its active form is free thyroxine (FT4). **FT4** concentration peaks during the first trimester and from the second trimester declines slowly until delivery. Maternal FT4 in early pregnancy crosses the placental membrane and from about week 20 the fetal thyroid gland is able to produce its own T4, however for the production of its own thyroid hormones, the fetus stays dependent on the maternal iodine supply until delivery. FT4 regulates the metabolic rate of the mother, and therefore it is involved in the efficiency of placental transport and substrate availability to the fetus. Downregulated maternal thyroid hormones in early pregnancy would delay nutrient transport and may also contribute to an inadequate development of the fetal thyroid gland. Fetal thyroxine regulates uptake of oxygen by fetal tissues. Hypothyroidic sheep fetuses have decreased oxygen uptake, which slows down the fetal oxidative metabolic rates, involving glucose cellular uptake and the fetal levels of glucose and insulin. This situation is reversed by an external thyroxine supply. Hypothyroidic fetuses are growth retarded (*Fowden et al. 1995*). Thus, maternal thyroid hormones earlier in pregnancy may positively affect the fetal growth rate during the third trimester. Further, FT3, generated from FT4, is known to stimulate bone formation (activates GH and IGF-1 signalling; *O'Shea et al. 2005*).

Proinsulin (**PI2**) as a precursor of insulin, an anabolic hormone, should stimulate the fetal growth rate. PI (or INS) of V2 will be more effective, as their levels increase during the course of pregnancy. Insulin increases the fetal umbilical glucose uptake, glucose utilisation and oxidation (*Fowden et al. 1988*). Increased **fetal insulin** levels lead to fetal macrosomia (such as in maternal diabetes – fetal insulin levels rise due to high maternal glucose levels), while lack of insulin impairs fetal growth (body weight and length of limbs seen in neonatal sheep after fetal pancreatectomy; *Fowden 1992*). SGA neonates had low levels of venous cord blood insulin levels (*Murphy 2006*). However, neither maternal insulin levels nor glucose levels correlated with fetal growth rate before delivery and therefore it is also possible that PI may have some direct effects on placental glucose transport or some other placental function. A biological role for PI has been demonstrated by Kamoda et al (*2006),* who investigated serum levels of PI in obese children. The authors discovered that PI, as well as INS, can participate in the suppression of hepatic IGF BP-1 production. Although there was no correlation between maternal FT4 at V1 and PI at V2, an effect of thyroid hormones on β-cell function was shown in the study of Wu et al (*1998*). In this study, thyroxine therapy of hypothyroid patients elevated their previously suppressed levels of proinsulin and, in hyperthyroid patients, antithyroid drugs decreased the proinsulin levels.

GRRT D-Ba2: The postnatal infant growth rate assessed between delivery and the last postnatal visit Ba2 showed independent positive contributions of **fetal gender** and **INS RES2**. The boys in the current study showed higher postnatal growth rate; $p < 0.001$, $AR^2 = 0.210$, $F = 20.1$ by ANOVA (see Chapter 3.12; *Gilbert et al. 2007).* A gender difference in the fetal growth rate was not found in the current study. However, growth

charts constructed by various large studies for monitoring fetal growth, demonstrated sex differences in the second half of pregnancy with higher growth rate for male fetuses (*Maršál et al. 1996*; See Chapter 1.4.1).

The increase in the insulin resistance (**INS RES2**) allows for enhanced placental glucose transport and supply to the fetus, stimulating thus the fetal growth (*Dunne 1999*; *Jansson et al. 2001*). Elevated insulin levels stimulate the glucose entry into fetal tissues and the subsequent glucose utilization and oxidation (*Hay et al. 1986*). This would explain the positive association between maternal insulin resistance at V2 and baby's growth rate after birth, which is the continuation of the fetal growth before delivery.

4.5.2.2 Birth size

BW: In regression model, correcting for confounders, the strongest effect on **BW** was produced purely by these confounders. **BW** was associted with maternal weight (**MW 1 and 2**), **maternal height** and the **birth age** and the **BW** was significantly higher in boys. As the pre-selected variables that correlated with the **BW** (see Appendix I) included **INSF2** and **LEPT2**, the model had to be adjusted for maternal **weight/ BMI**. After adjustment, no influence of maternal pregnancy hormones was found.

BHC and BL: The associations obtained for **BL** and **BHC** were similar for both variables and included **MW**, **gestational age**, maternal leptin at V1 (**LEPT1**), which correlated positively and the **IGF BP1 at V1/V2**, which correlated negatively. Transplancental transfer of maternal leptin to the fetus increases during late pregnancy as reported by McMillen et al (*2006*) and cord leptin levels were found to be correlated with

birth weight, ponderal index, birth length and head circumference in the study of Ong et al. (*1999;* ALSPAC cohort of the Avon Longitudinal Study of Pregnancy and Childhood). Also, as reported earlier (Chapter 4.5.1.4), according to the study of Ogueh et al (*1999*) leptin had a positive role in fetal bone development, as it was associated with decreases in bone resorption, with a resulting increase in bone mass.

During pregnancy, the **IGF BP1** is secreted by the liver and maternal decidua and it is the main controlling factor for IGF-1 action (*Murphy 2006*). A negative correlation between the IGF BP1 with BW was reported by other studies (*Murphy 2006*), whilst the IGF-1 and IGF BP3 were correlated positively. Therefore, the indirect association of **IGF-BP1** with the neonatal size found in the current study would be expected, especially as the fetal parameters involve bone growth. Thus, elevated concentrations of maternal IGF-BP1 have been associated with IUGR fetuses in connection with delayed bone ossification (*Verhaeghe et al. 1993*; *Lagha et al. 2006*).

4.5.2.3 Postnatal infant anthropometry

BMI Ba1: The infant neonatal BMI Ba1 was, as for birth weight, determined by the baby's age (**Age Ba1**) and maternal pregnancy weight (**MW1 and 2**) and further negatively associated with the maternal proinsulin levels (**PIF1 and PI 30min at V1** and **PI F2 and PI 60min at V2**). Higher baby's **BMI** will reflect the mother's **BMI,** maternal nutrition and also may be connected to the increasing maternal pregnancy insulin levels. The negative correlation with proinsulin levels is not completely clear. Low proinsulin may

reflect increased conversion of **PI** to **INS** in the environment of high glucose levels. Glucose crosses to the fetus and drives fetal insulin secretion. However, no significant relationship was found for maternal insulin or glucose concentration of V1 with neonatal **BMI Ba1**.

BMI Ba2: The infant **BMI Ba2** at six months after delivery reflected more the fetal **gender**, with boys having greater **BMI** values. Although the maternal pregnancy weight did not show a significant association with **BMI Ba2**, maternal leptin at the earlier pregnancy visit (**LEPT1)**, which correlated with maternal weight (see above), had a strong positive relationship. **BA BMI2** was also positively related to maternal **INS RES2** levels. A similar mechanism as for the **GRRT D-Ba2** is expected, with insulin stimulating fetal growth. Both infant variables (**BaBMI2** and **GRRT D-Ba2**) are strongly correlated ($R = 0.680$, $p<0.001$), which is mediated via infant weight. A weak negative association of **BMI Ba2** was observed with maternal levels of **IGF BP1 (V2).** A negative association would be expected, as circulating levels of IGF BP1 have been shown to be inversely correlated with leptin in non-obese adults (*Söderberg et al 2001*) and also inversely correlated with insulin (*Hwang et al 2003*) and insulin resistance. Also, the study of Kamoda et al (*2006*) found that the best predictive parameters for IGF-BP1 in obese non-diabetic children were BMI and proinsulin and that proinsulin was involved in the suppression of hepatic IGF-BP1 production.

Further, a negative association of the baby's **BMI** with maternal glucose of V1 (**GLC F1**) was observed. The reason for this inverse correlation is unclear, as the fetal glucose reflects maternal glucose levels and stimulates fetal growth (see above). Therefore, similarly as with the proinsulin correlation, some other factors may be involved in this

relationship. High glucose concentrations in culture media with amino acids (in vitro experiments with isolated fetal rat islets of Langerhanse) in the study of Hogg et al. (*1993*) stimulated IGF-BPs release in a dose-dependant manner, while increased concentration of amino acids in the media stimulated release of growth factors, especially IGF-2. With increasing concentrations of both the amino acids and glucose, insulin secretion rose. The negative relationship of maternal glucose with baby's **BMI** may thus reflect the balance between the concentrations of the growth factors and their binding proteins, which will depend on the ratio of protein and maternal glucose concentrations.

4.5.2.4 Postnatal infant metabolic variables

GLC F1: Baby's neonatal fasting glucose (**GLC F Ba1**) was proportionally related to baby's age (**Age Ba1**) and weight (**W Ba1**) and mother's fasting glucose at V1 (**GLC F1**). In addition, maternal **HPL2** and **CORT2** showed a direct relationship, whilst the maternal **FT4 (V2)** concentrations were related negatively**.** All three hormones were associated independently, as they were all part of one regression model. The **HPL2** was significant only in the analysis unadjusted for confounders.

Maternal cortisol was measured only at the second pregnancy visit (V2) and the last postpartum study visit (V3) and its levels at V2 were more than double the levels after delivery (Table 3.4).

In animal studies, external glucocorticoids administered in late pregnancy are known to reduce BW, resulting in hypertension and elevated glucose levels in adulthood (*Nyirenda et al. 1998; Ozanne2001*). Glucocorticoids can cross the placenta and their

excessive amount may override the control mechanism of the placental 11ß-hydroxysteroid dehydrogenase2 (HSD2), the activity of which is also downregulated by maternal low protein intake (see Chapter 1.4.2.4.4; *Langley-Evans et al. 1996*). Glucocorticoids can inhibit insulin release and β-cell replication (see Chapter 1.2.5.6), therefore exposure of the fetus to higher levels of maternal glucocorticoids may lead to elevated glucose levels during the early postnatal life. The protein intake of the study participants was found low (see Dietary intakes), which would also support this explanation.

An association of the neonatal fasting glucose (**GLC F1**) with maternal placental lactogen (**HPL 2)** may result from the stimulatory effects of maternal **HPL** on glucose delivery to fetus (*Fowden 2004*). This would support fetal growth and relatively larger neonates would present with relatively higher levels of glucose, although within a normal range.

GLC F Ba2: An association of the infant 6-month postnatal fasting glucose (**GLC F2**) with maternal placental lactogen from both pregnancy visits (**HPL1 and 2)** supports the previous association with **GLC F Ba1**.

The inverse relationship with maternal proinsulin levels was also part of the regression outcome for **Glc F Ba2** and **HOMA-IR Ba1**. Again, low **PI** may reflect higher **PI** conversion to insulin in the mother due to high maternal glucose levels.

Metabolic parameters: Fasting baby's neonatal insulin (**INS F Ba1**) and also the homeostatic index (**HOMA-IR Ba1**) were inversely related to maternal thyroid hormones of the first pregnancy visit (**FT4 V1).** The recombinant human IGF-1 administered to rats diminished FT4 concentrations in these rats (*Pellizas et al. 1998*), possibly due to decreased

number of TH receptor expression which lead to downregulated metabolic action of FT4 in the liver. Therefore, elevated blood levels IGF1 concentrations (still in normal range range) may cause a decline in the maternal serum FT4 concentration still supporting the fetal growth, with lower levels of FT4 indicating higher levels of the growth factor.

There was a significant **gender** difference in the infant postnatal leptin concentrations (**LEPT1 and 2**) with higher levels in females (see Chapter 3.12). With increasing **age**, as the infant is gaining weight **(W Ba 1 and 2)**, leptin levels also increase (*Harigaya et al. 1997*). The correlation between BMI and leptin is well known (*McMillen et al. 2006*), as is its gender difference (*Helland et al. 1998*). Maternal leptin blood levels before birth were associated with fetal leptin blood levels from the umbilical vessels in a study in Brazil (*Castro et al. 2006*). A high carbohydrate diet given to pregnant sows increased the neonatal leptin levels (*Litten et al. 2004*), therefore an elevated maternal glucose level (**Glc 60' V1**) may contribute to the elevated neonatal leptin concentrations as observed in the current study.

Some of the effects of maternal hormones on infant markers of β-cell function may be mediated by maternal adiponectin, which is known to modulate glucose tolerance and insulin sensitivity. It is also present in the breast milk, where it is believed it may have a preventive role against obesity, if found in lower amounts (*Savino et al. 2008*).

Summary:

The regression analysis has shown that maternal anthropometry and maternal hormones during the second and also the third trimester do influence the rate of fetal or infant growth and early metabolic health. These relationships are probably very complex, as there will also be influences of variables, which were not measured in the current study. Many hormones mediate the effects of maternal nutrient intake, their processing and partitioning between mother, placenta and fetus, therefore these interactions may also reflect in the analysis outcomes. Certain associations, especially with birth weight may be an inverted U-shape, or some studies report also J shaped curves, however, proving such a relationship requires larger subject numbers.

4.5.3 Discussion on the correlations with Socio-Economic Status (SES)

Mother's and father's education showed significant relationships with family income. There was a very strong relationship between mother's and father's educational status (Chapter 3.11), women were more likely to be unemployed as were their partners (see Chapter 3.1.3.2). Maternal employment positively correlated with her physical activity.

The mothers who grew up with many siblings in the family (and thus had fewer opportunities to study), were also the women with lower education, lower family income, less physically active during their gestation and more stressed. On the other hand, the higher education of mothers and also fathers was associated with lower maternal cortisol levels measured at V2. However, this relationship was not associated with family income, as the income did not show any correlation with maternal cortisol.

Employed mothers showed higher weight, **BMI** and leptin levels during pregnancy, elevated serum insulin concentration and insulin resistance index at V1 and plasma glucose, area under the glucose curve and proinsulin levels at V2 (see Table 3.14A). However, all these associations were attributable to the correlations with maternal weight and **BMI**, except for the **PI F** of **V2** (evaluated by the regression analysis).

The neonates of employed women had lower fasting glucose levels (Table 3.14A). The published expected glucose range for infants over 7 days of age are between 3.00 mmol/l and 6.49mmol/l (*Soldin et al. 2007*). The fasting glucose range in the current study was 2.8mmol/l to 5.3 mmol/l. Based on the regression equation, the employed mother had a baby with 3.86 mmol/l fasting glucose concentration in average, whilst an unemployed mother's baby had fasting glucose levels of 4.08 mmol/l.

Both mother's and father's employment were also directly related to the neonatal length and also employed fathers had offspring of a lower ponderal index (**PONDIX**), however there was no significant correlation between the **PONDIX** and income.

Although the family income did not directly correlate with maternal weight, it seems to be an explanation, together with the inverse significant correlation between maternal weight and cortisol, which was also associated negatively with maternal and paternal education. More educated parents may provide better for the family (better job and better income), resulting in less stress (Table 3.15) and better nutrition. As there were no diabetics included into the study, the "high" maternal glucose and hormonal levels of proinsulin, insulin and insulin resistance index reflect "healthy" adaptation to the pregnancy. Similarly, the inverse associations of infant fasting glucose and **PONDIX** with

the employment of parents rather represents a baby with parameters on the "healthier" side of the study population range. On the other hand, stressed mothers may experience inadequate elevation of insulin, poor delivery of glucose to the fetus, resulting in low adipose tissue mass and possibly shorter birth length. High cortisol levels in mothers were shown to downregulate the fetal growth (*Benediktsson et al. 1993*).

No correlation was observed between birth weight and any socio-economic indicators selected in this study. This may be due to a narrow range of the socio-economic differences in this studied population. However, in the Helsinki cohort, the links between the poor material wealth and future disease were found only in those born thin (*Barker 2004).*

CHAPTER 5

SUMMARY AND CONCLUSIONS

To the authors' knowledge, this study is the first in Africa that followed a group of pregnant (urban) Black African women in order to identify factors during pregnancy, which may predispose their offspring to future Type 2 diabetes.

The study has evaluated the effects of the maternal dietary intake of energy and macronutrients and hormonal levels during pregnancy on fetal growth, birth weight and

size and infant postnatal glucose tolerance and β-cell function, which are among the known risk factors predisposing subjects to Type 2 diabetes later in life.

1. **The first aim of the study was to assess if the maternal pregnancy dietary intake of macronutrients affect fetal, birth or postnatal size or growth.**

The timing of the first pregnancy visit fell into the period of major brain development and of the linear growth which both require protein and polyunsaturated fatty acids. The third pregnancy trimester is characterized by accelerated fetal growth and fetal fat accretion.

The nutritional intake of mothers during pregnancy in this study was characterized by rather low energy intake, mainly due to a low consumption of dietary protein, especially of plant protein. The total crude amount of fat intake was higher than was the intake observed in South African non-pregnant women of child-bearing age, with the main contribution from saturated fat and lowest contribution from polyunsaturated fat. The intake of total carbohydrate adjusted for the energy intake was well in range of the RDA.

The effects of the above maternal dietary intake on fetal biometry were analysed. The gestational age adjusted models “explained” over 90% of variation in the fetal biometry variables with the effect of maternal dietary intake in these models being very weak. However, there were some significant relationships and these will be discussed below.

Adjusting for gestational age (and other confounders), associations between total maternal energy intake and fetal parameters were found, with maternal protein and carbohydrate acting as the major energy sources for fetal growth. Although correlations between maternal **TOT ENERGY2** intake and fetal weight and length at V2 were also significant, from the adjusted results it was not obvious what nutrient contributed the most. Among unadjusted parameters this effect would be attributable to the **PUFA1** intake.

The strongest association observed was of **HC2** with maternal **TOT PROT** intake at **V1** and it was negative. However, the results do no indicate, which source of protein contributed the most towards this association. A suggestion was made, that the negative relationship may be due to a decreasing supply of **PL PROT** or possibly this may represent a negative feedback system that ensures that excessive skull growth does not occur at high levels of energy intake. However, we cannot currently prove this hypothesis.

No significant correlation between maternal intake of energy or macronutrients was found for fetal growth rate.

In conclusion, maternal total energy intake was associated with fetal weight, length and size of abdomen, whilst maternal protein intake was related to fetal head development and animal protein intake was reflected in the concurrent size of the fetal abdomen at the end of pregnancy. Polyunsaturated fat intake may be related to fetal fat accumulation (**FW2, AC2**) and linear growth (**FL2**). However, not many significant correlations of maternal dietary intake with fetal biometry were observed. This confirms that the effects of maternal nutrition during pregnancy were not as very obvious during fetal life as during early postnatal life.

The current study has found a positive relationship between the maternal total dietary protein intake (expressed as a percentage of the total energy intake) during the third trimester and the infant **birth weight**. A similar finding was reported in the study conducted in Haryana, India (*Rao et al. 2007*).

Comparing maternal nutrient intakes during pregnancy with the V3, the postpartum maternal dietary intakes of some nutrients significantly changed. The **TOT ENERGY** intake significantly increased reflecting elevations in **PL PROT**. Increased intakes were seen for **MUFA** and **PUFA** (and so **TOT FAT**) and for the total carbohydrate. The **TOT PROT** contribution toward energy intake, however, decreased. These nutrient changes may reflect the maternal adaptation to the increased requirements for breast-feeding.

Whilst the plant protein intake at V2 did not produce any significant association with fetal biometry or neonatal size, it showed many correlations with the postnatal infant **BMI** and the metabolic variables and possibly stimulated growth in length. Maternal fat intake during the second trimester also did not produce many significant correlations with the ultrasound data, however, postnatally, from maternal pregnancy intakes the most effective was **PUFA** intake, which on one side may have contributed to the growth in length, whilst together with **SAFA** it was also related to increase in the postnatal **BMI.**

Although there were no significant differences in the maternal macronutrient intake during pregnancy, the effects of the dietary intake of V1 and V2 differed, which indicates the importance of timing for the effect of a particular nutrient. It was interesting that from the maternal dietary intakes at V3, only **TOT ENERGY3** showed a relationship with

concurrent infant anthropometrical variables, which was a weak association with infant weight.

From the mothers' dietary intakes and their effects on fetal biometry, BW, infant postnatal anthropometry and infant birth and postnatal z-scores, the following conclusion can be drawn:

In early pregnancy, the maternal dietary supplies of total energy, protein and fat were probably sufficient for normal fetal growth and development. Maternal **PL PROT1** and the **PUFA1** intakes were associated directly or indirectly (e.g. via calcium and other minerals intake) with growth of the fetal skull. Maternal **PL PROT1** intake also positively affected the fetal liver development (based on the correlation with fetal **AC**). The **AN PROT** intake during early pregnancy was associated with neonatal **BMI**, and thus possibly contributing to fetal fat accretion during later prenatal development.

The **PUFA1** intake, may have contributed to the building of maternal adipose stores (not measured in the current study), utilized by fetus in the later stages of pregnancy. This may have included abdominal organ growth and fetal fat accretion, being reflected in the increased **AC2** and **FW2**. Also **PUFA1** was still involved in linear growth, showing strong associations with **FL2** and neonatal length.

Maternal **SAFA** intake was positively linked to neonatal **BMI**, possibly via the route of fat accumulation. Also, as the intake of **SAFA** was relatively high during pregnancy, the negative associations with early fetal biometry may point towards its inhibitory role in linear growth. Taking into account low levels of maternal **PL PROT** intake, the likely outcomes could be lighter and shorter offspring with a relatively larger **BMI.**

Protein intake **during the last pregnancy trimester**, when there are increased requirements for energy, seem inadequate. The nutrient reserves may therefore be directed to the development of the fetal brain (brain sparing effect) in the current study (and in the literature), which was negatively associated with the fetal bone growth. This could eventually lead to a shorter, lighter baby with a relatively larger head. The decline in z-scores for weight/age and especially for length/age at Ba1 visit could correspond to the switch from the maternal placental **PUFA** supply to the breast/formula feeding, when the baby could not fully support its growth. An improvement in these scores at Ba 2 visit may then reflect a mobilisation of the infant fat reserves and its own production of **PUFAs**.

2. **The second aim of the study was to assess if the maternal pregnancy dietary intake of macronutrients affect infant glucose and markers of infant β-cell function i.e. INS, PI, HOMA-IR.**

The data analysis showed significant correlations between maternal nutrient intake and postnatal glucose and markers of infant β-cell function.

The third trimester **TOT ENERGY** intake was inversely related to the neonatal and the 6-month postnatal infant fasting **GLC** and positively to neonatal fasting **INS**. These outcomes are the result of the combined effects of protein, fat and carbohydrate energy sources.

Maternal intake of **TOT CHO** (including added sugar) at the first pregnancy visit (V1) was related to the **size of fetus/infant**, whilst carbohydrate at the second pregnancy visit (V2) was related to infant **markers of β-cell function**.

Maternal protein, specifically animal protein at both pregnancy visits seems to be involved in the regulation of the infant postnatal concentrations of **IGF-BP3**. Maternal protein intake (animal protein intake) at the first visit was positively related to the infant neonatal insulin and **HOMA-IR** index, whilst the plant protein intake at V2 was correlated negatively with the neonatal and postnatal infant **β-cell function**. This indicates a protective role for the plant protein.

There is some involvement of infant **BMI** in the protein and carbohydrate results, although, the direct influence of protein and carbohydrate still remained after adjusting for **BMI** in the regression analysis. Infant **BMI** correlated with fat intake and also showed significant correlation with the infant **β-cell function**, which reflected possible protective effect of **PUFA**.

A concurrent inverse association between maternal total energy intake at V3 and levels of infant **INS** and **HOMA-IR2** and a positive correlation with infant leptin (**LEPT2)** were found, indicating a direct effect of maternal energy intake on infant **β-cell function** and adipose tissue.

3. **The third aim of the study was to assess if the maternal pregnancy hormonal levels affect fetal, birth or postnatal size or growth.**

Fetal growth rate. Contrary to the lack of effect of maternal dietary intakes on the fetal growth rate, positive correlations with maternal anthropometry and her hormonal

levels of placental lactogen, leptin, free thyroxine and proinsulin were found. Postnatal growth rate was associated with fetal gender and mother's level of insulin resistance at V2.

Birth parameters. In addition to maternal protein intake, the significant association with the baby's birth weight was also found with maternal weight, fetal gender and the gestational length. Similar correlations also applied to the birth length, with the exception of baby's gender and a positive effect of maternal leptin and negative effect of the **IGF BP1** on **BL**. The correlations with baby's birth head circumference were as for the birth length. The association of **BL** and **BHC** with the **IGF-BP1** may reflect a delayed bone ossification.

Postnatal infant anthropometry.

The major factors positively influencing neonatal **BMI** were maternal weight and baby's age, and negatively, maternal proinsulin levels, whilst in older babies there was a major influence of the gender and maternal leptin concentrations.

4. **The fourth aim of the study was to assess if the maternal pregnancy hormonal levels affect infant glucose and markers of infant β-cell function i.e. INS, PI, HOMA-IR.**

The outcomes of the current study show that maternal hormones can affect the neonatal and early postnatal infant glucose levels, **β-cell function** and are also linked to the programming of early obesity. This involves maternal free thyroxin levels, glucose at the

first pregnancy visit, **HPL** at the both pregnancy visits and maternal cortisol, proinsulin and insulin of the third-trimester visit. The maternal thyroid hormones seem to play an important role in fetal and postnatal growth and insulin metabolism.

The regression analysis has shown that maternal anthropometry and maternal hormones during the second, and also third trimester of pregnancy do influence the rate of fetal or infant growth and early metabolic health. These relationships are probably very complex, as there may also be variables that will have additional effects but which were not measured in the current study.

It is also possible that some associations between particular variables were not identified, due to insufficient **power.** The sample size assessment was based on the general rule for the relationship between the sample size and the number of explanatory variables entering the multiple regression models (*Petrie et al 2000a;* Chapter 2.2.1). However, the loss to follow-up was underestimated. The retrospective evaluation of the study power with regard to some key infant variables indicated a wide power range between 70-95%. (The power calculator used was:
http://hedwig.mgh.harvard.edu/sample_size/js/js_associative_quant.html).
One of the strongest outcomes included the relationship between maternal **FT4 (1)** and neonatal **INS F1**.

LIMITATIONS OF THE CURRENT STUDY

1. The sample size of pregnant women and infants included in this study is comparatively small. It is therefore possible that some associations were missed due to a lack of power. However, the study was of sufficient power to uncover a number of important associations, which will be the focus of future investigations.
2. Food frequency questionnaires are considered to be a less accurate measure of the absolute intakes of nutrients compared to dietary recalls or diet records, as the FFQs may be biased by the respondent's memory. However, they are better in assessing habitual dietary intakes, they are less expensive and require a shorter period of time for their administration. The FFQ in the current study was supplemented with a 24-h dietary history crosscheck. It may, however, have been better to support it with a full 24-h dietary recall questionnaire.
3. The correlation and regression analysis used in this study identified significant associations between variables of interest. However, it could not explain the full mechanisms behind these associations.
4. As the current study aimed to investigate crude associations and how they are mediated, it did not adjust for multiple testing in the analytical part of the analysis.
5. The statistical analyses in the current study were exploratory. Although certain steps to correct for the Type I statistical error were performed (the Bonferroni correction in the linear mixed models of the Descriptive part of the analysis and grading outcomes according to the p levels in the analytical part of analysis; comparison of results with existing literature), the study had many variables and full adjustment for

multiple testing was not done, and we therefore cannot exclude the fact that some results may have gained their significance by pure chance.

6. There were too many variables to be tested separately for confounding, therefore the study used a literature based set of well-established confounders for birth weight. It was assumed, that these confounders are common for all the other tested variables. However, in some instances these confounders were not significant and for some variables they may have acted as modifiers.

IMPLICATIONS OF THE STUDY

The study revealed low maternal intakes of total energy largely as a result of low intakes of protein. The offspring of this study cohort were born lighter and shorter, but with a larger head circumference in comparison with the standard population z-scores (WHO 2010). Clearly there is a potential area for future interventions.

This study also shows that infant neonatal insulin sensitivity could be improved by an increase in neonatal length and in the lean body mass. This could be achieved in a number of ways.

1. Changing the composition of the maternal diet. The process of urbanisation is generally associated with dietary changes, leading to diet rich in animal protein and saturated fat, includes elevated ratio of n-6 PUFA to n-3 PUFA and increased levels of carbohydrate.

Although the levels of essential amino acids found in animal protein are more suitable for human requirements than that of plant protein (*O'Brien et al. 1987*), it is not supportive of linear growth. Linear growth in the present study benefited from plant rather than animal protein consumption. Therefore, increasing consumption of the plant protein in early pregnancy would be suggested. However, some natural sources of plant protein found in corn, wheat and rice, which are among the frequently consumed food categories, have similar sulphur content to animal proteins, and therefore would not stimulate linear growth. However, the calcium in milk, the plant protein in fruits and vegetables (e.g. legumes, grains) can stimulate linear growth due to their mineral content and stimulatory effect on IGF-I concentrations (Chapter 4.5.1.3).

A further potential area for intervention is to decrease the dietary n-6 PUFA to n-3 PUFA ratio in order to decrease production of PGE2 and thus stimulate bone cell-derived growth factors. Intervention early in pregnancy is required, as the PUFA contributes to maternal adipose tissue generation, which serves as the main energy source during later pregnancy for accelerated infant growth. The richest natural sources of n-3 fatty acids are oily fish, or the precursor of n-3 PUFA, alpha linolenic acid, is found in plants such as green leafy vegetables or rape-seed oil (*Williams et al 2006*).

2. Increasing the total energy intake. Even with improved protein consumption, if total dietary energy intake is low, protein malnutrition cannot be improved (*O'Brien et al 1987*). Therefore increasing the energy intake is important. The intake of energy is known to be positively associated with the physical activity, which among the South African urban women is low (Chapter 4.3.3). In the current study the dietary intake of energy was also linked to the parental education and employment. This intervention is difficult, as it is not

achievable without improvement of the educational level and employment status of the parents.

3. Regulating maternal hormonal levels. The combined effect of maternal nutrition and hormones will be investigated in future research. However, improvements in the composition of the maternal diet and increasing the physical activity of urban women will lead to changes in their anthropometry and thus to beneficial changes in hormonal levels.

FOLLOW-ON RESEARCH FROM THIS PROJECT

• Investigation of the effects of the n-6 and n-3 PUFA ratio on fetal and neonatal growth.

• As the effects of the macronutrients on the fetal development are not isolated, the interaction of macronutrients and hormones will be carried out in the regression analysis (interactions terms).

• The effects of maternal intake of micronutrients as assessed by the food frequency questionnaire, will be explored.

• Influence of other determinants of fetal development/ birth weight will be investigated, such as the number of previous pregnancies, maternal birth size, mother's health history using appropriate statistical tools for low numbers and unequal balance

• The information collected by means of the food frequency questionnaire will be coded and analysed according to food groups (such as milk and dairy consumption, fish,

fruits and vegetables..) to support the findings related to the individual nutrients (e.g. PUFA).

- Complex model development employing statistical modelling tools

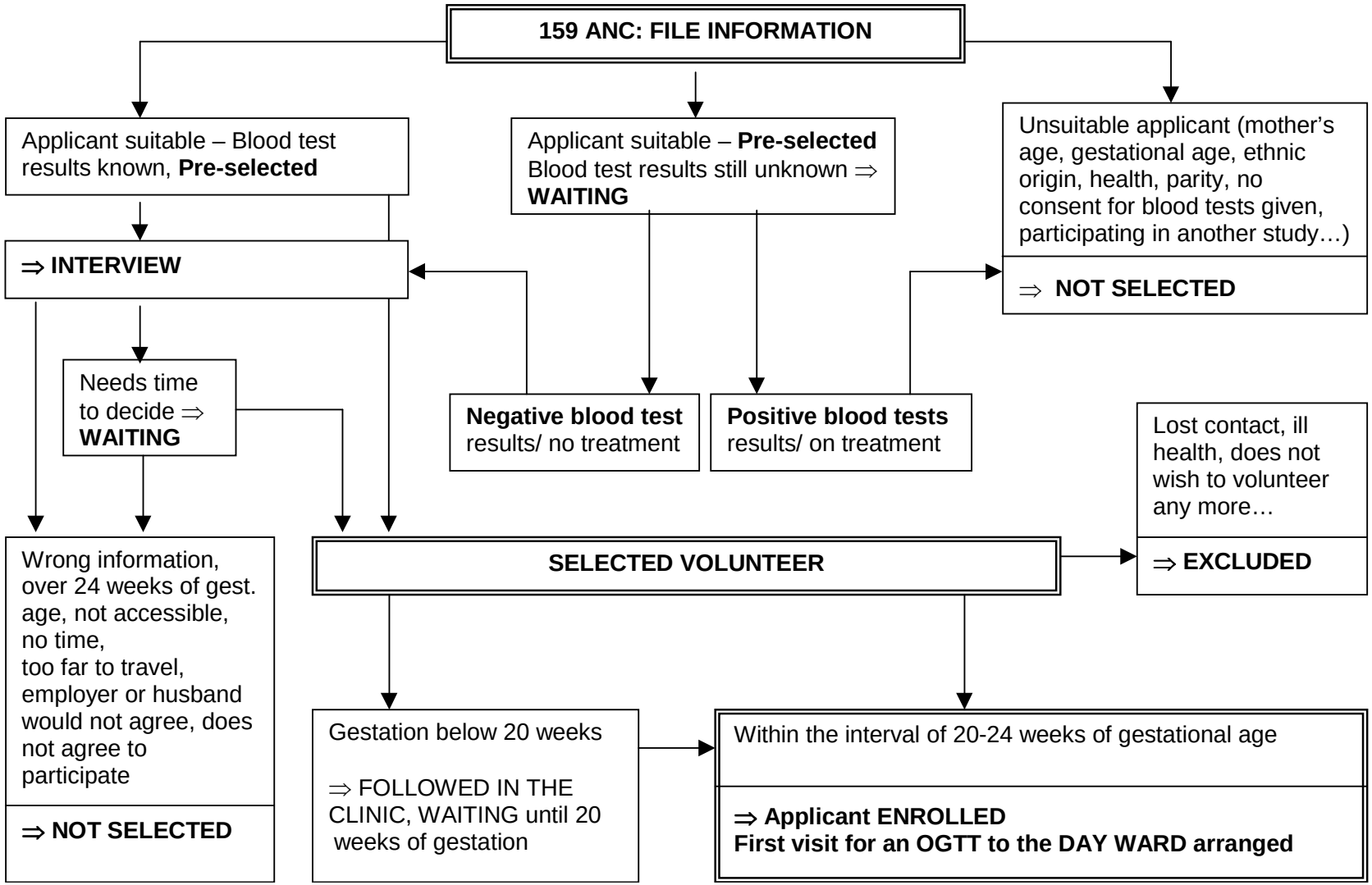
---

APPENDICES

Pg

A	**Enrolment diagram**	**283**
B	**Inclusion criteria questionnaire**	**284**
C	**Food frequency questionnaire**	**287**
D,E,F	**Maternal energy and macronutrients dietary inatake regression analyses and the list of used confounders**	**291 - 293**
D	**Maternal protein intake analysis**	**294**
E	**Maternal fat intake analysis**	**305**
F	**Maternal energy, carbohydrate, added sugar intake analysis**	**317**
G	**List of variables and their transformations**	**330 - 331**
H	**Maternal dietary intakes summary tables**	**332**
I	**Correlations used in the regression models listed in Appendix J**	**337**
J	**Linear regression models**	**344**
K	**Tables of outcomes from the linear mixed model analyses**	**349**
L	**Permission to reprint the RDA/DRI values**	**355**
M	**Ethics Clearance**	**356**

APPENDIX A: **Enrolment diagram**



APPENDIX B. Inclusion Criteria Questionnaire.

