

THE EFFECTS OF URSOLIC ACID ON NEONATAL PROGRAMMING AND ITS POTENTIAL TO PREVENT THE DEVELOPMENT OF METABOLIC DYSFUNCTION IN SPRAGUE DAWLEY RATS

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DECLARATION

I, Nyasha Charity Mukonowenzou, declare that the work contained in this dissertation is my own, except where others have helped as indicated in the acknowledgements and the reference list. This dissertation is being submitted for the degree of Master of Science in Medicine in the Faculty of Health Sciences at the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted before for any degree or examination at any University. I certify that all the experimental procedures used in this dissertation were approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (AESC number: 2014/49/D).

.....

Nyasha Charity Mukonowenzou

Signed on the.....day of.....2016

DEDICATION

To Kumbulani Yose,
who saw a diamond in the rough,
believed in it and polished it until it sparkled

CONFERENCE PRESENTATION

Data from this study was presented as a poster at the 43rd Congress of the Physiology Society of Southern Africa (PSSA), held at Khaya Ibhubesi, Free State from 6-9 September 2015. The poster was awarded the first prize in the Johnny Van der Walt student poster competition and is offered in support of this dissertation:

1. **Mukonowenzou N.C.**, Dangarembizi R., Chivandi E and Erlwanger K.H. The effects of ursolic acid administered to neonatal rats on the subsequent development of high fructose diet-induced metabolic dysfunction (poster PJ6 – conference book of abstracts).

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ABSTRACT

Fructose-rich diets and the early nutritional environment have been implicated in the increase in metabolic disorders worldwide. The “two hit” hypothesis has also come under the spotlight as consequences of early nutritional interventions have been shown to appear either spontaneously or after induction by a second intervention leading to worsened disease states. Current research is exploring the potential use of pharmacologically diverse phytochemicals such as ursolic acid (UA) to promote metabolic programming thereby imparting positive health benefits later in life. This study examined the effects of early administration of UA on the subsequent development of complications associated with diet-induced metabolic dysfunction in Sprague Dawley rats.

One hundred and seven suckling, six-day old male and female Sprague Dawley rats randomly received 10 ml/kg of either 0.5% dimethylsulphoxide (control), UA, 50% fructose solution or a mixture of 50% fructose and UA orogastrically for 14 days. They were then weaned onto normal rat chow and plain drinking water on day 21. At adulthood (day 70), half the number of rats in each treatment group either continued on plain drinking water or they received a 20% fructose solution as drinking fluid for eight weeks. Food and fluid intake, body mass gain, fasting blood triglyceride, and oral glucose tolerance were assessed before termination. On termination blood and tissue samples were collected to assess the effect of UA on growth, organ morphometry, adiposity, hepatic lipid storage and surrogate markers of health.

The effects of fructose were found to be dependent on the time of intervention and sex. In males, fructose consumption in adulthood resulted in a 7% increase in body mass and a 35% increase in circulating blood triglycerides which were not observed in females. A single fructose hit and fructose consumption both neonatally and in adulthood caused increased hepatic lipid storage in females by 32% and 67% respectively. In both sexes, fructose intake in adulthood caused decreases in food intake whilst increases in fluid intake were observed in female rats ($P < 0.05$). Fructose consumption had no effect on glucose tolerance, visceral adiposity, organ morphometry and surrogate health markers. Neonatal administration of UA caused a 6% increase in body mass in female rats and prevented excessive fructose-induced hepatic lipid storage in both male and female rats.

Although fructose administration had adverse effects in the liver, especially in female rats, neonatal intervention with UA was found to alter metabolism so as to protect against hepatic lipid accumulation. Therefore, UA is a phytochemical that shows great potential in the control of hepatic lipid metabolism and its metabolic complications.

TABLE OF CONTENTS

COVER PAGE	i
DECLARATION	ii
DEDICATION.....	iii
CONFERENCE PRESENTATION.....	iv
ACKNOWLEDGEMENTS	v
ABSTRACT	vii
TABLE OF CONTENTS	ix
LIST OF FIGURES	xii
LIST OF TABLES.....	xiii
LIST OF ABBREVIATIONS	xiv
CHAPTER 1 : INTRODUCTION	1
1.1 Dissertation structure and study background.....	2
1.1.1 Dissertation structure	2
1.1.2 Background	2
1.2 Neonatal Programming.....	3
1.2.1 Neonatal programming: preconceptual, periconceptual and preimplantation periods.....	4
1.2.2 Neonatal programming: uterine period.....	5
1.2.3 Neonatal programming: postnatal period.....	6
1.3 Metabolic syndrome	6
1.3.1 Definitions of metabolic syndrome	7
1.3.2 Metabolic syndrome and the dual burden of disease	8
1.3.3 Metabolic syndrome and fructose.....	9
1.3.3.1 Fructose metabolism	10
1.4 Treatment of metabolic syndrome	12
1.4.1 Ursolic acid	13
1.4.1.1 Anti-hyperglycaemic and anti-diabetic effects of ursolic acid.....	14
1.4.1.2 Anti-obesity and anti-hyperlipidaemic effects of ursolic acid	14
1.4.1.3 Hepatoprotective effects of ursolic acid.....	15
1.4.1.4 Anti-inflammatory and anti-cancer effects of ursolic acid	15
1.4.1.5 Other pharmacological effects of ursolic acid.....	16
1.5 Justification of the study.....	16
1.6 Aim of the study.....	17
1.7 Hypotheses.....	17

CHAPTER 2 : MATERIALS AND METHODS	19
2.1 Ethical clearance for the study	20
2.2 Housing and general care of the animals	20
2.3 Chemicals and reagents used	20
2.4 Study design.....	21
2.5 Measurement of body mass	23
2.6 Oral glucose tolerance tests	23
2.7 Terminal procedures.....	24
2.7.1 Tissue harvesting	24
2.8 Determination of hepatic lipid content	24
2.9 Clinical biochemistry assays.....	25
2.10 Determination of bone linear growth and estimation of bone density	25
2.11 Statistical analysis	26
CHAPTER 3 : RESULTS	27
3.1 Effect of neonatal intake of ursolic acid on growth performance.....	28
3.1.1 Body mass measurements	28
3.1.2 Linear growth	31
3.2 Effect of neonatal intake of ursolic acid on the development of metabolic dysfunction	35
3.2.1 Circulating metabolites	35
3.2.2 Tolerance to an oral glucose load in adulthood.....	39
3.2.3 Food and fluid intake in adulthood.....	44
3.2.4 Adiposity	49
3.2.5 Hepatic storage of lipids	51
3.3 Effect of neonatal intake of ursolic acid on the morphometry of the gastrointestinal tract and accessory organs.....	54
3.3.1 Gastrointestinal tract (GIT) organs	54
3.3.2 Accessory organs.....	57
3.4 Effect of neonatal intake of ursolic acid on the general health profile	60
3.4.1 Surrogate markers of liver function.....	60
3.4.2 Surrogate markers of renal function in adulthood.....	62
3.4.3 Clinical biochemistry	64
CHAPTER 4 : DISCUSSION	66
4.1 Growth performance	67
4.1.1 Body mass	67

4.1.2 Linear growth	68
4.2 The development of metabolic dysfunction	69
4.2.1 Circulating metabolites	69
4.2.2 Tolerance to an oral glucose load	70
4.2.3 Food and fluid intake	71
4.2.4 Adiposity	72
4.2.5 Hepatic storage of lipids	72
4.3 Morphometry of the GIT and accessory organs	74
4.3.1 Gastrointestinal tract organs	74
4.3.2 Accessory organs	74
4.4 General health profile	75
4.4.1 Surrogate markers of liver function	75
4.4.2 Surrogate markers of kidney function	75
4.4.3 Clinical biochemistry	76
CHAPTER 5 : CONCLUSION AND RECOMMENDATIONS	78
5.1 Conclusion	79
5.2 Limitations and recommendations	79
CHAPTER 6 : REFERENCES	82
APPENDIX 1: Ethics clearance certificate	123

LIST OF FIGURES

Figure 1.1:Fructose and glucose metabolism in the liver	11
Figure 2.1: A diagrammatic representation of the study design.....	22
Figure 3.1: Induction, weaning and terminal masses of male (A) and female (B) rats given different treatments.	29
Figure 3.2: Representative radiograph images of femora and tibiae of male rats.	33
Figure 3.3: Representative radiograph images of femora and tibiae of female rats.	34
Figure 3.4: Effect of ursolic acid on glucose tolerance in male (A) and female (B) rats. ...	40
Figure 3.5: Effect of ursolic acid on the total area under the curve of oral glucose tolerance test in male (A) and female (B) rats.....	42
Figure 3.6: Hepatic lipid content in male (A) and female (B) rats in adulthood.....	52

LIST OF TABLES

Table 1.1: Definitions of metabolic syndrome	7
Table 3.1: Effect of ursolic acid on tibial and femoral masses, lengths and Seedor indices in male and female rats	32
Table 3.2: Effect of ursolic acid on circulating metabolites; fasting blood glucose and triglyceride concentrations (P35).....	36
Table 3.3:Effect of ursolic acid on circulating metabolites in male and female rats in adulthood.....	38
Table 3.4: Food intake in male and female rats in adulthood	45
Table 3.5: Fluid intake in male and female rats in adulthood	47
Table 3.6: Effect of ursolic acid on adiposity.....	50
Table 3.7: Effect of ursolic acid on the masses and lengths of GIT organs.....	55
Table 3.8: Effect of ursolic acid on masses of GIT accessory organs	58
Table 3.9: Effect of ursolic acid on surrogate markers of liver function	61
Table 3.10: Effect of ursolic acid on surrogate markers for renal function in male and female rats	63
Table 3.11: Effect of ursolic acid on general health markers in male and female rats	65

LIST OF ABBREVIATIONS

Acetyl Co-A:	Acetyl coenzyme A
AD:	Alzheimer's disease
ADP:	Adenosine diphosphate
AESC:	Animal Ethics Screening Committee
AIDS:	Acquired Immune Deficiency Syndrome
ALB:	Albumin
ALP:	Alkaline phosphatase
ALT:	Alanine aminotransferase
AMP:	Adenosine monophosphate
AMPK:	AMP-activated kinase
AMY:	Amylase
ANOVA:	Analysis of variance
AST:	Aspartate aminotransferase
ATP III:	Adult treatment panel III
ATP:	Adenosine triphosphate
BUN:	Blood urea nitrogen
CAS:	Central animal services
CO ₂ :	Carbon dioxide
DMSO:	Dimethylsulphoxide
FAO:	Food and Agriculture Organisation
FW:	Fructose water
GIT:	Gastrointestinal tract
GLUT:	Glucose transporter
HDL:	High density lipoprotein
HFCS:	High fructose corn syrup
HIV:	Human Immunodeficiency Virus
IDF:	International Diabetes Federation
IUGR:	Intrauterine growth restriction
LDL:	Low density lipoprotein
LI:	Large intestines
LKB1:	Liver kinase binding -1
MS:	Metabolic syndrome
NAFLD:	Non- alcoholic fatty liver disease

NCD:	Non- communicable disease
NCEP:	National cholesterol education program
NF- κ B:	Nuclear factor kappa-B
OGTT:	Oral glucose tolerance test
PKA:	Protein kinase A
PPAR- α :	Peroxisome proliferator activated receptor-alpha
PPAR- γ :	Peroxisome proliferator activated receptor-gamma
PW:	Plain water
ROS:	Reactive oxygen species
rTL:	Relative to tibial length
SI:	Small intestines
TBIL:	Total bilirubin
TP:	Total protein
UA:	Ursolic acid
UHC:	Universal Health Coverage
VLDL:	Very low density lipoprotein
w/v:	weight/volume
WHA:	World Health Assembly
WHO:	World Health Organisation

CHAPTER 1 : INTRODUCTION

1.1 Dissertation structure and study background

1.1.1 Dissertation structure

This dissertation is comprised of six chapters; introduction, materials and methods, results, discussion, conclusions and recommendations and references. The introduction includes the literature review; exploring the concepts of neonatal programming, metabolic syndrome and the pharmacology of ursolic acid. The justification of the study, aims of the study and the hypotheses used are also included in the introduction. The materials and methods used in the study, results obtained, discussion and recommendations arising from the study follow in subsequent chapters.

1.1.2 Background

The indiscriminatory increase in metabolic syndrome (MS), characterised by several metabolic abnormalities, in children and adults worldwide is alarming (Harris, 2013, Ahima et al., 2016). Genetic factors have been implicated in the aetiology of the syndrome as epidemiological and animal studies have shown transgenerational and multigenerational links (Heindel et al., 2015, Abou and Mani, 2016). The rate at which the syndrome is spreading, however, defies a solely genetic cause (Li et al., 2011, Lillycrop and Burdge, 2011, Vickers, 2011). Therefore, the contribution of environmental factors (nutrition, exercise and early life conditions) is under the spotlight (Gadgil et al., 2015, Padmanabhan et al., 2016, Xiao et al., 2016).

As the pathogenesis of the syndrome is yet to be fully elucidated and the syndrome is multifactorial, there is no specific treatment available (Bruce and Byrne, 2009, Mahajan et al., 2010). Current interventions have resulted in the discontinued use of some pharmaceutical agents whilst marginal success rates have accompanied lifestyle changes (Giugliano et al., 2008, Kaur, 2014). Consequently, focus has shifted to the potential of phytochemicals abundant in fruits, herbs and vegetables (Graf et al., 2010, Akaberi and Hosseinzadeh, 2016). Pentacyclic triterpenes of the ursane group, such as ursolic acid (UA), have been shown to ameliorate metabolic syndrome associated abnormalities in adults (Rao et al., 2011, Sundaresan et al., 2012, Li et al., 2014). However, no work has been done to show the potential preventive effects of UA, if administered during the critical periods of sensitivity, against the development of metabolic syndrome later in life.

This study, therefore, investigated the effects of UA on early programming of subsequent diet-induced metabolic dysfunction in adulthood.

1.2 Neonatal Programming

Neonatal programming is a phenomenon that explains how the environment experienced in early life can have a long-term effect on the physiology, metabolism and therefore health of an individual (Martin-Gronert and Ozanne, 2012). The pre-conception, uterine and immediate postnatal periods are critical windows of physiological sensitivity (Aiken and Ozanne, 2013a, Wells, 2014). Environmental perturbations during these critical periods can trigger changes in organ and system development and if the changes are permanent, programming results (Gluckman et al., 2005, Barker, 2007, Cota and Allen, 2010). The foetus and neonate reprogram to favour early survival and improve success in an expected postnatal environment (Gluckman et al., 2008). A mismatch between the expected postnatal environment and the prevailing postnatal environment, however, can increase susceptibility to disease later in life (Cagampang et al., 2011, Velkoska and Morris, 2011, Reynolds et al., 2015).

Epidemiological and animal studies have shown that the outcomes of the early programming depend on the timing, duration, type and severity of insult and are at times sex-specific (Rinaudo and Wang, 2012, Aiken and Ozanne, 2013a, Goran et al., 2014). Changes in organ morphology and function programmed prenatally can be ameliorated or exacerbated postnatally (Ross and Desai, 2005). This is because neonatal programming can have either adverse or beneficial effects to disease later in life (Koletzko et al., 2011, Zohdi et al., 2012, Lewis et al., 2014). For instance, male rat pups receiving leptin supplementation during lactation then a high fat diet post-weaning were found to have lower body mass, fat accumulation and feed intake preventing metabolic dysfunction later in life (Pico et al., 2007, Priego et al., 2010). In addition, the consequences of an early/perinatal intervention can appear either spontaneously or they can be induced by another intervention after a period of latency (Heindel et al., 2015, Sun et al., 2015). The latter phenomenon is recognised in the “two hit” hypothesis for disease (Knudson, 1971, Day and James, 1998).

The two hit hypothesis states that a primary intervention (“first hit”) may sensitise an organ and lead to physiological alterations (Knudson, 1971, Erdélyi et al., 2013, Morris et al., 2015). The alterations may be immediately expressed leading to organ malfunction and

ultimately disease or may be suppressed (Bayer et al., 1999, Lahiri et al., 2007, Lahiri et al., 2009, Heindel et al., 2015). A second intervention (“second hit”), however, may unmask the suppressed effects leading to disease or amplify the effects of the “first hit” (Tsukamoto et al., 2009, Howard, 2013). For example, in the progression of non-alcoholic fatty liver disease (NAFLD), steatosis as a result of insulin resistance and/or obesity is usually the “first hit” (Dowman et al., 2010, Fabbrini et al., 2010, Tiniakos et al., 2010). Steatosis makes the liver vulnerable to a number of “second hits” including inflammatory cytokines, mitochondrial dysfunction and gut microbiota which ultimately lead to steatohepatitis and fibrosis (Tilg and Moschen, 2010, Pais et al., 2011, Lau et al., 2015). In studies using sheep, maternal obesity in the periconceptual period (“first hit”) has been found to increase fat mass (Rattanatrak et al., 2010), alter hepatic lipid metabolism (Nicholas et al., 2014) and insulin signalling (Nicholas et al., 2013) in the offspring. Maternal obesity in late gestation (“second hit”) results in the increased expression of lipogenic and adipogenic genes in the offspring (Muhlhausler et al., 2007, Long et al., 2015).

1.2.1 Neonatal programming: preconceptional, periconceptional and preimplantation periods

The maternal health status is important before, during and after conception as it has lasting metabolic effects on the offspring (Cardozo et al., 2011, Smith and Ryckman, 2015). In the preconception period, maternal metabolic syndrome alters the biochemical composition of follicular fluid, adversely affecting oocyte quality predisposing the resulting offspring to metabolic abnormalities (Leroy et al., 2012, Gu et al., 2015, Hou et al., 2016). Maternal over- and undernutrition result in epigenetic modifications which adversely alter oocyte maturity, blastocyst development and prenatal survival during the periconceptional and preimplantation periods (Robker, 2008, Ashworth et al., 2009, Niakan et al., 2012). Changes during these periods can alter whole cell lineages (Burdge et al., 2011, Aiken and Ozanne, 2013b). The extent to which the placenta can mitigate or exacerbate these changes, however, is unknown (Godfrey, 2002, Fowden et al., 2006). Undernutrition in these periods has been shown to increase susceptibility to cardiovascular anomalies including hypertension, small heart mass, excess in angiotensin converting enzyme and altered arterial vasodilation (Ashworth et al., 2005, Sinclair et al., 2007, Watkins et al., 2008, Watkins et al., 2010). Maternal overnutrition and obesity alter cardiovascular and behavioural mechanisms evidenced by distorted arterial contraction and dilation, pulse

pressure and stress responses in offspring (Gardner et al., 2004, Torrens et al., 2009, Hernandez et al., 2010). During embryonic development, maternal obesity and insulin resistance alter various metabolic pathways predisposing offspring to insulin resistance, hypercholesterolaemia and hyperleptinaemia in adulthood (Doblado and Moley, 2007, Hwang et al., 2010, Cardozo et al., 2011). Studies during this period have mostly been done in animals but are also important in the context of human *in vitro* reproduction where cardio-metabolic dysfunction is evident (Bertram and Hanson, 2001, Ceelen et al., 2008, Odom and Segars, 2010).

1.2.2 Neonatal programming: uterine period

Both maternal under and over-nutrition have adverse programming effects *in utero*. The uterine period is marked by vast gametogenesis as well as organogenesis such that nutritional manipulations cause permanent structural and reproductive alterations which affect functionality (Rhind et al., 2001). For example, in both altricial species and humans, the islets of the pancreas develop during gestation (Robb, 1961, Bouwens et al., 1997, Fowden and Hill, 2001). They then undergo remodelling during the first 2-3 postnatal weeks in altricial species and up to 4 years of age in humans (Robb, 1961, Hellerström and Swenne, 1991, Hill et al., 2000). Poor nutrition during these periods can alter islet morphology and function which may lead to increased susceptibility to diabetes in adulthood (Dahri et al., 1995, Fowden and Hill, 2001, McMillen and Robinson, 2005).

In under-nourished mothers, intrauterine growth restriction (IUGR) is a foetal coping mechanism which has been associated with epigenetic changes (Thompson et al., 2010, Xu et al., 2013). These changes lead to low birth weight babies who are likely to develop impaired glucose tolerance (Yajnik and Deshmukh, 2008), type-2 diabetes (Kanaka-Gantenbein, 2010), hypertension (Hinchliffe et al., 1992, Mackenzie and Brenner, 1995) and an increased risk of coronary heart disease (Eriksson et al., 2000) in adulthood. The loss of structural and functional units such as nephrons, cardiomyocytes and pancreatic cells due to IUGR may be instrumental in the metabolic complications experienced later in life (McMillen and Robinson, 2005, Dumortier et al., 2007, Zohdi et al., 2012).

One would expect that if the mother eats an obesogenic diet during pregnancy then the growing foetus reprograms and does not suffer from ill health after exposure to an obesogenic diet later in life. Studies, however, have shown that maternal overnutrition

leads to obesity and cardiovascular disorders in the offspring later in life (Li et al., 2011, Simmons, 2011, Brenseke et al., 2013). Obesity has been linked to leptin resistance and increased orexigenic neuropeptide production as foetal neural pathways, particularly those affecting feed intake, are reprogrammed in response to maternal overnutrition (Howie et al., 2009). Adipogenesis takes place during the late gestational phase and during the early postnatal period (Widdowson, 1968). Maternal overnutrition promotes fat accumulation in foetus which predisposes the offspring to obesity later in life (Samuelsson et al., 2008, Shankar et al., 2008). Excess circulating maternal lipids have also been associated with increasing the susceptibility of non-alcoholic fatty liver disease in offspring (Oben et al., 2010, Stewart et al., 2013).

1.2.3 Neonatal programming: postnatal period

In some mammals, organ development is incomplete at birth and continues in the early postnatal period (Patel and Srinivasan, 2011, Moore et al., 2015). Nutritional insults encountered during this suckling period can act as triggers for the induction of lasting programming effects (Guilloteau et al., 2009, Langley-Evans, 2009, Portha et al., 2011). Over-nutrition during the postnatal period is thought to program central appetite regulators as well as glucose and lipid metabolism increasing the risk of metabolic dysfunction in adulthood (Chen et al., 2009). Thus, an altered milieu in the immediate postnatal period with metabolic perturbations may cause epigenetic modifications resulting in changes in gene promoter region methylation thereby affecting gene expression in pathways associated with a range of physiologic processes (Jirtle and Skinner, 2007, Simmons, 2011). The phenotypic effects of these epigenetic modifications may not manifest until later in life, especially if they affect genes modulating responses to later environmental challenges such as dietary change (Gicquel et al., 2008, Szyf, 2009). Neonatal programming can be a result of interactions between various environmental stressors which can lead to increased susceptibility to some similar diseases (Heindel et al., 2015). It has therefore been implicated in the aetiology of the metabolic syndrome.

1.3 Metabolic syndrome

The metabolic syndrome is a cluster of cardiovascular risk determinants, including abdominal adiposity, glucose intolerance, hypertriglyceridaemia, non-alcoholic fatty liver disease (NAFLD) and decreased high density lipoprotein cholesterol (Alberti and Zimmet, 1998, National Cholesterol Education Program, 2002, Nomura and Yamanouchi, 2012).

Depending on sex, race, age, the population studied, setting (rural or urban area) and the definition of the syndrome used, global prevalence of metabolic syndrome can range from as low as less than 10% to as high as above 60% (Erasmus et al., 2012, Kaduka et al., 2012, Bhat et al., 2015).

1.3.1 Definitions of metabolic syndrome

Including those for children and adolescents, as many as 40 definitions exist for metabolic syndrome with the three most widely used shown below in Table 1.1 (Ford and Li, 2008, Kassi et al., 2011, Thaman and Arora, 2013). Being a public health concern, the many definitions have led to confusion in the identification, diagnosis and quantification of individuals at risk and sufferers of the syndrome (Alberti and Zimmet, 1998, Blaha and Elasy, 2006).

Table 1.1: Definitions of metabolic syndrome

World Health Organisation (WHO)	International Diabetes Federation (IDF)	National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III)
- Emphasises the presence of insulin resistance and any two of the following: ✓ Obesity ✓ Hypertension ✓ High triglyceride levels ✓ Reduced high density lipoprotein cholesterol (HDL) ✓ Microalbuminuria (Alberti and Zimmet, 1998)	- Emphasises the presence of central obesity (waist circumference with ethnicity specific values) plus any two of the following: ✓ Raised triglycerides ✓ Raised blood pressure ✓ Reduced HDL ✓ Raised fasting plasma glucose (International Diabetes Federation, 2005)	- Any three of the following: ✓ Abdominal obesity ✓ Elevated triglycerides ✓ Reduced HDL ✓ Raised blood pressure ✓ Raised fasting glucose (National Cholesterol Education Program, 2002)

Currently, a harmonised criteria is used to reduce disparities in research and to establish the incidence and prevalence of MS worldwide (Alberti et al., 2009). In tandem with the

NCEP definition, it emphasises that there be no mandatory component and that any three out of five abnormal components be considered as MS. However, it was agreed that waist circumference (with ethnicity specific values) be used as a preliminary screening tool (Alberti et al., 2009).

1.3.2 Metabolic syndrome and the dual burden of disease

Traditionally, the cardio-metabolic risk factors of MS have been known to be widespread in developed nations whilst communicable diseases and undernutrition have mostly affected developing nations (Horton, 2007, Miranda et al., 2008, Ellulu et al., 2014). This has been partly due to the different nutritional and lifestyle trends. Globalisation, however, has and continues to bring a number of transitions in its wake: nutritional, epidemiological and health (Popkin, 2002, Popkin et al., 2012, Azuike et al., 2013). The nutrition transition promoted the global availability of cheap sugar (high corn fructose syrup), vegetable oils and fats resulting in direct competition with grain products (Drewnowski and Popkin, 1997, Chopra et al., 2002, Hawkes, 2005). Consequently, there have been changes in nutrition and disease trends.

In developing nations, a decline in coarse grain consumption has fuelled increased consumption of refined grains and calorie-rich food whilst fruit and vegetable intake remain inadequate (Popkin and Ng, 2007, Kearney, 2010, Khan and Talukder, 2013). This has concomitantly led to the epidemiological and health transitions in which MS and other non-communicable diseases (NCDs) have been on the rise (Popkin et al., 2012). In some countries, NCDs surpass mortality of infectious diseases like HIV/AIDS (Kelly and Fuster, 2010). Globalisation also gives rise to urbanisation which paves way for sedentary lifestyles which worsen MS (Ruel et al., 2008).

The nutrition transition promotes undernutrition in the form of micronutrient deficiency. Micronutrient deficiency weakens natural immunity which increases the susceptibility to communicable diseases exacerbating mortality rates (Katona and Katona-Apte, 2008, França et al., 2009, Singer, 2013). Despite agricultural technological advances, some countries in Africa, particularly war-torn nations (South Sudan and Democratic Republic of Congo) and those suffering from periodic droughts (parts of Ethiopia, Kenya, Uganda and Somalia, Eritrea), undernutrition in the form of macronutrient deficiency is still a problem (World Health Organisation, 2007, Louis and Hess, 2008, Bain et al., 2014). As a

result, most developing countries suffer the double burden of disease; both communicable and non-communicable diseases at household, community and population levels (Popkin, 2003, Kennedy et al., 2006). Most of these nations already have frail healthcare policies and systems and disintegrating social service structures (Sikosana et al., 1997, Bradshaw and Ndegwa, 2000, Foley, 2009). The emergence and contribution of MS to the burden of disease in developing countries therefore threatens to overwhelm the health sector (Evans, 2009, Bloom et al., 2012).

1.3.3 Metabolic syndrome and fructose

With the increase in fructose consumption coinciding with increases in metabolic irregularities, fructose has been implicated in the genesis and progression of MS (Douard and Ferraris, 2008, Khitan and Kim, 2013). Fructose is the natural sugar found in honey and in many fruits (Shils and Shike, 2006). It is also a constituent of table sugar, sucrose (Harvey and Ferrier, 2011). Sweetness values are a measure of the relative sweetness of a substance with the standard being sucrose with a value of 100 (Shallenberger and Acree, 1971). Crystalline fructose has a sweetness value of 175 making it the sweetest natural sugar (Shallenberger and Acree, 1971, Shallenberger, 2012). The commercial hydrolysis of maize/corn yields glucose which is enzymatically isomerised to give fructose in varying glucose:fructose ratios in the production of high fructose corn syrup (HFCS) (Considine, 2012, Marie and Piggott, 2013). It is used as an ingredient in sweetened beverages and many processed foods as it is a cheap source of sugar with long shelf life and moisterisation benefits (Hanover and White, 1993, Berdanier et al., 2007, Tappy and Lê, 2010).

The consumption of commercially produced fructose is increasing whilst natural fructose consumption is decreasing or remaining constant globally (Marriott et al., 2009). Being sweet, fructose is highly palatable and is thus favoured by consumers resulting in overfeeding (Tappy and Lê, 2010, Gibney et al., 2013). High fructose consumption, however, leads to metabolic dysfunction; increased concentrations of plasma free fatty acids, leptin, triglycerides, uric acid and abdominal adiposity (Hallfrisch, 1990, Melanson et al., 2008, Alzamendi et al., 2009). Impaired insulin sensitivity also results (Aeberli et al., 2013, Khitan and Kim, 2013). In a bid to curb the detrimental metabolic effects of sugars such as fructose, “sugar-tax” has been introduced in countries like Mexico (sugar-sweetened beverages), Finland (sweets, ice-cream and soft drinks) and France (sweetened

beverages) (Escobar et al., 2013, Cornelsen and Carreido, 2015). Many countries like South Africa are in the process of implementing the same policy (National Department of Health, 2012). Among all the monosaccharides, fructose has received widespread attention mainly due to the way it is metabolised in the body.

1.3.3.1 Fructose metabolism

The metabolic pathways of fructose and glucose in the liver are shown in Figure 1.1 overleaf. At low concentrations in the small intestine lumen, fructose is converted to glucose (Mayes, 1993, Douard and Ferraris, 2013). At high concentrations, however, it is absorbed and metabolised in the liver resulting in very small amounts of fructose (about 0.01 mmol/L) entering systemic circulation (Mayes, 1993, Bray, 2007). Non-insulin dependent glucose transporters, GLUT-5 and GLUT-2, facilitate the movement of fructose into intestinal cells and into the liver respectively (Keembiyehetty et al., 2006, Manolescu et al., 2007, Thorens and Mueckler, 2010). The testes, spermatozoa, kidneys, adipose tissue, muscle and to a lesser extent the brain express GLUT- 5 (Gropper and Smith, 2012, Roy and Krishna, 2013). In cells, fructose is converted to fructose-1-phosphate by the enzyme fructokinase (Ishimoto et al., 2012). Through catalysis by aldolase B, fructose-1-phosphate is then converted to the trioses: glyceraldehyde-3-phosphate and dihydroxyacetone phosphate, which are the precursors for phospholipid and triacylglycerol synthesis (Harvey and Ferrier, 2011, Seidler, 2013).

Glucose, in comparison, is transported into cells by an insulin dependent transporter, GLUT-4 (Huang and Czech, 2007, Douard and Ferraris, 2013). It is converted to glucose-6-phosphate by hexokinase then to the triose phosphates and finally into pyruvate during glycolysis (Harvey and Ferrier, 2011, Bender, 2014). Glucose metabolism is highly regulated by the energy status of the cell through negative feedback mechanisms (Lieberman et al., 2006, Gropper and Smith, 2012). Phosphofructokinase, a rate limiting enzyme, catalyses the formation of fructose-1,6-bisphosphate (precursor of the triose phosphates) and is inhibited by citrate and adenosine triphosphate (Berg et al., 2002, Bender, 2014). The formation of triose phosphates in fructose metabolism, however, is void of this regulatory step thus it is a source of abundant and unregulated carbon atoms (Elliott et al., 2002, Rippe, 2014).

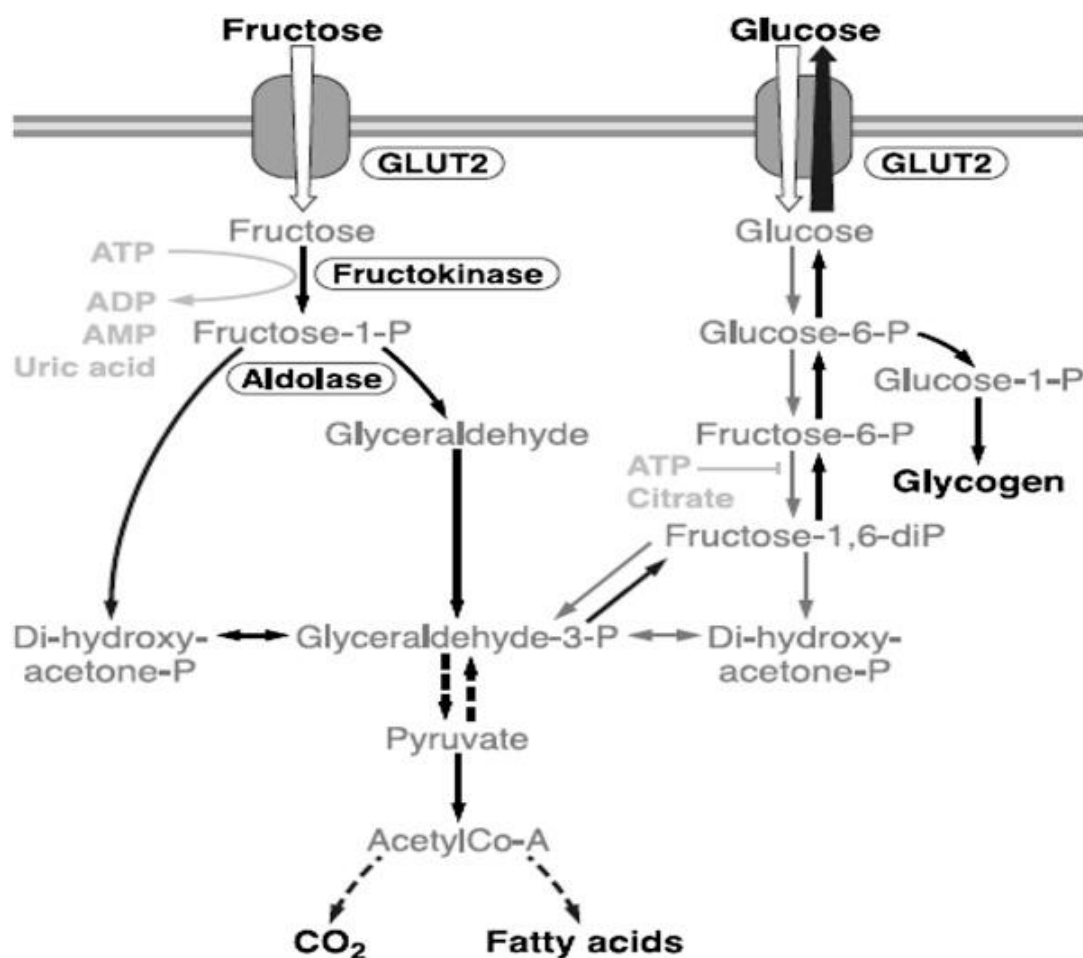


Figure 1.1:Fructose and glucose metabolism in the liver (Tappy and Lê, 2010). Abbreviations: -P; Phosphate, Acetyl Co-A; Acetyl coenzyme A, CO₂; carbon dioxide, ATP; Adenosine triphosphate, ADP; Adenosine diphosphate, AMP; Adenosine monophosphate GLUT 2; glucose transporter 2.

