

https://dspace.org/

Screening and diagnosis of gestational diabetes mellitus in an African population

Khambule, Lungile

2025
University of the Witwatersrand, Johannesburg
https://hdl.handle.net/10539/49995

Khambule, Lungile. (2025). Screening and diagnosis of gestational diabetes mellitus in an African population [PhD thesis, University of the Witwatersrand, Johannesburg]. WIReDSpace. https://hdl.handle.net/10539/49995

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



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

Screening and diagnosis of gestational diabetes mellitus in an African population.

By

Lungile Khambule

Student no: 458524

28/03/2025

Supervised by:

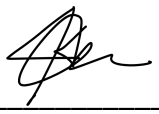
Prof. Jaya A. George

Prof. Nigel J. Crowther

Prof. Lawrence Chauke

DECLARATION

I, Lungile Khambule, hereby declare this thesis is my own. It is being submitted for the Doctor of Philosophy of Health Sciences degree at the University of the Witwatersrand, Johannesburg. It has not been previously submitted for any degree or examination at this or any other University. Please find the signed declaration in Appendix A



16/10/2025

Lungile Khambule *Date*

LIST OF PUBLICATIONS EMANATING FROM THE STUDY

The research outcomes from this PhD project were written up into four independent publications. One of these articles is a review article whilst the other three are research papers that make up the 'Results' chapters of this thesis. Two articles were accepted for publication in peer-reviewed journals, one has been submitted to a peer-reviewed scientific journal and is currently under review and the last paper is written in a scientific journal format and will be submitted to BJOG for consideration. Below is a list of original publications and the student's contribution to each. Appendix B contains the signed co-author agreement for all papers.

1. **Khambule, L**., George, J.A. (2019). The Role of Inflammation in the Development of GDM and the Use of Markers of Inflammation in GDM Screening. In: Guest, P. (eds) Reviews on Biomarker Studies of Metabolic and Metabolism-Related Disorders. Advances in Experimental Medicine and Biology, vol 1134. Springer, Cham. https://doi.org/10.1007/978-3-030-12668-1_12

2. **Khambule L**, Chikomba C, Y. Adam, L. Khan, C. Haldane, B. Vetter, J. George. Performance of point-of-care glucose testing for the diagnosis of gestational diabetes in South Africa. *Int J Gynecol Obstet*. 2024; 00:1-10. doi:10.1002/ijgo.15914

3. **Khambule L**, Chauke L, Crowther N.J, George J.A. Fasting plasma glucose performs better than glycated haemoglobin and glycated albumin for diagnosing gestational diabetes. Submitted to the *Int J Gynecol Obstet* and waiting for feedback.

4. **Khambule L**, Crowther N.J, Chauke L George J.A. The use of novel biomarkers for the diagnosis of gestational diabetes mellitus in Black African women. Written in a scientific journal format and will be submitted to *BJOG*.

Contributions of the PhD student

The student was responsible for the management of the project, which included data collecting, sample collection and analysis, method development and validation. Additionally, the student was in charge of the general design of the study and the conceptualisation of each sub-study. In addition to writing and revising each publication, the student was in charge of cleaning the data, doing statistical analysis, and interpreting the results. The manuscripts were critically reviewed, and the co-authors contributed with methodological advice and statistical assistance. Appendix B contains the signed co-author agreement for each publication used in the current thesis.

LIST OF PRESENTATIONS EMANATING FROM THE STUDY

1. **Khambule L**, Chauke L, Crowther N.J, George J.A. *Fasting plasma glucose is a cost-effective alternate method for diagnosing GDM in Black African women at high risk.* Oral presentation at the Johannesburg Health District Conference, JHB, South Africa, August 2024.
2. **Khambule L**, Chikomba C, Y. Adam, L. Khan, C. Haldane, B. Vetter, J. George. *Evaluation of glucose point-of-care glucometers against the laboratory hexokinase method for gestational diabetes mellitus diagnosis.* Oral presentation at the Society for Endocrinology, Metabolism, and Diabetes of South Africa (SEMDSA) Congress, Johannesburg, South Africa, August 2023.
3. **Manga J**, Odell N, Khambule L, Mohammed F. *Glycaemic characteristics, maternal and neonatal outcomes in antenatal patients with pregestational and gestational diabetes attending Charlotte Maxeke Johannesburg Academic Hospital, South Africa*. Poster presentation at the SEMDSA Congress Johannesburg, South Africa, August 2023.
4. **Khambule L**, Chikomba C, Y. Adam, L. Khan, C. Haldane, B. Vetter, J. George. *Performance of glucometers for the diagnosis of gestational diabetes.* Oral presentation at the Johannesburg Health District Conference, Johannesburg, South Africa, November 2023.
5. **Khambule L**, Chauke L, Crowther N.J, George J.A. *Glycated haemoglobin and fasting glucose levels are sensitive markers for gestational diabetes in the Black African population.* Oral presentation at SEMDSA Congress, Johannesburg, South Africa, September 2022.
6. **Khambule L**, Chauke L, Crowther N.J, George J.A. *Glycated haemoglobin and fasting glucose levels are sensitive markers for gestational diabetes in the black African population.* Poster presentation at Faculty of Health Sciences Research Day, Wits University, Johannesburg, South Africa, September 2022.
7. **Khambule L.** *Gestational Diabetes screening.* Oral presentation at The BRICS Young Scientist Forum. Virtual, September 2021.
8. **Khambule L.** *Gestational Diabetes.* Seminar presentation at the Department of Chemical Pathology, Wits University, Johannesburg, South Africa, May 2021

Non–scientific presentation

- Competed at the Wits Science Slam in July 2019 at Wits University and presented Gestational Diabetes to High School learners.

LIST OF GRANTS AND AWARDS EMANATING FROM THE STUDY

1. Recipient of a University of the Witwatersrand Staff Bursary for PhD fees (2018-2024).
2. Co-recipient with Prof N Crowther of National Health Laboratory Service (NHLS) Research Trust -R99 938.00 (2024).
3. Co-recipient with Prof N Crowther of Iris Ellen Hodges Cardiovascular Research Trust- R40 000.00 (2023).
4. Co-recipient with Prof J George of Foundation for Innovative New Diagnostics (FIND) grant - R500 000.00 (2022).
5. Recipient of SEMDSA Research Top-up Bursary - R50 000.00 (2022).
6. Recipient of SEMDSA best presentation Award - R5000.00 (2022).
7. Recipient of Female Academic Leadership Fellowship (FALF) Research grant - R211359.00 (2021).
8. Recipient of University of the Witwatersrand Faculty Research Committee (FRC) individual research grant - R4200.00 (2019).
9. Recipient of University of the Witwatersrand Early Career Academic Development (ECAD) - R25 000.00 (2019).
10. Recipient of National Research Foundation (NRF) Thuthuka- R514 894.00 (2018).

SUMMARY

Background: Gestational diabetes mellitus (GDM) is defined as hyperglycaemia that is discovered for the first time during pregnancy and is not overt. The prevalence of GDM in South Africa varies between 9.1% and 25%. Mothers with GDM are at increased risk of preeclampsia, caesarean delivery, and preterm birth. Similarly, babies born to GDM mothers are at increased risk of macrosomia, hypoglycaemia, respiratory distress, hypocalcaemia, hypomagnesaemia and Type 2 diabetes(T2D) later in life. Therefore, efficient screening and diagnosis remain the cornerstone for reducing GDM-related maternal and neonatal morbidity and mortality. The oral glucose tolerance test (OGTT) is the current gold standard for screening for GDM. However, in South Africa, the test is not universally accessible as only pregnant women with GDM-related risk factors are tested. This approach is likely to miss a significant number of women with GDM.

Aim: The overall aim of this research is to improve the current screening and diagnostic methods for GDM. Consequently, our first objective was to evaluate the performance of several point-of-care (POC) glucometers against the standard laboratory-based glucose measurements during an OGTT. The second objective was to evaluate the use of glycaemic markers namely, fasting plasma glucose (FPG), glycated albumin (GA) and glycated haemoglobin (HbA1c), for the diagnosis of GDM. The third objective was to determine the associations of various candidate biomarkers with glycaemia in both early and late pregnancy in Black African pregnant women.

Methods: This is a prospective cross-sectional study that was carried out at five different health facilities in Johannesburg, South Africa. Pregnant Black African women under 21 weeks of gestation (early pregnancy) and at 24-32 weeks of gestation (late pregnancy) were recruited and stratified into three groups. The women in late pregnancy (groups 1 and 2) were tested for GDM using the 75 g OGTT and classified into GDM and non-GDM following the International Association of Diabetes and Pregnancy Study Group (IADPSG) guidelines. Seven glucometers were compared to laboratory-based glucose measurements for GDM diagnosis using blood samples from the OGTT (Group 1). In the second group of late-pregnancy women (Group 2), candidate biomarkers (TNFα, IL-6, free fatty acids (FFAs), branched-chain amino acids and acylcarnitines) and known markers of glycaemia (FPG, HbA1c and GA) were assessed for their ability to diagnose GDM using the OGTT as the reference. Fasting and random blood samples were taken in women in early pregnancy (group 3), and candidate biomarkers and blood glucose were measured. Univariate correlations and multivariate regression analysis were performed to determine the relationship between glycaemia and

candidate biomarkers in early and late pregnancy, and their discriminatory power was assessed by receiver-operator characteristic (ROC) curve analysis.

Results: A total of 1716 women were recruited: 1076 from pregnancy (group 1), 477 also from late pregnancy (group 2), and 163 from early pregnancy (group 3).

For the glucometer study (Group 1), 83 (7.7%) were classified as GDM. The sensitivity of the glucometers for GDM diagnosis ranged from 17.6% to 87.18% and their specificity from 62.7% to 99.8%. The area under the ROC curve (AUC) was between 0.59 and 0.79. However, the laboratory-based FPG showed better performance than the glucometers in terms of sensitivity, specificity, and AUC, with levels of 94.0%, 100%, and 0.98, respectively.

Building on the observations from the Group 1 study, which showed potential for diagnosing GDM using a single test i.e. FPG, we subsequently compared the efficacy of FPG, glycated haemoglobin (HbA1c), and glycated albumin (GA), for the diagnosis of GDM in the Group 2 women. Overall, FPG at a 5.1mmol/L cut point performed better than HbA1c and GA, with sensitivity and specificity of 87% and 100% respectively. At the 5.5.% cut-off point, HbA1c had sensitivity and specificity of 54% and 80% respectively and GA, had a sensitivity and specificity of 54% and 83%, respectively at a cut point of 37.6%. Based on the selective screening criteria currently used, all the women in group 2 had one or more risk factors for GDM and as a result, would have qualified for OGTT. If FPG was then used as a second screening test, it would have averted the need for OGTTs in 77% of these women.

Thereafter, we sought to identify novel biomarkers for GDM diagnosis. Metabolites and proteins known to be linked to GDM were measured in 163 women below 21 weeks of pregnancy (Group 3) and 477 women between 24-32 weeks of pregnancy (Group 2). Free fatty acids (FFAs), and isoleucine were positively associated with GDM whilst acylcarnitine C2 demonstrated an inverse association. The sensitivity and specificity of these candidate biomarkers for the diagnosis of gestational diabetes mellitus (GDM) were inadequate, and they did not exhibit a significant association with fasting or random plasma glucose levels during early pregnancy, with the exception of valine, which demonstrated a significant correlation with random plasma glucose. (p=0.0029)

Conclusions: The current study showed that the performance of laboratory-based FPG measurements for GDM diagnosis is better than HbA1c, GA, and glucometers. Furthermore, we observed that a two-tiered approach utilising FPG would reduce the number of OGTTs required for

women diagnosed with GDM. In addition, valine has the potential to be used as an early marker of GDM, however, this would need to be validated in longitudinal studies.

ACKNOWLEDGEMENTS

- To my Spirit guides for the love, strength, and words of wisdom throughout my journey.
- Thank you to my family for the patience, love, and support.
- To my supervisor, Professor Jaya Anna George, thank you for seeing the greatness in me when I didn't. Thank you for your encouragement and for shaping the scientist in me.
- To my co-supervisors, Professor Nigel Crowther and Professor Lawrence Chauke, thank you for your gentle teaching, encouragement and patience.
- To Dr Wise, Dr. Ohaju and Dr. Ramogale-Zungu, thank you for supporting the study and for allowing me to come into the lives of the participants for the greater good.
- To Dr. Marketa Toman and Mrs. Tracy Snyman, thank you for taking the time to train me and for imparting your knowledge to me.
- To the staff at the antenatal clinic of Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), Rahima Moosa Mother and Child Hospital (RMMCH), Hillbrow Community Health Centre (HCHC) and Edenvale Hospital, thank you for your contribution to the study.
- To the staff at the National Health Laboratory Service (NHLS), CMJAH and RMMCH, thank you for your assistance.
- To my cheerers, at home and work who always gave me hope and strength to push through.
- A big thanks to the participants in the study, I am forever grateful for this experience, and I will forever keep it close to my heart.
- The study would not be possible without financial assistance from the following institutions and organisations:

- NHLS, University of the Witwatersrand, Iris Ellen Hodges Cardiovascular Research Trust, SEMDSA, FALF, NRF and FIND.

Contents

DECLARATION ii
LIST OF PUBLICATIONS EMANATING FROM THE STUDY i
LIST OF PRESENTATIONS EMANATING FROM THE STUDY i
Non–scientific presentation i
LIST OF GRANTS AND AWARDS EMANATING FROM THE STUDY i
SUMMARY i
ACKNOWLEDGEMENTS iv
LIST OF FIGURES ix
LIST OF TABLES xi
LIST OF ABBREVIATIONS xii
THESIS FORMAT 1
CHAPTER 1: LITERATURE REVIEW 2
1.1 EPIDEMIOLOGY 3
1.1.1 Diabetes in pregnancy definitions 3
1.1.2 Prevalence 4
1.1.3 Gestational Diabetes in South Africa 5
1.1.4 Impact of GDM on Maternal and Foetal Health 7
1.2 PATHOPHYSIOLOGY 9
1.2.1 Insulin Resistance and β-cell Dysfunction 9
1.2.2 The Role of Hormones in Inducing Insulin Resistance. 11
1.2.3 Low-grade Chronic Inflammation and the development of GDM 11
1.2.4 Branched-Chain Amino Acids' Role in Insulin Resistance 13
1.2.5 Free Fatty Acids Role in Insulin Resistance 14
1.2.6 Genetic and Epigenetic Factors Contributing to GDM 15
1.3 DIAGNOSTIC METHODS FOR GDM 17
1.3.1 Definitions 17
1.3.2 Overview of Conventional Diagnostic Criteria for GDM 17
1.3.3 Limitations and Challenges of Current Diagnostic Methods 20
1.3.4 Recent Advancements and Modifications in Diagnostic Criteria 22
1.4 SUMMARY OF THE LITERATURE REVIEW 25
1.5 AIMS, OBJECTIVES AND HYPOTHESES OF THE STUDY 25
CHAPTER 2: STUDY DESIGN AND METHODS 27
2.1 STUDY DESIGN 27
2.1.1 Women in late pregnancy 27
2.1.2 Women in early pregnancy 30

2.2 SAMPLE COLLECTION 31
2.2.1 Oral glucose tolerance test and GDM diagnostic criteria 32
2.3 SAMPLE ANALYSIS 32
2.3.1 Plasma glucose and glycated albumin analysis by automated instruments 32
2.4 DATA ANALYSIS 33
2.5 ETHICS 33
CHAPTER 3: PERFORMANCE OF POC GLUCOSE TESTING FOR THE DIAGNOSIS OF GESTATIONAL DIABETES IN SOUTH AFRICA 34
3.1 ABSTRACT 34
3.2 INTRODUCTION 35
3.3 MATERIALS AND METHODS 35
3.3.1 Study design 35
3.3.2 Study population 36
3.3.3 Medical history and sample collection 36
3.3.4 Sample collection 36
3.3.5 Oral glucose tolerance test 36
3.3.6 Statistical analysis 36
3.4 RESULTS 37
3.4.1 Study population 37
3.4.2 Method agreement and reliability analysis 39
3.4.3 Accuracy of POC glucometers 48
3.5. DISCUSSION 50
3.6 CONCLUSION 53
3.7 SUPPLEMENTARY MATERIAL 54
CHAPTER 4: FASTING PLASMA GLUCOSE PERFORMS BETTER THAN GLYCATED HAEMOGLOBIN AND GLYCATED ALBUMIN FOR DIAGNOSING GESTATIONAL DIABETES 60
4.1 ABSTRACT 60
4.2 INTRODUCTION 61
4.3 METHODOLOGY 62
4.3.1 Study design and population 62
4.3.2 Ethics 62
4.3.3 Sample Collection and Analysis 62
4.3.4 Oral glucose tolerance test 63
4.3.5 Glycated haemoglobin (HbA1c) and glycated albumin (GA) measurement 63
4.3.6 Statistical analysis 63
4.4 RESULTS 64
4.4.1 Participants 64
4.4.2 Clinical characteristics of GDM and non-GDM women 65

4.4.3 Comparison of GDM risk factors between those with and without GDM 66
4.4.4 Sensitivity and specificity of fasting plasma glucose, glycated haemoglobin and glycated albumin 68
4.5 DISCUSSION 70
4.6 CONCLUSION 73
CHAPTER 5: THE USE OF NOVEL BIOMARKERS FOR THE DIAGNOSIS OF GESTATIONAL DIABETES MELLITUS IN BLACK AFRICAN WOMEN 74
5.1 ABSTRACT 74
5.2 INTRODUCTION 75
5.3 METHODOLOGY 77
5.3.1 Study design 77
5.3.3 Sample and data collection 77
5.3.4 Biochemical analysis 78
5.3.5 Data analysis 80
5.4 RESULTS 81
5.4.1 Study population description 81
5.4.2 Biomarkers for GDM in late pregnancy 84
5.4.3 Sensitivity and specificity of the biomarkers 85
5.4.4 Correlations of biomarkers with fasting and random blood glucose in early pregnancy 88
5.5 DISCUSSION 89
5.6 CONCLUSION 92
5.7 SUPPLEMENTARY MATERIAL 93
CHAPTER 6: CONSOLIDATED DISCUSSION 107
6.1 SUMMARY OF THE STUDY OUTCOMES 107
6.2 HYPOTHESES REVISITED 109
6.3 CONSOLIDATED DISCUSSION 109
6.3.1 Strengths and limitations 111
6.3.2 Future studies and recommendations 112
6.3.3 Conclusion 114
REFERENCES 115
APPENDIX A: PLAGIARISM DECLARATION 129
APPENDIX B: CO-AUTHOR DECLARATION 130
APPENDIX C: QUESTIONNAIRE 132
APPENDIX D: INFORMATION SHEET AND CONSENT FORM 133
APPENDIX E: ETHIC CERTIFICATES 136
APPENDIX F: PUBLISHED PAPERS 138
APPENDIX G: TURN -IT-IN REPORT 181

LIST OF FIGURES

Figure 1.1 Median (interquartile range) prevalence (%) of gestational diabetes mellitus (GDM) by WHO region, 2005–2018 (map generated from WHO website) [4].. 5

Figure 1.2 BMI distribution in adults by gender in South Africa. 6

Figure 1.3 This figure illustrates the main steps of insulin action including insulin binding, signalling cascade, GLUT-4 exocytosis and translocation. Created in BioRender. (2025) https://BioRender.com/t32q818.....10

Figure 1.4 The chemical structure of branched-chain amino acids Created in BioRender. (2025) https://BioRender.com/o24h764 13

Figure 1.5 Mean (±SD) insulin sensitivity during a euglycaemic-hyperinsulinaemic clamp in pregnant women. GDM=Gestational diabetes mellitus, Pt= p value for individual longitudinal changes with time, Pg= p value for difference between groups. Taken from Catalano (1991) [115]... 16

Figure 1.6 The general reaction for glycation of a protein. Taken from Anguizola et al. 2013 (129)..... 23

Figure 2.1 Participants' recruitment flow diagram. Created in https://BioRender.com.....29

Figure 3.1. Diagram of participant recruitment, OGTT = oral glucose tolerance test ……40

Figure 3.2. Scatter plots with the least squares best-fit line + 95% confidence band (blue) and line y=x (orange) of each glucometer against the laboratory at fasting ……..42

Figure 3.3. Scatter plots with the least squares best-fit line + 95% confidence band (blue) and line y=x (orange) of each glucometer against the laboratory at 1-hour post glucose load……43

Figure 3.4. Scatter plots with the least squares best-fit line + 95% confidence band (blue) and line y=x (orange) of each glucometer against the laboratory at 2-hour post glucose load……44

Figure 3.5. Bland-Altman plots ((mean difference (blue) and 95% limit of agreements (red)) of each point of care glucometer against laboratory-based measurements at fasting. A1= Accucheck active, B1 = Codefree, C1= I-STAT, D1= LDX, E1 = Statstrip, F1= Vivacheck and G1=Xpress……46

Figure 3.6. Bland-Altman plots ((mean difference (blue) and 95% limit of agreements (red)) of each point of care glucometer against laboratory-based measurements at 1-hour post glucose load. A2= Accucheck active, B2 = Codefree, C2= I-STAT, D2= LDX, E2 = Statstrip, F2= Vivacheck and G2=Xpress……47

Figure 3.7. Bland-Altman plots ((mean difference (blue) and 95% limit of agreements (red)) of each point of care glucometer against laboratory-based measurements at 1-hour post glucose load. A3= Accucheck active, B3 = Codefree, C3= I-STAT, D3= LDX, E3 = Statstrip, F3= Vivacheck and G3=Xpress……..48

Figure 4.1 Flow diagram of the recruitment process ……..65

Figure 4.2 ROC curve analysis of GDM diagnosis by fasting plasma glucose (FPG), glycated albumin (GA) and glycated haemoglobin (HbA1c)……69

Figure 4.3 The comparison between a two-tiered diagnostic method (A) and the OGTT (B), created in https://BioRender.com.....72

Figure 5.1. Flow diagram of the recruitment process ……84

Figure 5.2 Receiver operating characteristic (ROC) curve analysis for GDM diagnosis by glycated albumin (GA), free fatty acids (FFAs), isoleucine and C2-carnitine. The OGTT is the reference method……88

Figure 5.3. Illustration of the catabolic process of branched-chain amino acids (BCAA) and free fatty acid (FFAs), and the TCA cycle. Key enzymes, transporters, and intermediates are shown, highlighting the transportation of acyl-CoA to the mitochondrial matrix for oxidation. CAT= carnitine acylcarnitine transferase, FAO=Fatty acid oxidation. Created in https://BioRender.com.....92

LIST OF TABLES

Table 1.1 Short- and long-term complications observed in mother and child following GDM diagnosis. 8

Table 1.2 Conventional diagnostic criteria for GDM before "harmonization". 18

Table 1.3 Latest recommendations from International and national bodies 20

Table 1.4 Risk factors for GDM. 22

Table 3.1 Clinical characteristics in all women and by GDM status 39

Table 3.2 Median glucose measured by glucometers at each OGTT time point 41

Table 3.3 Average difference, Bland–Altman 95%-limits of agreement, intraclass correlation 45

Table 3.4 The accuracy of each glucometer when compared to laboratory-based measurements 50

Table 3.5 The comparison of missed GDM cases by Statstrip with true GDM cases 51

Table 4.1 The prevalence of GDM by site 66

Table 4.2 The clinical characteristics of all women and by GDM status 67

Table 4.3 The GDM risk factors, overall and by GDM status 68

Table 4.4 Accuracy of glycated haemoglobin, glycated albumin and FPG when compared to all OGTT time points 70

Table 5.1 Demographic and clinical characteristics of participants 85

Table 5.2 Demographic and clinical characteristics of women in late pregnancy by GDM 86

Table 5.3 Potential biomarkers for GDM screening stratified by GDM. 87

Table 5.4 The association of the discriminatory biomarkers with glycaemia in late pregnancy. 88

Table 5.5 Multivariate logistic regression model for GDM 89

Table 5.6 Accuracy of biomarkers in late pregnancy. 89

Table 5.7 Correlations of the biomarkers with fasting and random plasma glucose in early pregnancy. 90

Table 6.1 Summary of the Consolidated Discussion 109

LIST OF ABBREVIATIONS

AAA	Aromatic amino acid
AC	Acylcarnitine
AUC	Area under ROC curve
Acyl-CoA	Acyl-Coenzyme A
β-cells	Beta-cells
BCAAs	Branched-chain amino acids
BCAT	Branched chain aminotransferase
BCKA	Branched chain α keto acid
BCKDC	Branched chain α-keto acid dehydrogenase complex
BMI	Body mass index
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
CVD	Cardiovascular disease
ELISA	Enzyme-linked immunosorbent assay
FAO	Fatty acid oxidation
FFAs	Free fatty acids
FPG	Fasting plasma glucose
GA	Glycated albumin
GDM	Gestational diabetes melltus
GLUT-4	Glucose Transporter Type 4
HAPO	Hyperglycaemia and Adverse Pregnancy Outcome
HbA1c	Glycated albumin
IADPSG	International Association of Diabetes and Pregnancy Study Group
IL-6	Interleukin-6
IQC	Internal quality controls
IR	Insulin resistance
IRS-1	insulin receptor substrate
KIC	ketoisocarprate
KIM	α-keto-β-methylglutarate
KIV	α-ketoisovalerate
LCMS/MS	Liquid chromatography-tandem mass spectrometry
LMICs	Low-middle-income countries
MCP-1	Macrophage chemotactic protein-1
mmHg	Millimetres of mercury
mTOR	Mammalian target of rapamycin
OGTT	Oral glucose tolerance test
POC	Point-of-care
ROC	Receiver operating characteristic
RMMCH	Rahima Moosa Mother and Child Hospital
SA	South Africa
SAA	Sub- Saharan Africa
SCI	systematic chronic inflammation

SEMDSA	South African Society of Endocrinology, Metabolism and Diabetes of South Africa
T1D	Type 1 diabetes
T2D	Type 2 Diabetes
TCA	Tricarboxylic acid cycle
TNF-α	Tumor necrosis factor-α
WHO	World Health Organization
WHR	Waist-to-hip ratio

Units and symbols

The International System of Units (SI) has been used throughout this thesis.

%	Percent
°C	Degrees Celcius
g	Gram
g/L	Gram per litre
kg	Kilogram
L	Litre
mmol/L	Millimol per litre
µL	Microlitre
>	Greater than
<	Smaller than
≥	Greater than or equal to
±	Plus/minus α alpha β beta

THESIS FORMAT

The thesis begins with a detailed literature review (Chapter 1), followed by a methods chapter (Chapter 2) describing the procedures that were common to all the papers that make up three results chapters (Chapters 3 to 5). Each of the results chapters includes a description of the methods that were specific to that study and refers to the methods chapter for those methods that are shared across the studies. Finally, as part of the integrated discussion (Chapter 6), each paper's results are summarized. In addition, an overview of the thesis and how the papers within it address the challenges in the field of study.

CHAPTER 1: LITERATURE REVIEW

Overview

This literature review begins with a description of the epidemiology and the definitions of gestational diabetes mellitus (GDM). This section also addresses GDM in the context of South Africa, focusing on the factors that may lead to the development of GDM among women living in urban areas. This information seeks to deepen the readers' understanding of the demographics studied. Subsequently adverse effects associated with GDM are outlined.

The second section of the literature review outlines the pathophysiology of GDM and examines the potential role of selected biomarkers in its development.

The third section deals with an examination of current screening and diagnostic methods for GDM including limitations. This section aims to elucidate the significance of exploring alternative methods for GDM screening and diagnosis, especially for public health facilities. Thereafter, current literature on the associations between selected biomarkers and GDM is reviewed while highlighting existing gaps in the research.

The chapter concludes with a list of aims, objectives, and hypotheses.

1.1 EPIDEMIOLOGY

1.1.1 Diabetes in pregnancy definitions

Diabetes can manifest itself in a variety of ways during pregnancy. In this section, each type of diabetes in pregnancy including GDM is described.

Pregestational diabetes refers to diabetes that develops before pregnancy. Affected women undergo therapy very early on, even before conception in preparation for pregnancy. Hyperglycaemia in pregnancy, also known as diabetes in pregnancy is a collective term for overt diabetes and gestational diabetes discovered for the first time during pregnancy. Thus, GDM can be classified as hyperglycaemia that is discovered in pregnancy, and which does not meet the diagnostic criteria for overt diabetes.

The criteria that are used to distinguish GDM from overt diabetes are the levels of glucose from the oral glucose tolerance test (OGTT). Following the criteria from the International Association of Diabetes and Pregnancy Study Group (IADPSG), women with GDM must have one or more of the following glucose cut-off levels: 5.1 - 6.9 mmol/L fasting, 10.0 mmol/L at 1 hour after glucose load, or 8.5 - 10.9 mmol/L after glucose load at 2 hours at 24 to 28 weeks of gestation. This was formulated from the Hyperglycaemia and Adverse Pregnancy Outcome (HAPO) study which found that at these levels the odds for birth weight, cord C-peptide, and percent body fat being >90th percentile reached 1.75 [1].

The women with overt diabetes should have fasting blood glucose (FPG) and a 2-hour post-glucose load above the cut-off levels for GDM [1]. The cut-off levels for GDM which were formulated following the outcome from the

The distinction between GDM and overt diabetes is important because of the different therapeutic approaches. Women with overt diabetes are likely to be treated by insulin therapy [1] because of their risk for diabetes-related complications such as nephropathy and retinopathy and their offspring are at high risk for congenital anomalies. Despite that GDM is associated with a lesser degree of complications than overt diabetes, its complications are those that should be prevented as they impact both the mother and the child in the long-term. Therefore, screening and diagnostic methods for GDM should be accurate and precise to reduce complications in those affected by GDM.

1.1.2 Prevalence

Historically, infectious diseases were the predominant cause of mortality in South Africa, primarily attributed to HIV/AIDS. However, the prevalence of non-communicable disease, particularly diabetes, is rising rapidly and is now ranked second globally and nationally based on the systematic analysis of the global and national disability-adjusted life years (DALY) systematic analysis from 1990 to 2021 [2]. Non-communicable diseases were second to Coronavirus disease 2019 (COVID-19). The DALY is for objectively assessing the impact of diverse diseases and conditions on individuals' productivity and well-being. It incorporated both mortality and morbidity estimates. The international diabetes federation (IDF) estimates that 19.7% of live births in 2024 were affected by hyperglycaemia in pregnancy, with 79% of cases due to gestational diabetes mellitus (GDM) [3]. Globally, there are considerable variations in the prevalence of GDM amongst different groups, ranging from 1% to 15%, with low and middle-income countries (LMICS) contributing more than 90% of the women with GDM (see Figure 1.1 for the prevalence by World Health Organization (WHO) regions) [4,5]. The highest prevalence is in the Middle East and North Africa at 15.2%, and the least in Europe at 6.1%. The disparities in the prevalence of GDM globally is mainly driven by the differences in ethnicities but the environment and socio-economic determinants of health such as access to healthcare, economic stability, discrimination, migration, lifestyle and other sociocultural factors contribute to the variations in prevalence.

This varying prevalence is also evident among subpopulations in sub-Saharan Africa. This could be contributed to the varying methodologies and diagnostic criteria making estimation of the true prevalence very difficult. Macauley et al.[6] in a meta-analysis of 14 African studies estimated a prevalence ranging from 0% in Tanzania to 13·9% in Nigeria. In contrast, a meta-analysis of 22 African studies by Mwanri et al. [7] estimated the prevalence of GDM to be between 2% and 6% in Africa. In South Africa (SA), the prevalence of GDM is estimated to range between 5,7% and 25,8% [8–10]. Globally, in attempt to harmonise the GDM criteria, several health organisations have adopted the IADPSG criteria [11–13]. Nevertheless, there is a significant amount of disparity in South Africa with respect to the screening and diagnostic methodologies for GDM. This will be discussed later in this chapter.

The transition from other criteria to IAPDSG criteria will likely cause a spike in the prevalence of GDM because of the lower cut-off points of the IADPSG criteria compared to the other

criteria. The diagnostic criteria are discussed later in this chapter. Considering that GDM is among the most common complications during pregnancy and is estimated to rise globally with the rise in T2D and obesity, efficient GDM diagnostics and early management of the disease need to be prioritised.

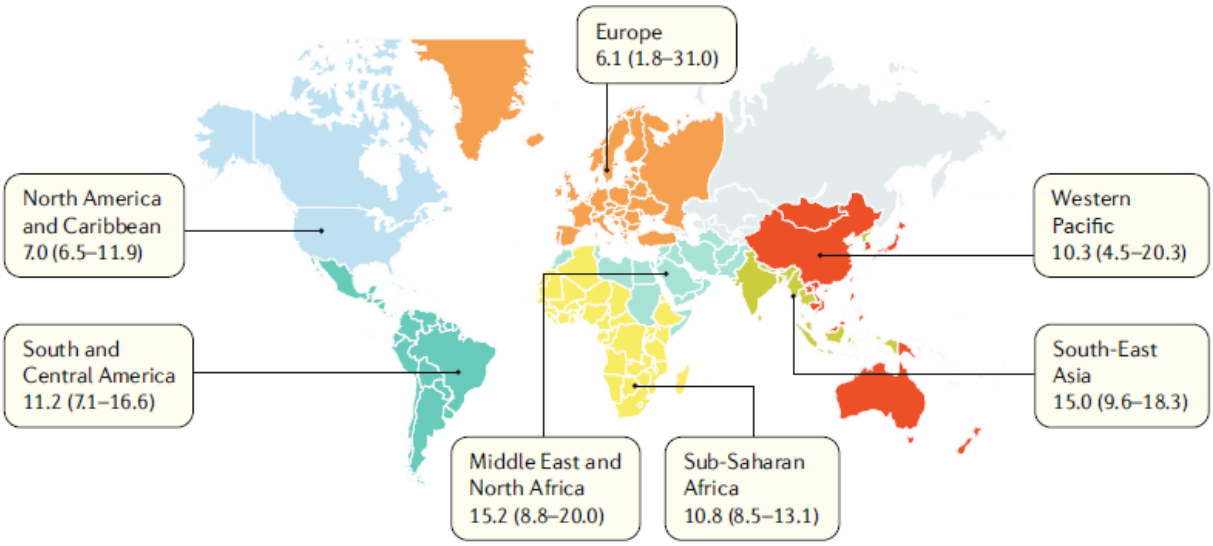


Figure 1.1 *Median (interquartile range) prevalence (%) of gestational diabetes mellitus (GDM) by WHO region, 2005–2018 (map generated from WHO website) [5].*

1.1.3 Gestational Diabetes in South Africa

There has been a noticeable rise in the occurrence of GDM in multiple countries over time. This increase appears to be linked to the increasing prevalence of obesity globally. The increase in the prevalence of obesity and GDM may be largely driven by the nutrition transition [14]. The term nutrition transition refers to changes in food intake and energy expenditure that occur in conjunction with economic, demographic, and epidemiological transitions [15]. Generally, nutrition transition is observed when individuals transition from consuming ethnic food to adopting more Western diets and engaging in fewer physical activities. In SA, there is a trend where people from rural areas move to cities (urban areas) in search for job opportunities[16]. The move from rural to urban environments coincides with changes in food and increased dependence on technology, for example, people in the cities drive more often than walk. As a result, they engage in less physical exercise, which increases their risk of obesity [17].