QUESTIONNAIRE (inclusion criteria)					*PND Study* Pg1	
Study No	***Hosp. No***	***First Names***	***Surename***	***Age***	***Ethnic group***	***Occupation***

Home Address	***Tel No - home***	***Cell phone No***	***Neighbour- tel.***	***Note:***

Employer - address	***Tel No - work***	***Husband / close relative (w/home)***	***Tel. No***

FAMILY:		
Soc.-econom. Status:	No of people in a household/flat	
	Average income/ month	
	How many brothers and sisters do you have?	
Genetic Status:	Any diseases which commonly occur in your family?	
	Any Down Syndrom or neural tube defect? Other?	
	Is/was your mother short? Thin?	
	If you know: Were you born premature?	
	If you know: Were you a small/thin baby?	

Mothers background							Pg 2	Resrv. for changes
Education:								
Previous pregnancies:								
	Spont. Abortions?		Stillbirth?		Other?			
Other children:	How many?							
	How old are they?							
Medical history:	Do you suffer from any chronic disease?							
	Have you ever been diagnosed with following:							
	{tuberculosis, HIV, syphilis - other STD?							
	chronic respiratory disease (asthma), chronic renal disease							
	congenital heart disease, high blood pressure							
	cystic fibrosis, anaemia, thyroid disease, toxoplasmosis}							
	If yes, state which one and when:							
	Have you been irradiated during pregnancy or shortly before? (X-ray, …)							

Mothers present state				
General health:		Are you taking any MUTI?		
Any present treatment?				
What medicaments?		Any allergy?		
Stress?	Do you have any stress?			
	Do you have any occupational hazards?			
	Are you working standing?			
Are you a smoker?	How many cigarettes do you smoke/day?			
Do you drink alcohol?	How often, how much?			

Baby's father			Pg 3	Resrv. for changes
Ethnic origin:				
Age:				
Education:				
Occupation:				
General health				
Height & Weight:				
Genetic status:	Is there any genetic disease in baby's father family?			
	If yes, which one?			

Further information			
Do you plan to attend all visits in the 159 ANC?	Y	N	
Do you plan to stay at the present address?	Y	N	
Do you plan to move out of Johannesburg? When?	Y	N	
For how long?			
Do you plan to deliver your baby in this hospital?	Y	N	
Do you plan to stay with your newborn baby in Johannesburg?	Y	N	
Will you look after your baby yourself? (Somebody else?)	Y	N	
Do you plan to come with your baby for both visits: after 2-3 weeks	Y	N	
after 5 months	Y	N	

What is the best way to contact you?	**Tel:**			
Any comments? *Please note: If you need any help, do not hessitate to ask the interviewer.*				

APPENDIX C. *FOOD FREQUENCY QUESTIONNAIRE* 1

NAME_____
DATE OF BIRTH_____ AGE (YEAR AND MONTHS)_____
SEX _____
ETHNIC GROUP_____
DAY OF INTERVIEW_____
DATE OF INTERVIEW_____

INSTRUCTIONS

The following questions are about the foods you USUALLY eat during an average week.
Please indicate the number of days per week that you eat item on average. Ring the answer as in the example:

If you eat the food every day, ring 7	7 6 5 4 3 2 1 M R
If you eat the food 3 days / week, ring 3	7 6 5 4 3 2 1 M R
If you eat the food only monthly, ring M	7 6 5 4 3 2 1 M R
If you never or rarely eat the food, ring R	7 6 5 4 3 2 1 M R

GRAIN AND CEREAL GROUP

	No. days / week
Bread	7 6 5 4 3 2 1 M R
Provitas/ cream crackers etc.	7 6 5 4 3 2 1 M R

Do you mainly eat white, brown or wholewheat bread? _____
How many slices of bread do you have per day? _____

BREAKFAST CEREALS AND PORRIDGES

No. days / week	
Cereals (Rice Krispies, Cornflakes)	7 6 5 4 3 2 1 M R
Sugar coated cereals (Coco pops, Frosties)	7 6 5 4 3 2 1 M R
Whole wheat cereals (All Bran, Weetbix)	7 6 5 4 3 2 1 M R
Porridge (Oats, Maltabella, Maize meal)	7 6 5 4 3 2 1 M R
Muesli	7 6 5 4 3 2 1 M R
Pronutro	7 6 5 4 3 2 1 M R

OTHER STARCHES

No. days / week		
Rice, pasta		7 6 5 4 3 2 1 M R
Stiff maize meal	- with amasi (sour milk)	7 6 5 4 3 2 1 M R
	- without amasi	7 6 5 4 3 2 1 M R
Samp/mielie rice	- with beans	7 6 5 4 3 2 1 M R
	- without beans	7 6 5 4 3 2 1 M R

MEATS AND MEAT SUBSTITUTES

No. days / week	
Read meat (beef, lamb, pork or mince)	7 6 5 4 3 2 1 M R
Processed meat (bacon, sausages, polony)	7 6 5 4 3 2 1 M R
Chicken	7 6 5 4 3 2 1 M R
Fish	7 6 5 4 3 2 1 M R
Eggs and egg dishes	7 6 5 4 3 2 1 M R
Cheese, cheese spread and cheese dishes	7 6 5 4 3 2 1 M R
Nuts, including peanut butter	7 6 5 4 3 2 1 M R
Dried peas, beans, baked beans or legumes	7 6 5 4 3 2 1 M R

VEGETABLES AND FRUIT 2

	No. days / week
Green and/ or yellow vegetables	7 6 5 4 3 2 1 M R
Potatoes	7 6 5 4 3 2 1 M R
Fresh fruit	7 6 5 4 3 2 1 M R
Canned fruit	7 6 5 4 3 2 1 M R
Dried fruit (raisins, prunes, dates)	7 6 5 4 3 2 1 M R
Fresh fruit juice (Ceres, Liquifruit)	7 6 5 4 3 2 1 M R

Name some of the vegetables you have eaten this past week

_____

Do you eat your vegetables most frequently cooked or raw?
_____

Do you mainly eat your potatoes as boiled, baked in jacket, mashed, roasted or as chips? _____

What types of fresh fruit have you eaten this past week?

_____

FATS AND OILS

	No. days / week
Oil/butter/margarine	7 6 5 4 3 2 1 M R
Salad dressing/mayonnaise	7 6 5 4 3 2 1 M R
Cream	7 6 5 4 3 2 1 M R
Non dairy creamers (Cremora)	7 6 5 4 3 2 1 M R
Ice-cream	7 6 5 4 3 2 1 M R

Please estimate, on average, the total amount of fat (oil/butter/margarine/dressing/cream/ non-dairy creamers) you use per day in teaspoons. _____ tsp.
(includes the fat on bread, in cooking and frying and on vegetables/salads)

	No. days / week
How often do you fry your food?	7 6 5 4 3 2 1 M R

MILK AND MILK PRODUCTS

	No. days / week
Milk	7 6 5 4 3 2 1 M R
Yoghurt - plain	7 6 5 4 3 2 1 M R
- flavoured	7 6 5 4 3 2 1 M R
Milo/ Nesquick/cocoa/ Horlicks	7 6 5 4 3 2 1 M R
Custard	7 6 5 4 3 2 1 M R

Please estimate, on average, how much milk you have per day, including that on cereal, in tea or coffee and milk drinks
_____ ml or _____ cups

What type of milk do you use: 3

Full cream _____
Low-fat (2 % fat) _____
Skimmed _____
Condensed _____

MISCILLANEOUS

Please estimate how many teaspoons of sugar you have, in total, per day? _____ tsp.
(in tea/coffee/milk drinks, on cereal/ porridge, added to vegetables)

	No. days / week
Sweets (sucking/ jelly type/ fudge/ toffee)	7 6 5 4 3 2 1 M R
Chocolates/ chocolate bars	7 6 5 4 3 2 1 M R
Jam, syrup, honey	7 6 5 4 3 2 1 M R
Jelly	7 6 5 4 3 2 1 M R
Sweet biscuits/ cakes/ pastries/ doughnuts tarts/ scones/ pancakes	7 6 5 4 3 2 1 M R
Crisps	7 6 5 4 3 2 1 M R
Popcorn (plain or candied)	7 6 5 4 3 2 1 M R
Puddings (trifle, baked puddings etc.)	7 6 5 4 3 2 1 M R
Coldrinks (Coke, Fanta etc.) or cordials	
- sweetened	7 6 5 4 3 2 1 M R
- diet	7 6 5 4 3 2 1 M R
Tea	7 6 5 4 3 2 1 M R
Coffee	7 6 5 4 3 2 1 M R
Spreads (bovril, marmite, fish paste, sandwich)	7 6 5 4 3 2 1 M R

How many meals do you have per day ie. Breakfast, lunch and/or dinner? _____

How many inbetween snacks do you have per day
ie. mid-morning, mid-afternoon and or late evening? _____

Please indicate how many **portions** of the following food groups you **ate yesterday** by circling the appropriate number.

Eg. If you had **1 egg** for breakfast, **cheese** for lunch, and **chicken** for dinner, ring 3 for number 2.
If you had **cereal** and **1 slice of toast** for breakfast, a sandwich for lunch (**2 slices of bread**) and **rice** for dinner, ring 5 for number 4.

	No. Portions yesterday
1. milk, yoghurt (1 portion = 1 cup/ 200 ml)	1 2 3 4 5 6 7 8 9 10
2. meat/ fish/ chicken/ cheese/ eggs/ nuts/ legumes	1 2 3 4 5 6 7 8 9 10
3. fruit/ fruit juice	1 2 3 4 5 6 7 8 9 10
4. vegetables (green and yellow)	1 2 3 4 5 6 7 8 9 10
5. potatoes	1 2 3 4 5 6 7 8 9 10
6. bread/ cereal/ porridge/ rice/ pasta/ maize meal/ samp/ mielie rice	1 2 3 4 5 6 7 8 9 10
7. oil/ butter/ margarine/ cream/ non dairy creamers/ salad dressings (1 portion = 1 tspn)	1 2 3 4 5 6 7 8 9 10

Were these number of portions typical of what you would normally consume on an average day ?

Yes _____ No _____

If you answered No, was the difference due to:

Illness _____

A party _____

Eating out _____

Other reasons _____

Appendix D, E, F.

Maternal energy and macronutrients dietary inatake regression analyses.

The Appendices D, E, F include tables of maternal energy and macronutrient dietary intakes and their effects on selected fetal, birth and infant parameters.

The variables were analysed in the multilinear regression models with and without adjustment for possible confounders (see also Chapter 2.8.3.3).

The following confounders were used in adjustment:

Due to restricted space in the result tables, a short abbreviation was used for the variables as described below. For analyses related to the gestation period, gestational age and maternal weight were included as confounders and for the postnatal period, the analyses were corrected for the infant age and weight. The analysis of birth parameters took into account maternal height, maternal weight and birth age. All analyses were corrected for infant gender. Abbreviations:

A (gestational/ birth/ infant age)

w (maternal/infant weight)[1]

s (sex)

h (maternal height)

Significant results ($p<0.05$) are in red type, whilst the results near significance ($p\geq5$ and $p<0.10$) are in black, bold type.

[1] Please note: Baby's BMI cannot be corrected for the baby's weight, as it is the part of the formula. The similar applies for the growth rate.

Outliers on or above three standard residuals (SR) were excluded. If exclusion of the outlier produced a change in the non-standardised B-coefficient greater than 10%, both outcomes are presented. The same applies for the instances where outlier exclusion led to a change in the outcome (significant/non significant). The result including the outlier is presented below the main table.

For the units of the B-coefficients see Appendix G.

Significance of confounders in individual regression models.

Due to restricted space in the regression outcome tables, the significance of confounders used in adjustment of the individual regression models is listed below:

Significant confounders associated with the following variables:

Maternal dietary intakes and fetal biometry:

FW1	Gestational age /(maternal weight*)
BPD1	Gestational age
AC1	Gestational age, sex /(maternal weight*)
HC1	Gestational age,
FL1	Gestational age, sex /(maternal weight*)
FW2	Gestational age, maternal weight
BPD2	Gestational age, maternal weight
AC2	Gestational age, maternal weight
HC2	Gestational age
FL2	Gestational age

(* significant association with the fetal biometry variable if gestational age removed)

Birth weight: Birth age, maternal weight, sex

Postnatal anthropometry:

Weight1	infant age, sex
Length1	infant age
BMI1	infant age
Weight2	sex
Length2	infant age
BMI2	sex

Infant metabolic parameters:

GLCF1	infant weight
INSF1	-
PIF1	-
HOMA1	infant weight
LEPT	infant weight, age, sex
IGF-BP3 (1)	infant weight
GLCF2	-
INSF2	infant weight, sex
PIF2	infant weight
HOMA2	infant weight, sex
LEPT2	infant weight, sex
IGF-BP3 (2)	infant weight

APPENDIX D. Maternal protein intake analysis.

TABLE OF RESULTS: D1 **Maternal protein intake at V1 vs fetal weight and size at US1.**

Protein intake V1	*FW1*		*BPD1*		*AC1*		*HC1*		*FL1*	
Adjusted for conf.	None	A,h,w,s	None	A,h,w,s	None	A,h,w,s	None	A,h,w,s	None	A,h,w,s
N number	106	104	106	103	106	102	97	95	104	103
TOT Prot (%E)										
B coef	-5.823	-0.936	-0.075	0.372	0.022	0.007	-2.620	-0.315	-0.522	0.225
Beta coef	-0.138	-0.023	-0.075	0.054	0.127	0.047	-0.111	-0.013	-0.087	0.038
p value	0.159	**0.079**	0.446	**0.024**[#1]	0.194	0.134	0.284	0.513	0.374	0.141
PL Prot (%E)										
B coef	5.300	-0.458	0.967	0.074	-0.026	-0.003	3.70	0.245	0.744	-0.060
Beta coef	0.187	-0.016	0.207	0.016	-0.229	-0.032	0.222	0.015	0.186	-0.015
p value	**0.055**	0.208	**0.033**	0.512	**0.018**	0.298	**0.029**	0.456	**0.057**	0.539
AN Prot (%E)										
B coef	-1.480	-0.080	-0.193	0.046	0.007	0.002	-0.904	-0.080	-0.160	0.043
Beta coef	-0.204	-0.011	-0.162	0.039	0.225	0.062	-0.214	-0.020	-0.156	0.042
p value	**0.036**	0.394	**0.098**	0.109	**0.021**	**0.050**[#2]	**0.035**	0.331	0.111	**0.085**[#3]

Analysis including outliers:

	#1	#2	#3
N number	104	104	104
B coef	0.305	0.001	0.042
Beta coef	0.044	0.044	0.041
p value	**0.077**	0.175	0.119

TABLE OF RESULTS: D2 **Maternal protein intake at V1 vs fetal weight and size at US2.**

Protein intake V1	*FW2*		*BPD2*		*AC2*		*HC2*		*FL2*	
Adjusted for conf.	None	A,h,w,s	None	A,h,w,s	None	A,h,w,s	None	A,h,w,s	None	A,h,w,s
N number	104	102,101*	104	102	103	102	95	92,93*	104	102
TOT Prot (%E)										
B coef	-0.188	-0.032	-0.784	-0.213	0.028	0.009	-4.322	-2.882	-0.785	-0.043
Beta coef	-0.205	-0.036	-0.169	-0.046	0.181	0.057	-0.276	-0.187	-0.194	-0.011
p value	**0.037**	0.572	**0.087**	0.538	**0.067**	0.450	**0.007**	**0.014**[#2]	**0.049**	0.871
PL Prot (%E)										
B coef	0.049	0.023	0.150	0.164	-0.007	-0.008	-0.419	-0.396	0.010	0.069
Beta coef	0.080	0.038	0.048	0.052	-0.071	-0.072	-0.041	-0.039	0.004	0.025
p value	0.417	0.531	0.626	0.471	0.479	0.321	0.694	0.615*	0.968	0.687
AN Prot (%E)										
B coef	-0.032	-0.006	-0.137	-0.055	0.005	0.002	-0.511	-0.372	-0.100	-0.003
Beta coef	-0.202	-0.040	-0.172	-0.069	0.181	0.076	-0.195	-0.142	-0.144	-0.004
p value	**0.040**	0.525*	**0.081**	0.354	**0.068**[#1]	0.310	**0.058**	**0.063**	0.145	0.946

Analysis including outliers:

	#1	#2
N number	104	93
B coef	0.006	-2.084
Beta coef	0.205	-0.136
p value	**0.037**	**0.085**

TABLE OF RESULTS: D3 **Maternal protein intake at V2 vs fetal weight and size at US2.**

Protein intake V2	*FW2*		*BPD2*		*AC2*			*HC2*		*FL2*	
Adjusted for conf.	None	A,h,w,s	None	A,h,w,s	None	Age	A,h,w,s	None	A,h,w,s	None	A,h,w,s
N number	105	102	105	103	104	102	104	95	93	105	103
TOT Prot (%E)											
B coef	-0.346	-0.133	0.316	1.412	0.156	0.134	0.092	8.711	1.137	0.762	0.690
Beta coef	-0.060	-0.025	0.011	0.050	0.169	0.145	0.094	0.096	0.049	0.032	0.028
p value	0.523	0.683	0.909	0.485	0.087	**0.048**[#1]	0.192	0.357	0.619	0.750	0.649
PL Prot (%E)											
B coef	0.004	-0.002	0.032	0.010	0.000	0.001	0.001	0.054	0.012	0.016	-0.005
Beta coef	0.057	-0.024	0.103	0.031	0.001	0.050	0.049	0.054	0.049	0.059	-0.019
p value	0.567	0.739	0.295	0.669	0.990	0.496	0.513	0.607	0.624	0551	0.773
AN Prot (%E)											
B coef	0.006	0.015	-0.210	-0.176	-0.014	-0.016	-0.013	-1.185	-0.184	-0.163	-0.054
Beta coef	0.011	0.028	-0.076	-0.064	-0.149	-0.169	-0.140	-0.133	-0.081	-0.068	-0.023
p value	0.916	0.649	0.441	0.373	0.133	**0.021**[#2]	**0.054**	0.200	0.414	0.489	0.715

Analysis including outliers:

	#1	#2
N number	105	105
B coef	0.120	-0.012
Beta coef	0.124	-0.125
p value	**0.085**	**0.085**

TABLE OF RESULTS: D4 **Maternal protein intake at V1 vs birth parameters.**

Protein intake V1	*BW*		*BL*		*PONDIX*		*BW/PLW*	
Adjusted for conf.	None	A,h,w,s	None	A,h,w,s	None	A,h,w,s	None	A,h,w,s
N number	103	103	100	100	100	100	86, 88*	86
TOT Prot (%E)								
B coef	0.101	-0.003	2.728	0.270	0.007	0.002	-0.014	-0.008
Beta coef	0.179	-0.005	0.110	0.011	0.014	0.003	-0.037	-0.031
p value	**0.071**	0.953	0.278	0.909	0.889	0.978	0.738	0.781
PL Prot (%E)								
B coef	0.043	0.055	2.857	2.542	-0.005	-0.001	-0.012	-0.006
Beta coef	0.111	0.137	0.164	0.146	-0.010	-0.004	-0.044	-0.023
p value	0.264	**0.096**	0.102	0.116	0.900	0.970	0.690	0.839
AN Prot (%E)								
B coef	0.010	-0.009	0.084	-0.293	1.419	0.000	0.001	0.000
Beta coef	0.100	-0.093	0.020	-0.068	0.020	0.005	0.017	-0.002
p value	0.316	0.272	0.847	0.470	0.844	0.963	0.874*	0.984

TABLE OF RESULTS: D5 **Maternal protein intake at V2 vs birth parameters.**

Protein intake V2	*BW*			*BL*		*PONDIX*		*BW/PLW*	
Adjusted for conf.	None	A	A,h,w,s	None	A,h,w,s	None	A,h,w,s	None	A,h,w,s
N number	103	103	103	100	100	100	100	87	85,87*
TOT Prot (%E)									
B coef	-0.647	-0.716	-0.608	-13.142	-16.338	-0.116	-0.140	0.386	0.412
Beta coef	-0.187	-0.207	-0.171	-0.090	-0.111	-0.040	-0.045	0.134	0.181
p value	**0.059**	**0.021**[#1]	**0.038**[#2]	0.379	0.234	0.711	0.665	0.220	0.105
PL Prot (%E)									
B coef	-0.003	-0.003	0.001	-0.077	-0.049	-0.001	0.000	0.000	0.000
Beta coef	-0.066	-0.067	0.017	-0.050	-0.031	-0.010	-0.006	-0.008	-0.014
p value	0.504	0.456	0.841	0.637	0.748	0.894	0.956	0.940	0.903*
AN Prot (%E)									
B coef	0.049	0.052	0.037	0.835	1.153	1.414	0.011	-0.036	-0.037
Beta coef	0.144	0.147	0.106	0.058	0.080	0.033	0.036	0.134	-0.156
p value	0.147	0.101	0.205	0.566	0.393	0.741	0.730	0.220	0.164

Analysis including outliers:

	#1	#2
N number	104	104
B coef	-0.624	-0.575
Beta coef	-0.172	-0.159
p value	**0.054**	**0.062**

TABLE OF RESULTS: D6 **Maternal protein intake at V1 vs infant anthropometry (Ba1 and Ba2).**

Protein intake V1	*Weight 1*		*Length 1*		*BMI 1*		*Weight 2*		*Length 2*		*BMI 2*	
Adjusted for conf.	None	A, s	None	A, s	None	A, s	None	A, s	None	A, s	None	A, s
N number	77	76	77	77	76	77	73	73	72	73	72	72
TOT Prot (%E)												
B coef	351.1	621.4	-7.770	-0.072	0.071	0.093	0.120	0.129	0.844	1.057	-0.016	-0.016
Beta coef	0.059	0.104	-0.263	-0.050	0.291	0.353	0.210	0.227	0.030	0.035	-0.288	-0.287
p value	0.613	0.238	**0.021**	0.635	**0.011**[#1]	**0.001**	**0.074**	**0.038**	0.801	0.754	**0.014**	**0.012**
PL Prot (%E)												
B coef	838.0	517.5	1.529	-0.362	0.036	0.027	0.013	0.020	0.402	2.027	0.000	0.001
Beta coef	0.199	0.123	0.072	-0.017	0.206	0.143	0.031	0.047	0.020	0.092	0.009	0.019
p value	**0.084**	0.159	0.535	0.870	**0.074**[#2]	0.203	0.798	0.675	0.871	0.416	0.941	0.871
AN Prot (%E)												
B coef	-44.68	79.86	-1.430	-0.998	0.007	0.013	0.015	0.016	0.078	-0.023	-0.002	-0.002
Beta coef	-0.042	0.075	-0.273	-0.191	0.158	0.275	0.154	0.170	0.016	-0.005	-0.231	-0.238
p value	0.716	0.400	**0.016**	**0.062**	0.172[#3]	**0.012**	0.193	0.122	0.891	0.968	**0.051**	**0.039**

Analysis including outliers:

	#1	#2	#3
N number	77	77	77
B coef	0.086	0.036	0.010
Beta coef	0.328	0.188	0.209
p value	**0.004**	0.101	**0.068**

TABLE OF RESULTS: D7 **Maternal protein intake at V2 vs infant anthropometry (Ba1 and Ba2).**

Protein intake V2	*Weight 1*		*Length 1*		*BMI 1*		*Weight 2*		*Length 2*		*BMI 2*	
Adjusted for conf.	None	A, s	None	A, s	None	A, s	None	A, s	None	A, s	None	A, s
N number	76	76	76	76	75	76	72	72	71	72	71	71
TOT Prot (%E)												
B coef	-2177.3	517.1	-51.194	-44.737	0.327	0.398	0.139	0.452	-17.444	2.351	-0.025	-0.042
Beta coef	-0.059	0.014	-0.289	-0.252	0.221	0.247	0.039	0.128	-0.104	0.013	-0.073	-0.124
p value	0.610	0.876	**0.012**	**0.014**	**0.057**[#1]	**0.026**	0.743	0.262	0.389	0.911	0.548	0.301
PL Prot (%E)												
B coef	14.887	-22.845	0.513	0.408	-0.002	-0.005	-0.006	-0.009	0.046	-0.090	0.001	0.001
Beta coef	0.039	-0.060	0.277	0.220	-0.142	-0.283	-0.166	-0.238	0.026	-0.047	0.218	0.269
p value	0.739	0.510	**0.016**	**0.032**	0.225[#2]	**0.010**	0.164	**0.033**	0.833	0.683	**0.069**	**0.021**
AN Prot (%E)												
B coef	-38.900	54.484	0.905	1.309	-0.017	-0.008	0.012	0.006	0.548	-0.177	-0.002	-0.002
Beta coef	-0.011	0.016	0.053	0.077	-0.119	-0.050	0.036	0.018	0.033	-0.010	-0.064	-0.059
p value	0.924	0.862	0.649	0.453	0.309	0.656	0.763	0.873	0.782	0.929	0.595	0.615

Analysis including outliers:

	#1	#2
N number	76	76
B coef	0.309	-0.004
Beta coef	0.191	-0.216
p value	**0.098**	**0.061**

TABLE OF RESULTS: D8 **Maternal protein intake at V1 vs infant glucose, HOMA index and metabolics (Ba1).**

Protein intake V1	*Glc F 1*		*Ins F 1*		*PI F 1*		*HOMA-IR 1*		*Leptin 1*		*IGF BP3 (1)*	
Adjusted for conf.	None	A, s, w	None	A, s, w	None	A, s, w	None	A, s, w	None	A, s, w	None	A, s, w
N number	77	75	76	76	76	76	75,74*	75,74*	76	76	76,77*	76,77*
TOT Prot (%E)												
B coef	-0.186	-0.036	-0.037	-0.037	-0.184	-0.246	0.610	0.612	0.164	-0.054	707.9	558.6
Beta coef	-0.039	-0.008	-0.346	-0.348	-0.112	-0.140	0.379	0.381	0.021	-0.007	0.231	0.182
p value	0.738	0.945	**0.002**	**0.003**	0.337	0.237	**0.001**	**0.001**	0.859	0.933	**0.045**[#1]	**0.095**[#2]
PL Prot (%E)												
B coef	0.367	0.182	-0.006	-0.005	0.072	0.059	0.204	0.169	0.782	-0.125	-69.10	-226.6
Beta coef	0.106	0.055	-0.081	-0.063	0.061	0.047	0.181	0.149	0.138	-0.022	-0.029	-0.096
p value	0.359	0.627	0.489	0.599	0.603	0.700	0.124*	0.204*	0.234	0.788	0.800*	0.391*
AN Prot (%E)												
B coef	-0.107	-0.059	-0.006	-0.006	-0.048	-0.059	0.092	0.100	-0.067	0.003	136.2	133.6
Beta coef	-0.125	-0.073	-0.307	-0.323	-0.165	-0.188	0.318	0.345	-0.048	0.002	0.236	0.231
p value	0.277	0.516	**0.007**	**0.006**	0.154	0.115	**0.005**	**0.003**	0.680	0.981	**0.039***	**0.036***

Analysis including outliers:

	#1	#2
N number	77	77
B coef	673.7	552.3
Beta coef	0.207	0.169
p value	**0.071**	0.123

TABLE OF RESULTS: D9 **Maternal protein intake at V2 vs infant glucose, HOMA index and metabolics (Ba1).**

Protein intake V2	*Glc F 1*		*Ins F 1*		*PI F 1*		*HOMA-IR 1*		*Leptin 1*		*IGF BP3 (1)*	
Adjusted for conf.	None	A, s, w	None	A, s, w	None	A, s, w	None	A, s, w	None	A, s, w	None	A, s, w
N number	76	75	75	75	76	76	74,73*	73	75	74	75,76*	76
TOT Prot (%E)												
B coef	2.319	3.436	-0.075	-0.095	-1.036	-0.989	1.166	1.137	6.370	4.712	-1290.0	-918.9
Beta coef	0.079	0.117	-0.114	-0.145	-0.103	-0.098	0.118	0.122	0.132	0.097	-0.064	-0.049
p value	0.500	0.309	0.330	0.228	0.376	0.416	0.317	0.300	0.260	0.232	0.581*	0.662
PL Prot (%E)												
B coef	0.048	0.039	0.002	0.002	0.002	0.001	-0.024	-0.023	-0.028	-0.008	-41.48	-39.31
Beta coef	0.155	0.126	0.298	0.329	0.021	0.011	-0.233	-0.237	-0.056	-0.017	-0.211	-0.188
p value	0.180	0.272	**0.010**	**0.005**	0.854	0.928	**0.046**	**0.042**[#1]	0.632	0.840	**0.070**[#2]	**0.093**
AN Prot (%E)												
B coef	-0.537	-0.516	-0.006	-0.006	0.031	0.031	0.045	0.039	-0.385	-0.249	365.7	361.9
Beta coef	-0.190	-0.183	-0.101	-0.098	0.032	0.032	0.051	0.041	-0.083	-0.054	0.190	0.188
p value	0.100	0.103	0.389	0.407	0.785	0.790	0.671*	0.727	0.480	0.505	0.100*	**0.085**

Analysis including outliers:

	#1	#2
N number	74	76
B coef	-0.028	-39.16
Beta coef	-0.269	-0.189
p value	**0.022**	0.103

TABLE OF RESULTS: D10 **Maternal protein intake at V1 vs infant glucose, HOMA index and metabolics (Ba2).**

Protein intake V1	***Glc F 2***		***Ins F 2***		***PI F 2***		***HOMA-IR 2***		***Leptin 2***		***IGF BP3 (2)***	
Adjusted for conf.	None	A, s, w	None	A, s, w	None	A, s, w	None	A, s, w	None	A, s, w	None	A, s, w
N number	74	73	72	71	74	72	75	71	74	73	73,74*	73
TOT Prot (%E)												
B coef	-0.460	-0.529	0.193	0.159	0.139	-0.010	0.610	0.257	0.139	-0.153	618.3	170.5
Beta coef	-0.081	-0.093	0.259	0.211	0.076	-0.006	0.379	0.158	0.058	-0.065	0.115	0.031
p value	0.495	0.457	**0.028**	**0.078**	0.519	0.963	**0.001**	0.190	0.621	0.524	0.330*	0.786
PL Prot (%E)												
B coef	0.279	0.269	-0.019	-0.026	-0.321	-0.355	0.204	-0.016	-0.006	-0.040	-481.8	-713.2
Beta coef	0.066	0.064	-0.034	-0.046	-0.237	-0.262	0.181	-0.013	-0.004	-0.023	-0.129	-0.178
p value	0.578	0.601	0.777	0.696	**0.042**	**0.021**	0.124	0.911	0.976	0.817	0.277	0.112
AN Prot (%E)												
B coef	-0.073	-0.077	0.032	0.027	0.045	0.028	0.092	0.044	0.020	-0.019	130.7	98.40
Beta coef	-0.075	-0.080	0.254	0.211	0.145	0.089	0.318	0.160	0.048	-0.048	0.153	0.107
p value	0.528	0.516	**0.031**	**0.075**	0.217	0.443	**0.005**	0.178	0.684	0.633	0.197	0.347

TABLE OF RESULTS: D11 **Maternal protein intake at V2 vs infant glucose, HOMA index and metabolics (Ba2).**

Protein intake V2	*Glc F 2*		*Ins F 2*		*PI F 2*		*HOMA-IR 2*		*Leptin 2*		*IGF BP3 (2)*	
Adjusted for conf.	None	A, s, w	None	A, s, w	None	A, s, w	None	A, s, w	None	A, s, w	None	A, s, w
N number	73	72	71	70	72,73*	71	74,73*	70	73	72	73,72*	72
TOT Prot (%E)												
B coef	0.138	0.630	-0.201	-0.562	1.246	1.087	1.166	-1.048	2.256	1.508	-5201.1	-7972.4
Beta coef	0.004	0.018	-0.045	-0.120	0.115	0.097	0.118	-0.103	0.158	0.103	-0.172	-0.236
p value	0.973	0.887	0.712	0.328	0.332*	0.417	0.317	0.398	0.183	0.315	0.149*	**0.040**
PL Prot (%E)												
B coef	0.019	0.025	-0.019	-0.016	-0.032	-0.007	-0.024	-0.029	-0.024	0.001	-62.58	-47.20
Beta coef	0.050	0.069	-0.379	-0.318	-0.273	-0.060	-0.233	-0.268	-0.152	0.007	-0.178	-0.134
p value	0.675	0.591	**0.001**	**0.008**	**0.020**[#1]	0.620	**0.046**	**0.027**	0.199	0.948	0.131	0.254
AN Prot (%E)												
B coef	-0.239	-0.294	0.113	0.118	-0.038	-0.057	0.045	0.202	-0.123	-0.165	839.3	860.8
Beta coef	-0.071	-0.088	0.256	0.264	-0.036	-0.053	0.051	0.210	-0.088	-0.118	0.263	0.267
p value	0.551	0.473	**0.031**	**0.024**	0.763*	0.646	0.671*	**0.073**	0.460	0.233	**0.024**	**0.016**

Analysis including outliers:

	#1
N number	73
B coef	-0.016
Beta coef	-0.138
p value	0.246

APPENDIX E. Maternal fat intake analysis.