Fructose facilitates *de novo* hepatic lipogenesis, lactate production, glycogen synthesis and gluconeogenesis (Park et al., 1992, Shils and Shike, 2006). This puts it at the crux of the progression of MS as accumulation of hepatic lipids contributes to dyslipidaemia, NAFLD and tissue-specific insulin resistance (Birkenfeld and Shulman, 2014, Perry et al., 2014). Furthermore, overconsumption as a result of fructose feeding contributes to obesity (Bray et al., 2004, Brown et al., 2008). Fructose metabolism also results in the depletion of hepatic adenosine triphosphate (ATP), which results in the production of adenosine monophosphate (AMP) and uric acid fuelling hyperuricaemia (Van den Berghe, 1985, Hallfrisch, 1990, Johnson et al., 2013). Hyperuricaemia has been implicated in the pathogenesis of insulin resistance leading to diabetes and hypertension (Gustafsson and Unwin, 2013, Johnson et al., 2013, Soltani et al., 2013). Putative mechanisms include endothelial dysfunction through reduction of nitric oxide, induction of the renin

angiotensin system and smooth muscle cell proliferation (Khosla et al., 2005, Sanchez-Lozada et al., 2005, Corry et al., 2008).

1.4 Treatment of metabolic syndrome

There is no specific treatment for MS (Reddy and Rao, 2006, Giugliano et al., 2008). As such, lifestyle modifications and pharmaceutical agents are being used to manage MS (Marvasti and Adeli, 2010, Brenseke et al., 2013). Lifestyle changes such as body mass reduction and increased physical activity are normally the first-line of therapy in the management of MS (Ricanati et al., 2011, De Lorgeril, 2012). This is because they can improve all facets of the syndrome (Dalle Grave et al., 2010, Yamaoka and Tango, 2012, Hartley, 2014). With increasing severity of MS, however, pharmaceutical agents are used together with lifestyle modifications (Rubio-Ruiz et al., 2013).

Lipid lowering drugs which reduce low density lipoprotein cholesterol (statins) and increase high density lipoprotein cholesterol (niacin and fibrates) have been used to manage MS (Michos et al., 2012, Elkes, 2016). Insulin sensitising drugs such as metformin and peroxisome proliferator activated receptor gamma (PPAR- γ) agonists with anti-diabetic properties (thiazolidinediones) are also used to reduce blood glucose in the management of MS (Derosa and Maffioli, 2010, Rojas and Gomes, 2013, Song, 2016). The undesirable side effects, low efficacy and high cost associated with the use of pharmaceutical agents have made them inaccessible and have led to their discontinued use leaving few drugs that can be used (Elangbam, 2009, Rodgers et al., 2012). This is a hindrance to one of WHO's overarching goals of universal health coverage (UHC).

The UHC “seeks to ensure that all people have access to promotive, preventive, curative and rehabilitative health services, of sufficient quality to be effective, while also ensuring that they do not suffer financial hardship when paying for these services” (WHO, 2015). With WHO predicting that deaths from NCDs such as MS will increase from 38 million to 52 million by 2030, there has been an increased use of traditional medicine such as ethnomedicines (Eddouks et al., 2012, WHO, 2014, WHO, 2015). The increased use of ethnomedicines was evident in Korea where annual expenditure on traditional medicine rose from US\$ 4.4 billion in 2004 to US\$ 7.4 billion in 2009 (Johnson, 2016). This trend was also apparent globally as an estimated US\$83 billion was spent on traditional medicine in 2008 and an exponential rate of increase observed (Robinson and Zhang, 2011). Consumers believe that “natural means safe” and ethnomedicines are cheaper, more locally

and readily available than conventional medicine (Cooper and Yamaguchi, 2013, WHO, 2015). In most developing countries ethnomedicines provide the bulk of primary health care (Payyappallimana, 2010, WHO, 2015). Additionally, traditional medicine is also being widely used in developed countries with 70% of Canadians, 82% of Australians and over 100 million users in Europe (Johnson, 2016). The World Health Assembly (WHA) and WHO have therefore put in place strategies to facilitate the safe use, regulation and promotion of ethnomedicines (World Health Assembly, 2009, WHO, 2015). These strategies have facilitated the integration of traditional and complementary medicine into the health system in countries such as China, Korea and Switzerland (Government of China, 2010, Swiss Confederation, 2011, Frass et al., 2012).

With regards to MS, researchers are currently investigating the therapeutic potential of naturally occurring phytochemicals which are abundant in fruits and vegetables (Kim et al., 2011, Holubková et al., 2012). Many of the phytochemicals display pharmacological and biochemical effects that include inhibition of several different enzyme systems related to absorption and metabolism of carbohydrates and lipids (Yoshizumi et al., 2006, de Melo et al., 2010, Gupta and Prakash, 2014). Pentacyclic triterpenes belonging to the lupane, oleanane and ursane series are some of the phytochemicals being explored (Hasani-Ranjbar et al., 2009, Alqahtani et al., 2013, Castellano et al., 2013). As most of these phytochemicals are taken orally, their interaction with the gastrointestinal tract (GIT) is of great importance (Gavhane and Yadav, 2012, McClements et al., 2015). Studies have shown that although development of the GIT is pre-programmed, nutritional manipulation in the intrauterine and early postnatal periods can affect it resulting in precocious maturation leading to higher body mass gains and enhanced bone development (Linderöth et al., 2005, Puzio et al., 2007). Ursolic acid is one of the phytochemicals being explored in the management and treatment of MS (Li et al., 2014, Nazaruk and Borzym-Kluczyk, 2014).

1.4.1 Ursolic acid

Ursolic acid (3 β -hydroxy-urs-12-en-28-oic acid) is an ursane pentacyclic triterpenoid that exists naturally in plants as a free acid or aglycone (Liu, 1995, Sun et al., 2006). It has been isolated from a number of fruits including apples (He and Liu, 2007), guavas (Begum et al., 2004), loquats (Zhou et al., 2007) and olives (Somova et al., 2003). It has also been isolated from medicinal herbs such as rosemary (Huang et al., 1994), basil (Chiang et al.,

2005), sage (Le Men and Pourrat, 1952) and thyme (Ismaili et al., 2001). Being biologically active both topically and internally, UA exhibits a wide range of pharmacological effects.

1.4.1.1 Anti-hyperglycaemic and anti-diabetic effects of ursolic acid

Diabetes mellitus is a result of altered insulin secretion and/or insulin action which perturbs fat, protein and carbohydrate metabolism resulting in chronic hyperglycaemia (Alberti and Zimmet, 1998). Lowering postprandial blood glucose concentration is one of the therapeutic strategies employed in managing diabetes mellitus (Nathan et al., 2009, Nyenwe et al., 2011). Ursolic acid extracted from *Cornus officinalis* Sieb. et Zucc (Gao et al., 2008) and *Osmanthus fragrans* (Kang et al., 2012) was found to lower fasting blood glucose and postprandial glucose concentrations in diabetic rats.

1.4.1.2 Anti-obesity and anti-hyperlipidaemic effects of ursolic acid

Adipocytes secrete hormones (leptin and adiponectin), store lipids and are insulin sensitive (Camp et al., 2002, Havel, 2004). Alterations in adipocyte metabolism have adverse effects, with obesity being marked by hyperplasia and hypertrophy of adipocytes (Drolet et al., 2008, Jo et al., 2009, Stephens, 2012). Ursolic acid stimulates lipolysis in mature 3T3-L1 adipocytes through a cyclic-AMP dependent protein kinase A (PKA) pathway (Li et al., 2010). Furthermore, UA suppresses pre-adipocyte differentiation and adipogenesis through the liver kinase binding-1/AMP-activated protein kinase (LKB1/AMPK) pathway in 3T3-L1 adipocytes, exhibiting anti-obesity properties (He et al., 2013).

Dietary fat is thought to promote body fat storage more effectively than dietary carbohydrates leading to metabolic anomalies (Consultation, 1998, Brown et al., 2008). However, fat absorption is only possible after fat digestion by lipase (Gargouri et al., 1997). Following oral administration of lipid emulsion in rats, UA has been shown to have lipase-inhibiting properties which prevent increases in plasma triacylglycerol concentrations (Kim et al., 2009). In non-obese mice, UA promotes thermogenesis, energy expenditure increase of brown fat which aids obesity management strategies (Kunkel et al., 2012).

1.4.1.3 Hepatoprotective effects of ursolic acid

The liver is the body's metabolic hub (Wall and Porter, 2014, Feldman et al., 2015). It is responsible for detoxification and the synthesis, metabolism, storage and redistribution of carbohydrates, proteins and lipids (Berg et al., 2002, Rui, 2014). Hypercaloric diets, sedentary lifestyles and drug use have deleterious effects on the liver resulting in NAFLD, cholestasis and liver injury (Epstein et al., 1998, Lee, 2003, Zelber-Sagi et al., 2007, Masarone et al., 2014). Thioacetamide (Akhtar and Sheikh, 2013), galactosamine (Keppler et al., 1968) and carbon tetrachloride (Hübner, 1965, Shi et al., 1998) are used to induce hepatotoxicity in rats as they decrease hepatocyte viability as well as disrupt bile secretion. Pre-treatment of rats with UA improves hepatocyte viability as well bile secretion in a similar manner as the hepatoprotective drug silymarin (Binduja et al., 1996, Feher and Lengyel, 2012). Ursolic acid ameliorates paracetamol-induced hepatotoxicity in rats exhibiting its anti-cholestatic and anti-choleretic effects in rats (Shukla et al., 1992).

Thought to be the hepatic facet of MS, NAFLD encompasses steatosis, inflammation, hepatocellular injury and fibrosis ultimately progressing to liver cirrhosis and hepatocellular carcinoma (Angulo, 2002, Dowman et al., 2010, Takahashi and Fukusato, 2014). Endoplasmic reticulum stress has been implicated in fuelling the progression of steatosis to more aggressive forms of NAFLD (Gentile et al., 2011, Lake et al., 2014). Li et al. (2015) administered UA to diabetic mice and L02 palmitic acid stimulated cells. Hepatic steatosis, liver mass and concentrations of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were reduced in the diabetic rats whilst lipid accumulation was reduced in L02 cells. Through enhanced lipid β -oxidation and inhibition of endoplasmic reticulum stress, UA exhibits hepatoprotective properties which may be crucial in NAFLD therapy.

1.4.1.4 Anti-inflammatory and anti-cancer effects of ursolic acid

Inflammation is a non-specific, adaptive immune response to any stimuli that cause cell injury (Markiewski and Lambris, 2007). In response to tissue damage, inflammation allows for repair of damaged tissue (Krafts, 2010) whilst in pathological conditions such as cancer, chronic inflammation may result in tissue damage and organ dysfunction (Coleman and Tsongalis, 2009, Dubois, 2015). Ursolic acid exhibits its anti-inflammatory property through attenuation of inducible nitric oxide synthase and cyclooxygenase-2 expression in the murine macrophage cell line, RAW264.7 (Suh et al., 1998, Ryu et al., 2000). Ursolic

acid also has anti-leukaemic and anti-cancer properties through its suppression of nuclear factor kappa-B (NF- κ B) activation (Najid et al., 1992, Shishodia et al., 2003). The anti-cancer effect of UA has also been illustrated in lung cancer (Kim et al., 2015), prostate cancer (Park et al., 2013), ovarian cancer (Wang et al., 2009), breast cancer (Gim et al., 2010) and hepatocellular carcinoma (Yang et al., 2010) cell lines through apoptosis. Ursolic acid has been used in cancer Phase I clinical trials (Zhu et al., 2013, Qian et al., 2015).

1.4.1.5 Other pharmacological effects of ursolic acid

Ursolic acid displays cardiogenic (Somova et al., 2004), anti-ulcer (Shih et al., 2004) and nephroprotective (Pai et al., 2012) properties in studies using rodents. Studies have demonstrated UA's anti-viral effects against human immunodeficiency virus (Quéré et al., 1996) and hepatitis C (Kong et al., 2013) in NS5B cells. Extracts from UA containing plants *Satureja parvifolia*, *Morinda lucida* and *Mimusops caffra* exhibited anti-malaria activity against chloroquine-sensitive *Plasmodium falciparum* strains (Baren et al., 2006, Cimanga et al., 2006, Simelane et al., 2013). In mice, oral administration of UA was found to reduce parasitic loads during the acute phase of *Trypanosoma cruzi* infections (da Silva Ferreira et al., 2013). In the nematodes that cause elephantiasis, *Brugia malayi* and *Wuchereria bancrofti*, UA induced apoptosis by altering enzyme activity in worms isolated from infected people (Saini et al., 2014). Ursolic acid has also been shown to be neuroprotective exhibiting anxiolytic (Pemminati et al., 2011), anti-nociceptive (González-Trujano et al., 2012, Verano et al., 2013) and anti-depressant like (Machado et al., 2012, Colla et al., 2014) properties in rodents.

1.5 Justification of the study

Most studies assessing the effects of UA on metabolic dysfunction used adult animals primarily and used UA as a treatment method (Jayaprakasam et al., 2006, Jang et al., 2009, Rao et al., 2011). Metabolic dysfunction, however, has the potential to be reversed by nutritional and therapeutic interventions during physiologically sensitive periods (Vickers, 2011). Additionally, the prevalence of MS seems to be sex-biased with females being more prone to the syndrome than males (Tonstad et al., 2007, Houti et al., 2016). With the manifestation of the metabolic abnormalities in different organs being sex-specific and with most studies having been done in males, there is need for sex-specific studies (Beigh and Jain, 2012, Tsai et al., 2014). Therefore, although UA displays great pharmacological

potential to ameliorate MS, there is a paucity of data of intervention strategies using UA during the period of developmental plasticity. As such, this study aimed to investigate the effects of UA on neonatal programming and to assess if it could protect against the development of diet-induced metabolic dysfunction in adulthood in Sprague Dawley rats in a sex-dependent manner.

1.6 Aim of the study

The main aim of this study was to determine the effect of UA on neonatal programming of metabolic dysfunction in rats fed a high fructose diet and to assess its potential to protect against the subsequent development of metabolic syndrome in adulthood. The specific objectives of this study were to determine the effects of UA on;

1. neonatal programming of diet-induced metabolic dysfunction and the subsequent health outcomes in adulthood.
2. growth performance of rats by measuring;
 - a. body mass gain.
 - b. linear growth (length, mass and density of the long bones: femur and tibia).
3. the development of metabolic dysfunction by measuring;
 - a. circulating levels of metabolites: glucose, cholesterol and triglycerides.
 - b. tolerance to an oral glucose load.
 - c. food and fluid intake.
 - d. adiposity.
 - e. hepatic storage of lipids.
4. the morphometry of the GIT (and accessory organs) by measuring lengths and masses of visceral organs.
5. the general health profile of the rats by measuring;
 - a. surrogate markers of liver function: alanine transaminase (ALT), alkaline phosphatase (ALP), total bilirubin and albumin.
 - b. surrogate markers of renal function: creatinine and blood urea nitrogen.
 - c. clinical biochemistry: total protein, calcium, phosphate, amylase and globulins.

1.7 Hypotheses

The null hypothesis (H_0) and alternate hypothesis (H_1) for the study were:

H₀: Ursolic acid has no effect on neonatal programming and does not protect against the subsequent development of metabolic dysfunction in Sprague Dawley rats fed a high fructose diet in the neonatal period and/or in adulthood. In addition, that no differences exist between male and female responses to UA treatment.

H₁: Ursolic acid has an effect on neonatal programming and protects against the subsequent development of metabolic dysfunction in Sprague Dawley rats fed a high fructose diet in the neonatal period and/or in adulthood. In addition, that differences exist between male and female responses to UA treatment.

CHAPTER 2 : MATERIALS AND METHODS

2.1 Ethical clearance for the study

All animal experiments were carried out according to protocols approved by the Animal Ethics Screening Committee (AESC) of the University of the Witwatersrand, AESC number 2014/49/D (see appendix for certificate).

2.2 Housing and general care of the animals

Sprague Dawley dams with suckling male and female rat pups from the Central Animal Services (CAS) at the University of the Witwatersrand were used in the study. Each dam, with a litter of 9-12 pups, was housed in perspex cages lined with wood shavings in temperature ($24^{\circ}\text{C} \pm 2^{\circ}\text{C}$), light (12h light:12h dark; 0700:1900 clock time) and ventilation controlled rooms. A commercial rat chow (Epol®, Johannesburg, South Africa) and clean drinking water were provided *ad libitum* for the dams. After weaning, the weaned rats were housed individually as described above.

2.3 Chemicals and reagents used

Dimethylsulphoxide (DMSO; Sigma-Aldrich, France) was reconstituted in distilled water to a final concentration of 0.5% and used as vehicle. Ursolic acid (Sigma-Aldrich, France) was prepared by dissolving 2 mg of ursolic acid in 10 μl of DMSO and making up to 2 ml with distilled water. The UA was prepared in bulk, aliquoted to 2 ml portions and stored in microtubules (Eppendorf) at -20°C until use. Fructose (Nature's choice, South Africa) was used to induce metabolic dysfunction. According to the manufacturer's product information label, the nutritional composition of the fructose was 1680 kJ energy, 0 g protein, 0.2 mg/100 g sodium, 99.8 g/100 g carbohydrates, 0 g fat and 0 g fibre. Fructose (20% w/v) was prepared by dissolving 20 g of fructose in tap water and making it up to 100 ml. Fructose (50% w/v) was prepared by dissolving 50 g of fructose in distilled water and making it up to 100 ml. The commercial rat chow (Epol®, Johannesburg, South Africa) had the following nutritional composition: 170 g/kg protein, 25 g/kg fat, 70 g/kg fibre, 25 g/kg calcium, 6 g/kg phosphorus and 6.5 g/kg lysine. Glucose solution (50% w/v; Radchem, South Africa) was made by dissolving 5 g of glucose in distilled water and filling it up to the 10 ml mark. A drop of food colouring (no nutritional value) (Robertsons, Retailer Brands (Pty) Ltd, South Africa) was added to 5 l of the drinking fluids and used to distinguish the fluids from one another.

2.4 Study design

Figure 2.1 overleaf shows a diagrammatic representation of the study design which aimed to simulate a ‘one hit’ and ‘two hit’ interventional study. A total of one hundred and seven male and female suckling pups were used (from 11 litters; 9-12 pups per litter). In the first stage of the study (which was from postnatal day 6 (P6) to P20), the first nutritional intervention “first hit” was introduced to induce neonatal programming. The pups in each litter were randomly assigned to four main treatment groups, each with a minimum of 26 pups in each treatment group. The rat pups were uniquely identified by marking with colour coded, non-toxic permanent marker ink on their tails. The rat pups received an oral administration of one of the following treatments;

Group 1 (control): *0.5 % DMSO (10 ml/kg b.w). The DMSO was used as a vehicle to dissolve the UA (Sundaresan et al., 2012) (n =27).

Group 2: *UA (10 mg/kg b.w) reconstituted in DMSO. We used this dose of UA because previous studies reported its effectiveness in reversing the symptoms of metabolic syndrome (visceral adiposity, blood glucose concentrations and plasma lipids) in mice fed a high fat diet (Rao et al., 2011) (n =27).

Group 3: 50% fructose solution (10 ml/kg b.w) (n =27)

Group 4:*UA (10 mg/kg b.w) + 50% fructose solution (n =26).

*= 10 ml/kg b.w

Orogastric administration of the treatments was done once daily between 09:00 and 11:00 using an orogastric tube mounted on a 1 ml syringe. In-between gavaging, the pups were allowed to suckle freely on their respective dams.

In the second phase of the study (non-interventional phase), the pups were weaned on P21 and housed individually. They were fed a commercially supplied rat chow and supplied with clean plain drinking water *ad libitum* until P69. In order to assess if the interventions in the first phase had any early metabolic effects on the pups, metabolic assays were done on P35. After overnight fasting, blood was taken from a pin prick at the tip of alcohol-swab sterilised tails of the rats (Parasuraman et al., 2010). The blood was used to determine fasting glucose and triglyceride concentrations. A calibrated glucometer (Ascensia, Ireland) was used to determine blood glucose concentration whilst a calibrated Accutrend

triglyceride meter (Roche Diagnostics, Germany) was used to determine blood triglyceride concentration as per manufacturer's instructions.

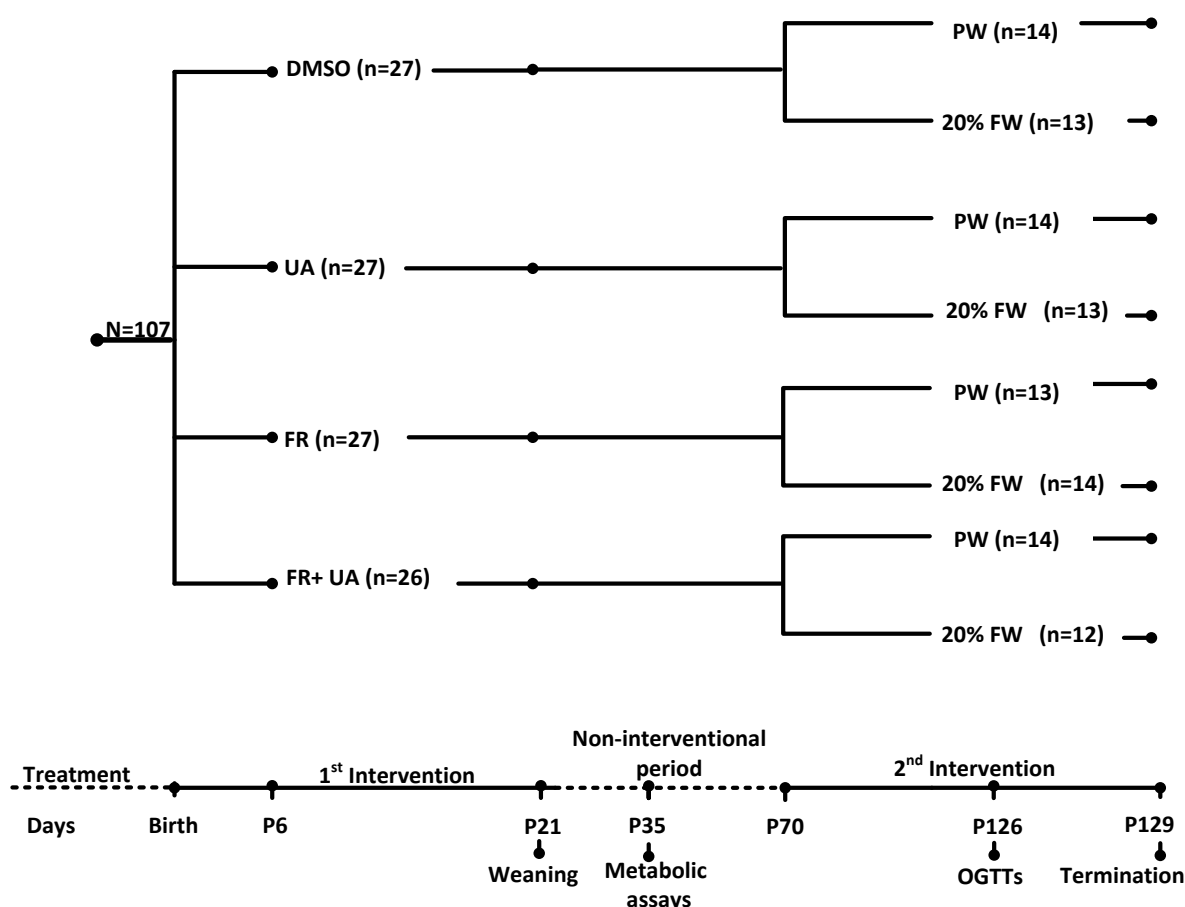


Figure 2.1: A diagrammatic representation of the study design. Abbreviations – DMSO; dimethylsulphoxide, UA; ursolic acid, FR; fructose, OGTTs; oral glucose tolerance tests, PW; plain drinking water and FW; fructose in drinking water, P; postnatal day.

The third phase of the study (second intervention; “second hit”) started on P70 until termination (P129). Normal rat chow was given to all the rats, however, half the number of rats in each group were assigned to one of two treatments (with males and females in each group) wherein they received either plain drinking water or 20% fructose in drinking water. Administration of 20% fructose for eight weeks has been shown to induce obesity, dyslipidaemia, hyperglycaemia, hyperinsulinaemia and hypertension in rats (Barros et al., 2007, Mamikutty et al., 2014). Twice a week, the drinking bottles were washed and changed and fresh solutions were prepared and provided. A drop of food colouring was added to 500 ml of either plain drinking water or 20% fructose in drinking water. This was in order to differentiate the fluids from one another. During this phase of the study, food

and fluid intake were measured weekly using modification of Mamikutty et al. (2014) formulae:

Average daily food intake = [initial feed mass (g) – final feed mass (g)]/ number of days the feed was supplied.

Average daily fluid intake = [initial fluid volume (ml) – final fluid volume (ml)]/ number of days the fluid was supplied.

Both daily food and fluid intake were then expressed and reported as a percentage of body mass as g/ 100 g and ml/100g respectively (Ghezzi et al., 2012).

The feed was weighed using a balance (Snowrex Electronic Scale, Clover Scales, Johannesburg) whilst a calibrated measuring cylinder was used to determine the volume of the fluids.

2.5 Measurement of body mass

In the first phase, the pups were weighed (Snowrex Electronic Scale, Clover Scales, Johannesburg) daily to ensure that the correct dosage of the various treatments was administered. Post-weaning, the rats were weighed twice every week in order to assess growth. The dams were also weighed twice every week as part of routine health checks.

2.6 Oral glucose tolerance tests

After eight weeks of intervention, tolerance to an oral glucose load was assessed on P126. The animals were habituated in perspex restrainers for an hour for three consecutive days prior to the procedure. The rats were fasted overnight but had *ad libitum* access to drinking water. Following sterilisation of the tail as described in Section 2.4, a fasting blood sample was taken following a pin-prick at the tip of the tail (time=0). Glucose (2 g/kg b.w) was then administered orogastrically and blood samples were collected from the tip of the tail after 15, 30, 60, 120 and 180 minutes to determine systemic glucose concentrations (Ghezzi *et al.*, 2012). Blood glucose was determined using a calibrated glucometer (Ascensia, Ireland) according to the manufacturer's instructions. The rats were returned to their designated feeding regime for 48 hours prior to termination. The total area under the curve (AUC) was calculated from the OGTT results.

2.7 Terminal procedures

The rats were terminated on P129 after an overnight fast but had *ad libitum* access to drinking water. Fasting triglyceride concentrations were assessed using blood taken from a pin prick at the tip of the sterilised tail as previously described. A calibrated Accutrend triglyceride meter (Roche Diagnostics, Germany) was used to determine triglyceride concentration as per manufacturer's instructions. The rats were euthanased using sodium pentobarbitone (200 mg/kg b.w).

2.7.1 Tissue harvesting

After euthanasia, the thorax was opened for organ harvesting and blood collection via cardiac puncture. Blood was collected into heparinised and plain tubes (BD Vacutainer, Plymouth, UK) and centrifuged (Rotofix 32A, Hettich Zentrifugen, Germany) at 4 000 G for 15 minutes. The supernatant (plasma/serum) was then pipetted into microtubes (Eppendorf) before being stored at -20°C for further analysis. The heart, liver, stomach, pancreas, caecum, small and large intestines, visceral and epididymal fat were carefully dissected out. The stomach, small intestines, caecum and large intestine were weighed after being gently squeezed to remove any wastes. The lengths of large and small intestines were measured using a ruler mounted on a dissection board. The masses of visceral (and epididymal in males) fat pads, heart, pancreas, liver and kidneys were also measured using a balance (Presica 310M, Switzerland). The liver was stored at -20°C for further analysis.

All organ masses were corrected relative to tibial length by using the formula;

Organ mass relative to tibial length = organ mass (g) / length of tibia (mm) (Nunes-Souza et al., 2016).

2.8 Determination of hepatic lipid content

Determination of the liver lipid content was done by solvent extraction at the Agricultural Research Council (Irene Analytical Services Laboratory) using the Tecator Soxtec method (Official Methods of Analysis of Analytical Chemists, 2005). The liver samples were freeze-dried, milled and 1 g was placed into a pre-weighed extraction thimble. Fat-free cotton wool was used to plug the thimble before it was placed on a thimble holder. Petroleum ether was added to the extraction cups before the cups were placed onto heating pads. The extraction process involved four stages: boiling (30 minutes), rinsing (30

minutes), petroleum ether recovery (10 minutes) and drying (30 minutes at $90 \pm 5^{\circ}\text{C}$). The extraction cups were then allowed to cool in a dessicator before the amount of the oil was determined using the following formula:

$$\% \text{ fat} = 100[(\text{mass of cup plus fat} - \text{mass of cup}) \div (\text{mass of sample})]$$

The test was done in triplicate.

2.9 Clinical biochemistry assays

An IDEXX VetTest Chemistry Analyser (IDEXX VetTest® Clinical Chemistry Analyser, IDEXX Laboratories Inc., USA) was used to measure the serum concentrations of alanine aminotransferase, alkaline phosphatase, blood urea nitrogen, creatinine, phosphate, calcium, total protein, albumin, globulin, total bilirubin and amylase as per manufacturer's instructions.

2.10 Determination of bone linear growth and estimation of bone density

The femur and tibia from the right leg of each rat were cleaned of non-calcified tissue and oven-dried (Salvis®, Switzerland) for five days at a temperature of 50°C . Bone dry mass was measured using a balance (Presica 310M Laser, Johannesburg, South Africa) whilst bone length was measured using venier calipers (Hi-impact, Dejuca, South Africa) were used to measure bone length. Tibia length was measured between the tibia head and medial malleolus. Femur length was measured between the greater trochanter and medial condyle. The Seedor index was used to estimate bone density and was calculated using the following formula;

$$\text{Seedor index} = \text{mass of bone (mg)} / \text{length of bone (mm)} \text{ (Seedor, 1991, Almeida et al., 2008).}$$

Radiographs of the bones were also taken to subjectively assess bone density using a Fuji film X-ray machine (Industrial X-ray film FR; Fuji Photo Film Co., Ltd, Tokyo, Japan). The bones were placed on a photographic plate, 1 metre away from the X-ray light source set at 4.8 kVp, 0.71 mA per plate for 10 seconds.

2.11 Statistical analysis

All data are expressed as mean and standard deviation and were analysed using Graph Pad Prism 5 (Graph Pad Software, San Diego, California, USA). Statistical significance was set at 5%. Linear growth, visceral organ mass, concentrations of circulating metabolites, liver lipids and general health profile markers were analysed using one-way analysis of variance (ANOVA). Body mass changes, oral glucose tolerance tests and food and fluid intake were analysed using the two-way repeated measures ANOVA with treatment and time as main effects. Unpaired student t-tests were used to analyse sex differences of the above parameters. The Bonferroni *post hoc* test was used to detect differences between and or within groups whenever the ANOVA showed significant differences or significant main effects.

CHAPTER 3 : RESULTS

3.1 Effect of neonatal intake of ursolic acid on growth performance

3.1.1 Body mass measurements

Figure 3.1 shows the induction, weaning and terminal masses of male (A) and female (B) rats across all the treatment groups. The treatments given in the neonatal phase had no adverse effects on growth of the rats across the treatment groups ($P > 0.05$). There was significant growth, from induction to weaning and from weaning to termination within all treatment groups in both sexes ($P < 0.0001$). In males, a late fructose hit, that is, rats receiving dimethylsulphoxide (DMSO) in the neonatal phase and fructose in adulthood (DMSO+FW) had increased terminal mass compared to rats receiving DMSO neonatally and plain water in adulthood (DMSO+ PW) (main effects of time ($P < 0.0001$), treatment ($P = 0.079$), and their interaction ($P = 0.0073$)). No differences were observed in rats receiving an early fructose hit (fructose neonatally and water in adulthood; FR+FW) as well as a double fructose hit (fructose neonatally and in adulthood) ($P > 0.05$). Ursolic acid (alone and in combination with fructose) had no effects on body mass ($P > 0.05$).

In females, UA administration alone promoted increases in terminal body mass; rats receiving UA neonatally with fructose in adulthood (UA+FW) had significantly higher terminal masses than the group receiving dimethylsulphoxide in the neonatal phase and fructose in the third phase (DMSO+FW) (main effects of time ($P < 0.0001$) treatment ($P = 0.21$), and their interaction ($P = 0.37$)). No differences were observed in rats receiving a late fructose hit as well as a double fructose hit (fructose neonatally and in adulthood; FR+FW) ($P > 0.05$). A comparison of the sexes revealed that male rats had significantly greater body mass gains than female rats across all treatments ($P < 0.0001$).