The discussion of the nutrition transition is incomplete without acknowledging fundamental

socio-economic changes in SA that may play a role in increasing obesity rates. The unemployment rate is currently at its highest level, with 47.3% (95%CI 49.9-55.6) of household heads being unemployed. This is more pronounced for women of childbearing age (60.7% in females vs. 33.2% in males) [18]. There was a significant rise in the unemployment rate from 32.6% in 2021 to 47.3% in 2023 following the COVID-19 pandemic in 2019 due to working people being retrenched from work [19]. Unemployment raises poverty rates and negatively impacts the country's economy. As a result, an increasing number of people choose to buy less expensive food, which may not always be conducive to their health due to the low nutritional value. Many individuals end up putting on weight increasing their risks of developing T2D and for women, GDM. Studies suggest that impoverished communities are likely to have poor health and lower life expectancy [20,21].

In SA, there are more women who are obese than men (41.3 vs 15.3%) as shown in Figure 1.2 [18]. Furthermore, a greater percentage of women have a waist-to-hip ratio (WHR) greater than 0.85, (52% vs. 11.2%). A WHR greater than 0.85 is linked with an increased risk of developing T2D and hypertension [18]. Together, these figures indicate that majority of women in SA are obese and those in the reproductive age group are likely at risk of developing GDM during pregnancy.

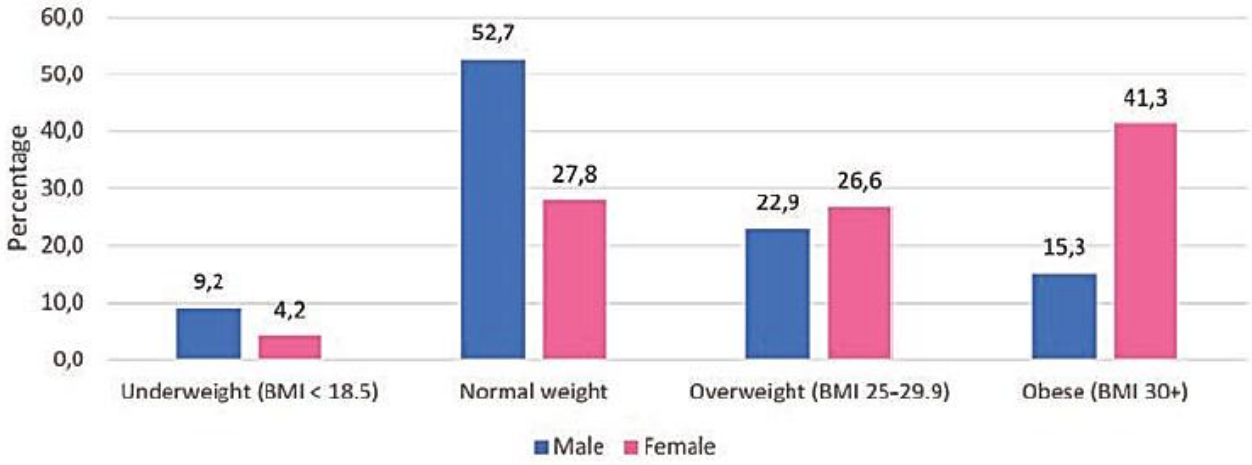


Figure 1.2 *BMI distribution in adults by gender in South Africa [18].*

Access to healthcare is one of the socioeconomic factors that influence health outcomes. Even though antenatal care services are accessible at no cost to women in South Africa,

testing for hyperglycemia in pregnancy is limited to a few public health facilities which are likely tertiary hospitals [22]. When screening for GDM using the risk factors criteria is used at the local clinics, the women are often referred to the nearest hospitals equipped to perform diagnostic testing. However, these hospitals are often far from where the women live, forcing them to spend money on transport, take time off work, and make additional arrangements to attend the test. In some hospitals, women are even required to purchase their own glucose powder from local pharmacies. These logistical and financial barriers create significant inconvenience, leading some women to forgo GDM diagnosis altogether. The referral system is not without challenges as many hospitals are overcrowded and often health workers have to stretch their limited resources to do their daily tasks. As a result, many women choose to stay at home. Based on the above and other public health system challenges, pregnant women with GDM are likely to be missed within the South African health system [23]. Although some hospitals are equipped for GDM testing, resources are not distributed equally across facilities. As a result, hospitals often adopt diagnostic criteria that align with their available budget and resources, leading to inconsistencies in testing practices and standards of care [24]. Aside from the logistics of administering the GDM test, there appears to be a knowledge gap regarding the significant indirect costs such as absence from work or from usual activities, childcare, and travel costs, which in some countries may be greater than the direct costs of testing. Therefore, GDM screening and diagnostic tools in SA should take into consideration cost implications both direct and indirect.

1.1.4 Impact of GDM on Maternal and Foetal Health

Women who have GDM typically return to euglycaemia following childbirth. However, the diagnosis of GDM can lead to complications for both the mother and the infant, both in the short and long term [25,26]. Therefore, it is crucial to ensure proper testing and treatment. If women are not identified during their antenatal visits, they may not be screened for long-term complications after pregnancy.

According to the HAPO study outcomes, moderate glucose intolerance in pregnancy correlated with birth weight, cord-blood serum C-peptide level, primary caesarean delivery, neonatal hypoglycaemia and maternal blood glucose levels [27]. Other known complications of GDM are preeclampsia, preterm delivery (defined as delivery before 37 weeks gestation),

shoulder dystocia or birth injury, hyperbilirubinaemia, and neonatal intensive care admission [28] (Table 1.1).

Other studies demonstrated that infants of women with GDM have higher odds of respiratory distress, hypocalcaemia, hypomagnesaemia and birth trauma [5,7,29]. Natural delivery of a foetus with macrosomia is linked with a high risk of shoulder dystocia, postpartum haemorrhaging, fourth-degree lacerations and uterine rupture in the mother because of the large size of the foetus [30]. For this reason, most mothers with GDM are compelled to have a caesarean delivery which also carries anaesthetic and surgical risks. The incidence of preeclampsia, a common cause of maternal and foetal morbidity in Africa has been shown to be influenced by the severity of GDM and the woman's prenatal body mass index (BMI) [31,32].

After pregnancy, women diagnosed with GDM are at higher odds of developing GDM in subsequent pregnancies. The risk rises with the number of previous GDM-affected pregnancies [33]. Furthermore, both the mother and child are at risk of obesity, T2D and cardiovascular diseases later on in life [25,26,34]. It is estimated that between 22 and 28 years following the first pregnancy, T2D will develop in 70% of women with GDM [25,35,36] and the risk is more pronounced in certain populations [37]. Table 1.1. below is a summary of the short and long-term complications of GDM

Table 1.1 Short- and long-term complications observed in mother and child following GDM diagnosis.

Short-term maternal complications	**Short-term foetal complications**
• Gestational hypertension • Assisted delivery, caesarean section, and preterm delivery • Pre-eclampsia	• LGA or macrosomia • Neonatal complications including shoulder dystocia, neonatal hypoglycaemia, hypocalcaemia hyperbilirubinemia, and hypomagnesaemia, • Neonatal respiratory distress. • Elevated cord-blood serum C-peptide levels • Neonatal intensive care admission

Long-term maternal complications	Long-term foetal complications
• T2D and recurrent GDM • cardiovascular complications • T1D (depends on the population)	• T2D • obesity • cardiovascular complications

1.2 PATHOPHYSIOLOGY

1.2.1 Insulin Resistance and β-cell Dysfunction

The aetiology of GDM is not fully understood, however, the prevailing common understanding is that it is a multifaceted disorder identified by reduced insulin sensitivity and β-cell function[38]. Insulin is a polypeptide hormone composed of 51 amino acids that is synthesised and secreted by the β-cells of the pancreatic islets of Langerhans when the concentration of blood glucose increases [39]. Normally, insulin promotes glucose removal from the circulation by mediating the translocation of Glucose Transporter Type 4 (GLUT-4) from the cytoplasm to the membrane in skeletal muscle and adipose tissues (Figure 1.3) [40].

The signalling pathway for insulin-induced glucose uptake is initiated by the binding of insulin or insulin-like growth factor to the insulin receptor which initiates a downstream signalling pathway. The signalling pathway is primarily regulated through the tyrosine phosphorylation of the insulin receptor substrate-1 (IRS-1) by the insulin receptor. Tyrosine phosphorylation of IRS-1 will result in the stimulation of the phosphatidylinositol 3-kinase (PI3K)-protein kinase B (PKB/AKT) or extracellular signal-regulated kinase 1 or 2/mitogen-activation protein kinase (ERK/MAPK) pathways, inducing the translocation of GLUT-4 from the cytoplasm to the membrane of that cell [41–43], whilst the ERK/MAPK pathway mediates in part processes involved in cell proliferation, differentiation and stress responses [44].

In the liver, insulin increases glucose uptake by activating the enzyme glucokinase (GCK) enzyme. This enzyme catalyses the phosphorylation of glucose to glucose-6-phosphate, a substrate for glycogen production [45].

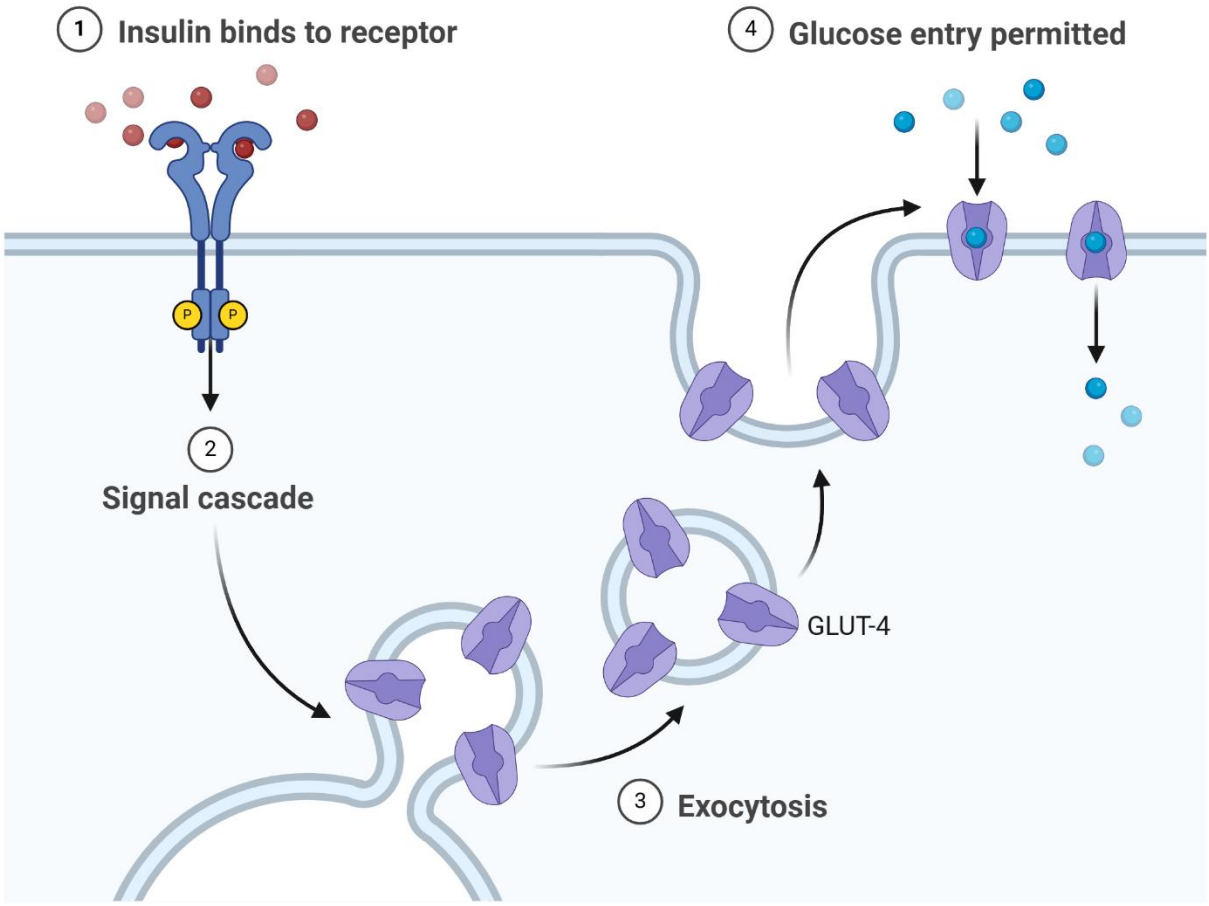


Figure 1.3 *This figure illustrates the main steps of insulin action including insulin binding, signalling cascade, GLUT-4 exocytosis and translocation. Created in BioRender. (2025) https://BioRender.com/t32q818*

The rate of glucose uptake stimulated by insulin is estimated to decline by 32% in the late stage of normal pregnancy [38]. This pregnancy-induced insulin resistance (IR) is the body's strategy to supply energy to both the growing foetus and the mother by reserving glucose for the foetus and using the stored lipids in the form of fatty acids for the mother [46]. The body achieves this by reducing glucose transport and/or tyrosine phosphorylation of IRS-1 which result in some degree of IR [47]. Pancreatic β-cells normally compensate for IR stimulated by pregnancy by increasing insulin production in response to the elevated circulatory glucose levels [38]. However, if the demand exceeds the supply of insulin, glucose will accumulate in circulation, ultimately leading to hyperglycaemia.

Below is a review of some of the common processes that are thought to contribute to the development of GDM.

1.2.2 The Role of Hormones in Inducing Insulin Resistance.

Pregnancy is regulated in part by pregnancy hormones. As the pregnancy advances, hormones such as oestrogen, progesterone, cortisol, human placental lactogen (hPL) and human placental growth hormone (hPGH) also rise [48].
The rise in these hormones coincides with the decline in insulin sensitivity [49]. Although the mechanisms by which these hormones regulate insulin action are not entirely clear, the literature suggests that these hormones play a critical role in the development of pregnancy-induced IR [50–54]. This theory is also supported by the fall in hormone levels just after delivery [55,56].

In pregnancy, the increase in progesterone and hPGH is associated with low rates of GLUT4 translocation which also inhibits IRS-1 action and downstream signalling pathways [51,52]. In animal studies, glucocorticoids such as cortisol directly suppress insulin release in β-cells [50]. However, in humans, cortisol stimulates gluconeogenesis to increase glucose levels while simultaneously inhibiting glucose uptake in tissues during periods of stress, including pregnancy.

The precise role of hPL in pregnancy is not fully understood. However, it is thought that hPL has multiple effects. One function involves enhancing insulin secretion, synthesis, β-cell proliferation, and promoting glucose oxidation and glycogen storage. The other function is to induce IR by inhibiting insulin from binding to insulin receptors. It has also been shown to promote lipolysis in adipose tissues, facilitating the mother's use of stored lipids [58].

This process of inducing IR during pregnancy by way of pregnancy hormones is part of the changes that occur during normal pregnancies. Under normal circumstances the pregnancy-induced IR is compensated by increased insulin secretion.

1.2.3 Low-grade Chronic Inflammation and the development of GDM

Inflammation is the body’s natural defence mechanism against toxins, infections, and other stress-inducing processes. However, persistent release of cytokines and chemokines results in tissue damage. This process of sustained inflammation is known as chronic inflammation. Normal pregnancy is associated with low-grade chronic inflammation. This process is critical during the development of human embryos. During embryo implantation, trophoblast invasion and placentation there is an increase in cytokine levels [41]. The success or failure of

the embryo implantation depends on the balance between the release of pro-to-anti-inflammatory cytokine release. This process is orchestrated by the trophoblast cells together with pregnancy hormones [59,60]. Pro-inflammatory cytokines such as TNF-α and IL-6 induce IR in part by stimulating the c-JUN N-terminal kinase (JNK) pathway and IκB kinase (IkappaB kinase or IKK) of the nuclear factor-kappa B (NF-κB) signalling pathway. The JNK signalling pathway, when activated can inhibit insulin signalling by initiating serine phosphorylation of IRS-1 [61,62]. Furthermore, TNF-α was found to downregulate the transcription factor peroxisome proliferator-activated receptor (PPAR-γ), a ligand-activated transcription factor that functions to induce insulin action and enhance glucose metabolism [63,64].

Adipose tissue may also stimulate low-grade-chronic inflammation. Adipocytes are the main storage site for lipids. However, adipocytes also possess the ability to function as endocrine cells and thus can release adipocytokines, hormones, and other peptides that regulate hunger to maintain energy homeostasis. However, in obesity, the extent to which the adipokines are released is imbalanced and the scale is shifted more towards pro-inflammatory adipokines [65]. Thus, if pro-inflammatory cytokines can inhibit insulin action as mentioned above, then the development of IR in pregnant women who are obese is a combination of pregnancy-induced-IR and IR induced by adipokines which places them at a much higher odds of developing GDM [66]. The correlation between adipose tissue and insulin action in obese women is further supported by findings indicating that loss of weight improves insulin sensitivity [67].

The levels of TNF-α correlate positively with IR in late pregnancy. [68]. This effect is more defined in women with GDM compared to healthy pregnant women [68,69]. Similarly, the level of interleukin-6 (IL-6), a pro-inflammatory biomarker, is greater in women with GDM compared to healthy pregnant women and women with GDM [70,71]. In addition, a direct association have been found between elevated IL-6 levels and pregravid BMI, fasting plasma glucose, and postprandial glucose levels [72], and inversely associated with insulin action [73].

However, studies reporting the association of IL-6 and TNF-α with GDM are inconsistent. According to López-Tinoco et al. [74], TNF-α and not IL-6 is linked to GDM. In addition, it is unclear whether the correlation between TNF- α and GDM is independent of BMI. According

to Winkler et al. [75], BMI attenuated the association of TNF-α with GDM in a multivariate model. Therefore, more research is required to determine whether TNF-α and IL-6 can predict GDM.

In addition to the aforementioned, other factors that play a part in the development of chronic low-grade inflammation include cigarette smoking, poor diet, psychological stress, sleep disturbances, altered circadian rhythm, and physical inactivity [76].

1.2.4 Branched-Chain Amino Acids' Role in Insulin Resistance

Branched-chain amino acids (BCAAs) are made up of three amino acids namely, leucine, isoleucine, and valine. These amino acids are essential amino acids, which means they cannot be synthesised endogenously. The elevated levels in circulation are linked to IR [77–80]. The structures of BCAAs are illustrated in Figure 1.4. The association of BCAAs with IR does not explain whether BCAA elevation is a result of or the cause of IR. In literature, there is a body of work that claims that elevated BCAAs cause IR [47,77,81,82]. In the studies, BCAAs are thought to induce IR through the stimulation of the mammalian target of rapamycin (mTOR) signalling pathway. The mTOR signalling pathway regulates protein synthesis and breakdown in cells and is a key signalling mediator in the crosstalk between amino acids and insulin [83]. The mTOR can exist as two complexes: mTORC1 and mTORC2. mTORC1 is primarily related to cell growth and proliferation, protein synthesis, suppression of autophagy and hypoxia, and transcription. On a theoretical level, researchers suggest that sustained elevated levels of BCAAs, observed in obese and T2D patients, may lead to overstimulation of the mTOR pathway. This overstimulation subsequently activates the mTORC1 signalling pathway, which initiates a negative feedback loop that inhibits insulin by inducing serine phosphorylation of IRS-1 (73,76,77). Serine phosphorylation of IRS-1 inhibits interaction between insulin receptors and IRS-1, while also initiating its degradation.

In addition, elevated levels of BCAAs are believed to interfere with the normal processes of catabolism of BCAA, subsequently disrupting mitochondrial β-oxidation to a degree that may lead to mitochondrial stress [47,77,84,85]. This theory claims that first there has to be BCAA overload/ nutrition overload, which leads to the disruption of normal BCAA catabolism in adipose tissue, in turn decreasing BCAA oxidation [84]. Secondly, circulatory BCAAs may further be elevated by other factors such as gene variation or increased BCAA synthesis by the gut microbiome [47]. The elevated circulatory BCAAs are thought to spill into the liver

and skeletal muscles, where they too will have impaired BCAA catabolism and mitochondrial oxidation, in turn, increasing circulatory BCAAs [77,81,82]. The combination of mitochondrial stress from different tissues ultimately leads to impaired insulin action [86–88]. However, whether they play a role in the aetiology of GDM is currently not known.

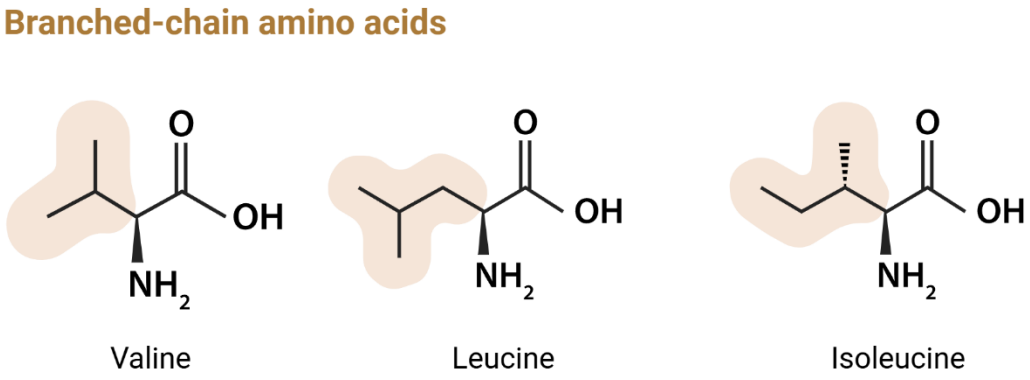


Figure 1.4 The chemical structure of branched-chain amino acids Created in BioRender. (2025) https://BioRender.com/o24h764

1.2.5 Free Fatty Acids Role in Insulin Resistance

Free fatty acids (FFAs) are important source of energy for body tissues and circulatory levels are elevated during times of starvation, prolonged exercise, and pregnancy. In pregnancy, particularly, late pregnancy, there is an increase in the level of FFA levels to compensate for the pregnancy-induced IR. This increase shifts the maternal energy source from glucose to lipids to reserve glucose for the growing foetus. Structurally, the FFAs are key components of esterified lipids such as triglycerides and phospholipids and are classified as saturated or unsaturated based on their length [89].

Normally, insulin regulates lipolysis (the breakdown of triglycerides into glycerol and FAs) in adipocytes, the main storage depot for triglycerides, by suppressing lipolysis during meals and promoting lipolysis in the fasting state (35). As mentioned previously, in pregnancy, hPL further stimulates lipolysis as compensation for low maternal glucose uptake induced by pregnancy hormones. This process preserves glucose for the foetus resulting in the elevation of FFAs. The above is more profound in pregnant women who are overweight or obese [90,91].

Some research indicates that unusually high FFAs are associated with decreased IR and glucose uptake [92–95]. It is thought that impaired FFA metabolism initiated by the increase in circulatory fatty acids disrupts the normal fatty acid oxidation (FAO) processes. This is shown by the increase in FAO enzymes that are not matched with an increase in the tricarboxylic acid cycle (TCA) enzymes [47,96] which are required for the metabolism of acetyl-CoA, which is the major breakdown product resulting from FFA catabolism. The disparity between the two processes (FAO and TCA) is thought to form incomplete FAO which increases fatty acid intermediates such as acylcarnitine (ACs). The impaired FAO may also lead to mitochondrial stress ultimately impairing insulin action [96,97]. In addition, it is thought that in the liver, excess FFAs are shifted from oxidative to esterification pathways, which create lipids with signalling characteristics such as diacylglycerol which blocks tyrosine phosphorylation of IRS-1 [98].

Metabolomic studies have found associations between FFAs and their metabolites with GDM [99–104]. This explains why women with high pregravid BMI are at increased risk of GDM in pregnancy.

1.2.6 Genetic and Epigenetic Factors Contributing to GDM

The prevalence of GDM is believed to be influenced to a lesser extent by genetic factors. There is less than 5% of women with GDM have monogenic(single gene mutation) disorders[54,105] and approximately 10% of GDM patients have autoantibodies that target specific β-cell antigens [106–108]. In addition, the pathophysiology of genetic defects related to the development of GDM is not widely studied and literature in this area is scarce. However, from existing studies available, it appears that GDM may arise from mutation of genes involved in glucose metabolism, insulin secretion and/ or β-cell function. For example, the *GCK* heterozygous mutation may result in a mild elevation of blood glucose levels, potentially leading to GDM in individuals with this mutation during pregnancy[109]. According to a systematic review by Zhang et al. [110], gene modifications of the following genes *TCF7L2, GCK, KCNJ11, CDKAL1, IGF2BP2* and *MTNR1B*, are significantly associated with GDM and linked to pancreatic islet β-cell function. Studies outside of pregnancy found that gene modifications of the two genes, *PPARG* and *IRS1*, contribute to IR development. In addition, studies suggest that the degree of the IR formed may be population-dependent as seen with the GLY1057 Asp variation of the IRS-2 gene. This variation was strongly linked with

IR and T2D but the degree to which the IR manifested was not the same across all populations studied [111–113]. Variations in the gene encoding zinc transporter 8 (ZnT8), a zinc regulator in β-cells, were identified as being linked to an increased risk for GDM in the Chinese population [114].

According to Steyn et al. [115], the degree of methylation in the promoter region of the glucose-6-phosphate dehydrogenase gene (*G6PD*) in both blood and placenta tissue is considerably great in women with GDM when compared to controls, and this was found to be correlated with reduced levels of *G6PD* mRNA expression.

There is the belief that women who develop GDM enter pregnancy with an already IR albeit asymptotic. Therefore, there is a high probability that the underlying processes described above, which may exist before pregnancy, are exacerbated by pregnancy-induced IR that occurs in the later stages of pregnancy. This results in an increased demand for insulin production, ultimately resulting in the depletion of β-cells and consequently, the development of GDM (Figure 1.5) [116].

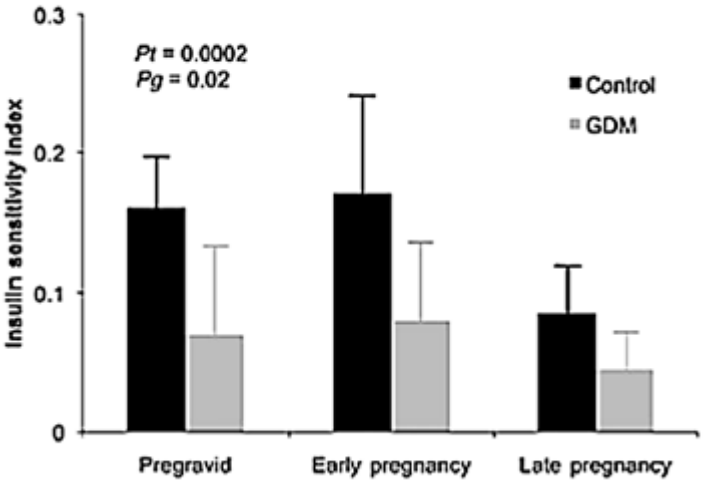


Figure 1.5. Mean (±SD) insulin sensitivity during a euglycaemic-hyperinsulinaemic clamp in pregnant women. GDM=Gestational diabetes mellitus, Pt= p value for individual longitudinal changes with time, Pg= p value for difference between groups. Taken from Catalano (1991) *[117]*.

1.3 DIAGNOSTIC METHODS FOR GDM

1.3.1 Definitions

The primary objective of a diagnostic test is to determine whether the disease is present or not and to guide treatment options for individuals showing symptoms or identified as high risk through screening. On the contrary, a screening test aims to identify individuals who are at higher odds of developing a disease, thereby facilitating early intervention to avert the onset of the disease and its associated complications[118] .

Target 3.8 of the sustainable developmental goals (SDG) established by the United Nations General Assembly in 2017 to be achieved by 2030 states that by 2030 all countries should achieve universal health coverage[119,120]. This is aligned with the World Health Organization's recommendations of universal screening for GDM[11]. Universal screening means that all persons in a given group, regardless of whether they have risk factors or symptoms for the disease are tested. This is currently not practised in many countries due to limited resources. Instead, countries that can implement some form of screening use selective screening. Selective screening uses risk factors to screen individuals considered to be at risk for a given condition, in this case, GDM.

1.3.2 Overview of Conventional Diagnostic Criteria for GDM

Following the HAPO study, where hyperglycaemia in pregnancy was linked to adverse maternal and neonatal outcomes, at moderate glucose levels [121], the IADPSG formulated new diagnostic criteria for the diagnosis of GDM [1]. The revised recommendations received substantial endorsement from multiple organisations, such as the World Health Organization (WHO) [11], American Diabetes Association (ADA) [12], Endocrine Society [122], International Federation of Gynaecology and Obstetrics [13] and Society of Endocrinology, Metabolism and Diabetes of South Africa (SEMDSA) [123] . However, there are also push-backs from various health organizations such as the National Institutes of Health (NIH) and the American Congress of Obstetricians and Gynaecologists (ACOG) who feel that there is not enough evidence to support the IADPSG criteria [12], therefore its use could lead to unnecessary treatment and increased burden on women who are likely low risk. The organizations also feel that the criteria will likely raise the prevalence of GDM which likely poses an extra burden to those women who have mild gestational diabetes [124,125]. Apart

from the logistics of GDM management and testing, other less researched implications include the psychological burden of being diagnosed with the disease. Moreover, the authors caution that the findings may not be transferable to other populations which were not included in the HAPO study. Thus, studies similar to the HAPO study in non-Caucasians are necessary to establish cut-offs specific to those populations.

Another commonly applied diagnostic criteria in SA are the United Kingdom National Institute for Health and Care Excellence (NICE) guidelines which recommend a selective screening method for GDM. Women with GDM risk factors are advised to go through a diagnostic 75-g 2-hour OGTT at 26 to 28 weeks of pregnancy (Table 1.3) using slightly higher cut-off levels than the IADPSG criteria [126]. The conventional risk factors recommended by NICE are BMI > 30 kg/m^2, previous macrosomia (≥4500 g), previous GDM, family history of diabetes, and ethnic origin with a high prevalence of diabetes (South Asian, Black Caribbean, Middle Eastern) (Table 1.4). One of the major contrasts of NICE guidelines from IADPSG is that they have higher cut -off levels which consequently play a role in the prevalence of GDM.

There are a few public health centres in SA that follow the ADA 2-step guidelines for GDM screening (Table 1.2), particularly in women with GDM risk factors in early pregnancy. However, there are an ongoing debate about whether it is appropriate to expose expectant mothers to a significant glucose challenge like the one suggested by the 2 Steps approaches mentioned above [127]. Also it should be considered that the glucose challenge test (GCT), the first step of the 2 -step approach, is generally only 85% sensitive with markedly less sensitivity among ethnic groups whose GDM is associated more with fasting hyperglycaemia [128].

For these reasons, there is still no consensus on the diagnostic criteria for GDM. The diagnostic criteria are shown in Table 1.2 and Table 1. 3.

Table 1.2. Conventional diagnostic criteria for GDM before "harmonization".

Diagnostic criteria	**Glucose load (g) using the OGTT**	**Glucose threshold (mmol/L)**				**Recommendation**
		Fasting	1 h	2 h	3h	

WHO; 1999	75	≥7.0	-	≥7.8	-	One or more of the cut-offs to be diagnosed with GDM
ADA 2 step	50 (GCT)	-	≥ 10			If 1hr ≥10mM then proceed to do the 3hr, 100g OGTT according to Carpenter and Coustan criteria
IADPSG; 2010	75	≥ 5.1	≥ 10	≥ 8.5	-	One or more of the cut-offs to be diagnosed with GDM
NICE; 2015	75	≥ 5.6	-	≥ 7.8	-	One or more of the cut-offs to be diagnosed with GDM
Carpenter and Coustan	100	≥ 5.3	≥ 10	≥ 8.6	≥ 7.8	More than one of the cut-offs to be diagnosed with GDM
NDDG	100	≥ 5.8	≥ 10.6	≥ 9.2	≥ 8.0	More than one of the cut-offs to be diagnosed with GDM

WHO= World Health Organization, ADA= American Diabetes Association, IADPSG= International Association of the Diabetes and Pregnancy Study Groups, NICE= United Kingdom-based National Institute for Health and Care Excellence, GCT= glucose challenge test; OGTT= oral glucose tolerance test; NDDG= National Diabetes Data Group. Adapted from Khambule and George (2019)[83].

Table 1.3 Latest recommendations from International and national bodies

Guidelines	Recommendations
WHO 2013	IADPSG criteria for GDM anytime
SEMDSA 2017	Selective screening and IADPSG diagnostic criteria
ADA	1. One-step screening approach with IADPSG criteria
	2. Two-step screening approach with 50-g GCT and 100-g OGTT with the Carpenter and Coustan criteria or the NDDG criteria
ACOG	Two-step screening strategy with 50-g GCT and 100-g OGTT with the Carpenter and Coustan criteria or the NDDG criteria
NICE	The selective screening approach, whereby women with risk factors for GDM are recommended to undergo a diagnostic 75-g 2-hour OGTT at 26 to 28 weeks gestation, with diagnostic (fasting or 2-hour post glucose load) glucose thresholds higher than the IADPSG diagnostic criteria for

	GDM, 5.1 mmol/L vs. 5.3mmol/L for fasting and 8.5mmol/L vs. 8.6mmol/L for 2-hour post glucose load.

WHO= World Health Organization; ADA= American Diabetes Association; SEMDSA= Society of Endocrinology, Metabolism and Diabetes of South Africa; ACOG: American College of Obstetricians and Gynecologists; IADPSG: the International Association of Diabetes and Pregnancy Study Groups; GCT: glucose challenge test; OGTT: oral glucose tolerance test; NDDG: National Diabetes Data Group

1.3.3 Limitations and Challenges of Current Diagnostic Methods

The OGTT is a cumbersome test which involves the patient drinking a concentrated glucose solution, after which blood glucose levels are measured at specified time intervals, fasting, after 1 and 2 hours respectively. This requires multiple blood withdrawals, and the glucose solution can cause nausea. While the OGTT is considered the gold standard for the diagnosis of GDM, it has some drawbacks including a high chance of preanalytical errors and the requirement of well-trained personnel to conduct the test. Preanalytical errors are largely caused by mishandling of glucose samples [129,130]. Preservatives such as fluoride and citrate stop ex-vivo glycolysis, leading to glucose consumption. However, a 0,5mmol/l decrease in glucose over a 2-4 h period is noted with fluoride tubes, and most of the decline occurs during the first post-collection [129]. Pre-analytical issues also include physical activity, gastric emptying, stress and sleep, and length of time spent in the fasting state [131,132]. There is also research which has shown that the OGTT is not reliable and reproducible [133,134]. Thus, according to Pintaudi et al (2022), GDM prevalence varied significantly depending on the OGTT glucose level estimates at the lowest (4.5%) or highest (25.3%) allowed value according to the total maximum allowable error [133]. As a result, there is a growing interest in practical solutions, particularly in developing countries and healthcare facilities with high patient loads and limited resources. Another drawback is that the conventional method for glucose measurement is lab-based thus the site at which the OGTT is performed should be near a laboratory. This is the main reason why in SA only a few health centres can perform the test.

Regarding universal screening, there are concerns that testing all pregnant women with the OGTT may increase costs and there is an increased burden of imposing excessive testing on women who would otherwise have been classified as low risk.

Although universal screening is currently not practical in SA, the risk-factor approach is not entirely effective in practice either and has failed to identify over 50% of women who would later develop GDM [8,10,135,136]. A study performed in SA showed that out of 554 women tested for GDM using the IADPSG criteria, 59 (10.6%) would be missed if the risk-factor approach was applied [137]. Furthermore, 170 (30.7%) of the 554 women tested had one or more of the risk factors mentioned below but tested negative for GDM. A study from Nigeria, showed that 31% of the women with GDM would be missed if selective screening was used [135]. Another South African study showed that 43.9% of GDM cases would be missed if the selective screening was used in a black African population [8]. Similarly, a study in Uganda found that 57% of the GDM cases would have been missed based on the selective screening [136].