TABLE OF RESULTS: E1 **Maternal dietary fat intake at V1 vs fetal weight and size at US1.**

FAT Intake V1	*FW1*		*BPD1*		*AC1*		*HC1*		*FL1*	
Adjusted for conf.	None	A,h,w,s	None	A,h,w,s	None	A,h,w,s	None	A,h,w,s	None	A,h,w,s
N number	105,106*	104	106	104	105	102	97	93	106	103
Total Fat (%E)										
B coef	-0.042	0.010	-0.011	-0.002	0.000	0.000	-0.034	0.000	-0.006	0.000
Beta coef	-0.094	0.023	-0.144	-0.032	0.136	0.008	-0.134	0.000	-0.100	-0.001
p value	0.340*	**0.074**	0.140	0.178	0.166	0.807	0.193	0.990	0.309	0.983
SAFA (% E)										
B coef	-7.389	0.721	-1.158	-0.131	0.029	0.002	-4.372	-0.541	-0.853	0.086
Beta coef	-0.232	0.022	-0.214	-0.024	0.227	0.014	-0.225	-0.029	-0.184	0.019
p value	**0.018**[#1]	0.106	**0.028**	0.369	**0.020**[#2]	0.675	**0.026**	0.174	**0.060**	0.475
MUFA (% E)										
B coef	-2.401	0.765	-0.596	-0.164	0.014	-0.002	-2.082	-0.289	-0.317	0.119
Beta coef	-0.079	0.025	-0.119	-0.032	0.118	-0.012	-0.113	-0.016	-0.074	0.027
p value	0.420*	**0.052**	0.225	0.181	0.232	0.686	0.269	0.426	0.453	0.262
PUFA (% E)										
B coef	0.635	0.207	-0.030	-0.074	-0.002	-0.001	0.381	0.227	0.069	-0.009
Beta coef	0.036	0.015	-0.010	-0.026	-0.023	-0.007	0.037	0.022	0.027	-0.004
p value	0.717*	0.229	0.917	0.288	0.815	0.826	0.718	0.273	0.780	0.884

Analysis including outliers:

	#1	#2
N number	106	106
B coef	-5.866	0.026
Beta coef	-0.179	0.196
p value	**0.066**	**0.044**

TABLE OF RESULTS: E2 **Maternal dietary fat intake at V1 vs fetal weight and size at US2.**

FAT Intake V1	*FW2*		*BPD2*		*AC2*		*HC2*		*FL2*	
Adjusted for conf.	None	A,h,w,s	None	A,h,w,s	None	A,h,w,s	None	A,h,w,s	None	A,h,w,s
N number	103,104*	101,102*	104	102	103,104*	102	95	93	103,104*	102
Total Fat (%E)										
B coef	0.001	0.000	0.003	-0.007	0.000	0.000	0.025	-0.005	0.013	0.001
Beta coef	0.137	-0.061	0.057	-0.134	0.137	0.029	0.154	-0.028	0.308	0.023
p value	0.165*	0.408*	0.563	**0.073**	0.167*	0.702	0.134	0.722	**0.002**[#5]	0.725
SAFA (% E)										
B coef	-0.035	-0.060	-0.033	-0.396	0.008	0.017	0.019	-0.502	0.019	-0.088
Beta coef	-0.050	-0.086	-0.009	-0.108	0.064	0.135	0.002	-0.042	0.006	-0.028
p value	0.614*	0.182[#2]	0.926	0.154	0.519	**0.077**	0.988	0.600	0.950*	0.677
MUFA (% E)										
B coef	0.110	0.023	0.292	-0.294	-0.014	-0.002	1.694	0.006	0.741	0.216
Beta coef	0.168	0.035	0.088	-0.085	-0.127	-0.013	0.152	0.001	0.255	0.071
p value	**0.089***	0.538	0.377	0.256	0.200	0.866	0.141	0.995	**0.010***	0.268
PUFA (% E)										
B coef	0.102	0.034	0.201	-0.095	-0.017	-0.008	1.302	0.173	0.455	0.088
Beta coef	0.272	0.091	0.103	-0.049	-0.254	-0.121	0.202	0.027	0.267	0.052
p value	**0.006**[#1]	0.150[#3]	0.299	0.522	**0.010**[#4]	0.109	**0.049**	0.734	**0.006***	0.430

Analysis including outliers:

	#1	#2	#3	#4	#5
N number	104	102	102	104	104
B coef	0.088	-0.098	0.025	-0.018	0.011
Beta coef	0.229	-0.136	0.064	-0.267	0.253
p value	**0.020**	**0.066**	0.388	**0.006**	**0.010**

TABLE OF RESULTS: E3 **Maternal dietary fat intake at V1 vs fetal weight and size at US2.**

FAT Intake V2	*FW2*		*BPD2*		*AC2*		*HC2*		*FL2*	
Adjusted for conf.	None	A,h,w,s	None	A,h,w,s	None	A,h,w,s	None	A,h,w,s	None	A,h,w,s
N number	105	103,102*	105	103	105,104*	103	95	92,93*	105	103
Total Fat (%E)										
B coef	-0.001	0.000	-0.007	-0.001	0.000	0.000	-0.016	0.001	-0.002	0.002
Beta coef	-0.093	0.019	-0.123	-0.019	0.120	0.002	-0.094	0.007	-0.051	0.051
p value	0.343	0.795	0.212	0.794	0.224	0.981	0.367	0.933*	0.604	0.435
SAFA (% E)										
B coef	-0.013	-0.008	-0.089	-0.091	0.000	0.001	-0.494	-0.498	-0.009	-0.034
Beta coef	-0.068	-0.042	-0.093	-0.095	0.003	0.032	-0.162	-0.167	-0.012	-0.042
p value	0.488	0.493*	0.344	0.189	0.976*	0.661	0.118	**0.029**[#1]	0.907	0.512
MUFA (% E)										
B coef	-0.001	0.001	-0.004	0.007	0.000	0.000	-0.016	0.006	0.002	0.012
Beta coef	-0.027	0.046	-0.026	0.042	0.058	-0.020	-0.032	0.013	0.013	0.794
p value	0.786	0.515	0.790	0.564	0.556	0.781	0.760	0.873*	0.894	0.168
PUFA (% E)										
B coef	-0.019	0.027	-0.182	0.102	0.006	-0.005	0.033	0.751	-0.097	0.165
Beta coef	-0.041	0.065	-0.077	0.043	0.067	-0.060	0.004	0.099	-0.050	0.081
p value	0.675	0.294	0.434	0.553	0.500	0.413	0.967	0.201*	0.615	0.199

Analysis including outliers:

	#1
N number	93
B coef	-0.382
Beta coef	-0.127
p value	0.105

TABLE OF RESULTS: E4 **Maternal dietary fat intake at V1 vs birth parameters.**

FAT Intake V1	*BW*		*BL*		*PONDIX*		*BW/PLW*	
Adjusted for conf.	None	A,h,w,s	None	A,h,w,s	None	A,h,w,s	None	A,h,w,s
N number	103,104*	103,104*	100	100	100	100	86,88*	86,88*
Total Fat (%E)								
B coef	-0.001	-0.001	-0.021	-0.004	-0.001	-0.001	0.000	0.000
Beta coef	-0.152	-0.100	-0.079	-0.016	-0.086	-0.094	-0.007	-0.036
p value	0.123*	0.268*	0.438	0.864	0.396	0.370	0.947*	0.747*
SAFA (% E)								
B coef	-0.007	-0.069	-1.699	-3.251	0.013	0.000	-0.017	0.020
Beta coef	-0.007	-0.150	0.084	-0.160	0.030	0.000	-0.054	-0.063
p value	0.944	**0.084**[#1]	0.408	**0.099**	0.768	0.999	0.624	0.592
MUFA (% E)								
B coef	-0.140	-0.059	-1.483	-0.750	-0.017	-0.020	0.004	-0.008
Beta coef	-0.140	-0.132	-0.081	-0.041	-0.043	-0.051	0.015	-0.027
p value	0.158	0.113	0.422	0.663	0.670	0.628	0.892	0.813
PUFA (% E)								
B coef	-0.112	-0.001	0.459	1.096	-0.022	-0.022	-0.018	-0.023
Beta coef	-0.112	-0.004	0.045	0.104	-0.101	-0.099	-0.108	-0.136
p value	0.261	0.968	0.657	0.267	0.318	0.345	0.324	0.230

Analysis including outliers:

	#1
N number	104
B coef	-0.066
Beta coef	-0.139
p value	0.122

TABLE OF RESULTS: E5 **Maternal dietary fat intake at V2 vs birth parameters.**

FAT Intake V2	*BW*		*BL*		*PONDIX*		*BW/PLW*	
Adjusted for conf.	None	A,h,w,s	None	A,h,w,s	None	A,h,w,s	None	A,h,w,s
N number	104,103*	103,104*	100	100	100	100	85,87*	85,87*
Total Fat (%E)								
B coef	0.000	0.000	0.012	0.008	0.000	0.000	-0.001	-0.001
Beta coef	0.031	0.006	0.041	0.027	-0.007	-0.022	-0.118	-0.103
p value	0.755	0.946*	0.688	0.779	0.944	0.841	0.283	0.359
SAFA (% E)								
B coef	-0.001	-0.012	-0.170	-0.201	-0.001	-0.002	-0.014	-0.014
Beta coef	-0.012	-0.102	-0.034	-0.040	-0.009	-0.020	-0.151	-0.154
p value	0.903*	0.226	0.737	0.670	0.933	0.849	0.164*	0.174*
MUFA (% E)								
B coef	0.001	0.000	-0.011	0.000	0.002	0.001	-0.002	0.002
Beta coef	0.047	0.005	-0.014	-0.001	0.096	0.061	-0.100	-0.113
p value	0.636	0.956	0.888	0.997	0.343	0.567	0.357*	0.318*
PUFA (% E)								
B coef	0.020	0.010	1.183	0.888	1.421	-0.001	0.008	0.001
Beta coef	0.070	0.032	0.094	0.070	0.007	-0.003	0.039	0.053
p value	0.483*	0.697	0.354	0.449	0.945	0.979	0.725	0.636

TABLE OF RESULTS: E6 **Maternal dietary fat intake at V1 vs infant anthropometry (Ba1 and Ba2).**

FAT Intake V1	*Weight 1*		*Length 1*		*BMI 1*		*Weight 2*		*Length 2*		*BMI 2*	
Adjusted for conf.	None	A,s	None	A,s	None	A,s	None	A,s	None	A,s	None	A,s
N number	76,77*	77	77	77	76,77*	77	73	73	73	73	72	72
Total Fat (%E)												
B coef	-11.19	-5.476	0.022	0.034	-0.001	-0.001	0.000	0.000	-0.029	-0.039	0.000	0.000
Beta coef	-0.181	-0.084	0.069	0.107	-0.241	-0.217	-0.029	-0.033	-0.085	-0.115	-0.040	-0.058
p value	0.118	0.340	0.550	0.293	**0.035***	**0.047**	0.810	0.766	0.477	0.307	0.740	0.622
SAFA (% E)												
B coef	-852.9	-203.7	-4.452	-1.915	-0.018	0.002	0.080	0.063	-0.722	-1.954	-0.012	-0.011
Beta coef	-0.174	-0.042	-0.185	-0.079	-0.091	0.011	0.176	0.138	-0.033	-0.082	-0.269	-0.254
p value	0.130*	0.650	0.108	0.451	0.436	0.922	0.135	0.211	0.786	0.465	**0.022**	**0.027**
MUFA (% E)												
B coef	-648.7	-317.6	-0.190	-0.119	-0.018	-0.020	-0.030	-0.044	-5.776	-6.373	-0.005	-0.004
Beta coef	-0.147	-0.070	-0.008	-0.005	-0.090	-0.099	-0.066	-0.099	-0.246	-0.271	-0.113	-0.095
p value	0.205	0.433	0.942	0.959	0.435*	0.372	0.578	0.371	**0.036**	**0.014**	0.344	416.0
PUFA (% E)												
B coef	-15.46	4.374	3.474	3.535	-0.025	-0.029	-0.016	-0.007	1.014	0.858	0.003	0.002
Beta coef	-0.006	0.002	0.270	0.275	-0.233	-0.252	-0.063	-0.029	0.083	0.066	0.140	0.102
p value	0.959*	0.985	**0.018**	**0.006**	**0.043**[#1]	**0.022**	0.598	0.794	0.487	0.562	0.241	0.385

Analysis including outliers:

	#1
N number	77
B coef	-0.029
Beta coef	-0.256
p value	**0.025**

TABLE OF RESULTS: E7 **Maternal dietary fat intake at V2 vs infant anthropometry (Ba1 and Ba2).**

FAT Intake V2	*Weight 1*		*Length 1*		*BMI 1*		*Weight 2*		*Length 2*		*BMI 2*	
Adjusted for conf.	None	A,s	None	A,s	None	A,s	None	A,s	None	A,s	None	A,s
N number	76,75*	76	76	76	75,76*	76	72	72	72,71*	72	71	71
Total Fat (%E)												
B coef	-1.763	1.373	-0.104	-0.101	0.001	0.001	0.001	0.001	-0.064	-0.028	0.000	0.000
Beta coef	-0.026	0.021	-0.319	-0.310	0.262	0.309	0.106	0.204	-0.207	-0.084	-0.279	-0.339
p value	0.822	0.822	**0.005**	**0.002**	**0.023***	**0.005**	0.374	**0.072**	**0.083**[#2]	0.471	**0.018**	**0.004**
SAFA (% E)												
B coef	-125.2	-42.60	-1.244	-0.976	0.001	0.005	0.002	0.009	-1.203	-0.822	-0.003	-0.003
Beta coef	-0.105	-0.036	-0.215	-0.168	0.022	0.093	0.018	0.080	-0.202	-0.138	-0.246	-0.277
p value	0.369	0.691	**0.063**	**0.099**	0.855	0.402	0.883	0.482	**0.089**	0.229	**0.038**	**0.018**
MUFA (% E)												
B coef	-6.282	0.938	-0.175	-0.162	0.001	0.001	0.001	0.003	-0.084	-0.053	0.000	0.000
Beta coef	-0.033	0.005	-0.188	-0.174	0.143	0.176	0.067	0.138	-0.096	-0.056	-0.177	-0.226
p value	0.779	0.957	0.103	**0.090**	0.217*	0.115	0.575	0.220	0.426*	0.624	0.140	**0.055**
PUFA (% E)												
B coef	418.4	317.5	-3.582	-3.804	0.038	0.051	0.059	0.084	-1.843	0.435	-0.008	-0.009
Beta coef	0.143	0.104	-0.241	-0.256	0.297	0.377	0.193	0.275	-0.127	0.028	-0.262	-0.305
p value	0.220*	0.251	**0.036**	**0.012**	**0.010**[#1]	**0.001**	0.105	**0.014**	0.291*	0.807	**0.027**	**0.009**

Analysis including outliers:

	#1	#2
N number	76	71
B coef	0.047	-0.057
Beta coef	0.349	-0.173
p value	**0.002**	0.148

TABLE OF RESULTS: E8 **Maternal dietary fat intake at V1 vs infant glucose, HOMA index and metabolics (Ba1).**

FAT Intake V1	*Glc F 1*		*Ins F 1*		*PI F 1*		*HOMA-IR 1*		*Leptin 1*		*IGF BP3 (1)*	
Adjusted for conf.	None	A, s,w	None	A, s,w	None	A, s,w	None	A, s,w	None	A, s,w	None	A, s,w
N number		75			32		25	25				43
Total Fat (%E)												
B coef	-0.002	-0.004	0.000	0.000	0.000	0.000	-0.005	-0.004	0.005	0.010	-2.472	-0.620
Beta coef	-0.036	-0.071	0.263	0.249	0.004	0.004	-0.271	-0.245	0.060	0.116	-0.070	-0.019
p value	0.758	0.524	**0.022**	**0.033**	0.974	0.976	**0.019**	**0.034**	0.608	0.147	0.547	0.866
SAFA (% E)												
B coef	-0.627	-0.485	0.003	0.002	-0.136	-0.187	-0.140	-0.099	-0.604	0.600	236.7	377.8
Beta coef	-0.161	-0.130	0.037	0.022	-0.101	-0.130	-0.113	-0.080	-0.095	0.093	0.089	0.142
p value	0.161	0.258	0.753	0.857	0.385	0.287	0.337	0.505	0.414	0.258	0.443	0.211
MUFA (% E)												
B coef	-0.149	-0.375	0.006	0.006	-0.168	-0.188	-0.112	-0.087	0.396	0.875	3.117	59.00
Beta coef	-0.041	-0.104	0.068	0.070	-0.125	-0.141	-0.090	-0.074	0.066	0.142	0.001	0.025
p value	0.725	0.350	0.560	0.554	0.278	0.237	0.443[#1]	0.522	0.573	**0.073**	0.991	0.819
PUFA (% E)												
B coef	-0.056	-0.169	0.013	0.013	0.024	0.059	-0.225	-0.224	0.460	0.169	-207.8	-210.5
Beta coef	-0.027	-0.084	0.287	0.282	0.033	0.077	-0.319	-0.319	0.135	0.049	-0.146	-0.148
p value	0.818	0.456	**0.012**	**0.015**	0.777	0.517	**0.005**	**0.006**[#2]	0.244	0.544	0.205	0.179

Analysis including outliers:

	#1	#2
N number		
B coef	-0.092	-0.194
Beta coef	-0.079	-0.293
p value	0.502	**0.010**

TABLE OF RESULTS: E9 **Maternal dietary fat intake at V2 vs infant glucose, HOMA index and metabolics (Ba1).**

FAT Intake V2	*Glc F 1*		*Ins F 1*		*PI F 1*		*HOMA-IR 1*		*Leptin 1*		*IGF BP3 (1)*	
Adjusted for conf.	None	A, s,w	None	A, s,w	None	A, s,w	None	A, s,w	None	A, s,w	None	A, s,w
N number											43	43
Total Fat (%E)												
B coef	-0.010	-0.004	0.000	0.000	-0.004	-0.004	0.005	0.005	0.013	0.005	6.351	6.656
Beta coef	-0.179	-0.223	-0.327	-0.364	-0.223	-0.223	0.253	0.287	0.142	0.057	0.173	0.181
p value	0.121	**0.063**	**0.004**	**0.002**	**0.053**	**0.063**	**0.029**	**0.014**	0.226	0.486	0.135[#2]	0.105[#3]
SAFA (% E)												
B coef	-0.169	-0.031	-0.003	-0.003	-0.030	-0.031	0.019	0.030	0.021	0.050	94.52	101.7
Beta coef	-0.176	-0.093	-0.128	-0.155	-0.092	-0.093	0.057	0.093	0.014	0.032	0.144	0.165
p value	0.129	0.437	0.275	0.193	0.427	0.438	0.627	0.430	0.908	0.697	0.214	0.133
MUFA (% E)												
B coef	-0.031	-0.004	-0.001	-0.001	-0.004	-0.004	0.011	0.013	0.023	0.007	26.87	28.22
Beta coef	-0.020	-0.071	-0.312	-0.340	-0.076	-0.071	0.221	0.249	0.093	0.028	0.256	0.269
p value	**0.082**	0.556	**0.007**	**0.004**	0.516	0.556	**0.058**	**0.034**	0.426	0.736	**0.026**	**0.014**
PUFA (% E)												
B coef	-0.210	-0.186	-0.018	-0.019	-0.188	-0.186	0.261	0.267	0.716	0.187	-0.343	-79.98
Beta coef	-0.085	-0.220	-0.335	-0.355	-0.223	-0.220	0.314	0.322	0.178	0.046	0.000	-0.048
p value	0.464	**0.068**	**0.003**	**0.003**	**0.053**	**0.068**	**0.006**	**0.006**	0.127	0.575	0.999	0.674

Analysis including outliers:

	#1	#2	#3
N number			
B coef	713.1	7.048	7.070
Beta coef	0.254	0.204	0.205
p value	**0.052**	**0.080**	**0.065**

TABLE OF RESULTS: E10 **Maternal dietary fat intake at V1 vs infant glucose, HOMA index and metabolics (Ba2).**

FAT Intake V1	*Glc F 2*		*Ins F 2*		*PI F 2*		*HOMA-IR 2*		*Leptin 2*		*IGF BP3 (2)*	
Adjusted for conf.	None	A,s,w	None	A,s,w	None	A,s,w	None	A,s,w	None	A,s,w	None	A,s,w
N number												
Total Fat (%E)												
B coef	-0.002	-0.001	-0.002	-0.002	-0.001	0.000	-0.004	-0.004	0.002	0.003	-2.967	-1.700
Beta coef	-0.032	-0.011	-0.214	-0.224	-0.026	-0.014	-0.212	-0.214	0.090	0.116	-0.049	-0.028
p value	0.790	0.928	**0.071**	**0.056**	0.830	0.905	**0.074**	**0.068**	0.447	0.239	0.682	0.803
SAFA (% E)												
B coef	-0.689	-0.657	0.002	-0.015	0.157	0.081	-0.076	-0.105	-0.019	-0.108	412.1	123.7
Beta coef	-0.150	-0.144	0.003	-0.025	0.108	0.055	-0.058	-0.080	-0.010	-0.057	0.095	0.029
p value	0.201	0.239	0.983	0.832	0.362	0.639	0.628	0.502	0.932	0.565	0.419	0.802
MUFA (% E)												
B coef	-0.077	0.022	-0.091	-0.072	-0.213	-0.185	-0.197	-0.150	-0.046	0.124	-182.62	-113.40
Beta coef	-0.017	0.005	-0.150	-0.118	-0.149	-0.128	-0.151	-0.115	-0.024	0.067	-0.043	-0.027
p value	0.886	0.968	0.208	0.314	0.204	0.266	0.206	0.328	0.837	0.500	0.717	0.814
PUFA (% E)												
B coef	0.135	0.162	-0.072	-0.082	-0.071	-0.063	-0.130	-0.147	0.105	0.097	-296.8	-296.8
Beta coef	0.054	0.065	-0.217	-0.247	-0.089	-0.078	-0.182	-0.206	0.101	0.094	-0.125	-0.125
p value	0.648	0.596	**0.068**	**0.036**	0.450	0.503	0.126	**0.082**	0.394	0.342	0.287	0.287

TABLE OF RESULTS: E11 **Maternal dietary fat intake at V2 vs infant glucose, HOMA index and metabolics (Ba2).**

FAT Intake V2	*Glc F 2*		*Ins F 2*		*PI F 2*		*HOMA-IR 2*		*Leptin 2*		*IGF BP3 (2)*	
Adjusted for conf.	None	A,s,w	None	A,s,w	None	A,s,w	None	A,s,w	None	A,s,w	None	A,s,w
N number					48		56					
Total Fat (%E)												
B coef	-0.008	-0.010	0.002	0.002	0.001	-0.001	0.004	0.003	0.005	0.002	1.694	-1.500
Beta coef	-0.127	-0.151	0.276	0.225	0.034	-0.037	0.205	0.152	0.184	0.077	0.028	-0.025
p value	0.283	0.238	**0.020**	**0.067**	0.777	0.764	**0.086**	0.220	0.119	0.459	0.814	0.834
SAFA (% E)												
B coef	-0.208	-0.266	0.025	0.020	0.004	-0.015	0.029	0.013	-0.014	-0.059	10.63	2.700
Beta coef	-0.182	-0.233	0.169	0.134	0.011	-0.041	0.088	0.038	-0.029	-0.125	0.010	0.003
p value	0.124	**0.057**	0.160	0.265	0.927	0.731	0.466	0.751	0.808	0.214	0.935	0.983
MUFA (% E)												
B coef	-0.009	-0.014	0.008	0.007	0.003	0.000	0.014	0.012	0.009	0.000	9.473	6.100
Beta coef	-0.052	-0.075	0.321	0.272	0.058	0.000	0.276	0.224	0.112	-0.001	0.055	0.036
p value	0.663	0.545	**0.006**	**0.022**	0.625[#1]	0.998	**0.020**[#2]	**0.060**	0.346	0.995	0.641	0.756
PUFA (% E)												
B coef	0.060	0.102	0.051	0.021	0.061	-0.011	0.107	0.052	0.392	0.291	23.53	-268.4
Beta coef	0.020	0.033	0.133	0.052	0.066	-0.011	0.128	0.059	0.319	0.229	0.008	-0.092
p value	0.865	0.798	0.269	0.678	0.578	0.927	0.290	0.638	**0.006**	**0.028**	0.944	0.446

Analysis including outliers:

	#1	#2
N number		
B coef	0.013	0.015
Beta coef	0.217	0.332
p value	**0.068**	**0.005**

TABLE OF RESULTS: E12 **Maternal dietary fat intake at V3 vs infant glucose, HOMA index and metabolics (Ba2).**

FAT Intake V3	*Glc F 2*		*Ins F 2*		*PI F 2*		*HOMA-IR 2*		*Leptin 2*		*IGF BP3 (2)*	
Adjusted for conf.	None	A, s,w	None	A, s,w	None	A, s,w	None	A, s,w	None	A, s,w	None	A, s,w
N number											76	
Total Fat (%E)												
B coef	0.009	0.008	-0.001	-0.001	0.001	0.001	0.000	0.000	0.001	0.000	-8.790	-7.100
Beta coef	0.135	0.117	-0.063	-0.056	0.042	0.042	-0.010	-0.011	0.023	14.00	-0.153	-0.114
p value	0.251	0.335	0.602	0.633	0.725	0.715	0.937	0.924	0.848	0.891	0.196	0.308
SAFA (% E)												
B coef	0.009	0.003	0.002	0.002	0.119	0.002	0.006	0.005	0.015	0.013	-24.15	-14.00
Beta coef	0.045	0.013	0.088	0.090	0.030	0.024	0.098	0.087	0.171	0.149	-0.134	-0.071
p value	0.702	0.916	0.460	0.444	0.801	0.833	0.412	0.458	0.146	0.125	0.258	0.524
MUFA (% E)												
B coef	0.012	0.009	0.000	0.001	0.003	0.002	0.002	0.002	-0.005	-0.006	-5.029	-5.500
Beta coef	0.063	0.049	0.013	0.024	0.045	0.033	0.031	0.036	-0.068	-0.073	-0.032	-0.032
p value	0.592	0.688	0.917	0.841	0.703	0.773	0.799	0.764	0.567	0.456	0.791	0.773
PUFA (% E)												
B coef	0.014	0.014	-0.002	-0.002	0.001	0.001	-0.003	-0.003	-0.001	-0.001	-13.46	-12.10
Beta coef	0.138	0.133	-0.163	-0.159	0.020	0.029	-0.100	-0.099	-0.025	-0.024	-0.136	-0.122
p value	0.241	0.272	0.171	0.173	0.869	0.802	0.403	0.399	0.834	0.810	0.247	0.275

APPENDIX F. Maternal intake of total energy, total CHO and added sugar.