All data presented as mean $\pm$ standard deviation. *** = significant growth from induction to weaning and from weaning to termination ($P < 0.0001$). μ = significantly greater terminal masses in male rats receiving DMSO+FW than those receiving DMSO+PW ($P < 0.05$). γ = significantly greater terminal masses in female rats receiving UA+FW than those receiving DMSO+FW ($P < 0.05$). DMSO + PW = 10 mg/kg b.w dimethylsulphoxide in neonatal phase + plain water in adulthood (n=14; 8 M, 6 F); DMSO + FW = 10 mg/kg b.w dimethylsulphoxide + 20% fructose in drinking water (n=13; 7 M, 6 F); UA + PW = 10 mg/kg b.w ursolic acid + plain water (n=14; 7 M, 7 F); UA + FW = 10 mg/kg b.w ursolic acid + 20% fructose in drinking water (n=13; 7 M, 6 F); FR + PW = 10 mg/kg b.w fructose + plain water (n=13; 6 M, 7 F); FR + FW = 10 mg/kg b.w fructose + 20% fructose in drinking water (n=14; 6 M, 8 F); UA+FR + PW = 10 mg/kg b.w ursolic acid and fructose + plain water (n=14; 7 M, 7 F); UA+FR + FW = 10 mg/kg b.w ursolic acid and fructose + 20% fructose in drinking water (n=12; 6 M, 6 F).

3.1.2 Linear growth

Table 3.1 shows the masses, lengths and densities of the femora and tibiae of male and female rats in adulthood across treatments. Neither UA nor fructose affected bone growth as there were no significant differences in the masses, lengths and Seedor indices of the bones across the treatment groups ($P > 0.05$). Male rats, however, had significantly heavier masses, longer lengths and greater Seedor index values than female rats across treatment groups ($P < 0.05$).

Figures 3.2 and 3.3 show representative radiographic images of the femora and tibiae of the male and female rats respectively.

Table 3.1: Effect of ursolic acid on tibial and femoral masses, lengths and Seedor indices in male and female rats

Parameter	Sex	DMSO+PW	DMSO+FW	UA+PW	UA+FW	FR + PW	FR + FW	UAFR+PW	UAFR+FW
Tibia mass (mg)	Male	718.80±42.97	747.40±56.28	764.60±64.19	773.00±100.50	761.20±59.40	797.70±75.99	739.90±32.18	773.30±51.40
	Female	546.50±22.39 ^β	546.30±24.74 ^β	569.70±37.73 ^β	594.30±47.36 ^β	563.10±26.28 ^β	555.40±44.00 ^β	571.10±35.09 ^β	564.80±34.84 ^β
Tibia length (mm)	Male	43.30±0.88	44.01±0.52	43.61±0.54	44.06±0.77	43.83±0.91	44.05±1.08	43.16±0.50	43.78±0.48
	Female	39.80±0.75 ^β	39.28±0.73 ^β	39.76±0.56 ^β	39.58±0.84 ^β	39.71±0.49 ^β	39.40±0.96 ^β	39.97±0.59 ^β	39.27±1.26 ^β
Seedor index	Male	16.51±0.84	16.98±1.22	17.52±1.40	17.53±2.08	17.35±1.11	18.09±1.41	17.14±0.67	17.66±1.06
	Female	13.73±0.32 ^β	13.91±0.49 ^β	14.32±0.77 ^β	15.00±0.96 ^β	14.18±0.56 ^β	14.08±0.82 ^β	14.29±0.75 ^β	14.38±0.67 ^β
Femur mass (mg)	Male	859.30±40.55	903.60±94.57	917.30±85.64	936.10±69.74	897.80±68.34	943.00±96.13	874.70±53.47	917.00±57.09
	Female	655.30±32.31 ^β	649.70±39.21 ^β	678.30±44.86 ^β	691.70±40.74 ^β	676.70±31.38 ^β	663.90±55.20 ^β	672.70±36.70 ^β	673.30±48.30 ^β
Femur length (mm)	Male	38.63±0.80	39.33±0.81	38.94±0.48	38.89±0.23	39.45±0.64	38.93±0.48	38.77±0.68	38.80±0.56
	Female	34.70±0.50 ^β	35.32±1.12 ^β	34.94±0.53 ^β	34.95±0.32 ^β	35.17±0.72 ^β	34.54±0.63 ^β	34.94±0.64 ^β	35.00±1.00 ^β
Seedor index	Male	22.24±0.84	22.95±2.03	23.56±2.20	24.07±1.74	22.75±1.56	24.21±2.32	22.56±1.29	23.63±1.34
	Female	18.89±0.91 ^β	18.39±0.80 ^β	19.42±1.34 ^β	19.79±1.15 ^β	19.24±0.79 ^β	19.21±1.33 ^β	19.25±0.84 ^β	19.22±1.00 ^β

All data presented as mean ± standard deviation. β = male rats had significantly heavier, longer and denser femora and tibiae than female rats ($P < 0.05$). DMSO + PW = 10 mg/kg b.w dimethylsulphoxide in neonatal phase + plain water in adulthood (n=14; 8 M, 6 F); DMSO + FW = 10 mg/kg b.w dimethylsulphoxide + 20% fructose in drinking water (n=13; 7 M, 6 F); UA + PW = 10 mg/kg b.w ursolic acid + plain water (n=14; 7 M, 7 F); UA + FW = 10 mg/kg b.w ursolic acid + 20% fructose in drinking water (n=13; 7 M, 6 F); FR + PW = 10 mg/kg b.w fructose + plain water (n=13; 6 M, 7 F); FR + FW = 10 mg/kg b.w fructose + 20% fructose in drinking water (n=14; 6 M, 8 F); UAFR + PW = 10 mg/kg b.w ursolic acid and fructose + plain water (n=14; 7 M, 7 F); UAFR + FW = 10 mg/kg b.w ursolic acid and fructose + 20% fructose in drinking water (n=12; 6 M, 6 F), S.I = Seedor Index.

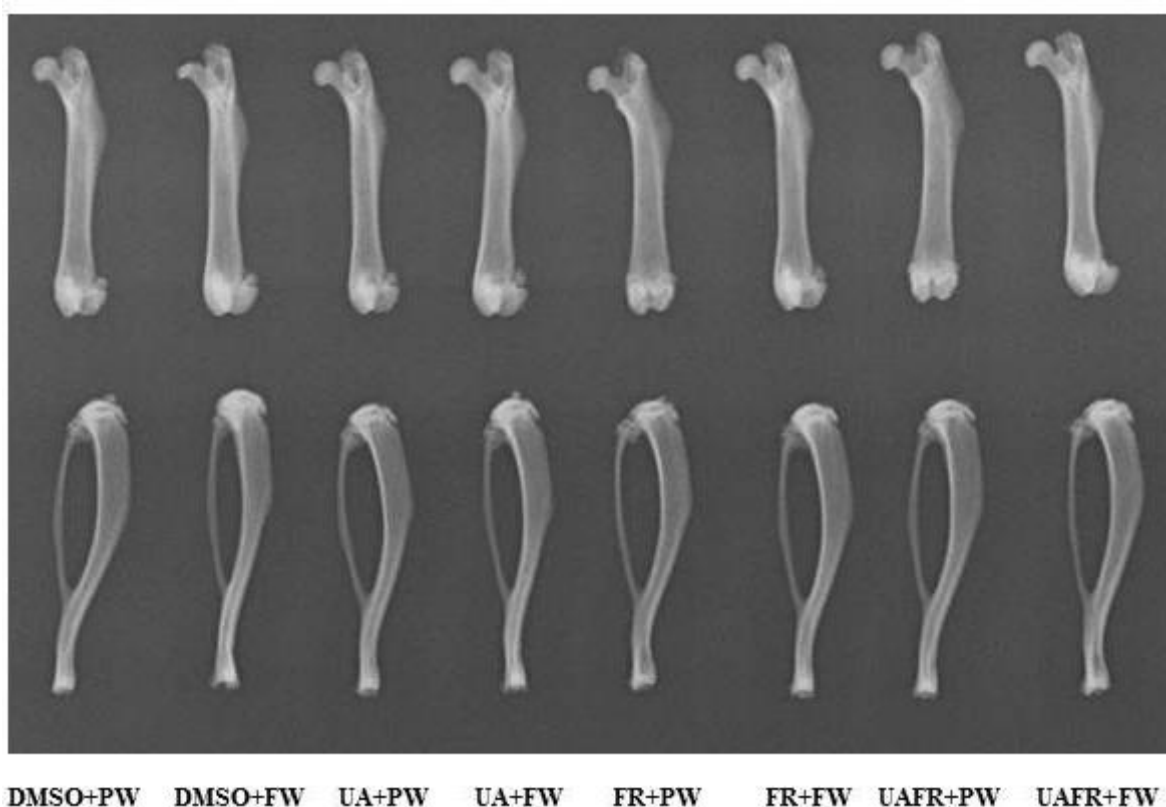


Figure 3.2: Representative radiograph images of femora and tibiae of male rats.

DMSO + PW =10 mg/kg b.w dimethylsulphoxide in neonatal phase + plain water in adulthood (n=8); DMSO + FW =10 mg/kg b.w dimethylsulphoxide + 20% fructose in drinking water (n=7); UA + PW =10 mg/kg b.w ursolic acid + plain water (n=7); UA + FW =10 mg/kg b.w ursolic acid + 20% fructose in drinking water (n=7); FR + PW =10 mg/kg b.w fructose + plain water (n=6); FR + FW =10 mg/kg b.w fructose + 20% fructose in drinking water (n=6); UAFR + PW =10 mg/kg b.w ursolic acid and fructose + plain water (n=7); UAFR + FW =10 mg/kg b.w ursolic acid and fructose + 20% fructose in drinking water (n=6).

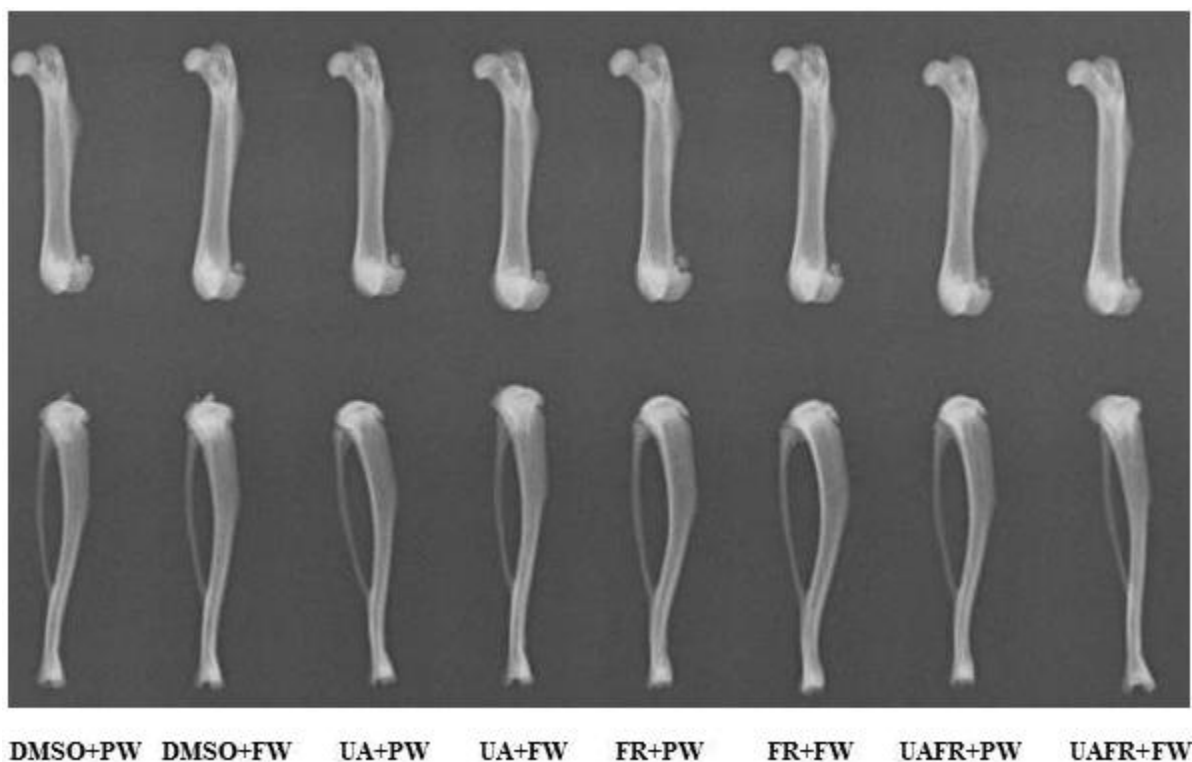


Figure 3.3: Representative radiograph images of femora and tibiae of female rats.

DMSO + PW =10 mg/kg b.w dimethylsulphoxide in neonatal phase + plain water in adulthood (n= 6); DMSO + FW =10 mg/kg b.w dimethylsulphoxide + 20% fructose in drinking water (n=6); UA + PW =10 mg/kg b.w ursolic acid + plain water (n=7); UA + FW =10 mg/kg b.w ursolic acid + 20% fructose in drinking water (n=6); FR + PW =10 mg/kg b.w fructose + plain water (n=7); FR + FW =10 mg/kg b.w fructose + 20% fructose in drinking water (n=8); UA+FR + PW =10 mg/kg b.w ursolic acid and fructose + plain water (n=7); UA+FR + FW =10 mg/kg b.w ursolic acid and fructose + 20% fructose in drinking water (n=6).

3.2 Effect of neonatal intake of ursolic acid on the development of metabolic dysfunction

3.2.1 Circulating metabolites

Table 3.2 shows the fasting blood glucose and triglyceride concentrations of male and female rats on P35 across the treatment groups. Early administration of fructose and UA had no effects on fasting blood glucose and triglyceride concentrations on P35 as there were no significant differences across treatment groups and between the sexes ($P > 0.05$).

Table 3.2: Effect of ursolic acid on circulating metabolites; fasting blood glucose and triglyceride concentrations (P35)

Parameter	Sex	DMSO	UA	FR	UAFR
Glucose (mmol/L)	Male	3.51±0.66	3.63±0.50	3.76±0.55	3.45±0.50
	Female	3.55±0.57	3.59±0.33	3.39±0.43	3.58±0.43
Triglycerides(mmol/L)	Male	1.31±0.27	1.33±0.24	1.30±0.37	1.41±0.41
	Female	1.46±0.50	1.37±0.10	1.54±0.37	1.59±0.51

DMSO + PW =10 mg/kg b.w dimethylsulphoxide in neonatal phase + plain water in adulthood (n=14; 8 M, 6 F); DMSO + FW =10 mg/kg b.w dimethylsulphoxide + 20% fructose in drinking water (n=13; 7 M, 6 F); UA + PW =10 mg/kg b.w ursolic acid + plain water (n=14; 7 M, 7 F); UA + FW =10 mg/kg b.w ursolic acid + 20% fructose in drinking water (n=13; 7 M, 6 F); FR + PW =10 mg/kg b.w fructose + plain water (n=13; 6 M, 7 F); FR + FW =10 mg/kg b.w fructose + 20% fructose in drinking water (n=14; 6 M, 8 F); UAFR + PW =10 mg/kg b.w ursolic acid and fructose + plain water (n=14; 7 M, 7 F); UAFR + FW =10 mg/kg b.w ursolic acid and fructose + 20% fructose in drinking water (n=12; 6 M, 6 F).

Table 3.3 shows concentrations of circulating metabolites (triglycerides and cholesterol) in serum at the end of the study. In male rats, a late fructose hit (administration of DMSO neonatally phase and fructose consumption in adulthood; DMSO+FW) resulted in greater triglyceride concentrations than in rats receiving DMSO neonatally and plain water in adulthood (DMSO+ PW) ($P < 0.05$). No differences were observed in rats receiving an early fructose hit (fructose neonatally and water in adulthood; FR+FW) as well as a double fructose hit (fructose neonatally and in adulthood; FR+FW) ($P > 0.05$). In female rats, no significant differences were observed across the treatment groups in triglyceride concentration ($P > 0.05$). Neonatal administration of UA and fructose alone and in combination did not have any effects on concentrations of circulating triglycerides ($P > 0.05$). No significant differences were observed in triglyceride concentration between the sexes ($P > 0.05$).

Neither UA administration neonatally nor fructose administration neonatally and/or in adulthood had any effects on cholesterol concentration in male and female rats ($P > 0.05$). Generally, female rats had higher cholesterol concentrations than their corresponding males ($P < 0.05$).

Table 3.3:Effect of ursolic acid on circulating metabolites in male and female rats in adulthood

Parameter	Sex	DMSO + PW	DMSO + FW	UA + PW	UA + FW	FR + PW	FR + FW	UAFR + PW	UAFR + FW
Triglycerides (mmol/l)	Male	1.33±0.15	2.06±0.52 ^μ	1.26±0.15	1.99±0.63	1.41±0.20	2.14±0.40	1.39±0.17	1.91±0.60
	Female	1.39±0.24	1.65±0.43	1.40±0.46	2.03±0.61	1.56±0.50	2.19±0.96	1.40±0.16	2.04±0.65
Cholesterol (mmol/l)	Male	1.70± 0.16	1.50± 0.35	1.50± 0.21	1.70±0.27	1.70± 0.19	1.80± 0.34	1.45± 0.24	1.83± 0.27
	Female	1.85± 0.25	2.09± 0.27 ^β	1.96± 1.18 ^β	2.03± 0.33	1.94± 0.28	1.99± 0.26	1.94± 0.44 ^β	2.13± 0.33

All data presented as mean ± standard deviation. μ = significantly increased triglyceride concentration in rats receiving DMSO+FW compared to the rats DMSO+PW ($P < 0.05$). β = females have significantly higher cholesterol concentrations than males ($P < 0.05$). DMSO + PW =10 mg/kg b.w dimethylsulphoxide in neonatal phase + plain water in adulthood (n=14; 8 M, 6 F); DMSO + FW =10 mg/kg b.w dimethylsulphoxide + 20% fructose in drinking water (n=13; 7 M, 6 F); UA + PW =10 mg/kg b.w ursolic acid + plain water (n=14; 7 M, 7 F); UA + FW =10 mg/kg b.w ursolic acid + 20% fructose in drinking water (n=13; 7 M, 6 F); FR + PW =10 mg/kg b.w fructose + plain water (n=13; 6 M, 7 F); FR + FW =10 mg/kg b.w fructose + 20% fructose in drinking water (n=14; 6 M, 8 F); UAFR + PW =10 mg/kg b.w ursolic acid and fructose + plain water (n=14; 7 M, 7 F); UAFR + FW =10 mg/kg b.w ursolic acid and fructose + 20% fructose in drinking water (n=12; 6 M, 6 F).

3.2.2 Tolerance to an oral glucose load in adulthood

Figure 3.4 shows the glucose handling profiles of the male (A) and female (B) rats at 0, 15, 30, 60, 120 and 180 minutes post-gavage with 50% glucose (2 g/kg b.w) after 8 weeks of fructose consumption. There were no significant differences in basal fasting glucose concentrations across the treatment groups (mean values; 3.8 and 4.0 mmol/L in both sexes) ($P > 0.05$). Sixty minutes post-gavage, all the male rats across the treatments had reached peak glucose concentrations between 5.3 and 6.3 mmol/L with no significant differences across the groups ($P > 0.05$). In females, with the exception of the group receiving ursolic acid in the neonatal phase and fructose in the third phase (UA+FW), there were no significant differences in peak blood glucose concentrations (6.0 and 7.4 mmol/L) which were achieved 30 minutes post-gavage ($P > 0.05$). Females that received ursolic acid as neonates and fructose in adulthood (UA+FW) had significantly higher blood glucose concentrations at 15 minutes post-gavage compared to their controls that received DMSO neonatally and fructose in adulthood ($P < 0.05$). In both sexes, 180 minutes post-gavage, blood glucose concentrations returned to normal (3.8-4.4 mmol/L). In males; main time effects ($P < 0.0001$), treatment ($P = 0.67$) and interaction ($P = 0.21$). In females; main time effects ($P < 0.0001$), treatment ($P = 0.018$) and interaction ($P = 0.16$). Fructose consumption, therefore, did not have any adverse effects on glucose handling in both sexes ($P > 0.05$).

Figure 3.5 shows the area under the curve of the glucose tolerance tests in male (A) and female (B) rats across the treatments. The area under the curve (concentration versus time) was calculated by the trapezoidal rule to obtain a summary of glucose tolerance. No significant differences were observed in the area under the curve in both sexes ($P > 0.05$).

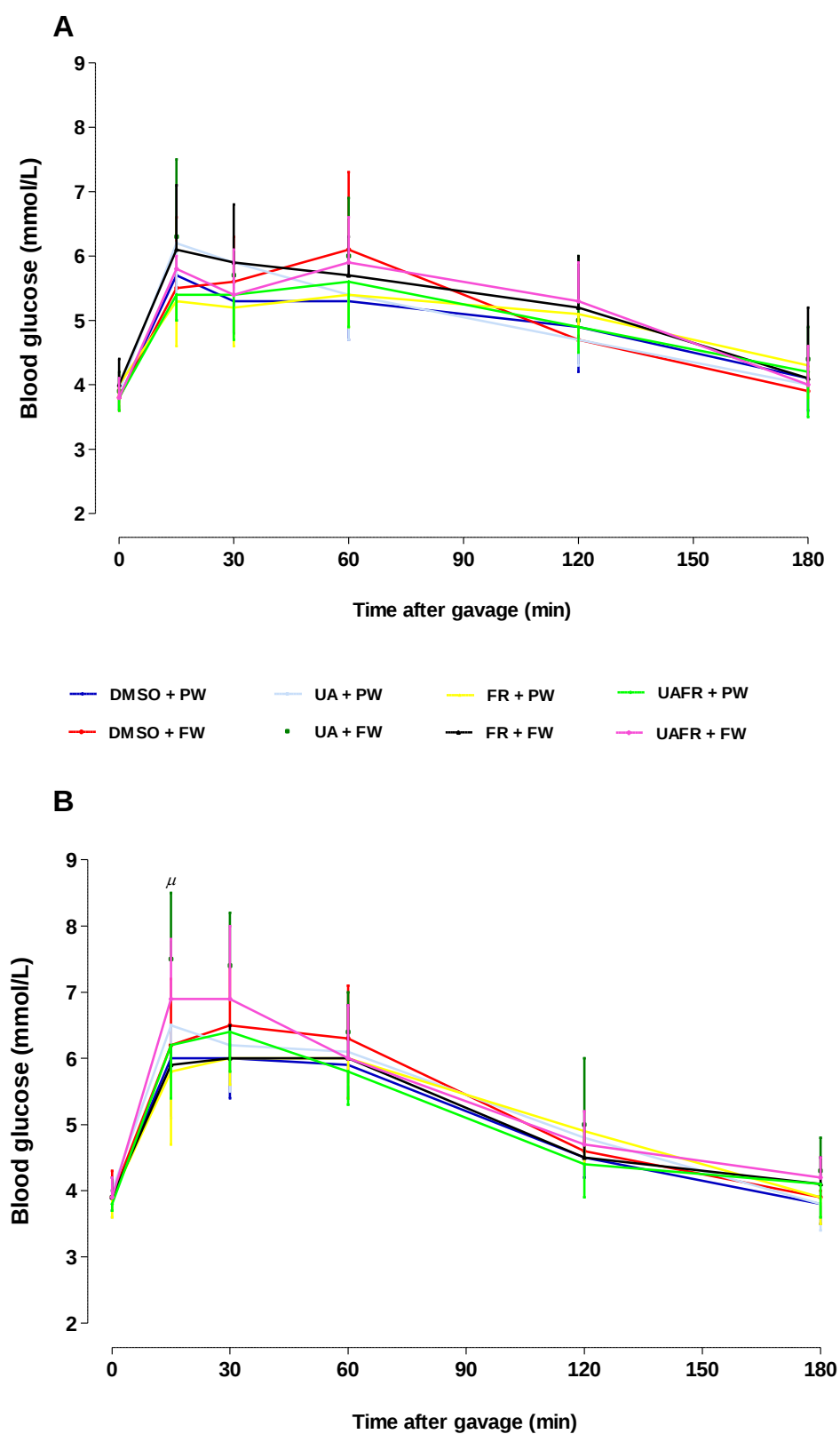


Figure 3.4: Effect of ursolic acid on glucose tolerance in male (A) and female (B) rats.

All data presented as mean $\pm$ standard deviation. μ = significant differences in blood glucose concentrations between UA + FW and DMSO+FW ($P < 0.05$). DMSO + PW = 10 mg/kg b.w dimethylsulphoxide in neonatal phase + plain water in adulthood (n=13; 8 M, 5 F); DMSO + FW = 10 mg/kg b.w dimethylsulphoxide + 20% fructose in drinking water (n=13; 7 M, 6 F); UA + PW = 10 mg/kg b.w ursolic acid + plain water (n=14; 7 M, 7 F); UA + FW = 10 mg/kg b.w ursolic acid + 20% fructose in drinking water (n=12; 7 M, 5 F); FR + PW = 10 mg/kg b.w fructose + plain water (n=13; 6 M, 7 F); FR + FW = 10 mg/kg b.w fructose + 20% fructose in drinking water (n=14; 6 M, 8 F); UAFR + PW = 10 mg/kg b.w ursolic acid and fructose + plain water (n=14; 7 M, 7 F); UAFR + FW = 10 mg/kg b.w ursolic acid and fructose + 20% fructose in drinking water (n=11; 6 M, 5 F).

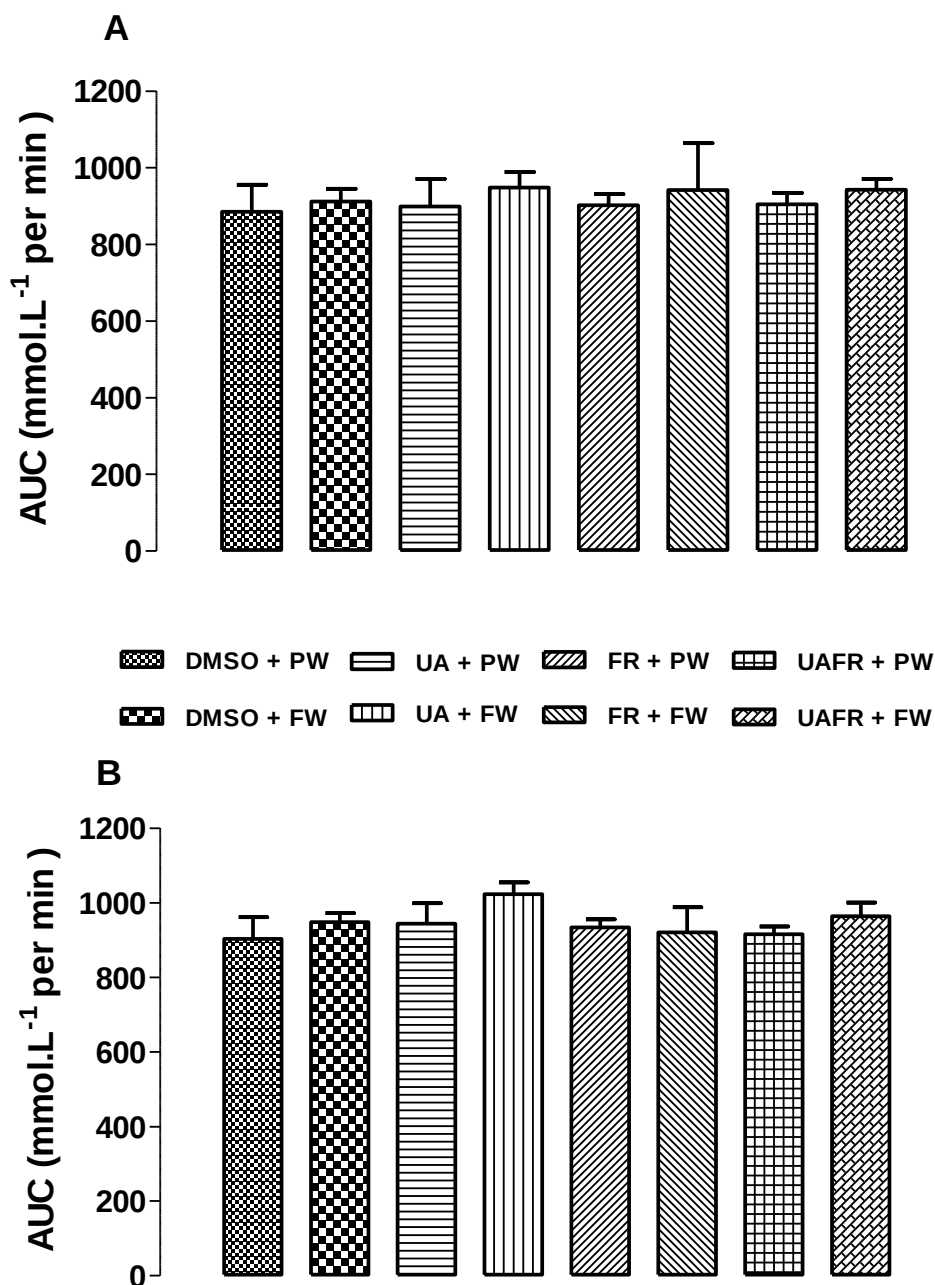


Figure 3.5: Effect of ursolic acid on the total area under the curve of oral glucose tolerance test in male (A) and female (B) rats.

DMSO + PW = 10 mg/kg b.w dimethylsulphoxide in neonatal phase + plain water in adulthood (n=13; 8 M, 5 F); DMSO + FW = 10 mg/kg b.w dimethylsulphoxide + 20% fructose in drinking water (n=13; 7 M, 6 F); UA + PW = 10 mg/kg b.w ursolic acid + plain water (n=14; 7 M, 7 F); UA + FW = 10 mg/kg b.w ursolic acid + 20% fructose in drinking water (n=12; 7 M, 5 F); FR + PW = 10 mg/kg b.w fructose + plain water (n=13; 6 M, 7 F); FR + FW = 10 mg/kg b.w fructose + 20% fructose in drinking water (n=14; 6 M, 8 F);

UAFR + PW = 10 mg/kg b.w ursolic acid and fructose + plain water (n=14; 7 M, 7 F);
UAFR + FW = 10 mg/kg b.w ursolic acid and fructose + 20% fructose in drinking water
(n=11; 6 M, 5 F).

3.2.3 Food and fluid intake in adulthood

Table 3.4 shows the food intake of adult male and female rats. In both male and female rats, a late fructose hit resulted in reduced food intake in rats receiving DMSO neonatally and fructose in adulthood (DMSO+FW) compared to rats that received DMSO neonatally and plain water in adulthood (DMSO+PW) (males; main effects of time ($P < 0.0001$), treatment ($P < 0.0001$), and their interaction ($P = 0.019$), females; main effects of time ($P < 0.0001$), treatment ($P < 0.0001$), and their interaction ($P = 0.74$). An early fructose hit and a double fructose hit did not have any effects on food intake in both sexes ($P > 0.05$). Neonatal UA administration had no effect on food intake ($P > 0.05$). No significant differences in food intake were observed between the sexes ($P > 0.05$).

Table 3.5 shows the fluid intake of adult male and female rats respectively. In females, a late fructose hit resulted in increased fluid intake as rats receiving DMSO neonatally and fructose in adulthood (DMSO+FW) consumed significantly increased fluid than those receiving DMSO neonatally and plain water in adulthood (DMSO+PW) during weeks 4 and 7 ($P < 0.05$). Fructose consumption both neonatally and in adulthood had no effect on fluid consumption in male rats (males; main effects of time ($P < 0.0001$), treatment ($P = 0.0024$) and their interaction ($P = 0.83$), females; main effects of time ($P = 0.2978$), treatment ($P = 0.014$), and their interaction ($P = 0.61$). Neonatal fructose administration had no effect on fluid intake. Female rats had significantly higher fluid intake than their male counterparts ($P < 0.05$).