The diagnosis of GDM in early pregnancy (<24 weeks) is not well established. The WHO 2013 guidelines stipulate that GDM may be diagnosed at any time using the IADPSG criteria [11], however, they acknowledge that the evidence supporting this recommendation is insufficient. Another option could be the possible use of biomarkers, which could be a cheaper and less invasive way to diagnose. Furthermore, there is a need for compelling data indicating how preventative interventions, notably routine GDM screening, can improve long-term health outcomes for both women and their children.

Table 1.4 Risk factors for GDM.

	Risk factors for GDM
1.	Repeated glycosuria
2.	Previous GDM
3.	First-degree relative with diabetes
4.	History of stillbirths of unknown origin, previous congenital anomalies and suspicion of polyhydramnios in present pregnancy.
5.	History of high-birth weight infant ≥4.5 kg
6.	Obesity (BMI >30 kg/m^2)
7.	History of polycystic ovarian syndrome

8.	History of unexpected perinatal death
9.	Women of South-Asian descent

In the following section, the recent advancements in the discovery of GDM-related biomarkers are discussed.

1.3.4 Recent Advancements and Modifications in Diagnostic Criteria

Point-of-care glucose analysis

One promising solution to address some of the drawbacks of OGTT is point-of-care (POC) glucose testing. The POC glucometers may provide numerous operational benefits, including shorter turnaround times, fewer health visits, timely health interventions and eliminating preanalytical sample handling errors associated with glucose stability [138]. The International Federation of Gynaecology and Obstetrics (FIGO) guidelines also encourage using glucometers to test for GDM in resource-limited settings because of the associated low cost, convenience of use, availability, and ability to identify and treat GDM as soon as possible [1]. To adopt this recommendation POC should be comparable with laboratory results. There has been conflicting data regarding the glucometers' performance in different clinical settings [139,140]. A study by Adams et al. [141] indicated that the Roche AccuChek active glucometer was not ideal for utilisation in GDM diagnosis when using the FIGO criteria. In a 2020 publication by Gracia-Claver et al.[140] on the performance of the AccuChek or Contour Next for diagnosis of GDM using the National Diabetes Data Group (NDDG) criteria showed good, acceptable performance for fasting sample and met the ISO 151917:2013 specification. The ISO 151917:2013 specifies the analytical performance specification of self-monitoring capillary glucose compared to the plasma venous glucose methods.

Glycaemic markers for GDM diagnosis

Given that most women diagnosed with GDM in the HAPO study were identified using fasting plasma glucose (FPG) alone, using FPG instead of the complete OGTT for diagnosing GDM may be beneficial for countries with limited resources[121]. However, patients would still need to come fasted for this test, therefore glycated albumin (GA) and glycated haemoglobin

(HbA1c) may be more desirable, as they can be measured in random blood samples. These glycaemic markers are formed through a naturally occurring process known as glycation. Glycation is a non-enzymatic addition of a reducing sugar to a protein. The first stage of the glycation process entails the attachment of the sugar to the amine group of the N-terminal or lysine residue on the proteins, resulting in the production of the intermediate Schiff base [142,143]. The Schiff base is an unstable, reversible product that leads to the formation of the irreversible Amadori product. This process is also known as early-stage glycation [142,143] and is illustrated in Figure 1.6. The benefit of using these glycaemic markers is that POC devices are available, but as mentioned previously, they would need to be validated and compared with the laboratory-based measurements before use [139,144,145].

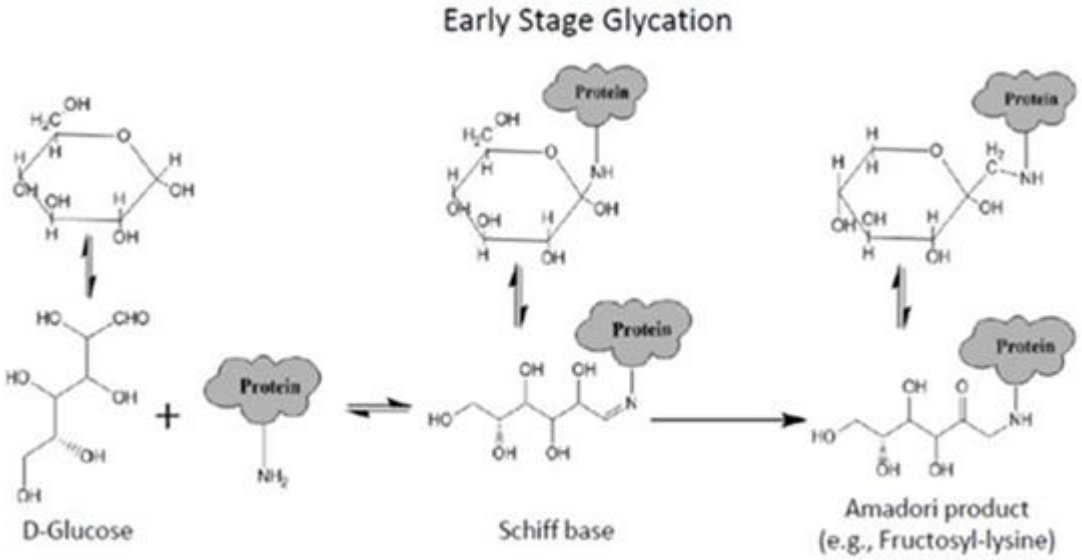


Figure 1.6 *The general reaction for glycation of a protein. Taken from Anguizola et al. [142]*

Metabolomics and proteomics

Metabolomics and proteomics use techniques such as liquid chromatography-tandem mass spectrometry (LCMS/MS) and gas chromatography-tandem mass spectrometry (GCMS/MS) to investigate metabolites and proteins in a biological system at a certain stage and time point. These techniques have fast-tracked biomarker research and have been used to gain insights into the pathophysiology of a disease that was not possible previously. These techniques can be used to discover biomarkers for GDM in early pregnancy. This is important

as it will facilitate prompt treatment and may prevent the adverse outcomes associated with GDM.

Metabolomics and proteomics have significantly expanded our understanding of diabetes. Before that, analysing individual amino acids, FFAs, and proteins simultaneously was a challenge, and the commonly used methods provided minimal opportunity for the discovery of new biomarkers.

The main drawback in metabolomics and proteomics is the likelihood of uncertainty in identified analytes, which may undermine the significance of the biomarkers, and this is possible even when standard operating procedures are utilised. Uncertainty can be generated by intrinsic biological variety among individual participants, participant compliance with instructions, clinical state fluctuations, and analyser/researcher experience. Therefore, to confirm the validity of the biomarkers, validation should be performed. However, this step is frequently by-passed in many studies due to the expense implications of performing the validation [146]. Another disadvantage is that reference standards for the biomarkers are required for quantitation and in some cases the reference standard is not commercially available.

Metabolomic studies aimed at GDM biomarker discovery are inconsistent and this might be caused by the varying diagnostic criteria and analytical platforms applied. Moreover, the differences in the genetic makeup of the population may influence the outcome of the studies [99,100,147–152]. There has not been many metabolomic studies conducted in the African population.

While metabolomics and proteomics have significantly enhanced our understanding of the pathophysiology of GDM, the existing studies have yet to identify reproducible, and clinically useful diagnostic biomarkers. However, the previous metabolomics and proteomics research has added knowledge on certain dysregulated proteins and metabolic pathways related to the pathophysiology of GDM [153].

1.4 SUMMARY OF THE LITERATURE REVIEW

- GDM shares similar pathophysiological characteristics with T2D including insulin resistance and β-cell dysfunction however the aetiology of these processes is still not fully understood
- There are not many studies conducted on GDM in SA and Africa, even though developing countries are estimated to contribute 99 % to maternal and childbirth mortality (141) and within developing countries, the rural and poor communities are mostly affected. The few published studies on GDM in Africa differ in methodology which makes the interpretation of results difficult.
- To date, there has not been a consensus on which screening method to apply for GDM. Although the OGTT test defined by the IADPSG is advocated by some international organisations such as WHO, its use as a screening test is not practical in resource-limited environments. Thus, within LMICs pre-screening tests are used which pick out women with GDM risk factors to whom the OGTT is then applied. However, these tests have low sensitivity and specificity.
- While South African women have access to antenatal care, there is a shortage of public health centres that can make the diagnosis of hyperglycaemia during pregnancy due to the lack of resources [23].
- The diagnosis of GDM is based entirely on the identification of hyperglycaemia in pregnancy through performing the OGTT. Therefore, other biological markers such as GA, HbA1c, BCAA and FFAs and metabolites and markers of inflammation, could also be investigated for the screening and diagnosis of GDM as they are markers of glycaemia and/or are implicated in the development of pregnancy-related hyperglycaemia.
- Metabolic and proteomic methodologies for screening for novel biomarkers for GDM have not yet identified such biomarkers but they have contributed to our understanding of the metabolic dysfunction that underpins the development of GDM

1.5 AIMS, OBJECTIVES AND HYPOTHESES OF THE STUDY

- In light of the above, the study was aimed at investigating alternative methods for detecting dysglycaemia and diagnosing GDM in African women.

- The specific **objectives** of this study are as follows:
    1. To determine the accuracy of seven POC glucometers in diagnosing GDM among high-risk pregnant women in South Africa using the OGTT and laboratory-based measures of glucose as the gold standard.
    2. To compare the accuracy of FPG, HbA1c and GA against the OGTT for diagnosing GDM in a high-risk black African population.
    3. To determine whether metabolites and proteins chosen from the literature as markers of GDM (IL-6, TNF-α, FFAs, BCAAs, and ACs), are associated with glycaemia at early and late stages of pregnancy and whether they may be used for diagnosing GDM and for uncovering more information on the pathophysiology of GDM in this population.

- The study **hypotheses** based on the objectives above are as follows:
    1. POC glucometers are comparable to laboratory-based glucose measurements for GDM diagnosis.
    2. FPG, HbA1c and GA performance is comparable to OGTT when used to diagnose GDM.
    3. Selected biomarkers, IL-6, TNF-α, FFAs, BCAAs and ACs are associated with glycaemia in early and late pregnancy in black African pregnant women and contribute to the pathophysiology of the disease.

CHAPTER 2: STUDY DESIGN AND METHODS

This chapter describes the procedures that were common to all the results chapters. The overall study design is explained, and the sample analysis is described in detail.

2.1 STUDY DESIGN

This study focused on three primary groups: women between 24 and 32 weeks recruited for the POC study and biomarker study designated as Group 1 and Group 2, respectively and women in early pregnancy (between 8 and 20 weeks), designated as Group 3. The study design for each study is discussed below and a flow diagram illustrating the participant recruitment is shown in Figure 2.1. Moreover, a detailed description of the testing procedures is also included.

2.1.1 Women in late pregnancy

Group 1 participants comprised pregnant women in late pregnancy who participated in the comparison of glucose POC devices with the laboratory-based glucose measurements obtained from OGTTs. Group 2 included pregnant women who underwent OGTTs and whose blood samples were used for comparing diagnostic accuracy of glycaemic markers and biomarkers.

Sample size calculation for Group 1

Using an estimated prevalence of GDM at 10%, the minimum required sample size was determined to be 1070. This sample size is necessary to achieve a sensitivity and specificity of at least 0.80, with a statistical power of 80% and an alpha threshold of 0.05 [154].

Sample size calculation for Group 2

The sample size for Group 2 was calculated based on the ability to detect a significant correlation of the selected blood biomarkers with fasting or random glucose levels, respectively. Thus, to detect an effect size (r) of 0.3 at $p<0.05$ at 80% power, we would need a sample size of 55 [155]. This was increased to 75 for each of the two groups (fasted and non-fasted). Therefore, the total sample size was 150.

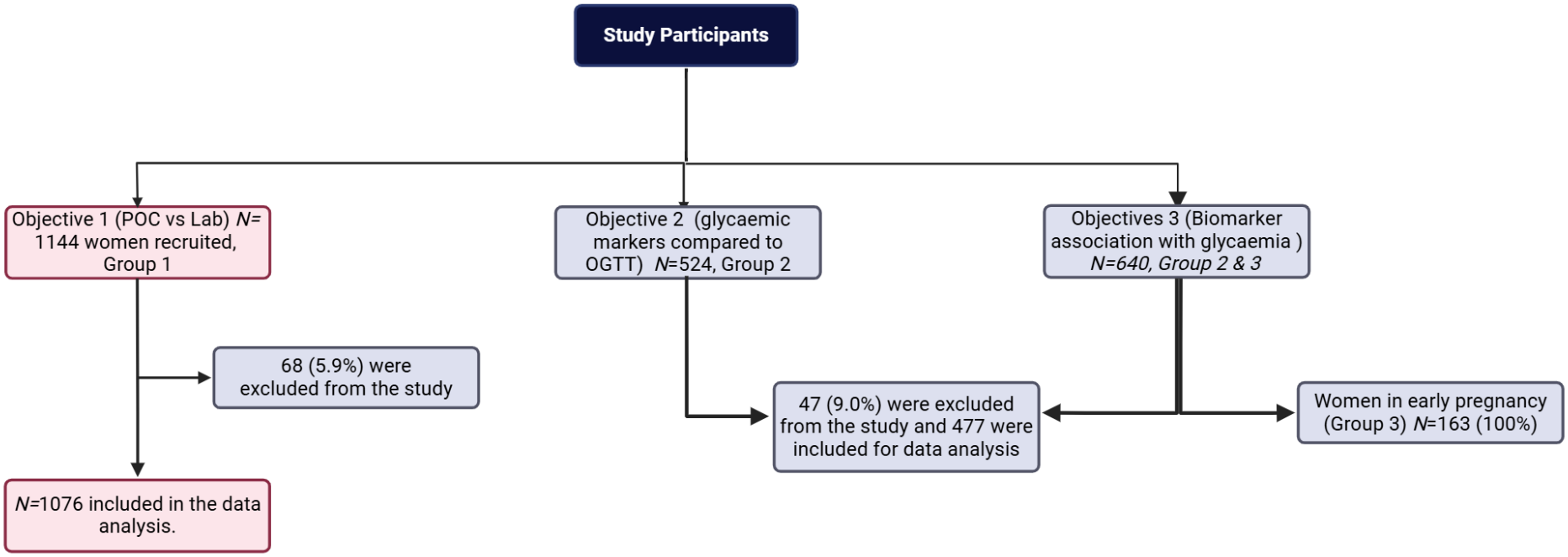


Figure 2.1 Participants' recruitment flow diagram. Created in https://BioRender.com

Inclusion and exclusion criteria for Groups 1 and 2

Groups 1 and 2 had the same inclusion and exclusion criteria. All Black African pregnant women visiting the ante-natal clinic who were scheduled for OGTT were informed about the study and those between 24-30 weeks of pregnancy and 18 years or older were invited to participate in the study. Women who were pre-diabetics, who did not fast for at least 8-12 hours before the OGTT, who smoked during that period, who had overt acute illness or infection were excluded. Overt diabetes/prediabetes was defined as [11]:

- Random plasma glucose ≥ 11.1mmol/L,
- Fasting plasma glucose ≥ 7mmol/L OR
- HbA1c ≥ 6.5%

Those who expressed interest in the study and fulfilled the inclusion criteria had their blood drawn. Gestational age was confirmed by ultrasound.

In order for a woman to be scheduled for an OGTT, she must have had one or more of the following risk factors for GDM: repeated glycosuria, previous GDM, first-degree relative with GDM, history of stillbirths of unknown origin, previous congenital anomalies and suspicion of polyhydramnios in present pregnancy, history of high-birth weight infant (≥4.5 kg), obese (body mass index (BMI) ≥30 kg/m^2), history of polycystic ovarian syndrome (PCOS) and history of unexpected perinatal death. These risk factors were recommended according to the guidelines of the Society of Endocrinology, Metabolism and Diabetes of South Africa (SEMDSA), the American College of Obstetricians and Gynecologists (ACOG) and the National Institute for Health and Care Excellence (NICE) [123,156,157].

Study sites

Participants were recruited from ante-natal clinics in Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), Chris Hani Baragwaneth Academic Hospital (CHBAH) Edenvale Hospital and Rahima Moosa Mother and Child Hospital (RMMCH). The central public hospital, CMJAH has 1088 beds serving patients from across Gauteng and neighbouring provinces [158]. The third largest hospital in the world, CHBAH is involved in research and the training of healthcare professionals. The maternity hospital treats approximately 60,000 patients, with an average of 1400-1600 deliveries per month [159]. The RMMC is a maternity

hospital which also trains health care professionals with 80-120 beds and reports 15000 deliveries every year, the second highest number of neonates in SA, after CHBAH [160,161]. The Edenvale Regional Hospital is a hospital that treats patients from the surrounding catchment area including nearby townships.

2.1.2 Women in early pregnancy

The participant's blood samples were analysed for several proposed biomarkers of GDM, namely GA, BCAAs (leucine, isoleucine and valine), FFAs, ACs (C2, C4, C5 and C8-carnitine) and inflammatory markers. The correlation of fasting and random glucose with each of these potential biomarkers was investigated.

Sample size calculation

The sample size for Group 3 was calculated based on the ability to detect a significant correlation of the selected blood biomarkers with fasting or random glucose levels. Thus, to detect an effect size (r) of 0.3 at $p<0.05$ at 80% power, we would need a sample size of 55 [155]. This was increased to 75 for each of the two groups (fasted and non-fasted). Therefore, the total sample size was 150.

Inclusion and exclusion criteria

All Black African pregnant women visiting the ante-natal clinic who were between 8-20 weeks gestation and 18 years or older were invited to participate in the study. The information sheet and consent form is shown in Appendix D. Women who were overt diabetics and were from ethnic groups other than Black African were excluded. The criteria for overt diabetes was as defined previously [11].
Gestational age was reported by using the participants' last menstrual period (LMP).
Those who expressed interest in the study and fulfilled the inclusion criteria had their blood drawn and information documented on questionnaires (Appendix C).

Study sites

Participants were recruited from the Hilbrow Community Health Care Center (Hillbrow CHC) antenatal clinic and CMJAH antenatal clinic. The Hillbrow CHC offers a variety of services,

such as family planning, antenatal and postnatal services, pregnancy termination, mother and child health services, 24-hour casualty services, a polyclinic, and outpatient services [162].

2.2 SAMPLE COLLECTION

The participant's demographic characteristics, and anthropometric data such as body weight, height and blood pressure measurements were collected using questionnaires (Appendix C) by the principal investigator (LK). An electronic scale and a wall-mounted stadiometer were used to measure the participant's body weight and height. The blood pressure was measured once in the right arm while the participant was seated. For women in late pregnancy, the mean blood pressure calculated from the measurements taken at the two most recent visits was utilised and one reading at the first visit was used for women in early pregnancy. Women who were HIV positive were included in this study as the prevalence of HIV is relatively high (16.8%) in South African women [163]. The HIV status of all the study participants was known due to routine clinic-based screening tests, and therefore HIV status was included in all the statistical tests to determine whether it affects glucose levels and risk for GDM at 16-20 and ≥24 weeks of gestation, respectively. To determine the HIV status, an in vitro qualitative immunoassay (Alere Determine HIV -1/2, Alere Medical, MA, United States) was utilised, which detects antibodies to HIV-1 and HIV-2 in capillary blood. Meditape UC-12S dipsticks (Sysmex, ZRG, Switzerland) were utilised to analyse the glucose levels in the urine in late pregnancy, and the presence of glycosuria was defined as a glucose level of 2+ or above. The anthropometry and biological fluid analysis for HIV and glycosuria were done by clinical staff at the designated site.

For all women in early pregnancy (Group 3) and those in the biomarker study in late pregnancy (Group 2), a qualified nurse collected 15 ml of venous blood from each participant at one time-point into one 5ml EDTA tube, one 5ml serum separating tube (SST) and one 5ml sodium fluoride tube. EDTA samples were used to measure HbA1c and glycated albumin, and SST samples were used to analyse BCAAs, FFAs, ACs and inflammatory markers. The sodium fluoride samples were used to measure blood glucose. After HbA1c analysis, the EDTA samples were fractionated by centrifugation at a speed of 1300 - 1400 x g for 15

minutes at 4°C. The SST tubes were allowed to clot before fractionation and were fractionated as described above. The serum and plasma samples were aliquoted into cryogenic tubes and stored at -80°C

The group 3 (early pregnancy) participants were classified into fasting and non-fasting (random glucose) groups. The participants who had not eaten or drank fluids in the last eight hours were classified as fasted and the rest were classified as non-fasted.

2.2.1 Oral glucose tolerance test and GDM diagnostic criteria

All the participants in Groups 1 and 2 underwent OGTT as per IADPSG guidelines [1]. Venous blood samples were taken at fasting, 1 hour, and 2 hours post glucose solution ingestion using the standard WHO phlebotomy technique in sodium fluoride tubes and glucose levels were measured on the same day of collection at the on-site laboratory.

Diagnosis of GDM was according to the IADPSG criteria, where one or more of the following cut-off levels of glucose had to be met: 5.1 - 6.9 mmol/l at fasting, ≥10.0 mmol/l at 1 hour of the OGTT or 8.5 - 10.9 mmol/l at 2 hours [1].

2.3 SAMPLE ANALYSIS

2.3.1 Plasma glucose and glycated albumin analysis by automated instruments

Plasma glucose was measured using the Cobas 8000 (Roche Diagnostics, Mannheim, DE) in CMJAH and CHBAH and the au480 chemical analyser (Beckman Coulter, Riyadh, KSA) in RHMMCH and Edenvale Hospital; both instruments used the glucose hexokinase method. Briefly, the principle of the method is, that hexokinase converts glucose to glucose-6-phosphate (G-6-P) in the presence of ATP. Glucose-6-phosphate dehydrogenase then transforms G-6-P to gluconate-6-phosphate in the presence of NADP+. As the NADP+ is reduced to NADPH during this reaction, the resulting increase in absorbance at 340 nm (secondary wavelength = 700 nm) is measured. Internal quality controls (IQC) were monitored regularly. The intra- assay CV for glucose was 1.00 and the inter-assay CV was 0.68.

Glycated albumin was measured using an auto-analyser Cobas 8000, 502 module (Roche, Basal, Switzerland) with spectrophotometric detection, at the National Health Laboratory

Service in CMJAH. GA was measured in serum samples. The quantILab Glycated Albumin enzymatic assay (Werfen, Milano, Italy, Ref 0018256640) was used to measure GA and albumin simultaneously on the Roche Cobas auto-analyser strictly following the manufacturer's instructions [164].

Briefly, the principle of the method is, that samples react with albumin-specific protease which converts GA protein to glycated amino acids. Then the glycated amino acids are reacted with ketoamine oxidase. The resultant solution (glucosone, amino acids and hydrogen peroxide) is oxidised using peroxidase in the presence of N, N-Bis (4-Sulfobutyl)-3-Methylaniline Disodium salt, and yields a blue-purple coloured product. The colour was assessed using absorbance measurements. Albumin was measured by the bromocresol purple colourimetric technique simultaneously. The results were utilised to determine the GA/albumin ratio which was expressed as a percentage. Internal quality controls (IQC) were monitored regularly. The intra-assay and inter-assay CV for the low-quality control (QC) sample were 0.10 and 2.04, and for high QC were 0.12 and 0.35, respectively.

2.4 DATA ANALYSIS

Data was collected on questionnaires (Appendix C) and transferred to Microsoft Excel spreadsheets. The data was only available to the principal investigator (LK) and supervisors. Statistical analyses were run using Stata (StataCorp, version 16) and MedCalc Software Ltd v22.021. Details of the statistical tests used are provided in each of the results chapters.

2.5 ETHICS

Ethics clearance for the PhD study was obtained from the University of the Witwatersrand Human Research Ethics Committee (HREC) (Medical); ethics clearance certificate M230675 for the project title ‘The use of novel biomarkers to improve screening for gestational diabetes in a Black African population’. In addition, an ethics clearance certificate, M220219 for the project title ‘A comparison between whole blood point of care testing and plasma glucose determination for the diagnosis of gestational diabetes mellitus’ was obtained. Copies of all two ethics clearance certificates can be found in Appendix E.

CHAPTER 3: PERFORMANCE OF POC GLUCOSE TESTING FOR THE DIAGNOSIS OF GESTATIONAL DIABETES IN SOUTH AFRICA

Khambule L, Chikomba C, Adam Y, Khan L, Haldane C, Vetter B, George J.A. Performance of point-of-care glucose testing for the diagnosis of gestational diabetes in South Africa. Int J Gynecol Obstet. 2024; 00:1-10. doi:10.1002/ijgo.15914

3.1 ABSTRACT

Objective: Lack of accessibility to oral glucose tolerance tests (OGTT) in South Africa (SA) means many pregnant women go without testing for gestational diabetes mellitus (GDM). This study evaluated point-of-care (POC) glucometers against the laboratory-based glucose method in pregnant women.

Methods: A cross-sectional study on pregnant women attending the prenatal clinic in Johannesburg who were recommended for the OGTT. OGTTs were conducted as per International Association of Diabetes and Pregnancy Study Groups (IADPSG) guidelines. Women who consented to the study donated both venous blood and capillary blood for lab-based and POC glucose measurements using seven POC glucometers: I-STAT, Xpress, LDX, VivaChek-Ino, Accu-Chek Active, StatStrip, and Codefree. By assessing sensitivity, specificity, and area under the receiver operating characteristic curve (AUC) and comparing Bland-Altman plots, the diagnostic accuracy of each glucose meter was compared to the reference method, the laboratory-based glucose method.

Results: Data were analysed for 1076 pregnant women. Based on OGTT testing, 83 women had GDM (7.7%). Overall, the POC glucometers performed poorly with sensitivity ranging from 17.6% to 87.18 and specificity ranging between 62.7% to 99.8%. The AUC ranged from 0.59 to 0.79. All POC glucometers showed moderate to poor reliability. Lab-based fasting plasma glucose (FPG) surpassed the POC glucometers in sensitivity, specificity, and AUC, with values of 94.0%, 100%, and 0.98, respectively.

Conclusion: We demonstrated that lab-based FPG has the potential to be used as a diagnostic test for GDM and that the POC glucometers cannot replace OGTT lab-based measurements.

3.2 INTRODUCTION

Gestational diabetes mellitus (GDM), defined as hyperglycaemia first discovered in pregnancy, is one of the most common complications of pregnancy, affecting about 16% of pregnant women worldwide [165], with the majority of women with GDM (87.6%) in low- and middle-income countries (LMICs) [166]. The first step to optimum GDM care and T2DM prevention/delay is the diagnosis [121]. In 2015, the International Federation of Gynaecology and Obstetrics (FIGO) published GDM consensus guidelines recommending a universal screening approach, the oral glucose tolerance test (OGTT) being the gold standard for screening and diagnosing GDM [121]. While these recommendations are widely recognised, LMICs like South Africa (SA), face many challenges to GDM screening, including individual, healthcare work-related, and health-system barriers [167].

However, the test involves laboratory-based glucose readings and there are only a few facilities that provide this service [8]. The point-of-care (POC) testing offers many operational advantages to the OGTT such as reduced turnaround time, eradication of preanalytical sample handling, stability errors, and prompt health interventions [138]. In SA, strip-based glucometers are readily available for self-monitoring among diabetics, however, strips are affected by external variables such as humidity, temperature, haematocrit, medicines and hypotension [168]. Cassette-based devices minimise the limitations of strips [169]. However, the evidence for the performance of the glucometers in the various clinical scenarios has been inconsistent [139,140]. Therefore, this study aimed to determine the accuracy of seven POC glucometers in diagnosing GDM among pregnant women in SA. We anticipate that the study will identify POC glucometers that accurately detect GDM.

3.3 MATERIALS AND METHODS

3.3.1 Study design

This cross-sectional study was conducted between the 13th of July 2022 and the 28th of February 2023, among pregnant women who attend the antenatal clinic at Chris Hani Baragwanath Academic Hospital (CHBAH).

The study followed the Standards for Reporting Diagnostic Accuracy Studies (STARD) guidelines, please see Table S1 for the checklist.

3.3.2 Study population

The sample size and study design is described in Chapter 2. In addition, women who were acutely ill, or on high dose vitamin C (>2000mg/day) or corticosteroid therapy, were excluded as were those with previous bariatric surgery, and those who smoked during the OGTT.

3.3.3 Medical history and sample collection

Data on women's age, and gestational age by last date of menstrual cycle and confirmed by ultrasound scans, parity, gravidity, history of miscarriages, medication and their weight were collected on the day of the OGTT test by LK and LK.

3.3.4 Sample collection

Capillary and venous plasma glucose samples were collected at three OGTT time points: fasting, 1-hour, and 2-hour post-glucose load. Each time, a finger was pricked once, and the blood was collected using a capillary tube to analyse the glucometers except for I-STAT and LDX glucometers as it required a larger volume of blood. Venous blood was collected in sodium fluoride and sodium citrate tubes and immediately analysed on I-STAT and LDX and the rest of the blood was used for lab-based glucose measurement. Information on the POC glucometers is reported in the supplementary files, Table S2.

3.3.5 Oral glucose tolerance test

The OGTTs were conducted by qualified nurses and diagnosed as per International Association of Diabetes and Pregnancy Study Groups (IADPSG) guidelines as described in Chapter 2. However, in this study, samples were kept in an ice slurry and separated within 10 minutes after the OGTT ended. Glucose was measured using the hexokinase method in the central lab by the Roche Cobas 8000/502 (Roche Diagnostics, Mannheim, Germany). Details of the glucose method are described in Chapter 2.

3.3.6 Statistical analysis

Statistical analyses and graphical representations for method agreement and reliability were conducted using MedCalc software v22.021. (Ostend, Belgium) and the rest was performed using STATA 18 software (StataCorp, College Station, TX, USA). A p-value <0.05 was assumed

to be statistically significant. The Shapiro-Wilk test was used to test for normality, normally and distributed data was reported as means ± standard deviation (SD) and non-normally distributed data were reported as medians with interquartile range (IQR). Categorical variables were expressed as sample numbers and percentages. Demographic and clinical characteristics in the GDM and non-GDM groups were compared using the student's t-test (for normally distributed data) or the Wilcoxon rank-sum test (for skewed data). Categorical data were analysed using a chi-squared or Fisher's exact test where expected frequencies were <5.

Methods were compared by Pearson correlation coefficient (PCC) and scatter plots with the least squares best-fit line and 95% confidence intervals (CI) band, intra-rater class coefficient (ICC) and Bland–Altman (BA) plots [170]. The ICC estimates and their 95% CI were based on single measurements absolute-agreement, 2-way random-effects model.

Sensitivity, specificity, negative predictive values (NPVs), positive predictive values (PPVs) and area under the receiver operating characteristic curve (AUC) were calculated to determine the diagnostic accuracy of each POC glucometer. The lab-based OGTT method and GDM diagnosis by IADPSG criteria were used as reference methods. Three criteria for GDM diagnosis were analysed, 1) GDM diagnosis by OGTT (as per IADPSG criteria), 2) FPG at 5.1 mmol/L and 3) FPG at 4.8 mmol/L. Both reference and classification methods were binary variables (classified as GDM yes /no). Criteria 2 and 3 were guided by literature that indicated that FPG can potentially diagnose GDM [8,138].

An ICC, PCC and AUC near the value of 1.0 suggest a high degree of method reliability, correlation or accuracy, respectively. For BA plots, POC glucometers with a mean difference of 1.3% or less and with narrow 95% limits of agreements (LOA) were deemed optimum [171].

3.4 RESULTS

3.4.1 Study population

In total, 1144 women were recruited, of whom 68 (5.94%) were excluded from the study. Reasons for exclusion were incomplete OGTT (6 (0.52%)), withdrew from the study (2 (0.17%)), vomited (=1(0.09%)), <18 years (1(0.09%)), and prediabetic (9 (0.79%)). Among the

1125 women included in the study, 49 (4.36%) had incomplete lab-based glucose measurements due to the following reasons: the specimen was insufficient, grossly haemolyzed, lipidemic, or clotted. Therefore, data were analysed for 1082 women (Figure 3.1).

The mean age of this population was 35 years (IQR: 29, 39). Mean gestational age was 25 weeks (IQR: 21, 29). Based on OGTT testing using the IADPSG criteria, 83 (7.7%) women had GDM.

Women with GDM had a significantly higher median (IQR) weight (89 kg [76, 104] vs. 80 [67, 95], p-value =0.001). Of note, there were no significant differences between groups in age, parity, and miscarriages, p value>0.05) (Table 3.1).

Table 3.1 Clinical characteristics in all women and by GDM status

Variable	**Total observations**	**All**	**Non-GDM**	**GDM**	**p-value**
Age (years)	1048	35 (29;39)	35 (29;39)	37 (31;39)	0.125
Gestational age (weeks)	1015	25 (21;29)	25 (21;29)	24 (20;28)	0.492
Miscarriages (n)	1034	0 (0;1)	0 (0;1)	0 (0;1)	0.889
Parity (n)	1034	2 (1;3)	2 (1;3)	2 (1;2)	0.198
Gravidity (n)	1034	4 (2;5)	4 (3;5)	3 (2;4)	0.255
Weight (kg)	1062	80 (68;95)	80 (67;95)	89 (73;101)	**0.001**
Fasting glucose from the laboratory (mmol/L)	1076	4.2 (3.9;4.5)	4.1 (3.9;4.4)	5.5 (5.2;5.8)	**<0.0001**
OGTT, 1-hour glucose from the laboratory (mmol/L)	1076	5.7 (4.9;6.8)	5.6 (4.9;6.6)	8.2 (6.2;9.7)	**<0.0001**
OGTT, 2-hour glucose from the laboratory (mmol/L)	1076	5.2 (4.5;6.0)	5.1 (4.5;5.8)	7.0 (5.8;8.6)	**<0.0001**

Data presented are median (IQR) unless stated otherwise. GDM, gestational diabetes mellitus; OGTT, oral glucose tolerance test.

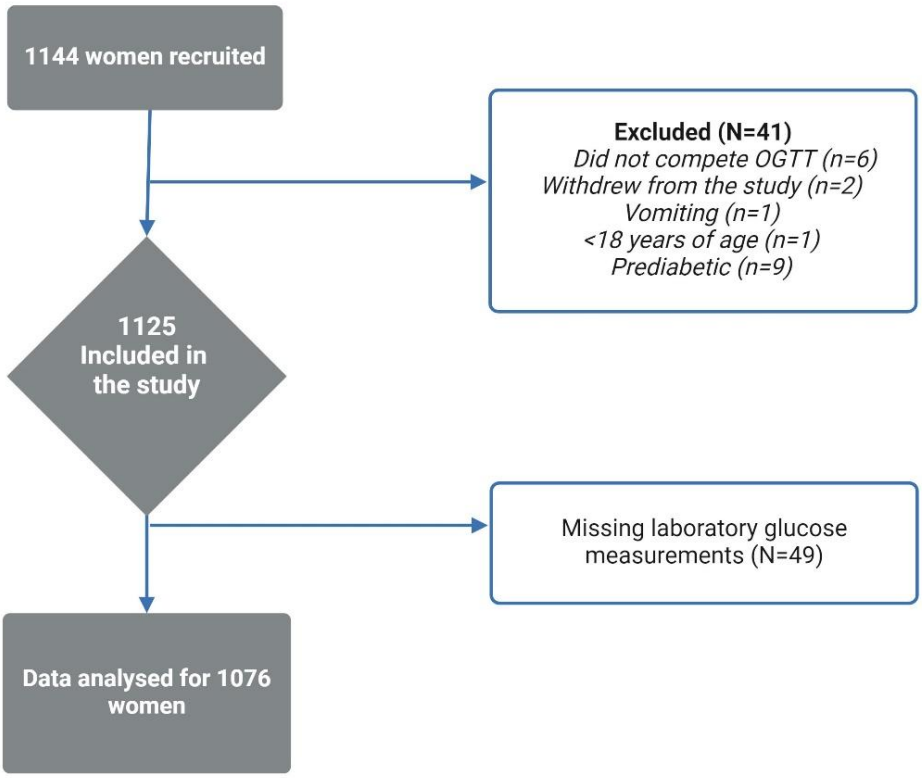


Figure 3.1. Diagram of participant recruitment, OGTT = oral glucose tolerance test

3.4.2 Method agreement and reliability analysis

Average glucose levels measured with the seven POC glucometers are shown in Table 3.2. Data was normally distributed, and the PCC ranged from 0.62 for Codefree at fasting to 0.87 for I-STAT and LDX at the 1-hour time point (p-value <0.001 for all, Figures 3.2- 3.4).