TABLE OF RESULTS: F1 **Maternal energy and carbohydrate intakes at V1 vs fetal weight and size at US1.**

E & CHO Intake V1	*FW1*		*BPD1*		*AC1*		*HC1*		*FL1*	
Adjusted for conf.	None	A,h,w,s	None	A,h,w,s	None	A,h,w,s	None	A,h,w,s	None	A,h,w,s
N number	106	104	106	103,104*	106,105*	104,101*	96	93,95*	106	103,104*
Total Energy										
B coef	-1.890	0.465	-0.547	-0.196	0.003	-0.007	-0.922	-0.049	-0.336	-0.004
Beta coef	-0.081	0.020	-0.141	-0.052	0.031	-0.087	-0.070	-0.004	-0.101	-0.001
p value	0.411	0.124	0.148	**0.036**[#1]	0.758*	**0.004**[#3]	0.502	0.857*	0.305	0.960
Total CHO (%E)										
B coef	0.081	-0.009	0.013	0.002	0.000	0.000	0.047	0.001	0.008	-0.002
Beta coef	0.183	-0.019	0.175	0.023	-0.200	-0.029	0.183	0.007	0.127	-0.024
p value	**0.061**	0.143	**0.073**	0.361*	**0.041**[#2]	0.372	0.075[#5]	0.722	0.194	0.328
Added Sugar										
B coef	-0.098	0.008	-0.022	-0.009	0.000	0.000	-0.039	0.007	-0.019	-0.005
Beta coef	-0.061	0.005	-0.082	-0.034	0.022	-0.065	-0.032	0.008	-0.083	-0.022
p value	0.535	0.689	0.404	0.163	0.823	**0.027**[#4 *]	0.674	0.697	0.399	0.385*

Analysis including outliers:

	#1	#2	#3	#4	#5
N number	104	105	104	104	96
B coef	-0.173	0.000	-0.008	0.000	0.045
Beta coef	-0.045	-0.185	-0.085	-0.050	0.166
p value	**0.078**	**0.058**[#2]	**0.009**	0.117	0.104[#5]

TABLE OF RESULTS: F2 **Maternal energy and carbohydrate intakes at V1 vs fetal weight and size at US2.**

E & CHO Intake V1	*FW2*		*BPD2*		*AC2*		*HC2*		*FL2*	
Adjusted for conf.	None	A,h,w,s	None	A,h,w,s	None	A,h,w,s	None	A,h,w,s	None	A,h,w,s
N number	104	101,102*	104	102	104,103*	102,101*	95,93*	92,93*	103	102
Total Energy										
B coef	0.063	0.039	0.107	-0.004	-0.008	0.000	0.970	1.183	0.350	0.158
Beta coef	0.124	0.080	0.042	-0.004	-0.095	-0.101	0.112	0.139	0.162	0.070
p value	0.209	0.204	0.675	0.984	0.339*	0.175	0.281	**0.065**[#1]	0.102	0.274
Total CHO (%E)										
B coef	0.000	0.000	0.000	0.008	-0.014	0.013	-0.012	0.010	-0.007	-0.001
Beta coef	-0.056	0.086	0.009	0.150	-0.056	0.057	-0.072	0.060	-0.157	-0.022
p value	0.570	0.230*	0.928	**0.041**	0.576	0.450*	0.491*	0.438*	0.114[#2]	0.740
Added Sugar										
B coef	0.001	0.001	0.003	0.013	0.000	0.000	0.038	0.066	0.004	0.002
Beta coef	0.023	0.033	0.014	0.072	0.011	-0.059	0.065	0.114	0.029	0.012
p value	0.814	0.596	0.888	0.320	0.909	0.415	0.532	0.134*	0.773	0.852

Analysis including outliers:

	#1	#2
N number	93	103
B coef	0.929	-0.008
Beta coef	0.109	-0.189
p value	0.163	**0.055**

TABLE OF RESULTS: F3 **Maternal energy and carbohydrate intakes at V2 vs fetal weight and size at US2.**

E & CHO Intake V2	*FW2*		*BPD2*		*AC2*		*HC2*		*FL2*	
Adjusted for conf.	None	A,h,w,s	None	A,h,w,s	None	A,h,w,s	None	A,h,w,s	None	A,h,w,s
N number	103	102,103*	103	103	104,105*	103,102*	95	93	105,104*	103
Total Energy										
B coef	-0.016	0.081	-0.199	0.267	0.001	-0.015	-0.778	0.697	0.099	0.352
Beta coef	-0.031	0.162	-0.076	0.101	0.009	-0.165	-0.093	0.085	0.013	0.155
p value	0.752	**0.011**[#1]	0.443	0.183	0.932	**0.031**	0.369	0.305	0.898*	**0.018**
Total CHO (%E)										
B coef	0.000	0.000	0.006	0.001	0.006	-0.017	0.018	-0.001	0.002	-0.002
Beta coef	0.066	-0.051	0.121	0.015	0.026	-0.078	0.116	-0.005	0.058	-0.058
p value	0.501	0.484*	0.220	0.836	0.793	0.303*	0.263	0.953	0.556	0.370
Added Sugar										
B coef	0.000	0.000	0.002	0.001	0.000	0.000	0.001	0.002	0.000	0.001
Beta coef	-0.023	0.007	0.048	0.075	0.039	-0.002	0.020	0.036	0.015	0.035
p value	0.820	0.924*	0.627	0.306	0.697*	0.974	0.845	0.644	0.883	0.585

Analysis including outliers:

	#1
N number	103
B coef	0.082
Beta coef	0.158
p value	**0.032**

TABLE OF RESULTS: F4 **Maternal energy and carbohydrate intakes at V1 vs birth parameters.**

E & CHO Intake V1	*BW*		*BL*		*PONDIX*		*BW/PLW*	
Adjusted for conf.	None	A,h,w,s	None	A,h,w,s	None	A,h,w,s	None	A,h,w,s
N number	103,104*	103	100	100	100	100	86,88*	86,88*
Total Energy								
B coef	-0.001	-0.002	1.426	1.323	-0.022	-0.032	0.004	0.006
Beta coef	-0.043	-0.005	0.105	0.097	-0.078	-0.111	0.020	0.027
p value	0.965	0.950	0.299	0.312	0.438	0.301	0.855	0.821
Total CHO (%E)								
B coef	0.001	0.001	0.014	0.008	0.001	0.001	0.000	0.000
Beta coef	0.107	0.146	0.052	0.029	0.080	0.092	0.004	0.022
p value	0.280*	**0.077**[#1]	0.605	0.758	0.430	0.376	0.971*	0.842*
Added Sugar								
B coef	-0.002	0.000	-0.028	-0.012	0.001	0.001	0.001	0.001
Beta coef	-0.070	0.017	-0.030	-0.012	0.030	0.025	0.065	0.084
p value	0.484	0.835	0.768	0.895	0.766	0.812	0.550	0.458

Analysis including outliers:

	#1
N number	104
B coef	0.001
Beta coef	0.102
p value	0.235

TABLE OF RESULTS: F5 **Maternal energy and carbohydrate intakes at V2 vs birth parameters.**

E & CHO Intake V2	*BW*		*BL*		*PONDIX*		*BW/PLW*	
Adjusted for conf.	None	A,h,w,s	None	A,h,w,s	None	A,h,w,s	None	A,h,w,s
N number	104,103*	104,103*	100	100	100	100	85,87*	85,87*
Total Energy								
B coef	-0.004	0.008	2.117	2.513	-0.026	-0.033	-0.020	-0.016
Beta coef	-0.012	0.023	0.152	0.181	-0.090	-0.114	-0.088	-0.073
p value	0.901*	0.789*	0.130	**0.061**	0.371	0.295	0.424	0.541
Total CHO (%E)								
B coef	-0.001	-0.001	0.101	-0.020	0.000	0.000	0.001	0.001
Beta coef	-0.092	-0.088	-0.073	-0.079	-0.017	-0.011	0.167	0.157
p value	0.352	0.320	0.469	0.410	0.864	0.917	0.128[#1]	0.174[#2]
Added Sugar								
B coef	0.000	0.000	0.003	0.002	0.000	0.000	0.000	0.000
Beta coef	-0.010	-0.045	0.037	0.023	-0.039	-0.057	-0.070	-0.060
p value	0.922	0.611	0.718	0.809	0.746	0.590	0.522*	0.597*

Analysis including outliers:

	#1	**#2**
N number	87	87
B coef	0.000	0.000
Beta coef	0.082	0.089
p value	0.449	0.443

TABLE OF RESULTS: F6 **Maternal energy and carbohydrate intakes at V1 vs infant anthropometry (Ba1 and Ba2).**

E & CHO Intake V1	*Weight 1*		*Length 1*		*BMI 1*		*Weight 2*		*Length 2*		*BMI 2*	
Adjusted for conf.	None	A,s	None	A,s	None	A,s	None	A,s	None	A,s	None	A,s
N number	77,76*	77	77	77	76,77*	77	73	73	73,72*	73	72	72
Total Energy												
B coef	-352.2	-203.6	-0.670	-0.157	-0.008	-0.009	-0.018	-0.012	-1.949	-2.067	-0.001	-0.002
Beta coef	-0.109	-0.063	-0.042	0.010	-0.063	-0.064	-0.059	-0.039	-0.128	-0.127	-0.028	-0.052
p value	0.346	0.476	0.717	0.923	0.589	0.561	0.619	0.724	0.282*	0.257	0.813	0.655
Total CHO (%E)												
B coef	9.957	3.196	0.008	-0.012	0.001	0.000	0.000	0.000	0.028	0.035	0.002	0.000
Beta coef	0.159	0.048	0.025	-0.037	0.174	0.107	-0.039	-0.043	0.081	0.104	0.000	0.150
p value	0.172*	0.588	0.828	0.715	0.132	0.333	0.744	0.699	0.497	0.359	0.126	0.200
Added Sugar												
B coef	-36.12	-18.65	0.036	0.103	-0.002	-0.002	-0.003	-0.003	0.001	0.055	0.001	0.046
Beta coef	-0.153	-0.079	0.031	0.089	-0.208	-0.172	-0.143	-0.117	0.001	0.047	0.246	0.229
p value	0.184	0.372	0.791	0.385	**0.069***	0.119	0.228	0.292	0.994	0.673	**0.037**	**0.046**

TABLE OF RESULTS: F7 **Maternal energy and carbohydrate intakes at V2 vs infant anthropometry (Ba1 and Ba2).**

E & CHO Intake V2	*Weight 1*		*Length 1*		*BMI 1*		*Weight 2*		*Length 2*		*BMI 2*	
Adjusted for conf.	None	A,s	None	A,s	None	A,s	None	A,s	None	A,s	None	A,s
N number	76	76	76	76	76	76	72	72	72	72	71	71
Total Energy												
B coef	-260.1	50.40	-0.042	0.264	-0.009	0.000	-0.012	0.016	-0.605	-0.019	0.001	-0.001
Beta coef	-0.080	0.016	0.716	0.017	-0.062	0.001	-0.038	0.049	-0.036	-0.001	0.039	-0.042
p value	0.491	0.866	0.665	0.874	0.600	0.995	0.755	0.672	0.764	0.992	0.749	0.733
Total CHO (%E)												
B coef	2.231	-0.658	0.061	0.056	0.000	-0.001	0.000	0.000	0.040	0.024	0.000	0.000
Beta coef	0.038	-0.011	0.217	0.198	-0.154	-0.197	-0.074	-0.144	0.138	0.083	0.210	0.258
p value	0.742	0.901	**0.060**	**0.054**	0.185	**0.079**	0.536	0.202	0.247	0.469	**0.080**	**0.027**
Added Sugar												
B coef	-3.537	0.413	-0.016	-0.002	0.000	0.000	0.000	0.000	0.017	0.018	0.000	0.000
Beta coef	-0.162	0.019	-0.149	-0.016	-0.061	0.037	0.047	0.093	0.153	0.168	0.081	0.037
p value	0.161	0.838	0.198	0.880	0.603	0.747	0.693	0.407	0.198	0.140	0.501	0.758

TABLE OF RESULTS: F8

Maternal energy and carbohydrate intakes at V3 vs infant anthropometry (Ba2).

E & CHO Intake V3	*Weight 2*		*Length 2*		*BMI 2*	
Adjusted for conf.	None	A,s	None	A,s	None	A,s
N number	73	73	72	73	72	72
Total Energy						
B coef	0.054	0.084	0.756	1.710	-0.006	-0.008
Beta coef	0.146	0.228	0.042	0.088	-0.160	-0.215
p value	0.219	**0.040**	0.728	0.440	0.179	**0.066**
Total CHO (%E)						
B coef	0.000	0.000	0.011	0.019	0.000	0.000
Beta coef	0.053	0.045	0.030	0.052	-0.008	-0.014
p value	0.657	0.683	0.804	0.646	0.946	0.908
Added Sugar						
B coef	0.002	0.004	0.107	0.177	0.000	0.000
Beta coef	0.082	0.147	0.080	0.123	-0.002	-0.059
p value	0.488	0.186	0.507	0.278	0.986	0.622

TABLE OF RESULTS: F9 **Maternal energy and carbohydrate intakes at V1 vs infant glucose, HOMA index and metabolics (Ba1).**

E & CHO Intake V1	*Glc F 1*		*Ins F 1*		*PI F 1*		*HOMA-IR 1*		*Leptin 1*		*IGF BP3 (1)*	
Adjusted for conf.	Out	A, s, w	Out	A, s, w	Out	A, s, w	Out	A, s, w	Out	A, s, w	Out	A, s, w
N number		75			32	32		25				43
Total Energy												
B coef	-0.293	-0.330	-0.002	-0.003	0.049	0.042	-0.182	0.071	0.199	0.251	21.57	115.1
Beta coef	-0.113	-0.133	-0.036	-0.055	0.678	0.047	-0.204	0.087	0.046	0.058	0.012	0.069
p value	0.328	0.231	0.761	0.640	**0.043**[#1]	0.691	**0.086**	0.453	0.691	0.468	0.916	0.526
Total CHO (%E)												
B coef	0.003	0.004	0.000	-0.001	0.002	0.002	0.002	0.002	-0.004	-0.010	-0.276	-1.659
Beta coef	0.051	0.068	-0.147	-0.131	0.087	0.091	0.117	0.116	-0.050	-0.110	-0.008	-0.046
p value	0.659	0.545	0.207	0.268	0.453	0.446	0.330	0.340	0.669	0.168	0.948	0.678
Added Sugar												
B coef	-0.028	-0.019	0.000	0.000	0.009	0.009	-0.006	0.002	-0.038	-0.009	2.856	4.660
Beta coef	-0.147	-0.099	0.009	-0.014	0.128	0.133	-0.104	0.027	-0.122	-0.030	0.022	0.038
p value	0.201	0.380	0.941	0.908	0.268	0.265	0.384	0.819	0.296	0.707	0.848	0.728

Analysis including outliers:

	#1
N number	104
B coef	0.007
Beta coef	0.007
p value	0.951

TABLE OF RESULTS: F10 **Maternal energy and carbohydrate intakes at V2 vs infant glucose, HOMA index and metabolics (Ba1).**

E & CHO Intake V2	*Glc F 1*		*Ins F 1*		*PI F 1*		*HOMA-IR 1*		*Leptin 1*		*IGF BP3 (1)*	
Adjusted for conf.	Out	A, s, w	Out	A, s, w	Out	A, s, w	Out	A, s, w	Out	A, s, w	Out	A, s, w
N number								25/56				43
Total Energy												
B coef	-0.685	-0.617	-0.015	-0.018	-0.070	-0.064	0.047	0.172	0.426	0.079	99.60	129.1
Beta coef	-0.259	-0.234	-0.253	-0.306	-0.078	-0.072	0.051	0.208	0.098	0.018	0.056	0.073
p value	**0.024**	**0.044**	**0.029**	**0.011**	0.501	0.556	0.672	**0.081**[#1]	0.403	0.828	0.630	0.523
Total CHO (%E)												
B coef	0.009	0.009	0.000	0.000	0.003	0.003	-0.004	-0.005	-0.007	-0.001	-6.170	-6.577
Beta coef	0.195	0.181	0.321	0.354	0.194	0.192	-0.273	-0.328	-0.087	-0.014	-0.194	-0.207
p value	**0.091**	0.113	**0.005**	**0.002**	**0.094**	0.109	**0.021**	**0.006**[#2]	0.457	0.869	**0.093**	**0.062**[#3]
Added Sugar												
B coef	-0.003	-0.001	0.000	0.000	0.001	0.001	0.000	0.000	0.001	0.004	-0.631	-0.366
Beta coef	-0.138	-0.060	-0.107	-0.154	0.116	0.132	0.013	0.096	0.028	0.124	-0.053	-0.031
p value	0.234	0.612	0.362	0.211	0.318	0.283	0.914	0.428	0.813	0.134	0.650	0.789

Analysis including outliers:

	#1	#2	#3
N number	104	104	104
B coef	0.195	-0.002	-6.830
Beta coef	0.221	-0.256	-0.228
p value	**0.068**	**0.036**	**0.038**

TABLE OF RESULTS: F11 **Maternal energy and carbohydrate intakes at V1 vs infant glucose, HOMA index and metabolics (Ba2).**

E & CHO Intake V1	*Glc F 2*		*Ins F 2*		*PI F 2*		*HOMA-IR 2*		*Leptin 2*		*IGF BP3 (2)*	
Adjusted for conf.	None	A, s, w	None	A, s, w	None	A, s, w	None	A, s, w	None	A, s, w	None	A, s, w
N number	**76**											
Total Energy												
B coef	37.41	-0.065	-0.089	-0.093	-0.063	-0.044	-0.182	-0.187	-0.073	-0.058	-0.673	81.30
Beta coef	0.014	-0.021	-0.217	-0.226	-0.063	-0.044	-0.204	-0.211	-0.056	-0.045	0.000	0.028
p value	0.909	0.864	**0.067**	**0.052**	0.595	0.701	**0.086**	**0.071**	0.636	0.647	0.998	0.806
Total CHO (%E)												
B coef	0.003	0.001	0.001	0.001	-0.001	0.000	0.002	0.003	-0.003	-0.003	-0.366	-0.100
Beta coef	0.040	0.022	0.104	0.136	-0.025	-0.013	0.117	0.139	-0.117	-0.100	-0.006	-0.002
p value	0.736	0.859	0.384	0.250	0.830	0.912	0.330	0.239	0.320	0.311	0.960	0.990
Added Sugar												
B coef	28.10	-0.002	-0.004	-0.003	0.008	0.011	-0.006	-0.006	-0.005	-0.001	27.35	40.00
Beta coef	0.146	-0.007	-0.123	-0.105	0.110	0.154	-0.104	-0.096	-0.051	-0.016	0.133	0.193
p value	0.216	0.955	0.304	0.376	0.352	0.181	0.384	0.418	0.668	0.870	0.260	**0.085**

TABLE OF RESULTS: F12 **Maternal energy and carbohydrate intakes at V2 vs infant glucose, HOMA index and metabolics (Ba2).**

E & CHO Intake V2	*Glc F 2*		*Ins F 2*		*PI F 2*		*HOMA-IR 2*		*Leptin 2*		*IGF BP3 (2)*	
Adjusted for conf.	Out	A, s, w	Out	A, s, w	Out	A, s, w	Out	A, s, w	Out	A, s, w	Out	A, s, w
N number					48		56				76	
Total Energy												
B coef	-0.763	-0.810	0.063	0.043	-0.099	-0.115	0.047	0.002	-0.085	-0.193	-93.44	-84.70
Beta coef	-0.236	-0.253	0.149	0.101	-0.097	-0.114	0.051	0.002	-0.063	-0.145	-0.033	-0.028
p value	**0.044**	**0.041**	0.215	0.408	0.414	0.338	0.672	0.985	0.597	0.155	0.785	0.813
Total CHO (%E)												
B coef	0.008	0.008	-0.003	-0.002	0.001	0.002	-0.004	-0.004	-0.003	-0.001	-3.745	-4.000
Beta coef	0.143	0.152	-0.355	-0.312	0.059	0.112	-0.273	-0.232	-0.122	-0.031	-0.077	-0.076
p value	0.227	0.220	**0.002**	**0.008**	0.622	0.343	**0.021**[#2]	**0.051**	0.303	0.756	0.522	0.508
Added Sugar												
B coef	-0.002	-0.002	0.000	0.000	0.001	0.001	0.000	0.000	0.001	0.000	-1.176	-1.600
Beta coef	-0.101	-0.116	0.058	0.006	0.207	0.182	0.013	-0.040	0.061	-0.026	-0.064	-0.080
p value	0.395	0.346	0.630	0.960	**0.078**[#1]	0.116	0.914	0.741	0.607	0.793	0.595	0.484

Analysis including outliers:

	#1	#2
N number	104	104
B coef	0.002	-0.005
Beta coef	0.260	-0.328
p value	**0.027**	**0.006**

TABLE OF RESULTS: F13 **Maternal energy and carbohydrate intakes at V3 vs infant glucose, HOMA index and metabolics (Ba2).**

E & CHO Intake V3	*Glc F 2*		*Ins F 2*		*PI F 2*		*HOMA-IR 2*		*Leptin 2*		*IGF BP3 (2)*	
Adjusted for conf.	None	A, s, w	None	A, s, w	None	A, s, w	None	A, s, w	None	A, s, w	None	A, s, w
N number											**76**	
Total Energy												
B coef	0.713	0.649	-0.100	-0.163	0.062	-0.049	-0.124	-0.254	0.566	0.321	-379.8	-603.4
Beta coef	0.191	0.175	-0.205	-0.334	0.052	-0.041	-0.117	-0.241	0.364	0.209	-0.108	-0.171
p value	0.104	0.164	**0.084**	**0.005**	0.665	0.735	0.326	**0.048**	**0.001**	**0.039**	0.361	0.142
Total CHO (%E)												
B coef	-0.012	-0.010	0.000	0.000	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001	9.078	5.700
Beta coef	-0.159	-0.140	0.031	0.015	-0.044	-0.051	-0.028	-0.035	-0.016	-0.024	0.143	0.084
p value	0.176	0.248	0.795	0.900	0.714	0.658	0.816	0.768	0.892	0.807	0.227	0.454
Added Sugar												
B coef	0.046	0.042	-0.003	-0.006	0.018	0.013	-0.001	-0.007	0.032	0.018	-9.936	4.100
Beta coef	0.168	0.154	-0.088	-0.169	0.201	0.149	-0.016	-0.096	0.283	0.161	-0.041	0.016
p value	0.152	0.212	0.463	0.158	**0.089**	0.200	0.895	0.427	**0.015**	0.106	0.732	0.890

Appendix: G1 List of variables and their transformations for data on mothers

DATA ON MOTHERS	V1	V2	V3
Variable	*Transformation*		
Age, anthropom., BP			
AGE			
HEIGHT			
WEIGHT	log	log	
BMI	log	log	
BP SYS			
BP DIA			
Metabolics			
GLC F	sqrt	rec	
GLC AUC	log	log	
INS F	log	log	
INS AUC	log	ln	
PI F	log	log	
PI AUC	log	log	
CPEP F	log		
CPEP AUC		sqrt	
HGB			
HPL	log	rec, sqrt	
IGF1	log, sqrt		
IGFBP1	log	log	
FT4	rec	rec	
CORTISOL		log	
LEPTIN	log	sqrt	
HOMA-IR	log	log	

DATA ON MOTHERS	V1	V2	V3
Variable	*Transformation*		
Nutritional intake			
ENERGY	log	log	log
TOTAL PROTEIN	log	rec	
PLANT PROTEIN	log		
ANIMAL PROTEIN	sqrt	log	
TOTAL FAT			
SAFA	log	sqrt	log
MUFA	log		
PUFA	log	log	sqrt
CHOLESTEROL			
TOTAL AVAIL. CHO			
ADDED SUGAR	sqrt		sqrt
PROTEIN (% EN.)	log		
FAT (% ENERGY)			
TOTAL CHO (% EN.)			

BMI - body mass index
BP - blood pressure
GLC - glucose
AUC - area under the curve
INS - insulin
PI - proinsulin
CPEP - C-peptide
HGB - haemoglobin
HPL - human placental lactogen
IGF-1 - insulin like growth factor 1
IGF-BP1 - IGF-binding protein 1
FT4 - free thyroxine
SAFA - saturated fatty acids
MUFA - monounsaturated fatty acids
PUFA - polyunsaturated fatty acids
CHO - carbohydrate

Appendix: G2 List of variables and their transformations for data from ultrasound, delivery and on infants

ULTRASOUND DATA	US1	US2
Variable	*Transformation*	
GEST AGE		log
EFW	sqrt	log
BPD		
AC	rec, sqrt	rec, log
HC		
FL		
RI		sqrt
AFI		
GRRT	sqrt	rec, sqrt
FL/AC		

DELIVERY DATA	
Variable	*Transf.*
BIRTH AGE	
B WEIGHT	log
B LENGTH	
HEAD_CIR	
SEX	
PLAC_W	rec, sqrt
PONDIX	log
BW/PLAC W	rec, sqrt
MATER W GAIN	

INFANT DATA	Ba1	Ba2
Variable	*Transformation*	
Age, anthropom. GRRT		
AGE (FROM DEL.)	log	rec, ^2
WEIGHT		log
LENGTH		
BMI	log, sqrt	rec
D-Ba1/2 GRRT		log
Ba1 - Ba2 GRRT		log
Metabolic data (fasting)		
GLC		
INS	rec	sqrt
PI	log	log
HOMA-IR	log	log
LEPTIN	log	log
IGF BP3		

EFW - estimated fetal weight
BPD - biparietal diameter
AC - abdomnal circumference
HC - head circumference
FL - femur length
RI - resistance index
AFI - amniotic fluid index
GRRT - growth rate
PONDIX - ponderal index

BMI - body mass index
D-Ba 1 GRRT - delivery to Ba1 growth rate
Ba1-Ba2 GRRT - Ba1 to Ba2 growth rate

US - ultrasound
Ba1, Ba2 - babies' visits

GLC - glucose
INS - insulin
PI - proinsulin
HOMA-IR - homeostasis index
IGF-BP3 - insulin-like growth factor binding protein 3

APPENDIX H. Maternal dietary intakes summary tables

Table H 1 contains results corrected for infant BMI, replacing the infant weight as a confounder (see the data in grey font). Correcting for BMI in many instances produced a weaker effect (lower p value and β-coefficient and increased AR^2) for maternal dietary protein intake on the particular infant metabolic parameter. This means, that the model with BMI explains more variation than the model with infant weight. However, in most cases BMI did not completely negate the relationship between the maternal dietary intake and the metabolic variable, with the p-value remaining < 0.05. The only cases when p value was greater than 0.05 was for IGF BP3 (Ba1 and Ba2) and HOMA-IR1.

Table H1 Maternal protein dietary intake summary tables vs fetal biometric and postnatal anthropometric and metabolic parameters and birth weight. (Table 1 for total protein intake, Table 2 for plant protein intake and Table 3 for animal protein intake).

Table H1.1

TOT PROT [%E]		**Intake V1**				**Intake V2**	
Variable	p	β	AR^2	**Variable**	p	β	AR^2
BPD 1	0.024	0.054	0.946	AC 2	0.048	0.145	0.459
HC 2	0.014	-0.187	0.537	BW	0.038	0.171	0.347
BMI 1	0.001	0.353	0.208	LENG 1	0.014	0.252	0.284
INS F 1	0.003	0.348	0.098	BMI 1	0.026	-0.247	0.146
	0.014	0.296	0.117				
HOMA 1	0.001	0.381	0.155	IGFBP3 (2)	0.040	0.236	0.161
	0.005	0.330	0.173		0.068	0.224	0.041
WEIG 2	0.038	0.227	0.180				
BMI 2	0.012	0.287	0.128				

Orange font indicates fetal biometric measurements and the blue font indicates infant postnatal parameters of visit 1 or visit2 (antenatal or postnatal). Grey font indicates outcomes of models corrected for infant BMI instead of the weight.

Table H 1.2

PLPROT [%E]		Intake V1				Intake V2	
Variable	p	β	AR^2	**Variable**	p	β	AR^2
PI F 2	0.021	-0.262	0.152	LENG 1	0.032	0.220	0.269
	0.045	-0.244	0.032	BMI 1	0.010	-0.283	0.165
				INS F 1	0.005	-0.329	0.084
					0.019	-0.280	0.110
				HOMA 1	0.042	-0.237	0.099
					0.081	-0.209	0.114
				WEIG 2	0.033	-0.238	0.180
				BMI 2	0.021	-0.269	0.118
				INS F 2	0.008	-0.318	0.141
					0.006	-0.336	0.125
				HOMA 2	0.027	-0.268	0.133
					0.024	-0.264	0.116

Blue font indicates the infant postnatal parameters of visit 1 or visit 2. Grey font indicates outcomes of models corrected for infant BMI instead of the weight.

Table H 1.3

ANPROT [%E]		Intake V1				Intake V2	
Variable	p	β	AR^2	**Variable**	p	β	AR^2
BMI 1	0.012	0.275	0.156	INS F 2	0.024	0.264	0.116
					0.017	0.283	0.101
INS F 1	0.006	0.323	0.079	IGF BP3 (2)	0.016	0.267	0.181
	0.021	0.273	0.108		0.015	0.288	0.078
HOMA 1	0.003	0.345	0.127				
	0.012	0.294	0.156				
IGFBP3 (1)	0.036	0.231	0.150				
	0.099	0.194	0.088				
BMI 2	0.039	0.238	0.102				

TABLE H 2 Fat dietary intake summary tables vs. fetal biometric and postnatal anthropometric and metabolic parameters. (Table 1 includes total fat intake, Table 2 saturated fat, Table 3 monounsaturated and Table D polyunsaturated fat intakes.)

Table H 2.1

TOT FAT [%E]		**Intake V1**				**Intake V2**	
Variable	p	β	AR^2	**Variable**	p	β	AR^2
BMI 1	0.047	-0.217	0.128	LENG 1	0.002	-0.310	0.316
INS F1	0.033	-0.249	0.037	BMI 1	0.005	0.309	0.180
	0.075	-0.206	0.081				
HOMA 1	0.034	-0.245	0.068	INS F1	0.002	0.364	0.107
	0.076	-0.203	0.116		0.010	0.311	0.125
				HOMA 1	0.014	0.287	0.030
					0.071	0.218	0.117
				BMI 2	0.004	0.339	0.157

Blue font indicates the infant postnatal parameters of visit 1 or visit 2. Grey font indicates outcomes of models corrected for infant BMI instead of the weight.

Table H 2.2

SAFA [%E]		**Intake V1**				**Intake V2**	
Variable	p	β	AR^2	**Variable**	p	β	AR^2
BMI 2	0.027	0.254	0.110	HC 2	0.029	-0.167	0.530
				BMI 2	0.018	0.277	0.121

Orange font indicates fetal biometric measurements.

Table H 2.3

MUFA [%E]		**Intake V1**				**Intake V2**	
Variable	p	β	AR^2	**Variable**	p	β	AR^2
LENG 2	0.014	-0.271	0.177	INS F 1	0.004	0.340	0.093
					0.009	0.302	0.127
				HOMA 1	0.034	0.249	0.070
					0.039	0.238	0.146
				IGFBP3 (1)	0.014	0.269	0.170
					0.052	0.223	0.102
				INS F2	0.022	0.272	0.118
					0.032	0.264	0.085

Table H 2.4

PUFA [%E]		Intake V1				Intake V2	
Variable	p	β	AR^2	**Variable**	p	β	AR^2
LENG 1	0.006	0.275	0.298	LENG 1	0.012	-0.256	0.286
BMI 1	0.022	-0.252	0.144	BMI 1	0.001	0.377	0.227
INS F1	0.015	-0.282	0.055	INS F1	0.003	0.355	0.100
	0.054	-0.227	0.085		0.015	0.303	0.116
HOMA 1	0.006	-0.319	0.110	HOMA 1	0.006	0.322	0.111
	0.025	-0.260	0.139		0.035	0.260	0.132
INS F 2	0.036	-0.247	0.105	BMI 2	0.009	0.305	0.137
	0.053	-0.234	0.071				
				LEPT 2	0.028	0.229	0.361
					0.851	0.021	0.193

Blue font indicates the infant postnatal parameters of visit 1 or visit 2. Grey font indicates outcomes of models corrected for infant BMI instead of the weight.

Table H 3 Total energy, total CHO and added sugar dietary intake summary tables vs fetal biometric and postnatal anthropometric and metabolic parameters. (Table 1 includes total energy intake, table 2 total carbohydrate intake and Table 3 an intake of added sugar.)

Table H 3.1

TOT CHO [%E]		Intake V1				Intake V2	
Variable	p	β	AR^2	**Variable**	p	β	AR^2
BPD 2	0.041	0.150	0.504	INS F1	0.002	-0.354	0.101
					0.007	-0.313	0.133
				HOMA 1	0.006	-0.328	0.094
					0.057	-0.221	0.122
				BMI 2	0.027	-0.258	0.111
				INS F2	0.008	-0.312	0.141
					0.010	-0.316	0.113

Orange font indicates fetal biometric measurements and the blue font indicates infant postnatal parameters of visit 1 or visit2 (antenatal or postnatal). Grey font indicates outcomes of models corrected for infant BMI instead of the weight.

Table H 3.2

ADDED SUGAR[g]		Intake V1				Intake V2	
Variable	p	β	AR^2	**Variable**	p	β	AR^2
AC 1	0.027	0.065	0.919	*No*			
BMI 2	0.046	-0.229	0.098	*associations*			

Table H 3.3

TOTAL ENERGY		Intake V1				Intake V2	
Variable	p	β	AR^2	**Variable**	p	β	AR^2
BPD 1	0.036	-0.052	0.945	EFW 2	0.011	0.162	0.659
AC 1	0.009	0.087	0.929	AC 2	0.031	0.165	0.503
				FL2	0.018	0.155	0.635
				GLC F1	0.044	-0.234	0.119
					0.048	-0.231	0.103
				INS F1	0.011	0.306	0.065
					0.008	0.308	0.131
				GLC F2	0.041	-0.253	0.020
					0.036	-0.263	0.185

Orange font indicates fetal biometric measurements and the blue font indicates infant postnatal parameters of visit 1 or visit2 (antenatal or postnatal). Grey font indicates outcomes of models corrected for infant BMI instead of the weight.

Table H 3.4

TOTAL ENERGY		Intake V3	
Variable	p	β	AR^2
WEIG 2	0.040	0.228	0.179
INS F2	0.005	-0.334	0.149
	0.008	-0.327	0.119
HOMA 2	0.048	-0.241	0.098
	0.058	-0.235	0.067
LEPT 2	0.039	0.209	0.355
	0.023	0.248	0.255

APPENDIX I.

Correlations used in the regression models listed in APPENDIX J.