Table 3.4: Food intake in male and female rats in adulthood

Week	Sex	Food intake (g/100 g of body mass)							
		DMSO+PW	DMSO+FW	UA+PW	UA+FW	FR+PW	FR+FW	UAFR+PW	UAFR+FW
1	Male	9.15±0.40	6.38±1.05 ^μ	8.97±0.62	6.25±0.91	8.47±0.95	5.84±1.23	9.91±0.81	5.80±0.71
	Female	10.01±0.99	6.82±1.43	9.42±1.13	7.03±2.28	9.45±1.36	6.72±1.45	9.05±1.30	6.57±1.36
2	Male	8.23±0.53	4.53±0.21 ^μ	7.73±0.47	4.28±0.27	7.91±0.81	4.43±0.68	8.06±0.32	4.19±0.33
	Female	8.71±1.17	5.01±1.28 ^μ	8.41±0.70	4.95±0.51	8.22±1.06	4.34±1.16	8.04±0.55	4.81±1.94
3	Male	8.04±0.52	4.45±0.49 ^μ	7.68±0.71	4.12±0.44	8.43±0.34	4.04±0.51	7.04±1.56	4.15±0.44
	Female	8.35±1.18	5.09±1.84 ^μ	8.71±1.15	4.51±0.71	8.31±0.69	4.52±0.96	8.11±0.63	4.55±1.86
4	Male	7.68±0.79	4.12±0.99 ^μ	7.05±0.90	3.64±0.63	7.26±0.56	3.89±0.90	7.64±0.61	3.62±0.62
	Female	7.71±1.13	4.80±0.93	7.84±0.59	4.87±2.62	7.83±0.99	3.56±0.75	7.46±1.10	3.33±0.74
5	Male	7.26±0.49	3.70±0.60 ^μ	6.83±0.64	3.44±0.35	7.10±0.35	3.84±0.56	6.97±0.59	3.66±0.21
	Female	8.02±0.96	4.23±1.13 ^μ	8.05±0.92 ^β	4.09±0.63	7.95±0.89	3.32±1.04	7.50±0.66	3.58±1.08
6	Male	7.36±0.66	3.68±0.86 ^μ	6.83±0.55	3.16±0.39	6.82±0.31	3.26±0.89	6.99±0.47	3.44±0.50
	Female	7.44±0.58	4.41±2.43	7.56±0.68	3.87±0.86	7.33±1.02	3.21±0.39	6.93±0.43	3.43±0.25
7	Male	6.97±0.68	3.23±0.43 ^μ	6.53±0.63	2.92±0.29	6.47±0.45	3.22±0.50	6.39±0.75	3.22±0.32
	Female	8.25±2.61	3.58±1.17 ^μ	6.76±0.66	3.57±0.36	6.87±0.67	3.11±0.61	6.84±0.56	3.13±0.38
8	Male	6.83±0.77	3.46±0.36 ^μ	6.29±0.68	3.04±0.36	6.32±0.68	3.18±0.64	6.23±0.55	3.49±0.56
	Female	8.12±2.12	3.66±1.51 ^μ	6.85±0.65	3.68±0.44	7.10±1.33	3.59±0.84	6.48±1.13	3.39±0.18

All data presented as mean $\pm$ standard deviation. μ = significant decreases in food intake in rats receiving DMSO+FW compared to rats receiving DMSO+PW ($P < 0.05$). DMSO + PW = 10 mg/kg b.w dimethylsulphoxide in neonatal phase + plain water in adulthood (n=14; 8 M, 6 F); DMSO + FW = 10 mg/kg b.w dimethylsulphoxide + 20% fructose in drinking water (n=13; 7 M, 6 F); UA + PW = 10 mg/kg b.w ursolic acid + plain water (n=14; 7 M, 7 F); UA + FW = 10 mg/kg b.w ursolic acid + 20% fructose in drinking water (n=13; 7 M, 6 F); FR + PW = 10 mg/kg b.w fructose + plain water (n=13; 6 M, 7 F); FR + FW = 10 mg/kg b.w fructose + 20% fructose in drinking water (n=14; 6 M, 8 F); UA+FR + PW = 10 mg/kg b.w ursolic acid and fructose + plain water (n=14; 7 M, 7 F); UA+FR + FW = 10 mg/kg b.w ursolic acid and fructose + 20% fructose in drinking water (n=12; 6 M, 6 F).

Table 3.5: Fluid intake in male and female rats in adulthood

Week	Sex	Fluid intake (ml /100 g of body mass)							
		DMSO+PW	DMSO+FW	UA+PW	UA+FW	FR+PW	FR+FW	UAFR+PW	UAFR+FW
1	Male	16.71±3.33	17.49±3.25	17.33±4.62	20.07±2.53	16.00±3.61	19.13±4.11	14.32±3.40	20.50±4.58
	Female	19.37±3.13	22.45±5.26	18.61±3.52	21.38±9.52	21.34±3.49	20.29±4.25	20.04±4.42	21.20±3.56 ^β
2	Male	13.92±3.99	17.6±67.04	13.50±2.14	18.45±4.26	14.12±2.22	19.76±6.87	13.61±4.09	17.30±2.49
	Female	15.30±1.93	23.87±5.30	16.31±4.42	21.69±6.54	16.93±2.51	22.27±4.78	18.18±3.70	25.48±3.14 ^β
3	Male	13.36±2.52	18.21±3.86	12.4±02.52	16.95±1.53	14.16±3.51	18.52±5.35	13.30±3.55	20.36±6.17
	Female	13.61±2.66	21.76±6.85	15.87±3.57	23.52±7.29	17.26±4.38	20.20±5.79	18.72±3.86	24.72±6.20
4	Male	13.87±2.62	16.32±3.78	12.94±2.23	19.22±4.10	12.90±2.52	18.69±2.50	12.35±2.55	17.75±3.15
	Female	15.74±3.34	26.09±8.21 ^μ	16.78±2.92	24.97±7.89	17.79±6.05	21.45±7.95	17.76±4.20	24.04±4.84
5	Male	13.53±3.16	18.53±5.41	13.26±3.26	18.43±3.65	12.78±4.06	18.77±4.84	11.25±1.91	16.37±3.31
	Female	14.97±1.47	22.29±4.54	15.48±4.27	22.52±8.24	17.44±4.50	22.78±7.96	17.99±3.99 ^β	24.32±5.41 ^β
6	Male	13.42±3.37	16.70±6.17	11.88±4.42	17.24±3.47	11.38±2.00	16.43±4.37	11.57±3.65	15.83±3.16
	Female	15.89±3.37	24.49±7.68	17.70±3.89	19.79±8.93	18.27±3.83 ^β	21.79±7.26	18.28±5.12 ^β	20.89±5.58
7	Male	13.29±3.50	16.63±5.61	12.48±3.33	16.64±5.21	11.13±1.32	17.80±4.75 ^γ	11.45±3.68	17.54±3.25
	Female	14.97±3.55	24.35±10.62 ^μ	17.24±5.05	17.94±7.92	17.27±2.68 ^β	19.75±6.53	18.23±5.46 ^β	20.93±5.27
8	Male	12.84±2.67	16.28±4.73	12.95±2.15	15.85±4.96	11.72±1.67	15.75±4.37	11.80±4.41	17.35±3.46
	Female	14.74±2.61	21.70±9.10	19.27±5.43	19.51±6.25	18.26±2.45 ^β	20.63±6.12	18.53±4.54 ^β	21.26±3.34

All data presented as mean $\pm$ standard deviation. μ = significant increases in fluid intake in rats receiving DMSO+FW compared to rats receiving DMSO+PW ($P < 0.05$). β = females consumed significantly more fluid than males ($P < 0.05$). DMSO + PW = 10 mg/kg b.w dimethylsulphoxide in neonatal phase + plain water in adulthood (n=14; 8 M, 6 F); DMSO + FW = 10 mg/kg b.w dimethylsulphoxide + 20% fructose in drinking water (n=13; 7 M, 6 F); UA + PW = 10 mg/kg b.w ursolic acid + plain water (n=14; 7 M, 7 F); UA + FW = 10 mg/kg b.w ursolic acid + 20% fructose in drinking water (n=13; 7 M, 6 F); FR + PW = 10 mg/kg b.w fructose + plain water (n=13; 6 M, 7 F); FR + FW = 10 mg/kg b.w fructose + 20% fructose in drinking water (n=14; 6 M, 8 F); UAFR + PW = 10 mg/kg b.w ursolic acid and fructose + plain water (n=14; 7 M, 7 F); UAFR + FW = 10 mg/kg b.w ursolic acid and fructose + 20% fructose in drinking water (n=12; 6 M, 6 F).

3.2.4 Adiposity

Table 3.6 shows the masses of visceral and epididymal fat pads. In both male and female rats, no significant differences were observed in visceral fat pad mass across the treatment groups ($P > 0.05$). Although the males rats showed a tendency to an increased visceral and epididymal fat mass following intake of fructose as adults, the differences were not statistically significant across the treatment groups ($P > 0.05$). No significant differences were observed in visceral fat pad mass between the sexes except for females receiving fructose both neonatally and in adulthood (FR+FW) that had greater visceral fat pad masses compared to their male counterparts ($P < 0.05$).

Table 3.6: Effect of ursolic acid on adiposity

Fat pad	Sex	Mass							
		DMSO + PW	DMSO+ FW	UA + PW	UA + FW	FR + PW	FR + FW	UAFR + PW	UAFR + FW
Visceral (g)	Male	11.28±3.69	17.39±5.13	11.40±2.76	21.53±6.46	13.24±3.29	24.18±4.71	10.79±3.83	21.70±6.07
	Female	10.92±3.60	17.66±4.09	11.21±4.48	18.52±7.01	10.64±2.42	18.98±3.81 ^β	12.30±5.20	19.51±5.40
Visceral (rTL)	Male	0.26±0.08	0.39±0.11	0.26±0.06	0.49±0.14	0.30±0.07	0.55±0.10	0.25±0.09	0.49±0.14
	Female	0.27±0.09	0.45±0.11	0.28±0.11	0.47±0.17	0.27±0.06	0.48±0.09	0.30±0.13	0.50±0.14
Epididymal (g)	Male	4.41±1.56	5.64±1.48	4.17±1.18	7.28±2.29	4.25±0.82	7.71±1.94	4.72±0.73	6.97±1.89
Epididymal(rTL)	Male	0.10±0.04	0.13±0.03	0.10±0.03	0.16±0.05	0.10±0.02	0.18±0.05	0.11±0.02	0.16±0.04

All data presented as mean ± standard deviation. β = significant differences in fat pad masses between male rats and female rats ($P < 0.05$).

DMSO + PW = 10 mg/kg b.w dimethylsulphoxide in neonatal phase + plain water in adulthood (n=14; 8 M, 6 F); DMSO + FW = 10 mg/kg b.w dimethylsulphoxide + 20% fructose in drinking water (n=13; 7 M, 6 F); UA + PW = 10 mg/kg b.w ursolic acid + plain water (n=14; 7 M, 7 F); UA + FW = 10 mg/kg b.w ursolic acid + 20% fructose in drinking water (n=13; 7 M, 6 F); FR + PW = 10 mg/kg b.w fructose + plain water (n=13; 6 M, 7 F); FR + FW = 10 mg/kg b.w fructose + 20% fructose in drinking water (n=14; 6 M, 8 F); UAFR + PW = 10 mg/kg b.w ursolic acid and fructose + plain water (n=14; 7 M, 7 F); UAFR + FW = 10 mg/kg b.w ursolic acid and fructose + 20% fructose in drinking water (n=12; 6 M, 6 F), rTL = relative to tibial length.

3.2.5 Hepatic storage of lipids

Figure 3.6 shows the terminal hepatic lipid content in male (A) and female (B) rats. In males, fructose administration in adulthood did not have adverse effects on hepatic lipid accumulation ($P > 0.05$). Neonatal administration of UA in combination with fructose with subsequent fructose consumption in adulthood (UAFR+FW) resulted in lower hepatic lipid content compared to the groups receiving DMSO neonatally and fructose or plain water in adulthood (DMSO+FW and DMSO+PW) ($P < 0.05$).

In female rats, a single fructose hit in adulthood resulted in a significant increase in hepatic lipid accumulation but not in the neonatal phase ($P < 0.0001$). However, a double hit of fructose (consumption both neonatally and in adulthood) resulted in even greater accumulation of hepatic lipids compared to the single hit in adulthood ($P < 0.0001$).

Neonatal UA administration alone and in combination with fructose with subsequent fructose feeding in adulthood (UA+FW and UAFR+FW) significantly prevented the accumulation of lipids as a result of a single late hit of fructose ($P < 0.05$) and a double hit of fructose ($P < 0.0001$). A comparison of the sexes revealed that the fructose effect was quite pronounced in females as they had significantly increased hepatic lipid accumulation than male rats ($P < 0.05$).

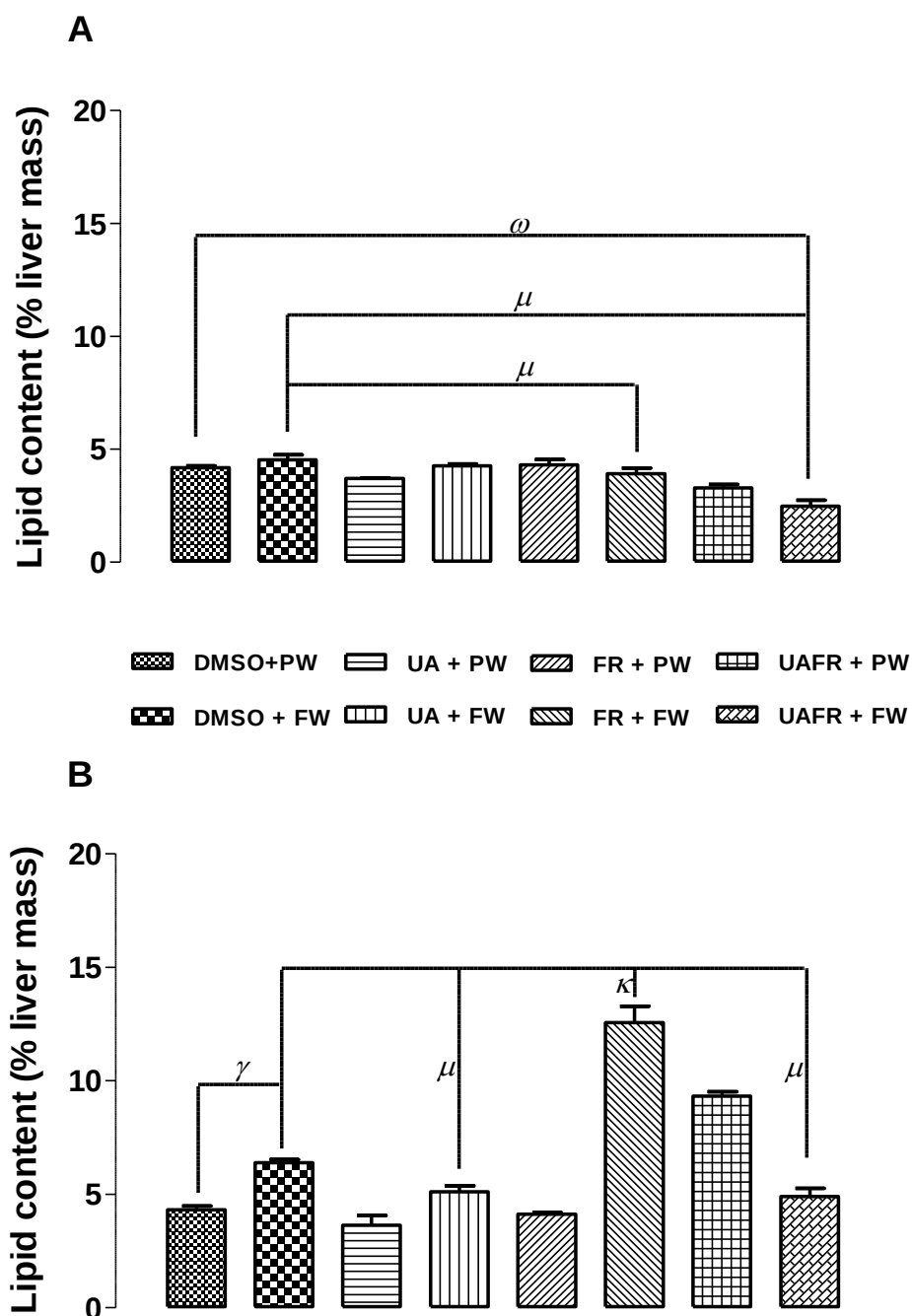


Figure 3.6: Hepatic lipid content in male (A) and female (B) rats in adulthood

All data presented as mean $\pm$ standard deviation. κ = significantly increased hepatic lipids in female rats receiving FR+FW compared to those receiving DMSO+FW ($P < 0.0001$). μ = significantly lower hepatic lipid content in rats receiving FR+FW (males; $P < 0.05$), UA+FW (females; $P < 0.05$) and UAFR+FW (males; $P < 0.0001$, females; $P < 0.001$) compared to rats receiving DMSO+FW. γ = significantly increased hepatic lipids in animals receiving DMSO+FW compared to those receiving DMSO+PW ($P < 0.05$). ω =

significantly lower hepatic lipid content in rats receiving UAFR+FW than in DMSO+PW ($P<0.05$). DMSO + PW =10 mg/kg b.w dimethylsulphoxide in neonatal phase + plain water in adulthood (n=14; 8 M, 6 F); DMSO + FW =10 mg/kg b.w dimethylsulphoxide + 20% fructose in drinking water (n=13; 7 M, 6 F); UA + PW =10 mg/kg b.w ursolic acid + plain water (n=14; 7 M, 7 F); UA + FW =10 mg/kg b.w ursolic acid + 20% fructose in drinking water (n=13; 7 M, 6 F); FR + PW =10 mg/kg b.w fructose + plain water (n=13; 6 M, 7 F); FR + FW =10 mg/kg b.w fructose + 20% fructose in drinking water (n=14; 6 M, 8 F); UAFR + PW =10 mg/kg b.w ursolic acid and fructose + plain water (n=14; 7 M, 7 F); UAFR + FW =10 mg/kg b.w ursolic acid and fructose + 20% fructose in drinking water (n=12; 6 M, 6 F).

3.3 Effect of neonatal intake of ursolic acid on the morphometry of the gastrointestinal tract and accessory organs

3.3.1 Gastrointestinal tract (GIT) organs

Table 3.7 shows the effect of fructose on the GIT organs in male and female Sprague Dawley rats across all treatment groups. Fructose and UA in the neonatal and adult phases had no adverse effects in the organ masses and lengths in male and female rats across the treatment groups ($P > 0.05$). Generally, male rats had significantly heavier and longer absolute organ masses and lengths than their corresponding females ($P < 0.05$). The females, however, had significantly heavier and longer organ masses and lengths respectively relative tibial length ($P < 0.05$).

Table 3.7: Effect of ursolic acid on the masses and lengths of GIT organs

Organ	Sex	Organ morphometry							
		DMSO + PW	DMSO + FW	UA + PW	UA + FW	FR + PW	FR + FW	UAFR + PW	UAFR + FW
Stomach (g)	Male	2.06±0.13	2.14±0.22	2.11±0.10	2.35±0.26	2.17±0.15	2.38±0.17	2.10±0.14	2.23±0.15
	Female	1.50±0.07 ^β	1.57±0.24 ^β	1.58±0.13 ^β	1.64±0.15 ^β	1.59±0.10 ^β	1.59±0.10 ^β	1.62±0.16 ^β	1.59±0.13 ^β
Stomach (rTL)	Male	0.05±0.00	0.05±0.01	0.05±0.00	0.05±0.01	0.05±0.00	0.05±0.00	0.05±0.00	0.05±0.00
	Female	0.04±0.00 ^β	0.04±0.00 ^β	0.04±0.00 ^β	0.04±0.00 ^β	0.04±0.00 ^β	0.04±0.00 ^β	0.04±0.00 ^β	0.04±0.00 ^β
SI (g)	Male	8.35±0.62	8.89±1.10	8.08±0.61	9.44±1.04	8.85±1.11	9.54±0.87	8.14±0.89	9.20±0.76
	Female	6.08±0.40 ^β	6.39±1.11 ^β	6.44±0.56 ^β	6.55±0.84 ^β	6.21±0.46 ^β	6.39±0.43 ^β	6.50±0.92 ^β	6.74±0.86 ^β
SI (rTL)	Male	0.19±0.02	0.20±0.03	0.19±0.01	0.21±0.02	0.20±0.02	0.22±0.02	0.19±0.02	0.21±0.02
	Female	0.15±0.01 ^β	0.16±0.02 ^β	0.16±0.01 ^β	0.17±0.02 ^β	0.16±0.01 ^β	0.16±0.01 ^β	0.16±0.02 ^β	0.17±0.02 ^β
SI (mm)	Male	1434±59.51	1439±93.53	1427±56.48	1470±129.60	1502±80.35	1452±103.20	1413±64.73	1548±107.20
	Female	1270±108.30 ^β	1200±76.16 ^β	1258±69.69 ^β	1258±65.01 ^β	1311±104.30 ^β	1248±36.15 ^β	1284±61.76 ^β	1284±47.90 ^β
LI (g)	Male	2.22±0.22	1.96±0.23	2.31±0.25	1.88±0.24	2.20±0.25	2.07±0.37	2.00±0.24	2.10±0.25
	Female	1.66±0.09 ^β	1.46±0.21 ^β	1.72±0.23 ^β	1.64±0.27	1.73±0.33 ^β	1.53±0.21 ^β	1.79±0.32	1.59±0.26 ^β
LI (rTL)	Male	0.05±0.01	0.04±0.01	0.05±0.01	0.04±0.01	0.05±0.00	0.05±0.01	0.05±0.01	0.05±0.01
	Female	0.15±0.01 ^β	0.16±0.02 ^β	0.16±0.01 ^β	0.17±0.02	0.16±0.01	0.16±0.01 ^β	0.16±0.02	0.17±0.02 ^β
LI (mm)	Male	260.00±28.28	248.60±13.45	265.70±11.34	245.70±28.78	260.80±19.60	250.80±30.40	247.90±9.06	259.20±20.10
	Female	217.50±4.18 ^β	215.00±13.78 ^β	235.00±9.57 ^β	222.50±15.41	234.30±24.40	207.50±15.81 ^β	230.70±20.50	216.70±13.66 ^β
Caecum (g)	Male	1.52±0.16	1.15±0.53	1.46±0.18	1.18±0.22	1.56±0.16	1.23±0.25	1.51±0.21	1.10±0.18
	Female	0.98±0.13 ^β	0.84±0.14	1.17±0.23 ^β	0.89±0.10 ^β	1.11±0.12 ^β	0.83±0.10 ^β	1.11±0.16 ^β	0.93±0.18

Caecum (rTL)	Male	0.04±0.00	0.03±0.01	0.03±0.00	0.03±0.00	0.04±0.00	0.03±0.01	0.03±0.00	0.03±0.00
	Female	0.02±0.00 ^β	0.02±0.00	0.03±0.01	0.02±0.00	0.03±0.00 ^β	0.02±0.00 ^β	0.03±0.00 ^β	0.02±0.00

All data presented as mean ± standard deviation. ^β = significant differences in organ masses and lengths between male rats and female rats ($P < 0.05$). DMSO + PW = 10 mg/kg b.w dimethylsulphoxide in neonatal phase + plain water in adulthood (n=14; 8 M, 6 F); DMSO + FW = 10 mg/kg b.w dimethylsulphoxide + 20% fructose in drinking water (n=13; 7 M, 6 F); UA + PW = 10 mg/kg b.w ursolic acid + plain water (n=14; 7 M, 7 F); UA + FW = 10 mg/kg b.w ursolic acid + 20% fructose in drinking water (n=13; 7 M, 6 F); FR + PW = 10 mg/kg b.w fructose + plain water (n=13; 6 M, 7 F); FR + FW = 10 mg/kg b.w fructose + 20% fructose in drinking water (n=14; 6 M, 8 F); UA + FR + PW = 10 mg/kg b.w ursolic acid and fructose + plain water (n=14; 7 M, 7 F); UA + FR + FW = 10 mg/kg b.w ursolic acid and fructose + 20% fructose in drinking water (n=12; 6 M, 6 F), rTL = relative to tibial length, SI = small intestines, LI = large intestines.

3.3.2 Accessory organs

Table 3.8 shows the effect of neonatal administration of UA on GIT accessory organs at the end of the study. Neither neonatal UA administration nor fructose consumption neonatally and in adulthood had any effects on the masses of accessory organs across the treatment groups in both sexes ($P > 0.05$). Males had significantly heavier organ masses than females across the treatments ($P < 0.05$).

Table 3.8: Effect of ursolic acid on masses of GIT accessory organs

Organ	Sex	Organ morphometry							
		DMSO + PW	DMSO+ FW	UA + PW	UA + FW	FR + PW	FR + FW	UAFR + PW	UAFR + FW
Liver (g)	Male	12.18±1.24	13.21±1.19	11.93±0.74	13.67±1.51	12.53±1.24	14.15±2.04	11.40±1.11	14.08±1.39
	Female	6.84±0.82 ^β	7.54±0.75 ^β	7.31±0.71 ^β	7.79±0.64 ^β	7.26±0.89 ^β	7.53±0.85 ^β	7.49±0.72 ^β	8.10±0.83 ^β
Liver (rTL)	Male	0.28±0.03	0.30±0.03	0.27±0.01	0.31±0.03	0.29±0.02	0.32±0.04	0.26±0.02	0.32±0.03
	Female	0.17±0.02 ^β	0.19±0.02 ^β	0.18±0.02 ^β	0.20±0.01 ^β	0.18±0.02 ^β	0.19±0.02 ^β	0.19±0.02 ^β	0.21±0.02 ^β
Pancreas (g)	Male	1.54±0.38	1.68±0.29	1.51±0.30	1.76±0.28	1.84±0.36	1.81±0.50	1.64±0.54	1.47±0.29
	Female	1.11±0.36 ^β	1.22±0.17 ^β	1.26±0.21	1.28±0.29 ^β	1.24±0.18 ^β	1.21±0.12 ^β	1.18±0.23	1.25±0.36
Pancreas (rTL)	Male	0.04±0.01	0.04±0.01	0.03±0.01	0.04±0.01	0.04±0.01	0.04±0.01	0.04±0.01	0.03±0.01
	Female	0.03±0.01	0.03±0.01	0.03±0.00	0.03±0.01 ^β	0.03±0.01 ^β	0.03±0.00 ^β	0.03±0.00	0.03±0.01
Kidney (g)	Male	2.76±0.16	2.80±0.30	2.69±0.22	2.87±0.35	2.79±0.23	2.86±0.35	2.69±0.32	2.89±0.26
	Female	1.62±0.14 ^β	1.85±0.50 ^β	1.71±0.14 ^β	1.71±0.15 ^β	1.66±0.14 ^β	1.70±0.22 ^β	1.77±0.13 ^β	1.74±0.16 ^β
Kidney (rTL)	Male	0.06±0.00	0.06±0.01	0.06±0.00	0.07±0.01	0.06±0.00	0.06±0.01	0.06±0.01	0.07±0.01
	Female	0.04±0.00 ^β	0.05±0.01	0.04±0.00	0.04±0.00 ^β	0.04±0.00 ^β	0.04±0.00 ^β	0.04±0.00 ^β	0.04±0.00 ^β
Heart (g)	Male	1.47±0.12	1.59±0.16	1.49±0.08	1.60±0.11	1.45±0.10	1.65±0.20	1.44±0.13	1.53±0.19
	Female	0.99±0.07 ^β	0.97±0.08 ^β	1.00±0.06 ^β	1.04±0.14 ^β	1.02±0.07 ^β	1.07±0.08 ^β	1.04±0.09 ^β	1.08±0.09 ^β
Heart (rTL)	Male	0.03±0.00	0.04±0.00	0.03±0.00	0.04±0.00	0.03±0.00	0.04±0.00	0.03±0.00	0.03±0.00
	Female	0.02±0.00	0.02±0.00	0.03±0.00	0.03±0.00	0.03±0.00	0.03±0.00	0.03±0.00	0.03±0.00

β = significant differences in organ masses between male rats and female rats ($P < 0.05$). DMSO + PW = 10 mg/kg b.w dimethylsulphoxide in neonatal phase + plain water in adulthood (n=14; 8 M, 6 F); DMSO + FW = 10 mg/kg b.w dimethylsulphoxide + 20% fructose in drinking water (n=13; 7 M, 6 F); UA + PW = 10 mg/kg b.w ursolic acid + plain water (n=14; 7 M, 7 F); UA + FW = 10 mg/kg b.w ursolic acid + 20% fructose in drinking water (n=13; 7 M, 6 F); FR + PW = 10 mg/kg b.w fructose + plain water (n=13; 6 M, 7 F); FR + FW = 10 mg/kg b.w fructose + 20% fructose in drinking water (n=14; 6 M, 8 F); UAFR + PW = 10 mg/kg b.w ursolic acid and fructose + plain water (n=14; 7 M, 7 F); UAFR + FW = 10 mg/kg b.w ursolic acid and fructose + 20% fructose in drinking water (n=12; 6 M, 6 F).

3.4 Effect of neonatal intake of ursolic acid on the general health profile

3.4.1 Surrogate markers of liver function

Table 3.9 shows the effect of UA on surrogate markers of liver function; alanine aminotransferase (ALT), alkaline phosphatase (ALP), albumin (ALB) and total bilirubin (TBIL). There were no significant differences across the treatments in both sexes ($P > 0.05$). Generally, female rats had significantly lower concentrations of ALP compared to their corresponding males ($P < 0.05$).

Table 3.9: Effect of ursolic acid on surrogate markers of liver function

Parameter	Sex	DMSO + PW	DMSO + FW	UA + PW	UA + FW	FR + PW	FR + FW	UAFR + PW	UAFR + FW
ALT (U/L)	Male	93.50±46.02	91.57±31.36	131.10±89.08	100.00±58.59	74.00±22.47	77.00±49.85	82.71±12.63	119.20±84.3
	Female	68.83±31.94	60.83±26.21	88.29±38.34	63.50±16.93	93.29±44.39	70.00±37.91	85.43±21.41	72.33±33.20
ALP (U/L)	Male	132.10±29.85	116.70±34.7	129.00±29.91	105.00±27.18	113.30±41.7	108.30±26.8	127.60±40.2	110.00±8.60
	Female	105.00±54.59	80.83±18.1 ^β	84.14±15.91 ^β	73.83±10.94 ^β	89.00±13.04	77.63±11.99 ^β	84.86±15.84 ^β	70.83±14.84 ^β
ALB (g/dL)	Male	28.00±2.00	30.14±2.85	29.57±2.37	32.00±3.16	29.17±3.76	31.33±2.88	28.43±2.44	30.33±2.34
	Female	29.33±3.01	30.83±2.14	28.71±1.89	30.00±2.76	30.71±3.64	30.75±4.40	32.00±5.51	34.83±7.94
TBIL(g/dL)	Male	3.20±1.10	3.57±0.98	4.50±3.70	5.75±4.27	24.33±37.82	4.80±3.03	nd	5.83±2.71
	Female	6.00±4.55	6.75±3.86	4.40±2.41	4.40±1.95	6.80±3.63	4.60±3.21	5.50±2.52	6.20±3.56

All data presented as mean ± standard deviation. β = significantly higher ALP concentrations in female rats than in male rats ($P < 0.05$).

DMSO + PW =10 mg/kg b.w dimethylsulphoxide in neonatal phase + plain water in adulthood (n=14; 8 M, 6 F); DMSO + FW =10 mg/kg b.w dimethylsulphoxide + 20% fructose in drinking water (n=13; 7 M, 6 F); UA + PW =10 mg/kg b.w ursolic acid + plain water (n=14; 7 M, 7 F); UA + FW =10 mg/kg b.w ursolic acid + 20% fructose in drinking water (n=13; 7 M, 6 F); FR + PW =10 mg/kg b.w fructose + plain water (n=13; 6 M, 7 F); FR + FW =10 mg/kg b.w fructose + 20% fructose in drinking water (n=14; 6 M, 8 F); UAFR + PW =10 mg/kg b.w ursolic acid and fructose + plain water (n=14; 7 M, 7 F); UAFR + FW =10 mg/kg b.w ursolic acid and fructose + 20% fructose in drinking water (n=12; 6 M, 6 F).

3.4.2 Surrogate markers of renal function in adulthood

Table 3.10 shows the effect of UA on surrogate markers of renal function: creatinine and blood urea nitrogen (BUN). In both male and female rats, a late fructose hit in adulthood resulted in significantly lower BUN in rats receiving DMSO neonatally and fructose in adulthood (DMSO+FW) compared rats receiving DMSO neonatally and plain water in adulthood (DMSO+PW) ($P < 0.05$). No significant differences were observed in creatinine concentrations across the treatment groups in both sexes ($P > 0.05$). Female rats generally had significantly lower BUN and creatinine concentrations than their corresponding males ($P < 0.05$).

Table 3.10: Effect of ursolic acid on surrogate markers for renal function in male and female rats

Parameter	Sex	DMSO + PW	DMSO + FW	UA + PW	UA + FW	FR + PW	FR + FW	UAFR + PW	UAFR + FW
Creatinine ($\mu\text{mol/l}$)	Male	47.38 $\pm$ 8.25	53.00 $\pm$ 7.35	47.86 $\pm$ 7.08	47.86 $\pm$ 4.81	44.17 $\pm$ 9.50	51.50 $\pm$ 6.78	44.00 $\pm$ 5.20	44.00 $\pm$ 5.69
	Female	44.00 $\pm$ 5.69	47.00 $\pm$ 4.65	38.86 $\pm$ 4.8 ^{β}	47.00 $\pm$ 7.35	44.00 $\pm$ 5.20	46.25 $\pm$ 6.36	40.14 $\pm$ 7.08	45.50 $\pm$ 6.78
BUN (mmol/l)	Male	7.65 $\pm$ 1.33	5.19 $\pm$ 1.12 ^{μ}	7.34 $\pm$ 1.03	5.57 $\pm$ 0.85	7.57 $\pm$ 0.98	4.75 $\pm$ 0.39	6.94 $\pm$ 1.13	5.40 $\pm$ 0.94
	Female	6.55 $\pm$ 1.48	4.17 $\pm$ 0.86 ^{μ}	5.81 $\pm$ 0.97 ^{β}	4.88 $\pm$ 0.72	6.23 $\pm$ 1.09 ^{β}	4.75 $\pm$ 0.89	6.07 $\pm$ 0.95	4.58 $\pm$ 0.83

All data presented as mean $\pm$ standard deviation. μ = significantly lower BUN concentrations in rats receiving DMSO+FW than in the rats receiving DMSO+PW ($P < 0.05$). β = significantly lower creatinine and BUN concentrations in females rats compared to male rats ($P < 0.05$). DMSO + PW = 10 mg/kg b.w dimethylsulphoxide in neonatal phase + plain water in adulthood (n=14; 8 M, 6 F); DMSO + FW = 10 mg/kg b.w dimethylsulphoxide + 20% fructose in drinking water (n=13; 7 M, 6 F); UA + PW = 10 mg/kg b.w ursolic acid + plain water (n=14; 7 M, 7 F); UA + FW = 10 mg/kg b.w ursolic acid + 20% fructose in drinking water (n=13; 7 M, 6 F); FR + PW = 10 mg/kg b.w fructose + plain water (n=13; 6 M, 7 F); FR + FW = 10 mg/kg b.w fructose + 20% fructose in drinking water (n=14; 6 M, 8 F); UAFR + PW = 10 mg/kg b.w ursolic acid and fructose + plain water (n=14; 7 M, 7 F); UAFR + FW = 10 mg/kg b.w ursolic acid and fructose + 20% fructose in drinking water (n=12; 6 M, 6 F).