Table 3.2 Median glucose measured by glucometers at each OGTT time point

Device	Time point	Median glucose level (mmol/L)		
		All participants	**Non-GDM**	**GDM**
Laboratory	Fasting	4.2 (3.9; 4.5)	4.1 (3.9; 4.4)	5.5 (5.2; 6.0)
	OGTT, 1-hour	5.8 (6.9; 5.8)	5.7 (4.9; 6.6)	8.2 (6.2; 10.4)
	OGTT, 2-hour	5.2 (4.5; 6.0)	5.1 (4.5;5.8)	7.1 (5.9; 8.8)
I-STAT	Fasting	3.7 (3.4; 4.0)	3.6 (3.4; 3.9)	4.5 (4.1; 5.1)
	OGTT, 1-hour	4.9 (4.1; 5.9)	4.8 (4.1; 5.7)	7.2 (5.6; 8.8)
	OGTT, 2-hour	4.4 (3.9; 5.1)	4.3 (3.8; 5.0)	5.9 (4.9; 7.4)
Xpress	Fasting	4.5 (4.1;4.9)	4.4 (4.1; 4.9)	5.7 (4.9; 6.3)
	OGTT, 1-hour	7.2 (6.3; 8.3)	7.0 (6.2; 8.0)	9.0 (7.7; 11.2)
	OGTT, 2-hour	6.4 (5.7; 7.3)	6.3 (5.6; 7.0)	8.4 (7.0; 10.1)
LDX	Fasting	3.8 (3.5; 4.1)	3.7 (3.5; 4.0)	4.6 (4.2; 5.2)
	OGTT, 1-hour	5.1 (4.2; 5.9)	4.9 (4.2; 5.7)	7.2 (5.7; 8.5)
	OGTT, 2-hour	4.5 (3.9; 5.1)	4.4 (3.9; 5.0)	6.0 (5.1; 7.3)
Vivachek Ino	Fasting	4.7 (4.3; 5.1)	4.6 (4.3; 5.0)	6.1 (5.2; 6.5)
	OGTT, 1-hour	7.5 (6.5; 8.6)	7.3 (6.5; 8.3)	9.7 (8.3; 11.3)
	OGTT, 2-hour	6.6 (5.9; 7.5)	6.5 (5.8; 7.3)	9.0 (7.3; 10.6)
AccuChek Active	Fasting	4.5 (4.1; 4.9)	4.4 (4.1; 4.8)	5.5 (4.8; 6.3)
	OGTT, 1-hour	7.1 (6.1; 8.2)	7.0 (6.0; 8.0)	9.6 (8.1; 10.9)
	OGTT, 2-hour	6.2 (5.4; 7.1)	6.2 (5.3; 6.9)	8.1 (6.8; 10.0)
Statstrip	Fasting	4.3 (3.9; 4.8)	4.2 (3.8; 4.7)	5.4 (4.9; 5.9)
	OGTT, 1-hour	6.9 (6.0; 8.0)	6.3 (5.9; 7.8)	9.0 (7.6; 10.7)
	OGTT, 2-hour	6.1 (5.4; 7.0)	6.1 (5.3; 6.8)	8.0 (6.8; 9.5)
Codefree	Fasting	4.8 (4.5;5.3)	4.7 (4.4; 5.2)	5.8 (5.3; 6.6)
	OGTT, 1-hour	7.6 (6.6, 8.9)	7.5 (6.5; 8.6)	10.0 (8.0; 11.7)
	OGTT, 2-hour	6.7 (6.0; 7.6)	6.6 (6.0; 7.4)	8.7 (7.5; 11.0)

Data presented are median (IQR). GDM, gestational diabetes; OGTT, oral glucose tolerance test.

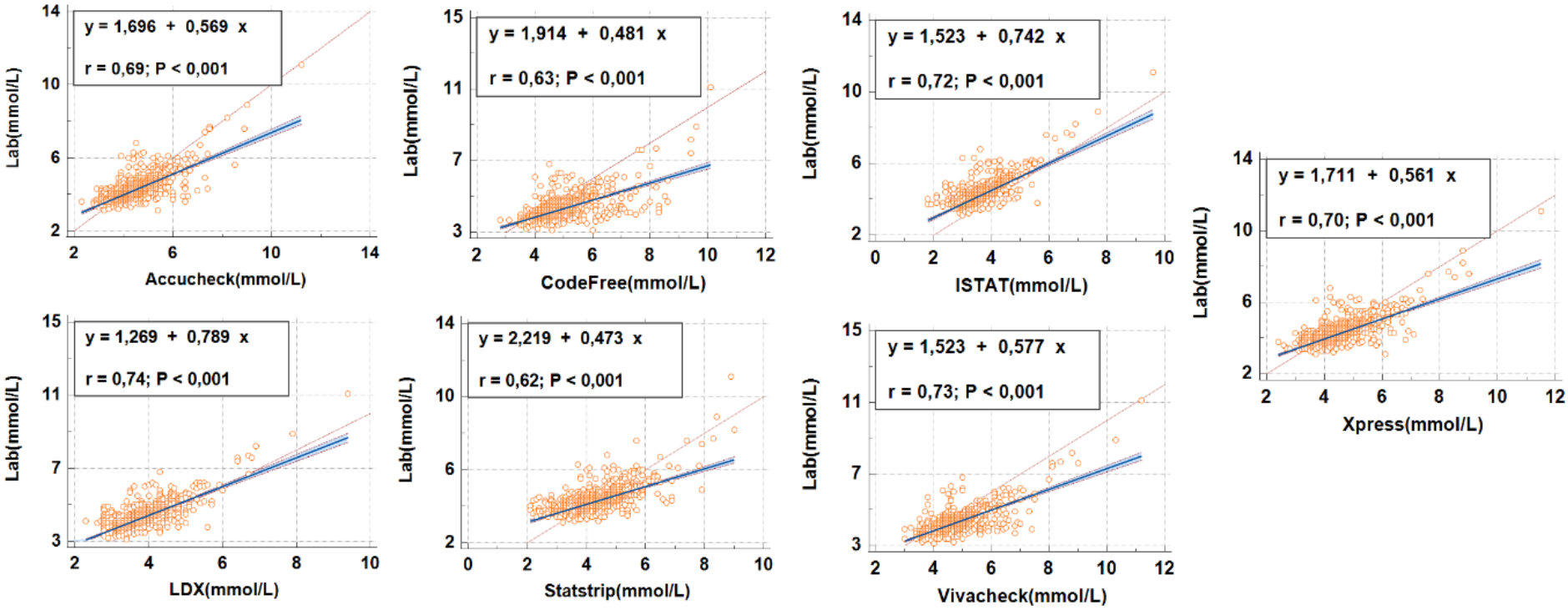


Figure 3.2. Scatter plots with the least squares best-fit line + 95% confidence band (blue) and line y=x (orange) of each glucometer against the laboratory at fasting.

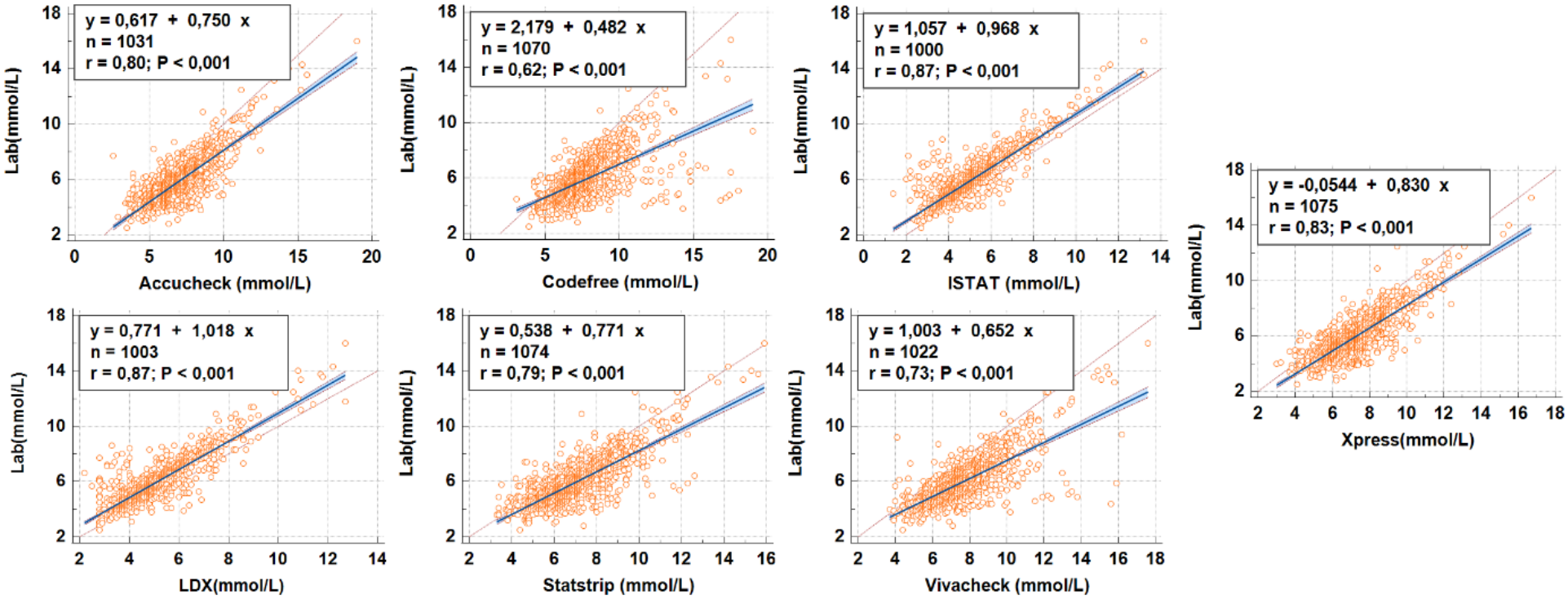


Figure 3.3. Scatter plots with the least squares best-fit line + 95% confidence band (blue) and line y=x (orange) of each glucometer against the laboratory at 1-hour post glucose load

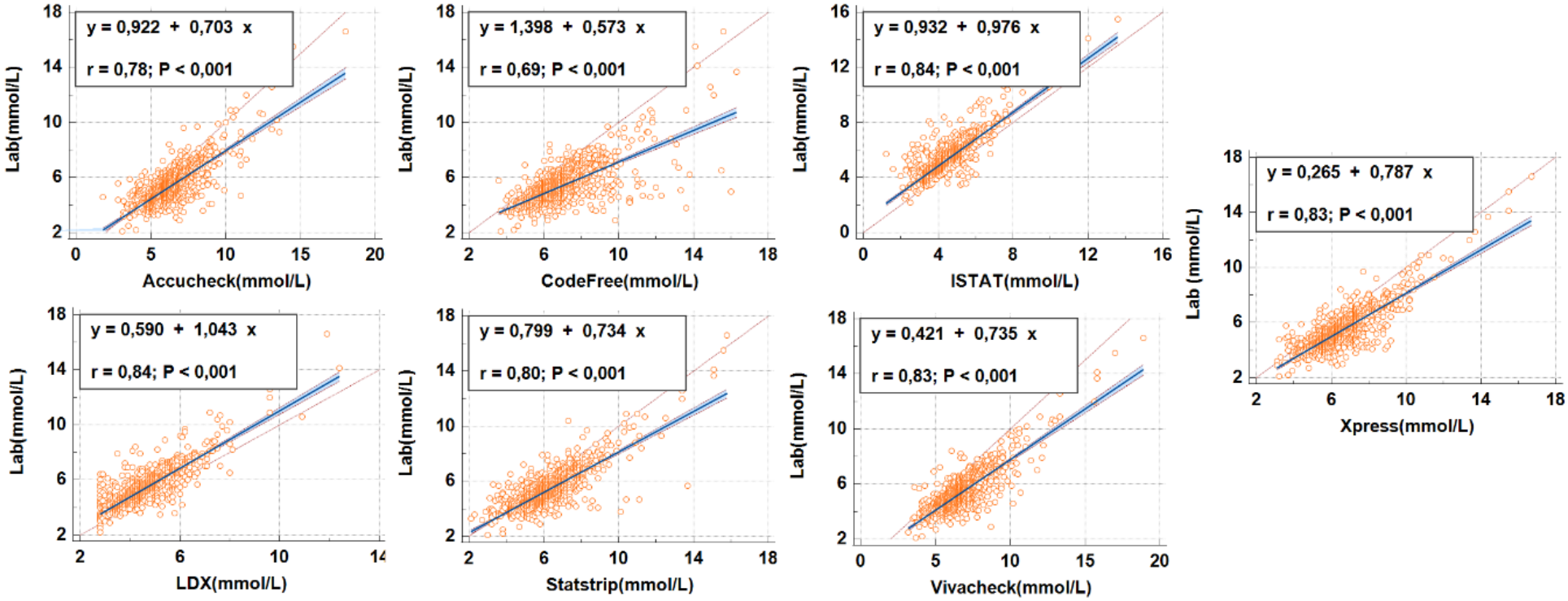


Figure 3.4. Scatter plots with the least squares best-fit line + 95% confidence band (blue) and line y=x (orange) of each glucometer against the laboratory at 2-hour post glucose load

According to the BA plots, Statstrip had the optimum mean differences (0.20%) at fasting and a desirable mean difference (-2.82%) at 1-hour post glucose load. Codefree had the highest mean difference (23.1%) at 1-hour post glucose load. All POC glucometers including Statstrip, had wide 95% LOA at all time points. The postprandial glucose readings showed the biggest mean percentage difference. According to the ICC estimates, all POC glucometers had moderate to poor reliability with a wide 95% CIs (Table 3.3 and Figure 3.5-3.7).

Table 3.3 Average difference, Bland–Altman 95%-limits of agreement, intraclass correlation coefficient (ICC, 95% CI) of point of care glucometers at three-time points.

At fasting (0 hr)				
	Observations (n)	**Differences % (SD)**	**95%-Limits of agreement (%)**	**ICC (95% CI)**
Accucheck Active	1021	-4.86 (12.1)	-28.5 to 19.0	0,525 (0,364 -0,639)
Codefree	1036	-12.2 (10.8)	-33.3 to 9.10	0,332 (-0,024 - 0,572)
I-STAT	976	16.6 (15.6)	-17.6 to 50.8	0,385 (-0,067 - 0,657)
LDX	987	13.0 (13.0)	-12.5 to 38.5	0,458 (0,013 - 0,703)
StatStrip	1062	0.20 (17.7)	-34.7 to 35.1	0,500 (0,452 - 0,544)
VivaChek	1001	-9.62 (10.3)	-29.9 to 10.6	0,450 (0,042 - 0,674)
Xpress	1071	-5.34 (12.2)	-29.2 to 18.5	0,521 (0,332 - 0,648)
At 1 hr post glucose load				
Accucheck Active	1008	-15.8 (16.2)	-47.5 to 16.0	0,592 (-0,011 - 0,811)
Codefree	1049	-23.1 (15.0)	-52.4 to 6.21	0,344 (-0,066 - 0,613)
I-STAT	978	20.0 (26.0)	-31.0 to 70.9	0,706 (0,098 - 0,875)
LDX	981	18.1 (21.2)	-23.5 to 59.7	0,708 (0,126- 0,873)
StatStrip	1051	-2.82 (22.3)	-46.6 to 41.0	0,599 (0,058 - 0,806)
VivaChek	999	-21.2 (14.4)	-49.6 to 7.03	0,438 (-0,079 - 0,717)
Xpress	1052	-17.8 (12.9)	-43.1 to 7.57	0,587 (-0,068 to 0,830)
At 2 hrs post glucose load				
Accucheck active	988	-14.1 (15.7)	-44.8 to 16.6	0,569(0,049 - 0,784)
Codefree	1033	-21.7 (13.2)	-47.5 to 4.0	0,361 (-0,085 - 0,647)
I-STAT	980	20.0 (23.6)	-26.2 to 66.2	0,624 (0,015 - 0,834)
LDX	976	18.0 (18.3)	-17.9 to 54.0	0,631 (0,045 - 0,833)
StatStrip	1051	-12.9 (14.4)	-41.1 to 15.2	0,606 (0,096 - 0,804)
VivaChek	993	-19.8 (12.0)	-43.3 to 3.69	0,490 (-0,094 - 0,777)
Xpress	1057	-16.8 (12.2)	-40.7 to 7.01	0,559 (-0,073 to 0,812)

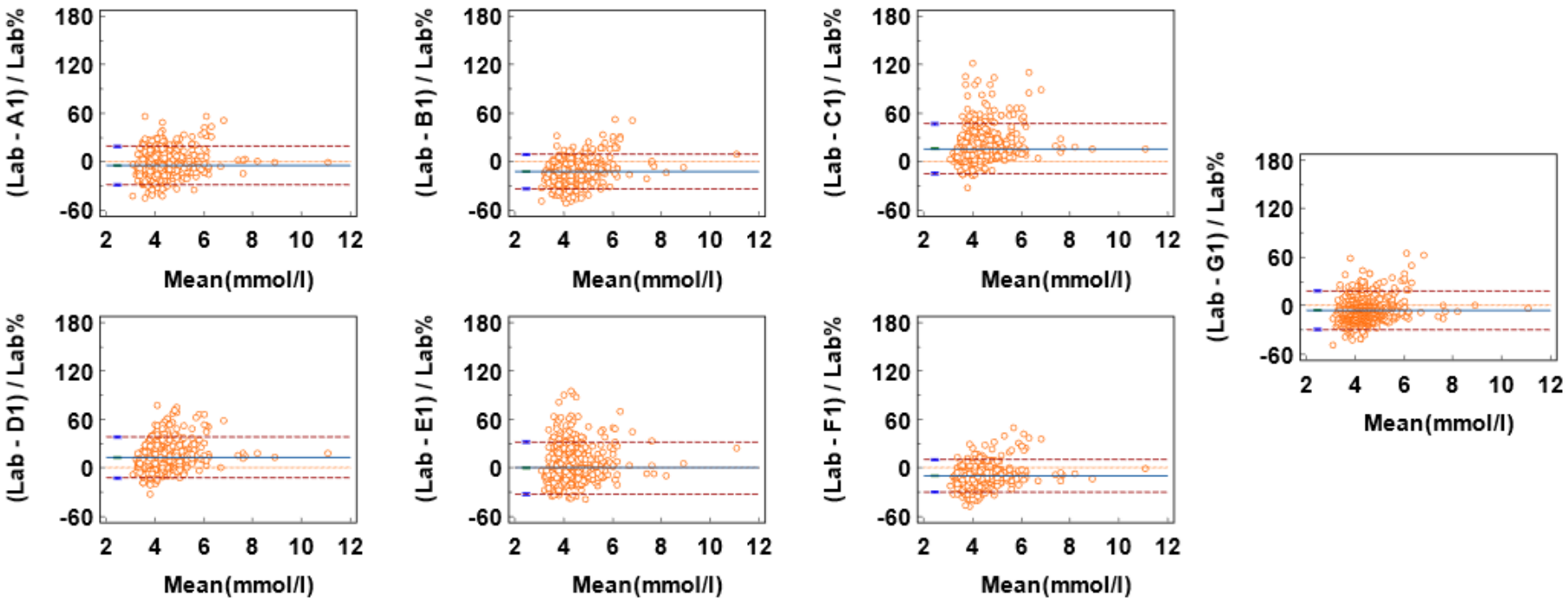


Figure 3.5. Bland-Altman plots ((mean difference (blue) and 95% limit of agreements (red)) of each point of care glucometer against laboratory-based measurements at fasting. A1= Accucheck active, B1 = Codefree, C1= I-STAT, D1= LDX, E1 = Statstrip, F1= Vivacheck and G1=Xpress.

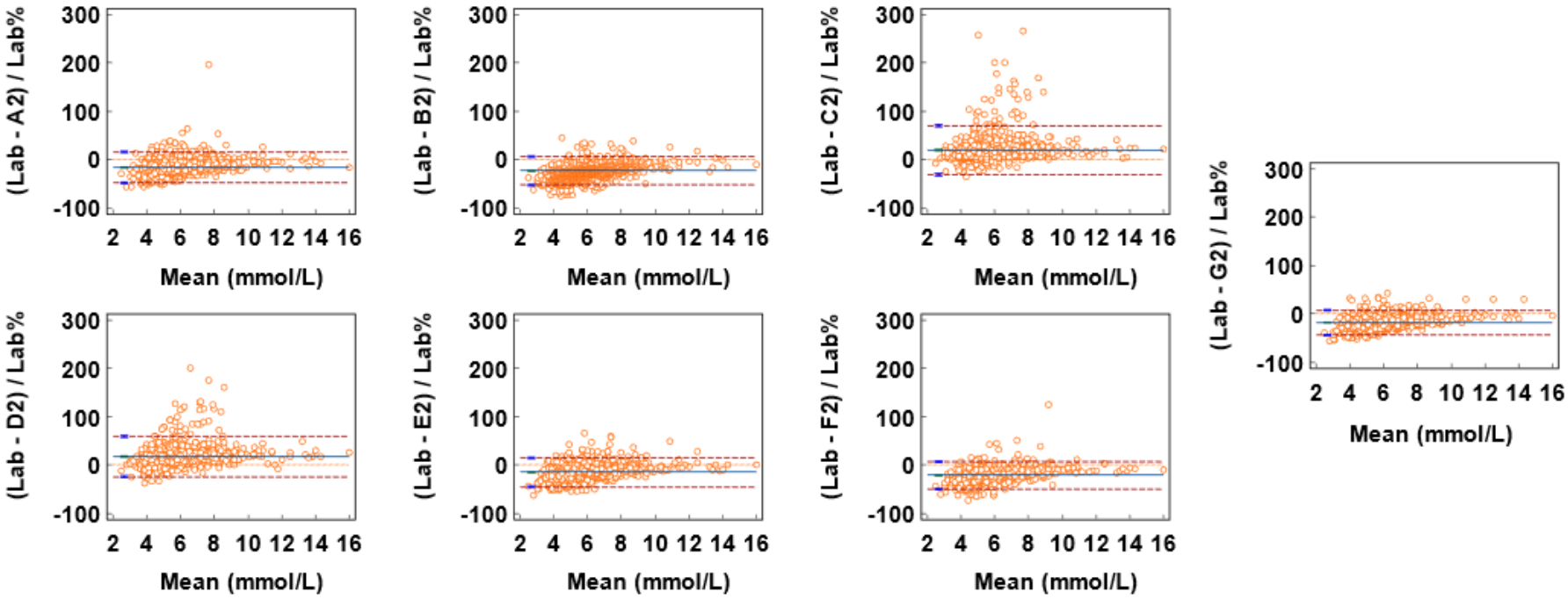


Figure 3.6. Bland-Altman plots ((mean difference (blue) and 95% limit of agreements (red)) of each point of care glucometer against laboratory-based measurements at 1-hour post glucose load. A2= Accucheck active, B2 = Codefree, C2= I-STAT, D2= LDX, E2 = Statstrip, F2= Vivacheck and G2=Xpress

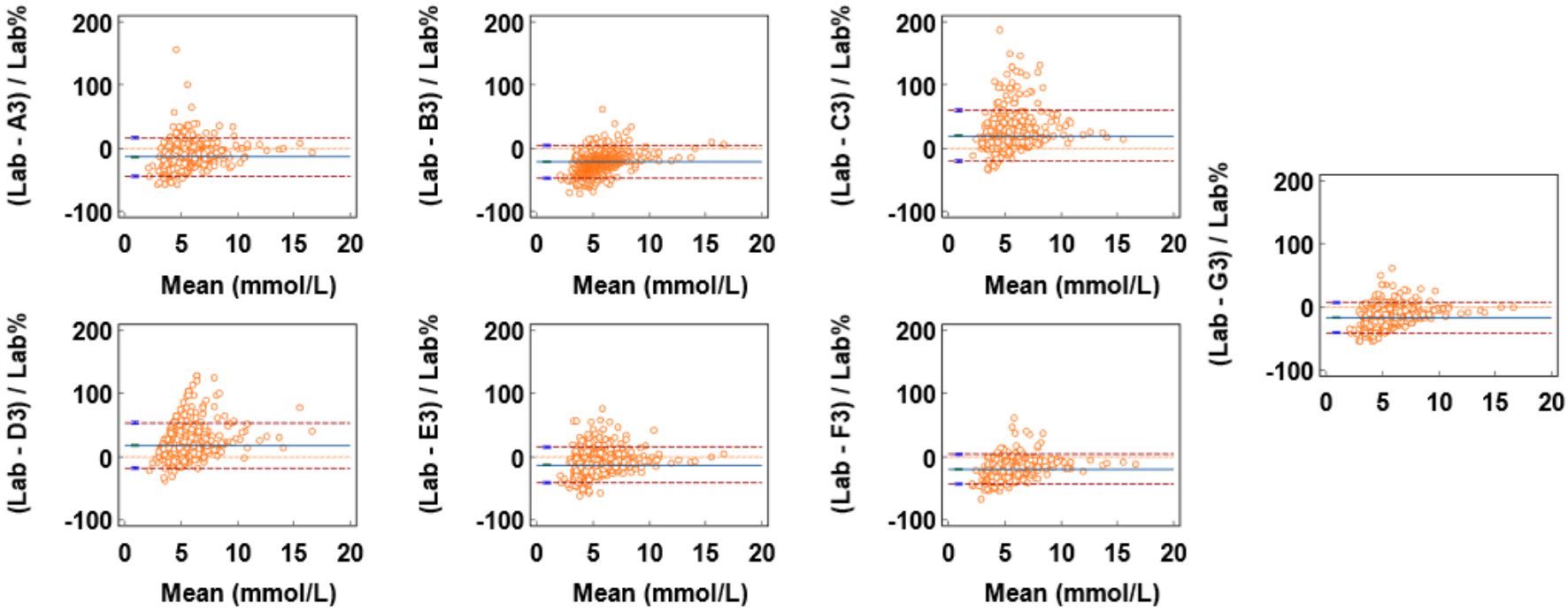


Figure 3.7. Bland-Altman plots ((mean difference (blue) and 95% limit of agreements (red)) of each point of care glucometer against laboratory-based measurements at 1-hour post glucose load. A3= Accucheck active, B3 = Codefree, C3= I-STAT, D3= LDX, E3 = Statstrip, F3= Vivacheck and G3=Xpress

3.4.3 Accuracy of POC glucometers

For criteria 1) GDM diagnosis by OGTT (as per IADPSG criteria, sensitivity (95%CI) varied from 17.57 (15.17-19.96) for I-STAT to 87.18(85.14- 89.22) for Codefree (Table 3.4).

Specificity (95%CI) was poorest for Codefree at 62.71(59.76-65.66) and was highest for I-STAT at 99.78 (99.48-100). The NPV (95% CI) were good for all POC devices and ranged from 93.76 (92.24 to 95.15) for LDX to 98.35 (97.58 to 99.13) for the Codefree. PPVs (95% CI) ranged between 16.08 (13.83-18.32) for Codefree to 86.67 (84.53-88.81) for the I-STAT. The AUC (95%CI) ranged from 0.59 (0.54-0.63) for I-STAT to 0.79 (0.74-0.85) for Accucheck.

The accuracy of POC glucometers when using FPG glucose alone was lower than when all three time points were used (Table S3 and S4). However, for lab-based FPG at 5.1 mmol/L threshold, sensitivity (95% CI) and specificity (95% CI) were 100 (100-100) and 93.98 (92.55; 95.40) respectively (Table S4).

Table 3.4 The accuracy of each glucometer when compared to laboratory-based measurements

	Accucheck active	**Codefree**	**I-STAT**	**LDX**	**Statstrip**	**Vivacheck**	**Xpress**
Observations	982	1030	969	964	1076	983	1052
PPV, % (95% CI)	28.25 (25.44; 31.07)	16.08 (13.83; 18.32)	86.67 (84.53; 88.81)	83.33 (80.98; 85.69)	27.06 (24.41;29.72)	17.96 (15.56; 20.36)	24.36)21.77; 26.96)
NPV, % (95% CI)	98.02 (97.15;98.89)	98.35 (97.58; 99.13)	93.61 (92.07; 95.15)	93.76 (92.24; 95.29)	97.20 (96.22;98.19)	98.00 (97.12; 98.87)	98.07 (97.24 ;98.90)
Sensitivity, % (95% CI)	80.77 (78.30; 83.23)	87.18 (85.14; 89.22)	17.57 (15.17; 19.96)	20.27 (17.73; 22.81)	71.08 (68.38; 73.79)	82.19 (79.80; 84.58)	81.71 (79.37; 84.04)
Specificity, % (95% CI)	82.30 (79.91; 84.69)	62.71 (59.76; 65.66)	99.78 (99.48; 100.00)	99.66 (99.30; 100.00)	83.99 (81.80; 86.18)	69.89 967.02; 72.76)	78.56 (76.08; 81.04)
False +ve, n	160	355	2	3	159	274	208
False -ve, n	15	10	61	59	24	13	15
True +ve, n	63	68	13	15	59	60	67
True -ve, n	744	597	893	887	834	636	762
AUC	0.79 (0.74; 0.85)	0.74 (0.69; 0.79)	0.59 (0.54; 0.63)	0.59 (0.55; 0.64)	0.76 (0.69; 0.82)	0.74 (0.68; 0.80)	0.78 (0.73; 0.84)

AUC, area under the curve; CI, confidence interval; OGTT, oral glucose tolerance test

We then compared the GDM cases that were missed by Statstrip (the glucometer that performed better than the other glucometers) with all GDMs using the laboratory method as the reference method. The GDM cases that were missed by Statstrip had higher mean laboratory-based fasting glucose measurements than the overall mean glucose measurement of all GDM cases (Table 3.5).

Table 3.5 The comparison of missed GDM cases by StatStrip with true GDM cases (as diagnosed by laboratory method)

OGTT time point	Missed GDM cases by Stratstrip			GDM		p-value
	Observations (n)	StatStrip measurement	Laboratory measurement	Observations (n)	Laboratory measurement	
Fasting glucose (mmol/L)	21	4.39 (0.58)	5.74 (0.45)	83	5.46 (0.52)	0.026*

All glucose measurements expressed as mean (SD); *comparisons between the missed GDM cases (lab-based glucose) and all GDM cases.

3.5. DISCUSSION

This study, conducted among pregnant women in a tertiary care hospital in SA, investigated the accuracy of seven POC glucometers in diagnosing GDM. Compared with laboratory diagnosis, the POC glucose testing performed with a sensitivity ranging between 18 and 87% and a specificity ranging between 63 and 99%. The number of diagnoses that would have been missed with the POC glucose testing ranged from 10 to 61.

In vitro glycolysis is a key consideration with laboratory GDM diagnosis and pre-analytical protocols should be designed to minimise glycolysis. As a result, the HAPO research included strict pre-analytical procedures for sample collection and processing [27]. In SA, most OGTT testing during pregnancy is performed in tertiary academic or provincial tertiary hospitals with variable proximity to a laboratory. Nurses are unable to centrifuge samples immediately and transport on ice slurry rarely happens. Pre-analytical error is likely significant in these settings, underpinning the need for POC testing. Considering that POC glucometers are readily available and, in most cases, less expensive than conventional lab-based measurements, POC testing is an economically viable choice [139]. Handheld glucometers are simple to use and rarely require calibration, while bench-top platforms like the LDX and I-STAT need regular calibration and technical oversight. Ensuring readiness

requires clear guidelines for maintenance, spare parts, and laboratory supervision. Operator training is crucial alongside competency checks, refresher courses, and troubleshooting support as part of the day-to-day operation of the instruments. Bench-top glucometers require more advanced training, including calibration and quality control. Therefore, to maintain accuracy and reliability across sites, these requirements must be integrated into existing quality management systems with regular QC, external assurance, and supervision even for the handheld glucometers.

However, our study shows a lack of diagnostic accuracy among POC glucometers, with the number of women with GDM diagnosed based on glucose meter readings varying widely, which indicates that the POC glucose testing cannot be used interchangeably. Such variation has been reported previously in a comparative performance of 5 POC glucometers [139].

Our results are in line with several other studies which suggest that POC glucose testing compares poorly to laboratory measures for the diagnosis of GDM [172,173]. In a study among 529 pregnant women attending a primary healthcare clinic in Johannesburg, the Accuchek Active glucose meter diagnosed GDM with a sensitivity of 27.0% and a specificity of 89.4% which is not very different to our PPV and NPV [10]. In contrast to the above study, Gallardo-Rincón et al. [174], reported a sensitivity and specificity of 89.5% and 66.6%, respectively for Accucheck Instant. According to Daly et al. [138], lowering the diagnostic threshold for FPG from 5.1 to 4.8mmol/L increased sensitivity from 51% to 92.5% but decreased specificity from 100% to 76.5% [138]. Interestingly, the same authors also used a regression equation to predict plasma glucose levels based on glucose meter readings, indicating that such an approach may be acceptable in LMICs where plasma samples are subject to glycolysis [138]. In our study, the greatest differences between POC-measured and laboratory-measured glucose levels were at the 1-hour time point which may be an indication of poor performance of the POC glucose testing at higher glucose concentrations.

In addition, when comparing the fasting glucose levels in GDM cases diagnosed with the laboratory method but not by the StatStrip glucometer it was found that the glucose levels from the laboratory method were higher than the mean glucose level for all the GDM cases. Similar results were found for VivaCheck and Codefree. This discovery is concerning because it suggests that GDM cases with fasting glucose levels well above the diagnostic cut point of

5.1 mmol/L may go unnoticed, potentially leading to health complications associated with GDM for the mother and the child. Possible reasons for this bias include device variability between strip lots (despite our efforts to limit testing to a single lot), humidity, temperature, haematocrit, medicines and hypotension [168]. However, previous literature showed that StatStrip is not affected by haematocrit levels or impacted by substances such as maltose, galactose, dopamine, or uric acid [175].

Another option for GDM screening is the use of FPG measurements. In the current study, the measurement of FPG showed good sensitivity and specificity for diagnosing GDM when samples were analysed in the laboratory. These findings are in line with those from several other studies conducted in SA which showed high accuracy of laboratory fasting glucose in diagnosing GDM [139,141]. In addition, the FPG may be a better GDM screening test than universal OGTT testing due to its quick turnaround and simplicity of use. However, it is important to determine the diagnostic performance and optimal cut-off of FPG in the screening of GDM. In addition, it must be noted that pre-analytical errors and glycolysis are also risks with laboratory analysis of FPG. In addition, the use of lab-based FPG would not address the geographical barriers in the country as the women would still need to travel to the clinic to get tested.