I.1.1 Associations with fetal growth rates:

Maternal visit 1 (V1) and GRR1 (between V1 and V2):

GRRT1	Valid N	Pearson R	p-level
WEIGHT1	105	0.213	0.029
BMI1	105	0.216	0.027
GLC1_30	105	0.202	0.039
GLC1_60	105	0.212	0.030
GLC1_120	105	0.201	0.039
AREA_GLC1	105	0.235	0.016
INS1_60	105	0.184	0.061
CPEPF1	79	0.168	0.086
CPEP1_60	79	0.187	0.099
CPEP1_120	79	0.225	0.047
HPL1	105	0.322	0.001
LEPT1	105	0.178	0.070

Maternal visit 1 (V1) and GRR2 (between V2 and delivery):

GRRT2	Valid N	Pearson R	p-level
HEIGHT	101	0.185	0.065
FT4V1	101	0.188	0.060

I.1.2. Associations with the birth parameters:

Maternal V1 and BW:

BW	Valid N	Pearson R	p-level
WEIGHT1	105	0.348	<0.001
HEIGHT	105	0.237	0.015
BMI1	105	0.279	0.004
INS1_0	105	0.186	0.057
INS1_60	105	0.187	0.099
AREA_INS1	105	0.189	0.053
IGFBP1_V1	105	-0.256	0.008
LEPT1	105	0.295	0.002
INS_RES1	105	0.191	0.051

Maternal V1 and BL:

BL	Valid N	Pearson R	p-level
WEIGHT1	101	0.287	0.004
BMI1	101	0.255	0.010
INS1_0	101	0.196	0.050
IGFBP1_V1	101	-0.257	0.010
LEPT1	101	0.262	0.008
INS_RES1	101	0.208	0.037

Maternal V1 and BHC:

BHC	Valid N	Pearson R	p-level
WEIGHT1	102	0.275	0.005
BMI1	102	0.334	0.003
GLC1_120	102	0.209	0.035
INS1_0	102	0.213	0.032
INS1_60	102	0.236	0.017
INS1_120	102	0.213	0.032
AREA_INS1	102	0.216	0.029
IGFBP1_V1	102	-0.359	<0.001
LEPT1	102	0.343	<0.001
INS_RES1	102	0.216	0.029

I.1.3 Associations with the postnatal parameters:

Maternal V1 and the postnatal growth rate between delivery and Ba2 (GRRT Del-Ba2):

No correlations found

Maternal V1 and baby's BMI at the first visit (Ba1):

BMI_Ba1	Valid N	Pearson R	p-level
WEIGHT1	77	0.309	0.006
BMI1	77	0.284	0.012
PI1_0	77	-0.261	0.022
PI1_30	76	-0.261	0.042
LEPT1	77	0.222	0.053

Maternal V1 and baby's BMI at the second visit (Ba2):

BMI_Ba2	Valid N	Pearson R	p-level
GLC1_O	72	-0.267	0.024
FT4_V1	72	0.205	0.083
IGFBP1_V1	72	-0.236	0.046
LEPT1	72	0.242	0.040

Maternal V1 and baby's fasting glucose levels at the first visit (Ba1):

GLC F Ba1	Valid N	Pearson R	p-level
GLC1_O	77	0.228	0.047
HAEMOGLOBIN	77	-0.194	0.092

Maternal V1 and baby's fasting glucose levels at the second visit (Ba2):

GLC F Ba2	Valid N	Pearson R	p-level
C-PEP1_30	74	-0.258	0.051
HPL1	74	0.279	0.016

Maternal V1 and baby's fasting insulin levels at the first visit (Ba1):

INS F Ba1	Valid N	Pearson R	p-level
BMI1	76	0.202	0.081
FT4_V1	76	-0.371	0.004

Maternal V1 and baby's fasting insulin levels at the first visit (Ba2):

No correlations found

Maternal V1 and baby's HOMA-IR index at the first visit (Ba1):

HOMA Ba1	Valid N	Pearson R	p-level
BMI_1	75	0.236	0.072
FT4_V1	75	-0.423	0.001

Maternal V1 and baby's HOMA-IR index at the second visit (Ba2):

No correlations found

Maternal V1 and baby's fasting leptin levels at the first visit (Ba1):

No correlations found

Maternal V1 and baby's fasting leptin levels at the second visit (Ba2):

LEPT F Ba2	Valid N	Pearson R	p-level
FT4_V1	74	0.291	0.012

I.2.1 Associations with fetal growth rates:

Maternal visit 2 (V2) and GRR1 (between V1 and V2):

GRRT 1	Valid N	Pearson R	p-level
WEIGHT2	105	0.193	0.049
BMI2	105	0.192	0.049
BP_SYS2	105	0.271	0.005
BP_DIA2	105	0.247	0.011
INS2_60	105	-0.163	0.096
PI2_60	105	-0.082	0.067
HPL2	103	0.217	0.028
LEPT	103	0.253	0.010

Maternal visit 2 (V2) and GRR2 (between V2 and delivery):

GRRT2	Valid N	Pearson R	p-level
BP_DIA2	101	-0.178	0.075
INS2_120	101	0.173	0.083
PI2_0	101	0.187	0.064

I.2.2 Associations with the birth parameters:

Maternal V2 and BW:

BW	Valid N	Pearson R	p-level
WEIGHT2	104	0.345	<0.001
BMI2	104	0.266	0.006
INS2_0	104	0.170	0.085
IGFBP1_V2	104	-0.205	0.038
LEPT2	104	0.212	0.031
INS_RES2	104	0.193	0.050

Maternal V2 and BL:

BL	Valid N	Pearson R	p-level
WEIGHT2	100	0.298	0.003
BMI2	100	0.259	0.009
GLC2_O	100	0.217	0.030
IGFBP1_V2	100	-0.278	0.005
LEPT2	100	0.241	0.016
INS_RES2	100	0.212	0.035

Maternal V2 and BHC:

BHC	Valid N	Pearson R	p-level
WEIGHT2	101	0.255	0.010
BMI2	101	0.308	0.002
INS2_0	101	0.197	0.049
IGFBP1_V2	101	-0.315	0.002
LEPT2	101	0.240	0.017
INS_RES2	101	0.211	0.037

I.2.3 Associations with the postnatal parameters:

Maternal V2 and the postnatal growth rate between delivery and Ba2 (GRRT Del-Ba2):

GRRT D-Ba2	Valid N	Pearson R	p-level
INS_RES2	72	0.222	0.063

Maternal V2 and baby's BMI at the first visit (Ba1):

BMI Ba1	Valid N	Pearson R	p-level
WEIGHT2	76	0.278	0.015
BMI2	76	0.251	0.029
PI2_0	76	-0.323	0.005
PI2_30	76	-0.224	0.052
PI2_60	76	-0.336	0.003
PI2_120	76	-0.238	0.040
AREA_PI2	76	-0.274	0.017

Maternal V2 and baby's BMI at the second visit (Ba2):

BMI Ba2	Valid N	Pearson R	p-level
IGF1_BP1_V2	71	0.252	0.034
LEPT_2	71	0.210	0.080

Maternal V2 and baby's fasting glucose levels at the first visit (Ba1):

GLC F Ba1	Valid N	Pearson R	p-level
GLC2_O	76	0.195	0.092
HPL2	76	0.223	0.053
FT4_V2	76	-0.212	0.067
CORT2	76	0.225	0.052

Maternal V2 and baby's fasting glucose levels at the second visit (Ba2):

GLC F Ba2	Valid N	Pearson R	p-level
INS2_60	73	-0.243	0.039
AREAINS_2	73	-0.216	0.067
PI2_30	73	-0.206	0.082
PI2_60	73	-0.228	0.054
AREA_PI2	73	-0.201	0.090
HPL_2	73	0.273	0.020
CORT_2	73	0.249	0.035
IGFBP1_V2	73	0.201	0.089

Maternal V2 and baby's fasting insulin levels at the first visit (Ba1):

INS F Ba1	Valid N	Pearson R	p-level
PI2_0	75	-0.216	0.062

Maternal V2 and baby's fasting insulin levels at the first visit (Ba1):

INS Ba1	Valid N	Pearson R	p-level
PI2_0	71	0.221	0.064

Maternal V2 and baby's fasting insulin levels at the second visit (Ba2):

No correlations found

Maternal V2 and baby's HOMA-IR index at the first visit (Ba1):

HOMA Ba1	Valid N	Pearson R	p-level
BMI_2	74	0.212	0.070
PI2_0	74	-0.246	0.036

Maternal V2 and baby's HOMA-IR index at the second visit (Ba2):
No correlations found

Maternal V2 and baby's fasting leptin levels at the first visit (Ba1):

LEPT F Ba1	Valid N	Pearson R	p-level
BP_SYS2	75	0.209	0.071

Maternal V2 and baby's fasting leptin levels at the second visit (Ba2):

LEPT F Ba2	Valid N	Pearson R	p-level
INS2_30	73	-0.214	0.069

APPENDIX J

Table J1.1: Fetal growth rate **Maternal parameters at visit 1**

GRR1			
Adjusted	***B***	***BETA***	***p***
N=75; AR^2=0.243			
Intercept	*-0.848*		
MW1	2.266	0.396	<0.001
HPL1	1.334	0.390	<0.001
Not Adjusted	***B***	***BETA***	***p***
N=76; AR^2=0.243			
Intercept	*-0.392*		
MW1	2.282	0.398	<0.001
HPL1	1.328	0.388	<0.001

GRR2			
Adjusted	***B***	***BETA***	***p***
N=101; AR^2=0.054			
Intercept	*0.320*		
HEIGHT	0.104	0.194	0.049
FT4 (V1)	0.456	0.197	0.046
Not Adjusted	***B***	***BETA***	***p***
same outcome			

Table J1.2: Birth weight and postnatal growth rate

BW			
Adjusted	***B***	***BETA***	***p***
N=104; AR^2=0.248			
Intercept	*2.478*		
BIRTH AGE	0.016	0.379	<0.001
MW1	0.210	0.281	0.002
Not Adjusted	***B***	***BETA***	***p***
N=104; AR^2=0.115			
Intercept	*3.014*		
MW1	0.263	0.351	<0.001

GRRT D-Ba2			
Adjusted	***B***	***BETA***	***p***
N=101; AR^2=0.054			
Intercept	*1.031*		
SEX	0.076	0.434	<0.001
Adjusted	***B***	***BETA***	***p***
no correlations			

Table J1.3: Birth parameters

BL			
Adjusted	***B***	***BETA***	***p***
N=101; AR^2=0.168			
Intercept	*23.049*		
BIRTH AGE	0.609	0.346	<0.001
LEPT 1	1.695	0.199	0.035
Not Adjusted	***B***	***BETA***	***p***
N=101; AR^2=0.073			
Intercept	*33.045*		
MW1	8.782	0.287	0.004

BHC			
Adjusted	***B***	***BETA***	***p***
N=102; AR^2=0.228			
Intercept	*22.805*		
BIRTH AGE	0.316	0.292	0.002
LEPT1	1.037	0.199	0.045
IGF BP1 V1	-1.143	-0.202	0.044
Not Adjusted	***B***	***BETA***	***p***
N=102; AR^2=0.154			
Intercept	*35.679*		
LEPT1	1.192	0.229	0.027
IGF BP1 V1	-1.458	-0.258	0.013

Table J1.4: Postnatal anthropometric parameters

BMI Ba1			
Adjusted	***B***	***BETA***	***p***
N=76; AR^2 =0.274			
Intercept	*0.352*		
PI F1	-0.010	-0.314	0.002
AGE Ba1	0.125	0.354	0.001
MW1	0.113	0.354	0.001
Not Adjusted	***B***	***BETA***	***p***
N=76; AR^2=0.163			
Intercept	*0.385*		
MW1	0.114	0.357	0.001
PI 30' V1	-0.033	-0.302	0.006

BMI Ba2			
Adjusted	***B***	***BETA***	***p***
N=72; AR^2=0.263			
Intercept	*0.037*		
SEX	0.004	0.339	0.002
LEPT1	0.007	0.358	0.001
GLC F1	-0.018	-0.410	<0.001
Not Adjusted	***B***	***BETA***	***p***
N=72; AR^2=0.157			
Intercept	*0.035*		
LEPT1	0.007	0.346	0.003
GLC F1	-0.016	-0.365	0.002

Table J1.5: Postnatal glucose

GLC Ba1			
Adjusted	***B***	***BETA***	***p***
N=76; AR^2=0.082			
Intercept	*3.029*		
WEIG Ba1	0.0002	0.228	0.047
Not Adjusted	***B***	***BETA***	***p***
N=77; AR^2=0.039			
Intercept	*2.309*		
GLC F1	0.086	0.228	0.047

GLC Ba2			
Adjusted	***B***	***BETA***	***p***
N=72; AR^2=0.146			
Intercept	*3.586*		
HPL1	1.668	0.398	0.001
Not Adjusted	***B***	***BETA***	***p***
N=73; AR^2=0.132			
Intercept	*3.602*		
HPL1	1.605	0.379	0.001

Table J1.6: Neonatal insulin and HOMA-IR index

INS Ba1			
Adjusted	***B***	***BETA***	***p***
N=76; AR^2=0.106			
Intercept	*0.059*		
FT4 V1	-0.355	-0.343	0.002
Not Adjusted	***B***	***BETA***	***p***
N=76 ; AR^2=0.106			
Intercept	*0.059*		
FT4 V1	-0.355	-0.343	0.002
same outcome			

HOMA-Ba1			
Adjusted	***B***	***BETA***	***p***
=74; AR^2 =0.227			
Intercept	*-0.888*		
WEIG Ba1	<0.001	0.344	0.002
FT4 V1	-6.109	0.417	<0.001
Not Adjusted	***B***	***BETA***	***p***
N=101; AR^2=0.054			
Intercept	*-0.508*		
FT4 (V1)	-5.686	0.364	0.001

Table J1.7: Postnatal leptin

LEPT Ba1			
Adjusted	***B***	***BETA***	***p***
N=75; AR^2=0.539			
Intercept	*-0.970*		
SEX	-0.729	-0.465	<0.001
WEIG Ba1	0.001	0.473	<0.001
AGE Ba1	2.743	0.264	0.012
Not Adjusted	***B***	***BETA***	***p***
Outcome not significant			

LEPT Ba2			
Adjusted	***B***	***BETA***	***p***
N=73; AR^2=0.302			
Intercept	*-8.111*		
SEX	-0.201	-0.430	<0.001
WEIG Ba2	2.315	0.557	<0.001
Not Adjusted	***B***	***BETA***	***p***
N=74; AR^2=0.072			
Intercept	*1.041*		
FT4 V1	-6.163	-0.291	0.012

Table J2.1: Fetal growth rate **Maternal parameters at visit 1**

GRR1			
Adjusted	***B***	***BETA***	***p***
N=101; AR^2=0.223	*(2 outliers excluded)*		
Intercept	*3.732*		
BP SYS2	0.011	0.278	0.002
LEPT2	0.090	0.334	0.001
HPL2	2.174	0.341	<0.001
Not Adjusted	***B***	***BETA***	***p***
N=103; AR^2=0.223	*(2 outliers excluded)*		
Intercept			
BP SYS2			
LEPT2	*same outcome*		
HPL2			

GRR2			
Adjusted	***B***	***BETA***	***p***
N=100; AR^2=0.066			
Intercept	*0.390*		
HEIGHT	0.119	0.231	0.021
PI F V2	0.035	0.221	0.027
Not Adjusted	***B***	***BETA***	***p***
Outcome not significant			

Table J2.2: Birth weight

BW			
Adjusted	***B***	***BETA***	***p***
N=104; AR^2=0.252			
Intercept	*2.416*		
BIRTH AGE	0.017	0.390	<0.001
MW2	0.229	0.277	0.002
Not Adjusted	***B***	***BETA***	***p***
N=104; AR^2=0.110			
Intercept	*2.967*		
MW2	0.284	0.345	<0.001

BW			
Adjusted	***B***	***BETA***	***p***
N=103; AR^2=0.336	*(one outlier excluded)*		
Intercept	*2.289*		
BIRTH AGE	0.018	0.417	<0.001
MW2	0.259	0.315	<0.001
SEX	0.020	0.164	0.046
Not Adjusted	***B***	***BETA***	***p***
no outliers present			

Table J2.3: Birth parameters

BL			
Adjusted	***B***	***BETA***	***p***
N=100; AR^2=0.193			
Intercept	*27.513*		
BIRTH AGE	0.642	0.366	<0.001
IGF BP1 V2	-1.967	-0.231	0.013
Not Adjusted	***B***	***BETA***	***p***
N=99 ; AR^2=0.073			
Intercept	*31.547*		
MW2	9.487	0.287	0.004

BHC			
Adjusted	***B***	***BETA***	***p***
N=101; AR^2=0.195			
Intercept	*22.422*		
BIRTH AGE	0.371	0.341	<0.001
IGF BP1 V2	-1.390	-0.266	0.004
Not Adjusted	***B***	***BETA***	***p***
N=77; AR^2=0.257			
Intercept	*37.500*		
IGF BP1 V2	-1.626	-0.311	0.002

Table J2.4: Postnatal growth rate

GRRT D-Ba2			
Adjusted	***B***	***BETA***	***p***
N=71; AR^2=0.026			
Intercept	*1.282*		
SEX	0.074	0.422	<0.001
INS RES2	0.142	0.275	0.010
Not Adjusted	***B***	***BETA***	***p***
N=71; AR^2=0.093			
Intercept	*1.389*		
INS RES2	0.168	0.325	0.006

Table J2.5: Postnatal anthropometric parameters

BMI Ba1			
Adjusted	***B***	***BETA***	***p***
N=76; AR^2=0.254			
Intercept	*0.395*		
AGE Ba1	0.124	0.354	0.001
PI F V2	-0.046	-0.327	0.002
MW2	0.086	0.247	0.017
Not Adjusted	***B***	***BETA***	***p***
N=76; AR^2=0.195			
Intercept	*0.390*		
MW2	0.116	0.333	0.002
PI 60' V2	-0.040	-0.376	0.001

BMI Ba2			
Adjusted	***B***	***BETA***	***p***
N=71; AR^2=0.071			
Intercept	*0.062*		
SEX	-0.003	-0.291	0.014
Not Adjusted	***B***	***BETA***	***p***
N=71; AR^2=0.030			
Intercept	*0.060*		
LEPT2	-0.001	-0.210	0.000

Table J2.6: Postnatal glucose

GLC Ba1			
Adjusted	***B***	***BETA***	***p***
N=75; AR^2=0.217			
Intercept	*-1.516*		
AGE Ba1	2.069	0.324	0.003
CORT2	1.562	0.367	0.001
FT4 V2	-12.223	-0.332	0.003
Not Adjusted	***B***	***BETA***	***p***
N=76; AR^2=0.156			
Intercept	*-1.491*		
HPL2	1.611	0.224	0.044
CORT2	1.247	0.293	0.012
FT4 V2	-12.310	-0.347	0.003

GLC Ba2			
Adjusted	***B***	***BETA***	***p***
N=72; AR^2=0.049			
Intercept	*5.197*		
HPL2	2.090	0.250	0.034
Not Adjusted	***B***	***BETA***	***p***
N=73; AR^2=0.096			
Intercept	*5.638*		
HPL2	2.275	0.271	0.018
PI 30' V2	-0.577	-0.228	0.046

Table J2.7: Neonatal insulin and HOMA-IR index

INS Ba2			
Adjusted	***B***	***BETA***	***p***
N=74; AR^2=0.063			
Intercept	-0.475		
WEIG Ba2	0.260	0.346	0.041
SEX	-0.041	-0.271	0.034
Not Adjusted	***B***	***BETA***	***p***
Intercept			
No significant outcome			

HOMA Ba1			
Adjusted	***B***	***BETA***	***p***
N=74; AR^2=0.053			
Intercept	*0.006*		
PI F V2	-0.224	-0.258	0.027
Not Adjusted	***B***	***BETA***	***p***
Intercept			
PI F V2	*same outcome*		

Table J2.8: Postnatal leptin

LEPT Ba1			
Adjusted	***B***	***BETA***	***p***
N=74; AR^2=0.539			
Intercept	*-0.986*		
SEX	-0.726	-0.460	<0.001
WEIG Ba1	0.001	0.473	<0.001
AGE Ba1	2.736	0.263	0.013
Not Adjusted	***B***	***BETA***	***p***
Intercept			
No significant outcome			

LEPT Ba2			
Adjusted	***B***	***BETA***	***p***
N=7; AR^2=0.353			
Intercept	*-7.946*		
SEX	-0.207	-0.438	<0.001
WEIG Ba2	2.416	0.580	<0.001
INS 30' V2	-0.203	-0.237	0.017
Not Adjusted	***B***	***BETA***	***p***
Intercept			
No significant outcome			

APPENDIX K

Tables of outcomes from the linear mixed model analyses.

(Where applicable, the varialbes were used in their transformed form as described in the Appendix G. Results are expressed in units of particular transformation.)

Table K1. Maternal anthropometry and blood pressure.

Variable	Coef.	SE	z	P value	Adj. P*	95% CI
Weight [kg]						
V1 vs V2	-4.38	0.40	-11.00	0.000	<0.01	-5.16; -3.60
V1 vs V3	1.28	0.46	0.76	0.006	0.018	0.37; 2.19
V2 vs V3	5.66	0.47	12.2	0.000	<0.01	1.75; 6.57
BMI [kgm^{-2}]						
V1 vs V2	-1.75	0.16	-11.0	0.000	<0.01	-2.06; -1.44
V1 vs V3	0.51	0.18	2.72	0.006	0.018	0.14; 0.86
V2 vs V3	2.25	0.19	12.2	0.000	<0.01	1.89; 2.61
SYSTOLIC BP [mmHg]						
V1 vs V2	-1.16	1.27	-0.92	0.359	1.000	-3.63; 1.32
V1 vs V3	-8.29	1.44	-5.74	0.000	<0.01	-11.12; -5.46
V2 vs V3	-7.13	1.45	-4.92	0.000	<0.01	-9.97; -4.29
DIASTOLIC BP [mmHg]						
V1 vs V2	-1.66	1.01	-1.64	0.100	0.300	-3.65; 0.32
V1 vs V3	-9.35	1.16	-8.08	0.000	<0.01	-11.62; -7.08
V2 vs V3	-7.68	1.16	-6.63	0.000	<0.01	-9.95; -5.41

*Adjustment with the Bonferroni correction.

Table K2. Maternal glucose in the OGTT.

GLUCOSE						
Time points	Coef.	SE	z	P value	Adj. P*	95% CI
0 min						
V1 vs V2	-0.14	0.12	-1.21	0.228	0.684	-0.38; 0.09
V1 vs V3	-0.66	0.13	-4.95	0.000	<0.01	-0.92;-0.40
V2 vs V3	-0.52	0.13	-3.86	0.000	<0.01	-0.78; -0.25
30 min						
V1 vs V2	-0.51	0.12	-4.26	0.000	<0.01	-0.74; -0.27
V1 vs V3	-0.66	0.13	-4.99	0.000	<0.01	-0.92; -0.40
V2 vs V3	-0.16	0.13	-1.17	0.242	0.726	-0.42; 0.11
60 min						
V1 vs V2	-0.74	0.12	-6.23	0.000	<0.01	-0.98; -0.51
V1 vs V3	-0.25	0.13	-1.89	0.058	0.174	-0.51; 0.01
V2 vs V3	0.49	0.13	3.68	0.000	<0.01	-0.23; 0.75
120 min						
V1 vs V2	-0.45	0.12	-3.78	0.000	<0.01	-0.68; -0.22
V1 vs V3	-0.27	0.13	-2.02	0.044	0.132	-0.53; -0.01
V2 vs V3	0.18	0.13	1.36	0.172	0.516	-0.08; 0.44

*Adjustment with the Bonferroni correction.

Table K3. Maternal insulin in the OGTT.

INSULIN						
Time points	Coef.	SE	z	P value	Adj. P*	95% CI
0 min						
V1 vs V2	-8.31	32.40	-0.256	0.798	1.000	-71.81; 55.20
V1 vs V3	-19.77	36.17	-0.547	0.585	1.000	-90.67; 51.13
V2 vs V3	-11.46	36.26	-0.316	0.752	1.000	-82.53; 59.60
30 min						
V1 vs V2	-140.66	32.40	-4.34	0.000	<0.01	-204.16; -77.15
V1 vs V3	-7.50	36.33	-0.206	0.836	1.000	-78.71; 63.71
V2 vs V3	133.16	36.41	3.66	0.000	<0.01	61.79; 204.53
60 min						
V1 vs V2	-253.39	32.40	-7.82	0.000	<0.01	-316.89; -189.88
V1 vs V3	42.26	36.33	1.16	0.245	0.735	-28.95; 113.47
V2 vs V3	295.64	36.41	8.12	0.000	<0.01	224.27; 367.01
120 min						
V1 vs V2	-125.25	32.40	-3.87	0.000	<0.01	-188.75; -61.74
V1 vs V3	8.62	36.33	0.237	0.813	1.000	-62.59; 79.83
V2 vs V3	133.87	36.41	3.68	0.000	<0.01	62.49; 205.24

*Adjustment with the Bonferroni correction.

Table K4. Maternal C-peptide in the OGTT.

C-PEPTIDE						
Time points	Coef.	SE	z	P value	Adj. P*	95% CI
0 min						
V1 vs V2	-0.01	0.11	-0.081	0.936	1.000	-0.22; 0.20
V1 vs V3	0.03	0.12	0.252	0.801	1.000	-0.20; 0.26
V2 vs V3	0.04	0.12	0.323	0.747	1.000	-0.20; 0.27
30 min						
V1 vs V2	-0.17	0.12	-1.46	0.144	0.432	-0.40; 0.06
V1 vs V3	0.12	0.13	0.948	0.343	1.000	-0.13; 0.37
V2 vs V3	0.29	0.12	2.41	0.016	0.048	0.05; 0.52
60 min						
V1 vs V2	-0.44	0.12	-3.81	0.000	<0.01	-0.67; -0.21
V1 vs V3	0.39	0.13	3.08	0.002	0.006	0.14; 0.64
V2 vs V3	0.84	0.12	6.98	0.000	<0.01	0.60; 1.07
120 min						
V1 vs V2	-0.37	0.12	-3.15	0.002	0.006	-0.59; -0.14
V1 vs V3	0.41	0.13	3.2	0.001	0.003	0.16; 0.66
V2 vs V3	0.77	0.12	6.45	0.000	<0.01	0.54; 1.01

*Adjustment with the Bonferroni correction.

Table K5. Maternal proinsulin in the OGTT.

PROINSULIN						
Time points	Coef.	SE	z	P value	Adj. P*	95% CI
0 min						
V1 vs V2	-0.32	0.46	-0.695	0.487	1.000	-1.22; 0.58
V1 vs V3	-0.77	0.52	-1.49	0135	0.405	-1.78; 0.24
V2 vs V3	-0.45	0.52	-0.871	0.384	1.000	-1.47; 0.56
30 min						
V1 vs V2	-0.52	0.46	-1.12	0.264	0.792	-1.42; 0.39
V1 vs V3	-2.76	0.52	-5.32	0.000	<0.01	-3.77; -1.74
V2 vs V3	-2.24	0.52	-4.33	0.000	<0.01	-3.26; -1.23
60 min						
V1 vs V2	-1.08	0.46	-2.32	0.020	0.060	-1.99; -0.17
V1 vs V3	-2.83	0.52	-5.45	0.000	<0.01	-3.85; -1.81
V2 vs V3	-1.75	0.52	-3.38	0.001	0.003	-2.77; -0.74
120 min						
V1 vs V2	-1.43	0.46	-3.09	0.002	0.006	-2.33; -0.52
V1 vs V3	-3.51	0.52	-6.78	0.000	<0.01	-4.53; -2.50
V2 vs V3	-2.08	0.52	-4.02	0.000	<0.01	-3.10; -1.07

*Adjustment with the Bonferroni correction.

Table K6. Maternal hormones and the HOMA-IR index.

Variable	Coef.	SE	z	P value	Adj. P*	95% CI
IGF 1						
V1 vs V2	-125.35	8.10	-15.50	0.000	<0.01	-141.22; -109.48
V1 vs V3	81.03	9.24	8.77	0.000	<0.01	62.93; 99.13
V2 vs V3	206.38	9.26	22.30	0.000	<0.01	188.23; 224.54
IGF BP1						
V1 vs V2	8.72	3.12	2.79	0.005	0.015	2.60; 14.84
V1 vs V3	70.70	3.57	19.8	0.000	<0.01	63.70; 77.71
V2 vs V3	61.99	61.99	17.3	0.000	<0.01	54.96; 69.02
FT4						
V1 vs V2	1.08	0.17	6.50	0.000	<0.01	0.76; 1.41
V1 vs V3	0.10	0.19	0.54	0.593	1.000	-0.27; 0.48
V2 vs V3	-0.98	0.19	-5.13	0.000	<0.01	-1.36; -0.61
LEPT						
V1 vs V2	-2.63	1.13	-2.33	0.020	0.060	-4.85; -0.41
V1 vs V3	0.211	1.30	0.16	0.871	1.000	-2.34; 2.76
V2 vs V3	2.85	1.31	2.18	0.029	0.087	0.29; 5.40
HOMA-IR						
V1 vs V2	-0.26	0.10	-2.72	0.007	0.021	-0.45; -0.07
V1 vs V3	-0.57	0.11	-5.14	0.000	<0.01	-0.78; -0.35
V2 vs V3	-0.30	0.11	-2.74	0.006	0.018	-0.52; -0.09

*Adjustment with the Bonferroni correction.;

Table K7a. Maternal dietary intakes (a)

Variable	Coef.	SE	z	P value	Adj. P*	95% CI
ENERGY						
V1 vs V2	-0.02	0.04	-0.43	0.667	1.000	-0.10; 0.06
V1 vs V3	-0.12	0.05	-2.67	0.008	0.024	-0.21; -0.03
V2 vs V3	-0.11	0.05	-2.28	0.023	0.069	-0.20; -0.02
TOT PROT						
V1 vs V2	-0.03	0.05	-0.60	0.549	1.000	-0.12; 0.06
V1 vs V3	-0.01	0.05	-0.26	0.792	1.000	-0.11; 0.09
V2 vs V3	0.01	0.05	0.27	0.790	1.000	-0.09; 0.11
PL PROT						
V1 vs V2	-0.06	0.05	-1.23	0.220	0.660	-0.15; 0.03
V1 vs V3	-0.16	0.05	-3.14	0.002	0.006	-0.27; -0.06
V2 vs V3	-0.11	0.05	-2.05	0.040	0.120	-0.21; -0.01
AN PROT						
V1 vs V2	-0.02	0.06	-0.29	0.78	1.000	-0.13; 0.09
V1 vs V3	0.06	0.06	1.00	0.32	0.960	-0.06; 0.19
V2 vs V3	0.08	0.06	1.25	0.21	0.630	-0.05; 0.21
TOT FAT						
V1 vs V2	-0.02	0.06	-0.33	0.742	1.000	-0.14; 0.10
V1 vs V3	-0.20	0.07	-3.01	0.003	0.009	-0.34; -0.07
V2 vs V3	-0.19	0.07	-2.72	0.007	0.021	-0.32; -0.05
SAFA						
V1 vs V2	-0.01	0.06	-0.11	0.91	1.000	-0.12; 0.11
V1 vs V3	-0.07	0.07	-1.02	0.31	0.930	-0.20; 0.06
V2 vs V3	-0.06	0.07	-0.92	0.36	1.000	-0.19; 0.07
MUFA						
V1 vs V2	-0.04	0.06	-0.68	0.496	1.000	-0.16; 0.08
V1 vs V3	-0.24	0.07	-3.50	0.000	<0.01	-0.38; -0.11
V2 vs V3	-0.20	0.07	-2.89	0.004	0.012	-0.34; -0.07
PUFA						
V1 vs V2	-0.03	0.08	-0.42	0.678	1.000	-0.19; 0.13
V1 vs V3	-0.34	0.09	-3.71	0.000	<0.01	-0.51; -0.16
V2 vs V3	-0.30	0.09	-3.34	0.001	0.003	-0.48; -0.13
TOT CHO						
V1 vs V2	-0.02	0.04	-0.39	0.700	1.000	-0.09; 0.06
V1 vs V3	-0.10	0.04	-2.42	0.015	0.045	-0.19; -0.02
V2 vs V3	-0.09	0.04	-2.08	0.037	0.111	-0.17; -0.01
TOT AV CHO						
V1 vs V2	-0.01	0.04	-0.32	0.752	1.000	-0.09; 0.06
V1 vs V3	-0.11	0.04	-2.47	0.014	0.042	-0.19; -0.02
V2 vs V3	-0.09	0.04	-2.19	0.029	0.087	-0.18; -0.01

*Adjustment with the Bonferroni correction.

Table K7b. Maternal dietary intakes (b).

Variable	Coef.	SE	z	P value	Adj. P*	95% CI
CHOLEST. [mg]						
V1 vs V2	0.03	0.07	0.43	0.667	1.000	-0.11; 0.17
V1 vs V3	-0.07	0.08	-0.89	0.372	1.000	-0.23; 0.08
V2 vs V3	-0.10	0.08	-1.27	0.205	0.615	-0.26; 0.06
DIET. FIBRE [g]						
V1 vs V2	-0.06	0.05	-1.27	0.202	0.606	-0.16; 0.03
V1 vs V3	-0.06	0.06	-1.03	0.303	0.909	-0.16; 0.05
V2 vs V3	0.01	0.06	0.10	0.922	1.000	-0.10; 0.11
ADDED SUGAR [g]						
V1 vs V2	0.06	0.25	0.25	0.806	1.000	-0.44; 0.56
V1 vs V3	-0.61	0.29	-2.12	0.034	0.102	-1.17; -0.05
V2 vs V3	-0.67	0.29	-2.33	0.020	0.060	-1.23; -0.11
TOT PROT [%E]						
V1 vs V2	-0.16	0.38	-0.41	0.680	1.000	-0.91; 0.59
V1 vs V3	1.49	0.43	3.49	0.000	<0.01	0.66; 2.33
V2 vs V3	1.65	0.43	3.86	0.000	<0.01	0.81; 2.49
TOT FAT [%E]						
V1 vs V2	0.16	1.12	0.14	0.887	1.000	-2.03; 2.35
V1 vs V3	-2.83	1.24	-2.28	0.023	0.069	-5.27; -0.40
V2 vs V3	-2.99	1.24	-2.40	0.016	0.048	-5.43; -0.55
TOT CHO [%E]						
V1 vs V2	-0.15	1.09	-0.14	0.892	1.000	-2.29; 1.99
V1 vs V3	1.32	1.22	1.08	0.279	0.937	-1.07; 3.72
V2 vs V3	1.47	1.22	1.20	0.229	0.687	-0.93; 3.87

*Adjustment with the Bonferroni correction.

APPENDIX L



THE NATIONAL ACADEMIES PRESS

Marketing Department
Rights & Permissions

December 14, 2009 **Update: Reference #: 12140900**

Marketa Toman
Dept. Chemical Pathology
NHLS-WITS Medical School
Johannesburg
South Africa

Dear Ms. Toman:

You have requested permission to reprint the following material copyrighted by the National Academy of Sciences:

Tables: "Dietary Reference Intakes: Recommended Intakes for Individuals, Vitamins"
"Dietary Reference Intakes: Recommended Intakes for Individuals, Macronutrients"
Dietary Reference Intakes: The Essential Guide to Nutrient Requirements, 2006

Your request is granted for the material cited above. The charge for this permission is **$15.00 US** provided that credit is given to the copyright holder.

Suggested credit (example):
Reprinted with permission from (title), (year) by the National Academy of Sciences, Courtesy of the National Academies Press, Washington, D.C. (This credit may be edited pursuant to the publisher's house style and format so long as the essential elements are included).