3.4.3 Clinical biochemistry

Table 3.11 shows the serum concentrations of total protein (TP), calcium, globulin, phosphate and amylase. Ursolic acid administration and fructose consumption neonatally and in adulthood had no significant effects across the treatments in both sexes ($P > 0.05$). The females generally had significantly lower amylase and phosphate concentrations than their corresponding males ($P < 0.05$). There were no significant differences between males and females in TP, calcium and globulin concentrations ($P > 0.05$).

Table 3.11: Effect of ursolic acid on general health markers in male and female rats

Parameter	Sex	DMSO + PW	DMSO + FW	UA + PW	UA + FW	FR + PW	FR + FW	UAFR + PW	UAFR + FW
TP (g/L)	Male	57.25±3.50	59.29±4.19	55.86±5.31	60.86±4.78	61.00±8.88	63.83±7.81	49.29±5.82	62.17±4.07
	Female	58.50±5.47	61.17±2.23	55.43±8.08	59.83±3.43	57.14±5.46	58.88±4.79	55.14±8.36	59.33±4.13
Calcium (mmol/L)	Male	2.57±0.17	2.59±0.18	2.54±0.09	2.69±0.14	2.52±0.09	2.86±0.26	2.51±0.19	2.69±0.21
	Female	2.58±0.32	2.63±0.06	2.52±0.20	2.63±0.11	2.59±0.16	2.60±0.11	2.52±0.23	2.74±0.19
Globulin (g/L)	Male	28.88±4.36	29.14±5.87	26.14±6.15	29.14±5.64	31.67±7.69	32.17±8.80	20.86±4.63	32.00±4.60
	Female	28.67±4.89	30.50±2.51	26.71±7.34	29.83±5.60	26.29±6.42	28.13±6.71	23.14±7.45	24.50±6.98
Phosphate (mmol/L)	Male	2.05±0.11	2.16±0.23	2.20±0.30	2.04±0.26	2.15±0.23	2.08±0.18	1.92±0.18	2.12±0.18
	Female	1.77±0.30 ^β	1.93±0.24	1.79±0.18 ^β	1.89±0.26	1.83±0.15 ^β	1.70±0.23 ^β	1.74±0.20	1.81±0.12 ^β
Amylase (U/L)	Male	1497±214.3	1770±271.7	1563±373.1	1964±224.7	1458±165.3	2020±299.2	1590±182.6	1900±248.5
	Female	891.5±230.4 ^β	1526±361.1	859.7±190.2 ^β	1186±229.8 ^β	900.1±120.9 ^β	1446±446.3 ^β	959.6±272 ^β	1597±174.6 ^β

All data presented as mean ± standard deviation. ^β = significantly lower amylase and phosphate concentrations in female rats compared to male rats ($P < 0.05$). DMSO + PW =10 mg/kg b.w dimethylsulphoxide in neonatal phase + plain water in adulthood (n=14; 8 M, 6 F); DMSO + FW =10 mg/kg b.w dimethylsulphoxide + 20% fructose in drinking water (n=13; 7 M, 6 F); UA + PW =10 mg/kg b.w ursolic acid + plain water (n=14; 7 M, 7 F); UA + FW =10 mg/kg b.w ursolic acid + 20% fructose in drinking water (n=13; 7 M, 6 F); FR + PW =10 mg/kg b.w fructose + plain water (n=13; 6 M, 7 F); FR + FW =10 mg/kg b.w fructose + 20% fructose in drinking water (n=14; 6 M, 8 F); UAFR + PW =10 mg/kg b.w ursolic acid and fructose + plain water (n=14; 7 M, 7 F); UAFR + FW =10 mg/kg b.w ursolic acid and fructose + 20% fructose in drinking water (n=12; 6 M, 6 F).

CHAPTER 4 : DISCUSSION

To our knowledge, this is the first study to investigate the effects of neonatal administration of UA on the susceptibility of adult rats to diet-induced metabolic dysfunction. The effects of fructose were shown to be dependent on the time of intervention. An early fructose hit (fructose administered in the neonatal phase) without a secondary fructose hit later in life did not seem to have any discernible effects on metabolic dysfunction. A late fructose hit (fructose consumption in adulthood) resulted in differences in body mass, triglyceride concentration and food and fluid intake. Administration of fructose neonatally with the subsequent consumption of fructose in adulthood (double fructose hit) increased hepatic lipid accumulation. Fructose consumption neonatally and/or in adulthood did not affect linear growth, adiposity, glucose handling, organ morphometry and general health markers. Ursolic acid administration was shown to be hepatoprotective as it prevented hepatic lipid accumulation. Sex differences in response to the interventions were also observed.

4.1 Growth performance

4.1.1 Body mass

In the present study, a late fructose hit in adulthood resulted in increases in body mass although an early fructose hit did not have any effects in males. In females, fructose consumption both neonatally and in adulthood did not have any effects on body mass (Figure 3.1). Mamikutty et al. (2014) (20% fructose in drinking water for 8 weeks in male Wistar rats) and Barros et al. (2007) (10% and 20% fructose in drinking water for 2 months in hamsters) also found fructose consumption in adulthood to promote increases in body mass. The increases observed may have been due to fructose altering satiety signalling pathways and triggering minimal changes in blood glucose concentration thereby promoting overconsumption (Teff et al., 2004, Allen et al., 2005, Scarpace and Zhang, 2009, Rizkalla, 2010).

Administration of UA (alone) to neonates resulted in increases in body mass in female rats in the present study (Figure 3.1). In studies where UA has been used to ameliorate the effects of calorie-dense diets, it has been found to lower body mass. Rao et al. (2011) found UA (10 mg/kg b.w) to lower the body mass of 6 week old Swiss mice fed a high fat diet for 15 weeks. Li et al. (2014) and Sundaresan et al. (2012) also found UA to reduce body mass in mice consuming high fat diets. The effects of UA (alone) administered in the

neonatal phase with subsequent fructose consumption in adulthood in increasing body mass observed in this study warrant further investigation.

In the present study, fructose consumption resulted in higher body mass gains in male rats compared to their female counterparts. This could have been due to the anabolic effects of testosterone (Gibney et al., 2003). Body mass is used as an indicator of growth, however, it is affected acutely by factors such as hydration and postprandial status (MacCracken and Stebbings, 2012, Mahan et al., 2012, Stookey, 2016). To minimize the potential effects of gut fill on body mass, in the present study the rats were fasted overnight so as to facilitate emptying of particulate matter from the GIT. To investigate the long-term growth, hormone-regulated growth indicators such as linear growth were also used to assess growth.

4.1.2 Linear growth

Indicators of bone growth are increases in length, width and mass which all respond to growth hormone in a dose-dependent manner (Albright and Reifenshtein Jr, 1949, Venken et al., 2008, Orwoll et al., 2009). Consequently, bone length (particularly tibial length) is often used as an indicator of growth (Panchal et al., 2013, Mamikutty et al., 2014). In this study, consumption of 20% fructose had no adverse effects on femur and tibia length, mass and Seedox indices across treatments in both sexes. This is in line with reports that fructose consumption does not directly cause adverse effects on bone growth (Tsanzi et al., 2008).

As the consumption of 20% fructose had no adverse effects on bone, neonatal administration of ursolic acid had no effects on bone growth later in life. Ursolic acid administration in adulthood, however, has been reported to have protective effects on bone *in vivo*; in streptozocin-induced diabetic 6-week old rats, UA ameliorated the deleterious bone effects of diabetes by suppressing osteoclast differentiation (Yu et al., 2015). *In vitro*, Lee et al. (2008) and Tan et al. (2015) also found UA to stimulate osteoblast differentiation and suppress osteoclast genesis in mouse cells using cell culture techniques.

Male rats had larger masses, lengths and Seedox indices of femora and tibiae compared to their corresponding females (Table 3.1). This may have been due to the fact that in humans and rodents, males naturally have larger cortical bone sizes than females (Seeman, 1998, Orwoll et al., 2009). In humans, the larger bones are due to the process of periosteal apposition which is inhibited in females after epiphyseal fusion at the onset of puberty

(Orwoll et al., 2009). Epiphyseal closure does not occur in female rodents, however, the secretion of testosterone in males at puberty contributes to enhanced bone growth by inducing osteoblast proliferation (Kung, 2003, Vanderschueren et al., 2004).

4.2 The development of metabolic dysfunction

4.2.1 Circulating metabolites

Neonatal administration of fructose and UA did not have any effects on fasting blood glucose and triglyceride concentrations (P35 assays) in the early stages of this study following the ‘first hit’ (Table 3.2). Most studies only assay for these metabolites after the second intervention as early programming effects may be observed after a latent period (which can last up to years) or after a second intervention (Rhind et al., 2001, Barouki et al., 2012, Heindel et al., 2015). Thus, this could explain why in the current study, the “first hit” did not manifest in overtly discernible changes two weeks after stopping the intervention. In the absence of a second intervention, subtle changes may be impossible to detect without employing molecular techniques (Heindel et al., 2015). Unfortunately I did not do any molecular studies on the rats and this may be the subject for future studies.

In our study, the “second hit” in adulthood did not affect the circulating concentrations of triglycerides in both sexes. However, a single late fructose hit in adulthood resulted in increased triglyceride concentrations in male rats (Table 3.3). Baena et al. (2015) showed that consumption of 10% fructose for 8 weeks in female weanling rats increased the concentration of circulating triglycerides. This suggests that the age of rats and timing of fructose consumption are critical when assessing triglyceride concentration in female rats. Fructose feeding did not have any adverse effects on total cholesterol concentration in this study in both males and females although Mamikutty et al. (2014) observed increases in cholesterol concentrations using a similar model in male Wistar rats. Stanhope et al. (2011) also found similar results using fructose and high fructose corn syrup in humans. The increases in cholesterol were attributed to fructose-induced elevation of low density lipoprotein (LDL) (Seneff et al., 2011). Due to technical limitations, I was unable to assay the types of cholesterol i.e. LDL and HDL.

Neonatal UA administration (alone or in combination with fructose) did not have apparent effects in triglyceride concentrations in both sexes. In adult mice, UA treatment for 8 weeks at 50 mg/kg and 200 mg/kg was found to reduce triglyceride concentrations in a

dose-dependent manner after consumption of high fat diets (Li et al., 2014, Jia et al., 2015). Using a model that induces increases in triglyceride concentration, the dosages used to lower triglyceride concentration may need to be investigated further. Administration of UA neonatally (alone and in combination with fructose) did not have any effects on cholesterol concentration. In male Sprague Dawley rats, UA (50 mg/kg) was found to have no effect on cholesterol concentrations when used alone but had synergistic cholesterol lowering effects with artesunate after 4 weeks of supplementation (Yuliang et al., 2015). In rabbits, UA (25 mg/kg) had cholesterol lowering effects both alone and in combination with artesunate after 8 weeks of feeding (Wang et al., 2013). Therefore, the effect of UA on cholesterol seems to be species, time and dose dependent.

Although no differences were noted between the sexes in triglyceride concentration, female rats had higher concentrations of cholesterol than male rats (Table 3.3). Generally, oestrogen increases HDL- cholesterol while decreasing triglycerides and may explain the sex-differences observed in cholesterol concentrations in the present study although HDL was not assayed for (Fowler, 2006, Kumar et al., 2010). Studies by Korićanac et al. (2013) also suggest that the effects of fructose-induced metabolic perturbations may depend on timing and duration of fructose consumption in female rats.

4.2.2 Tolerance to an oral glucose load

Prolonged fructose consumption is generally associated with impaired glucose handling in rodents. Impaired glucose handling can be measured using an oral glucose tolerance test and is characterised by elevated basal fasting glucose concentrations and insufficient or no insulin effects therefore delaying glucose uptake and prolonging the hyperglycaemic state following a glucose load (American Diabetes Association, 2013, Nussey and Whitehead, 2013). Fructose consumption for 8 weeks had no adverse effects on glucose handling in both male and female rats (Figure 3.4). The basal fasting glucose concentrations of rats on a fructose diet in adulthood were comparable with those drinking plain water (Figure 3.4). Similarly, the time taken to reach peak glucose concentrations and the time taken to return to normal glucose concentrations following an oral glucose load was not significantly different. Impaired glucose tolerance has been shown in 3-week old rats consuming 20% fructose in drinking water for 6 weeks and 25% fructose for an additional 6-weeks (Dupas et al., 2015). Differences in age of rats, genetic predisposition and the duration of fructose

consumption and well as the type of fructose consumed may account for the differences in glucose handling (Barbosa et al., 2007, de Moura et al., 2009).

Ursolic acid has been shown to improve tolerance in high caloric diet induced glucose intolerance. Jayaprakasam et al. (2006) and Jia et al. (2015) showed that UA administration (500 mg/kg b.w and 50/200 mg/kg b.w respectively) for 8 weeks in mice fed a high fat diet improved glucose handling. *In vitro* studies (Jung et al., 2007) using Chinese hamster ovary cells expressing human insulin receptor and *in vivo* studies using hyperglycaemic rats have shown UA to have insulomimetic, insulin sensitising and secretagogue properties (Castro et al., 2015) which may explain UA's direct hypoglycaemic effects. As fructose did not alter glucose tolerance, the potential protective role of UA against altered glucose handling was not apparent and may need to be further investigated.

4.2.3 Food and fluid intake

Food and fluid intake are affected by the satiety hormones insulin, ghrelin and leptin amongst others (Woods et al., 2006, Ahima et al., 2016). Fructose does not stimulate insulin and leptin secretion (Rodin, 1991, Lindqvist et al., 2008) and it inhibits ghrelin secretion affecting satiety control (Castro et al., 2011). Being highly palatable, fructose consumption facilitates central nervous system alterations in the reward system which encourages overfeeding (Spring et al., 2008, Davis et al., 2010). In the present study, a single fructose hit in adulthood resulted in decreased food intake (both sexes) and increased fluid intake (female rats). However, no effects were noted in rats that received a double fructose hit in comparison to those that received a single late hit in both sexes. (Tables 3.4 and 3.5). These results may account for the change observed in body mass changes between groups of rats of the same sex which received either a late fructose hit or a double fructose hit (section 4.1.1). Neonatal administration of fructose with subsequent fructose consumption in adulthood did not predispose the rats to consume more fluid than the corresponding controls receiving water. Neonatal UA acid administration, alone and in combination with fructose did not have any effects on food and fluid intake although triterpenoids have been found to suppress sweet taste thereby causing a reduction in food and fluid intake (Nazaruk and Borzym-Kluczyk, 2014).

Interestingly, female rats consumed more fructose in adulthood than their male counterparts (Table 3.3). Studies by Curtis et al. (2005) in Sprague Dawley rats suggest that female rats have a higher preference for sweet solutions than males possibly due to the

effects of oestrogen which increases the threshold for responding to sweet solutions. A study by Ackroff and Sclafani (2004) revealed that males are more sensitive to sweet taste than females following fructose consumption in rats. These two factors may have contributed to the higher fluid consumption in females compared to males.

4.2.4 Adiposity

Rats consuming fructose (both sexes) showed increased adiposity, however, the differences were not significant in rats receiving a late fructose hit as well as a double fructose hit although Mamikutty et al. (2014) observed increases in visceral fat pad depositions using a similar experimental model in male Wistar rats. Genetic differences between the two strains of rats may have played a role in the differences observed as Van Liefde et al. (1993) demonstrated that there are differences in β -adrenoceptor subtypes between Sprague Dawley and Wistar rats which affect lipolysis.

As fructose intake did not have any adverse effects on adiposity, the neonatal intake of ursolic acid did not seem to have any effects on visceral and epididymal fat deposition in the present study. No significant differences were observed between the sexes although fructose-induced adiposity is thought to be sex-specific (Korićanac et al., 2013). It is notable though there are conflicting reports on the role of oestrogen, age of rats and duration of fructose consumption in promoting adiposity which may need to be investigated further (Korićanac et al., 2013, Guerra et al., 2014).

4.2.5 Hepatic storage of lipids

The liver is a homeostatic organ largely responsible for carbohydrate, fat and protein metabolism, bile secretion and detoxification (Singh, 2008, Preston and Wilson, 2012). It plays a central role in fructose metabolism as the bulk of ingested fructose is metabolised there (Agarwal and Agarwal, 2007, Chatterjea and Shinde, 2012). In the present study, fructose intake in adulthood promoted hepatic lipid accumulation in female rats but not in males (Figure 3.6). This fructose effect was even more pronounced in animals that received fructose neonatally and in adulthood (first and second fructose hits) lending credence to the “two hit” hypothesis (section 1.2). As described in section 1.3.3.1, fructose is found in minimal concentrations in circulation due to its rapid uptake on first pass in the liver. As the bulk of fructose metabolism occurs in the liver, it is vulnerable to the deleterious effects of fructose as was shown the present study. Findings from the study

suggest that fructose consumption in adulthood has more pronounced effects than consumption in the neonatal phase. However, consumption of fructose neonatally and in adulthood (double hit) resulted in even greater lipid accumulation which suggests that although no differences were observed in the neonatal phase, the first fructose hit is instrumental in hepatic lipid accumulation and thus the effects of timing of fructose consumption on hepatic lipid accumulation may need further investigation. Baena et al. (2015) showed that 10% fructose consumption for 8 weeks in female rats led to increased hepatic lipid accumulation through decreased autophagy. Inhibition of autophagy in the liver increases lipid accumulation in hepatocytes (Singh et al., 2009, Amir and Czaja, 2011) which may have contributed to the enhanced hepatic lipid accumulation observed in this study. Additionally, the *de novo* lipogenesis described in section 1.3.3.1 also promotes storage of lipids in the liver. These effects of fructose may lead to the development and progression of steatosis to NAFLD (Zelber-Sagi et al., 2007, Ouyang et al., 2008, Abdelmalek et al., 2010, Sánchez-Lozada et al., 2010). In both children and adults, NAFLD is a major global health concern which has been implicated as being a precursor and mediator of the metabolic syndrome (Gutteridge, 1997, Ekeleme et al., 2005, Allabi et al., 2011).

A major and exciting finding from this study was that neonatal intake of UA in combination with fructose with subsequent fructose consumption in adulthood resulted in decreased hepatic lipid accumulation in both sexes in our study. This is may be because UA is a peroxisome proliferator-activated receptor alpha (PPAR α) agonist (similar to the commercially available lipid lowering agent fenofibrate) and induces hepatic autophagy (lipophagy) making it instrumental in hepatic lipid metabolism (Mandard et al., 2004, Jia et al., 2011). The PPAR α regulates genes responsible for fatty acid transport, utilisation and hepatic, mitochondrial and peroxisomal β fatty acid oxidation (Aoyama et al., 1998, Reddy and Rao, 2006, Souza-Mello, 2015). By inducing hepatic autophagy, UA has been shown to facilitate the breakdown of lipid droplets resulting in a reduction in lipid concentrations (Singh et al., 2009, Jia et al., 2015).

In the present study, female rats had enhanced hepatic lipid accumulation compared to their male counterparts. Vilà et al. (2011) showed that female rats were more prone to fructose-induced metabolic alterations including fatty livers after consuming 10% fructose for two-weeks due to altered leptin regulation. Due to technical challenges, leptin was not assayed for in the present study. The effects of androgens and oestrogens on liver

metabolism are age, species and strain specific and could have also contributed to the differences observed in the present study although further investigations are needed (Korićanac et al., 2013, Shen and Shi, 2015).

4.3 Morphometry of the GIT and accessory organs

4.3.1 Gastrointestinal tract organs

As the GIT is responsible for the digestion and absorption of nutrients, a dysfunctional GIT may be the starting point for many chronic diseases which may adversely affect the quality of life (Parkman et al., 2010, Guinane and Cotter, 2013). Consequently, GIT organ morphometry is used as an indicator of gut health (Luna, 1996, Haba et al., 2000). In this study, fructose consumption (in neonates and adult rats) had no effects on GIT components and therefore did not adversely affect gut health. Administration of ursolic acid during the neonatal phase did not have any effects on the GIT organ macro-morphometry. Males generally had larger GIT organ masses compared to their corresponding females this may have been due to the growth promoting effects of testosterone and growth hormone described above (section 4.1).

4.3.2 Accessory organs

Organ weight is a sensitive and useful tool in assessing the effect of various chemical compounds on specific body organs (Black, 2002, Bucci, 2002, Wooley, 2003). In toxicological studies, differences in organ weights between treatment groups may indicate early effects of a compound even in the absence of morphological changes (Bailey et al., 2004, Sellers et al., 2007). Elevated heart and kidney weights are normally indicative of myocardial and tubular hypertrophy respectively (Greaves, 2011). In our study, males generally had heavier organ masses probably due to the effects of testosterone as described in section 4.1.1. Fructose and UA did not adversely affect the masses of accessory organs suggesting that the general well-being of the organs was probably not affected. This was further supported by the rats' general health profiles (section 4.4) which showed that fructose consumption did not adversely alter surrogate markers of health.

4.4 General health profile

4.4.1 Surrogate markers of liver function

A liver biopsy is the ‘gold standard’ in assessing liver injury, inflammation and fibrosis (Bravo et al., 2001, Choudhury and Sanyal, 2004, Fallatah, 2014). However, it has a number of limitations and so biomarkers such as serum concentrations of albumin, TP, ALP, AST, ALT, and TBIL are used to assess liver injury (Wallach, 2007). Serum albumin concentration reflects nutritional status, the liver’s synthetic ability, and hydration making it an indicator of hepatobiliary irregularities (Lee, 2009, Brooks et al., 2015). Total protein is an estimation of the sum of albumin and globulin (Brooks et al., 2015). Although ALP is a measure of the integrity of the hepatobiliary system, it is not specific as serum concentrations are also elevated by osteoblasts, pregnancy and childhood growth (Schoch and Whiteman, 2007, Khurana, 2009, Lee, 2009, McCarthy and Frassica, 2014). Serum concentrations of ALT are used to assess active hepatic injury and the potential hepatic toxicity of various medicines (Green and Flamm, 2002, Brooks et al., 2015). Total bilirubin includes both direct and indirect bilirubin and is an indicator of hepatocellular injury (Schoch and Whiteman, 2007, Brooks et al., 2015). Fructose consumption in adulthood did not affect the serum concentrations of the surrogate markers of liver function and therefore it probably did not cause injury in this study.

Li et al. (2014) showed that UA (0.125%, 0.25% and 0.5%) alleviated liver injury and lowered ALT and AST concentrations in rats after six weeks of high fat feeding in a dose-dependent manner. As 20% fructose intake did not have adverse effects on surrogate markers of liver function in the current study, it is difficult to draw conclusions on the potential protective role of UA on the liver when subjected to fructose-induced metabolic stress may need to be further investigated.

4.4.2 Surrogate markers of kidney function

Creatinine and urea are nitrogen containing end products of metabolism; creatinine is a result of muscle creatine catabolism while urea is a result of blood and tissue protein turnover (Hosten, 1990, Wallach, 2007). The kidneys filter almost all the endogenous creatinine from blood and it is excreted in urine (Malarkey and McMorro, 2011). Therefore, creatinine and BUN are used to assess renal insufficiency (Traynor et al., 2006, Wallach, 2007, Lascano and Poggio, 2010). Although serum concentrations of creatinine

and BUN are useful indicators of renal function, they only become abnormal after 50% of renal function is lost (Wallach, 2007). In the present study, fructose intake had no adverse effects on plasma BUN and creatinine concentrations and thus fructose did not elicit any observable adverse effects on the kidney. At high concentrations (60%), chronic fructose ingestion has been shown to induce renal hypertrophy, glomerulosclerosis and tubulointerstitial disease (Kizhner and Werman, 2002, Nakayama et al., 2010). It has also been shown to exacerbate established kidney disease (Gersch et al., 2007). Uric acid accumulation as a result of fructose metabolism is a putative mechanism for the adverse effects in the kidneys (Sánchez-Lozada et al., 2007). This suggests that the effects of fructose on the kidney are affected by the concentration of fructose as well as the duration of fructose consumption.

In streptozocin-induced diabetic rats, UA has been shown to confer renal protection following 16 weeks of UA supplementation (Ling et al., 2013). In the present study, fructose did not seem to adversely affect the kidney thus the potential protective role of neonatal administration of UA may need to be ascertained in future studies where renal impairment is observed. The female rats had lower creatinine and BUN concentrations probably due to decreased muscle mass compared to male rats (Pagana and Pagana, 2013).

4.4.3 Clinical biochemistry

Total protein (TP) in circulation is a health marker that reflects nutritional status and is used in the diagnosis of oedema, kidney and liver disease (Singh et al., 2011, Mahan et al., 2012). Fructose consumption did not have any adverse effects of total protein in both sexes and no differences were observed between the sexes.

Calcium plays important roles in a number of physiological activities; blood clotting, nerve transmission, muscle contraction and maintenance of bone and teeth health (Fischbach and Dunning, 2009, Langley-Evans, 2015). Fructose intake in adulthood did not affect calcium concentration in the present study.

In the body, phosphorus is present as phosphate and is essential for bone mineralisation, generation of ATP, maintenance of cell structure and acid-base homeostasis (Penido and Alon, 2012). Fructose intake did not adversely affect circulating phosphate concentrations. Female rats had lower concentrations of phosphate compared to males and this may have been due to the fact that high fructose consumption causes a decrease in serum phosphate

concentration as most of the phosphate is lost in urine (Berdanier et al., 2007). The female rats consumed more fructose-rich fluid (Table 3.4) than their male counterparts which could have contributed to the differences observed.

Amylase is a hydrolytic enzyme which is instrumental in carbohydrate metabolism is found mainly in saliva and pancreatic juice (Stoker, 2012, Patton and Thibodeau, 2014). Serum amylase concentration is an indicator of acute pancreatitis or inflammation of the salivary glands (Hensleigh and Holaway, 1988, Matull et al., 2006, Brooks et al., 2015). In the present study, fructose consumption had no adverse effects on the serum amylase concentrations. Consequently, it was not possible to further explore the effects neonatal administration of UA on fructose associated effects on blood amylase concentrations. Ursolic acid, however, has been shown, to have inhibitory effects on α -amylase activity and is currently being explored as for use as alternative therapy in diabetes management (Ali et al., 2006, Kumar et al., 2013, Nazaruk and Borzym-Kluczyk, 2014).

Globulins are serum proteins that include carrier proteins, antibodies, complement and enzymes (Bhagavan, 2002, Thrall et al., 2012). Malnutrition, congenital immune deficiency and nephrotic syndrome lower the levels of globulins (Busher, 1990, Neto et al., 2009). Fructose consumption had no effect on globulin concentration. This was further supported by the total protein concentrations which were within normal concentrations.

Thus in general, in this study, 20% fructose intake had no adverse effects on the clinical biochemistry profile. Neonatal administration of UA also had no apparent effects on the above parameters.

CHAPTER 5 : CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

With most studies investigating UA as a treatment option, this study sought to determine the potential prophylactic role of UA by targeting a critical developmental period regarding metabolic dysfunction. The first nutritional intervention was aimed at neonatal programming whilst the second was to induce metabolic dysfunction as a “second hit” and to investigate whether the early interventions with UA would have an attenuating effect. Intake of fructose both neonatally and in adulthood resulted in increased hepatic lipid accumulation which was pronounced in female rats. In males, a late fructose hit in adulthood was shown to increase body mass and circulating triglyceride concentrations. Food (both sexes) and fluid (females) intake were also altered due to a late fructose hit. The interventions were well tolerated by the rats as no adverse health effects with regards to linear growth, organ morphometry, glucose handling and surrogate health markers were observed.

In the present study, UA was shown to be hepatoprotective evidenced by the decreased lipid accumulation in the rats receiving UA (alone and in combination with fructose) neonatally in both sexes. While there has been progress in awareness programs on the potential dangers of added sugars such as fructose as well as the introduction of “sugar tax”, decreasing sugar consumption will be no easy feat. With fructose-induced metabolic problems such as obesity and NAFLD affecting a large number of the population, including children, findings from this study show that phytochemicals like UA may play a crucial role in strategic preventative nutrition.

5.2 Limitations and recommendations

For future studies, manipulations of the maternal diet in addition to the dietary interventions employed in this study are recommended. As there are a number of physiologically sensitive periods from conception to the immediate postnatal phase, maternal dietary interventions may provide broader insights regarding the effects of fructose on the parameters tested in the current study. Broader studies to assay for insulin and leptin resistance, body mass index and blood pressure may also be instrumental as these are also markers of MS. Due to some technical challenges, LDL, HDL, VLDL and leptin concentrations could not be determined but analysis is recommended for future studies.

The use of molecular techniques could aid in identifying subtle changes that may not be exhibited in the phenotype. Additionally, these techniques can also aid in establishing the mechanisms involved. Although the current study was an experimental study, no molecular techniques were employed. However, their use is recommended for future studies so as to identify any potential alterations not apparent in the phenotype. Using an identical experimental model, it is recommended that 20% fructose be administered in feed. This is because some studies suggest that fructose in feed elicits a more aggressive form of MS than in drinking water. This may help in establishing a working model for future studies.

As UA was administered orally, bioavailability assays may also be done in the future. Poor absorption, intestinal microbiota, pancreatic secretions and low solubility lower the bioavailability of ingested phytonutrients. Responses to drugs are at times dose-specific necessitating the use of bioavailability assays.

Further studies are necessary to determine the optimum dose and duration for maximal UA action against metabolic dysfunction in the neonatal phase. Although consumption of 20% fructose resulted in metabolic alterations particularly in the liver, it is recommended to continue feeding until 10-12 weeks as this may induce MS-associated alterations not observed in the present study. In this study, hepatic lipid accumulation was observed which is believed to be the precursor of metabolic syndrome therefore feeding for a longer period of time may result in the occurrence of other metabolic anomalies associated with metabolic syndrome.

The present study focused on neonatal programming in rats, therefore, caution needs to be exercised when using this data as a predictor of potential human reactions. Studies in higher order animals such as primates are recommended to account for molecular, cellular, physiologic and immunologic differences between species. As the effects of the treatments were found to be time-dependent and at times sex-specific, it is recommended to do studies involving males and females of different ages where possible.

Hepatic lipid accumulation (as observed in the present study) is believed to be the precursor of metabolic dysfunction and ultimately, metabolic syndrome. Major drivers for hepatic lipid accumulation include; *de novo* lipogenesis, decreased lipophagy and β -oxidation of lipids (Shils and Shike, 2006, Singh et al., 2009) . As the causes of fatty liver

and MS multi-factorial, a multi-pronged approach involving a number pathways needs to be explored in future studies. Potential pathways include hepatic autophagy, fatty acid synthesis, β -oxidation of lipids and biochemical pathways that promote *de novo* lipogenesis such as glycolysis and the citric acid cycle.

CHAPTER 6 : REFERENCES

- ABDELMALEK, M. F., SUZUKI, A., GUY, C., UNALP-ARIDA, A., COLVIN, R., JOHNSON, R. J. & DIEHL, A. M. (2010). Increased fructose consumption is associated with fibrosis severity in patients with nonalcoholic fatty liver disease. *Hepatology*, 51(6):1961-1971.
- ABOU, Z. M. & MANI, A. (2016). Metabolic syndrome: genetic insights into disease pathogenesis. *Current Opinion in Lipidology*, 27(2):162-171.
- ACKROFF, K. & SCLAFANI, A. (2004). Fructose-conditioned flavor preferences in male and female rats: effects of sweet taste and sugar concentration. *Appetite*, 42(3):287-297.
- AEBERLI, I., HOCHULI, M., GERBER, P. A., SZE, L., MURER, S. B., TAPPY, L., SPINAS, G. A. & BERNEIS, K. (2013). Moderate amounts of fructose consumption impair insulin sensitivity in healthy young men: a randomized controlled trial. *Diabetes Care*, 36(1):150-156.
- AGARWAL, G. & AGARWAL, O. (2007). *Text book of Biochemistry*, Meerut, India, Krishna Prakashan Media, pp135-136.
- AHIMA, R. S., LEWIS, K. H., BASU, H., ASCHNER, P., AGYEMANG, C., BOATEMAA, S., AGYEMANG, F., AIKINS, A., NIKOLOSKI, Z., WILLIAMS, G., WALI, M. & RAM, V. S. (2016). *Metabolic Syndrome: A Comprehensive Textbook*, Cham, Springer International Publishing, pp3-100.
- AIKEN, C. E. & OZANNE, S. E. (2013a). Sex differences in developmental programming models. *Reproduction*, 145(1):R1-R13.
- AIKEN, C. E. & OZANNE, S. E. (2013b). Transgenerational developmental programming. *Human Reproduction Update*, 20(1):63-75.
- AKABERI, M. & HOSSEINZADEH, H. (2016). Grapes (*Vitis vinifera*) as a Potential Candidate for the Therapy of the Metabolic Syndrome. *Phytotherapy Research*, doi:10.1002/ptr.5570.
- AKHTAR, T. & SHEIKH, N. (2013). An overview of thioacetamide-induced hepatotoxicity. *Toxin Reviews*, 32(3):43-46.
- ALBERTI, K., ECKEL, R. H., GRUNDY, S. M., ZIMMET, P. Z., CLEEMAN, J. I., DONATO, K. A., FRUCHART, J.-C., JAMES, W. P. T., LORIA, C. M. & SMITH, S. C. (2009). Harmonizing the Metabolic Syndrome A Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and

- International Association for the Study of Obesity. *Circulation*, 120(16):1640-1645.
- ALBERTI, K. G. M. M. & ZIMMET, P. F. (1998). Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. *Diabetic Medicine*, 15(7):539-553.
- ALBRIGHT, F. & REIFENSTEIN JR, E. (1949). The parathyroid glands and metabolic bone disease. *Journal of Bone and Joint Surgery* 31(4):881-882.
- ALI, H., HOUGHTON, P. & SOUMYANATH, A. (2006). α -Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to *Phyllanthus amarus*. *Journal of Ethnopharmacology*, 107(3):449-455.
- ALLABI, A. C., BUSIA, K., EKANMIAN, V. & BAKIONO, F. (2011). The use of medicinal plants in self-care in the Agonlin region of Benin. *Journal of ethnopharmacology*, 133(1):234-243.
- ALLEN, L., PRENTICE, A. & CABALLERO, B. (2005). *Encyclopedia of Human Nutrition, Four-Volume Set*, Elsevier Science, pp355.
- ALMEIDA, P. I., MENDES, A. A., BALOG, A., VULCANO, L. C., BALLARIN, A. W., ALMEIDA, I., TAKAHASHI, S., KOMIYAMA, C., SILVA, M. & CARDOSO, K. (2008). Study on the bone mineral density of broiler suffering femoral joint degenerative lesions. *Revista Brasileira de Ciência Avícola*, 10(2):103-108.
- ALQAHTANI, A., HAMID, K., KAM, A., WONG, K., ABDELHAK, Z., RAZMOVSKI-NAUMOVSKI, V., CHAN, K., LI, K., GROUNDWATER, P. & LI, G. (2013). The pentacyclic triterpenoids in herbal medicines and their pharmacological activities in diabetes and diabetic complications. *Current Medicinal Chemistry*, 20(7):908-931.
- ALZAMENDI, A., GIOVAMBATTISTA, A., RASCHIA, A., MADRID, V., GAILLARD, R. C., REBOLLEDO, O., GAGLIARDINO, J. J. & SPINEDI, E. (2009). Fructose-rich diet-induced abdominal adipose tissue endocrine dysfunction in normal male rats. *Endocrine*, 35(2):227-232.
- AMERICAN DIABETES ASSOCIATION, A. (2013). Diagnosis and classification of diabetes mellitus. *Diabetes care*, 36(1):S67-S74.
- AMIR, M. & CZAJA, M. J. (2011). Autophagy in nonalcoholic steatohepatitis. *Expert Review of Gastroenterology & Hepatology*, 5(2):159-166.
- ANGULO, P. (2002). Nonalcoholic fatty liver disease. *New England Journal of Medicine*, 346(16):1221-1231.