Given the high NPV of the glucometers at fasting, which suggests the likelihood of not having GDM when tested negative, we recommend the use of POC for screening for GDM using FPG at 5.1 mmol/L and reserve OGTTs (by lab-based glucose measurements) for women who test positive. Furthermore, we recommend that future studies should perform modelling analyses to evaluate the impact of having access to POC GDM screening versus being undiagnosed/untreated for GDM entirely. Of note, if POC glucose testing is to be used for diagnosis, manufacturers should meet the performance specifications as recommended by the Milan hierarchy (179).

Our study had several limitations. First was the length of time between the collection of blood and analysis in the central laboratory. During this time, there was a risk of glycolysis. POC analysis was sometimes interrupted mid-OGTT due to POC glucometer malfunctions or insufficient cartridges/strips, especially for I-STAT and LDX, whose cartridges were stored far from the clinic. This contributed to the uneven observations across the POC glucometers.

Patient information such as singleton or twin pregnancy, foetal weight, and oligo/polyhydramnios was not collected. Finally, this study was conducted in a high-risk group, which may skew the results for the study population compared to the healthy pregnant women. Our study also had several notable strengths including the number of women participating in the study, as well as the wide range of POC glucometers. As such, these results are a valuable source of information on the accuracy of POC glucose testing in diagnosing GDM among women in SA.

3.6 CONCLUSION

Based on the present study, the use of these POC glucometers for the diagnosis of GDM cannot be recommended. Nonetheless, the POC glucometers have the potential to screen for GDM when a two-step screening process is considered. This may be beneficial in resource-constrained settings where access to laboratory measurement of glucose may be difficult. In addition, lab-based FPG has the potential to be used as a diagnostic test for GDM. Future research should look at why POC devices such as Statstrip, VivaCheck, and Codefree in some GDM cases were unable to accurately measure fasting glucose levels that were significantly higher than the diagnostic cut point of 5.1 mmol/L.

Building on these findings, we proceeded to explore the application of glycaemic indicators, specifically FPG, glycated albumin (GA), and glycated haemoglobin (HbA1c), in the diagnosis of GDM. We re-assessed the diagnostic accuracy of FPG in a new population to confirm the results from the current study, whilst GA and HbA1c were tested because they do not require the collection of fasting glucose samples. The glycaemic markers were evaluated in relation to the OGTT, and GDM was diagnosed according to the IADPSG criteria. This manuscript (Chapter 4) has been submitted to the International Journal of Gynaecology and Obstetrics.

3.7 SUPPLEMENTARY MATERIAL

Table S1: STARD 2015 Checklist

	Section & Topic	No	Item
	TITLE OR ABSTRACT		
		1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)
	ABSTRACT		
		2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)
	INTRODUCTION		
		3	Scientific and clinical background, including the intended use and clinical role of the index test
		4	Study objectives and hypotheses
	METHODS		
	Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)
	Participants	**6**	Eligibility criteria
		7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)

		8	Where and when potentially eligible participants were identified (setting, location and dates)
		9	Whether participants formed a consecutive, random or convenience series
	Test methods	**10a**	Index test, in sufficient detail to allow replication
		10b	Reference standard, in sufficient detail to allow replication
		11	Rationale for choosing the reference standard (if alternatives exist)
		12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory
		12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory
		13a	Whether clinical information and reference standard results were available to the performers/readers of the index test
		13b	Whether clinical information and index test results were available to the assessors of the reference standard
	Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy
		15	How indeterminate index test or reference standard results were handled
		16	How missing data on the index test and reference standard were handled
		17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory

		18	Intended sample size and how it was determined
	RESULTS		
	Participants	**19**	Flow of participants, using a diagram
		20	Baseline demographic and clinical characteristics of participants
		21a	Distribution of severity of disease in those with the target condition
		21b	Distribution of alternative diagnoses in those without the target condition
		22	Time interval and any clinical interventions between index test and reference standard
	Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard
		24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)
		25	Any adverse events from performing the index test or the reference standard
	DISCUSSION		
		26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability
		27	Implications for practise, including the intended use and clinical role of the index test
	OTHER INFORMATION		
		28	Registration number and name of registry
		29	Where the full study protocol can be accessed
		30	Sources of funding and other support; role of funders

Table S2: Information on the instruments used.

Before commencing the study, all POC glucometers were evaluated using the CLSI EP15 precision study protocol [176] and internal quality controls from Randox Laboratories (Co. Antrim, UK) and Nova Biomedical (Cheshire, UK) were used.

Manufacturer, country, year	**Instrument name, year, country**	**Instrument type**	**Sample type used for analysis in the study**	**Test principle**
Roche, Germany	Cobas c 502 (reference)	Automated analyser at the core laboratory	Plasma	Hexokinase with spectrophotometric detection.
Abbott, USA, 2021	I-STAT	Handheld multi-analyser, cartridge-based	Whole blood	Glucose oxidase
Nova Biomedical, USA, 2021	StatStrip Xpress 2	SMBG	Capillary	Glucose oxidase
Abbott, USA, 2021	Alere Cholestech LDX	Benchtop	Whole blood	Glucose oxidase
Vivachek Laboratories, USA, 2021	VivaChek Ino [177]	SMBG	Capillary	Glucose oxidase
Roche, Germany, 2021	Accu-Chek Active	SMBG	Capillary	Glucose dehydrogenase
Nova Biomedical, USA, 2018	StatStrip	Handheld multi-analyser, strip-based	Capillary	Glucose oxidase
SD Biosensor, Republic of Korea, 2021	SD Codefree [178]	SMBG	Capillary	Glucose oxidase

SMGB-self-monitoring of blood glucose, USA- United States of America

Table S3: The accuracy of each POC glucometer at fasting (4.8 mmol/L threshold) when compared to laboratory-based OGTT results

	Accucheck active	Codefree	I-STAT	LDX	Statstrip	Vivacheck	Xpress
Participants	1026	1055	979	991	1067	1008	1075
PPV, % (95% CI)	18.24 (15.85; 20.57)	12.57 (10.57; 14.57)	75.86 (73.18; 78.54)	60.00 (56.95; 63.05)	23.05 (20.52; 25.58)	14.12 (11.97; 16.27)	18.21 (15.90; 20.51)
NPV, % (95% CI)	97.25 (96.25; 98.25)	97.51 (96.57; 98.45)	94.32 (92.87; 95.77)	94.32 (92.88; 95.76)	97.49 (96.56; 98.43)	97.74 (96.83; 98.66)	97.88 (97.02; 98.74)
Sensitivity, % (95% CI)	76.25 (73.65; 78.850	83.75 (81.52; 85.98)	28.95 (26.11; 31.79)	30.77 (27.90; 33.64)	75.61 (73.03; 78.19)	82.43 (80.08; 84.78)	81.71 (79.40; 84.02)
Specificity, % (95% CI)	71.04 (68.26; 73.81)	52.21 (49.19; 55.22)	99.22 (98.68; 99.77)	95.25 (97.43; 99.06)	78.98 (76.54; 81.43)	60.28 (57.26; 63.30)	69.69 (66.94; 72.44)
False +ve, n	274	466	7	16	207	371	301
False -ve, n	19	13	54	54	20	13	15
True +ve, n	61	67	22	24	62	61	67
True -ve, n	672	509	896	897	778	563	692
AUC	0.74 (0.68; 0.79)	0.67 (0.62; 0.72)	0.65 (0.59; 0.71)	0.62 (0.57; 0.68)	0.77 (0.72; 0.83)	0.71 (0.66; 0.76)	0.76 (0.71; 0.81)

AUC, area under the curve; CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.

Table S4: The accuracy of each POC glucometer at fasting (5.1 mmol/L threshold) when compared to laboratory-based OGTT results

	Accucheck active	Codefree	I-STAT	LDX	Statstrip	Vivacheck	Xpress 2	Laboratory*
Participants	1013	1055	976	991	1067	1008	1075	1076
PPV, % (95% CI)	31.49 (28.65; 34.33)	17.40 (15.12; 19.69)	85.71 (83.52; 87.91)	78.95 (76.41; 81.49)	33.97 (31.13; 36.82)	20.65 (18.15; 23.15)	25.99 (23.37; 28.61)	100 (100; 100)
NPV, % (95% CI)	97.28 (96.28; 98.27)	97.55 (96.61; 98.48)	93.37 (91.81; 94.93)	93.52 (91.99; 95.05)	96.82 (95.76; 97.87)	97.68 (96.75; 98.61)	97.29 (96.32; 98.26)	99.50 (99.08; 99.92)
Sensitivity, % (95% CI)	71.25 (68.48; 74.02)	78.75 (76.28; 81.22)	15.79 (13.51; 18.07)	19.23 (16.78; 21.68)	64.63 (61.77; 67.50)	77.03 (74.43; 79.62)	71.95 (69.27; 74.64)	93.98 (92.55; 95.40)
Specificity, % (95% CI)	86.89 (84.83; 88.96)	69.33 (66.55; 72.12)	99.78 (99.48; 100.00)	99.56 (99.15; 99.97)	89.54 (87.71; 91.38)	76.55 (73.94; 79.17)	83.08 (80.84; 85.32)	100 (100; 100)
False +ve, n	124	299	2	4	103	219	168	0
False -ve, n	23	17	64	63	29	17	23	5
True +ve, n	57	63	12	15	53	57	59	78
True -ve, n	822	679	901	909	882	715	825	993
AUC	0.79 (0.73; 0.85)	0.73 (0.68; 0.79)	0.57 (0.53; 0.62)	0.57 (0.53; 0.61)	0.77 (0.70; 0.83)	0.76 (0.70; 0.82)	0.77 (0.71; 0.83)	0.98 (0.94; 1.00)

AUC, area under the curve; CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value. *glucose at 5.1 mmol/L fasting blood

CHAPTER 4: FASTING PLASMA GLUCOSE PERFORMS BETTER THAN GLYCATED HAEMOGLOBIN AND GLYCATED ALBUMIN FOR DIAGNOSING GESTATIONAL DIABETES

Khambule L, Chauke L, Crowther N.J., George J.A. Fasting plasma glucose performs better than glycated haemoglobin and glycated albumin for diagnosing gestational diabetes. Submitted to the *Int J Gynecol Obstet* (under review).

4.1 ABSTRACT

Objective: Universal screening for gestational diabetes mellitus (GDM) using the oral glucose tolerance test (OGTT) has been recommended. In resource-poor environments, OGTTs are only administered to women at high-risk for GDM. In countries such as South Africa, where female obesity is common, this translates into high numbers of tests. We therefore investigated glycated haemoglobin (HbA1c), glycated albumin (GA) and fasting plasma glucose (FPG) as OGTT alternatives.

Methods: This was a cross-sectional study in black South African women between 24-32 weeks of gestation who were at high risk for GDM (N=524). All women underwent an OGTT and were classified as non-GDM or GDM based on the International Association of Diabetes and Pregnancy Study Group (IADPSG) criteria. Demographic and anthropometric data were documented, and blood glucose, GA and HbA1c levels were measured using standardised laboratory procedures. Cut points for GDM diagnosis using HbA1c and GA were calculated using ROC curve analysis.

Results: The sensitivity and specificity of HbA1c at a 5.5% cut-point for GDM diagnosis were 54% and 80% and for GA at a 37.56% cut-point were 54% and 83% whilst FPG at a 5.1mmol/L cut-point were 87% and 100%, respectively, missing 10 of 78 women with GDM. Applying an FPG cut point of 4.6mmol/L, 9 of the 10 remaining women with GDM were identified, with sensitivity and specificity of 90% and 75%, respectively. This two-tiered approach missed 1 of 78 women with GDM.

Conclusion: Unlike GA and HbA1c, FPG is a viable method for GDM diagnosis that when used in a two-tiered modality reduces the number of required OGTTs by 77%.

4.2 INTRODUCTION

Over 90% of the women with GDM in the world are found in low- and middle-income countries (LMICs) [7] and in South Africa, GDM prevalence ranges from 9.1 to 25.8% [9,10]. Complications of GDM are preventable if it is diagnosed and treated in time.

A number of organisations recommend universal screening for GDM using the OGTT, but in resourced limited settings selective screening based on the risk factors for GDM is often used to reduce the number of OGTTs. Selective screening has however, displayed contradictory findings, with some research stating that it has high sensitivity and specificity and others indicating that it only averted a small number of OGTTs and created unnecessary complications of testing [118]. A South African study showed that 59 (10.6%) out of 554 women would be missed if selective screening was applied and 170 (30.7%) of the 554 women tested had one or more of the risk factors mentioned below but tested negative for GDM [137]. The inadequacy of the selective screening approach has also been observed in European, South American and South Asian populations [179–181]. Thus, the choice of screening and diagnostic methods for GDM must take into consideration the infrastructural and financial constraints of the healthcare system within each country.

The HbA1c test is a common method for monitoring glycaemic control and diagnosing diabetes in non-pregnant individuals. However, because of the changing erythrocyte count during pregnancy, HbA1c testing may inaccurately indicate the person's glycaemic status [143,182–185]. Unlike HbA1c, glycated albumin (GA) is unaffected by iron levels and, therefore, not altered by iron deficiency during pregnancy and maybe a more accurate measure of glycaemia through pregnancy than HbA1c [183]. In addition, a study conducted in a Japanese population showed an association between GA and neonatal health problems such as respiratory issues, hypoglycaemia, macrosomia, and polycythaemia [186]. The use of HbA1c or GA for GDM diagnosis is appealing because these tests do not require an OGTT, and HbA1c stability is superior to that of plasma glucose [187]. In addition, point-of-care (POC) instruments are available for both HbA1c and GA measurements. However, internationally accepted cut points for the diagnosis of GDM using HbA1c or GA are not currently available.

Using fasting plasma glucose (FPG) alone would reduce the cost implications of performing a full OGTT. In addition, POC instruments for glucose are readily available. In the HAPO study, out of the cohort, 16% had GDM, and FPG identified 8.3% of the cohort with GDM. Another 5.7% and 2.1% of the cohort were detected by 1-h and 2-h plasma glucose measurements [188].

In a South African black population, FPG at 5.1 mmol/L exhibited a sensitivity of 88% (95% CIs 74 - 96) and a specificity of 100% [8].

Therefore, this study aimed to compare the diagnostic accuracy of HbA1c, GA and FPG for GDM diagnosis in urban black African women as an alternative to the OGTT.

4.3 METHODOLOGY

4.3.1 Study design and population

This was a multicentre cross-sectional study of pregnant black African women between 24 and 32 weeks of gestation who were scheduled for an OGTT by the nurses as they had one or more risk factors for GDM. This study was conducted between November 2018 and May 2022. The sample size and study design are described in Chapter 2.

4.3.2 Ethics

Institutional ethics approval was obtained; details are available in Chapter 2.

4.3.3 Sample Collection and Analysis

Haemoglobin was tested using a HemoCue POCT Hb 201 analyser (Danaher, CA, USA). The HIV status and glucosuria were tested as described in Chapter 2. Anthropometric measurements were performed on the day of the OGTT by the healthcare workers and the PI collected the data onto questionnaires. Body mass index (BMI; kg/m^2) was calculated and categorised as normal or overweight (18.5–29.9 kg/m^2) or obese (≥30.0 kg/m^2). Medical and family history were recorded on questionnaires by the principal investigator and was taken from the participants' clinical records and by interview; participants were asked about their employment, alcohol use and smoking status. All participants had their gestation period confirmed by ultrasound.

4.3.4 Oral glucose tolerance test

All the participants went through OGTT as per IADPSG guidelines described in Chapter 2. Whole blood samples were collected in sodium fluoride tubes and glucose levels were measured within 30 minutes of collection. Glucose was measured using the Cobas 8000 (Roche Diagnostics, Mannheim, DE) in CMJAH and the au480 chemical analyser (Beckman Coulter, Riyadh, KSA) in RHMMCH and Edenvale Hospital; both instruments used the glucose hexokinase method.

4.3.5 Glycated haemoglobin (HbA1c) and glycated albumin (GA) measurement

Glycated haemoglobin (HbA1c) was measured using the Variant II kit and high-pressure liquid chromatography (HPLC) system from Bio-Rad (Hercules, CA). Briefly, the method uses the principle of ion-exchange HPLC. Haemoglobins are separated based on their ionic interactions with a pH gradient buffered cartridge, the separated haemoglobins are detected using absorbance at 415nm and the background was corrected using 690nm. The detection of haemoglobin variants, including HbS, HbC, and HbE was done by analysing chromatograms of each patient sample, none of which were observed in participants. The intra-assay and inter-assay CVs for HbA1c were 0.64 and 1.15, respectively.

Albumin and GA measurements were determined by the quantILab Glycated Albumin enzymatic assay (Werfen, Italy) on a Cobas 8000 autoanalyzer, as per the manufacturer's instructions [164]. The GA levels were expressed as a percentage derived from the GA/albumin ratio. Details pertaining to GA analysis are available in Chapter 2

4.3.6 Statistical analysis

Statistical analysis was performed using STATA 16.1 software (StataCorp, College Station, TX, USA). Continuous variables with a normal distribution were expressed as means and SDs and as medians with an interquartile range if not normally distributed. Categorical variables were expressed as sample numbers with percentages. The Student's t-test for normally distributed data and the Wilcoxon rank-sum test for skewed data were used to compare continuous variables between the women with GDM and the control group. The chi-squared test was performed for categorical data and Fisher's exact test was used in cases where counts were <5. The area under the receiver operator characteristic (ROC) curve (AUC) with 95% CI were determined for GDM diagnosis using HbA1c, GA and FPG as continuous

variables and the OGTT as the reference method. The optimum GA and HbA1c thresholds for diagnosing GDM were estimated using the Youden index [189]. Sensitivity (%), specificity (%), positive predictive value (PPV) and negative predictive value (NPV) were calculated for each test at the optimum threshold. A $p<0.05$ was assumed to be statistically significant.

4.4 RESULTS

4.4.1 Participants

Five hundred and twenty-four women met the inclusion criteria. Following the OGTT and HbA1c analysis, 47 (8.97%) women were excluded as a result of 28 (5.34%) women with missing OGTT results and 19 (3.63%) women classified as prediabetics leaving a final sample size of 477 (Figure 4.1). Within this group, 268 (56.2%) women had an HbA1c test, routinely performed only at CMJAH and RHMMAH. The GA test was performed on 200 randomly selected blood samples.

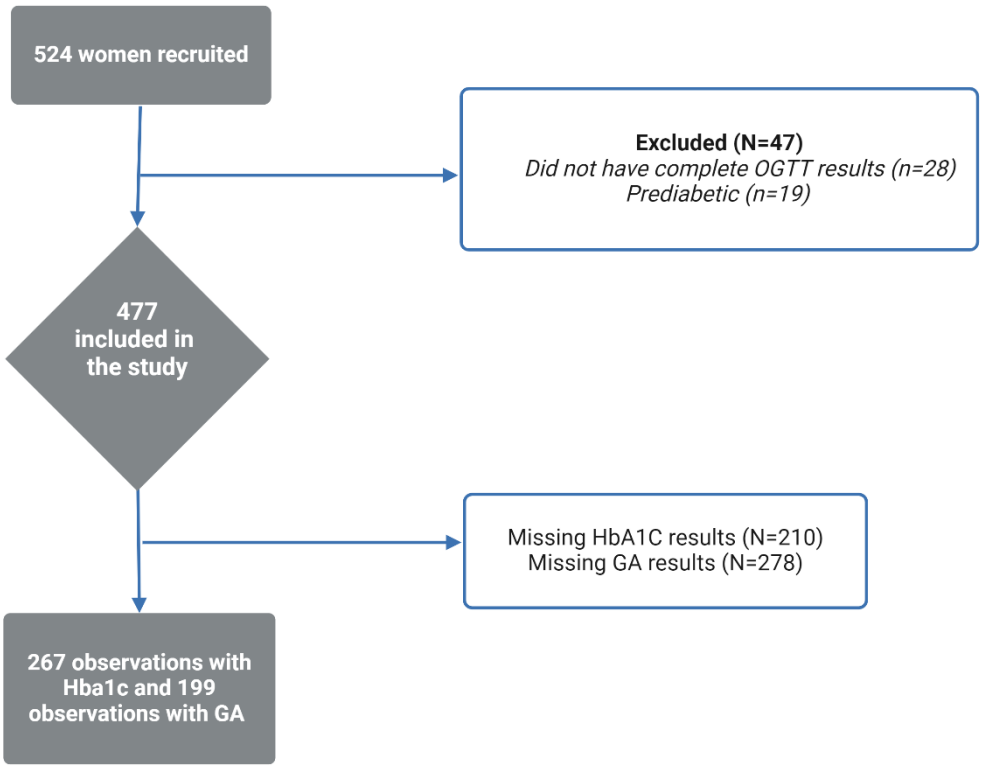


Figure 4.1 Flow diagram of the recruitment process.

4.4.2 Clinical characteristics of GDM and non-GDM women

Out of the 477 women in the study, 78 (16.35%) had GDM. The number of women with GDM by site was 37 (14.12%), 34 (18.58%) and 7(21.88%) for CMJAH, Edenvale Regional Hospital and RMMCH, respectively (Table 4.1).

Table 4.1 The prevalence of GDM by site

Study Site	**Prevalence (%)**
Charlotte Maxeke Johannesburg Academic Hospital	14.12
Edenvale Regional Hospital	18.58
Rahimah Moosa Mother and Child Hospital	21.88

The women diagnosed with GDM were younger (p-value = 0.02) and had significantly higher BMI (p-value = 0.01), blood glucose and glycated markers (p-value <0.0001 for all) but had a significantly lower proportion of HIV-positive women (p-value = 0.03) when compared to those without GDM (Table 4.2).

Table 4.2 The clinical characteristics of all women and by GDM status

Variable	All		Non-GDM		GDM		p-value
	N	Value	N	Value	N	Value	
Age (years)	477	37 (32, 40)	399	37 (33, 40)	78	36 (30, 38)	**0.02**
Gestational age (weeks)	477	28 (26, 32)	399	28 (26, 32)	78	28 (27, 31)	0.93
BMI (kg/m2)	469	33.02 (28.55, 38.30)	392	33.56 (28.43, 37.50)	77	34.41 (29.40, 41.50)	**0.01**
Alcohol consumers	470	28 (5.96)	394	21 (5.33)	76	7 (9.21)	0.19
Smokers	471	5 (1.06)	395	3 (0.76)	76	2 (2.63)	0.15
HIV positive	467	135 (28.91)	390	121 (31.02)	77	14 (18.18)	**0.03**
Haemoglobin (g/L)	331	11.95 ± 1.60	282	11.88 ± 1.62	49	12.3 ± 1.43	0.08
FPG (mmol/L)	477	4.4 (4.0, 4.7)	399	4.3 (4.0, 4.6)	78	5.3 (5.1, 5.6)	**<0.001**
Glucose 1hr after post load (mmol/L)	477	6.5 (5.4, 7.8)	399	6.2 (5.3, 7.2)	78	9.2 (7.8, 10.1)	**<0.001**
Glucose 2hr after post load (mmol/L)	477	5.5 (4.8, 6.5)	399	5.4 (4.7, 6.2)	78	7.45(6.1, 8.8)	**<0.001**
HbA1c (%)	268	5.2 (4.9, 5.5)	227	5.1 (4.9, 5.4)	41	5.5 (5.2, 5.9)	**<0.001**
Glycated Albumin (%)	199	22.98 (11.36, 38.62)	128	13.51 (10.99, 30.66)	71	36.12 (21.63, 51.26)	**<0.001**

Data expressed as n (%), mean ± SD or median (interquartile range); GDM=gestational diabetes, BMI=body mass index, HbA1c=glycated haemoglobin, HIV=human immunodeficiency virus, FPG=fasting plasma glucose

4.4.3 Comparison of GDM risk factors between those with and without GDM

The GDM risk factors were not significantly different between women diagnosed with GDM and controls with the exception of repeated glycosuria (p=0.02) and history of GDM (p=0.03) (Table 4.3).

Table 4.3 The GDM risk factors, overall and by GDM status

Risk Factors	All participants		Non-GDM		GDM		p-value
	N	Frequency	N	Frequency	N	Frequency	
Obese	469	315 (67.16)	392	258 (65.82)	77	57 (74.03)	0.16
Repeated glycosuria	475	15 (3.16)	397	9 (2.27)	78	6 (7.69)	**0.02**
History of GDM	475	5 (1.05)	397	2 (0.50)	78	3 (3.85)	**0.03**
Adverse pregnancy outcome	475	53 (11.16)	397	44 (11.8)	78	9 (11.54)	0.85
PCOS	476	0 (0.00)	398	0 (0.00)	78	0 (0.00)	n/a
Advanced maternal age	476	300 (63.03)	398	257 (64.57)	78	43 (55.13)	0.11
History of miscarriages	475	136 (28.63)	397	111 (27.96)	78	25 (32.05)	0.47
History of an LGA newborn	476	61 (12.82)	398	50 (12.56)	78	11 (14.10)	0.71
First-degree relative with T2D	476	91 (19.12)	398	72 (18.08)	78	19 (24.36)	0.20

Data expressed as n (%), N=number of observations, GDM=gestational diabetes mellitus, PCOS=polycystic ovarian syndrome, T2D=type 2 diabetes, LGA=large for gestational age

4.4.4 Sensitivity and specificity of fasting plasma glucose, glycated haemoglobin and glycated albumin

The ROC curve analysis (Figure 4.2) demonstrated that FPG had the highest AUC (95%CI), followed by HbA1c then GA at 0.98 (0.96, 1.00), 0.73 (0.65, 0.83) and 0.73 (0.66, 0.80), respectively. Using the ROC curves the optimum threshold for FPG, GA and HbA1c for diagnosing GDM were 5.1mmol/L, 37.56% and 5,5%, respectively.

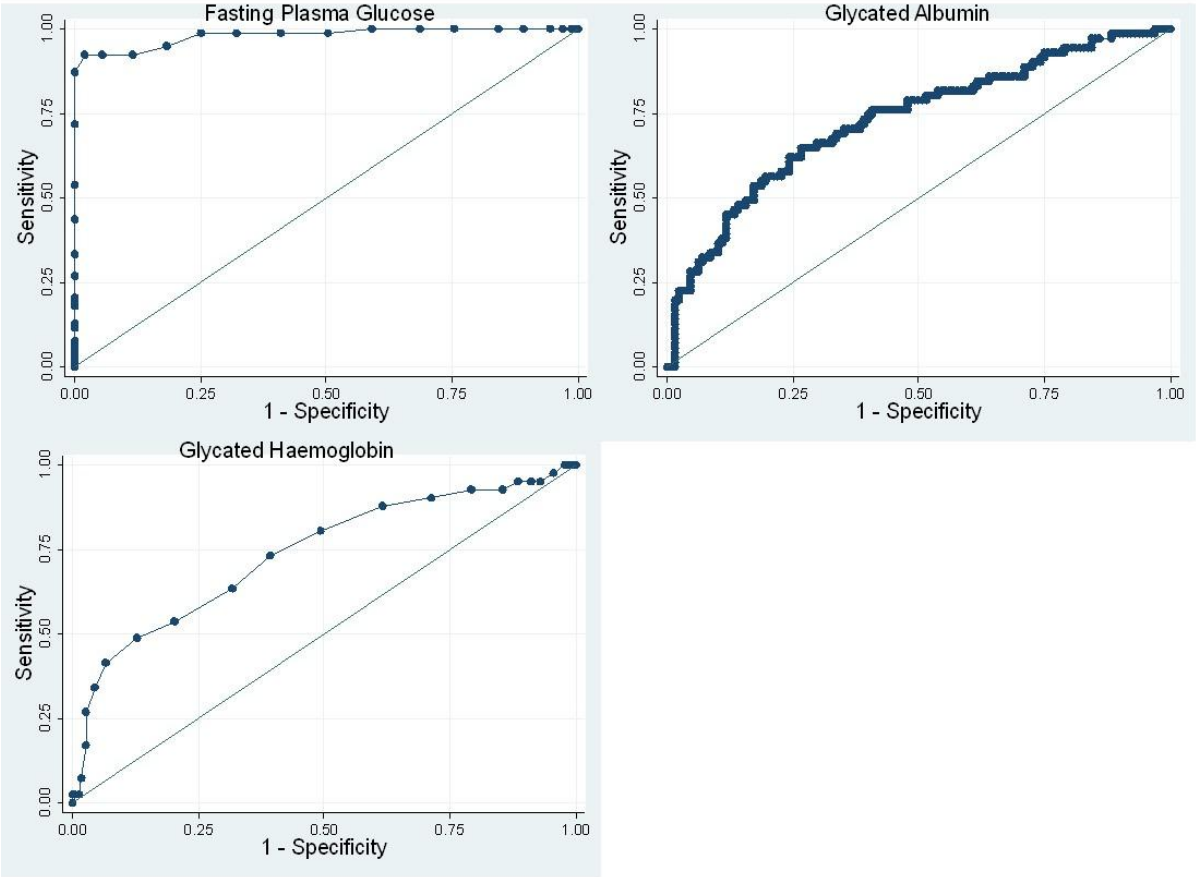


Figure 4.2 ROC curve analysis of GDM diagnosis by fasting plasma glucose (FPG), glycated albumin (GA) and glycated haemoglobin (HbA1c).

The FPG had the highest sensitivity and specificity, with values of 87.2% and 100% respectively. HbA1c and GA showed lower values, with sensitivities of 53.7% and 53.5% and specificities of 79.7% and 82.8% respectively (Table 4.3). Thus, FPG was superior to HbA1c or GA and was studied further. The cut point of 5.1mmol/L for FPG incorrectly identified 10 women as not being GDM. We, therefore, carried out a ROC curve analysis on all the women who were labelled as 'non-GDM' using the 5.1mmol/L FPG cut point (n=409) and obtained a

new cut point of 4.6mmol/L providing a sensitivity of 90.0%, a specificity of 74.9%, a positive predictive value of 8.3% and a negative predictive value of 99.7% (Table 4.4; also included are characteristics of the 4.6mmol/l cut point if applied to all 477 women). Therefore, from the 109 women with FPG≥4.6mmol/L, it correctly identified 9 of the 10 undiagnosed women with GDM as having GDM but misdiagnosed 100 women as having GDM. In addition, 300 women had FPG <4.6mmol/L of whom one woman was misdiagnosed at not having GDM.

Table 4.4 Accuracy of glycated haemoglobin, glycated albumin and FPG when compared to all OGTT time points

	FPG at ≥5.1 mmol/L	**HbA1c at ≥5.5 %**	**GA at ≥35.76%**	**FPG between 4.6 mmol/L and 5.0 mmo/L**	**FPG ≥ 4.6 mmol/L**
Participants	477	268	199	409	477
Sensitivity, % (95%CI)	87.2 (77.7, 93.7)	53.7 (37.4, 69.3)	53.5 (41.3, 65.5)	90.0 (55.5, 99.7)	98.7 (97.7,99.7)
Specificity, % (95% CI)	100 (99.1, 100)	79.7 (73.9, 84.8)	82.8 (75.1, 88.9)	74.9 (70.4, 79.1)	74.9 (71.1, 78.8)
PPV, % (95%CI)	100 (94.7, 100)	32.4 (21.5, 44.8)	63.3 (49.9, 75.4)	8.26 (3.85, 15.1)	43.5 (39.1, 48.0)
NPV, % (95%CI)	97.6 (95.5, 98.8)	90.5 (85.6, 94.2)	76.3 (68.3, 83.1)	99.7 (98.2, 100)	99.7 (99.2, 100)
False positive, N	0	46	22	100	100
False negative, N	10	19	33	1	1
True positive, N	68	22	38	9	77
True negative, N	399	181	106	299	299

PPV=positive predictive value; NPV=negative predictive value, FPG=fasting plasma glucose, GA=glycated albumin, HbA1c=glycated haemoglobin

Applying an OGTT to these 109 women would be necessary to single out the 9 women with GDM. Therefore, such a two-tiered approach would cut down the number of OGTTs by 77% (i.e., from 477 to 109) and would miss one woman with GDM. This is shown diagrammatically in Figure 4.3.

4.5 DISCUSSION

This cross-sectional study focused on black African women who were at high risk for GDM. Our findings indicate that HbA1c and GA are unacceptable diagnostic tests for GDM. However, FPG alone identified 87.2% (68 of 78) of women with GDM, but 12.8% (10 of 78) of women with GDM were not diagnosed, but 90% of these GDM cases were identified when using a FPG cut point of 4.6 mmol/L.

The IADPSG recommends that for environments where universal screening by OGTT is not applicable, FPG may be used and a full OGTT should be reserved for those women with a non-diagnostic FPG [190]. If we translate this to the current study, 409 of 477 women would have to be tested using the OGTT, but with our two-tiered approach, this would be reduced to 109 OGTTs.

Several studies agree with the use of FPG cut-points to identify pregnant women who should have an OGTT, using IADPSG diagnostic criteria [8,191–193]. A study from the United Arab Emirates on women with high risk for GDM (n=10283) used a FPG cut point of ≥4.4mmol/L and reported a reduction in the number of OGTTs of 50.6% and a sensitivity of 95.4% [30]. Other studies used FPG for universal screening of all pregnant women. Thus, an Australian study using a FPG cut-point of ≥4.7mmol/L in 4650 women reported an 82.9% reduction in OGTTs [192]. A study from Switzerland using a FPG cut-point of ≥4.4mmol/L in 2298 women reported 78.5% sensitivity and a reduction in OGTTs of 63.8% [194]. These studies demonstrate that FPG applied in a two-tier approach may be used for universal GDM screening. This would be particularly relevant in resource-limited environments where it would reduce the number of OGTTs. The variations in FPG levels across the studies may be attributed to the prevalence of obesity and the level of abnormal glucose tolerance within each population studied [193].