Thank you,

Barbara Murphy

APPENDIX M

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

COMMITTEE FOR RESEARCH ON HUMAN SUBJECTS (MEDICAL)
Ref: R14/49 Toman

CLEARANCE CERTIFICATE **PROTOCOL NUMBER** M991114

PROJECT Factors Duing Pregnancy Affecting The Susceptibility of OffSpring To Non Insulin Dependentt Diabetes Mellitus

INVESTIGATORS Mrs M Toman

DEPARTMENT Dept of Chemical Pathology, SAIMR

DATE CONSIDERED 991126

DECISION OF THE COMMITTEE *

Approved unconditionally

DATE 00/01/31 **CHAIRMAN**.....(Professor P E Cleaton-Jones)

* Guidelines for written "informed consent" attached where applicable.

c c Supervisor: Dr N Crowther
Dept of Dept of Chemical Pathology, SAIMR

Works2\lain0015\HumEth97.wdb\M 991114
==========
DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10001, 10th Floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee.

DATE 14.02.2000 SIGNATURE .....

PROTOCOL NO.: M 991114

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

REFERENCES

A web-based training in medical embryology. Online course in embryology for medicine students developed by the universities of Fribourg, Lausanne and Bern (Switzerland). [Accessed: 18.12.2008] http://www.embryology.ch/indexen.html

Al MD, van Houwelingen AC, Kester AD, Hasaart TH, de Jong AE et al (1995). Maternal essential fatty acid patterns during normal pregnancy and their relationship to the neonatal essential fatty acid status. *Br J Nutr*, 74:55-68.

Al MD, van Houwelingen AC, Hornstra G (2000). Long-chain polyunsaturated fatty acids, pregnancy and pregnancy outcome. *Am J Clin Nutr*, 71(suppl):285S-91S.

Allen LH (2001). Biological mechanisms that might underline iron's effects on fetal growth and preterm birth. *J Nutr*, 131:581S-589S.

American Diabetes Association (ADA; 2009). Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care*, 32(1):S62-S67.

American Diabetes Association (ADA; 2008). Statement. Economic costs of diabetes in the U.S. in 2007. *Diabetes Care*, 31(3):596-615.

Andralojc KM, Mercallia A, Nowak KW, Albarello L, Calcango R et al (2009). Ghrelin-producing epsilon cells in the developing and adult human pancreas. *Diabetologia*, 52(3):486-493.

Andreasyan K, Ponsonby AL, Dwyer T, Morley R, Riley R et al (2007). Higher maternal dietary protein intake in late pregnancy is associated with a lower ponderal index at birth. *Eur J Clin Nutr*, 61(4):498-508.

Armitage JA, Taylor PD, Poston L (2005). Experimental models of developmental programming: consequences of exposure to an energy rich diet during development. Topical Review. *J Physiol*, 565 (Pt.1): 3-8.

Arslanian S (1998). Insulin secretion and sensitivity in healthy African-American vs American white children. *Clin Pediatr (Phila*), 37(2):81-88.

Arslanian S, Danadian K (1998). Insulin secretion, insulin sensitivity and diabetes in black children. *Trends Endocrinol Metab*, 9(5):194-199.

Ayuk PT-Y, Theophanous D, D'Souza SW, Sibley CP and Glazier JD (2002). L-arginin transport by the microvillous plasma membrane of the syncytiotrophoblast from human placenta in relation to nitric oxide production: effects of gestation, preeclampsia, and intrauterine growth restriction. *J Clin Endocrinol Metab*, 87(2):747-751.

Barker DJP (1992). Fetal growth and adult disease. Commentaries. *Br J Obstet Gynaecol*, 99(4):275-276.

Barker DJP (1995). Education and debate. Fetal origins of coronary heart disease. *BMJ*, 311:171-174.

Barker DJP (1997). Fetal nutrition and cardiovascular disease in later life. *Br Med Bull*, 53(1):96-108.

Barker DJP(1998). *In utero* programming of chronic disease. *Clin Sci (Lond)*, 95(2):115-128.

Barker DJP (2002). Commentary: Components in the interpretation of the high mortality in the country of Finnmark. *Int J Epidemiol*, 31(2):309-310.

Barker DJP (2004). The developmental origins of well-being. *Phil Trans R Soc Lond B*, 359:1359-1366.

Barker DJP, Bull AR, Osmond C, Simmonds SJ (1990). Fetal and placental size and risk of hypertension in adult life. *BMJ*, 301:259-262.

Barker DJP, Eriksson JG, Forsén T, Osmond C (2002). Fetal origins of adult disease: strength of effects and biological basis. *Int J Epidemiol*, 31(6):1235-1239.

Barker DJP, Hales CN, Fall CHD, Osmond C, Phipps K et al. (1993a). Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. *Diabetologia,* 36:62-67.

Barker DJP, Martyn CN (1992). The maternal and fetal origins of cardiovascular disease. Review article. *J Epidemiol Community Health*, 46(1):8-11

Barker DJP, Osmond C (1986). Infant mortality, childhood nutrition and ischaemic heart disease in England and Wales. *Lancet,* 321(8489):1077-81

Barker DJP, Osmond C (1987). Death rates from stroke in England and Wales predicted from past maternal mortality. *BMJ,* 295:83-86.

Barker DJP, Osmond C, Simmonds SJ, Wield GA (1993b). The relation of small head circumference and thinness at birth to death from cardiovascular disease in adult life. *BMJ,* 306:422-426.

Bateson P, Barker D, Clutton-Brock T, Deb D, D'Udine B et al (2004). Developmental plasticity and human health. *Nature,* 430: 419-421. (Abstract; accessed 20.8.2009] http://www.nature.com/nature/journal/v430/n6998/abs/nature02725.html

Bavdekar A, Yajnik CS, Fall CH, Bapat S, Pandit AN et al (1999). Insulin Resistance Syndrome in 8-year-old Indian children. Small at birth, big at 8 years, or both? *Diabetes,* 48: 2422-2429.

Baylink DJ, Finkelman RD, Mohan S (1993). Growth factors to stimulate bone formation. *J Bone Mineral Res*, 8(2):S565-S572.

Bell AW, Hay WW Jr., Ehrhardt RA (1999). Placental transport of nutrients and its implication for fetal growth. *J Reprod Fertil Suppl,* 54:401-410.

Benediktsson R, Lindsay RS, Noble J, Seckl JR, Edwards CR (1993). Glucocorticoid exposure in utero: new model for adult hypertension. *Lancet*, 341:339-341.

Ben-Haroush A, Yogev Y, Hod M (2004). Epidemiology of gestational diabetes mellitus and its association with Type 2 diabetes. *Diabet Med*, 21(2):103-113.

Bergmann RL, Bergmann KE, Dudenhausen IW (2008). Undernutrition and growth restriction in pregnancy. *Nestle Nutr Workshop Ser Pediatr Program*, 61:103-121

Bergman RN, Van Citters GW, Mittelman SD, Dea MK, Hamilton-Wessler M et al (2001). Central role of the adipocyte in the metabolic syndrome. *J Investig Med*, 49(1):119-126.

Bergman RN, Ider YZ, Bowden CR, Cobelli C (1979). Quantitative estimation of insulin sensitivity. *Am J Physiol*, 236:E667-E677.

Bergman RN, Finegood DT, Ader M (1985). Assessment of insulin sensitivity in vivo. *Endocr Rev*, 6:45-86.

Bernard C, Berthault M-F, Saulnier C, Ktorza A (1999). Neogenesis vs. apoptosis as main components of pancreatic β-cell mass changes in glucose-infused normal and mildly diabetic adult rats. *FASEB J*, 13:1195-1205.

Bertin E, Gangnerau M-N, Bailbé D, Portha B (1999). Glucose metabolism and β-cell mass in adult offspring of rats protein and /or energy restricted during the last week of pregnancy. *Am J Physiol*, 277(1Pt1):E11-E17.

Barzel US, Massey LK (1998). Excess dietary protein can adversely affect bone. *J Nutr*, 128:1051-1053.

Basch CE, Shea S, Zybert P (1994).The reproducibility of data from a food frequency questionnaire among low-income Latina mothers and their children. *Am J Public Health*, 84(5): 861-864.

Bieswal F, Ahn MT, Reusens B, Holvoet P, Raes M et al (2006). The Importance of Catch-up Growth after Early Malnutrition for the Programming of Obesity in Male Rat. *Obesity*, 14: 1330-1343.

Billestrup N, Nielsen JH (1991). The stimulatory effect of growth hormone, Prolactin, and placental lactogen on beta-cell proliferation is not mediated by insulin-like growth factor-1. *Endocrinology*, 129(2):883-888.

Billewicz WZ, Thomson AM (2005). Birthweights in sonsecutive pregnancies. *BJOG*, 80(6):491-498.

Black AE (2000). Critical evaluation of energy intake using the Goldberg cut-off for energy intake: basal metabolic rate. A practical guide to its calculation, use and limitations. *Int J Obes*, 24:1119-1130.

Bloemberg BPM, Kromhout D, Obermann-De Boer GL, Van Kampen-Donker M. (1989). The reproducibility of dietary intake data assessment with the cross-check dietary history method. *Am J Epidemiol*,130 (5):1047-1056.

Bogardus C (1995). Agonist/Antagonist. Agonist: The case for insulin resistance as a necessary and sufficient cause of type II diabetes mellitus. *J Lab Clin Med*, 125: 556-558.

Bonjour JP, Ammann P, Chavalley T, Rizzoli R (2001). Protein intake and bone growth. *Can J Appl Physiol*, 26(Suppl):S153-156.

Bonner-Weir S (1990). Morphology or the endocrine pancreas. The pancreatic islets. In: Becker KL, editor. *Principles and practice of endocrinology and metabolism*. Part IX: Disorders of fuel metabolism. Chapter 135:pp 1064-1068.

Bonner-Weir S (1994). Brief Reviews. Islet cell growth and the growth factors involved. *Trends Endocrinol Metab*, 5(2):60-64.

Bonora E, Targher G, Alberiche M, Bonadonna RC, Saggiani F et al. (2000). Homeosasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity. *Diabetes Care*, 23:57-63.

Bourne LT, Lambert EV, Steyn K (2002). Where does the black population of South Africa stand on the nutrition transition? *Public Health Nutr*, 5(1A):157-162.

Bouwens L, Rooman I (2005). Regulation of pancreatic Beta-cell mass. *Physiol Rev*, 85:1255-1270.

Bradshaw D, Pieterse D, Norman R, Levitt NS (2007). Estimating the burden of disease attributable to diabetes in South Africa in 2000. *JEMDSA*, 12(2):65-71.

Brelje TC, Scharp DW, Lacy PE, Ogren L, Talamantes F et al (1993). Effect of homologous placental lactogens, prolactins, and growth hormones on islet β-cell division and insulin secretion in rat, mouse, and human islets: implication for placental lactogen regulation of islet function during pregnancy. *Endocrinology*, 132:879-887.

Brudenell JM (1989). Obstetric problems of fetal macrosomia. Diagnosis of fetal macrosomia. In: Sharp F, Fraser RB, Milner RDG, editors. *Fetal Growth*. Proceedings of the Twentieth Study Group of the Royal College of Obstetrics and Gynaecologiests. The Royal College of Obstatricians and Gynaecologists 1989.The Peacock Press Ltd, England, pp159-160.

Buchanan TA (2001). Pancreatic B-cell defects in gestational diabetes: Implications for the pathogenesis and prevention of Type 2 diabetes. *J Clin Endocrinol Metab*, 86(3):989-993.

Burdge GC, Calder PC (2006). Lipid metabolism: relevance to developmental origins of health and disease. The effects of impaired assimilation of polyunsaturated fatty acids on fetal development. The central nervous system. In: Gluckman PD, Hanson MA, editors. First Reprint 2007. *Developmental origins of health and disease*. New York: Cambridge University Press. Chapter 11: 166-168.

Burton GJ, Hempstock J, Jauniaux E (2001). Nutrition of the human fetus during the first trimester – a review. *Placenta*, 22(Suppl A):S70-S77.

Burton GJ, Jauniaux E, Watson AL (1999). Influence of oxygen supply on placental structure. In: O'Brien PMS, Wheeler T, Barker DJP, editors. *Fetal Programming.* Influences on Development and Disease in Later Life. Royal College of Obstetricians and Gynaecologists (RCOG), London. RCOG Press. Chapter 28: 326-341.

Burton GJ, Watson AL, Hempstock J, Skepper JN, Jauniaux E (2002). Uterine glands provide histotrophic nutrition for the human fetus during the first trimester of pregnancy. *J Clin Endocrinol Metab*, 87(6):2954-2959.

Byberg L, McKeigue PM, Zethelius B, Lithell HO (2000). Birth weight and the insulin resistance syndrome: association of low birth weight with truncal obesity and raised plasminogen activator inhibitor-1 but not with abdominal obesity or plasma lipid disturbances. *Diabetologia*, 43:54-60.

Byrne CD, Phillips DI (2000). Fetal origins of adult disease: epidemiology and mechanisms. *J Clin Pathol*, 53: 822-828.

Byrne MM, Struris J, Menzel S, Yamagata K, Fajans SS et al (1996). Altered insulin secretory responses to glucose in diabetic and nondiabetic subjects with mutations in the diabetes susceptibility gene MODY3 on chromosome 12. *Diabetes,* 45 (11):1503-1510.

Campbell AGM, Dawes GS, Fishman AP, Hyman AI (1967). Regional redistribution of blood flow in the mature fetal lamb. *Circ Res*, XX1:229-235

Campbell DM, Hall MH, Barker DJP, Cross J, Shiell AW and Godfrey KM (1996). Diet in pregnancy and the offspring's blood pressure 40 years later. *Br J Obstet Gynaecol*, 103: 237-280.

Campbell S (1989). The detection of intrauterine growth retardation. Detection of the SGA fetus. In: Sharp F, Fraser RB, Milner RDG, editors. *Fetal Growth.* Proceedings of the Twentieth Study Group of the Royal College of Obstetrics and Gynaecologiests. The Royal College of Obstatricians and Gynaecologists 1989.The Peacock Press Ltd, England, pp.251-252.

Campbell S, Thoms A (1977). Ultrasound measurement of the fetal head to abdomen circumference ratio in the assessment of growth retardation. *Br J Obstet Gynaecol*, 84(3):165-174.

Cao R, Brakenhielm E, Wahlestedt C, Thyberg J, Cao Y (2001). Leptin induces vascular permeability and synergistically stimulates angiogenesis with FGF-2 and VEGF. *Proc Natl Acad Sci*, 98(11): 6390-6395.

Casey BM, McIntire DD, Leveno KJ (2001). The continuing value of the Apgar score for the assessment of newborn infants. *N Engl J Med*, 344 (7):467-71

Cassel PG, Hitman GA (2003). Genetics of Type 2 diabetes mellitus: current progress and future directions. In: Raz I, Skyler JS, Shafrir E, editors. *Diabetes.* First edition. From Research to Diagnosis and Treatment.Martin Dunitz, an imprint of the Taylor & Francis Group, London, pp 35-54.

Castellucci M, Kosanke G, Verdenelli F, Huppertz B, Kaufmann P (2000). Villous sprouting: fundamental mechanisms of human placental development. *Hum Reprod Update*, 6(5):485-494.

Castellucci M, Scheper M, Scheffen I, Celona A, Kaufmann P (1990). The development of the human placental villous tree. *Anat Embryol (Berl),*181(2):117-128.

Castro FC, Leite HV, Pereira AK, Reis ZSN, Cabral ACV (2004). Association between anthropometry and circulationg leptin in maternal, fetal andplacental compartments, in healthy pregnancy. *Rev. Bras Ginecol Obstet* [online], 26(9):691-695.

Cetin I, Marconi AM, Bozzeti P, Sereni LP, Corbetta C, Pardi G, Battaglia FC (1988). Umbilical amino acid concentrations in appropriate and small for gestational age infants: a biochemical difference present in utero. *Am J Obstet Gynecol*, 158:120-126.

Chan J, Rabbitt EH, Innes BA, Bulmer JN, Stewart PM et al (2007). Glucocorticoid-induced apoptosis in human deciduas: a novel role for 11ß-hydroxysteroid dehydrogenase in late gestation. *J Endocrinol*, 195:7-15.

Chen H, Sullivan G, Quon MJ (2005). Assessing the predictive accuracy of QUICKI as a surrogate index for insulin sensitivity using a calibration model. *Diabetes*, 54:1914-1925.

Ciliberto CF and Marx GF (1998). Physiological changes associated with pregnancy. *Update in Anaesthesia*, section Physiology, 9(2):1-3; http://www.nda.ox.ac.uk/wfsa/html/u09/u09_003.htm. [Accessed: 22.8.2009]

Clark A, Grant AM (1983). Quantitative morphology of endocrine cells in human fetal pancreas. *Diabetologia,* 25:31-35.

Clayton PE, Cianfarani S, Czernichow P, Johannsson G, Rapaport R et al (2007). Consensus Statement: Management of the child born small for gestational age through to adulthood: A Consensus Statement of the International Societies of Pediatric Endocrinology and the Growth Hormone Research Society. *J Clin Endocrin Metab*, 92(3):804-810.

Corwin RL, Hartman TJ, Maczuga SA, Graubard BI (2005). Dietary saturated fat intake is inversely associated with bone densitiy in humans: analysis of NHANES III. *J Nutr*, 136: 159-165.

Coughlan A, McCarty DJ, Jorgensen LN, Zimmet P (1997). The Epidemic of NIDDM in Asian and Pacific Island populations: Prevalence and risk factors. *Horm Metab Res,* 29: 323-331.

Cox SE (1999). The Fetal origins hypothesis: An overview and implications. *Nutrition Abstracts and Reviews. Series A: Human and Experimental*, 69 (10):929-935.

Crespi EJ, Denver R (2005). Ancient origins of human developmental plasticity. Wiley-Liss Plenary Symposium. *Am J Hum Biol*, 17: 44-54.

Crowther NJ, Cameron N, Trusler J, Gray IP (1998). Association between poor glucose tolerance and rapid post natal weight gain in seven-year-old children. *Diabetologia,* 41(10):1163-1167.

Crowther NJ, Cameron N, Trusler J, Toman M, Norris SA et al (2008). Influence of catch-up growth on glucose tolerance and cell function in 7-year-old children: Results from the Birth to Twenty Study. *Pediatrics,* 121:e1715-e1722.

Crowther NJ, Ferris WF, Ojwang PJ, Rheeder P (2006). The effect of abdominal obesity on insulin sensitivity and serum lipid and cytokine concentrations in African women. *Clinical Endocrinology*, 64(5): 535-541.

Crowther NJ, Trusler J, Cameron N, Toman M, Gray IP (2000). Relation between weight gain and beta-cell secretory activity and non-esterified fatty acid production in 7-year-old African children: results from the Birth to Ten Study. *Diabetologia,* 43:978-985.

Cruz ML, Bergman RN, Goran MI (2002). Unique effect of visceral fat on insulin sensitivity in obese Hispanic children with a family history of type 2 diabetes. *Diabetes Care*, 2:1631-1636.

Dallal GE (2007, August8). Multiple linear regression. Regression diagnostics. Collinearity. In: *The little handbook of statistical practice.* http://www.StatisticalPractice.com Accessed: November 2010.

De Boo HA, Harding JE (2006). The developmental origins of adult disease (Barker) hypothesis. Invited Review. *Australian and New Zealand Journal of Obstetrics and Gynaecology*, 46:4-14.

DeFronzo RA, Tobin JD, Andres R (1979). Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol*, 237: E214-E223.

De Gasparo M, Milner GR, Norris PD, Milner RDG (1978). Effect of glucose and amino acids on foetal rat pancreatic growth and insulin secretion *in vitro*. *J Endocrinol*, 77:241-248.

De Geus EJ, Posthuma D, Ijzerman RG, Boomsma DI (2001). Comparing blood pressure of twins and their singleton siblins: being a twin does not affect adult blood pressure. *Twin Res*., 4(5):385-391.

Denver RJ, Crespi EJ (2006). Stress hormones and human developmental plasticity: Lessons from tadpoles. *NeoReviews*, 7(4):e183-e188.

Department of Health (1999). South Africa Demographic and Health Survey (SADHS) 1998, Pretoria: Department of Health, www.doh.gov.za/facts/sadhs-f.html.[Accessed: 11.3.2009]

Desai M, Crowther NJ, Lucas A, Hales N (1996). Organ-selective growth in the offspring of protein-restricted mothers. *Br J Nutr*, 76(4):591-603.

Desai M, Crowther NJ, Ozanne SE, Lucas A, Hales CN (1995). Adult glucose and lipid metabolism may be programmed during fetal life. *Biochem. Soc Trans*, 23: 331-335.

Diabetes Atlas, 3rd edition; IDF.[accessed: 2.9.2009]; http://www.diabetesatlas.org

Diabetes Atlas, 4th edition; IDF.[accessed: 30.11.2009]; http://www.diabetesatlas.org

Dildy GA, Jackson GM, Fowers GK, Oshiro BT, Varner BT et al. (1996). Very advanced maternal age: pregnancy after age 45. *Am J Obstet Gynecol*, 175(3 Pt1): 668-674.

Divon MY, Ferber A (2002). Doppler evaluation of the fetus. *Clin Obstet Gynecol*, 45(4):1015-1025.

Dodic M, Peers A, Coghlan JP, Wintour M (1999). Can excess glucocorticoid, in utero, predispose to cardiovascular and metabolic disease in middle age*? Trends Endocrinol Metab,* 10 (3): 86-91.

Dogra VS, Bhatt S (2006). Intrauterine growth retardation. [Accessed: 2.2.2009] http://Emedicine.medscape.com/article/404098-overview.

Duggleby SL, Jackson AA (2002). Protein, amino acid and nitrogen metabolism during pregnancy: how might the mother meet the needs of her fetus? *Cur Opin Clin Nutr Metab Care*, 5: 503-509.

Dugoff L, Lynch AM, Cioffi-Ragan D, Hobbins JC, Schultz LK et al (2005). First trimester uterine artery Doppler abnormalities predict subsequent intrauterine growth restriction. *Am J Obstet Gynecol*, 193:1208-1212.

Dunne FP (1999). Pregestational diabetes mellitus and pregnancy. *Trends Endocrinol Metab*, 10(5):179-182.

Durbin SA, Lee CW, Parker VG (2005). The Effect of Amniotic Fluid Index on the Accuracy of Sonographic Estimated Fetal Weight. *Journal of Diagnostic Medical Sonography*, 21(4):329-335.

Duttaroy AK (2009). Transport of fatty acids across the human placenta: A review. *Prog Lip Res*, 48:52-61.

El-Haddad MA, Ross MG (2006). The developmental environment: effect on fluid and electrolyte homeostasis. In: Gluckman PD, Hanson MA, editors. First Reprint 2007. *Developmental origins of health and disease*. New York: Cambridge University Press. Chapter 24: 323-335.

Emanuel I, Filakti H, Alberman E and Evans SJ (1992). Intergenerational studies of human birthweight from the 1958 birth cohort. 1. Evidence for a multigenerational effect. *Br J Obstet Gynaecol*, 99 (1):67-74.)

Ericsson A, Hamark B, Johansson BR, Powell TL, Jansson T (2004). Hormonal regulation of glucose and system A amino acid transport in the first trimester placental villous fragments. *Am J Physiol Regul Integr Comp Physiol*, 288(3):R656-662.

Eriksson J (2001). Commentary: Early "catch-up" growth is good for later health. *Int J Epidemiol*,30: 1330-1331.

Eriksson J, Franssila-Kallunki A, Ekstrand A, Saloranta C, Widen E et al (1989). Early metabolic defects in persons at increased risk for NIDDM. *N Engl J Med*, 321(6):337-43.

Eriksson JG, Forsén T, Osmond C, Barker DJP (2003b). Pathways of infant and childhood growth that lead to Type 2 diabetes. *Diabetes Care*, 26(11):3006-3010.

Eriksson JG, Forsén T, Tuomilehto J, Jaddoe VW, Osmond C, Barker DJP (2002). Effects of size at birth and childhood growth on the insulin resistance syndrome in elderly individuals. *Diabetologia*, 45: 342-348.

Eriksson JG, Forsén T, Tuomilehto J, Osmond C, Barker DJP (2001). Early growth and coronary heart disease in later life: longitudinal study. *BMJ*, 322:949-953.

Eriksson JG, Forsén T, Tuomilehto J, Osmond C, Barker DJP (2003a). Early adiposity rebound in childhood and risk of Type 2 diabetes in adult life. *Diabetologia* 46:190-194.

Eriksson JG, Forsén T, Tuomilehto J, Winter PD, Osmond C, Barker DJP (1999b). Catch-up growth in childhood and death from coronary heart disease: longitudinal study. *BMJ*, 318(7181): 427-431.

Erkkola M, Karppinen M, Javanainen J, Räsänen L, Knip M et al. (2001). Validity and reproducibility of a food frequency questionnaire for pregnant Finish women. *Am J Epidemiol*, 154:466-476.

Ethnic Groups and Language. South Africa Table of Contents. Language Groups. http://countrystudies.us/south-africa/45.htm [Accessed 11.7.2009]

Fall CH, Stein CE, Kumaran K, Cox V, Osmond C, Barker DJ, Hales CN (1998). Size at birth, maternal weight, and type 2 diabetes in South India. *Diabet Med*, 15(3):220-227. (Abstract)

Fall CHD (2001). Non-industrialised countries and affluence. *Br Med Bull*, 60:33-50.

Fall CHD (2003a). The Fetal and Early Life Origins of Adult Disease. *Indian Pediatrics*, 40:480-502.

Fall CHD, Yajnik CS, Rao S, Davies AA, Brown N et al. (2003b). Micronutrients and fetal growth. *J Nutr*, 133:1747S-1756S.

Ferris WF, Naran NF, Crowther NJ, Rheeder P, vand der Merwe L et al. (2005). The relationship between insulin sensitivity and serum adiponectin levels in theree population groups. *Horm Metab Res*, 37(11):695-701.

Fleming TP (2006). The periconceptional and embryonic period. Embryo metabolism in response to environmental conditions. In: Gluckman PD and Hanson MA, editors. *Developmental Origins of Health and Disease*. New York: Cambridge University Press. Chapter 4: 53-54.

Forsén T, Eriksson JG, Tuomilehto J, Teramo K, Osmond C, Barker DJP (1997). Mother's weight in pregnancy and coronary heart disease in a cohort of Finish men: follow up study. *BMJ*, 315:837-840.

Fowden AL (1992). The role of insulin in fetal growth. *Early Hum Dev*, 29(1-3):177-181.

Fowden AL, Silver M (1995). The effects of thyroid hormones on oxygen and glucose metabolism in the sheep fetus during late gestation. *J Physiol*, 482 (1):203-213.

Fowden AL, Forhead AJ (2004). Endocrine mechanisms of intrauterine programming. *Reproduction*, 127:515-526.

Fowden AL, Hay WW Jr. (1988). The effects of pancreatectomy on the rates of glucose utilisation, oxidation and production in the sheep fetus. *Quarterly Journal of Experimental Physiology*, 73:973-984.

Gauteng Department of Health. Gauteng Provincial Government. Directorate. *Mother and Child Health.* Ante Natal Care policy Document. (No issue date supplied on the document.)

Gallaher BW, Breier BH, Keven CL, Harding JE, Gluckman PD (1998). Fetal programming of insulin-like growth factor (IGF)-I and IGF-binding protein-3: evidence for an altered response to undernutrition in late gestation following exposure to periconceptual undernutrition in the sheep. *J Endocrinol*, 159:501-508.

Gesina E, Blondeau B, Milet A, Le Nin I, Duchene B et al (2006). Glucocorticoid signaling affects pancreatic development through both direct and indirect effects. *Diabetologia*, 49:2939-2947.

Ghafoorunissa, Ibrahim A, Natarajan S (2005). Substituting dietary linoleic acid with alpha-linolenic acid improves insulin sensitivity in sucrose fed rats. *Biochim Biophys Acta*, 1733(1):67-75.

Gilbert JS, Brandon E, Vera T (2007). Fetal insulin secretion in late gestation: does size matter*? J Physiol*, 585 (3): 651-652.

Girling JC (2006). Thyroid disorders in pregnancy. *Curr Obstet Gynaecol*, 16:47-53.

Gluckman PD (1995). The Endocrine Regulation of Fetal Growth in Late Gestation: The Role of Insulin-Like Growth Factors. Clinical Review 68. *J Clin Endocrinol Metab*, 80(4):1047-1050.

Gluckman PD, Hanson MA (2006). The conceptual basis for the developmental origins of health and disease. In: Gluckman PD, Hanson MA, editors. *Developmental origins of health and disease.* New York: Cambridge University Press. Chapter 3: 33-50

Gluckman PD, Hanson MA (2007). Developmental plasticity and human disease: research directions. Symposium. *J Intern Med*, 261:461-471.

Gluckman PD, Hanson MA, Spencer HG (2005a). Predictive adaptive responses and human evolution. *Trends Ecol Evol*, 20(10):527-533.

Gluckman PD, Hanson MA, Spencer HG and Bateson P (2005b). Environmental influences during development and their later consequences for health and disease: implications for the interpretation of empirical studies. *Proc Biol Sci*, 272 (1564):671-677.

Godfrey K (2006). The 'developmental origins' hypothesis: Epidemiology. In: Gluckman PD, Hanson MA, editors. *Developmental origins of health and disease*. New York: Cambridge University Press. Chapter 2, Figure 2.4.

Godfrey K and Robinson S (1998). Maternal nutrition, placental growth and fetal programming. Reproduction and Development Group Symposium on "Early environmental, genetic and nutritional influences on adult disease". *Proc Nutr Soc*, 57:105-111.

Godfrey KM, Redman CW, Barker DJ, Osmond C (1991). The effect of maternal anaemia and iron deficiency on the ratio of fetal weight to placental weight. *Br J Obstet Gynaecol*, 98(9):886-891.

Godfrey K, Robinson S, Barker DJP, Osmond C, Cox V (1996). Papers. Maternal nutrition in early and late pregnancy in relation to placental and fetal growth. *BMJ*,312(7028):410

Godfrey KM, Barker DJP (2000). Fetal nutrition and adult disease. *Am J Clin Nutr*, 71(suppl):1344S-1352S.

Godfrey KM, Redman CW, Barker DDJ, Osmond C (1991). The effect of maternal anaemia and iron deficiency on the ratio of fetal weight to placental weight. *Br J Obstet Gynaecol*, 98(9): 886-891.

Gramellini D, Fieni S, Verrotti C, Piantelli G, Cavallotti D et al (2004). Ultrasound evaluation of amniotic fluid volume: methods and clinical accuracy. Conference Report. *Acta Biomed,* 75 (Suppl 1):40-44.

Gray IP, Cooper PA, Cory BJ, Toman M, Crowther NJ (2002). The intrauterine environment is a strong determinant of glucose tolerance during the neonatal period, even in prematurity. *J Clin Endocrinol Metab*, 87(9): 1-5.

Gude NM, Roberts CT, Kalionis B, King RG (2004*)*. Growth and function of the normal human placenta. *Thrombosis Research*, 114: (5-6) 397-407.

Guidozzi F, Patel R, MacPhail AP (1995). A prospective study of iron status in white and black pregnant women in an urban hospital. *S Afr Med J*, 85(3):170-173.

Gurr MI (2000). Fats. Basics of fat metabolism. Digestion. In: Garrow JS, James WPT, Ralph A, editors. *Human Nutrition and Dietetics*. 10th edition. Churchill Livingstone, UK.Chapter 7:106-107.

Hadlock FP, Deter RL, Harrist RB, Park SK (1984). Estimated Fetal Age: Computer-assisted analysis of multiple fetal growth parameters. *Radiology*, 152: 497 (Sonoline Sienna User Reference)

Hadlock FP, Deter RL, Harrist RB, Roecker E, Park SK (1983). A Date-Independent Predictor of Intrauterine Growth Retardation: Femur length/abdominal circumference ratio. *Am J Roentgenol*, 141: 979-984.

Hadlock FP, Harrist RB, Sharman RS, Deter RL, Park SK (1985). Estimation of fetal weight with the use of head, body, and femur measurements – A prospective study. *Am J Obstet Gynecol*, 151: 333.

Haffner SM, Miettien H, Stern MP (1997). The homeostasis model in the San Antonio Heart Study. *Diabetes Care*, 20(7):1087-1092.

Hahn T, Barth S, Graf R, Engelmann M, Beslagic D et al (1999). Placental glucose transporter expression is regulated by glucocorticoids. *J Clin Endocrinol Metab*, 84(4):1445-1452.

Hales CN, Barker DJP (1992). Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. *Diabetologia*, 35: 595-601.

Hales CN, Barker DJP (2001). The thrifty phenotype hypothesis. *Br Med Bull*, 60:5-20.

Hales CN, Barker DJP, Clark PMS, Cox LJ, Fall C, Osmond C, Winter PD (1991). Fetal and infant growth and impaired glucose tolerance at age 64. *BMJ,* 303:1019-1022.

Handwerger S, Freemark M (2000). The roles of placental growth hormone and placental lactogen in the regulation of human fetal growth and development. *J Pediatr Endocrinol Metab*, 13(4):343-356.

Harigaya A, Nagashima K, Nako Y, Morikawa A (1997). Relationship between concentration of serum leptin and fetal growth. *J Clin Endocrinol Metab*, 82:3281-3284.