- AOYAMA, T., PETERS, J. M., IRITANI, N., NAKAJIMA, T., FURIHATA, K., HASHIMOTO, T. & GONZALEZ, F. J. (1998). Altered constitutive expression of fatty acid-metabolizing enzymes in mice lacking the peroxisome proliferator-activated receptor α (PPAR α). *Journal of Biological Chemistry*, 273(10):5678-5684.
- ASHWORTH, C., MCEVOY, T., ROOKE, J. & ROBINSON, J. (2005). Nutritional programming of physiological systems throughout development. *Trends in Developmental Biology*, 117-129.
- ASHWORTH, C. J., TOMA, L. M. & HUNTER, M. G. (2009). Nutritional effects on oocyte and embryo development in mammals: implications for reproductive efficiency and environmental sustainability. *Philosophical transactions of the royal society B: biological sciences*, 364(1534):3351-3361.
- AZUIKE, E., EMELUMADU, O., ADINMA, E., IFEADIKE, C., EBENEKE, U. & ADOGU, P. (2013). Nutrition Transition in Developing Countries: A Review. *Afrimedic Journal*, 2(2):1-5.
- BAENA, M., SANGÜESA, G., HUTTER, N., SÁNCHEZ, R. M., ROGLANS, N., LAGUNA, J. C. & ALEGRET, M. (2015). Fructose supplementation impairs rat liver autophagy through mTORC activation without inducing endoplasmic reticulum stress. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, 1851(2):107-116.
- BAILEY, S. A., ZIDELL, R. H. & PERRY, R. W. (2004). Relationships between organ weight and body/brain weight in the rat: what is the best analytical endpoint? *Toxicologic Pathology*, 32(4):448-466.
- BAIN, L. E., AWAH, P. K., GERALDINE, N., KINDONG, N. P., SIGA, Y., BERNARD, N. & TANJEKO, A. T. (2014). Malnutrition in Sub-Saharan Africa: burden, causes and prospects. *Pan African Medical Journal*, 15(1):120.
- BARBOSA, C., ALBUQUERQUE, E., FARIA, E., OLIVEIRA, H. & CASTILHO, L. (2007). Opposite lipemic response of Wistar rats and C57BL/6 mice to dietary glucose or fructose supplementation. *Brazilian Journal of Medical and Biological Research*, 40(3):323-331.
- BAREN, C. V., ANAO, I., LIRA, P. D. L., DEBENEDETTI, S., HOUGHTON, P., CROFT, S. & MARTINO, V. (2006). Triterpenic acids and flavonoids from *Satureja parvifolia*. Evaluation of their antiprotozoal activity. *Zeitschrift für Naturforschung C*, 61(3-4):189-192.

- BARKER, D. J. (2007). The origins of the developmental origins theory. *Journal of Internal Medicine*, 261(5):412-417.
- BAROUKI, R., GLUCKMAN, P. D., GRANDJEAN, P., HANSON, M. & HEINDEL, J. J. (2012). Developmental origins of non-communicable disease: implications for research and public health. *Environmental Health*, 11(42):doi:10.1186/1476-1069X-1111-1142
- BARROS, C. M. M., LESSA, R. Q., GRECHI, M. P., MOUÇO, T. L., SOUZA, M. D. G. C., WIERNSPERGER, N. & BOUSKELA, E. (2007). Substitution of drinking water by fructose solution induces hyperinsulinemia and hyperglycemia in hamsters. *Clinics*, 62(3):327-334.
- BAYER, T. A., FALKAI, P. & MAIER, W. (1999). Genetic and non-genetic vulnerability factors in schizophrenia: the basis of the “two hit hypothesis”. *Journal of Psychiatric Research*, 33(6):543-548.
- BEGUM, S., HASSAN, S. I., ALI, S. N. & SIDDIQUI, B. S. (2004). Chemical constituents from the leaves of *Psidium guajava*. *Natural Product Research*, 18(2):135-140.
- BEIGH, S. H. & JAIN, S. (2012). Prevalence of metabolic syndrome and gender differences. *Bioinformation*, 8(13):613-616.
- BENDER, D. A. (2014). *Introduction to nutrition and metabolism*, CRC Press, pp128.
- BERDANIER, C. D., DWYER, J. T. & FELDMAN, E. B. (2007). *Handbook of nutrition and food*, CRC Press, pp878.
- BERG, J., TYMOCZKO, J. & STRYER, L. (2002). *Biochemistry*, Freeman, ppsection 16.
- BERTRAM, C. E. & HANSON, M. A. (2001). Animal models and programming of the metabolic syndrome Type 2 diabetes. *British Medical Bulletin*, 60(1):103-121.
- BHAGAVAN, N. V. (2002). *Medical biochemistry*, California, USA, Academic press, pp951-953.
- BHAT, R., PARAY, I., ZARGAR, S., GANIE, A. & KHAN, I. (2015). Prevalence of the metabolic syndrome among North Indian adolescents using Adult Treatment Panel III and pediatric International Diabetic Federation definitions. *Archives of Medicine and Health Sciences*, 3(1):44.

- BINDUJA, S., VISEN, P., DAYAL, R., AGARWAL, D. & PATNAIK, G. (1996). Protective action of ursolic acid against chemical induced hepato-toxicity in rats. *Indian Journal of Pharmacology*, 28(4):232.
- BIRKENFELD, A. L. & SHULMAN, G. I. (2014). Nonalcoholic fatty liver disease, hepatic insulin resistance, and type 2 diabetes. *Hepatology*, 59(2):713-723.
- BLACK, H. (2002). Preparation of the report for a toxicology/pathology study. *Handbook of Toxicologic Pathology (WM Haschek, CG Rousseaux, and MA Wallig, eds.)*, 1419-433.
- BLAHA, M. & ELASY, T. A. (2006). Clinical use of the metabolic syndrome: why the confusion? *Clinical Diabetes*, 24(3):125-131.
- BLOOM, D. E., CAFIERO, E., JANÉ-LLOPIS, E., ABRAHAM-GESSEL, S., BLOOM, L. R., FATHIMA, S., FEIGL, A. B., GAZIANO, T., HAMANDI, A. & MOWAFI, M. 2012. The global economic burden of noncommunicable diseases. Program on the Global Demography of Aging.
- BOUWENS, L., LU, W. & DE KRIJGER, R. (1997). Proliferation and differentiation in the human fetal endocrine pancreas. *Diabetologia*, 40(4):398-404.
- BRADSHAW, Y. W. & NDEGWA, S. N. (2000). *The uncertain promise of Southern Africa*, Indiana University Press, pp349.
- BRAVO, A. A., SHETH, S. G. & CHOPRA, S. (2001). Liver biopsy. *New England Journal of Medicine*, 344(7):495-500.
- BRAY, G. A. (2007). How bad is fructose? *The American Journal of Clinical Nutrition*, 86(4):895-896.
- BRAY, G. A., NIELSEN, S. J. & POPKIN, B. M. (2004). Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. *The American Journal of Clinical Nutrition*, 79(4):537-543.
- BRENSEKE, B., PRATER, M. R., BAHAMONDE, J. & GUTIERREZ, J. C. (2013). Current thoughts on maternal nutrition and fetal programming of the metabolic syndrome. *Journal of Pregnancy*, doi:10.1155/2013/368461.
- BROOKS, A., SANOSKI, C., HAJJAR, E. & OVERHOLSER, B. (2015). *North American Pharmacist Licensure Examination (NAPLEX) : Strategies, Practice and Review*, USA, Kaplan Publishing, pp459.
- BROWN, C., DULLOO, A. & MONTANI, J. (2008). Sugary drinks in the pathogenesis of obesity and cardiovascular diseases. *International Journal of Obesity*, 32(6):S28-S34.

- BRUCE, K. & BYRNE, C. (2009). The metabolic syndrome: common origins of a multifactorial disorder. *Postgraduate Medical Journal*, 85(1009):614-621.
- BUCCI, T. (2002). *The Practice of Toxicologic Pathology: Basic Techniques*, Academic Press, pp681-784.
- BURDGE, G. C., HOILE, S. P., ULLER, T., THOMAS, N. A., GLUCKMAN, P. D., HANSON, M. A. & LILLYCROP, K. A. (2011). Progressive, transgenerational changes in offspring phenotype and epigenotype following nutritional transition. *PloS one*, 6(11):e28282.
- BUSHER, J. (1990). *Clinical methods: The History, Physical, and Laboratory Examinations*, Boston, USA, Elsevier, ppNBK204.
- CAGAMPANG, F. R., POORE, K. R. & HANSON, M. A. (2011). Developmental origins of the metabolic syndrome: body clocks and stress responses. *Brain, Behavior, and Immunity*, 25(2):214-220.
- CAMP, H. S., REN, D. & LEFF, T. (2002). Adipogenesis and fat-cell function in obesity and diabetes. *Trends in Molecular Medicine*, 8(9):442-447.
- CARDOZO, E., PAVONE, M. E. & HIRSHFELD-CYTRON, J. E. (2011). Metabolic syndrome and oocyte quality. *Trends in Endocrinology & Metabolism*, 22(3):103-109.
- CASTELLANO, J. M., GUINDA, A., DELGADO, T., RADA, M. & CAYUELA, J. A. (2013). Biochemical basis of the antidiabetic activity of oleanolic acid and related pentacyclic triterpenes. *Diabetes*, 62(6):1791-1799.
- CASTRO, A. J. G., FREDERICO, M. J. S., CAZAROLLI, L. H., MENDES, C. P., BRETANHA, L. C., SCHMIDT, É. C., BOUZON, Z. L., DE MEDEIROS PINTO, V. A., DA FONTE RAMOS, C. & PIZZOLATTI, M. G. (2015). The mechanism of action of ursolic acid as insulin secretagogue and insulinomimetic is mediated by cross-talk between calcium and kinases to regulate glucose balance. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 1850(1):51-61.
- CASTRO, G. S., CARDOSO, J. F., VANNUCCHI, H., ZUCOLOTO, S. & JORDÃO, A. A. (2011). Fructose and NAFLD: metabolic implications and models of induction in rats. *Acta Cirurgica Brasileira*, 26(2):45-50.
- CEELEN, M., VAN WEISSENBRUCH, M. M., VERMEIDEN, J. P., VAN LEEUWEN, F. E. & DELEMARRE-VAN DE WAAL, H. A. (2008). Growth and development of children born after in vitro fertilization. *Fertility and Sterility*, 90(5):1662-1673.

- CHATTERJEA, M. & SHINDE, R. (2012). *Textbook of Medical Biochemistry*, New Delhi, India, Jaypee Brothers Medical Publishers, pp373.
- CHEN, H., SIMAR, D. & MORRIS, M. J. (2009). Hypothalamic neuroendocrine circuitry is programmed by maternal obesity: interaction with postnatal nutritional environment. *PloS One*, 4(7):e6259.
- CHIANG, L. C., NG, L. T., CHENG, P. W., CHIANG, W. & LIN, C. C. (2005). Antiviral activities of extracts and selected pure constituents of *Ocimum basilicum*. *Clinical and Experimental Pharmacology and Physiology*, 32(10):811-816.
- CHOPRA, M., GALBRAITH, S. & DARNTON-HILL, I. (2002). A global response to a global problem: the epidemic of overnutrition. *Bulletin of the World Health Organization*, 80(12):952-958.
- CHOUDHURY, J. & SANYAL, A. J. (2004). Clinical aspects of fatty liver disease. *Seminars in liver disease*, 24(4):349-362.
- CIMANGA, R. K., TONA, G. L., MESIA, G. K., KAMBU, O. K., BAKANA, D. P., KALENDA, P. D., PENGE, A. O., MUYEMBE, J.-J. T., TOTTE, J. & PIETERS, L. (2006). Bioassay-Guided Isolation of Antimalarial Triterpenoid Acids from the Leaves of *Morinda lucida*. *Pharmaceutical Biology*, 44(9):677-681.
- COLEMAN, W. B. & TSONGALIS, G. J. (2009). *Molecular pathology: the molecular basis of human disease*, Academic Press, pp25-34.
- COLLA, A. R., OLIVEIRA, Á., PAZINI, F. L., ROSA, J. M., MANOSSO, L. M., CUNHA, M. P. & RODRIGUES, A. L. S. (2014). Serotonergic and noradrenergic systems are implicated in the antidepressant-like effect of ursolic acid in mice. *Pharmacology Biochemistry and Behavior*, 124(1):108-116.
- CONSIDINE, D. M. (2012). *Foods and Food Production Encyclopedia*, Springer Science & Business Media, pp1138.
- CONSULTATION, J. F. W. E. 1998. Carbohydrates in human nutrition (FAO Food and Nutrition Paper 66). Rome, Italy: FAO.
- COOPER, E. L. & YAMAGUCHI, N. (2013). *Complementary and Alternative Approaches to Biomedicine*, Springer pp9-15.
- CORNELSEN, L. & CARREIDO, A. (2015). Health-related taxes on food and beverages. *Food Research Collaboration Policy Brief*.
- CORRY, D. B., ESLAMI, P., YAMAMOTO, K., NYBY, M. D., MAKINO, H. & TUCK, M. L. (2008). Uric acid stimulates vascular smooth muscle cell proliferation and

- oxidative stress via the vascular renin–angiotensin system. *Journal of Hypertension*, 26(2):269-275.
- COTA, B. M. & ALLEN, P. J. (2010). The developmental origins of health and disease hypothesis. *Pediatric Nursing*, 36(3):157.
- CURTIS, K. S., STRATFORD, J. M. & CONTRERAS, R. J. (2005). Estrogen increases the taste threshold for sucrose in rats. *Physiology & Behavior*, 86(3):281-286.
- DA SILVA FERREIRA, D., ESPERANDIM, V. R., TOLDO, M. P. A., KUEHN, C. C., DO PRADO JUNIOR, J. C., CUNHA, W. R., E SILVA, M. L. A. & DE ALBUQUERQUE, S. (2013). *In vivo* activity of ursolic and oleanolic acids during the acute phase of *Trypanosoma cruzi* infection. *Experimental Parasitology*, 134(4):455-459.
- DAHRI, S., REUSENS, B., REMACLE, C. & HOET, J. (1995). Nutritional influences on pancreatic development and potential links with non-insulin-dependent diabetes. *Proceedings of the Nutrition Society*, 54(02):345-356.
- DALLE GRAVE, R., CALUGI, S., CENTIS, E., MARZOCCHI, R., EL GHOSH, M. & MARCHESINI, G. (2010). Lifestyle modification in the management of the metabolic syndrome: achievements and challenges. *Diabetes Metabolic Syndrome and Obesity: Targets and Therapy*, 3:373-385.
- DAVIS, J. F., CHOI, D. L. & BENOIT, S. C. (2010). Insulin, leptin and reward. *Trends in Endocrinology & Metabolism*, 21(2):68-74.
- DAY, C. P. & JAMES, O. F. (1998). Steatohepatitis: a tale of two “hits”? *Gastroenterology*, 114(4):842-845.
- DE LORGERIL, M. (2012). Commentary on the clinical management of metabolic syndrome: why a healthy lifestyle is important. *BMC Medicine*, 10(1):139.
- DE MELO, C. L., QUEIROZ, M. G. R., FONSECA, S. G., BIZERRA, A. M., LEMOS, T. L., MELO, T. S., SANTOS, F. A. & RAO, V. S. (2010). Oleanolic acid, a natural triterpenoid improves blood glucose tolerance in normal mice and ameliorates visceral obesity in mice fed a high-fat diet. *Chemico-biological Interactions*, 185(1):59-65.
- DE MOURA, R. F., RIBEIRO, C., DE OLIVEIRA, J. A., STEVANATO, E. & DE MELLO, M. A. R. (2009). Metabolic syndrome signs in Wistar rats submitted to different high-fructose ingestion protocols. *British Journal of Nutrition*, 101(08):1178-1184.

- DEROSA, G. & MAFFIOLI, P. (2010). Effects of thiazolidinediones and sulfonylureas in patients with diabetes. *Diabetes Technology & Therapeutics*, 12(6):491-501.
- DOBLADO, M. & MOLEY, K. H. (2007). Glucose metabolism in pregnancy and embryogenesis. *Current Opinion in Endocrinology, Diabetes and Obesity*, 14(6):488-493.
- DOUARD, V. & FERRARIS, R. P. (2008). Regulation of the fructose transporter GLUT5 in health and disease. *American Journal of Physiology-Endocrinology and Metabolism*, 295(2):E227-E237.
- DOUARD, V. & FERRARIS, R. P. (2013). The role of fructose transporters in diseases linked to excessive fructose intake. *The Journal of Physiology*, 591(2):401-414.
- DOWMAN, J. K., TOMLINSON, J. & NEWSOME, P. (2010). Pathogenesis of non-alcoholic fatty liver disease. *QJM*, 103(2):71-83.
- DREWNOWSKI, A. & POPKIN, B. (1997). The Nutrition Transition: New Trends in the Global Diet. *Nutrition Reviews*, 55(2):31-43.
- DROLET, R., RICHARD, C., SNIDERMAN, A., MAILLOUX, J., FORTIER, M., HUOT, C., RHEAUME, C. & TCHERNOF, A. (2008). Hypertrophy and hyperplasia of abdominal adipose tissues in women. *International Journal of Obesity*, 32(2):283-291.
- DUBOIS, R. N. (2015). The Jeremiah Metzger Lecture: Inflammation, Immune Modulators, and Chronic Disease. *Transactions of the American Clinical and Climatological Association*, 126:230.
- DUMORTIER, O., BLONDEAU, B., DUVILLIE, B., REUSENS, B., BRÉANT, B. & REMACLE, C. (2007). Different mechanisms operating during different critical time-windows reduce rat fetal beta cell mass due to a maternal low-protein or low-energy diet. *Diabetologia*, 50(12):2495-2503.
- DUPAS, J., GOANVEC, C., FERAY, A., GUERNEC, A., ALAIN, C., GUERRERO, F. & MANSOURATI, J. (2015). Progressive Induction of Type 2 Diabetes: Effects of a Reality-Like Fructose Enriched Diet in Young Wistar Rats. *PloS one*, 11(1):e0146821-e0146821.
- EDDOUKS, M., CHATTOPADHYAY, D., DE FEO, V. & CHO, W. C. (2012). Medicinal plants in the prevention and treatment of chronic diseases. *Evidence-based Complementary and Alternative medicine: eCAM*, doi:10.1155/2012/458274.

- EKELEME, F., CHIKOYE, D. & AKOBUNDU, I. (2005). Weed seedbank response to planted fallow and tillage in southwest Nigeria. *Agroforestry systems*, 63(3):299-306.
- ELANGBAM, C. (2009). Review paper: Current strategies in the development of anti-obesity drugs and their safety concerns. *Veterinary Pathology Online*, 46(1):10-24.
- ELKES, J. (2016). Primary prevention of cardiovascular disease with statins: assessing the evidence base behind clinical guidance. *Insulin*, 1504.
- ELLIOTT, S. S., KEIM, N. L., STERN, J. S., TEFF, K. & HAVEL, P. J. (2002). Fructose, weight gain, and the insulin resistance syndrome. *The American Journal of Clinical Nutrition*, 76(5):911-922.
- ELLULU, M., ABED, Y., RAHMAT, A., RANNEH, Y. & ALI, F. (2014). Epidemiology of obesity in developing countries: challenges and prevention. *Global Epidemic Obesity*, 2(1):2.
- EPSTEIN, F. H., TRAUNER, M., MEIER, P. J. & BOYER, J. L. (1998). Molecular pathogenesis of cholestasis. *New England Journal of Medicine*, 339(17):1217-1227.
- ERASMUS, R. T., SOITA, D. J., HASSAN, M. S., BLANCO-BLANCO, E., VERGOTINE, Z., KENGNE, A. P. & MATSHA, T. E. (2012). High prevalence of diabetes mellitus and metabolic syndrome in a South African coloured population: Baseline data of a study in Bellville, Cape Town. *South African Medical Journal*, 102(11):841-844.
- ERDÉLYI, K., PACHER, P., VIRÁG, L. & SZABÓ, C. (2013). Role of poly (ADP-ribose) polymerase 1 in a 'two-hit' model of hypoxia and oxidative stress in human A549 epithelial cells in vitro. *International Journal of Molecular Medicine*, 32(2):339-346.
- ERIKSSON, J., FORSEN, T., TUOMILEHTO, J., OSMOND, C. & BARKER, D. (2000). Fetal and childhood growth and hypertension in adult life. *Hypertension*, 36(5):790-794.
- ESCOBAR, M. A. C., VEERMAN, J. L., TOLLMAN, S. M., BERTRAM, M. Y. & HOFMAN, K. J. (2013). Evidence that a tax on sugar sweetened beverages reduces the obesity rate: a meta-analysis. *BMC Public Health*, 13(1):1.
- EVANS, A. 2009. The feeding of the nine billion. London: Chatham House.

- FABBRINI, E., SULLIVAN, S. & KLEIN, S. (2010). Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. *Hepatology*, 51(2):679-689.
- FALLATAH, H. I. (2014). Noninvasive Biomarkers of Liver Fibrosis: An Overview. *Advances in Hepatology*, doi:10.1155/2014/357287.
- FEHER, J. & LENGYEL, G. (2012). Silymarin in the prevention and treatment of liver diseases and primary liver cancer. *Current Pharmaceutical Biotechnology*, 13(1):210-217.
- FELDMAN, M., FRIEDMAN, L. S. & BRANDT, L. J. (2015). *Sleisenger and Fordtran's Gastrointestinal and Liver Disease: Pathophysiology, Diagnosis, Management*, Elsevier Health Sciences, pp1234-1235.
- FISCHBACH, F. T. & DUNNING, M. B. (2009). *A manual of laboratory and diagnostic tests*, Lippincott Williams & Wilkins, pp994.
- FOLEY, E. (2009). *Your pocket is what cures you: The politics of health in Senegal*, Rutgers University Press, pp37-90.
- FORD, E. S. & LI, C. (2008). Defining the metabolic syndrome in children and adolescents: will the real definition please stand up? *The Journal of Pediatrics*, 152(2):160-164.
- FOWDEN, A. L., GIUSSANI, D. A. & FORHEAD, A. J. (2006). Intrauterine programming of physiological systems: causes and consequences. *Physiology*, 21(1):29-37.
- FOWDEN, A. L. & HILL, D. J. (2001). Intra-uterine programming of the endocrine pancreas. *British Medical Bulletin*, 60(1):123-142.
- FOWLER, D. (2006). Traditional fever remedies: a list of Zambian plants. http://www.giftshealth.org/ritam/news/Traditional_Fever_remedies_1.pdf. Accessed April, 202010.
- FRANÇA, T., ISHIKAWA, L., ZORZELLA-PEZAVENTO, S., CHIUSO-MINICUCCI, F., DA CUNHA, M. & SARTORI, A. (2009). Impact of malnutrition on immunity and infection. *Journal of Venomous Animals and Toxins including Tropical Diseases*, 15(3):374-390.
- FRASS, M., STRASSL, R. P., FRIEHS, H., MÜLLNER, M., KUNDI, M. & KAYE, A. D. (2012). Use and acceptance of complementary and alternative medicine among the general population and medical personnel: a systematic review. *The Ochsner Journal*, 12(1):45-56.

- GADGIL, M. D., ANDERSON, C. A., KANDULA, N. R. & KANAYA, A. M. (2015). Dietary patterns are associated with metabolic risk factors in South Asians living in the United States. *The Journal of Nutrition*, jn207753.
- GAO, D., LI, N., LI, Q., LI, J., HAN, Z., FAN, Y. & LIU, Z. (2008). Study of the extraction, purification and antidiabetic potential of ursolic acid from *Cornus officinalis* Sieb. et Zucc. *Future Medicine*, 5(5):697-705.
- GARDNER, D. S., PEARCE, S., DANDREA, J., WALKER, R., RAMSAY, M. M., STEPHENSON, T. & SYMONDS, M. E. (2004). Peri-implantation undernutrition programs blunted angiotensin II evoked baroreflex responses in young adult sheep. *Hypertension*, 43(6):1290-1296.
- GARGOURI, Y., RANSAC, S. & VERGER, R. (1997). Covalent inhibition of digestive lipases: an in vitro study. *Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism*, 1344(1):6-37.
- GAVHANE, Y. N. & YADAV, A. V. (2012). Loss of orally administered drugs in GI tract. *Saudi Pharmaceutical Journal*, 20(4):331-344.
- GENTILE, C. L., FRYE, M. A. & PAGLIASSOTTI, M. J. (2011). Fatty acids and the endoplasmic reticulum in nonalcoholic fatty liver disease. *Biofactors*, 37(1):8-16.
- GERSCH, M. S., MU, W., CIRILLO, P., REUNGJUI, S., ZHANG, L., RONCAL, C., SAUTIN, Y. Y., JOHNSON, R. J. & NAKAGAWA, T. (2007). Fructose, but not dextrose, accelerates the progression of chronic kidney disease. *American Journal of Physiology-Renal Physiology*, 293(4):F1256-F1261.
- GHEZZI, A. C., CAMBRI, L. T., BOTEZELLI, J. D., RIBEIRO, C., DALIA, R. A. & ROSTOM DE MELLO, M. A. (2012). Metabolic syndrome markers in wistar rats of different ages. *Diabetology & Metabolic Syndrome*, 4(1):16.
- GIBNEY, J., WOLTERS, T., MALES, M., SMYTHE, G., UMPLEBY, A. & HO, K. (2003). Testosterone enhances the effect of growth hormone (GH) to increase IGF-I but exerts an anabolic effect that is independent of GH action. *Endocrine Abstracts*, 5:161.
- GIBNEY, M. J., LANHAM-NEW, S. A., CASSIDY, A. & VORSTER, H. H. (2013). *Introduction to Human Nutrition*, John Wiley & Sons.
- GICQUEL, C., EL-OSTA, A. & LE BOUC, Y. (2008). Epigenetic regulation and fetal programming. *Best Practice & Research Clinical Endocrinology & Metabolism*, 22(1):1-16.

- GIM, G.-H., CHOI, Y.-K., SHIN, Y.-C. & KO, S.-G. (2010). Growth inhibition effects of human breast cancer cell line by ursolic acid. *The FASEB Journal*, 24(1):1b496.
- GIUGLIANO, D., CERIELLO, A. & ESPOSITO, K. (2008). Are there specific treatments for the metabolic syndrome? *American Journal of Clinical Nutrition*, 87(1):8-11.
- GLUCKMAN, P. D., HANSON, M. A., BEEDLE, A. S. & SPENCER, H. G. (2008). Predictive adaptive responses in perspective. *Oikos*, 116:1957-1963.
- GLUCKMAN, P. D., HANSON, M. A. & PINAL, C. (2005). The developmental origins of adult disease. *Maternal & Child Nutrition*, 1(3):130-141.
- GODFREY, K. M. (2002). The role of the placenta in fetal programming—a review. *Placenta*, 23:S20-S27.
- GONZÁLEZ-TRUJANO, M. E., VENTURA-MARTÍNEZ, R., CHÁVEZ, M., DÍAZ-REVAL, I. & PELLICER, F. (2012). Spasmolytic and antinociceptive activities of ursolic acid and acacetin identified in *Agastache mexicana*. *Planta Medica*, 78(8):793-796.
- GORAN, M. I., TAPPY, L. & LÊ, K.-A. (2014). *Dietary Sugars and Health*, CRC Press.
- GOVERNMENT OF CHINA (2010). China Statistical Yearbook 2011: Chinese Medicine (1987-2010). The State Administration of Traditional Chinese Medicine, China.
- GRAF, B. L., RASKIN, I., CEFALU, W. T. & RIBNICKY, D. M. (2010). Plant-derived therapeutics for the treatment of metabolic syndrome. *Current Opinion in Investigational Drugs (London, England: 2000)*, 11(10):1107.
- GREAVES, P. (2011). *Histopathology of preclinical toxicity studies: interpretation and relevance in drug safety evaluation*, Academic Press, pp266,554.
- GREEN, R. M. & FLAMM, S. (2002). American Gastroenterological Association (AGA): A technical review on the evaluation of liver chemistry tests. *Gastroenterology*, 123(4):1367-1384.
- GROPPER, S. & SMITH, J. (2012). *Advanced nutrition and human metabolism*, Cengage Learning.
- GU, L., LIU, H., GU, X., BOOTS, C., MOLEY, K. H. & WANG, Q. (2015). Metabolic control of oocyte development: linking maternal nutrition and reproductive outcomes. *Cellular and Molecular Life Sciences*, 72(2):251-271.
- GUERRA, R. C., ZUÑIGA-MUÑOZ, A., GUARNER LANS, V., DÍAZ-DÍAZ, E., TENA BETANCOURT, C. A. & PÉREZ-TORRES, I. (2014). Modulation of the activities of catalase, Cu-Zn, Mn superoxide dismutase, and glutathione peroxidase in

- adipocyte from ovariectomised female rats with metabolic syndrome. *International Journal of Endocrinology*, doi:10.1155/2014/175080.
- GUILLOTEAU, P., ZABIELSKI, R., HAMMON, H. & METGES, C. (2009). Adverse effects of nutritional programming during prenatal and early postnatal life, some aspects of regulation and potential prevention and treatments. *Journal of Physiology and Pharmacology*, 60(3):17-35.
- GUINANE, C. M. & COTTER, P. D. (2013). Role of the gut microbiota in health and chronic gastrointestinal disease: understanding a hidden metabolic organ. *Therapeutic Advances in Gastroenterology*, 6(4):295-308.
- GUPTA, C. & PRAKASH, D. (2014). Phytonutrients as therapeutic agents. *Journal of Complementary and Integrative Medicine*, 11(3):151-169.
- GUSTAFSSON, D. & UNWIN, R. (2013). The pathophysiology of hyperuricaemia and its possible relationship to cardiovascular disease, morbidity and mortality. *BMC Nephrology*, 14(1):164.
- GUTTERIDGE, R. (1997). *Senna siamea* (Larnk) Irwin & Bameby. *Plant Resources of South-East Asia*, (1):232-236.
- HABA, F., KAMELINA, A., LABERCHE, J. & VASSILIEV, B. (2000). Structure de la feuille et sa variabilité intraspécifique chez les plantes ligneuses tropicales: *Cassia siamea* Lam.(Sempervirente) et *Cassia sieberiana* DC (Caducifoliee). *Revue de cytologie et de biologie végétales, Le Botaniste*, 23(3-4):35-40.
- HALLFRISCH, J. (1990). Metabolic effects of dietary fructose. *The FASEB Journal*, 4(9):2652-2660.
- HANOVER, L. M. & WHITE, J. S. (1993). Manufacturing, composition, and applications of fructose. *The American Journal of Clinical Nutrition*, 58(5):724S-732S.
- HARRIS, M. F. (2013). The metabolic syndrome. *Australian Family Physician*, 42(8):524-527.
- HARTLEY, M. (2014). Lifestyle Modification as First Line of Treatment for Chronic Disease. . *Journal of Diabetes, Metabolic Disorders & Control*, 1(2):00009.
- HARVEY, R. & FERRIER, D. (2011). *Lippincott's illustrated reviews: Biochemistry*.
- HASANI-RANJBAR, S., NAYEBI, N., LARIJANI, B. & ABDOLLAHI, M. (2009). A systematic review of the efficacy and safety of herbal medicines used in the treatment of obesity. *World Journal of Gastroenterology*, 15(25):3073.
- HAVEL, P. J. (2004). Update on adipocyte hormones regulation of energy balance and carbohydrate/lipid metabolism. *Diabetes*, 53(1):S143-S151.