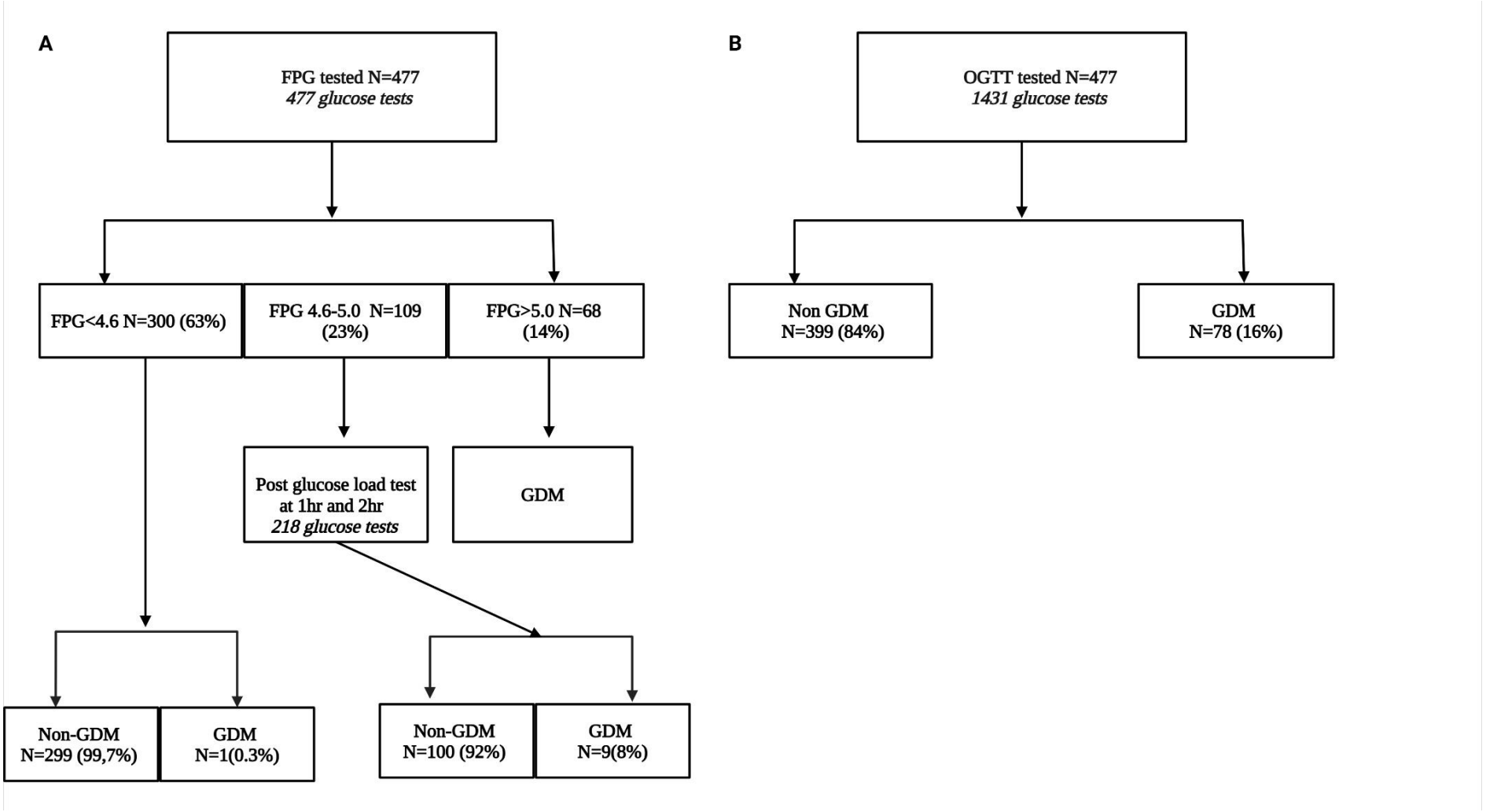


Figure 4.3. The comparison between a two-tiered diagnostic method (A) and the OGTT (B), created in https://BioRender.com.

As demonstrated in the current study, HbA1c for GDM diagnosis has poor performance [1,195–197]. A Belgium study reported an AUC of 0.73 and sensitivity, specificity, PPV and NPV of 12.0%, 99.4%, 20 and 0.88, respectively, for HbA1c at a 5.5% threshold [197]. Few publications are investigating GA as a diagnostic test for GDM however, two previous studies do report similar findings to the current study demonstrating AUC for the ROC curve of 0.53 [198] and 0.57 [199]. In both these studies, FPG was superior to HbA1c and GA for GDM diagnosis [198,199].

Furthermore, this study demonstrated that conventional risk factors discriminate poorly between women with and without GDM except for repeated glycosuria and a history of GDM. Previous studies from South Africa [8,10] have shown that selective screening carries the risk of missing significant proportions of the women with GDM, leading to OGTTs being performed in a large proportion (34.6% and 45.8%, respectively) of the screened individuals. Similarly, a study conducted in Nigeria reported that 14 out of 45 women with GDM were missed using selective screening [135].

One of the main reasons why selective screening using risk factors for GDM has poor sensitivity for detecting GDM may be due to the high prevalence of female obesity in South Africa. Thus, in 2017 the prevalence of obesity in women of childbearing age (18-49 years) was 35.2% and this high prevalence is mirrored in the present study where 67.2% of the subjects at increased risk for GDM were obese. However, although BMI was slightly higher in those women who were diagnosed with GDM, the prevalence of obesity did not differ between GDM cases and those without GDM. Thus, the high prevalence of obesity in this population will lead to a large number of pregnant women meeting the criteria for an OGTT, (one risk factor needed to meet the criteria), but as shown in this study only a small percentage will have GDM. This could be due to the fact that GDM is a multifactorial disease, and a combination of factors contributes to its development**.**

One of the strengths of our study is that we have reported sensitivity and specificity for three different glycaemic tests (FPG, HbA1c and GA) for diagnosing GDM and have estimated the optimum diagnostic thresholds for each test in an under-studied population. This is the first time that such an analysis has been undertaken on the African continent. In addition, we have confirmed that FPG is an appropriate screening test for GDM in this population and

have further shown that once screened OGTTs need only be performed in women with FPG between 4.6 and 5.1 mmol/L. This process reduces the number of required OGTTs by 77% in this high-risk group. This study is important in that it demonstrates that this screening process may dramatically reduce the number of OGTTs necessary within a financially constrained healthcare system and that HbA1c and GA are not appropriate tests for GDM diagnosis in this population. A limitation is that the study focused on women with high risk for GDM and GA and HbA1c measurements were not available for all study participants.

4.6 CONCLUSION

In conclusion, FPG used in a two-tiered process for identifying women with GDM amongst a high-risk population provides a diagnostic method of high sensitivity and specificity and reduces OGTTs.

Although this study shows that FPG may be a useful method of screening high risk individuals for GDM it still requires the collection of a fasting blood glucose sample whilst other markers of glycaemia that do not require fasting blood samples (GA and HbA1c) had poor diagnostic ability. Furthermore, none of these blood analytes have recognised cut points for diagnosing GDM in early pregnancy. We therefore believe that it is important to study prospective biomarkers that go beyond glycaemic markers, and which may provide important information on the pathophysiology of GDM. In the following study we investigated potential biomarkers of GDM in early and in late pregnancy. This manuscript (Chapter 5) will be submitted to the *British Journal of Obstetrics & Gynaecology*.

CHAPTER 5: THE USE OF NOVEL BIOMARKERS FOR THE DIAGNOSIS OF GESTATIONAL DIABETES MELLITUS IN BLACK AFRICAN WOMEN

Khambule L, Chauke L, Crowther N.J., George J.A. The use of novel biomarkers for the diagnosis of gestational diabetes mellitus in Black African women. Written in a scientific journal format.

5.1 ABSTRACT

Objective: This study aimed to evaluate whether IL-6, TNF-α, free fatty acids (FFAs), branched-chain amino acids (BCAAs; valine, leucine, isoleucine), phenylalanine, and acylcarnitines (C2, C4, C5, and C8-carnitines) were associated with glycaemia in early and late stages of pregnancy and whether they could be potential biomarkers of gestational diabetes mellitus (GDM) in African women.

Methods: This cross-sectional study involved two cohorts of women: one comprised of women in early pregnancy (≤20 weeks), and the other included women in late pregnancy (24-32 weeks). The women in late pregnancy were assessed for GDM using an oral glucose tolerance test (OGTT) following the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria. Potential biomarkers selected from the literature were analysed in both cohorts. Biomarker levels were compared between women who had and did not have GDM in late pregnancy, and their discriminatory power assessed by receiver-operator characteristic (ROC) curve analysis and their correlation with glycaemia levels was measured in both cohorts.

Results: A total of 477 and 170 women in late and early pregnancy, respectively, were part of the analysis. Among the 477 women, 78 (16.4%) had GDM. Women with GDM showed significantly higher levels of FFAs, and isoleucine, alongside significantly lower levels of C2, C4, and C5-carnitines, when compared to the non-GDM group (all p-values < 0.05) but ROC curves demonstrated sensitivities from 7.7% to 44.9% and specificities from 79.3% to 87.7% for GDM diagnosis. In early pregnancy, only valine correlated with blood glucose, independent of BMI and age (p=0.029).

Conclusion: The selected biomarkers are not suitable for diagnosis of GDM. Furthermore, they do not reflect levels of glycaemia in early pregnancy (except for valine) but do so in late pregnancy where they demonstrate altered FFA and BCAA metabolism in those with GDM.

5.2 INTRODUCTION

The aetiology of GDM has some similarities to that of type 2 diabetes (T2D), and like T2D, is greatly influenced by the high prevalence of female obesity that exists presently in South Africa [18]. The rising prevalence of obesity in South Africa can be attributed to the nation's rapid economic growth and increased migration from rural areas to cities, where lifestyles are characterised by high consumption of calorie-dense foods and less exercise [17].

The conventional selective screening procedure for GDM that is used in many low-and-middle-income countries such as South Africa, is known to have poor sensitivity and specificity for GDM [10]. One method that could be used to improve the screening procedure for GDM would be to make use of early biomarkers for GDM. This may be feasible as a number of studies have shown that metabolic differences are observed between women with and without GDM [100,200,201] and a significant body of literature has emerged focused on identifying early pregnancy biomarkers for GDM [100,103,202–204].

The majority of the GDM biomarker studies emanate from untargeted metabolomics or proteomic analysis, but none of them were conducted in African populations and none have been confirmed in validation studies, which are expensive to perform [146]. The biomarkers that are currently being investigated represent the pathophysiological pathways linked with GDM, one of the factors contributing to GDM development being inflammation. Adipose tissue, beyond storing lipids, acts as an endocrine organ releasing adipocytokines. In obesity, this release shifts toward pro-inflammatory adipokines, compounding pregnancy-induced insulin resistance (IR) and increasing GDM risk. The pro-inflammatory adipokines investigated in the current study are interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α). These were selected as they were found to be elevated in women diagnosed with GDM [68–71]. Moreover, TNF-α is thought to contribute to IR directly through the c-JUN N-terminal kinase (JNK) pathway and IκB kinase (IkappaB kinase or IKK) of the nuclear factor-kappa B

(NF-κB) signalling pathway. Elevated IL- 6 levels were found to be directly associated with pregravid BMI, FPG, and postprandial glucose levels [72], and conversely associated with insulin action in Pima Indians with GDM [73]. These inflammatory markers are more specific to GDM than other inflammatory markers linked to GDM such as creatine reactive protein (CRP) [205].

Impaired metabolism of branch-chained amino acids (BCAAs; valine, leucine, isoleucine), free fatty acids (FFAs) and their metabolites acylcarnitines have also been strongly linked to IR directly and indirectly. BCAAs are thought to induce IR through the stimulation of the mammalian target of rapamycin (mTOR) signalling pathway [47,77,81,82]. The mTOR signalling pathway regulates protein synthesis and breakdown in cells and is a key signalling mediator in the crosstalk between amino acids and insulin [83]. The BCAAs have been identified as novel biomarkers for T2D [206,207], IR[81], metabolic syndrome[208] obesity [77,82] and incident CVD [209]. BCAAs have also been associated with GDM [99,100,151,152,210]. Thus, maternal plasma, umbilical venous and arterial plasma BCAAs were higher in GDM cases than in controls mid-pregnancy [99,150,152] and early-pregnancy [100]. In pregnancy, human placental lactogen (hPL) further stimulates lipolysis as a compensation for the IR thus elevating FFAs in circulation [58]. Disrupted metabolism of FFAs and BCAAs results in abnormal accumulation of their metabolites such as acylcarnitines (ACs) and could possibly discriminate between GDM and healthy pregnancy. ACs have been reported to be associated with GDM in early pregnancy and late pregnancy [202,211–214]. One study showed a 30% increase in AC levels in GDM cases when compared to controls (p=0.005) [214]. In the present study, the following ACs were selected, isobutyryl- carnitine, isovaleryl-carnitine, acetyl-carnitine and octanoyl-carnitine as they showed stronger association with GDM in literature [147,211,213]. These inflammatory markers and metabolites are linked to IR, except for the ACs which are indicators of incomplete oxidation of BCAAs and FFAs metabolism, however they are all commonly found to be elevated in women with GDM.

Our main aim was to determine whether any of these chosen metabolites and proteins were associated with glycaemia at either of these stages of pregnancy and whether they may be possible biomarkers of GDM in this population. In addition, these analyses may provide important information on the pathophysiology of GDM in African women.

5.3 METHODOLOGY

5.3.1 Study design

This was a multicentre cross-sectional study which included two cohorts. One cohort was made up of black African women between 24 and 32 weeks of pregnancy who are at high risk of developing GDM [123] and had gone through an OGTT between November 2018 and May 2022. The second cohort included pregnant women visiting the Hillbrow Community Health Centre (CHC) ante-natal clinic in Johannesburg for the first time between 8-20 weeks of pregnancy. The study design and sample size estimates are described in Chapter 2. If the selected biomarkers can distinguish between GDM and non-GDM, it would be advantageous to evaluate their efficacy as indicators of poor glycaemic control in the early stages of pregnancy. The inclusion of the second cohort in the study aimed to investigate the relationship between potential biomarkers and glucose levels in early pregnancy. The OGTT was not conducted in the early pregnancy group because there are currently no guidelines that define glucose cut points for diagnosing GDM in the first or second trimester of pregnancy. Ideally, the early pregnant women would be followed up until late pregnancy where the OGTT can be performed, however, this was not done in current study as the estimated dropout rate of participants is about 40% as observed in a longitudinal study performed in a population similar to the current study [215]. It was hoped that the present study would identify biomarkers that would associate with glycaemia in early pregnancy and would distinguish between GDM and non-GDM cases later in pregnancy. Such molecules could then be used in a future longitudinal study to determine if they can identify GDM early in pregnancy.

5.3.3 Sample and data collection

A standardised questionnaire was used to collect information on age, gestational age, parity, and gravidity. The gestational age was estimated from the first day of the last menstrual period. HIV positive women were included in this study as the prevalence of HIV is relatively high (16.8%) in South African women [163]. The HIV status of all the study participants was known due to routine clinic-based screening tests, and therefore HIV status was included in all the statistical tests to determine whether it affects glucose levels and risk for GDM at 16-20 and ≥24 weeks of gestation, respectively.

Blood samples of participants in the late pregnancy cohort were collected during a 75g oral glucose tolerance test (OGTT), as per IADPSG guidelines described in Chapter 2.

5.3.4 Biochemical analysis

Plasma glucose and serum glycated albumin (GA) were measured using a Roche auto-analyser, Cobas 8100 system (Basal, Switzerland). The GA kits were purchased from QuantLab, Werfen (Milano, Italy). Details of the glucose and glycated albumin analysis are provided in Chapter 2. The intra-assay CV for glucose and GA were 1.00 and 0.10 % respectively. The inter-assay CV for glucose and GA were 0.68 and 2.87%, respectively.

The inflammatory markers, TNF-α and IL-6 were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits from ELabscience (Houston, TX, USA) and R&D systems (Bio-tech, Minneapolis, MN, USA), respectively. The analysis was carried out by strictly following the manufacturers' instructions. The assays used a quantitative sandwich enzyme immunoassay method. Absorption at 450nm was calculated using a Synergy HT microplate reader (Biotek, Vermont, USA) and the background was corrected using 540nm. The microplates were incubated and washed using the Thermostar incubator (BMG Labtech, Ortenberg, Germany) and ELX50 microplate washer (Biotek, Vermont, USA), respectively. The intra-assay and inter-assay CVs for TNF-α were 1.56 and 1.21 %, respectively. The intra-assay and inter-assay CVs for IL-6 were 1.6 and 3.3%, respectively.

Total FFAs were analysed using a fluorometric assay from Elabscience (TX, USA). The manufacturer's instructions were strictly followed. Briefly, standards and samples were pipetted onto a microplate followed by the enzyme acyl synthase to convert FFAs to acyl-CoA, which is then converted into hydrogen peroxide by acyl oxidase. Thereafter, the fluorescent substrate is added which reacts with hydrogen peroxide to form a fluorescent end-product. The fluorescence intensity at the 535nm excitation and 590nm emission wavelength was measured. The intensity was proportional to the concentration of FFA. Fluorescence was measured with the Synergy HT microplate reader (Biotek, Vermont, USA) and the microplates were incubated and washed using the Thermostar incubator (BMG Labtech, Ortenberg, Germany) and ELX50 microplate washer (Biotek, Vermont, USA), respectively. The average intra-assay and inter-assay CVs were 4.2 and 5.3 %, respectively.

The BCAA and acylcarnitine (ACs)s methods were optimised and validated. The methods for BCAAs and acylcarnitines are described below.

Sample and standards preparation

Two stock solutions were prepared, one containing the amino acids (valine, leucine, isoleucine and phenylalanine) and the other containing ACs (C2, C4, C5 and C8-carnitines) purchased from Toronto Research Chemicals (Toronto, Canada). Each stock solutions were prepared in 50/50/0.1 (v/v/v) methanol/de-ionised water/formic acid to make up a 4mM solution and stored at -4°C. On the day of analysis, standards and quality controls (QC) were prepared from each stock solution in 4 % bovine serum albumin (BSA). Standard concentration ranges of 0- 300.0 and 0-120 µM were prepared by serial diluting for amino acids and ACs, respectively. The QC samples were used during validation and clinical sample analysis.

Deuterated internal standards, namely leucine-d3, valine-d8 and phenylalanine-d5 for amino acids and carnitine-d3 for ACs were purchased from Toronto Research Chemicals (Toronto, Canada) and prepared in 50/50/0.1 (v/v/v) methanol/de-ionised water/formic acid to form two stock solutions, each at a concentration of 75µmol/l and aliquoted into polypropylene tubes and stored at − 80°C before use.

Samples were thawed, and 120µl of each standard/sample was aliquoted into polypropylene tubes, followed by 80µl of the deuterated internal standards. Thereafter 600µl of pure methanol (high-performance liquid chromatography grade) was added to precipitate the protein in the samples and vortex mixed. To separate the pellet and supernatant, the samples were centrifugated at 3 1486 X g for 10 minutes using the Mikro 22 centrifuge (Hettish, Tuttlingen, Germany) for 10 minutes, and the supernatant from each sample was transferred to its designated vial.

Liquid chromatography conditions

Separation of analytes was performed by the Nexera Series LC-40D XR liquid chromatography system (Shimadzu, Kyoto Japan) consisting of a gradient elution pump and CTC-PAL autosampler. The stationary phase was the Hypercarb 3 µm, 50× 2.1 mm column from Thermoscientific (MA, USA). The mobile phases for both methods were 0.1% trichloroacetic acid in de-ionised water (Mobile phase A) and 0.1% trichloroacetic acid in acetonitrile (Mobile phase B). A gradient at a flow rate of 200 µL/min was used for amino acid analysis such that mobile phase B was maintained at 10% for the first 3 min, then ramped up to 90% over 4 min and maintained at 90% for 1 min, followed by a reduction to 10% for 2 min for

column re-equilibration as shown in Table S1. For ACs, an isocratic flow was used and mobile phase B was kept constant at 30% for 4 min.

Mass spectrometry conditions

Tandem mass spectrometry detection was carried out using a 5500 linear ion trap quadrupole tandem mass spectrometer (QTRAP) from Sciex (MA, USA) with a Turbo IonSpray electrospray ionization (ESI) source operated in the positive ion mode. Data was collected and processed using Analyst® software (version 1.6.3). Mass spectrometric parameters were optimised by infusing each analyte with a mixture of 20/80 mobile phase A/B at a flow rate of 400 µL/min. The ESI spray voltage was + 5500 V. The source temperature was 500 °C. The curtain gas was 20.00, and the nebulizer gas settings 1 and 2 were 70 as shown in Table S2. The ion transitions of MS/MS detection for each analyte and internal standard are shown in Table S3.

Method Validation

The acylcarnitine and amino acids methods were validated following the Food and Drug Administration (FDA) guidelines and all passed the validation [216]. The method validation procedure and outcome are shown in the Supplementary material at the end of the chapter.

5.3.5 Data analysis

Statistical analysis utilised STATA 16.1 software (StataCorp, College Station, TX, USA). A p-value of < 0.05 was considered statistically significant. The distribution of the data was evaluated with the Shapiro–Wilk test and continuous variables displaying a normal distribution were reported as means and standard deviations, while those not normally distributed were presented as medians and interquartile ranges (IQRs). Categorical variables were presented as sample numbers along with their corresponding percentages. To compare the demographic, anthropometric, and biomarkers between the women with GDM and the control group, Student t-test was employed for normally distributed data and the Wilcoxon rank-sum test for skewed data. The chi-squared test was conducted for categorical data, while Fisher's exact test was applied in instances where numbers were < 5. Correlations of glucose levels from the OGTT with discriminatory biomarkers were analysed in late pregnancy using Spearman correlation. Correlations of fasting and random blood glucose

with biomarker levels were analysed in early pregnancy using linear regression, with blood glucose as the dependent variable and each biomarker as the independent variable adjusted for age, gestational age and BMI. In the women in late pregnancy, a logistic regression model was set up for GDM using independent variables that differed significantly ($p<0.05$) between GDM and non-GDM groups. The variance inflation factor (VIF) was calculated, and VIF >5 was used as an indication of multicollinearity; none of the variables had VIF >5. The area under the ROC curve (AUC) with 95% CI was determined for potential biomarkers of GDM that remained significant after the multivariate logistic regression alongside GA, which is known to be a good indicator of glycaemia during pregnancy [183]. The Youden index [189] was used to determine the biomarkers' optimum cut-off levels for detecting GDM, and sensitivity and specificity were calculated for all.

5.4 RESULTS

5.4.1 Study population description

Six hundred and ninety-four pregnant women were recruited of which 524 were women in late pregnancy and 170 were women in early pregnancy (Figure 5.1). From the late pregnancy cohort, 28 (5.34%) women had incomplete OGTT results, and 19 (3.62%) women had overt diabetes and were thus excluded from the study. Four hundred and seventy-seven (477; 91.0%) women were included in the data analysis. In the early pregnancy cohort, 7 (4.12%) women were classified as overt diabetes and were therefore excluded from the study, therefore, 163 (95.9%) women were included in the data analysis, 87 were fasting and 76 were non-fasted.

The prevalence of GDM in the late-pregnancy cohort was 16.4% (78/477). The body mass index (BMI) of women in late pregnancy was higher than that of women in early pregnancy, and they were older (Table 5.1). In addition, they had a higher proportion of women positive for human immunodeficiency virus (HIV), married and employed but fewer smokers. When comparing non-GDM and GDM groups in late pregnancy, women with GDM were significantly younger and had significantly higher median BMI and systolic blood pressure (SBP) but a significantly lower proportion were positive for HIV ($p<0.05$ for all) (Table 5.2). Gestational age, parity, gravidity, diastolic blood pressure (DBP), current employment, marital

status, alcohol intake and smoking status were not significantly different between the two groups (Table 5.2).

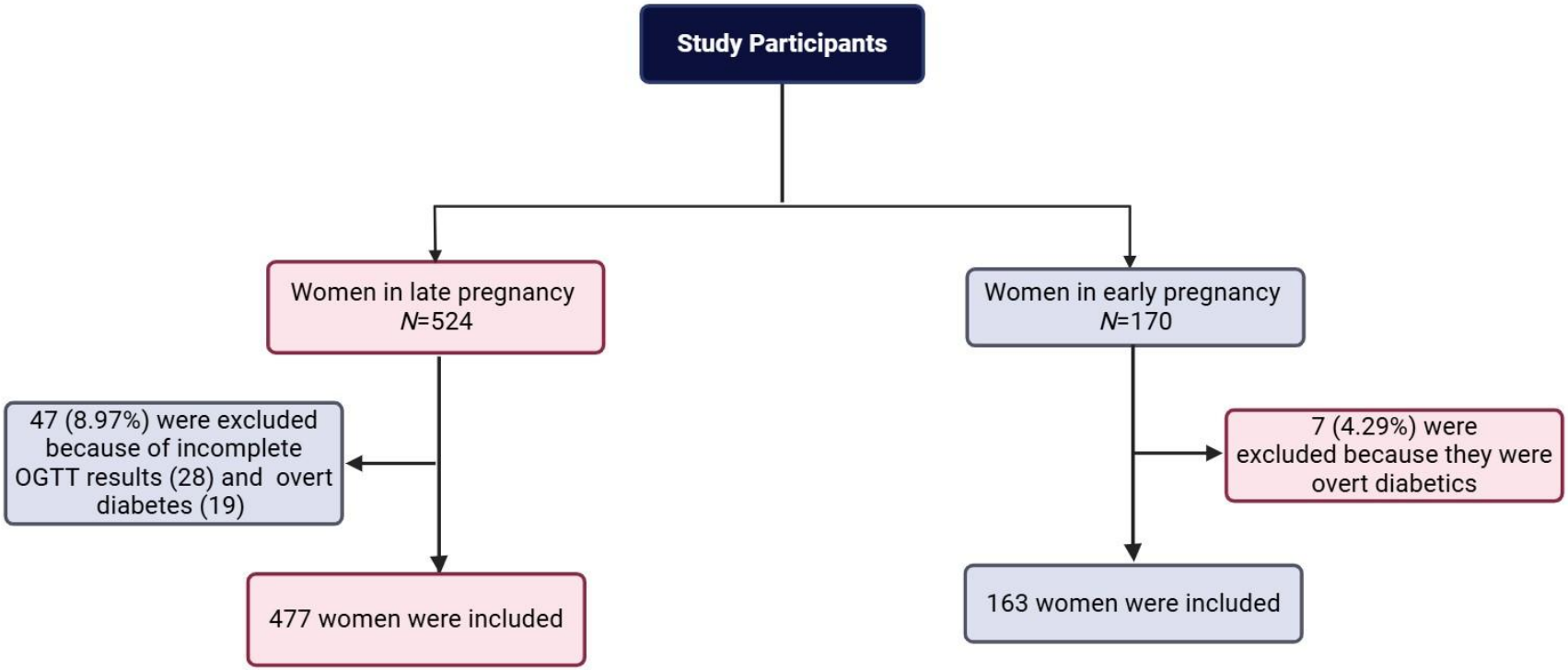


Figure 5.1. Flow diagram of the recruitment process

Table 5.1 Demographic and clinical characteristics of participants

Variable	All pregnant women in early pregnancy		All pregnant women in late pregnancy	
	N	Value	N	Value
Age (years)	162	33 (26, 38)	477	37 (32, 40)
Gestational age (weeks)	163	16 (12, 19)	477	28 (26, 32)
Parity	162	1 (0, 2)	456	2 (1,3)
Gravidity	161	3 (2, 4)	458	3 (3,4)
BMI (kg/m^2)	160	29.27 (25.54, 34.13)	469	33.02 (28.55, 38.30)
Systolic BP (mmHg)	162	123.98 ± 0.97	476	123.50 (115.00, 132.50)
Diastolic BP (mmHg)	163	73.00 (66.00, 80.00)	476	73.50 (67.50, 81.25)
Alcohol consumers	163	13 (7.98)	470	28 (5.96)
Current Smoker	163	7 (4.29)	471	5 (1.06)
Married	162	25 (15.43)	470	148 (31.49)
Employed	162	63 (38.89)	469	233 (49.68)
HIV positive	160	35 (21.88)	467	135 (28.91)
Repeated glycosuria	161	3 (1.86)	475	16 (3.37)

Data expressed as n (%), mean ± SD or median (interquartile range); GDM=gestational diabetes, BMI=body mass index, HIV=human immunodeficiency virus, BP= Blood pressure, HIV=human immunodeficiency virus

5.4.2 Biomarkers for GDM in late pregnancy

Women with GDM had significantly higher levels of FFAs and isoleucine and significantly lower acylcarnitines C2, C4 and C5 compared to the non-GDM group ($p<0.05$ for all; Table 5.3). All the above discriminatory biomarkers were significantly associated with glucose levels from the OGTT except for C2-carnitine ($p<0.05$ for all; Table 5.4). Thus, the FFAs showed a positive correlation with glucose at all three-time points, while isoleucine levels correlated with FPG and glucose at 2 hours post-load. The acylcarnitines displayed an inverse relationship, C4-carnitine correlated with glucose levels at 1 hour and 2 hours post-load, while C5-carnitine correlated with glucose only at 2 hours post-load. In a multivariate logistic regression model, only FFAs, isoleucine, C2-carnitine and BMI were significantly

associated with GDM (Table 5.5). In addition, the Spearman correlation showed a negative relationship between FFAs and C2-carnitine (r=-0.1642, p<0.01).

Table 5.1 Demographic and clinical characteristics of women in late pregnancy by GDM

Variable	Non-GDM		GDM		p-value
	N	Value	N	Value	
Age (years)	399	37 (33, 40)	78	36 (30, 38)	**0.02**
Gestational age (weeks)	399	28 (26, 32)	78	28 (27, 31)	0.93
Parity	381	2 (1, 3)	75	2 (1, 3)	0.33
Gravidity	383	3 (3, 4)	75	3 (2, 4)	0.90
BMI (kg/m^2)	392	33.56 (28.43, 37.50)	77	34.41 (29.40, 41.50)	**0.01**
Systolic BP (mmHg)	398	123.25 (114.00, 131.50)	78	126.50 (120.00, 135.00)	**0.01**
Diastolic BP (mmHg)	398	73.50 (67.50, 81.00)	78	75.50 (67.50, 82.00)	0.37
Alcohol consumers	394	21 (5.33)	76	7 (9.21)	0.19
Current Smoker	395	3 (0.76)	76	2 (2.63)	0.15
Married	395	121 (30.63)	75	27 (36.00)	0.36
Employed	393	198 (50.38)	76	35 (46.05)	0.49
HIV positive	390	121 (31.03)	77	14 (18.8)	**0.02**
Repeated glycosuria	397	9 (2.27)	78	7 (8.97)	**0.01**

Data expressed as n (%), mean ± SD or median (interquartile range); GDM=gestational diabetes, BMI=body mass index, HIV=human immunodeficiency virus, BP= Blood pressure, HIV=human immunodeficiency virus

5.4.3 Sensitivity and specificity of the biomarkers

Using ROC curve analyses, the discriminatory biomarkers from the multivariate regression model, namely C2-carnitine, isoleucine and FFAs were found to have AUCs of 0.42, 0.64, and 0.65, respectively (Figure 5.2). Sensitivity ranged from 7.74% for C2-carnitine at a cut point of 2.52 µmol/l to 44.94% for FFAs at a cut point of 569.75 µmol/l. Specificity ranged from 79.39% for C2-carnitine at the 2.52 µmol/l cut point to 87.73% for isoleucine at a cut point of 127.45 µmol/l (Table 5.6). This compares to data for GA which at a cut point of 35.76% had AUC, sensitivity and specificity of 0.73, 53.52 and 82.81% respectively.

Table 5.3 Potential biomarkers for GDM screening stratified by GDM.

Variable	Non-GDM		GDM		p-value
	N	Value	N	Value	
IL- 6 (pg/ml)	206	2.26 (1.36, 3.14)	62	2.47 (1.10, 3.33)	0.62
TNF-α (pg/ml)	188	43.85 (29.10, 71.72)	72	49.83(26.87, 74.72)	0.68
Free fatty acids (µmol/l)	179	296.33 (176.00, 672.20)	74	590.80 (274.00, 920.00)	**<0.001**
Valine (µmol/l)	393	116.65 (101.64, 133.01)	78	117.87 (103.51, 136.72)	0.54
Leucine (µmol/l)	393	55.85 (46.62, 63.65)	77	50.44 (45.17, 64.25)	0.17
Isoleucine (µmol/l)	393	106.72 (94.88, 121.10)	78	118.68 (101.82, 135.59)	**<0.001**
Phenylalanine (µmol/l)	393	64.20 (56.17, 75.17)	77	63.72 (54.51, 77.76)	0.65
C2-carnitine (µmol/l)	388	1.76 (0.89, 2.82)	76	1.45 (0.94, 1.97)	**0.03**
C4-carnitine (µmol/l)	388	0.13 (0.08, 0.17)	76	0.10 (0.04, 0.15)	**<0.001**
C5-carnitine (µmol/l)	388	0.15 (0.06, 0.19)	76	0.09 (0.04, 0.18)	**0.01**
C8-carnitine (µmol/l)	388	0.15 (0.11, 0.24)	76	0.14 (0.11, 0.19)	0.10
FPG (mmol/L)	399	4.3 (4.0, 4.60)	77	5.3 (5.1, 5.6)	**<0.001**
Glucose 1hr after post load (mmol/L)	399	6.2 (5.3, 7.2)	78	9.3 (7.8, 10.2)	**<0.001**
Glucose 2hr after post load (mmol/L)	399	5.4 (4.7, 6.2)	78	7.5 (6.2, 8.8)	**<0.001**

Data expressed as n (%), mean ± SD or median (interquartile range); GDM=gestational diabetes, IL-6=interleukin-6, TNF-α= tumor necrosis factor-α

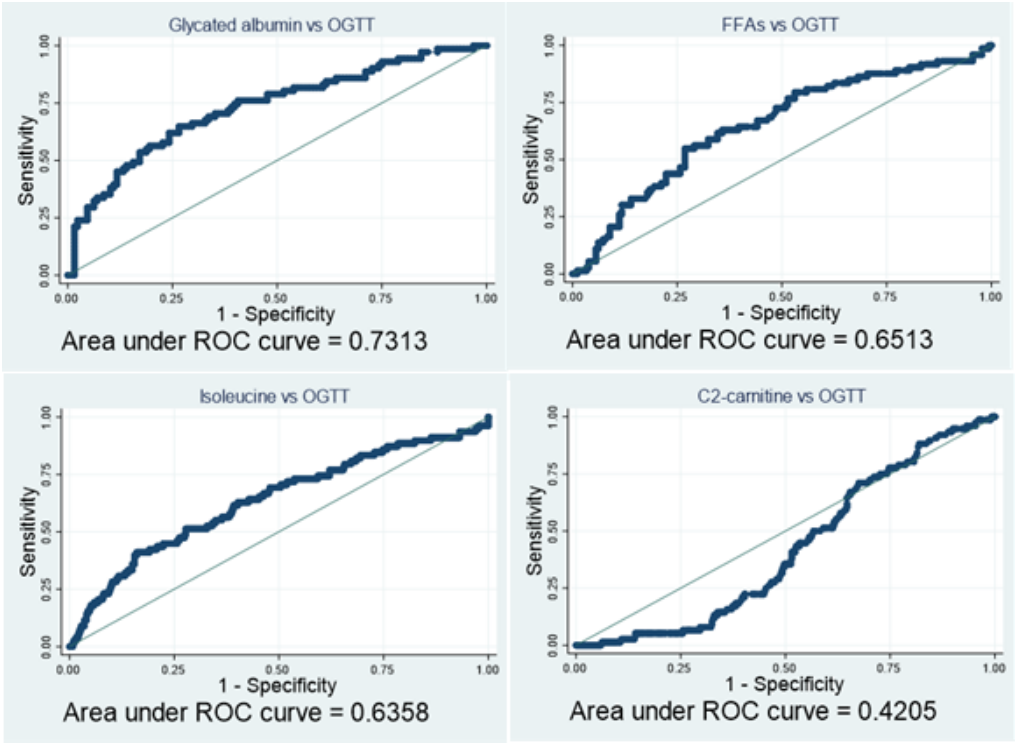


Figure 5.2 Receiver operating characteristic (ROC) curve analysis for GDM diagnosis by glycated albumin (GA), free fatty acids (FFAs), isoleucine and C2-carnitine. The OGTT as the reference method.

Table 5.4 The association of the discriminatory biomarkers with glycaemia in late pregnancy.

Variable		FPG (mmol/L)	Glucose 1hr after post-load (mmol/L)	Glucose 2hr after post-load (mmol/L)
Isoleucine (µmol/l)	Spearman's rho	0.1310	0.0582	0.1092
	p-value	**<0.01**	0.21	**0.02**
C2-carnitine (µmol/l)	Spearman's rho	-0.0795	0.0099	0.0485
	p-value	0.09	0.83	0.30
C4-carnitine (µmol/l)	Spearman's rho	-0.0518	-0.0920	-0.1565
	p-value	0.27	**0.05**	**<0.01**
C5-carnitine (µmol/l)	Spearman's rho	-0.0305	0.0765	-0.1396
	p-value	0.51	0.10	**<0.01**
Free fatty acids (µmol/l)	Spearman's rho	0.2587	0.1786	0.1368
	p-value	**<0.0001**	**<0.01**	**0.03**

Table 5.5 Multivariate logistic regression model for GDM.