Haugen G, Hanson M, Kiserud T, Crozier S, Inskip H et al (2005). Fetal liver-sparing cardiovascular adaptations linked to mother's slimness and diet. *Circ Res*, 96:12-14.

Hay WW (1994). Placental transport of nutrients to the fetus. *Horm Res,* 42(4-5):215-222.

Hay WW Jr (2006a). Placental-fetal glucose exchange and fetal glucose metabolism. *Trans Am Clin Climatol Assoc*, 117: 321-340.

Hay WW Jr (2006b). Recent observations on the regulation of fetal metabolism by glucose. Symposium Report. *J Physiol*, 572 (Pt 1): 17-24.

Hay WW Jr, Meznarich HK (1986). The effect of hyperinsulinaemia on glucose utilization and oxidation and on oxygen consumption in the fetal lamb. *Quarterly Journal of Experimental Physiology*, 71(689-698.

Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ et al (2008). Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proc Natl Acad Sci USA*, 105(44):17046-17049.

Helland IB, Reseland JE, Saugstad OD, Drevon CA (1998). Leptin levels in pregnant women and newborn infants: gender differences and reduction during the neonatal period. *Pediatrics.* [Accessed 8.7.2006]. http://pediatrics.aapublications.org/cgi/content/full/101/3/e12.

Hill DJ, Duvellie B (2000). Pancreatic development and adult diabetes. *Pediatr Res*, 48 (3): 269-274.

Hills FA, English J, Chard T (1996). Circulating levels of IGF-I and IGF-binding protein-1 through pregnancy: relation to birthweight and maternal weight. *J Endocrinol*, 148(2):303-9.

Hinchliffe SA, Lynch MRJ, Sargent PH, Howard CV, Van Velzen (1992). Fetal and Neonatal Medicine. The effect of intrauterine growth retardation on the development of renal nephrones. *Br J Obstet Gynaecol, 99:296-301*.

Hogg J, Han VKM, Clemmons DR, Hill DJ (1993). Interactions of nutrients, insulin-like growth factors (IGFs) and IGF-binding proteins in the regulation of DNA synthesis by isolated fetal rat islets of Langerhans. *J Endocrinol*, 138:401-412.

Hoque M, Hoque E, Kader SB (2009). Risk factors for anaemia in pregnancy in rural KwaZulu-Natal, South Africa: Implication for health education and health promotion. *SA Fam Pract*, 51(1):68-72.

Hoque M, Kader SB, Hoque E (2007). Prevalence of Anaemia in Pregnancy at Uthungulu Health of KwaZulu-Natal, South Africa-2003.3. [Abstract; Accessed: 2.11.2009], http://www.safpj.co.za/index.php/safpj/article/view/630

http://www.childrencount.ci.org.za) [Accessed: 5.11.2009].

Högström M, Nordström P, Nordström A (2007). n-3 Fatty acids are positively associated with peak bone mineral density and bone accrual in healthy men: the NO_2 Study. *Am J Clin Nutr*, 85:803-807.

Humphriss DB, Stewart MW, Berrish TS, Barriocanal LA, Trojano LR et al (1997). Multiple metabolic abnormalities in normal glucose tolerant relatives of NIDDM families. *Diabetologia*, 40:1185-1190.

Hwang DL, Huang SP, Lan WS, Lee PD (2003). Elevated insulin, proinsulin and insulin-like growth factor-binding protein-1 in liver disease. *Growth Horm IGF Res*, 13(6):316-321.

Iglesias-Barreira V, Ahn MT, Reusens B, Dahri S, Hoet JJ, Remacle C (1996). Pre-and postnatal low protein diet affect pancreatic islet blood flow and insulin release in adult rats. *Endocrinology*, 137(9) 3797-3801.

Iliadou A, Cnattingius S, Lichtenstein P (2004). Low birthweight and Type 2 diabetes: A study on 11 162 Swedish twins. *IJE*, 33:948-953.

Illsley NP (2000). Glucose transporters in the human placenta. *Placenta*, 21(1):14-22.

International Diabetes Federation (2002). Consensus Document. Consensus document of the aetiology of Type 2 diabetes. Colombo, Sri Lanka meeting, July 2002. [Accessed: August 2009] www.idf.org/webdata/docs/Consensus%20Document.doc

Jablonka E, Oborny B, Molnar I, Kisdi E, Hofbauer J et al. (1995). The adaptive advantage of phenotypic memory in changing environments. *Philos Trans R Soc Lond B Biol Sci,* 350:133-141.

Jaffe R, Jauniaux E, Hustin J (1997). Maternal circulation in the first-trimester human placenta-myth or reality? *Am J Obstet Gynecol*, 176(3):695-705.

James JL, Stone PR, Chamley LW (2006). The effects of oxygen concentration and gestational age on extravillous trophoblast outgrowth in a human first trimester villous explant model. *Hum Reprod*, 21(10): 2699-2705.

Jansson N, Greenwood SL, Johansson BR, Powell TL, Jansson T (2003). Leptin stimulates the activity of the system A amino acid transporter in human placental villous fragments. *J Clin Endocrinol Metab*, 88(3):1205-1211.

Jansson T, Ekstrand Y, Wennergren M and Powell TL (2001). Placental glucose transport in gestational diabetes mellitus. *Am J Obstet Gynecol*, 184:111-116.

Jansson T, Ekstrand Y, Björn C, Wennergren M and Powell TL (2002). Alterations in the activity of placental amino acid transporters in pregnancies complicated by diabetes. *Diabetes*, 51:2214-2219.

Jansson T, Wennergren M, Powell TL (1999). Placental glucose transport and GLUT1 expression in insulin-dependent diabetes. *Am J Obstet Gynecol*, 180:163-168.

Jaquet D, Gaboriau A, Czernichow P, Levy-Marchal C (2000). Insulin resistance early in adulthood in subjects born with intrauterine growth retardation. *J Clin Endocrinol Metab*, 85(4):1401-1406.

Jauniaux E, Gulbis B, Burton GJ (2003). The human first trimester gestational sac limits rather than facilitates oxygen transfer to the foetus. *Placenta*, 24(Suppl A):S86-93.

Jauniaux E, Watson AL, Hempstock J, Yi-Ping Bao, Skepper JN and Burton GJ (2000). Onset of maternal arterial blood flow and placental oxidative stress. A possible factor in human early pregnancy failure. *Am J Pathol*, 157(6):2111- 2122.

Jaya DS, Kumar NS, Bai LS (1995). Anthropometric indices, cord length and placental weight in newborns. *Indian Pediatrics*, 32(11):1183-1188.

Johnson LW and Weinstock RS (2006). The Metabolic Syndrome: Concepts and controversy. *Mayo Clin Proc* 81(12):1615-1620. http://www.mayoclinicproceedings.com [Accessed: 12.9.2009]

Jones CT, Parer JT (1983). The effect of alterations in placental blood flow on the growth of and nutrient supply to the fetal guinea-pig. *J Physiol*, 343(525-537.

Junien C, Nathanielsz P (2007). Complications of obesity. Report on the IASO Stock Conference 2006: early and lifelong environmental epigenomic programming of metabolic syndrome, obesity and type II diabetes. *Obesity Reviews*, 8(6):487-502.

Kabir I, Rahman MM, Haider R, Mazumder RN, Khaled MA et al (1998). Increased height gain of children fed a high-protein diet during convalescence from shigellosis: a six-month follow-up study. *J Nutr*, 128:1688-1691.

Kalhan SC, Schwartz R, Adam PAJ (1975). Placental barrier to human insulin-I^{125} in insulin-dependent diabetic mothers. *J Clin Endocrinol Metab*, 40(1):139-142.

Kamoda T, Saitoh H, Inudoh M, Miyazaki K, Matsui A (2006). The serum levels of proinsulin and their relationship with IGFBP-1 in obese children. *Diabetes Obes Metab*, 8(2):192-196.

Kassem SA, Ariel I, Thornton PS, Scheimberg I, Glaser B (2000). Beta-cell proliferation and apoptosis in the developing normal human pancreas and in hyperinsulinism of infancy. *Diabetes*, 49(8):1325-1333.

Katz A, Nambi SS, Mather K, Baron AD, Follmann DA et al (2000). Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab*, 85: 2402-2410.

Keskin M, Kurtoglu S, Kendirci M, Atabek ME, Yazizi C (2005). Homeostasis Model Assessment Is More Reliable Than the Fasting Glucose/Insulin Ratio and Quantitative Insulin Sensitivity Check Index for Assessing Insulin Resistance Among Obese Children and Adolescents. *Pediatrics*, 115(4): 500-503.

Khaliq A, Dunk C, Jiang J, Shams M, Li XF et al (1999). Hypoxia down-regulates placenta growth factor, whereas fetal growth restriction up-regulates placenta growth factor expression: molecular evidence for "placental hyperoxia" in intrauterine growth restriction. *Lab Invest*, 79(2):151-170.

Khan N, Couper JJ (1994). Low-birth-weight infants show earlier onset of IDDM. *Diabetes Care*, 17:653-656.

King JC (2003). The risk of maternal nutritional depletion and poor outcomes increases in early or closely spaced pregnancies. *J Nutr*, 133: 1732S-1736S.

Kingdom J, Huppertz B, Seaward G, Kaufmann P (2000). Development of the placental villous tree and its consequences for fetal growth. *Eur J Obstet Gynecol Reprod Biol*, 92(1):35-43.

Kingdom J, Kaufmann P (1999). Oxygen and placental vascular development. *Adv Exp Med Biol*, 474:259-275.

Kliman HJ. From Trophoblast to Human Placenta (from the Encyklopedia of Reproduction), Yale University School of Medicine. [Accessed 19. December 2008]. http://www.med.yale.edu/obgyn/kliman/placenta/articles/EOR_Placenta/Trophtoplacenta.html

Kosterlitz HW (1947). The effects of changes in dietary protein on the composition and structure of the liver cell. *J Physiol*, 106:194-210

Kramer MS (1987). Determinants of low birth weight: methodological assessment and meta-analysis. *Bulletin of the World Health Organization*, 65(5): 663-737.

Kramer MS (2003). Invited commentary: Association between restricted fetal growth and adult chronic disease: Is it causal? Is it important? *Am J Epidemil*, 152(7); 605-608.

Kramer MS (2003). The epidemiology of adverse pregnancy outcomes: An overview. *J Nutr*, 133: 1592S-1596S.

Krampl E, Kametas NA, McAuliffe F, Cacho-Zegarra AM, Nicolaides KH (2002). Maternal serum insulin-like growth factor binding protein-1 in pregnancy at high altitude. *Obstet Gynecol*, 99(4):594-598.

Kruger HS, Venter CS, Vorster HH (2003). Physical inactivity as a risk factor for cardiovascular disease in communities undergoing rural to urban transition: the THUSA study. *Cardiovasc J South Afr*, 14(1):16-23.

Kruger MC, Schollum LM (2005). Is docosahexaenoic acid more effective than eicosapentaenoic acid for increasing calcium bioavailability? *Prostaglandins Leukot Essent Fatty Acids*, 73(5):327-334.

Kuzawa CW (2005). Fetal origins of developmental plasticity: Are fetal cues reliable predictors of future nutritional environments? Wiley-Liss Plenary Symposium. *Am J Hum Biol*, 17: 5-21.

Lagha NB, Seurin D, Le Bouc Y, Binoux M, Berdal A et al (2006). Insulin-like growth factor binding protein (IGFBP-1) Involvement in intrauterine growth retardation: Study on IGFBP-1 Overexpressing transgenic mice. *Endocrinology*, 147(10):4730-4737.

Langenhoven ML, Kruger M, Gouws E, Faber M (1991a). Research Institute for Nutritional Diseases (RIND) Food Composition Tables, 3rd edition. Parow: South African Medical Research Council, pp248. In: MacKeown JM (1999). The impact of past and present energy, macronutrient and micronutrient intake on the incidence of dental caries among 5-year-old urban Black South African children. *Thesis*, University of the Witwatersrand, Ref 90.

Langenhoven ML, Conradie PJ, Wolmarans P, Faber M (1991b). National Research Programme for Nutritional Intervention. Food Quantities Manual, 2ndedition. Parow: South African Medical Research Council, pp213 In: MacKeown JM (1999). The impact of past and present energy, macronutrient and micronutrient intake on the incidence of dental caries among 5-year-old urban Black South African children. *Thesis*, University of the Witwatersrand, Ref 91.

Langley-Evans SC, Phillips GJ, Benediktsson R, Gardner DS, Edwards CRW et al (1996). Protein intake in pregnancy, placental glucocorticoid metabolism and the programming of hypertension in the rat. *Placenta*, 17:169-172.

Law CM, Barker DJP, Bull AR, Osmond C (1991). Maternal and fetal influences on blood pressure. *Arch Dis Child*, 66:1291-1295.

Leese HJ, Isherwood-Peel SG (1999). Early embryo nutrition and disorders in later life. In: O'Brien PMS, Wheeler T, Barker DJP, editors. *Fetal Programming. Influences on Development and Disease in Later Life.* Royal College of Obstetricians and Gynaecologists (RCOG), London. RCOG Press, Chapter 11:104-116,

Le Floch JP, Escuyer P, Baudin E, Baudon D, Perlemuter L (1990). Blood glucose area under the curve. Methodological aspects. *Diabetes Care*, 13(2):172-175.

Leon DA (1999). Fetal growth and later disease: epidemiological evidence from Swedish cohorts In: O'Brien PMS, Wheeler T, Barker DJP, editors. *Fetal Programming. Influences on Development and Disease in Later Life.* Royal College of Obstetricians and Gynaecologists (RCOG), London. RCOG Press, Chapter 2:12-29.

Leon DA, Lithell HO, Vågerö D, Koupilová I, Mohsen R et al (1998). Reduced fetal growth rate and increased risk of death from ischaemic heart disease: cohort study of 15 000 Swedish men and women born 1915-29. Papers. *BMJ*, 317:241-245

Leonce J, Brockton N, Robinson S, Venkatesan S, Bannister P et al (2006). Glucose production in the human placenta. *Placenta*, 27(Suppl A):S103-S108;

Levitt NS (1996). Diabetes Mellitus in Black Africans. *Int J Diab Dev Countries*, 16:41-44.

Levitt NS, Katzenellenbogen JM, Bradshaw D, Hoffman MN, Bonnici F (1993). The prevalence and identification of risk factors for NIDDM in urban Africans in Cape Town, South Africa. *Diabetes Care*, 16:601-607.

Levitt NS, Lambert EV, Norris SA (2005a). Early life origins of adult chronic diseases: A South African perspective. In: Chronic Diseases of Lifestyle in South Africa since 1995-2005. Chronic Diseases of Lifestyle Research Unit. Online documents/reports. *South African Medical Research Council0 (MRC).* Chapter 6: 58-64. http://www.ac.za/chronic/cdl1995-2005.htm. [Accessed: 17.10.2009]

Levitt NS, Lambert EV, Woods D, Hales NC, Andrew R et al (2000). Impaired glucose tolerance and elevated blood pressure in low birth weight, nonobese, young South African adults: early programming of cortisol axis. *J Clin Endocrinol Metab*, 85(12): 4611- 4618.

Levitt NS, Lambert EV, Woods D, Seckl JR, Hales NC (2005b). Adult BMI and fat distribution but not height amplify the effect of low birthweight on insulin resistance and increased blood pressure in 20-year-old South Africans. *Diabetologia*, 48:1118-1125.

Levitt NS, Steyn K, De Wet T, Morrell C, Edwards R et al (1999b). An inverse relation between blood pressure and birth weight among 5 year old children from Soweto, South Africa. *J Epidemiol Community Health*, 53:264-268.

Lewis TLT, Chamberlain GVP, editors (1990a). Normal Puerperium. Infant Feeding. *Obstetrics by Ten Teachers.* Fifteenth edition. Printed by Hodder and Stoughton Ltd, Great Britain, Chapter 6: 244-253.

Lewis TLT, Chamberlain GVP, editors (1990b). Abnormal pregnancy. Polyhydramnios and Oligohydramnios.*Obstetrics by Ten Teachers.* Fifteenth edition. Printed by Hodder and Stoughton Ltd, Great Britain, Chapter 3: 84-86.

Lewis TLT, Chamberlain GVP, editors (1990c). Abnormal pregnancy. The fetus at risk at late pregnancy. Tests of placental function. *Obstetrics by Ten Teachers*. Fifteenth edition. Printed by Hodder and Stoughton Ltd, Great Britain, Chapter 3: 130-132.

Lewis TLT, Chamberlain GVP, editors (1990d). Anatomy and physiology. *Obstetrics by Ten Teachers*. Fifteenth edition. Printed by Hodder and Stoughton Ltd, Great Britain, Chapter 1: 4-18

Li Y, Greiner RS, Salem N Jr, Watkins BA (2003). Impact of dietary n-3 FA deficiency on rat bone tissue FA composition. *Lipids*, 38(6):683-686.

Li Y, Seifert MF, Lim SY, Salem N Jr, Watkins BA (2010). Bone mineral content is positively correlated to n-3 fatty acids in the femur of growing rats. *Br J Nutr*, 104(5):674-685.

Li Y, Seifert MF, Ney DM, Grahn M, Grant A et al (1999). Dietary conjugated linoleic acids alter serum IGF-I and IGF binding protein concentrations and reduce bone formation in rats fed (n-6) or (n-3) fatty acids. *J Bone and Miner Res*, 14(7):1153-1162.

Lind T, Whittaker PG. The placenta as an endocrine regulator. In: Sharp F, Fraser RB, Milner RDG, editors. *Fetal Growth.* Proceedings of the Twentieth Study Group of the Royal College of Obstetrics and Gynaecologiests. The Royal College of Obstatricians and Gynaecologists 1989.The Peacock Press Ltd, England, pp.53-67.

Lithell HO, McKeigue PM, Berglund L, Mohsen R, Lithell UB, Leon DA (1996). Relation of size at birth to non-insulin dependent diabetes and insulin concentrations in men aged 50-60 years. Papers. *BMJ*, 312:406-410

Litten JC, Perkins KS, Corson AM, Clarke L (2004). The effects of maternal diet during late pregnancy and body shape at birth on postnatal plasma concentrations of insulin like growth factor 1 (IGF1) and leptin. *Endocrine Abstracts*, 8:p41. (Abstract).

Lucas A (1991). Programming by early nutrition in man. In: The childhood environment and adult disease. *CIBA Foundation Symposium* 156. Wiley, Chichester, UK, pp 38-55.

Lucas A (1998). Programming by early nutrition: An experimental approach. Symposium: The effects of childhood diet on adult health and disease. *J Nutr*, 128(2): 401S-406S.

Ludvigsson JF, Ludvigsson J (2004). Milk consumption during pregnancy and infant birthweight. *Acta paediatrica*, 93(11):1474-1478.

Luke B (1994). Nutritional Influences on Fetal Growth. *Clin Obstet Gynecol*, 37(3):538-549.

Lumey LH (1998a). Compensatory placental growth after restricted maternal nutrition in early pregnancy. *Placenta*, 19(1):105-111.

Lumey LH (1998b). Reproductive outcomes in women prenatally exposed to undernutrition: a review of findings from the Dutch famine birth cohort. *Proc Nutr Soc*, 57:129-135.

Macara L, Kingdom JC, Kaufmann P, Kohnen G, Hair J et al (1996). Structural analysis of placental terminal villi from growth-restricted pregnancies with abnormal umbilical artery Doppler waveforms. *Placenta*,17(1):37-48.

MacIntyre UE (1998). Dietary intakes of Africans in transition in the North West Province. *Thesis.* Potchefstroomse Universiteit. Energy and macronutrient intakes. Chapter 8.2.4, pp361.

MacIntyre UE, Venter CS, Vorster HH (2001a). A culture sensitive quantitative food frequency questionnaire used in an African population 1. Development and reproducibility. *Public health Nutrition*, 4: 53-62.

MacIntyre UE, Venter CS, Vorster HH (2001b). A culture sensitive quantitative food frequency questionnaire used in an African population 2. Relative validation by 7-day weight records and biomarkers. *Public Health Nutr*, 4: 63-71.

Mackenzie HS, Lawler EV, Brenner BM (1996). Congenital oligonephropathy: The fetal flaw in essential hypertension? *Kidney Int*, 49(Suppl 55):S30-S34.

MacKeown JM (1999). The impact of past and present energy, macronutrient and micronutrient intake on the incidence of dental caries among 5-year-old urban Black South African children. *Thesis*, University of the Witwatersrand, Food frequency questionnaire – 1995 Birth-To-Ten Interception. Appendix F and section 2.2.5.

MacKeown JM, Pedro TM, Norris SA (2007). Energy, macro- and micronutrient intake among a true longitudinal group of South African adolescents at two interceptions (2000 and 2003): the Birth-to-Twenty (Bt20) Study. *Public Health Nutrition.* 10(6): 635-643.

Mamabolo RL, Alberts M, Levitt NS, Delemarre-van de Waal HA, Steyn NP (2007). Prevalence of gestational diabetes mellitus and the effect of weight on measures of insulin secretion and insulin resistance in third-trimester pregnant rural women residing in the Central Region of Limpopo Province, South Africa. *Diabet Med*, 24:233-239.

Mamelle N, Cochet V, Claris O (2001). Definition of fetal growth restriction according to constitutional growth potential. Neonatology. Fetal and neonatal research. *Biol Neonate*, 80 (4) 277-285. (Abstract)

Margetts B M, Cade J E, Osmond C (1989). Comparison of Food Frequency Questionnaire with a Diet Record. *Inr J Epidemiol*, Vol. 18, No. 4, Pg 868-873.

Maršál K (1989). Fetal and placental blood flow. In: Sharp F, Fraser RB, Milner RDG, editors. *Fetal Growth*. Proceedings of the Twentieth Study Group of the Royal College of Obstetrics and Gynaecologiests. The Royal College of Obstatricians and Gynaecologists 1989.The Peacock Press Ltd, England, pp.297-305

Maršál K, Gardiner H (1999). Changes in fetal and placental circulations associated with intrauterine growth restriction in humans. In: O'Brien PMS, Wheeler T, Barker DJP, editors. *Fetal Programming.* Influences on Development and Disease in Later Life. Royal College of Obstetricians and Gynaecologists (RCOG), London. RCOG Press, Pg. 350.

Maršál K, Persson PH, Larsen T, Lilja H, Selbing A and Sultan B (1996). Intrauterine growth curves based on ultrasonically estimated foetal weights. *Acta Paediatr*, 85: 843-848.

Martin-Gronert MS, Ozanne SE (2006). Maternal nutrition during pregnancy and health of the offspring. *Biochem Soc Trans*, 34 (part 5):779-782.

Massey LK (2003). Dietary animal and plant protein and human bone health: a whole foods approach. *J Nutr,* 133:862S-865S.

Matthews DR, Hosker AS, Rudenski AS, Naylor BA, Treacher DF et al. (1985). Homeostasis model assessment: insulin resistance and ß-cell function from fasting plasma glucose and insulin concentration in man. *Diabetologia,* 28:412-419.

Mathews F, Yudkin P, Neil A (1999). Influence of maternal nutrition on outcome of pregnancy: prospective cohort study. *BMJ* 319: 339-343

Matsuda M, DeFronzo R (1999). Insulin Sensitivity Indices Obtained From Oral Glucose Tolerance Testing. *Diabetes Care,* 22 (9):1462-1470.

McArdle HJ and Ashworth CJ (1999). Micronutrients in fetal growth and development. *Br Med Bull,* 55(3):499-510.

McCance DR, Pettitt DJ, Hanson RL, Jacobsson LTH et al (1994). Birth weight and non-insulin dependent diabetes: thrifty genotype, thrifty phenotype, or surviving small baby genotype? *BMJ,* 308:942-945.

McCance RA (1962). Food growth and time. *Lancet,* 2:271-272.

McCarthy TL, Centrella M, Raisz LG, Canalis E (1991). Prostaglandin E2 stimulates insulin-like growth factor I synthesis in osteoblast-enriched cultures from fetal rat bone. *Endocrinology*, 128(6):2895-2900.

McMillen IC, Edwards LJ, Duffield J, Muhlhausler BS (2006). Regulation of leptin synthesis and secretion before birth: implications for the early programming of adult obesity. *Reproduction,*131; 415-427.

McMillen IC, Robinson JS (2005). Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. *Physiol Rev*, 85:571-633.

McNeil CJ (2007). Glucocorticoid exposure and tissue gene expression of 11ß-HSD1, 11ß-HSD2, and glucocorticoid receptor in a porcine model of differential fetal growth. *Reproduction,* 133:653-661.

McNeill (2000). Energy intake and expenditure. IN:Garrow JS, James WPT, Ralph A, editors. Chapter 3. *Human Nutrition and Dietetics.* Tenth Edition. Churchill Livingstone, UK. Chapter 3:25-36.

Mensink RP, Zock PL, Kester ADM, Katan MB (2003). Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *Am J Clin Nutr*, 77:12146-1155.

Meites S (1988). Skin-puncture and blood-collecting technique for Infants: Update and problems. *Clin Chem*, 34(9):1890-1894.

Mi J, Law C, Zhang KL, Osmond C, Stein C, Barker D (2000). Effects of infant birthweight and maternal body mass index in pregnancy on components of the insulin resistance syndrome in China. *Ann Intern Med*, 132 (4) 253-260.

Milman N (2008). Prepartum anaemia: prevention and treatment. *Ann Hematol*, 87:949-959.

Milner RDG (1989). Mechanisms of overgrowth. Experimental evidence. In: Sharp F, Fraser RB,

Milner RDG, editors. *Fetal Growth*. Proceedings of the Twentieth Study Group of the Royal College of Obstetrics and Gynaecologiests. The Royal College of Obstatricians and Gynaecologists 1989.The Peacock Press Ltd, England, pp.139-143.

Mitchell EA, Robinson E, Clark PM, Becroft DMO, Glavish N et al (2004). Maternal nutritional risk factors for small for gestational age babies in a developed country: a case-control study. *Arch Dis Child* Fetal neonatal Ed, 89:F431-F435.

Mitchell ML (2001). Fetal brain to liver weight ratio as a measure of intrauterine growth retardation: Analysis of 182 stillborn autopsies. *Mod Pathol*, 14(1):14-19.

Molag ML, de Vries JHM, Ocké MC, Dagnelie PC et al (2007). Design Characteristics of Food Frequency Questionnaires in Relation to Their Validity. Practice of Epidemiology. *Am J Epidemiol*, 166: 1468-1478.

Mollard RC, Kovacs HR, Fitzpatrick-Wong SC, Weiler HA (2005). Low levels of dietary arachidonic and docosahexaenoic acids improve bone mass in neonatal piglets, but higher levels provide no benefit. *J Nutr*, 135(3):505-512.

Mollentze WF, Levitt NS (2005). Diabetes mellitus and impaired glucose tolerance in South Africa. Chapter 10. *Chronic diseases of lifestyle in South Africa:1995-2005*. Chronic Diseases of Lifestyle Research Unit. Online documents/reports. South African Medical Research Council. http://www.ac.za/chronic/cdl1995-2005.htm [Accesed: 18.10.2009].

Moore KL, Persaud TVN (1993a). *The Developing Human*. Clinically Oriented Embryology. Fifth edition W.B. Saunders Company, Philadelphia. Chapter 2.

Moore KL, Persaud TVN (1993b). *The Developing Human*. Clinically Oriented Embryology. Fifth edition W.B. Saunders Company, Philadelphia. Chapter 6.

Moore KL, Persaud TVN (1993c). *The Developing Human*. Clinically Oriented Embryology. Fifth edition W.B. Saunders Company, Philadelphia. Chapter 7.

Moore KL, Persaud TVN (1993d). *The Developing Human*. Clinically Oriented Embryology. Fifth edition W.B. Saunders Company, Philadelphia. Figure 8-14.

MooreTR (1990). Superiority of the four-quadrant sum over the single deepest pocket technique in ultrasonographic identification of abnormal amniotic fluid volumes. *Am J Obstet Gynecol,* 163:762-767.

Moore VM, Davies MJ, Willson KJ, Worsley A and Robinson JS (2004). Dietary composition of pregnant women is related to size of the baby at birth. American Society for Nutritional Science. *J. Nut,* 134:1820-1826.

Moritz KM, Wintour EM, Dodic M (2006). The developmental environment, renal function and disease. Maternal diabetes. In: Gluckman PD, Hanson MA, editors. *Developmental origins of health and disease*. New York: Cambridge University Press. Chapter 22:315-316.

Moses RG, Luebcke M, Davis WS, Coleman KJ, Tapsell LC et al. (2006). Effect of a low-glycaemic index diet during pregnancy on obstetric outcomes. *Am J Clin Nutr,* 84:807-812.

Motala AA, Esterhuizen T, Gouws E, Pirie FJ, Omar MAK (2008). Diabetes and other disorders of glycemia in a rural South African community. Prevalence and associated risk factors. *Diabetes Care*, 31(9):1783-1788.

Muaku SM, Beauloye V, Thissen JP, Underwood LE, Fossion C et al. (1996). Long-term effects of gestational protein malnutrition on postnatal growth, insulin-like growth factors (IGF)-I, and IGF-binding proteins in rat progeny. *Pediatr Res*, 39(4):619-655.

Muniyappa R, Lee S, Chen H, Quon MJ (2008). Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage. *Am J Physiol Endocrinol Metab*, 294:E15-E26.

Muramatsu T, Yatsuya H, Toyoshima H, Sasaki S, Li Y et al. (2010). Higher dietary intake of alpha-linolenic acid is associated with lower insulin resistance in middle-aged Japanese. *Preventive Medicine,* 50(5-6):272-276.

Murphy VE, Smith R, WB Giles, Clifton VL (2006). Endocrine regulation of human fetal growth: The role of the mother, placenta, and fetus. *Endocrine Reviews*, 27(2):141-169.

Myatt L (2006). Placental adaptive responses and fetal programming. *Symposium report. J Physiol*, 572(1):25-30 .

Myatt L, Roberts V (2006). Placental mechanisms and developmental origins of health and disease. In: Gluckman PD and Hanson MA, editors. *Developmental Origins of Health and Disease*. New York: Cambridge University Press. Chapter 9: 130-142

Neel JV (1962). Diabetes Mellitus: a "Thrifty" genotype rendered detrimental by "progress"*? Am J Hum Genet*, 14(4):353-362.

Neggers Y, Goldenberg RL (2003). Some thoughts on body mass index, micronutrient intakes and pregnancy outcome. *J. Nutr*, 133:1737S – 1740S.

Nelson DM, Smith SD, Furesz TC, Sadovsky Y, Ganapathy V et al (2003). Hypoxia reduces expression and function of system A amino acid transporters in cultured term human trophoblasts. *Am J Cell Physiol*, 284(2):C310-C315.

Neumann CH, Carroll BA (1984). Fetal biometry and intrauterine growth retardation: Current concepts. *West J Med*, 140:414-420.

Nicolaides KH, Economides DL, Thorpe-Beeston G (1989). Clinical implications of fetal undergrowth. Treatment of fetal growth retardation. Blood glucose and plasma insulin. Sharp F, Fraser RB, Milner RDG, editors. *Fetal Growth.* Proceedings of the Twentieth Study Group of the Royal College of Obstetrics and Gynaecologiests. The Royal College of Obstatricians and Gynaecologists 1989.The Peacock Press Ltd, England, Section 6: 342-346

NNMB (The National Nutrition Monitoring Bureau of the National Institute of Nutrition in India; 1991);III-Nutrition and mortality outcomes. Maternal nutritional status. Pg.10-11. http://www.unsystem.org/scn/archives/india/ch07.htm. Approached: 8.2.2011.

Ntyintyane L, Panz V, Raal F, Gill G (2010). Comparison between surrogate indices of insulin sensitivity and resistance, and the hyperinsulinaemic euglycaemic glucose clamp in urban South African blacks with and without coronary artery disease. *Diabetes and Vascular Disease Research.* http://dvr.sagepub.com/content/7/2/151. Accessed on March 28, 2011.

Nwosu EC, Welch CR, Manasse PR, Walkinshaw SA (1993). Longitudinal assessment of amniotic fluid index. *Br J Obstet Gynaecol*, 100: 816-819.

Nyirenda MJ, Lindsay RS, Kenyon C, Burchell A, Seckl JR (1998). Glucocorticoid exposure in late gestation permanently programs rat hepatic phosphoenolpyruvate carboxykinase and glucocorticoid receptor expression and causes glucose intolerance in adult offspring. *J Clin Invest*, 101(10):2174-2181.

Ogueh O, Sooranna S, Nicolaides KH, Johnson MR (2000). The relationship between leptin concentration and bone metabolism in the human fetus. *J Clin Endocrinol Metab*, 85:1997-1999.

Olsen SF, Halldorsson TI, Willet WC, Knudsen VK, Gillman MW et al (2007). Milk consumption during pregnancy is associated with increase infant size at birth: prospective cohort study. *Am J Clin Nutr*, 86(4):1104-1110.

Olsen SF, Hansen HS, Sørensen TI, Jensen B, Secher NJ et al (1986). Intake of marine fat, rich in (n-3)-polyunsaturated fatty acids, may increase birthweight by prolonging gestation. *Lancet*, 2(8503):367-369.