- HAWKES, C. (2005). The role of foreign direct investment in the nutrition transition. *Public Health Nutrition*, 8(04):357-365.
- HE, X. & LIU, R. H. (2007). Triterpenoids isolated from apple peels have potent antiproliferative activity and may be partially responsible for apple's anticancer activity. *Journal of Agricultural and Food Chemistry*, 55(11):4366-4370.
- HE, Y., LI, Y., ZHAO, T., WANG, Y. & SUN, C. (2013). Ursolic acid inhibits adipogenesis in 3T3-L1 adipocytes through LKB1/AMPK pathway. *PloS one*, 8(7):e70135.
- HEINDEL, J. J., BALBUS, J., BIRNBAUM, L., BRUNE-DRISSE, M. N., GRANDJEAN, P., GRAY, K., LANDRIGAN, P. J., SLY, P. D., SUK, W. & CORY SLECHTA, D. (2015). Developmental origins of health and disease: integrating environmental influences. *Endocrinology*, 156(10):3416-3421.
- HELLERSTRÖM, C. & SWENNE, I. (1991). Functional maturation and proliferation of fetal pancreatic β -cells. *Diabetes*, 40(2):89-93.
- HENSLEIGH, T. & HOLAWAY, B. (1988). *Agroforestry Species for the Philippines*, Manila: AJA Printers, pp45-49.
- HERNANDEZ, C., MATTHEWS, L., OLIVER, M., BLOOMFIELD, F. & HARDING, J. (2010). Effects of sex, litter size and periconceptional ewe nutrition on offspring behavioural and physiological response to isolation. *Physiology & Behavior*, 101(5):588-594.
- HILL, D., STRUTT, B., ARANY, E., ZAINA, S., COUKELL, S. & GRAHAM, C. (2000). Increased and Persistent Circulating Insulin-Like Growth Factor II in Neonatal Transgenic Mice Suppresses Developmental Apoptosis in the Pancreatic Islets 1. *Endocrinology*, 141(3):1151-1157.
- HINCHLIFFE, S., LYNCH, M., SARGENT, P., HOWARD, C. & VELZEN, D. V. (1992). The effect of intrauterine growth retardation on the development of renal nephrons. *International Journal of Obstetrics & Gynaecology*, 99(4):296-301.
- HOLUBKOVÁ, A., PENESOVÁ, A., ŠTURDÍK, E., MOŠOVSKÁ, S. & MIKUŠOVÁ, L. (2012). Phytochemicals with potential effects in metabolic syndrome prevention and therapy. *Acta Chimica Slovaca*, 5(2):186-199.
- HORTON, R. (2007). Chronic diseases: the case for urgent global action. *The Lancet*, 370(9603):1881-1882.
- HOSTEN, O. A. (1990). *Clinical methods*, Boston.

- HOU, Y.-J., ZHU, C.-C., DUAN, X., LIU, H.-L., WANG, Q. & SUN, S.-C. (2016). Both diet and gene mutation induced obesity affect oocyte quality in mice. *Scientific Reports*, 6(18858).
- HOUTI, L., HAMANI-MEDJAOUI, I., LARDJAM-HETRAF, S. A., OUHAIBI-DJELLOULI, H., CHOUGRANI, S., GOUMIDI, L. & MEDIENE-BENCHEKOR, S. (2016). Prevalence of Metabolic Syndrome and its Related Risk Factors in the City of Oran, Algeria: the ISOR Study. *Ethnicity & Disease*, 26(1):99-106.
- HOWARD, J. (2013). The cytokine hypothesis: A neurodevelopmental explanation for the emergence of schizophrenia later in life. *Advances in Bioscience and Biotechnology*, 2013.
- HOWIE, G., SLOBODA, D., KAMAL, T. & VICKERS, M. (2009). Maternal nutritional history predicts obesity in adult offspring independent of postnatal diet. *The Journal of Physiology*, 587(4):905-915.
- HUANG, M.-T., HO, C.-T., WANG, Z. Y., FERRARO, T., LOU, Y.-R., STAUBER, K., MA, W., GEORGIADIS, C., LASKIN, J. D. & CONNEY, A. H. (1994). Inhibition of skin tumorigenesis by rosemary and its constituents carnosol and ursolic acid. *Cancer Research*, 54(3):701-708.
- HUANG, S. & CZECH, M. P. (2007). The GLUT4 glucose transporter. *Cell Metabolism*, 5(4):237-252.
- HÜBNER, G. (1965). Ultrastructural liver damage caused by direct action of carbon tetrachloride *in vivo* and *in vitro*. *Virchows Archiv für pathologische Anatomie und Physiologie und für klinische Medizin*, 339(3):187.
- HWANG, L. L., WANG, C. H., LI, T. L., CHANG, S. D., LIN, L. C., CHEN, C. P., CHEN, C. T., LIANG, K. C., HO, I. K. & YANG, W. S. (2010). Sex differences in high-fat diet-induced obesity, metabolic alterations and learning, and synaptic plasticity deficits in mice. *Obesity*, 18(3):463-469.
- INTERNATIONAL DIABETES FEDERATION, I. 2005
- IDF worldwide definition of the metabolic syndrome.
- ISHIMOTO, T., LANASPA, M. A., LE, M. T., GARCIA, G. E., DIGGLE, C. P., MACLEAN, P. S., JACKMAN, M. R., ASIPU, A., RONCAL-JIMENEZ, C. A. & KOSUGI, T. (2012). Opposing effects of fructokinase C and A isoforms on fructose-induced metabolic syndrome in mice. *Proceedings of the National Academy of Sciences*, 109(11):4320-4325.

- ISMAILI, H., TORTORA, S., SOSA, S., FKI-H-TETOUANI, S., ILIDRISSI, A., LOGGIA, R. D., TUBARO, A. & AQUINO, R. (2001). Topical anti-inflammatory activity of *Thymus wilddenowii*. *Journal of Pharmacy and Pharmacology*, 53(12):1645-1652.
- JANG, S.-M., YEE, S.-T., CHOI, J., CHOI, M.-S., DO, G.-M., JEON, S.-M., YEO, J., KIM, M.-J., SEO, K.-I. & LEE, M.-K. (2009). Ursolic acid enhances the cellular immune system and pancreatic β -cell function in streptozotocin-induced diabetic mice fed a high-fat diet. *International Immunopharmacology*, 9(1):113-119.
- JAYAPRAKASAM, B., OLSON, L. K., SCHUTZKI, R. E., TAI, M.-H. & NAIR, M. G. (2006). Amelioration of obesity and glucose intolerance in high-fat-fed C57BL/6 mice by anthocyanins and ursolic acid in Cornelian cherry (*Cornus mas*). *Journal of Agricultural and Food Chemistry*, 54(1):243-248.
- JIA, Y., BHUIYAN, M. J. H., JUN, H.-J., LEE, J. H., HOANG, M. H., LEE, H.-J., KIM, N., LEE, D., HWANG, K. Y. & HWANG, B. Y. (2011). Ursolic acid is a PPAR- α agonist that regulates hepatic lipid metabolism. *Bioorganic & Medicinal Chemistry Letters*, 21(19):5876-5880.
- JIA, Y., KIM, S., KIM, J., KIM, B., WU, C., LEE, J. H., JUN, H. J., KIM, N., LEE, D. & LEE, S. J. (2015). Ursolic acid improves lipid and glucose metabolism in high-fat-fed C57BL/6J mice by activating peroxisome proliferator-activated receptor alpha and hepatic autophagy. *Molecular Nutrition & Food Research*, 59(2):344-354.
- JIRTLE, R. L. & SKINNER, M. K. (2007). Environmental epigenomics and disease susceptibility. *Nature Reviews Genetics*, 8(4):253-262.
- JO, J., GAVRILOVA, O., PACK, S., JOU, W., MULLEN, S., SUMNER, A. E., CUSHMAN, S. W. & PERIWAL, V. (2009). Hypertrophy and/or hyperplasia: dynamics of adipose tissue growth. *PLoS Computational Biology*, 5(3):e1000324.
- JOHNSON, P. H. (2016). *Global Healthcare: Issues and Policies*, Jones & Bartlett Learning, pp293-293.
- JOHNSON, R. J., NAKAGAWA, T., SANCHEZ-LOZADA, L. G., SHAFIU, M., SUNDARAM, S., LE, M., ISHIMOTO, T., SAUTIN, Y. Y. & LANASPA, M. A. (2013). Sugar, uric acid, and the etiology of diabetes and obesity. *Diabetes*, 62(10):3307-3315.

- JUNG, S., HA, Y., SHIM, E., CHOI, S., JIN, J., YUN-CHOI, H. & LEE, J. (2007). Insulin-mimetic and insulin-sensitizing activities of a pentacyclic triterpenoid insulin receptor activator. *Biochemical Journal*, 403:243-250.
- KADUKA, L. U., KOMBE, Y., KENYA, E., KURIA, E., BORE, J. K., BUKANIA, Z. N. & MWANGI, M. (2012). Prevalence of metabolic syndrome among an urban population in Kenya. *Diabetes Care*, 35(4):887-893.
- KANAKA-GANTENBEIN, C. (2010). Fetal origins of adult diabetes. *Annals of the New York Academy of Sciences*, 1205(1):99-105.
- KANG, W., SONG, Y. & GU, X. (2012). α -glucosidase inhibitory in vitro and antidiabetic activity in vivo of *Osmanthus fragrans*. *Journal of Medicinal Plants Research*, 6(14):2850-2856.
- KASSI, E., PERVANIDOU, P., KALTSAS, G. & CHROUSOS, G. (2011). Metabolic syndrome: definitions and controversies. *BioMedCentral Medicine*, 9(1):48.
- KATONA, P. & KATONA-APTE, J. (2008). The interaction between nutrition and infection. *Clinical Infectious Diseases*, 46(10):1582-1588.
- KAUR, J. (2014). A comprehensive review on metabolic syndrome. *Cardiology Research and Practice*, doi:10.1155/2014/943162.
- KEARNEY, J. (2010). Food consumption trends and drivers. *Philosophical Transactions of the Royal society B: Biological Sciences*, 365(1554):2793-2807.
- KEEMBIYEHETTY, C., AUGUSTIN, R., CARAYANNOPOULOS, M. O., STEER, S., MANOLESCU, A., CHEESEMAN, C. I. & MOLEY, K. H. (2006). Mouse glucose transporter 9 splice variants are expressed in adult liver and kidney and are up-regulated in diabetes. *Molecular Endocrinology*, 20(3):686-697.
- KELLY, B. B. & FUSTER, V. (2010). *Promoting Cardiovascular Health in the Developing World:: A Critical Challenge to Achieve Global Health*, National Academies Press.
- KENNEDY, G., NANTEL, G. & SHETTY, P. (2006). The double burden of malnutrition: case studies from six developing countries. *Rome: Nutrition Planning, Assessment and Evaluation Service, Food and Agriculture Organization of the United Nations*.
- KEPPLER, D., LESCH, R., REUTTER, W. & DECKER, K. (1968). Experimental hepatitis induced by D-galactosamine. *Experimental and Molecular Pathology*, 9(2):279-290.
- KHAN, S. H. & TALUKDER, S. H. (2013). Nutrition transition in Bangladesh: is the country ready for this double burden. *Obesity Reviews*, 14(S2):126-133.

- KHITAN, Z. & KIM, D. H. (2013). Fructose: a key factor in the development of metabolic syndrome and hypertension. *Journal of Nutrition and Metabolism*, doi:10.1155/2013/682673.
- KHOSLA, U. M., ZHARIKOV, S., FINCH, J. L., NAKAGAWA, T., RONCAL, C., MU, W., KROTOVA, K., BLOCK, E. R., PRABHAKAR, S. & JOHNSON, R. J. (2005). Hyperuricemia induces endothelial dysfunction. *Kidney International*, 67(5):1739-1742.
- KHURANA, J. S. (2009). *The Surgical Pathology of Bone Tumors and Tumor-Like Lesions*, Springer, pp35.
- KIM, J., JANG, D. S., KIM, H. & KIM, J. S. (2009). Anti-lipase and lipolytic activities of ursolic acid isolated from the roots of *Actinidia arguta*. *Archives of Pharmacal Research*, 32(7):983-987.
- KIM, S.-H., RYU, H. G., LEE, J., SHIN, J., HARIKISHORE, A., JUNG, H.-Y., KIM, Y. S., LYU, H.-N., OH, E. & BAEK, N.-I. (2015). Ursolic acid exerts anti-cancer activity by suppressing vaccinia-related kinase 1-mediated damage repair in lung cancer cells. *Scientific Reports*, 5:(14570):doi:10.1038/srep14570.
- KIM, S., JIN, Y., CHOI, Y. & PARK, T. (2011). Resveratrol exerts anti-obesity effects via mechanisms involving down-regulation of adipogenic and inflammatory processes in mice. *Biochemical Pharmacology*, 81(11):1343-1351.
- KIZHNER, T. & WERMAN, M. J. (2002). Long-term fructose intake: biochemical consequences and altered renal histology in the male rat. *Metabolism*, 51(12):1538-1547.
- KNUDSON, A. G. (1971). Mutation and cancer: statistical study of retinoblastoma. *Proceedings of the National Academy of Sciences*, 68(4):820-823.
- KOLETZKO, B., BRANDS, B. & DEMMELMAIR, H. (2011). The Early Nutrition Programming Project (EARNEST): 5 y of successful multidisciplinary collaborative research. *American Journal of Clinical Nutrition*, 94(6):1749S-1753S.
- KONG, L., LI, S., LIAO, Q., ZHANG, Y., SUN, R., ZHU, X., ZHANG, Q., WANG, J., WU, X. & FANG, X. (2013). Oleanolic acid and ursolic acid: novel hepatitis C virus antivirals that inhibit NS5B activity. *Antiviral Research*, 98(1):44-53.
- KORIĆANAC, G., ĐORĐEVIĆ, A., ŽAKULA, Z., VOJNOVIĆ-MILUTINOVIĆ, D., TEPAVČEVIĆ, S., VELIČKOVIĆ, N., MILOSAVLJEVIĆ, T., STOJILJKOVIĆ, M., ROMIĆ, S. & MATIĆ, G. (2013). Gender modulates development of the

- metabolic syndrome phenotype in fructose-fed rats. *Archives of Biological Sciences*, 65(2):455-464.
- KRAFTS, K. P. (2010). Tissue repair: The hidden drama. *Organogenesis*, 6(4):225-233.
- KUMAR, S., KUMAR, V. & PRAKASH, O. (2010). Antidiabetic and anti-lipemic effects of Cassia siamea leaves extract in streptozotocin induced diabetic rats. *Asian Pacific Journal of Tropical Medicine*, 3(11):871-873.
- KUMAR, S., KUMAR, V., PRAKASH, O. & ALI, M. (2013). Enzymes Inhibition and Antidiabetic Effect of Isolated Constituents from Callistemon lanceolatus. *The Natural Products Journal*, 3(4):252-259.
- KUNG, A. W. (2003). Androgen and bone mass in men. *Asian Journal of Andrology*, 5(2):148-154.
- KUNKEL, S. D., ELMORE, C. J., BONGERS, K. S., EBERT, S. M., FOX, D. K., DYLE, M. C., BULLARD, S. A. & ADAMS, C. M. (2012). Ursolic acid increases skeletal muscle and brown fat and decreases diet-induced obesity, glucose intolerance and fatty liver disease. *PloS One*, 7(6):e39332.
- LAHIRI, D. K., MALONEY, B., RIYAZ BASHA, M., WEN GE, Y. & ZAWIA, N. H. (2007). How and when environmental agents and dietary factors affect the course of Alzheimer's disease: the "LEARn" model (latent early-life associated regulation) may explain the triggering of AD. *Current Alzheimer Research*, 4(2):219-228.
- LAHIRI, D. K., MALONEY, B. & ZAWIA, N. H. (2009). The LEARN model: an epigenetic explanation for idiopathic neurobiological diseases. *Molecular Psychiatry*, 14(11):992-1003.
- LAKE, A. D., NOVAK, P., HARDWICK, R. N., FLORES-KEOWN, B., ZHAO, F., KLIMECKI, W. T. & CHERRINGTON, N. J. (2014). The adaptive endoplasmic reticulum stress response to lipotoxicity in progressive human nonalcoholic fatty liver disease. *Toxicological Sciences*, 137(1):26-35.
- LANGLEY-EVANS, S. (2015). *Nutrition, Health and Disease: A Lifespan Approach*, Oxford, UK, John Wiley & Sons, pp306.
- LANGLEY-EVANS, S. C. (2009). Nutritional programming of disease: unravelling the mechanism. *Journal of Anatomy*, 215(1):36-51.
- LASCANO, M. E. & POGGIO, E. D. (2010). Kidney function assessment by creatinine-based estimation equations. *Disease Management Project: Nephrology*. Cleveland, Center for Continuing Education, 1.

- LAU, E., CARVALHO, D. & FREITAS, P. (2015). Gut Microbiota: Association with NAFLD and Metabolic Disturbances. *BioMed Research International*.
- LE MEN, J. & POURRAT, H. (1952). [The presence of ursolic acid in the leaves of *Vinca minor L.*, *Nerium oleander L.* and *Salvia officinalis L.*]. *Annales Pharmaceutiques Francaises*, 10349-351.
- LEE, M. (2009). *Basic skills in interpreting laboratory data*, American Society of Health-Systems Pharmacists, pp235-245.
- LEE, S.-U., PARK, S.-J., KWAK, H. B., OH, J., MIN, Y. K. & KIM, S. H. (2008). Anabolic activity of ursolic acid in bone: Stimulating osteoblast differentiation in vitro and inducing new bone formation in vivo. *Pharmacological Research*, 58(5):290-296.
- LEE, W. M. (2003). Drug-induced hepatotoxicity. *New England Journal of Medicine*, 349(5):474-485.
- LEROY, J., VALCKX, S., STURMEY, R., BOSSAERT, P., HOECK, V. V. & BOLS, P. (2012). Maternal metabolic health and oocyte quality: the role of the intrafollicular environment. *Animal Reproduction*, 9:777-788.
- LEWIS, A. J., GALBALLY, M., GANNON, T. & SYMEONIDES, C. (2014). Early life programming as a target for prevention of child and adolescent mental disorders. *BMC Medicine*, 12(1):33.
- LI, J.-S., WANG, W.-J., SUN, Y., ZHANG, Y.-H. & ZHENG, L. (2015). Ursolic acid inhibits the development of nonalcoholic fatty liver disease by attenuating endoplasmic reticulum stress. *Food & Function*, 6(5):1643-1651.
- LI, M., SLOBODA, D. & VICKERS, M. (2011). Maternal obesity and developmental programming of metabolic disorders in offspring: evidence from animal models. *Experimental Diabetes Research*, 2011.
- LI, S., MENG, F., LIAO, X., WANG, Y., SUN, Z., GUO, F., LI, X., MENG, M., LI, Y. & SUN, C. (2014). Therapeutic role of ursolic acid on ameliorating hepatic steatosis and improving metabolic disorders in high-fat diet-induced non-alcoholic fatty liver disease rats. *PloS One*, 9(1):e86724.
- LI, Y., KANG, Z., LI, S., KONG, T., LIU, X. & SUN, C. (2010). Ursolic acid stimulates lipolysis in primary-cultured rat adipocytes. *Molecular Nutrition & Food Research*, 54(11):1609-1617.
- LIEBERMAN, M., MARKS, A. D., SMITH, C. M. & MARKS, D. B. (2006). *Marks' Essential Medical Biochemistry*, Lippincott Williams & Wilkins, pp267.

- LILLYCROP, K. A. & BURDGE, G. C. (2011). Epigenetic changes in early life and future risk of obesity. *International Journal of Obesity*, 35(1):72-83.
- LINDEROTH, A., BIERNAT, M., PRYKHODKO, O., KORNILOVSKA, I., PUSZTAI, A., PIERZYNOWSKI, S. G. & BJÖRN, W. R. (2005). Induced growth and maturation of the gastrointestinal tract after *Phaseolus vulgaris* lectin exposure in suckling rats. *Journal of Pediatric Gastroenterology and Nutrition*, 41(2):195-203.
- LINDQVIST, A., BAELEMANS, A. & ERLANSON-ALBERTSSON, C. (2008). Effects of sucrose, glucose and fructose on peripheral and central appetite signals. *Regulatory Peptides*, 150(1):26-32.
- LING, C., JINPING, L., XIA, L. & RENYONG, Y. (2013). Ursolic acid provides kidney protection in diabetic rats. *Current Therapeutic Research*, 75:59-63.
- LIU, J. (1995). Pharmacology of oleanolic acid and ursolic acid. *Journal of Ethnopharmacology*, 49(2):57-68.
- LONG, N. M., RULE, D. C., TUERSUNJIANG, N., NATHANIELSZ, P. W. & FORD, S. P. (2015). Maternal obesity in sheep increases fatty acid synthesis, upregulates nutrient transporters, and increases adiposity in adult male offspring after a feeding challenge. *PloS one*, 10(4):e0122152.
- LOUIS, M. E. S. & HESS, J. J. (2008). Climate change: impacts on and implications for global health. *American Journal of Preventive Medicine*, 35(5):527-538.
- LUNA, R. K. (1996). *Plantation trees*, International Book Distributors, pp242.
- MACCRACKEN, J. G. & STEBBINGS, J. L. (2012). Test of a body condition index with amphibians. *Journal of Herpetology*, 46(3):346-350.
- MACHADO, D., NEIS, V., BALEN, G., COLLA, A., CUNHA, M., DALMARCO, J., PIZZOLATTI, M., PREDIGER, R. & RODRIGUES, A. (2012). Antidepressant-like effect of ursolic acid isolated from *Rosmarinus officinalis* L. in mice: evidence for the involvement of the dopaminergic system. *Pharmacology Biochemistry and Behavior*, 103(2):204-211.
- MACKENZIE, H. S. & BRENNER, B. M. (1995). Fewer nephrons at birth: a missing link in the etiology of essential hypertension? *American Journal of Kidney Diseases*, 26(1):91-98.
- MAHAJAN, R., GUPTA, K. & KAPOOR, V. (2010). A systematic account of pathogenesis, diagnosis and pharmacotherapy of metabolic syndrome: things we need to know. *International Journal of Pharmacology*, 6(4):338-345.

- MAHAN, L. K., ESCOTT-STUMP, S. & RAYMOND, J. L. (2012). *Krause's food & the nutrition care process*, USA, Elsevier Health Sciences, pp1082.
- MALARKEY, L. M. & MCMORROW, M. E. (2011). *Saunders Nursing Guide to Diagnostic and Laboratory Tests*, Elsevier Health Sciences.
- MAMIKUTTY, N., THEENT, Z. C., SAPRI, S. R., SAHRUDDIN, N. N., MOHD YUSOF, M. R. & HAJI SUHAIMI, F. (2014). The establishment of metabolic syndrome model by induction of fructose drinking water in male Wistar rats. *BioMed Research International*, doi:10.1155/2014/263897.
- MANDARD, S., MÜLLER, M. & KERSTEN, S. (2004). Peroxisome proliferator-activated receptor α target genes. *Cellular and Molecular Life Sciences* 61(4):393-416.
- MANOLESCU, A. R., WITKOWSKA, K., KINNAIRD, A., CESSFORD, T. & CHEESEMAN, C. (2007). Facilitated hexose transporters: new perspectives on form and function. *Physiology*, 22(4):234-240.
- MARIE, S. & PIGGOTT, J. R. (2013). *Handbook of sweeteners*, Springer Science & Business Media, pp68.
- MARKIEWSKI, M. M. & LAMBRIS, J. D. (2007). The role of complement in inflammatory diseases from behind the scenes into the spotlight. *The American Journal of Pathology*, 171(3):715-727.
- MARRIOTT, B. P., COLE, N. & LEE, E. (2009). National estimates of dietary fructose intake increased from 1977 to 2004 in the United States. *The Journal of Nutrition*, 139(6):1228S-1235S.
- MARTIN-GRONERT, M. S. & OZANNE, S. E. (2012). Mechanisms underlying the developmental origins of disease. *Reviews in Endocrine and Metabolic Disorders*, 13(2):85-92.
- MARVASTI, T. B. & ADELI, K. (2010). Pharmacological management of metabolic syndrome and its lipid complications. *DARU Journal of Pharmaceutical Sciences*, 18(3):146.
- MASARONE, M., FEDERICO, A., ABENAVOLI, L., LOGUERCI, C. & PERSICO, M. (2014). Non Alcoholic Fatty Liver: Epidemiology and Natural History. *Reviews on Recent Clinical Trials*, 9(3):126-133.
- MATULL, W., PEREIRA, S. & O'DONOHUE, J. (2006). Biochemical markers of acute pancreatitis. *Journal of Clinical Pathology*, 59(4):340-344.

- MAYES, P. A. (1993). Intermediary metabolism of fructose. *American Journal of Clinical Nutrition*, 58(5):754S-765S.
- MCCARTHY, E. F. & FRASSICA, F. J. (2014). *Pathology of Bone and Joint Disorders Print and Online Bundle*, Cambridge University Press, pp28.
- MCCLEMENTS, D. J., ZOU, L., ZHANG, R., SALVIA-TRUJILLO, L., KUMOSANI, T. & XIAO, H. (2015). Enhancing Nutraceutical Performance Using Excipient Foods: Designing Food Structures and Compositions to Increase Bioavailability. *Comprehensive Reviews in Food Science and Food Safety*, 14(6):824-847.
- MCMILLEN, I. C. & ROBINSON, J. S. (2005). Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. *Physiological Reviews*, 85(2):571-633.
- MELANSON, K. J., ANGELOPOULOS, T. J., NGUYEN, V., ZUKLEY, L., LOWNDES, J. & RIPPE, J. M. (2008). High-fructose corn syrup, energy intake, and appetite regulation. *The American Journal of Clinical Nutrition*, 88(6):1738S-1744S.
- MICHOS, E. D., SIBLEY, C. T., BAER, J. T., BLAHA, M. J. & BLUMENTHAL, R. S. (2012). Niacin and statin combination therapy for atherosclerosis regression and prevention of cardiovascular disease events: reconciling the AIM-HIGH (Atherothrombosis Intervention in Metabolic Syndrome With Low HDL/High Triglycerides: Impact on Global Health Outcomes) trial with previous surrogate endpoint trials. *Journal of the American College of Cardiology*, 59(23):2058-2064.
- MIRANDA, J., KINRA, S., CASAS, J., DAVEY SMITH, G. & EBRAHIM, S. (2008). Non-communicable diseases in low-and middle-income countries: context, determinants and health policy. *Tropical Medicine & International Health*, 13(10):1225-1234.
- MOORE, K. L., PERSAUD, T. V. N. & TORCHIA, M. G. (2015). *The developing human: clinically oriented embryology*, Elsevier Health Sciences, pp2-4.
- MORRIS, C. F., TAHIR, M., ARSHID, S., CASTRO, M. S. & FONTES, W. (2015). Reconciling the IPC and Two-Hit Models: Dissecting the Underlying Cellular and Molecular Mechanisms of Two Seemingly Opposing Frameworks. *Journal of Immunology Research*, doi:10.1155/2015/697193.
- MUHLHAUSLER, B. S., DUFFIELD, J. A. & MCMILLEN, I. C. (2007). Increased maternal nutrition stimulates peroxisome proliferator activated receptor- γ , adiponectin, and leptin messenger ribonucleic acid expression in adipose tissue before birth. *Endocrinology*, 148(2):878-885.

- NAJID, A., SIMON, A., COOK, J., CHABLE-RABINOVITCH, H., DELAGE, C., CHULIA, A. & RIGAUD, M. (1992). Characterization of ursolic acid as a lipoxygenase and cyclooxygenase inhibitor using macrophages, platelets and differentiated HL60 leukemic cells. *FEBS Letters*, 299(3):213-217.
- NAKAYAMA, T., KOSUGI, T., GERSCH, M., CONNOR, T., SANCHEZ-LOZADA, L. G., LANASPA, M. A., RONCAL, C., PEREZ-POZO, S. E., JOHNSON, R. J. & NAKAGAWA, T. (2010). Dietary fructose causes tubulointerstitial injury in the normal rat kidney. *American Journal of Physiology-Renal Physiology*, 298(3):F712-F720.
- NATHAN, D. M., BUSE, J. B., DAVIDSON, M. B., FERRANNINI, E., HOLMAN, R. R., SHERWIN, R. & ZINMAN, B. (2009). Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy a consensus statement of the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care*, 32(1):193-203.
- NATIONAL CHOLESTEROL EDUCATION PROGRAM, N. (2002). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation*, 106(25):3143.
- NATIONAL DEPARTMENT OF HEALTH, P. 2012. Strategic plan for the prevention and control of non-communicable diseases 2013-17. National Department of Health Pretoria.
- NAZARUK, J. & BORZYM-KLUCZYK, M. (2014). The role of triterpenes in the management of diabetes mellitus and its complications. *Phytochemistry Reviews*, 14(4):675-690.
- NETO, R., SALLES, N. & CARVALHO, J. F. D. (2009). The use of inflammatory laboratory tests in rheumatology. *Revista Brasileira de Reumatologia*, 49(4):413-430.
- NIAKAN, K. K., HAN, J., PEDERSEN, R. A., SIMON, C. & PERA, R. A. R. (2012). Human pre-implantation embryo development. *Development*, 139(5):829-841.
- NICHOLAS, L. M., RATTANATRAY, L., MACLAUGHLIN, S. M., OZANNE, S. E., KLEEMANN, D. O., WALKER, S. K., MORRISON, J. L., ZHANG, S., MUHLHÄUSLER, B. S. & MARTIN-GRONERT, M. S. (2013). Differential effects of maternal obesity and weight loss in the periconceptional period on the

- epigenetic regulation of hepatic insulin-signaling pathways in the offspring. *The FASEB Journal*, 27(9):3786-3796.
- NICHOLAS, L. M., RATTANATRAY, L., MORRISON, J. L., KLEEMANN, D. O., WALKER, S. K., ZHANG, S., MACLAUGHLIN, S. & MCMILLEN, I. C. (2014). Maternal obesity or weight loss around conception impacts hepatic fatty acid metabolism in the offspring. *Obesity*, 22(7):1685-1693.
- NOMURA, K. & YAMANOUCHI, T. (2012). The role of fructose-enriched diets in mechanisms of nonalcoholic fatty liver disease. *The Journal of Nutritional Biochemistry*, 23(3):203-208.
- NUNES-SOUZA, V., CÉSAR-GOMES, C. J., DA FONSECA, L. J. S., GUEDES, G. D. S., SMANIOTTO, S. & RABELO, L. A. (2016). Aging Increases Susceptibility to High Fat Diet-Induced Metabolic Syndrome in C57BL/6 Mice: Improvement in Glycemic and Lipid Profile after Antioxidant Therapy. *Oxidative Medicine and Cellular Longevity*, doi:10.1155/2016/1987960.
- NUSSEY, S. S. & WHITEHEAD, S. A. (2013). *Endocrinology: An Integrated Approach*, Oxford, UK, CRC Press, pp35-36.
- NYENWE, E. A., JERKINS, T. W., UMPIERREZ, G. E. & KITABCHI, A. E. (2011). Management of type 2 diabetes: evolving strategies for the treatment of patients with type 2 diabetes. *Metabolism*, 60(1):1-23.
- OBEN, J. A., MOURALIDARANE, A., SAMUELSSON, A.-M., MATTHEWS, P. J., MORGAN, M. L., MCKEE, C., SOEDA, J., FERNANDEZ-TWINN, D. S., MARTIN-GRONERT, M. S. & OZANNE, S. E. (2010). Maternal obesity during pregnancy and lactation programs the development of offspring non-alcoholic fatty liver disease in mice. *Journal of Hepatology*, 52(6):913-920.
- ODOM, L. N. & SEGARS, J. (2010). Imprinting disorders and assisted reproductive technology. *Current Opinion in Endocrinology, Diabetes, and Obesity*, 17(6):517.
- OFFICIAL METHODS OF ANALYSIS OF ANALYTICAL CHEMISTS, A. 2005. Method number 920:39- Fat (Crude) or Ether Extract
- ORWOLL, E. S., BILEZIKIAN, J. P. & VANDERSCHUEREN, D. (2009). *Osteoporosis in men: the effects of gender on skeletal health*, Academic Press, pp86-95.
- OUYANG, X., CIRILLO, P., SAUTIN, Y., MCCALL, S., BRUCHETTE, J. L., DIEHL, A. M., JOHNSON, R. J. & ABDELMALEK, M. F. (2008). Fructose consumption as a risk factor for non-alcoholic fatty liver disease. *Journal of Hepatology*, 48(6):993-999.

- PADMANABHAN, V., CARDOSO, R. C. & PUTTABYATAPPA, M. (2016). Developmental programming, a pathway to disease. *Endocrinology*, doi.org/10.1210/en.2016-1003
- PAGANA, K. D. & PAGANA, T. J. (2013). *Mosby's Manual of Diagnostic and Laboratory Tests*, Elsevier Science Health Science Division, pp191-193, 512.
- PAI, P. G., CHAMARI NAWARATHNA, S., KULKARNI, A., HABEEBA, U., REDDY C, S., TEERTHANATH, S. & SHENOY, J. P. (2012). Nephroprotective effect of ursolic acid in a murine model of gentamicin-induced renal damage. *Pharmacology*, doi:10.5402/2012/410902.
- PAIS, R., PASCALE, A., FEDCHUCK, L., CHARLOTTE, F., POYNARD, T. & RATZIU, V. (2011). Progression from isolated steatosis to steatohepatitis and fibrosis in nonalcoholic fatty liver disease. *Clinics and Research in Hepatology and Gastroenterology*, 35(1):23-28.
- PANCHAL, S. K., WARD, L. & BROWN, L. (2013). Ellagic acid attenuates high-carbohydrate, high-fat diet-induced metabolic syndrome in rats. *European Journal of Nutrition*, 52(2):559-568.
- PARASURAMAN, S., RAVEENDRAN, R. & KESAVAN, R. (2010). Blood sample collection in small laboratory animals. *Journal of Pharmacology & Pharmacotherapeutics*, 1(2):87.
- PARK, J.-H., KWON, H.-Y., SOHN, E. J., KIM, K. A., KIM, B., JEONG, S.-J., HO SONG, J., KOO, J. S. & KIM, S.-H. (2013). Inhibition of Wnt/ β -catenin signaling mediates ursolic acid-induced apoptosis in PC-3 prostate cancer cells. *Pharmacological Reports*, 65(5):1366-1374.
- PARK, O.-J., CESAR, D., FAIX, D., WU, K., SHACKLETON, C. & HELLERSTEIN, M. K. (1992). Mechanisms of fructose-induced hypertriglyceridaemia in the rat. Activation of hepatic pyruvate dehydrogenase through inhibition of pyruvate dehydrogenase kinase. *Biochemistry Journal*, 282:753-757.
- PARKMAN, H., FASS, R. & FOXX-ORENSTEIN, A. (2010). Treatment of patients with diabetic gastroparesis. *Gastroenterology and Hepatology*, 6(6: Supplement 9):1-16.
- PATEL, M. S. & SRINIVASAN, M. (2011). Metabolic programming in the immediate postnatal life. *Annals of Nutrition and Metabolism*, 58(2):18-28.
- PATTON, K. T. & THIBODEAU, G. A. (2014). *Anatomy & Physiology*, Missouri, USA, Elsevier Health Sciences, pp909.