Independent variable	Odds ratio (95% CI)	p-value
Age (years)	0.97 (0.92, 1.02)	0.23
BMI (kg/m2)	1.05 (1.01, 1.11)	**0.03**
Systolic BP (mmHg)	1.02 (0.99, 1.05)	0.13
Repeated glycosuria	1.95 (0.35, 10.72)	0.45
HIV positive	0.69 (0.31, 1.53)	0.36
Free fatty acids (µmol/l)	1.001 (1.00, 1.002)	**<0.01**
Isoleucine (µmol/l)	1.01 (1.00, 1.03)	**0.03**
C2-carnitine (µmol/l)	0.49 (0.33, 0.74)	**<0.01**
C4-carnitine (µmol/l)	0.02 (0.01, 9.78)	0.22
C5-carnitine (µmol/l)	2.04 (0.04, 111.63)	0.73

5.4.4 Correlations of biomarkers with fasting and random blood glucose in early pregnancy

Using linear regression analysis with adjustment for age, BMI and gestational age, only one significant association of biomarkers with FPG or random plasma glucose (RPG) in early pregnancy was identified (Table 5.7). Thus, valine was significantly associated with RPG (p=0.029) with an estimated 8.27 ± 3.71 µmol/l increase in valine for every 1mmol/l increase in RPG.

Table 5.6 Accuracy of biomarkers in late pregnancy.

	Isoleucine at 127.45 (µmol/l)	**Free fatty acids at 569.75 (µmol/l)**	**C2-carnitine at 2.52 (µmol/l)**	**GA (%) at 35.76%**
Observations	471	252	464	199
AUC (95% CI)	0.64 (0.56, 0.71)	0.65 (0.58, 0.73)	0.42 (0.36, 0.48)	0.73 (0.66, 0.81)
Sensitivity (95% CI)	33.33 (29.08, 37.59)	44.94 (38.80, 51.09)	7.74 (5.31, 10.17)	53.52 (46.59, 60.45)
Specificity (95% CI)	87.73 (84.77, 90.70)	79.75 (74.79, 84.72)	79.29 (75.60, 82.98)	82.81 (77.57, 88.05)

GA= glycated albumin, AUC= area under curve

5.5 DISCUSSION

In the current study, we found that blood levels of FFAs, isoleucine, and acylcarnitines C2, C4, and C5 levels differed between GDM and non-GDM women in late pregnancy and were all correlated with glycaemia, except for C2-carnitine. Apart from acylcarnitines C4 and C5, the metabolites and proteins listed above were also significantly associated with GDM after adjustment for possible confounding variables. However, these discriminatory biomarkers had poor sensitivity and specificity, thus, could not replace the OGTT for GDM diagnosis. Lastly, the biomarkers did not correlate with the levels of glycaemia in early pregnancy, except for valine.

Table 5.7 Correlations of the biomarkers with fasting and random plasma glucose in early pregnancy.

Dependent variable	Fasting plasma glucose		Random plasma glucose	
	Adjusted β-coefficient (SE)*	p-value	Adjusted β-coefficient (SE)*	p-value
GA (%)	-0.78 (2.63)	0.771	-0.51 (1.41)	0.718
IL-6 (pg/ml)	-0.08 (1.24)	0.947	-0.77 (1.72)	0.656
TNF-alpha (pg/ml)	-11.02 (14.89)	0.462	-2.68 (4.11)	0.517
FFAs (µmol/l)	-44.30 (80.01)	0.582	30.93 (39.97)	0.442
Valine (µmol/l)	8.40 (6.11)	0.173	8.27 (3.71)	**0.029**
Leucine (µmol/l)	2.46 (6.34)	0.669	4.96 (3.46)	0.157
Isoleucine (µmol/l)	4.48 (8.47)	0.599	6.22 (4.40)	0.162
Phenylalanine (µmol/l)	3.02 (4.19)	0.473	2.91 (3.26)	0.376
C2-carnitine (µmol/l)	-0.33 (0.60)	0.591	0.19 (0.18)	0.288
C4-carnitine (µmol/l)	0.03 (0.03)	0.294	-0.01 (0.01)	0.461
C5-carnitine (µmol/l)	0.02 (0.03)	0.444	<0.01 (0.01)	0.764
C8-carnitine (µmol/l)	-0.02 (0.07)	0.713	<0.01 (0.02)	0.907

*Models were all adjusted for body mass index and gestational age. SE=Standard error, GA= glycated albumin, IL-6=interleukin-6, TNF-α= tumor necrosis factor-α, FFAs= Free fatty acids

The current study showed that in late pregnancy fatty acid levels are higher in women with GDM compared to the non-GDM group. Similar results have been reported previously [100–102,104]. A body of literature suggests that elevated FFA causes IR [92,93,98,117,217] via

the accumulation of FFA metabolites, particularly diacylglycerol (DAG) [98], in the intramyocelluar regions of skeletal muscles. Diacylglycerol is known to lead to the attenuation of insulin receptor signalling [218]

Within the present study the high levels of fatty acids in the circulation, but low levels of acylcarnitines in women with GDM suggest that there may be a problem with the carnitine-dependent transport of fatty acids into the mitochondria (Figure 5.3). It is also interesting to note that we observed a negative correlation between serum fatty acid and acylcarnitine C2 levels. Research focusing on acylcarnitines in third trimester GDM is lacking, and follow-up studies are necessary to completely comprehend the observed decline in these metabolites. Similar to our findings, Roy et al. [202] indicated that C4 levels were lower in women with GDM compared to controls, but this difference was not statistically significant. However, in the same study, C2 was higher in women with GDM than controls. Heath et al. [213]demonstrated a decline in medium-chain acylcarnitines, including C8 in GDM individuals over time. The authors hypothesise that the dysregulated energy metabolism seen in GDM may affect carnitine octanoyltransferase activity, lowering plasma medium chain acylcarnitine levels. In contrast to our findings, C2 and C8 were higher in women with GDM than controls [147,219]. The inconsistent findings on acylcarnitine levels in GDM are likely caused by the differences in genetic background, dietary intakes, and samples collected at different time points and environmental impacts [220]. Furthermore, sample size variations may have contributed to the variability of research outcomes, the lowest being 20 women with GDM in the studies by Dudzik et al. [147] and Heath et al. [213] and the highest being 107 women with GDM in the study of Lin et al. [219].

In agreement with the current study, previous investigators have found that BCAAs correlate with GDM and with glycaemia in early pregnancy [100,200,202,221]. According to Enquobahrie et al. (2015), seventeen metabolites including valine, differentiated between GDM and non-GDM in early pregnancy and combining the metabolites with the conventional risk factors for GDM improved the prediction of GDM [100]. Similarly, Wang et al. [221] observed that BCAAs are higher in women who later develop GDM. In other studies, isoleucine and not valine were significantly higher in GDM than non-GDM women in early pregnancy [200,202]. It is also noteworthy that studies have suggested that BCAAs

may contribute to insulin resistance [220] and a recent meta-analysis demonstrates that BCAAs are predictive of incident T2D [222]. The reasons for the higher levels of BCAAs in women with GDM are not known however, noting that the subjects with GDM had higher BMIs than those without, previous studies have suggested that catabolism of BCAAs in adipose tissue is compromised in obesity [78,85] and higher levels of insulin resistance may attenuate protein anabolism [223].

Although there are statistically significant correlations between biomarkers and glycaemia, applying them for diagnosing GDM in this population is not recommended as their sensitivity and specificity, as assessed using ROC curve analysis, are low.

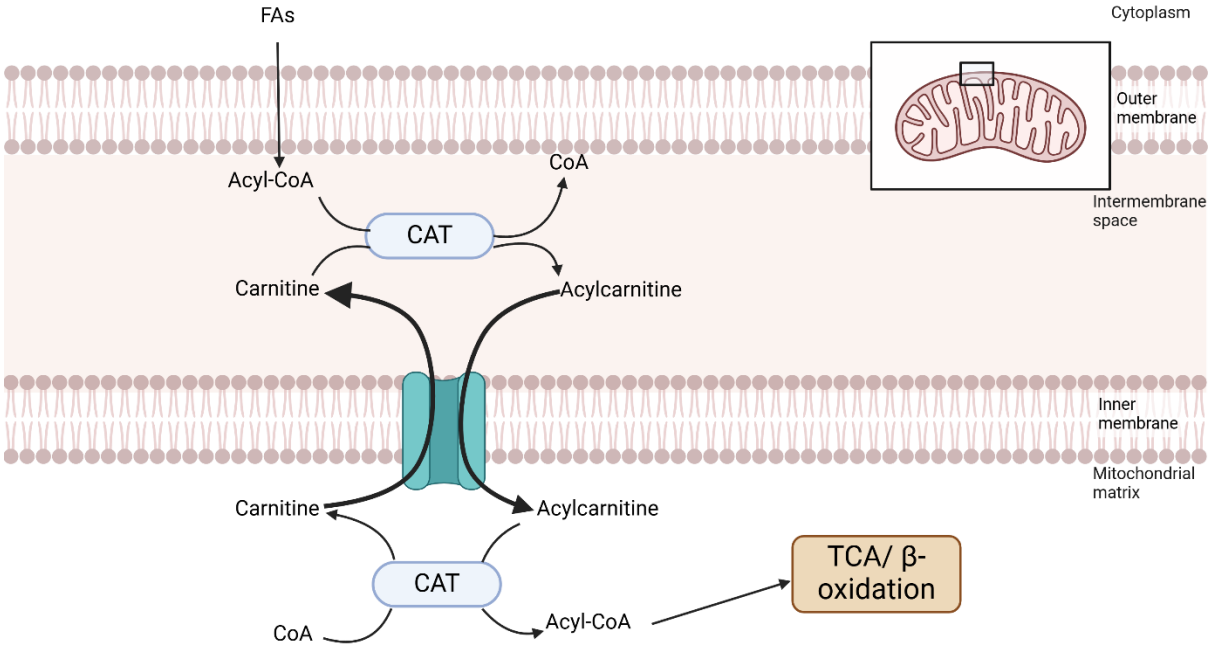


Figure 5.3. Illustration of the catabolic process of free fatty acid (FFAs), and the TCA cycle. Key enzymes, transporters, and intermediates are shown, highlighting the transportation of acyl-CoA to the mitochondrial matrix for oxidation. CAT= carnitine acylcarnitine transferase, FAO=Fatty acid oxidation. Created in https://BioRender.com

The study's strengths include a large sample size of participants from an understudied population. This is also one of the few studies that have investigated a broad array of candidate biomarkers including both protein- and metabolite-based biomarkers. In addition, we assessed the association of these biomarkers with glycaemia in both late and early pregnancy. The OGTT was not undertaken in the latter group because no guidelines currently exist that define glucose cut points for diagnosing GDM in the first or second trimester of pregnancy. There were some demographic differences between these 2 study groups, but we believe that these would not be sufficient to differentially alter the association of the biomarkers with glycaemia. The use of two cross-sectional study groups means that we could only observe statistical associations between variables rather than causal relationships. Another limitation was the overlap of the first and second trimesters in the women in early pregnancy. Previous studies that sought to discover early biomarkers for GDM and who collected samples in the second trimester [100,147,151,153] generally show fewer significant associations compared to those collected from the first and/or third trimesters [153]. In the current study two-thirds of the women in early pregnancy were in their second trimester. Lastly, insulin levels were not measured in this study which negates our ability to determine the relationship of the selected biomarkers with insulin resistance.

5.6 CONCLUSION

The study outcomes have expanded existing knowledge on GDM biomarkers, particularly among black Africans, by demonstrating that none of the selected biomarkers are suitable for GDM diagnosis. The study indicates that the metabolism of BCAAs and free fatty acids may contribute to the aetiology of GDM and that BCAAs, particularly valine, correlate with glycaemia in early pregnancy. This data can be used to inform the development of a longitudinal study for the identification of early biomarkers of GDM, and which may provide some mechanistic insight into the aetiology of this disease in the context of BCAA and free fatty acid metabolism.

5.7 SUPPLEMENTARY MATERIAL

Table S1: Gradient Elution

Time (min)	Flow (mL/min)	%A_2	%B_2
Initial	0.10	90.00	10.00
3.00	0.10	20.00	90.00
8.00	0.10	92.00	90.00
10.00	0.10	20.00	10.00

A_2= mobile phase A, B_2= mobile phase B

Table S3. Tandem mass spectrometer parameters

Parameter	**Value**
Ionization Mode	Electrospray Ionization (ESI), positive
ESI spray voltage	+ 5500 V
Gas setting 1	70
Gas setting 2	70
Curtain gas	20
Source Temperature	500°C

Table S3. MRM transitions

Analyte	Q1 Mass (Da)	Q3 Mass (Da)	Dwell Time (msec)	DP (volts)	CE (volts)
Valine 1	118.2	57.1	150.0	80.0	36.2
Valine 2	118.2	71.9	150.0	80.0	15.4
Leucine 1	132.1	43.1	150.0	100.0	30.0
Isoleucine 1	132.1	69.1	150.0	100.0	22.0
Phenylalanine 1	165.8	103.1	150.0	80.0	15.5
Phenylalanine 2	165.8	119.9	150.0	80.0	27.6
Valine-d8	125.2	57.1	150.0	80.0	36.2
Leucine-d3	135.10	46.1	150.0	120.0	30.0
Phenylalanine-d5	171.0	125.1	150.0	80.0	40.0
C2-carnitine 1	205.1	85.1	150.0	120.0	30.0
C2-carnitine 2	250.1	60.1	150.0	120.0	30.0
C4-carnitine 1	233.1	85.1	150.0	120.0	28.0
C4-carnitine 2	233.1	145.2	150.0	120.0	28.0
C5-carnitine 1	247.1	85.1	150.0	120.0	30.0
C5-carnitine 2	247.1	103.2	150.0	120.0	30.0
C8-carnitine 1	288.8	85.1	150.0	120.0	30.0
C8-carnitine 2	288.8	60.1	150.0	120.0	30.0
C0-carnitine-d3	166.1	86.2	150.0	120.0	40.0

Q1=quadruple 1, Q3= quadruple 3, DP=declustering potential, CE=collision energy.

METHOD VALIDATION FOR ACYLCARNITINES

The LC-MS/MS analytical method for acylcarnitines namely, c2, c4, c5 and c8-carnitines were validated in accordance with Food and Drug Administration (FDA) guidelines [216].The LC-MS/MS method was verified in terms of specificity, linearity, sensitivity, precision, accuracy, and recovery.

1.1 Linearity

A six-point calibration curve was used for linearity assessment; the concentration ranged from 0-120µM. The standards were prepared in a bovine serum albumin matrix, and the calibration curves included three replicates per calibration point, and linearity was assessed by linear regression.

1.2 Accuracy and precision

The intra- and inter-day accuracy and precision measurements were conducted using measurements of two quality controls (low and high controls) repeated three times within five days. According to the FDA, accuracy should be ± 15% of nominal concentrations, except for the LLOQ, which should be ± 20% of nominal concentrations and ± 15% CV, except ± 20% CV at LLOQ for precision.

1.3 Sensitivity

Sensitivity was assessed by calculating the lower limit of detection (LLOD) and the lower limit of quantitation (LLOQ). The limits of detection (LLOD) and quantification (LLOQ) were determined using the standard deviation of the lowest standard, using the following formula

$LOD = 3.3 * SD$

$LOQ = 10 * SD$

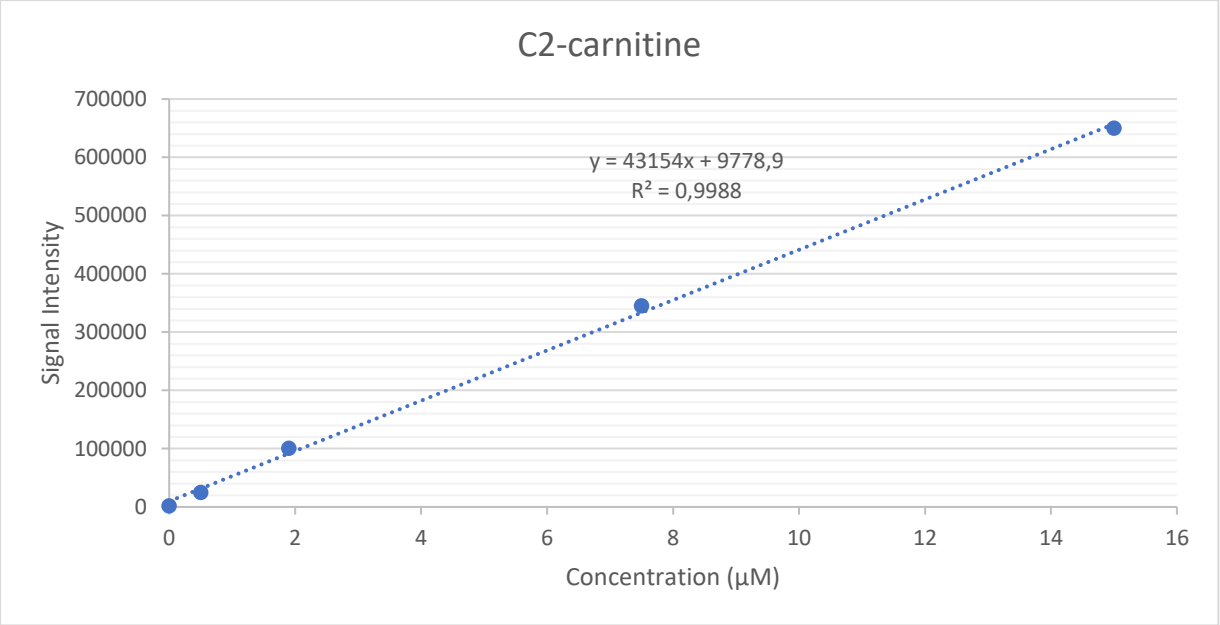
1.4 Specificity

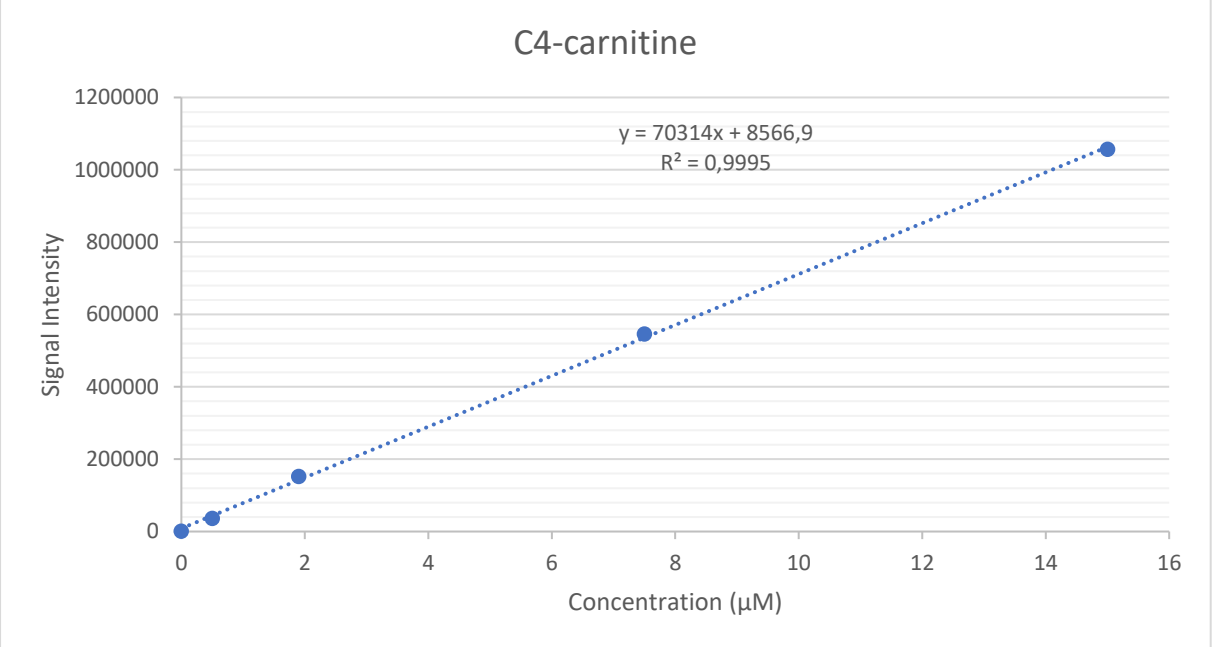
The Specificity was assessed by comparing chromatograms of blanks (50% acetonitrile and zero standards) with acylcarnitine standards. The chromatograms were used to determine if there was interference at the retention times of the analytes and the internal standards.

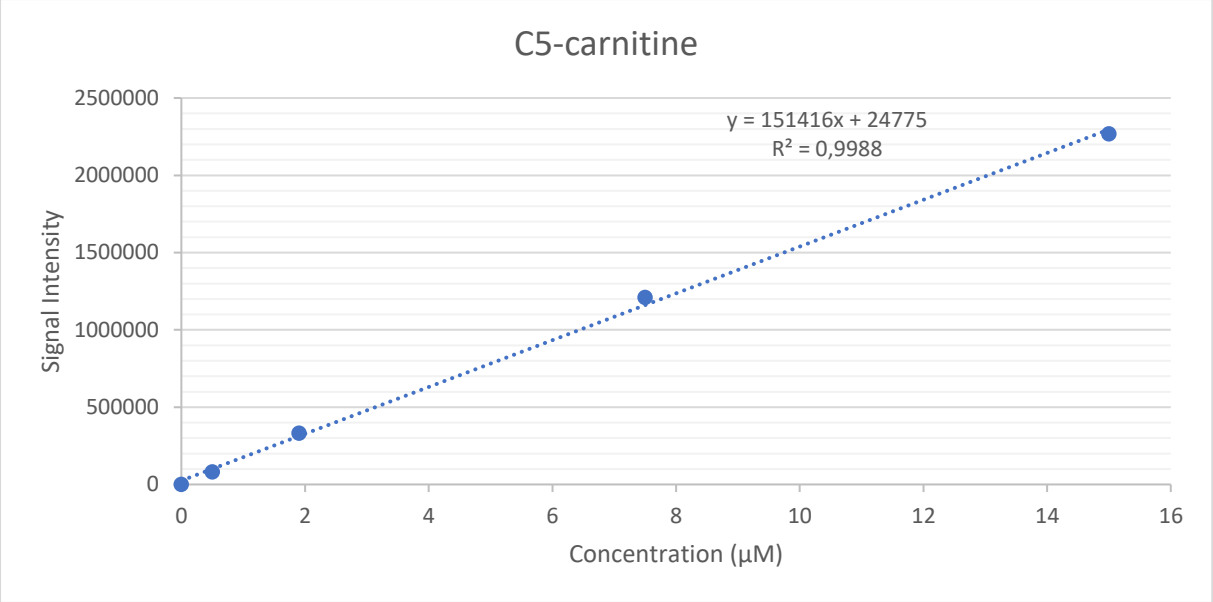
2. RESULTS

2.1 Linearity

The linearity was limiting and had to be narrowed down to 0-15µM. All the analytes displayed a linear relationship between 0.5-15µM. The regression coefficients were all above 0.99.


C2-carnitine
y = 43154x + 9778,9
R² = 0,9988
Signal Intensity
Concentration (µM)
0
100000
200000
300000
400000
500000
600000
700000
0
2
4
6
8
10
12
14
16


C4-carnitine
y = 70314x + 8566,9
R² = 0,9995
Signal Intensity
Concentration (µM)
0
200000
400000
600000
800000
1000000
1200000
0
2
4
6
8
10
12
14
16



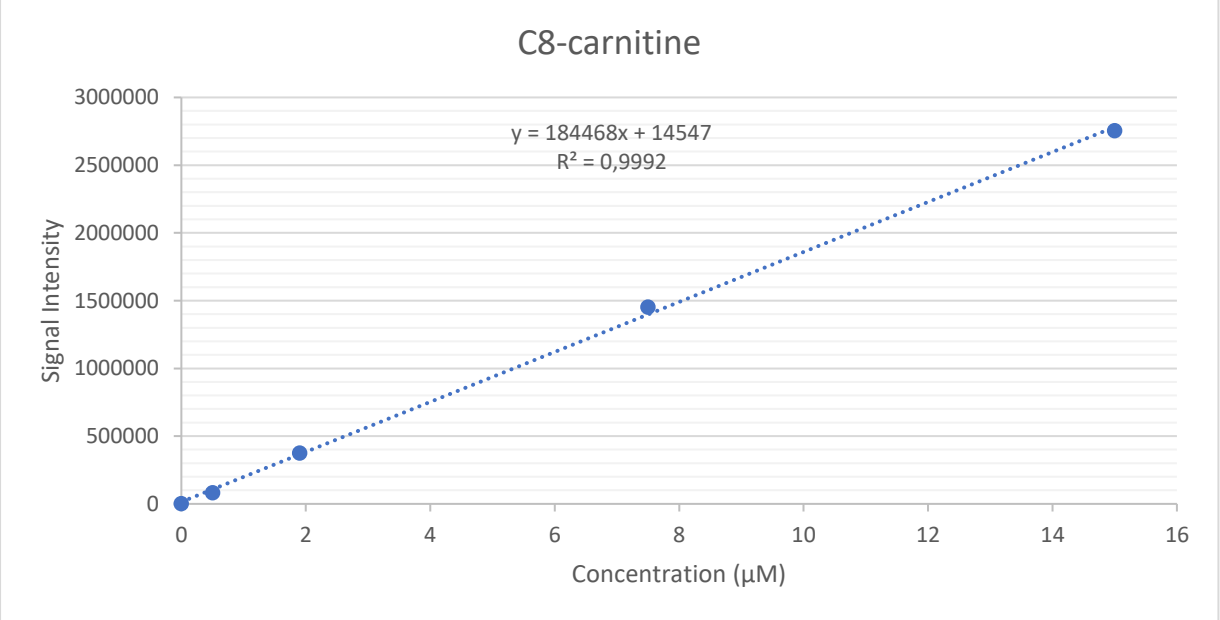


Figure 2.1 Calibration curves for the acylcarnitines.

2.2 Accuracy and Precision

The analytes were within ± 15% of the nominal concentration with accuracy between 91.83-112.44% for QC 1 and 88.06 -97.09% for QC 2 for inter-day.

The analytes had CVs below 15 ranging between 1.94 to 10.98% for QC1 and 1.44 to 10.11% for QC2 for inter-day.

Table 2.1 Inter-day precision and accuracy

	True Conc (µM)	Mean (µM)	SD	CV (%)	Accuracy (%)
C2-carnitine					

QC1	4,00	3,82	0,54	14,17	95,42
QC2	25,00	23,58	1,79	7,58	94,34
C4-carnitine					
QC1	4,00	3,67	0,34	9,17	91,83
QC2	25,00	24,27	1,32	5,43	97,09
C5-carnitine					
QC1	4,00	4,05	0,35	8,72	101,33
QC2	25,00	23,00	0,83	3,62	91,99
C8-carnitine					
QC1	4,00	4,50	0,14	3,12	112,44
QC2	25,00	22,01	1,95	8,86	88,06

The analytes were within ± 15% of the nominal concentration with accuracy between 94.28-113.69% for QC 1 and 88.55 -99.49% for QC 2 for intra-day.

The analytes had CVs below 15 ranging between 9.43 to 14.69% for QC1 and 0.24 to 12.10% for QC2 for intra-day.

Table 2.2 Intra-day precision and accuracy

	True Conc (µM)	Mean (µM)	SD	CV (%)	Accuracy (%)
C2-carnitine					
QC1	4,00	3,81	0,56	14,69	95,14
QC2	25,00	23,68	2,86	12,10	94,72
C4-carnitine					
QC1	4,00	3,77	0,40	10,62	94,28
QC2	25,00	24,34	1,43	5,87	97,37
C5-carnitine					
QC1	4,00	4,55	0,43	9,43	113,69
QC2	25,00	22,14	0,05	0,24	88,55
C8-carnitine					
QC1	4,00	4,03	0,49	12,12	100,72
QC2	25,00	24,87	2,61	10,48	99,49

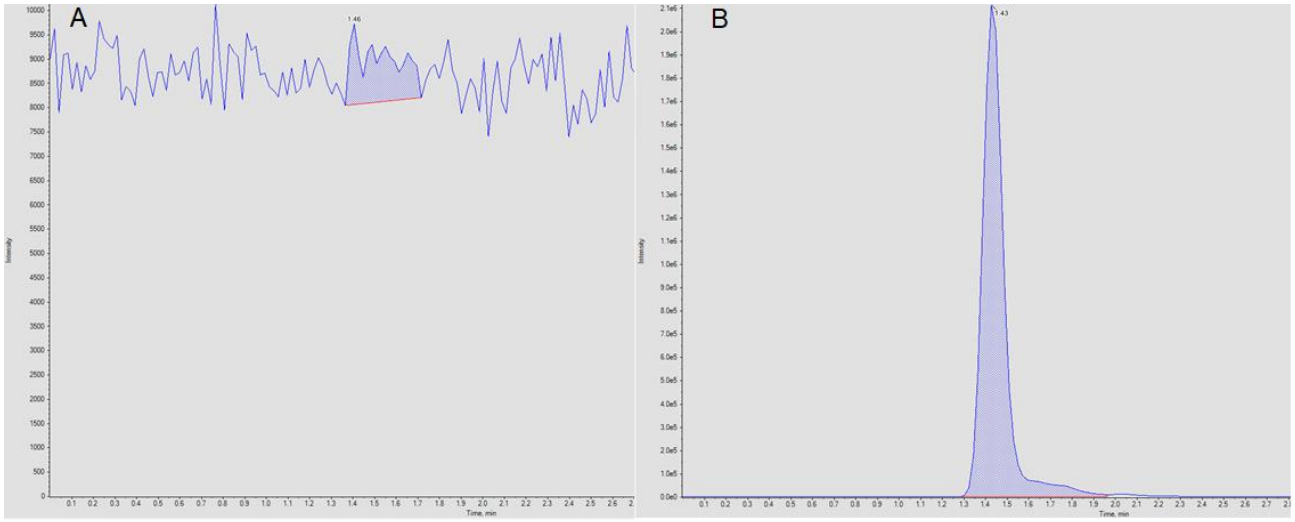
2.3 Sensitivity

The LLOQ and LLOD for c2-carnitine were calculated as 0.14 and 0.05 µM, respectively; for c4-carnitine, they were calculated as 0.35 and 0.12 µM; for c5-carnitine, they were calculated as 0.57 and 0.19 µM; and for c8-carnitine, they were calculated as 0.07 and 0.02 µM.

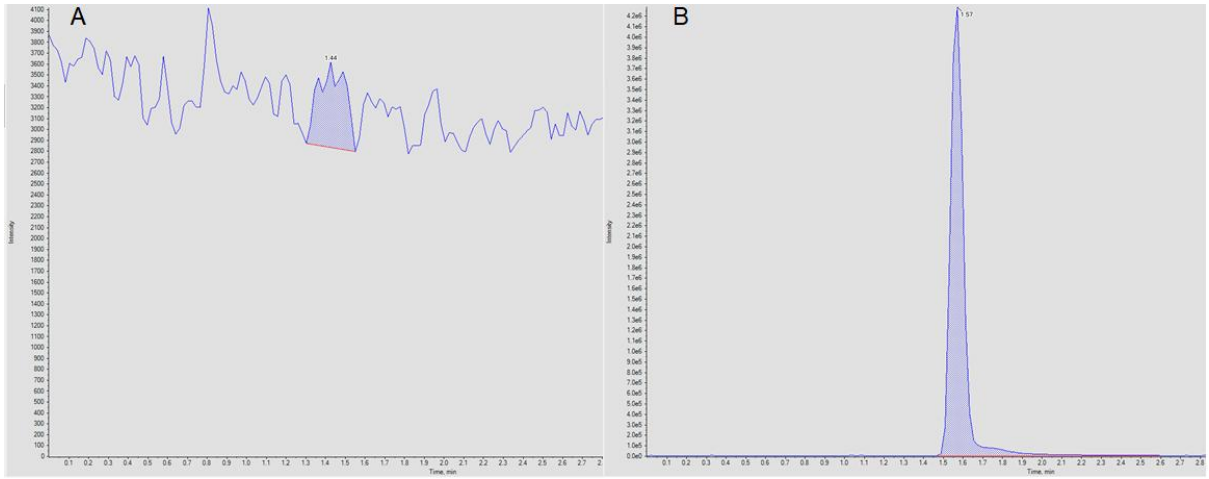
Table 2.3 Limits of detection and quantitation

Analyte	LLOQ (µM)	LLOD (µM)
C2-carnitine	0.14	0.05
C4-carnitine	0.35	0.12
C5-carnitine	0.57	0.19
C8-carnitine	0.07	0.02

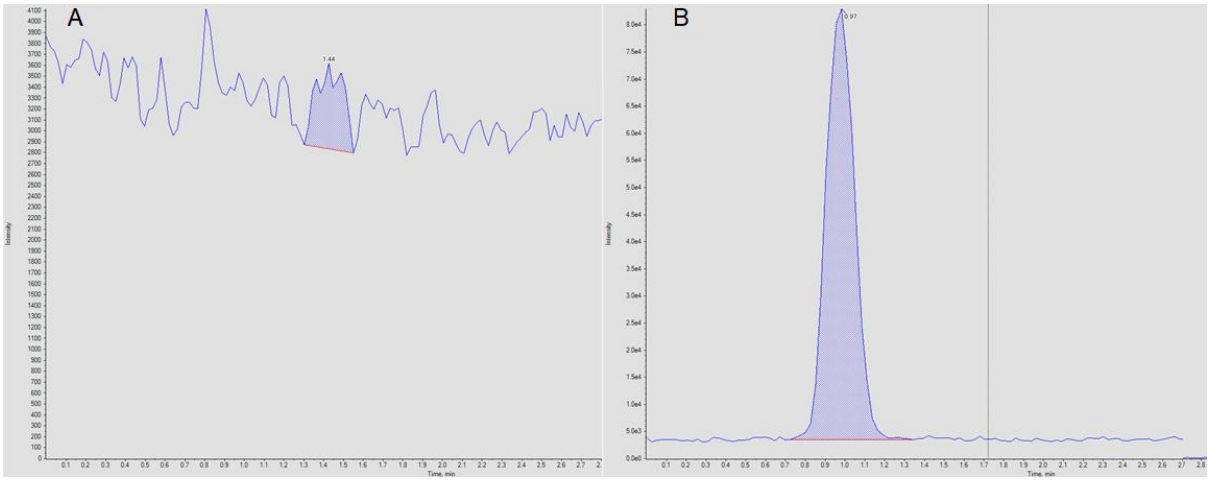
2.4 Specificity



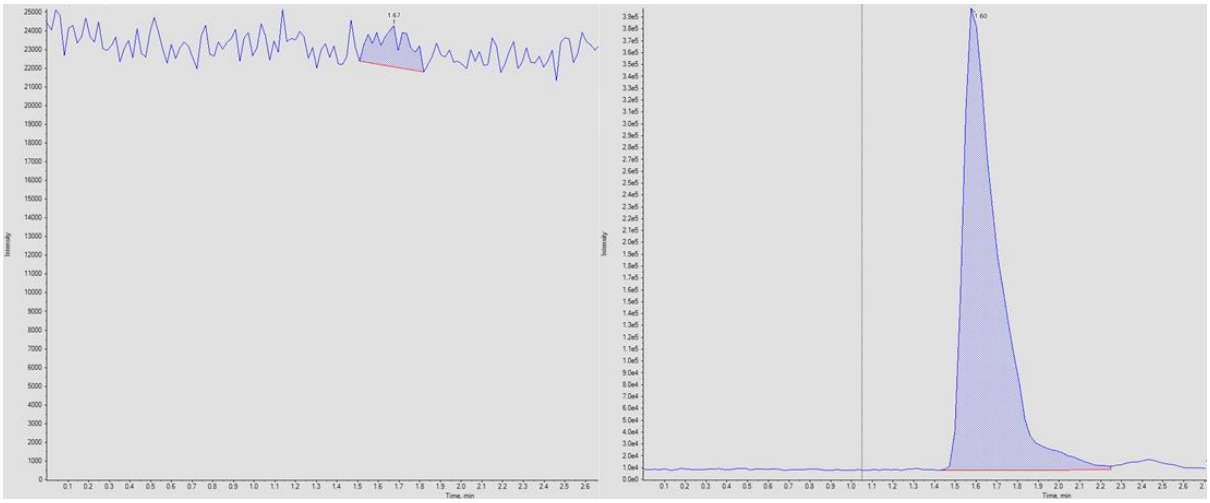
Chromatogram of blank (A) and c2-carnitine standard (B)



Chromatogram of blank (A) and c4-carnitine standard (B)



Chromatogram of blank (A) and c5-carnitine standard (B)



Chromatogram of blank (A) and c5-carnitine standard (B)

3. CONCLUSION

The method suggested in the present study proves to be suitable for accurate measurements of c2, c4, c5 and c8 concentrations in human serum.