Olsen SF, Secher NJ (2002). Low consumption of seafood in early pregnancy as a risk factor for preterm delivery: prospective cohort study. *BMJ*, 324:1-5

Omar MA, Seedat MA, Motala AA, Dyer RB, Becker P (1993). The prevalence of diabetes mellitus and impaired glucose tolerance in a group of urban South African blacks. *S Afr Med J*, 83:641-643.

Ong KK, Ahmed MI, Sheriff A, Woods KA, Watts A (1999). Cord blood leptin is associated with size at birth and predicts infancy weight gain in humans. *J Clin Endocrinol Metab*, 84:1145-1148.

Ong KK, Dunger DB (2004). Birth weight, infant growth and insulin resistance. *Eur J Endocrinol,* 151 (Suppl3): U131-139.

O' Brien D, Hambidge KM (1987). Normal childhood nutrition and its disorders. Proteins. In: Kempe CH, Silver HK, O'Brien D, Fulginiti VA, editors. *Current Pediatric Diagnostis & Treatment.* A LANGE medical book. Ninth Edition. Prentice-Hall International, Inc., USA. Chapter4, Pg 98-101.

O'Rahilly R, Müller F, editors (2001a). *Human Embryology & Teratology*. Third Edition. Wiley-Liss, Canada. Chapter 8: Figure 8.1.

O'Rahilly R, Müller F, editors (2001b). *Human Embryology & Teratology*. Third Edition. Wiley-Liss, Canada. Chapter 4.

O'Rahilly R, Müller F, editors (2001b). *Human Embryology & Teratology*. Third Edition. Wiley-Liss, Canada. Chapter 5.

O'Rahilly R, Müller F, editors (2001b). *Human Embryology & Teratology*. Third Edition. Wiley-Liss, Canada. Chapter 7.

O'Shea PJ, Sriskantharajan S, Yao H, Suzuki H, Cheng SY et al (2005). Thyroid hormone (T3) activates GH/IGF-1 signalling during skeletal development. *Endocrine Abstracts*, 9: P149

Ounsted M, Moar VA, Scott AA (1985). Risk factors associated with small-for-dates and large-for-dates infants. Obstetrical and Gynecological Survey. *Br J Obstet Gynaecol.,* 92 (3):226-232

Owens JA, Gatford KL, De Blasio MJ, Edwards LJ, McMillen IC et al (2007). Restriction of placental growth in sheep impairs insulin secretion but not sensitivity before birth. *J Physiol*, 584(3):935-949.

Ozanne SE (2001). Metabolic programming in animals. *Br Med Bull*, 60:143-152.

Ozanne SE and Hales CN (1999). The long-term consequences of intra-uterine protein malnutrition for glucose metabolism. *Proc Nutr Soc*, 58 (3): 615-619.

Ozcimen EE, Uckuyu A, Ciftci FC, Yanik FF, Bakar C (2008). Diagnosis of gestational diabetes mellitus by use of the homeostasis model assessment-insulin resistance index in the first trimester. *Gynecological Endocrinology*, 24(4):224-229.

Parretti E, Lapolla A, Dalfrà MG, Pacini G, Mari A et al. (2006). Preeclampsia in lean normotensive normotolerant pregnant women can be predicted by simple insulin sensitivity indexes. *Hypertension*, 47:449-453.

Parretti E, Mecacci F, Papini M, Cioni R, Carignani L et al (2001). Third-trimester maternal glucose levels from diurnal profiles in nondiabetic pregnancies. Correlation with sonographic parameters of fetal growth. *Diabetes Care*, 24 (8):1319-1323.

Peleg D, Colleen M, Kennedy MD, Hunter SK (1998). Intrauterine growth restriction: identification and management. AAFP (*American Family Physician*), [Accessed 2.2.2009]. http://www.aafp.org/afp/980800ap/peleg.html.

Pellizas CG, Coleoni AH, Costamanga ME, Di Fulvio M, Masini-Repiso AM (1998). Insulin-like growth factor I reduces thyroid hormone receptors in the rat liver. Evidence for a feed-back loop regulating the peripheral thyroid hormone action. *J Endocrinol*, 158:87-95.

Persson PH (1989). Fetal growth curves. Limits of normal fetal growth. In: Sharp F, Fraser RB, Milner RDG, editors. *Fetal Growth*. Proceedings of the Twentieth Study Group of the Royal College of Obstetrics and Gynaecologiests. November 1988. Published by The Royal College of Obstatricians and Gynaecologists. Printed by The Peacock Press Ltd, England, pp 21-22.

Perucchini D, Fischer U, Spinas GA, Huch R, Huch A et al (1999). Using fasting plasma glucose concentrations to screen for gestational diabetes mellitus: prospective population based study. *BMJ*, 319:812-815.

Petrie A, Sabin C (2000a). Choice of explanatory variables. Multiple linear regression. In: *Medical Statistics at a Glance*. Blackwell Science Ltd, UK. Reprint 2003. Chapter 29, Pg. 75-76.

Petrie A, Sabin C (2000b). Statistical modelling. Model selection. In: *Medical Statistics at a Glance*. Blackwell Science Ltd, UK. Reprint 2003. Chapter 31, Pg. 80.

Petrie A, Sabin C (2000c). Checking assumptions. Are two or more variances equal? In: *Medical Statistics at a Glance.* Blackwell Science Ltd, UK. Reprint 2003. Chapter 32, Pg. 82.

Petrie A, Sabin C (2000d). Methods for repeated measures. Repeated measures analysis of variance. In: *Medical Statistics at a Glance.* Blackwell Science Ltd, UK. Reprint 2003. Chapter 39, Pg.102.

Petrik J. Arany E, McDonald TJ, Hill DJ (1998). Apoptosis in the pancreatic islet cells of the neonatal rat is associated with a reduced expression of insulin-like growth factor II that may act as a survival factor. *Endocrinology*, 139(6):2994-3004.

Petrik J, Reusens B, Arany E, Remacle C, Coelho C et al (1999). A low protein diet alters the balance of islet cell replication and apoptosis in the fetal and neonatal rat and is associated with a reduced pancreatic expression of insulin-like growth factor-II. *Endocrinology*, 140(10):4861-4873.

Phelan JP, Smith CV, Broussard P, Small M (1987). Amniotic fluid volume assessment with the four-quadrant technique at 36-42 weeks' gestation. *J Reprod Med*, 32(7):540-542.

Phillips DIW (1996). Insulin resistance as a programmed response to fetal undernutrition. For debates. *Diabetologia*, 39 (1119-1122).

Phillips DI, Barker DJ, Hales CN, Hirst S, Osmond C (1994). Thinness at birth and insulin resistance in adult life. *Diabetologia*, 37(2):150-154.

Picciano MF (2003). Pregnancy and lactation: physiological adjustments, nutritional requirements and the role of dietary supplements. *J Nutr*, 133:1997S-2002S.

Piper K, Brickwood S, Turnpenny LW, Cameron IT, Ball SG et al (2004). Beta cell differentiation during early human pancreas development. *J Endocrinol*, 181:11-23.

Polonsky KS (1995). Lilly Lecture 1994. The beta-cell in diabetes: from molecular genetics to clinical research. *Diabetes*, 44 (6):705-717.

Pontiroli AE, Pizzocri P, Caumo A, Perseghin G, Luzi L (2004). Evaluation of insulin release and insulin sensitivity through oral glucose tolerance test: differences between NGT, IFG, IGT, and Type 2 diabetes mellitus. A cross-sectional and follow-up study. *Acta Diabetol*, 41(2):70-76.

Poulsen P, Kyvik KO, Vaag A and Beck-Nielsen H (1999a). Heritability of type II (non-insulin-dependent) diabetes mellitus and abnormal glucose tolerance: a population-based twin study. *Diabetologia,* 42:139-145.

Poulsen P, Vaag A, Beck-Nielsen H (1999b). Does zygocity influence the metabolic profile of twins? A population based cross sectional study. *BMJ, 319:151-154.*

Poulsen P, Vaag A (2006). The Intrauterine Environment as Reflected by Birth Size and Twin and Zygosity Status Influences Insulin Action and Intracellular Glucose Metabolism in and Age- or Time-Dependent Manner. *Diabetes*, 55:1819-1824.

Poulsen P, Grunnet LG, Pilgaard K, Storgaard, Alibegovic A et al (2009). Increased Risk of Type 2 Diabetes in Elderly Twins. *Diabetes,* 58:1350-1355.

Poulter NR, Chang CL, MacGregor AJ, Snieder H, Spector TD (1999). Association between birth weight and adult blood pressure in twins: historical cohort study. *BMJ*, 319:1330-1333.

Prado CL, Pugh-Bernard AE, Elghazi L, Sosa-Pineda B, Sussel L (2004). Ghrelin cells replace insulin-producing β cells in two mouse models of pancreas development. *Proc Natl Acad Sci USA*, 101(9):2924-2929.

Prentki M, Nolan CJ (2006). Islet β cell failure in type 2 diabetes. Review series. *J Clin Invest*, 116(7):1802-1812.

Qiu C, Vadachkoria S, Meryman L, Frederick IO, Williams MA (2005). Maternal plasma concentrations of IGF-I, IGFBP-I, and C-peptide in early pregnancy and subsequent risk of gestational diabetes mellitus. *Am J Obstet Gynecol*, 193: 1691-1697.

Qui XS, Huang TT, Deng HY, Shen ZY, Ke ZY et al (2004). Effects of early nutrition intervention on IGF1, IGFBP3, intestinal development, and catch-up growth of intrauterine growth retardation rats. *Chin Med Sci J*, 19(3):189-92.

Radziuk J (2000). Insulin Sensitivity and Its Measurement: STRUCTURAL Commonalities among the Methods. *J Clin Endocrinol Metab*, 85:4426-4433.

Rahier J, Wallon J, Henquin J-C (1981). Cell populations in the endocrine pancreas of human neonates and infants. *Diabetologia*, 20:540-546.

Ramakrishnan U (2004). Nutrition and low birth weight: from research to practice. *Am J Clin Nutr*, 79 (1):17-21

Rao BT, Aggarwal AK, Kumar R (2007). Dietary intake in third trimester of pregnancy and prevalence of LBW: A Community-based study in a rural area of Haryana. *Indian J Community Med*, 32(4): 272-276

Rao S, Yajnik CS, Kanade A, Fall CHD, Margetts BM et al (2001). Intake of micronutrient-rich foods in rural Indian mothers is associated with the size of their babies at birth: Pune Maternal Nutrition Study. *J Nutr*, 131:1217-1224.

Rasmussen KM (2001). Is there a causal relationship between iron defficiency or iron-defficiency anemia and weight at birth, length of gestation and perinatal mortality? *J Nutr* 131:590S-603S.

Rastogi T, Mathers C (2002). Global burden of iron deficiency anaemia in the year 2000. Obtained from: www.who.int/entity/healthinfo/statistics/bod_irondeficiencyanaemia.pdf; [Accessed: 2.3.2010]

Ravelli AC, van der Meulen JH, Michels RP, Osmond C, Barker DJ et al (1998). Glucose tolerance in adults after prenatal exposure to famine. *Lancet*, 351(9097): 173-177.

Ravelli AC, van der Meulen JH, Osmond C, Barker DJ, Bleker OP (1999). Obesity at age of 50 y in men and women exposed to famine prenatally. *Am J Clin Nutr*, 70(5): 811-816.

Rawlings J S, Rawlings V B, Read J A (1995). Prevalence of low birth weight and preterm delivery in relation to the interval between pregnancies among White and Black women. *NEJM,* 332:69-74.

Reaven GM (1988). Role of insulin resistance in human disease. *Diabetes,* 37:1595-1607

Reaven GM (1995). Pathophysiology of insulin resistance in human disease. *Physiol Rev,* 75(3):473-486.

Reece EA, Goldstein I, Hobbins JC (1993). *Fundamentals of Obstetrics & Gynecologic Ultrasound.* Prentice-Hall International Inc., USA.

Reik W, Dean W, Walter J (2001a). Epigenetic reprogramming in mammalian development. *Science,* 293(5532):1089-1093.

Reik W, Walter J (2001b). Evolution of imprinting mechanisms: the battle of the sexes begins in the zygote. *Nat Genet,* 27:255-256.

Retnakaran R, Qi Y, Sermer M, Connelly PW, Hanley AJG et al (2008). Glucose intlerance in pregnancy and future risk of pre-diabetes or diabetes. *Diabetes Care,* 31 (10):2026-2031.

Reusens B, Kalbe L, Remacle C (2006). The developmental environment and the endocrine pancreas. In: Gluckman PD and Hanson MA, editors. *Developmental Origins of Health and Disease*. New York: Cambridge University Press, Chapter 16: 233-243.

Rich-Edwards JW, Stampfer MJ, Manson JE, Rosner B, Hankinson SE et al (1997). Birth weight and risk of cardiovascular disease in a cohort of women followed up since 1976. Papers. *BMJ,* 315:396-400.

Richter LM, Norris SA, Swart TM, Ginsburg C (2006). In-migration and living conditions of young adolescents in greater Johannesburg, South Africa. UKPMC Funders Group, Author Manuscript. *Soc Dyn,* 32 (1):195-216.

Richter LM, Yach D, Cameron N, Griesel RD, De Wet T (1995): Enrolment into Birth to Ten (BTT): population and sample characteristics (Study progress). *Paediatr Perinat Epidemiol*, 9:109-120.

Rickard IJ, Lummaa V (2007). The Predictive Adaptive Response and metabolic syndrome: Challenges for the hypothesis. *Trends Endocrinol Metab*, 18 (3): 94-99.

Robinson R (2001). The fetal origins of adult disease: no longer just a hypothesis and may be critically important in south Asia. *BMJ*, 323:52.

Romundstad PR, Smith GD, Nilsen TIL, Vatten LJ (2007). Associations of Prepregnancy Cardiovascular Risk Factors with the Offspring's Birth Weight. *Am J Epidemiol*, 166:1359-1364.

Rosenbloom AL, Joe JR, Young RS, Winter WE (1999). Emerging epidemic of type 2 diabetes in youth. *Diabetes Care*, 22(2):345-354.

Sagawa N, Yura S, Itoh H, Kakui K, Takemura M (2002). Possible role of placental leptin in pregnancy: a review. *Endocrine*, 19(1):65-71.

Sadler TW. *Langman's Medical Embryology*. Eight Edition (2000). Lippincott Williams & Wilkins, USA. A Wolters Kluwer Company.

Salmerón J, Hu FB, Manson JE, Stampfer MJ, Colditz GA, Rimm EB et al. (2001). Dietary fat intake and risk of type 2 diabetes in women. *Am J Clin Nutr*, 73(6):1019-1026.

Sample size. Sample size and statistical power calculator. (Accessed: December 2010.) http://hedwig.mgh.harvard.edu/sample_size/js/js_associative_quant.html

Sardesai VM (1992). Biochemical and nutritional aspects of eicosanoids. *J Nutr Biochem*, 3(11): 562-579.

Savino F, Liguori SA, Fissore MF, Oggero R (2009). Breast milk hormones and their protective effect on obesity. *Int J Pediatr Endocrinol*. Online. [Accessed: 15.3.2010] http://www.ncbi.nhlm.nih.gov/pmc/articles/PMC2798107

Schaefer EJ, Augustin JL, Schaefer MM, Rasmussen H, Ordovas JM et al (2000). Lack of efficacy of a food-frequency questionnaire in assessing dietary macronutrient intake in subjects consuming diets of known composition. *Am J Clin Nutr*, 71:746-751.

Schäffer L, Vogel J, Breymann C, Gassmann M, Marti HH (2005). Preserved placental oxygenation and development during severe hypoxia. *Am J Physiol Regul Integr Physiol*, 290: R844-R851.

Scharfmann R, Xiao X, Heimberg H, Mallet J, Ravassard P (2008). Beta cells within single human islets originate from multiple progenitors. *PloS ONE*, 3 (10): 1-8. [Accessed: 12.9.2009] http://www.plosone.org/article/info:doi/10.1371/journal.pone.0003559

Schieve LA, Cogswell ME, Scanlon KS, Perry G, Ferre C et al (2000). Prepregnancy body mass index and pregnancy weight gain: Associations with preterm delivery. *Obstet Gynecol*, 96:194-200.

Scholl TO, Chen X, Gaughan C, Smith WK (2002). Influence of maternal glucose level on ethnic differences in birth weight and pregnancy outcome. *Am J Epidemiol*, 156: 498-506.

Scholl TO, Chen X, Khoo CS, Lenders C (2004). The dietary glycaemic index during pregnancy: influence on infant birth weight, fetal growth, and biomarkers of carbohydrate metabolism. *Am J Epidemiol*, 159:467-474.

Schrimmer DB, Moore TR (2002). Sonographic evaluation of amniotic fluid volume. *Clin Obstet Gynecol*, 45(4):1026-1038.

Seckl JR (2004). Prenatal glucocorticoids and long-term programming. *Eur J Endocrinol*, 151:U49-U62.

Seltzer HS, Allen FW, Herron AL, Brennan MT (1967). Insulin secretion in response to glycaemic stimulus: Relation of delayed initial release to carbohydrate intolerance in mild diabetes mellitus. *J Clin Invest*, 46:323-335.

Sempos CT, Liu K, Ernst ND (1999). Food and nutrient exposures: what to consider when evaluating epidemiologic evidence. *Am J Clin Nutr*, 69(Suppl): 1330S-8S.

Sharp D (2005). The fetal matrix: evolution, development and disease. *J R Soc Med*, 98(3):130-131.

Shiono PH, Klebanoff MA, Graubard BI, Berendes HW, Rhoads GG (1986). Birth weight among women of different ethnic groups. *Obstet Gynecol Surv*, 41(6): 348-349.

Simister J, Piesse J (2002). Household consumption decisions and nutrition in South Africa. In: *Centre for the Study of African Economies Conference*, St. Catherine's College, Oxford. www.csae.ox.ac.uk/conferences/2002-UPaGiSSA/papers/simister-csae2002.pdf
(Downloaded: October 2010).

Singla PN, Tyagi M, Shankar R, Dash D, Kumar A (1996). Fetal iron status in maternal anemia. *Acta Paediatr*, 85: 1327-1330.

Sladek R, Rocheleau G, Rung J, Dina C, Shen L et al (2007). A genome-wide association study identifies novel risk loci for type 2 diabetes. *Nature*, 445:881-885.

Sloan NL, Lederman SA, Leighton J, Himes JH and Rush D (2001). The effect of prenatal dietary protein intake on birth weight. *Nutr Res*, 21 (1):129-139.

Soldin SJ, Brugnara C, Wong EC, editors (2007). *Pediatric reference intervals*. Chemistry tests. Glucose. AACC (American Association for Clinical Chemistry) Press, Washington, DC.,USA. Pg 108.

Sonoline Sienna User Reference (2000). SONOLINE Sienna's instrument software manual, version 1.5, 2.0, and 3, Siemens Medical Systems, Inc., Chapter 15:20

Sood R, Zehnder JL, Druzin ML, Brown PO (2006). Gene expression patterns in human placenta. *Proc Natl Acad Sci USA (PNAS)*, 103(14): 5478-5483. Fig. 1 downloaded via PNAS open access: www.pnas.org/cgi/doi/10.1073/pnas.0508035103. [Accessed 25.8.2009]

Soonthornpun S, Setasuban W, Thamprasit A, Chayanunnukul W, Rattarasarn C et al (2003). Novel Insulin Sensitivity Index Derived from Oral Glucose Tolerance Test. *J Clin Endocrinol & Metab*, 88(3):1019-1023.

Sorenson RL, Brelje TC (1997). Adaptation of islets of Langerhans to pregnancy: beta-cell growth, enhanced insulin secretion and the role of lactogenic hormones. *Horm Metab Res*, 29(6):301-307.

Söderberg S, Ahrén B, Eliasson M, Dinesen B, Brismar K et al.(2001). Circulating IGF binding protein-1 is inversely associated with leptin in non-obese postmenopausal women. *Eur J Endocrinol*, 144(3):283-290.

Sørensen S, von Tabouillot D, Schiøler V, Greisen G, Petersen S et al (2000). Serial measurements of serum human placental lactogen (hPL) and serial ultrasound examinations in the evaluation of fetal growth. *Early Hum Dev* 60: 25-34.

Srinivasan M, Laychock SG, Hill DJ, Patel MS (2003). Neonatal nutrition: metabolic programming of pancreatic islets and obesity. Minireview. *Exp Biol Med*, 228:15-23.

Stanner SA, Yudkin JS (2001). Fetal programming and the Leningrad Siege study. *Twin Res*, 4(5): 287-292.

Stats Online. Statistics South Africa. Statistical release P0277.2, 12.4.2006. Quarterly employment statistics, November 2005. Part2: Estimation of average monthly earnings. http://www.statssa.gov.za/PublicationsHTML/P02772November2005/html/P02772November2005.html. [Aproached 3.11.2009].

Stein AD, Zybert PA, van de Bor M and Lumey LH (2004). Intrauterine famine exposure and body proportions at birth: the Dutch Hunger Winter. *Int J Epidemiol*, 33(4):831-836.

Steinhoff C, Paulsen M, Kielbasa S, Walter J, Vingron M (2009). Expression profile and transcription factor binding site exploration of imprinted genes in human and mouse. *BMC Genomics*, 10: 144-158.

Steyn NP (2005). Nutrition and chronic diseases of lifestyle in South Africa.Chapter4. *Chronic Diseases of Lifestyle in South Africa:1995-2005*. Chronic Diseases of Lifestyle Research Unit. Online documents/reports. South African Medical Research Council. [Accessed: 10.1.2009] http://www.ac.za/chronic/cdl1995-2005.htm

Steyn NP, Nel JH (2006). Dietary intake of adult women in South Africa and Nigeria with a focus on the use of spreads. *The MRC report*. http://www.mrc.ac.za/chronic/kenyareport.pdf [Accessed: 10.1.2009]

Strauss RS, Dietz WH (1999). Low maternal weight gain in the second or third trimester increases the risk for intrauterine growth retardation. Community and International Nutrition. *J Nutr*, 129: 988-993.

Strumvoll M, Mitrakou A, Pimenta W, Jenssen T, Yki-Järvinene H et al. (2000b). Assessment of Insulin Secretion From the Oral Glucose Tolerance Test in White Patients With Type 2 Diabetes. *Diabetes Care*, 23:1440-1441.

Strumvoll M, Mitrakou A, Pimenta W, Jenssen T, Yki-Järvinene H et al. (2000a). Use of the Oral Glucose Tolerance Test to Assess Insulin Release and Insulin Sensitivity. *Diabetes Care*, 23 (3): 295-301.

Swenne I (1992). Pancreatic Beta-cell growth and diabetes mellitus. *Diabetologia* 35: 193-201.

Thame M, Osmond C, Bennett F, Wilks R, Forrester T (2004). Fetal growth is directly related to maternal anthropometry and placental volume. Original Communication. *Eur J Clin Nutr*, 58:894-900.

Thame M, Osmond C, Wilks RJ, Bennett FI, McFarlane-Anderson N et al (2000). Blood pressure is related to placental and birth weight. *Hypertension*, 35(2):662-667

Thompson RS, Trudinger BJ, Cook CM (1988). Doppler ultrasound waveform indices: A/B ratio, pulsatility index and Pourcelot ratio. *British Journal of Obstetrics and Gynaecology*, 95(581-588).

Trichopoulou A, Georgiou E, Bassiakos Y, Lipworth L, Lagiou P et al (1997). Energy intake and monounsaturated fat in relation to bone mineral density among women and men in Greece. *Prev Med*, 26(3):395-400.

Turner RC, Levy JC, Clark A (1993). Complex genetics of Type 2 diabetes: thrifty genes and previously neutral polymorphism. *Q J Med*, 86 (7):413-417.

UltraSound-Technology Information Portal (US-TIP)- US Database [accessed 1.2.2008]. www.us-tip.com/serv1.php?type=db&search=S&set=9

UNSW Embryology, http://embryology.med.unsw.edu.au/ [Accessed December 2008].

Van Bogaert L-J (1999). Customised gravidogram and fetal growth chart in a South African population. *Int J Gynecol Obstet*, 66:129-136.

Van Winkle LJ (2001). Amino acid transport regulation and early embryo development. *Biol Reprod*, 64: 1-12.

Vatanparast H, Bailey DA, Baxter-Jones ADG, Whiting SJ (2007). The effects of dietary protein on bone mineral mass in young adults may be modulated by adolescent calcium intake. *J Nutr*, 137:2674-2679.

Verhaeghe J, Pintiaux A, van Herck E, Hennen G, Foidart J-M et al (2002). Placental GH, IGI, IGF-binding protein –1 and leptin during a glucose challenge test in pregnant women: Relation with maternal body weight, glucose tolerance, and birth weight. *J Clin Endocrinol Metab*, 87(6):2875-2882.

Verhaeghe J, Van Bree R, Van Herck E, Laureys J, Bouillon R et al (1993). C-peptide, insulin-like growth factors I and II, and insulin-like growth factor binding protein-1 in umbilical cord serum: correlations with birth weight. *Am J Obstet Gynecol*, 169(1):89-97.

Villar J and Belizan JM (1986). The evaluation of the methods used in the diagnosis of intrauterine growth retardation. Review. *Obstet Gynecol Surv*, 41(4): 187 –199.

Vinik A, Rafaeloff R, Pittenger G, Rosenberg L, Duguid W (1997). Induction of pancreatic islet neogenesis. *Horm Metab Res*, 29:278-293.

Vohr BR, Boney CM (2008). Gestational diabetes: the forerunner for the development of maternal and childhood obesity and metabolic syndrome? *J Matern Fetal Neonatal Med*, 21(3):149-157.

Waisberg R (2009a). Metabolic and Hormonal studies in South African women of Indian and African origin. *Thesis.* University of the Witwatersrand. Bioelectrical Impedance Analysis. Chapter 2.3.4, Pg 111.

Waisberg R (2009b). Metabolic and Hormonal studies in South African women of Indian and African origin. *Thesis.* University of the Witwatersrand. Food intake questionnaire assessment. Chapter 3.7. Table 3.14, Pg. 160.

Wallace TM, Levy JC, Matthews DR (2004). Use and abuse of HOMA modeling. *Diabetes Care,* 27(6): 1487-1495.

Watkins BA, Li Y, Allen KGD, Hoffmann WE, Seifert MF (2000a). Dietary ratio of (n-6)/(n-3) polyunsaturated fatty acids alters the fatty acid composition of bone compartments and biomarkers of bone formation in rats. *J Nutr*, 130:2274-2284.

Watkins BA, Li Y, Lippman HE, Seifert MF (2001). Omega-3 polyunsaturated fatty acids and skeletal health. *Exp Biol Med,* 226(6):485-497.

Watkins BA, Li Y, Lippman HE, Feng S (2003). Modulatory effect of omega-3 polyunsaturated fatty acids on osteoblast function and bone metabolism. *Prostaglandins Leukot Essent Fatty Acids*, 68(6):387-398.

Watkins BA, Li Y, Seifert MF (2006). Dieatry ratio of n-6/n-3 PUFAs and docosahexaenoic acid: actions on bone mineral and serum biomarkers in ovariectomized rats. *J Nutr Biochem*, 17(4):282-289.

Watkins BA, Seifert MF (2000b). Conjugated linoleic acid and bone biology. *Journal of the American College of Nutrition,* 19(4):478S-486S.

Westermarck T, Antila E (2000). In: Garrow JS, James WPT, Ralph A (Editors). Diet in relation to the nervous system. Development. *Human Nutrition and Dietetics*. 10th Edition. Churchill Livingstone, UK. Chapter 43:715-716.

Westwood M (1999). Role of insulin-like factors binding protein 1 in human pregnancy. *Rev Reprod*, 4(3), 160-167.

Whitelaw E, Garrick D. Epigenetic mechanisms (2007).In: Gluckman PD and Hanson MA, editors *Developmental Origins of Health and Disease*. New York: Cambridge University. Reprint 2007. Chapter 5: 62-65.

WHO Anthro. Anthropometric calculator. Software for assessing growth and development of the world's children. Geneva: World Health Organization, version 3.1.0, June 2010; http://www.who.int/childgrowth/software/en

Wigglesworth JS (1989). Aethiology of fetal undergrowth. Factors controlling normal fetal growth. In: *Fetal Growth*. Editors: Sharp F, Fraser RB, Milner RDG. Proceedings of the Twentieth Study Group of the Royal College of Obstetrics and Gynaecologiests. November 1988. Published by The Royal College of Obstatricians and Gynaecologists. Printed by The Peacock Press Ltd, England, pp185-186.

Williams CM, Burdge G (2006). Long-chain n-3 PUFA: plant v. marine sources. *Proc Nutr Soc*, 65(1):42-50.

Williams S, Poulton R (1999). Twins and maternal smoking: ordeals for the fetal origins hypothesis? A cohort study. *BMJ*, 318: 897-900.

Wild AE, Kwong WY, Roberts P, Fleming TP (1999). Factors influencing blastocyst development. Possible consequences of reduced ICM cell numbers. In: O'Brien PMS, Wheeler T, Barker DJP, editors. *Fetal Programming. Influences on Development and Disease in Later Life.* Royal College of Obstetricians and Gynaecologists (RCOG), London. RCOG Press, Chapter 12:127-128.

Wilkin TJ (1993). Early nutrition and diabetes mellitus. *BMJ* 306:283-284

Willet W, Stampfer MJ (1986). Total energy intake: implications for epidemiologic analyses. *Am J Epidemiol*, 124 (1):17-27.

Wohl GR, Loehrke L, Watkins BA, Zernicke RF (1998). Effects of high-fat diet on mature mineral content, structure, and mechanical properties. *Calcif Tissue Int*, 63(1):74-79.

Woo J, ultrasound net. History of ultrasound in obstetrics and gynecology. Parts 1-3; Obstetric ultrasound – a comprehensive guide to ultrasound scans Doppler ultrasound.
[Accessed: 20.4.2008] www.ob-ultrasound.net/doppler_a.html

World Health Organization (1999). Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications: Report of a WHO Consultation. Part1: Diagnosis and Classification of Diabetes Mellitus. Geneva, *World Health Organization*.

World Health Organization (2006). Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia: report of a WHO/IDF consultation. Geneva, *World Health Organization*.

Wu G, Bazer FW, Cudd TA, Meininger CJ, Spencer TE (2004). Maternal nutrition and fetal development, *J Nutr* 134:2169-2172.

Wu TJ, Huang SM, Taylor RI, Kao PC (1998). Abnormal proinsulin levels in thyroid dysfunction measured by a sensitive proinsulin immunochemiluminoassay. *Ann Clin Lab Sci*, 28(2):82-87

Yajnik CS (2002). The lifecycle effects of nutrition and body size on adult adiposity, diabetes and cardiovascular disease. *Obes Rev*, 3(3): 217-224.

Yajnik CS (2004). Early life origins of insulin resistance and Type 2 diabetes in India and other Asian countries. *J Nutr*, 134: 205-210.

Yajnik CS, Fall CH, Vaidya U, Pandit AN, Bavdekar A et al (1995). Fetal growth and glucose and insulin metabolism in four-year-old Indian children. *Diabet med*, 12(4):330-336. ABS

Yajnik CS, Fall CHD**,** Coyaji KJ, Hirve SS, Rao S et al (2003a). Neonatal anthropometry: the thin-fat Indian baby. The Pune Maternal Nutrition Study. *Int J Obes*, 27:173-180.

Yajnik CS, Joglekar CV, Pandit AN, Bavdekar AR, Bapat SA et al (2003b). Higher offspring birth weight predicts the metabolic syndrome in mothers but not fathers 8 years after delivery. The Pune Children's Study. *Diabetes*, 52:2090-2096.

Yasuhara D, Naruo T, Nagai N, Tanaka M, Muranaga M et al. (2003). Insulinogenic index at 15 min as a marker of nutritional rehabilitation in anorexia nervosa. *Am J Clin Nutr*, 77:292-299.

Yokoyama H, Emoto M, Fujiwara S, Motoyama K, Morioka et al (2003). Quantitative insulin sensitivity of homeostasis model assessment in normal range weight and moderately obese type 2 diabetic patients. *Diabetes Care*, 26(8):2426-32.

Zamudio S, Baumann MU and Illsley NP (2006). Effects of chronic hypoxia in vivo on the expression of human placental glucose transporters. *Placenta*, 27:49-55.

Zhang J, Brenner RA, Klebanoff MA (2001a). Differences in birth weight and blood pressure at age 7 years among twins. *Am J Epidemiol*, 153:779-782.

Zhang J, Klebanoff MA (2001b). Low blood pressure during pregnancy and poor perinatal outcomes: an obstetric paradox. *Am J Epidemiol*, 153:642-646.

Zhu BP, Rolfs RT, Nangle BE and Horan JM (1999). Effect of the interval between pregnancies on perinatal outcomes. *N Engl J Med*, 340(8):589-594.

Zimmet P, Alberti KGMM, Shaw J (2001). Global and societal implications of the diabetes epidemic. *Nature*, 414:782-787.

Zimmet P, Shaw J (2003). Diabetes on six continents – ethnic and geographic differences: views on the future. In: Raz I, Skyler JS, Shafrir E, editors. *Diabetes*. From Research to Diagnosis and Treatment. London and New York: Martin Dunitz, Taylor & Francis Group.Chapter 1:1-10