- PAYYAPPALLIMANA, U. (2010). Role of traditional medicine in primary health care. *Yokohama Journal of Social Sciences*, 14(6):57-78.
- PEMMINATI, S., GOPALAKRISHNA, H., RAI, A., SHETTY, S., VINOD, A., PAI, P. G. & SHENOY, A. K. (2011). Anxiolytic effect of chronic administration of ursolic acid in rats. *Journal of Applied Pharmaceutical Sciences*, 1(3):68-71.
- PENIDO, M. G. M. & ALON, U. S. (2012). Phosphate homeostasis and its role in bone health. *Pediatric Nephrology*, 27(11):2039-2048.
- PERRY, R. J., SAMUEL, V. T., PETERSEN, K. F. & SHULMAN, G. I. (2014). The role of hepatic lipids in hepatic insulin resistance and type 2 diabetes. *Nature*, 510(7503):84-91.
- PICO, C., OLIVER, P., SANCHEZ, J., MIRALLES, O., CAIMARI, A., PRIEGO, T. & PALOU, A. (2007). The intake of physiological doses of leptin during lactation in rats prevents obesity in later life. *International Journal of Obesity*, 31(8):1199-1209.
- POPKIN, B. & NG, S. W. (2007). The nutrition transition in high-and low-income countries: what are the policy lessons? *Agricultural Economics*, 37(s1):199-211.
- POPKIN, B. M. (2002). An overview on the nutrition transition and its health implications: the Bellagio meeting. *Public Health Nutrition*, 5(1A):93-103.
- POPKIN, B. M. (2003). The nutrition transition in the developing world. *Development Policy Review*, 21(5-6):581-597.
- POPKIN, B. M., ADAIR, L. S. & NG, S. W. (2012). Global nutrition transition and the pandemic of obesity in developing countries. *Nutrition Reviews*, 70(1):3-21.
- PORTHA, B., CHAVEY, A. & MOVASSAT, J. (2011). Early-life origins of type 2 diabetes: fetal programming of the beta-cell mass. *Experimental Diabetes Research*, doi:10.1155/2011/105076
- PRESTON, R. R. & WILSON, T. E. (2012). *Physiology*, Philadelphia, USA, Lippincott Williams & Wilkins, pp406.
- PRIEGO, T., SÁNCHEZ, J., PALOU, A. & PICO, C. (2010). Leptin intake during the suckling period improves the metabolic response of adipose tissue to a high-fat diet. *International Journal of Obesity*, 34(5):809-819.

- PUZIO, I., KAPICA, M., BIENKO, M., PIEDRA, J. V., GAJEWSKI, Z., WILCZAK, J., KULASEK, G. & ZABIELSKI, R. (2007). Dietary bioactive substances influenced perinatal bone development in piglets. *Livestock Science*, 108(1):72-75.
- QIAN, Z., WANG, X., SONG, Z., ZHANG, H., ZHOU, S., ZHAO, J. & WANG, H. (2015). A Phase I Trial to Evaluate the Multiple-Dose Safety and Antitumor Activity of Ursolic Acid Liposomes in Subjects with Advanced Solid Tumors. *BioMed Research International*, doi:10.1155/2015/809714.
- QUÉRÉ, L., WENGER, T. & SCHRAMM, H. J. (1996). Triterpenes as potential dimerization inhibitors of HIV-1 protease. *Biochemical and Biophysical Research Communications*, 227(2):484-488.
- RAO, V. S., DE MELO, C. L., QUEIROZ, M. G. R., LEMOS, T. L., MENEZES, D. B., MELO, T. S. & SANTOS, F. A. (2011). Ursolic acid, a pentacyclic triterpene from *Sambucus australis*, prevents abdominal adiposity in mice fed a high-fat diet. *Journal of Medicinal Food*, 14(11):1375-1382.
- RATTANATRAY, L., MACLAUGHLIN, S., KLEEMANN, D., WALKER, S., MUHLHAUSLER, B. & MCMILLEN, I. (2010). Impact of maternal periconceptional overnutrition on fat mass and expression of adipogenic and lipogenic genes in visceral and subcutaneous fat depots in the postnatal lamb. *Endocrinology*, 151(11):5195-5205.
- REDDY, J. K. & RAO, M. S. (2006). Lipid metabolism and liver inflammation. II. Fatty liver disease and fatty acid oxidation. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 290(5):G852-G858.
- REYNOLDS, C. M., GRAY, C., LI, M., SEGOVIA, S. A. & VICKERS, M. H. (2015). Early Life Nutrition and Energy Balance Disorders in Offspring in Later Life. *Nutrients*, 7(9):8090-8111.
- RHIND, S. M., RAE, M. T. & BROOKS, A. N. (2001). Effects of nutrition and environmental factors on the fetal programming of the reproductive axis. *Reproduction*, 122(2):205-214.
- RICANATI, E. H., GOLUBIĆ, M., YANG, D., SAAGER, L., MASCHA, E. J. & ROIZEN, M. F. (2011). Mitigating preventable chronic disease: Progress report of the Cleveland Clinic's Lifestyle 180 program. *Nutrition & Metabolism*, 8(1):1.
- RINAUDO, P. & WANG, E. (2012). Fetal programming and metabolic syndrome. *Annual Review of Physiology*, 74:107.

- RIPPE, J. M. (2014). *Fructose, High Fructose Corn Syrup, Sucrose and Health*, Springer, pp38-43.
- RIZKALLA, S. W. (2010). Health implications of fructose consumption: A review of recent data. *Nutrition & Metabolism*, 7(1):1.
- ROBB, P. (1961). The development of the islets of Langerhans in the human foetus. *Quarterly Journal of Experimental Physiology and Cognate Medical Sciences*, 46(4):335-343.
- ROBINSON, M. M. & ZHANG, X. (2011). The world medicines situation 2011. Traditional medicines: global situation, issues and challenges. *World Health Organization, Geneva*.
- ROBKER, R. L. (2008). Evidence that obesity alters the quality of oocytes and embryos. *Pathophysiology*, 15(2):115-121.
- RODGERS, R. J., TSCHÖP, M. H. & WILDING, J. P. (2012). Anti-obesity drugs: past, present and future. *Disease Models & Mechanisms*, 5(5):621-626.
- RODIN, J. (1991). Effects of pure sugar vs. mixed starch fructose loads on food intake. *Appetite*, 17(3):213-219.
- ROJAS, L. B. A. & GOMES, M. B. (2013). Metformin: an old but still the best treatment for type 2 diabetes. *Diabetology & Metabolic Syndrome*, 5(1):1.
- ROSS, M. G. & DESAI, M. (2005). Gestational programming: population survival effects of drought and famine during pregnancy. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 288(1):R25-R33.
- ROY, V. K. & KRISHNA, A. (2013). The expression pattern of the glucose transporter GLUT-5 in the testis during the spermatogenic cycle of the vesperilionid bat *Scotophilus heathi*. *General and Comparative Endocrinology*, 191:59-64.
- RUBIO-RUIZ, M., HAFIDI, M. E., PÉREZ-TORRES, I., BANOS, G. & GUARNER, V. (2013). Medicinal agents and metabolic syndrome. *Current Medicinal Chemistry*, 20(21):2626-2640.
- RUEL, M. T., GARRETT, J. L. & HADDAD, L. 2008. Rapid urbanization and the challenges of obtaining food and nutrition security. *Nutrition and Health in Developing Countries*. Springer, pp639-656.
- RUI, L. (2014). Energy metabolism in the liver. *Comprehensive Physiology*, 4(1):177-197.
- RYU, S. Y., OAK, M.-H., YOON, S.-K., CHO, D.-I., YOO, G.-S., KIM, T.-S. & KIM, K.-M. (2000). Anti-allergic and anti-inflammatory triterpenes from the herb of *Prunella vulgaris*. *Planta Medica*, 66(4):358-360.

- SAINI, P., GAYEN, P., KUMAR, D., NAYAK, A., MUKHERJEE, N., MUKHERJEE, S., PAL, B. C. & BABU, S. P. S. (2014). Antifilarial effect of ursolic acid from *Nyctanthes arborescens*: Molecular and biochemical evidences. *Parasitology International*, 63(5):717-728.
- SAMUELSSON, A.-M., MATTHEWS, P. A., ARGENTON, M., CHRISTIE, M. R., MCCONNELL, J. M., JANSEN, E. H., PIERSMA, A. H., OZANNE, S. E., TWINN, D. F. & REMACLE, C. (2008). Diet-induced obesity in female mice leads to offspring hyperphagia, adiposity, hypertension, and insulin resistance A novel murine model of developmental programming. *Hypertension*, 51(2):383-392.
- SÁNCHEZ-LOZADA, L. G., MU, W., RONCAL, C., SAUTIN, Y. Y., ABDELMALEK, M., REUNGJUI, S., LE, M., NAKAGAWA, T., LAN, H. Y. & YU, X. (2010). Comparison of free fructose and glucose to sucrose in the ability to cause fatty liver. *European Journal of Nutrition*, 49(1):1-9.
- SÁNCHEZ-LOZADA, L. G., TAPIA, E., JIMÉNEZ, A., BAUTISTA, P., CRISTÓBAL, M., NEPOMUCENO, T., SOTO, V., ÁVILA-CASADO, C., NAKAGAWA, T. & JOHNSON, R. J. (2007). Fructose-induced metabolic syndrome is associated with glomerular hypertension and renal microvascular damage in rats. *American Journal of Physiology-Renal Physiology*, 292(1):F423-F429.
- SANCHEZ-LOZADA, L. G., TAPIA, E., SANTAMARIA, J., AVILA-CASADO, C., SOTO, V., NEPOMUCENO, T., RODRIGUEZ-ITURBE, B., JOHNSON, R. J. & HERRERA-ACOSTA, J. (2005). Mild hyperuricemia induces vasoconstriction and maintains glomerular hypertension in normal and remnant kidney rats. *Kidney International*, 67(1):237-247.
- SCARPACE, P. J. & ZHANG, Y. (2009). Leptin resistance: a predisposing factor for diet-induced obesity. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 296(3):R493-R500.
- SCHOCH, L. & WHITEMAN, K. (2007). Monitoring liver function. *Nursing* 37(11):22-23.
- SEEDOR, J. G. (1991). The bisphosphonate alendronate (MK-217) inhibit bone loss due to ovariectomy in rats. *Journal of Bone and Mineral Research*, 6(4):339-346.
- SEEMAN, E. (1998). Growth in bone mass and size-are racial and gender differences in bone mineral density more apparent than real? *Journal of Clinical Endocrinology and Metabolism*, 83(5):1414-1419.

- SEIDLER, N. W. 2013. Basic biology of GAPDH. *GAPDH: Biological Properties and Diversity*. Springer, pp1-36.
- SELLERS, R. S., MORTAN, D., MICHAEL, B., ROOME, N., JOHNSON, J. K., YANO, B. L., PERRY, R. & SCHAFER, K. (2007). Society of Toxicologic Pathology position paper: organ weight recommendations for toxicology studies. *Toxicologic Pathology*, 35(5):751-755.
- SENEFF, S., WAINWRIGHT, G. & MASCITELLI, L. (2011). Review paper: Is the metabolic syndrome caused by a high fructose, and relatively low fat, low cholesterol diet? *Archives of Medical Sciences*, 7(1):8-20.
- SHALLENBERGER, R. & ACREE, T. 1971. Chemical structure of compounds and their sweet and bitter taste. *Taste*. Springer, pp221-277.
- SHALLENBERGER, R. S. (2012). *Taste chemistry*, Springer Science & Business Media, pp191-196.
- SHANKAR, K., HARRELL, A., LIU, X., GILCHRIST, J. M., RONIS, M. J. & BADGER, T. M. (2008). Maternal obesity at conception programs obesity in the offspring. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 294(2):R528-R538.
- SHEN, M. & SHI, H. (2015). Sex hormones and their receptors regulate liver energy homeostasis. *International Journal of Endocrinology*, doi:10.1155/2015/294278.
- SHI, J., AISAKI, K., IKAWA, Y. & WAKE, K. (1998). Evidence of hepatocyte apoptosis in rat liver after the administration of carbon tetrachloride. *The American Journal of Pathology*, 153(2):515-525.
- SHIH, Y.-H., CHEIN, Y.-C., WANG, J.-Y. & FU, Y.-S. (2004). Ursolic acid protects hippocampal neurons against kainate-induced excitotoxicity in rats. *Neuroscience Letters*, 362(2):136-140.
- SHILS, M. E. & SHIKE, M. (2006). *Modern nutrition in health and disease*, Lippincott Williams & Wilkins, pp63.
- SHISHODIA, S., MAJUMDAR, S., BANERJEE, S. & AGGARWAL, B. B. (2003). Ursolic acid inhibits nuclear factor- κ B activation induced by carcinogenic agents through suppression of I κ B α kinase and p65 phosphorylation correlation with down-regulation of cyclooxygenase 2, matrix metalloproteinase 9, and cyclin D1. *Cancer Research*, 63(15):4375-4383.

- SHUKLA, B., VISEN, P., PATNAIK, G., TRIPATHI, S., SRIMAL, R., DAYAL, R. & DOBHAL, P. (1992). Hepatoprotective activity in the rat of ursolic acid isolated from Eucalyptus hybrid. *Phytotherapy Research*, 6(2):74-79.
- SIKOSANA, P. L. N., DLAMINI, Q. & ISSAKOV, A. (1997). *Health sector reform in sub-Saharan Africa: A review of experiences, information gaps and research needs*, Division of Analysis, Research, and Assessment, World Health Organization.
- SIMELANE, M. B., SHONHAI, A., SHODE, F. O., SMITH, P., SINGH, M. & OPOKU, A. R. (2013). Anti-Plasmodial Activity of some Zulu medicinal plants and of some triterpenes isolated from them. *Molecules*, 18(10):12313-12323.
- SIMMONS, R. (2011). Epigenetics and maternal nutrition: nature v. nurture. *Proceedings of the Nutrition Society*, 70(01):73-81.
- SINCLAIR, K. D., ALLEGGRUCCI, C., SINGH, R., GARDNER, D. S., SEBASTIAN, S., BISPHAM, J., THURSTON, A., HUNTLEY, J. F., REES, W. D. & MALONEY, C. A. (2007). DNA methylation, insulin resistance, and blood pressure in offspring determined by maternal periconceptional B vitamin and methionine status. *Proceedings of the National Academy of Sciences*, 104(49):19351-19356.
- SINGER, M. (2013). Development, coinfection, and the syndemics of pregnancy in Sub-Saharan Africa. *Infectious Diseases of Poverty*, 2(1):26.
- SINGH, A., BHAT, K., TEJ & SHARMA, P., OM (2011). Clinical biochemistry of hepatotoxicity. *Journal of Clinical Toxicology*, S4:001(doi: 10.4172/2161-0495.S4-001).
- SINGH, I. (2008). *Anatomy and physiology for nurses*, New Dehli, India, Jaypee Brothers publishers, pp190.
- SINGH, R., KAUSHIK, S., WANG, Y., XIANG, Y., NOVAK, I., KOMATSU, M., TANAKA, K., CUERVO, A. M. & CZAJA, M. J. (2009). Autophagy regulates lipid metabolism. *Nature*, 458(7242):1131-1135.
- SMITH, C. J. & RYCKMAN, K. K. (2015). Epigenetic and developmental influences on the risk of obesity, diabetes, and metabolic syndrome. *Diabetes, Metabolic syndrome and Obesity: Targets and Therapy*, 8:295.
- SOLTANI, Z., RASHEED, K., KAPUSTA, D. R. & REISIN, E. (2013). Potential role of uric acid in metabolic syndrome, hypertension, kidney injury, and cardiovascular diseases: is it time for reappraisal? *Current Hypertension Reports*, 15(3):175-181.

- SOMOVA, L., SHODE, F. & MIPANDO, M. (2004). Cardiotonic and antidysrhythmic effects of oleanolic and ursolic acids, methyl maslinate and uvaol. *Phytomedicine*, 11(2):121-129.
- SOMOVA, L., SHODE, F., RAMNANAN, P. & NADAR, A. (2003). Antihypertensive, antiatherosclerotic and antioxidant activity of triterpenoids isolated from *Olea europaea*, subspecies *africana* leaves. *Journal of Ethnopharmacology*, 84(2):299-305.
- SONG, R. (2016). Mechanism of Metformin: A Tale of Two Sites. *Diabetes Care*, 39(2):187-189.
- SOUZA-MELLO, V. (2015). Peroxisome proliferator-activated receptors as targets to treat non-alcoholic fatty liver disease. *World Journal of Hepatology*, 7(8):1012-1019.
- SPRING, B., SCHNEIDER, K., SMITH, M., KENDZOR, D., APPELHANS, B., HEDEKER, D. & PAGOTO, S. (2008). Abuse potential of carbohydrates for overweight carbohydrate cravers. *Psychopharmacology*, 197(4):637-647.
- STANHOPE, K. L., BREMER, A. A., MEDICI, V., NAKAJIMA, K., ITO, Y., NAKANO, T., CHEN, G., FONG, T. H., LEE, V. & MENORCA, R. I. (2011). Consumption of fructose and high fructose corn syrup increase postprandial triglycerides, LDL-cholesterol, and apolipoprotein-B in young men and women. *Journal of Clinical Endocrinology & Metabolism*, 96(10):E1596-E1605.
- STEPHENS, J. M. (2012). The fat controller: adipocyte development. *PLoS Biology*, 10(11):e1001436.
- STEWART, M. S., HEERWAGEN, M. & FRIEDMAN, J. E. (2013). Developmental programming of pediatric nonalcoholic fatty liver disease: redefining the "first hit". *Clinical Obstetrics and Gynecology*, 56(3):577-590.
- STOKER, H. S. (2012). *General, organic, and biological chemistry*, Boston, USA, Cengage Learning, pp884-885.
- STOOKEY, J. J. (2016). Negative, Null and Beneficial Effects of Drinking Water on Energy Intake, Energy Expenditure, Fat Oxidation and Weight Change in Randomized Trials: A Qualitative Review. *Nutrients*, 8(1):19.
- SUH, N., HONDA, T., FINLAY, H. J., BARCHOWSKY, A., WILLIAMS, C., BENOIT, N. E., XIE, Q.-W., NATHAN, C., GRIBBLE, G. W. & SPORN, M. B. (1998). Novel triterpenoids suppress inducible nitric oxide synthase (iNOS) and inducible cyclooxygenase (COX-2) in mouse macrophages. *Cancer Research*, 58(4):717-723.

- SUN, A., MAYO, A. & TURNER, S. (2015). Metabolic syndrome unravelling or unravelled? *Paediatrics and Child Health*, 25(7):308-313.
- SUN, H., FANG, W.-S., WANG, W.-Z. & HU, C. (2006). Structure-activity relationships of oleanane-and ursane-type triterpenoids. *Botanical Studies*, 47:339-368.
- SUNDARESAN, A., HARINI, R. & VISWANATHAN PUGALENDI, K. (2012). Ursolic acid and rosiglitazone combination alleviates metabolic syndrome in high fat diet fed C57BL/6J mice. *General Physiology and Biophysics*, 31(3):323.
- SWISS CONFEDERATION (2011). Fünf Methoden der Komplementärmedizin werden unter bestimmten Bedingungen während sechs Jahren provisorisch vergütet (Five CAM methods eligible for reimbursement under specific conditions for a provisional period of six years). Bundesamt für Gesundheit (press release), Swiss Confederation.
- SZYF, M. (2009). Epigenetics, DNA methylation, and chromatin modifying drugs. *Annual Review of Pharmacology and Toxicology*, 49:243-263.
- TAKAHASHI, Y. & FUKUSATO, T. (2014). Histopathology of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *World Journal of Gastroenterology*, 20(42):15539.
- TAN, H., ASHOUR, A., KATAKURA, Y. & SHIMIZU, K. (2015). A structure–activity relationship study on antiosteoclastogenesis effect of triterpenoids from the leaves of loquat (*Eriobotrya japonica*). *Phytomedicine*, 22(4):498-503.
- TAPPY, L. & LÊ, K.-A. (2010). Metabolic effects of fructose and the worldwide increase in obesity. *Physiological Reviews*, 90(1):23-46.
- TEFF, K. L., ELLIOTT, S. S., TSCHÖP, M., KIEFFER, T. J., RADER, D., HEIMAN, M., TOWNSEND, R. R., KEIM, N. L., D’ALESSIO, D. & HAVEL, P. J. (2004). Dietary fructose reduces circulating insulin and leptin, attenuates postprandial suppression of ghrelin, and increases triglycerides in women. *The Journal of Clinical Endocrinology & Metabolism*, 89(6):2963-2972.
- THAMAN, R. G. & ARORA, G. P. (2013). Metabolic Syndrome: Definition and pathophysiology the discussion goes on. *Journal of Physiology & Pharmacology Advances*, 3(3):48-56.
- THOMPSON, R. F., FAZZARI, M. J., NIU, H., BARZILAI, N., SIMMONS, R. A. & GREALLY, J. M. (2010). Experimental intrauterine growth restriction induces

- alterations in DNA methylation and gene expression in pancreatic islets of rats. *Journal of Biological Chemistry*, 285(20):15111-15118.
- THORENS, B. & MUECKLER, M. (2010). Glucose transporters in the 21st Century. *American Journal of Physiology-Endocrinology and Metabolism*, 298(2):E141-E145.
- THRALL, M. A., WEISER, G., ALLISON, R. & CAMPBELL, T. W. (2012). *Veterinary hematology and clinical chemistry*, Iowa, USA, John Wiley & Sons.
- TILG, H. & MOSCHEN, A. R. (2010). Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. *Hepatology*, 52(5):1836-1846.
- TINIAKOS, D. G., VOS, M. B. & BRUNT, E. M. (2010). Nonalcoholic fatty liver disease: pathology and pathogenesis. *Annual Review of Pathological Mechanical Disease*, 5:145-171.
- TONSTAD, S., SANDVIK, E., LARSEN, P. L. & THELLE, D. (2007). Gender differences in the prevalence and determinants of the metabolic syndrome in screened subjects at risk for coronary heart disease. *Metabolic Syndrome and Related Disorders*, 5(2):174-182.
- TORRENS, C., SNELLING, T. H., CHAU, R., SHANMUGANATHAN, M., CLEAL, J. K., POORE, K. R., NOAKES, D. E., POSTON, L., HANSON, M. A. & GREEN, L. R. (2009). Effects of pre-and periconceptional undernutrition on arterial function in adult female sheep are vascular bed dependent. *Experimental Physiology*, 94(9):1024-1033.
- TRAYNOR, J., MACTIER, R., GEDDES, C. C. & FOX, J. G. (2006). How to measure renal function in clinical practice. *BMJ*, 333(7571):733-737.
- TSAI, Y.-J., WU, M.-P. & HSU, Y.-W. (2014). Emerging health problems among women: Inactivity, obesity, and metabolic syndrome. *Gynecology and Minimally Invasive Therapy*, 3(1):12-14.
- TSANZI, E., LIGHT, H. R. & TOU, J. C. (2008). The effect of feeding different sugar-sweetened beverages to growing female Sprague–Dawley rats on bone mass and strength. *Bone*, 42(5):960-968.
- TSUKAMOTO, H., MACHIDA, K., DYNKY, A. & MKRTCHYAN, H. (2009). “Second hit” models of alcoholic liver disease. *Seminars in Liver Disease*, 29(2):178.

- VAN DEN BERGHE, G. (1985). Fructose: metabolism and short-term effects on carbohydrate and purine metabolic pathways. *Progress in Biochemical Pharmacology*, 21:1-32.
- VAN LIEFDE, I., VAN ERMEN, A., VAN WITZENBURG, A., FRAEYMAN, N. & VAUQUELIN, G. (1993). Species and strain-related differences in the expression and functionality of beta-adrenoceptor subtypes in adipose tissue. *Archives Internationales de Pharmacodynamie et de Thérapie*, 327(1):69-86.
- VANDERSCHUEREN, D., VENKEN, K., BOUILLON, R. & LEGATO, M. J. 2004. Animal models for gender-based skeletal differences. *Principles of gender specific medicine*. Elsevier, pp1043-1045.
- VELKOSKA, E. & MORRIS, M. J. (2011). Mechanisms behind early life nutrition and adult disease outcome. *World Journal of Diabetes*, 2(8):127.
- VENKEN, K., CALLEWAERT, F., BOONEN, S. & VANDERSCHUEREN, D. (2008). Sex hormones, their receptors and bone health. *Osteoporosis International*, 19(11):1517-1525.
- VERANO, J., GONZÁLEZ-TRUJANO, M. E., DÉCIGA-CAMPOS, M., VENTURA-MARTÍNEZ, R. & PELLICER, F. (2013). Ursolic acid from *Agastache mexicana* aerial parts produces antinociceptive activity involving TRPV1 receptors, cGMP and a serotonergic synergism. *Pharmacology Biochemistry and Behavior*, 110:255-264.
- VICKERS, M. H. (2011). Developmental programming of the metabolic syndrome-critical windows for intervention. *World Journal of Diabetes*, 2(9):137.
- VILÀ, L., ROGLANS, N., PERNA, V., SÁNCHEZ, R. M., VÁZQUEZ-CARRERA, M., ALEGRET, M. & LAGUNA, J. C. (2011). Liver AMP/ATP ratio and fructokinase expression are related to gender differences in AMPK activity and glucose intolerance in rats ingesting liquid fructose. *The Journal of Nutritional Biochemistry*, 22(8):741-751.
- WALL, B. T. & PORTER, C. (2014). *Carnitine Metabolism and Human Nutrition*, Taylor & Francis, pp8.
- WALLACH, J. B. (2007). *Interpretation of Diagnostic Tests*, Lippincott Williams & Wilkins, pp34-81.
- WANG, X., LI, L., WANG, B. & XIANG, J. (2009). Effects of ursolic acid on the proliferation and apoptosis of human ovarian cancer cells. *Journal of Huazhong University of Science and Technology [Medical Sciences]*, 29:761-764.

- WANG, Y., WANG, Z., SHEN, H., YIN, M. & TANG, K. (2013). Effects of artesunate and ursolic acid on hyperlipidemia and its complications in rabbit. *European Journal of Pharmaceutical Sciences*, 50(3):366-371.
- WATKINS, A. J., LUCAS, E. S., TORRENS, C., CLEAL, J. K., GREEN, L., OSMOND, C., ECKERT, J. J., GRAY, W. P., HANSON, M. A. & FLEMING, T. P. (2010). Maternal low-protein diet during mouse pre-implantation development induces vascular dysfunction and altered renin–angiotensin-system homeostasis in the offspring. *British Journal of Nutrition*, 103(12):1762-1770.
- WATKINS, A. J., URSELL, E., PANTON, R., PAPENBROCK, T., HOLLIS, L., CUNNINGHAM, C., WILKINS, A., PERRY, V. H., SHETH, B. & KWONG, W. Y. (2008). Adaptive responses by mouse early embryos to maternal diet protect fetal growth but predispose to adult onset disease. *Biology of Reproduction*, 78(2):299-306.
- WELLS, J. C. (2014). Adaptive variability in the duration of critical windows of plasticity: Implications for the programming of obesity. *Evolution, Medicine, and Public Health*, 1:109-121.
- WHO 2014. Global status report on non-communicable diseases, 2014. *World Health Organization*.
- WHO 2015. WHO traditional medicine strategy 2014–2023. . *World Health Organization*.
- WIDDOWSON, E. (1968). Growth and composition of the fetus and newborn. *Biology of Gestation*, 2(1):1-49.
- WOODS, S. C., LUTZ, T. A., GEARY, N. & LANGHANS, W. (2006). Pancreatic signals controlling food intake; insulin, glucagon and amylin. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 361(1471):1219-1235.
- WOOLEY, A. (2003). *Determination—general and reproductive toxicology*, New York, Taylor and Francis, pp80-106
- WORLD HEALTH ASSEMBLY, W. 2009. Traditional medicine. *In sixty-second World Health Assembly, Geneva, 18-22 May. Resolutions and decisions, annexes.*: World Health Organization
- WORLD HEALTH ORGANISATION, W. (2007). The world health report 2007: a safer future: global public health security in the 21st century.

- XIAO, J., SHEN, C., CHU, M. J., GAO, Y. X., XU, G. F., HUANG, J. P., XU, Q. Q. & CAI, H. (2016). Physical Activity and Sedentary Behavior Associated with Components of Metabolic Syndrome among People in Rural China. *PloS one*, 11(1).
- XU, X.-F., LV, Y., GU, W.-Z., TANG, L.-L., WEI, J.-K., ZHANG, L.-Y. & DU, L.-Z. (2013). Epigenetics of hypoxic pulmonary arterial hypertension following intrauterine growth retardation rat: epigenetics in PAH following IUGR. *Respiratory Research*, 14(20):10.1186.
- YAJNIK, C. S. & DESHMUKH, U. S. (2008). Maternal nutrition, intrauterine programming and consequential risks in the offspring. *Reviews in Endocrine and Metabolic Disorders*, 9(3):203-211.
- YAMAOKA, K. & TANGO, T. (2012). Effects of lifestyle modification on metabolic syndrome: a systematic review and meta-analysis. *BMC Medicine*, 10(1):1.
- YANG, L., LIU, X., LU, Z., CHAN, J. Y.-W., ZHOU, L., FUNG, K.-P., WU, P. & WU, S. (2010). Ursolic acid induces doxorubicin-resistant HepG2 cell death via the release of apoptosis-inducing factor. *Cancer Letters*, 298(1):128-138.
- YOSHIKUNI, K., HIRANO, K., ANDO, H., HIRAI, Y., IDA, Y., TSUJI, T., TANAKA, T., SATOUCHI, K. & TERAOKA, J. (2006). Lupane-type saponins from leaves of *Acanthopanax sessiliflorus* and their inhibitory activity on pancreatic lipase. *Journal of Agricultural and Food Chemistry*, 54(2):335-341.
- YU, S.-G., ZHANG, C.-J., XU, X.-E., SUN, J.-H., ZHANG, L. & YU, P.-F. (2015). Ursolic acid derivative ameliorates streptozotocin-induced diabetic bone deleterious effects in mice. *International Journal of Clinical and Experimental Pathology*, 8(4):3681.
- YULIANG, W., ZEJIAN, W., HANLIN, S., MING, Y. & KEXUAN, T. (2015). The hypolipidemic effect of artesunate and ursolic acid in rats. *Pakistan Journal of Pharmaceutical Sciences*, 28(3):871-874.
- ZELBER-SAGI, S., NITZAN-KALUSKI, D., GOLDSMITH, R., WEBB, M., BLENDIS, L., HALPERN, Z. & OREN, R. (2007). Long term nutritional intake and the risk for non-alcoholic fatty liver disease (NAFLD): a population based study. *Journal of Hepatology*, 47(5):711-717.
- ZHOU, C., CHEN, K., SUN, C., CHEN, Q., ZHANG, W. & LI, X. (2007). Determination of oleanolic acid, ursolic acid and amygdalin in the flower of *Eriobotrya japonica* Lindl. by HPLC. *Biomedical Chromatography*, 21(7):755-761.

- ZHU, Z., QIAN, Z., YAN, Z., ZHAO, C., WANG, H. & YING, G. (2013). A phase I pharmacokinetic study of ursolic acid nanoliposomes in healthy volunteers and patients with advanced solid tumors. *International Journal of Nanomedicine*, 8:129.
- ZOHDI, V., SUTHERLAND, M. R., LIM, K., GUBHAJU, L., ZIMANYI, M. A. & BLACK, M. J. (2012). Low birth weight due to intrauterine growth restriction and/or preterm birth: effects on nephron number and long-term renal health. *International Journal of Nephrology*, doi:10.1155/2012/136942.

APPENDIX 1: Ethics clearance certificate



STRICTLY CONFIDENTIAL

ANIMAL ETHICS SCREENING COMMITTEE (AESC)

CLEARANCE CERTIFICATE NO. 2014/49/D

APPLICANT: Ms N Mukonowenzou

SCHOOL: Physiology

LOCATION: Faculty of Health Sciences

PROJECT TITLE: *The effects of ursolic acid on neonatal programming and its potential to prevent the development of metabolic dysfunction in Sprague Dawley rats*

Number and Species

142 Sprague Dawley rats

Approval was given for the use of animals for the project described above at an AESC meeting held on 26 August 2014. This approval remains valid until 25 August 2016.

The use of these animals is subject to AESC guidelines for the use and care of animals, is limited to the procedures described in the application form and is subject to any additional conditions listed below:

None.

Signed: _____
(Chairperson AESC)

Date: 12/9/2014

I am satisfied that the persons listed in this application are competent to perform the procedures therein, in terms of Section 23 (1) (c) of the Veterinary and Para-Veterinary Professions Act (19 of 1982)

Signed: _____
(Registered Veterinarian)

Date: 11th September 2014

cc: Supervisor: Prof K Erlwanger & Dr E Chivandi
Director: CAS

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