METHOD VALIDATION FOR BRANCHED-CHAIN AMINO ACIDS

1.METHODS

The LC-MS/MS analytical method for branched-chain amino acids (valine, leucine, isoleucine) and phenylalanine was validated following Food and Drug Administration (FDA) guidelines [216].The LC-MS/MS method was verified in terms of specificity, linearity, sensitivity, precision, accuracy, and recovery.

1.1 Linearity

A six-point calibration curve was used for linearity assessment; the concentration ranged of 9.4-300µM. The standards were prepared in a bovine serum albumin matrix and the calibration curves included three replicates per calibration point, and linearity was assessed by linear regression.

1.2 Accuracy and precision

The intra- and inter-day accuracy and precision measurements were conducted using measurements of two quality controls (low and high controls) repeated three times within five days. According to the FDA, accuracy should be ± 15% of nominal concentrations, except for the LLOQ, which should be ± 20% of nominal concentrations and ± 15% CV, except ± 20% CV at LLOQ for precision.

1.3 Sensitivity

Sensitivity was assessed by calculating the lower limit of detection (LLOD) and the lower limit of quantitation (LLOQ). The limits of detection (LLOD) and quantification (LLOQ) were determined using the standard deviation of the lowest standard, using the following formula

$LOD = 3.3 * SD$

$LOQ = 10 * SD$

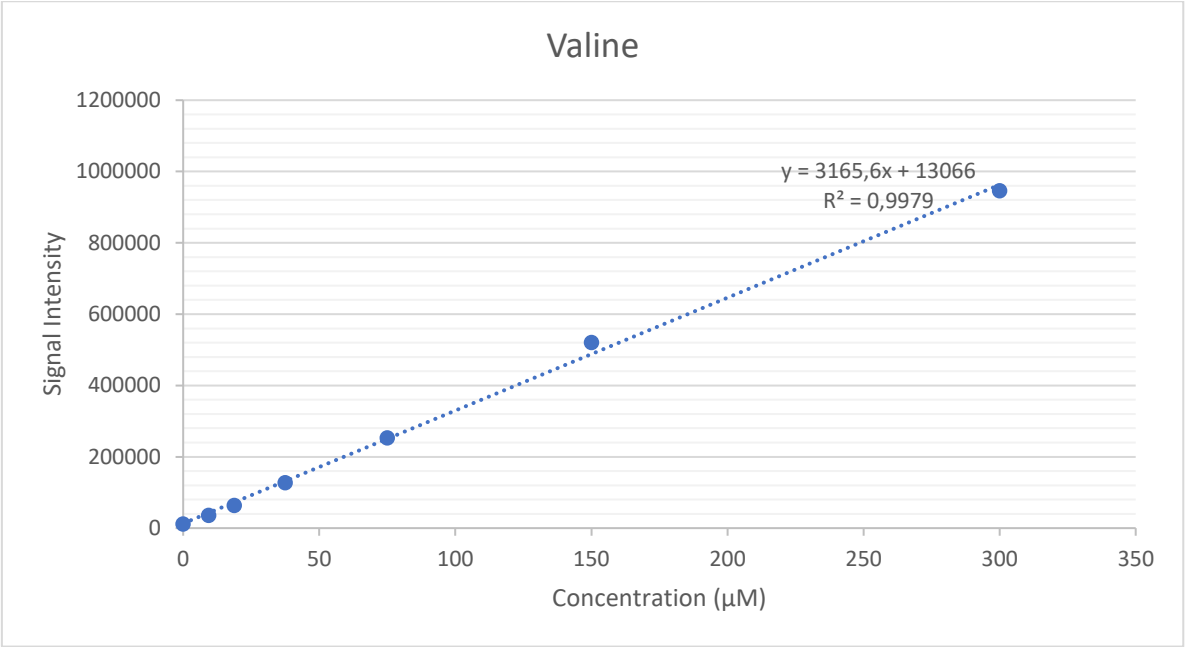
1.4 Specificity

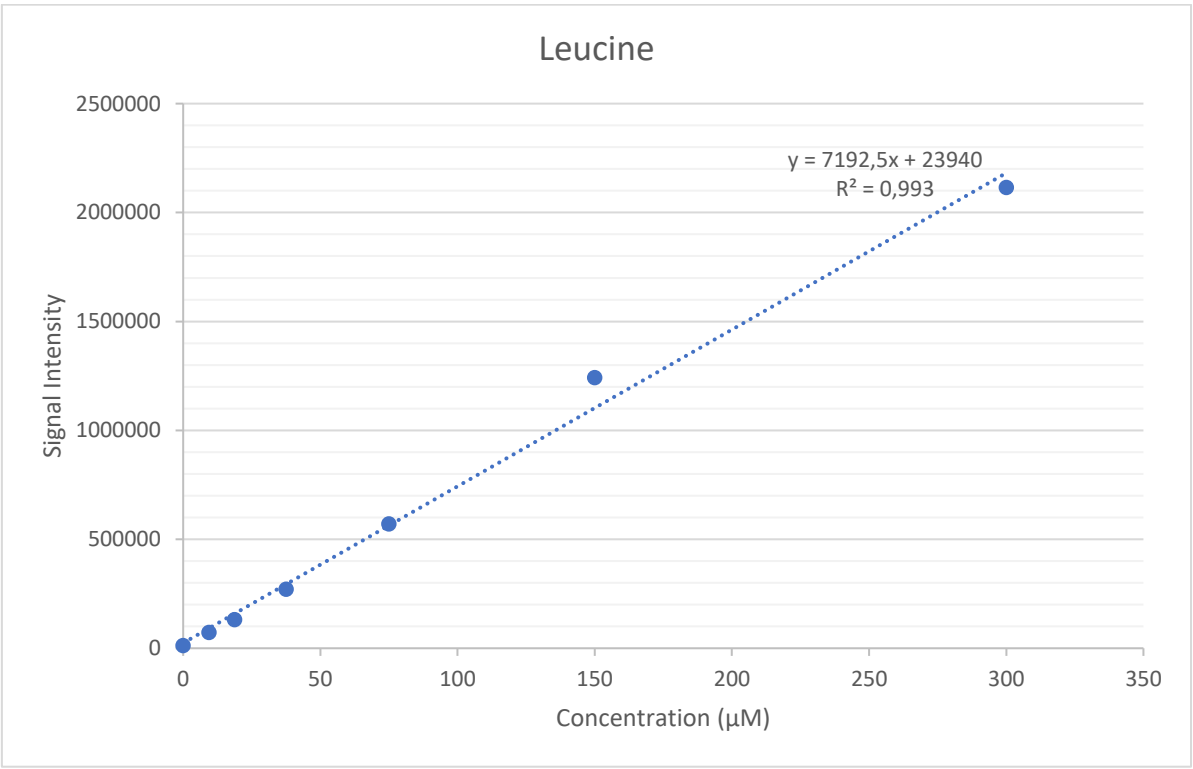
The Specificity was assessed by comparing chromatograms of blanks (50% acetonitrile and zero standards) with BCAAs and phenylalanine standards. The chromatograms were used to determine if there was interference at the retention times of the analytes and the internal standards.

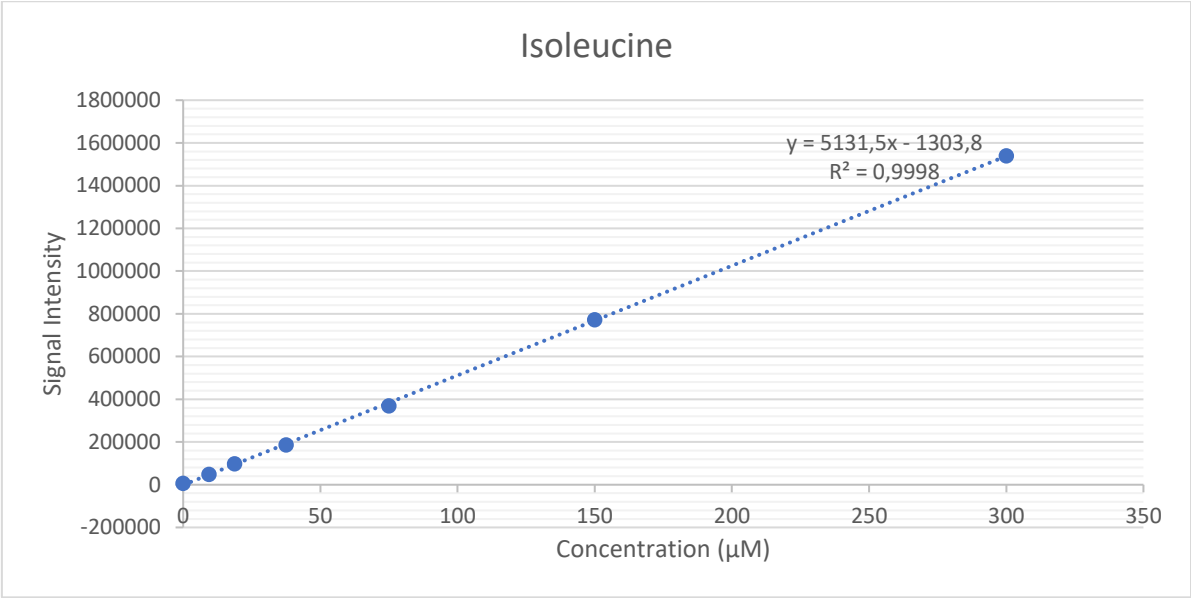
2. RESULTS

2.1 Linearity

All the analytes displayed a linear relationship between 0-300µM. The regression coefficients were all above 0.99.


Valine
y = 3165,6x + 13066
R² = 0,9979
Signal Intensity
Concentration (µM)
0
200000
400000
600000
800000
1000000
1200000
0
50
100
150
200
250
300
350


Leucine
y = 7192,5x + 23940
R² = 0,993
Signal Intensity
Concentration (µM)
0
500000
1000000
1500000
2000000
2500000
0
50
100
150
200
250
300
350



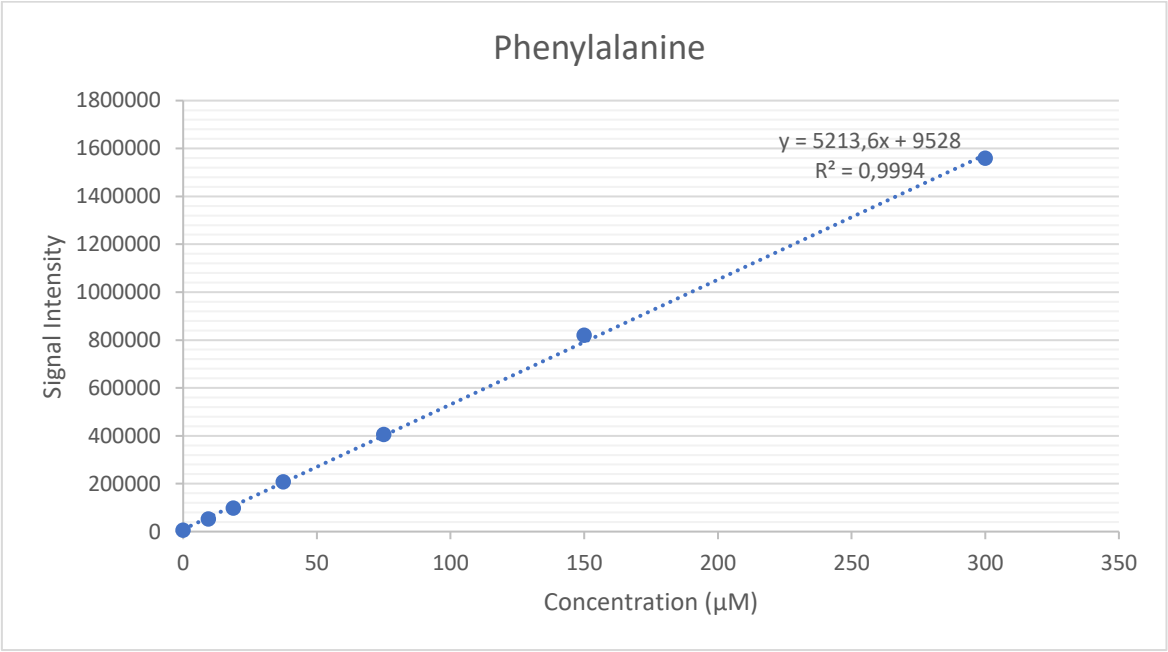


Figure 2.1 Calibration curves for the amino acids.

2.2 Accuracy and Precision

The analytes were within ± 15% of the nominal concentration, with accuracy ranging from 96.27% to 104.19% for QC 1 and from 99.50% to 105.83% for QC 2.

The analytes had CVs below 15 ranging from 1.94% to 10.98% for QC1 and from 1.44% to 10.11% for QC2.

Table 2.1 Inter-day precision and accuracy

	True Conc (µM)	Mean (µM)	SD	CV (%)	Accuracy (%)
Valine					
QC 1	50	48,99	2,05	4,19	97,99
QC 2	100	103,29	5,71	5,53	103,29
Leucine					
QC 1	50	51,01	1,75	3,43	102,03
QC 2	100	105,06	7,26	6,91	105,06
Isoleucine					
QC 1	50	50,46	0,98	1,94	100,91
QC 2	100	105,83	1,53	1,44	105,83
Phenylalanine					
QC 1	50	50,57	1,37	2,71	101,14
QC 2	100	104,41	3,19	3,05	104,41

Table 2.2 Intra-day precision and accuracy

	True Conc (µM)	Mean (µM)	SD	CV (%)	Accuracy (%)
Valine					
QC 1	50,00	49,41	5,43	10,98	98,82
QC 2	100,00	99,89	6,71	6,72	99,89
Leucine					
QC 1	50,00	48,14	5,00	10,39	96,27
QC 2	100,00	104,03	10,52	10,11	104,03
Isoleucine					
QC 1	50,00	49,68	1,99	4,01	99,36
QC 2	100,00	99,50	5,87	5,90	99,50
Phenylalanine					
CTL 2	50,00	52,10	3,10	5,96	104,19
CTL 3	100,00	100,76	5,45	5,41	100,76

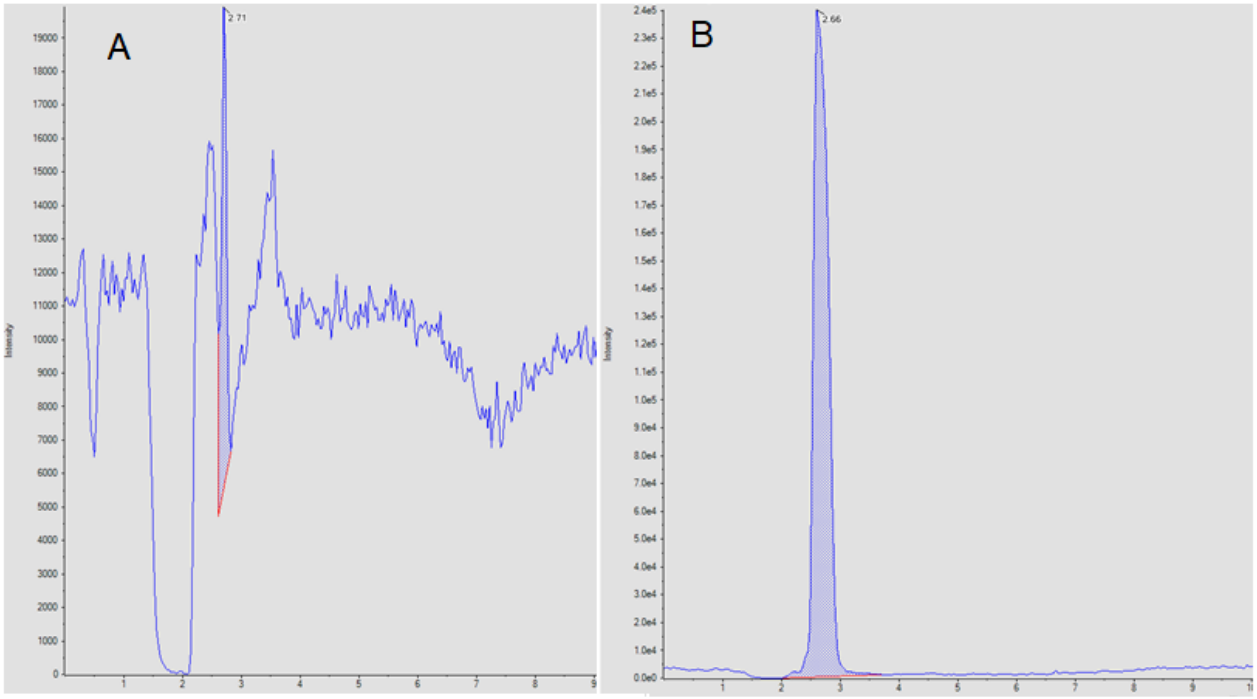
2.3 Sensitivity

The LLOQ and LLOD for valine were calculated as 4.80 and 1.58 µM, respectively; for leucine, they were calculated as 8.00 and 2.64 µM; for isoleucine, they were calculated as 4.44 and 1.47 µM; and for phenylalanine, they were calculated as 3.59 and 1.19 µM.

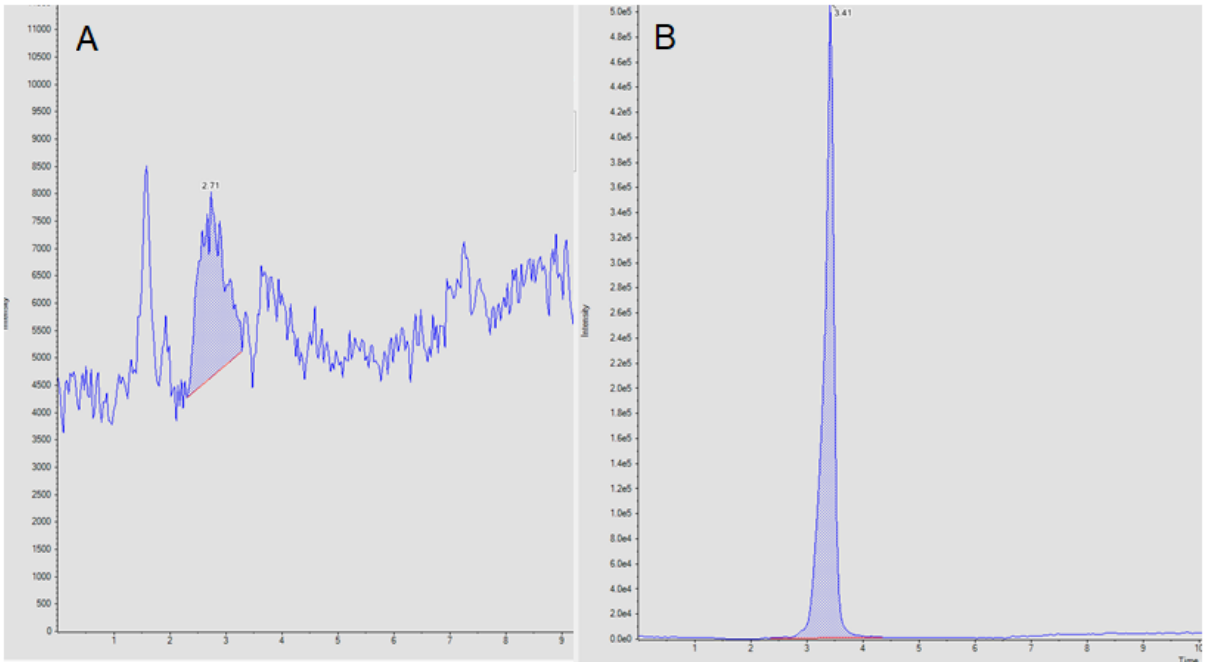
Table 2.3 Limits of detection and quantitation.

Analyte	LLOQ (µM)	LLOD (µM)
Valine	4.80	1.58
Leucine	8.00	2.64
Isoleucine	4.44	1.47
Phenylalanine	3.59	1.19

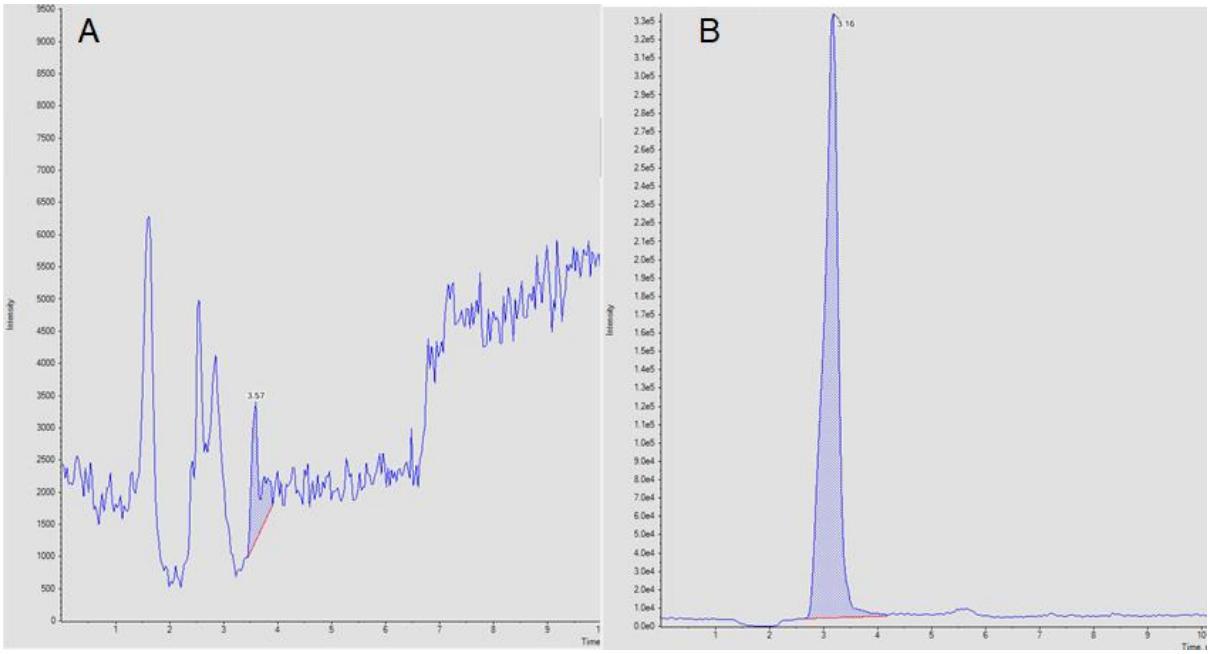
2.4 Specificity



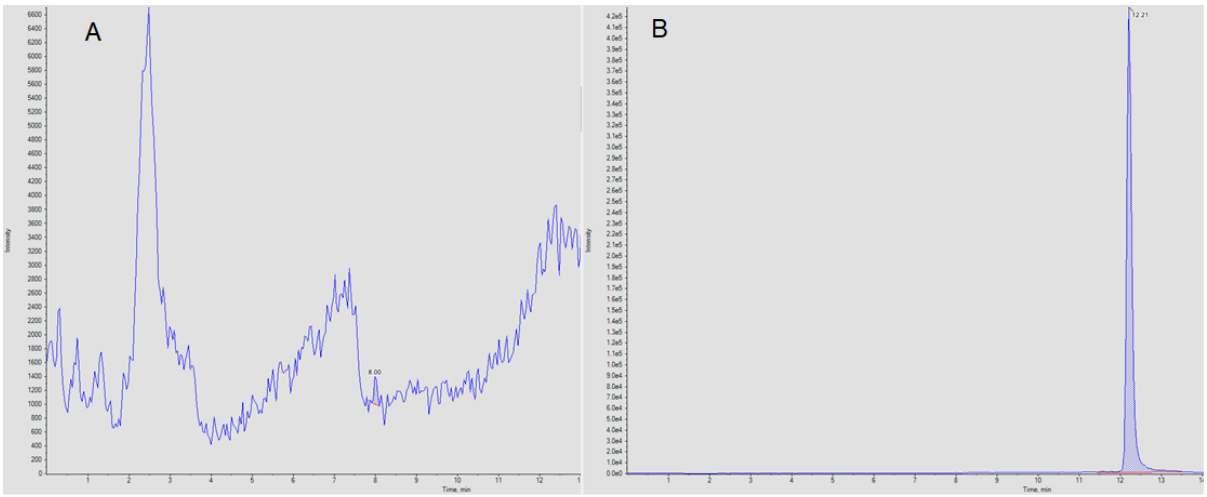
Chromatogram of blank (A) and valine standard (B)



Chromatogram of blank (A) and leucine standard (B)



Chromatogram of blank (A) and isoleucine standard (B)



Chromatogram of blank (A) and phenylalanine standard (B)

3. Conclusion

The method suggested in the present study proves to be suitable for accurate measurements of branched-chain amino acid concentrations in human serum.

CHAPTER 6: CONSOLIDATED DISCUSSION

This section summarises the important conclusions from the empirical articles discussed in Chapters 3 to 5. It also covers how the findings contribute to the body of knowledge on GDM. The PhD study's strengths and limitations are discussed, along with recommendations for GDM screening and diagnosis in South Africa. Ultimately, this chapter concludes with a summary of the core message and potential future research.

6.1 SUMMARY OF THE STUDY OUTCOMES

In this study, we aimed to establish a novel method for the screening or diagnosis of gestational diabetes mellitus (GDM) that is not only sensitive and specific but also considers resource availability, infrastructure, and patient compliance. The study showed that the seven glucometers evaluated are not ready to replace the lab-based glucose analytical method for GDM diagnosis. However, lab-based FPG has emerged as the most effective test when compared to other glycemic markers for GDM diagnosis. Furthermore, this study emphasises that most traditional risk indicators fail to differentiate between GDM and non-GDM except for repeated glycosuria and having a history of GDM. Candidates such as BCAAs, FFAs, and acylcarnitines correlated with GDM. Valine was the only biomarker that correlated with glycaemia in early pregnancy Table 6.1 gives a summary of the main outcomes of the study.

Table 6.1 Summary of the Consolidated Discussion

Empirical Paper	Chapter	Objective	Main Findings
I	3	To determine the accuracy of seven POC glucometers in diagnosing GDM among pregnant women in South Africa.	• The prevalence of GDM in this cohort was 7.7% when using the IADPSG criteria. • The seven glucometers performed poorly with sensitivity ranging between 18-87% and specificity ranging between 63-99%. • The glucometers cannot be used interchangeably as their performance varies. • Lab-based FPG showed a considerably high sensitivity and specificity, 100% and 99.50%, respectively.
II	4	To compare the accuracy of FPG, HbA1c and GA against the OGTT for diagnosing GDM in a black African population.	• The prevalence of GDM in this cohort was 16.4% when using the IADPSG criteria. • Conventional GDM risk factors were not significantly different between women with and without GDM. • FPG at 5.1mmol/l threshold had superior accuracy than HbA1c and GA with AUC (95%CI) of 0.98 (0.96, 1.00) and missed 10 out of 78 women with GDM. • Using FPG in a two-tiered method increases the specificity for GDM diagnosis and missed 1 out of 78 women with GDM.
III	5	To determine whether any of these chosen metabolites and proteins (IL-6, TNF-α, FFAs, BCAAs, and ACs), are associated with glycaemia at either of these stages of pregnancy and whether they may be possible biomarkers of GDM in this population. In addition, these analyses may provide important information on the pathophysiology of GDM in African women.	• FFAs, isoleucine and c2 carnitine were associated with GDM independent of BMI and age. • The above biomarkers, however, cannot replace OGTT. Their sensitivity ranged between 7.7 & 44.9% and specificity between 79.4 & 87.7%. • In early pregnancy, valine was associated with blood glucose.

6.2 HYPOTHESES REVISITED

The study hypotheses were as follows:

1. POC glucometers are comparable to laboratory-based glucose measurements for GDM diagnosis.

The study described in Chapter 3 has shown that overall, the POC glucometers had poor sensitivity and specificity and may not be used for GDM diagnosis. Therefore, the above hypothesis was rejected.

2. FPG, HbA1c and GA performance is comparable to OGTT when used to diagnose GDM.

The study described in Chapter 4 partially agrees with the above hypothesis. The study concluded that FPG and not HbA1c and GA are comparable to the OGTT in terms of sensitivity and specificity.

3. Selected biomarkers, IL-6, TNF-α, FFAs, BCAAs and ACs are associated with glycaemia in early and late pregnancy in black African pregnant women and contribute to the pathophysiology of the disease.

The study described in Chapter 5 partially agrees with the above hypothesis. The biomarkers that were significantly associated with glycaemia are FFAs, isoleucine and c2-carnitine in late pregnancy. However, only valine was associated with glycaemia in early pregnancy.

6.3 CONSOLIDATED DISCUSSION

As mentioned in the introduction, the WHO recommends universal testing for GDM, ideally by OGTT, following the data from the HAPO study [11]. The main challenges with universal screening/diagnosis by OGTT are the cost implications and the unnecessary screening intervention of women who would be classified as low risk. This burden is translated to the health practitioners who would need to perform the test. The current study outcomes indicate that FPG may be utilised for diagnosing GDM and is more beneficial when used as a two-tiered approach. The benefits of employing FPG as a two-tiered strategy include the reduction in the number of OGTTs to be performed. The current study indicated that the number of glucose tests would be halved if the two-tiered approach were used. Therefore,

this may reduce the costs associated with GDM diagnosis. Another benefit is that many of the women who tested negative for GDM using the two-tiered approach would not need to undergo the uncomfortable OGTT which would reduce the unnecessary testing associated with universal screening by OGTT. However, the study outcome would need to be confirmed in a general population. We foresee that reducing GDM testing costs may redirect the funds to opportunities such as universal screening for GDM which is aligned with the WHO and the sustainable goal number 3 implemented by the United Nations General Assembly [224]. The goal states that nations like South Africa should aim to '*Ensure healthy lives and promote well-being for all at all ages*'. Although universal screening incurs significant costs compared to selective screening, its use can mitigate the long-term consequences of GDM that would likely remain unseen in GDM women missed by selective screening thus reducing long-term costs to manage and treat adverse outcomes associated with GDM like T2D [225]. When compared to the OGTT, FPG does not necessarily reduce the overall burden of testing. Women are still required to take time off work, fast overnight, and travel to and from the hospital for the test. This creates both financial and logistical challenges, such as transport costs and the risk of losing their wages for that day, which in turn increases the likelihood of missed or delayed follow-up [24]. The use of POC devices at local clinics could help mitigate these challenges. By bringing testing closer to the community, POC instruments reduce travel and waiting times, encourage better follow-up, and improve early detection of gestational diabetes. Furthermore, POC testing can complement the OGTT as a confirmatory test, allowing for quicker turnaround of results and timely initiation of treatment, ultimately improving maternal and neonatal outcomes [226]. These women would also be encouraged to undergo retesting post-pregnancy. Health services in developing countries like South Africa, may struggle to fulfil these demands [24,227].

There is no clear indication why the current study showed varying prevalence estimations for GDM across the different study sites despite the use of the same criteria and sample collection procedure for GDM diagnosis. A study performed in a cohort recruited from CHBAH, similar to the cohort in the POC glucometer study in Chapter 3 reported a prevalence of 9.1% [9]. Similarly, Dickson et al. [8], reported a GDM prevalence of 7.0%. These results are similar to the prevalence we reported from the glucometer study at 7.7% (Paper 1). In contrast, Adam and Rheeder [10] reported a prevalence of 15% when selective

screening was used in a SA population, which was closer to the prevalence we reported in the glycaemic markers study at 16.4% (Paper 2). This variation in the prevalence of GDM may signify differences in the socioeconomic and demographic characteristics of the populations served by the different hospitals at which the studies were undertaken.

The study outcome emphasized the association of BCAAs, FFAs and acylcarnitines with glycaemia in pregnancy, particularly in later gestation. This variation in the prevalence of GDM may signify differences in the socioeconomic and demographic characteristics of the populations served by different hospitals at which the studies were undertaken.

6.3.1 Strengths and limitations

The study had several limitations. Thus, in the POC study (Chapter 3) we cannot be certain that all samples for laboratory glucose analysis were separated on time, which may have affected the results. Glucose concentrations in whole blood decline ex vivo due to glucose consumption, which is mostly by red and white blood cells. Such a drop in glucose levels will result in missed diagnoses of GDM in women with glucose concentrations close to the cut points for diabetes diagnosis. The current study used blood collection tubes containing sodium fluoride, which is a routinely used glycolysis inhibitor. Although, fluoride helps to maintain long-term glucose stability, the rates of decline of glucose in the first hour after sample collection in tubes with and without fluoride are almost comparable, and glycolysis continues for up to 4 hours in samples containing only fluoride [145,228]. The American Diabetes Association recommends collecting the sample in a tube containing a fast efficient glycolytic inhibitor, such as granulated citrate buffer [229].

In the biomarker study (Chapter5), we were unable to show a trend in candidate biomarkers across pregnancy as the cohort in early pregnancy was not followed longitudinally. However, one study showed that FFA levels are elevated before pregnancy and throughout pregnancy in women with GDM [230]. Moreover, the study indicated that the ability of insulin to suppress FFA release declines in pregnancy, and this is more profound for women with GDM. Also, we did not run OGTT tests for women in early pregnancy and therefore were not able to analyse the association of biomarkers with dysglycaemia. However, there are no current diagnostic guidelines for detecting GDM or any form of glucose intolerance in early pregnancy and therefore we believe that the use of FPG and random blood glucose

measurements was appropriate. Furthermore, we did not measure insulin in this study and therefore were not able to assess the association of the biomarkers with insulin sensitivity. In addition, this study was conducted on women who were chosen from all pregnant women using selective screening based on the presence of GDM risk factors. Thus, our data is only applicable to women at high risk for GDM and therefore we do not know whether FPG would provide the same discriminatory power in a general population of pregnant women. This is also true for the POC study, therefore the glucometers sensitivity for screening all pregnant women could not be analysed. Therefore, future studies should evaluate the glucometers performance as screening methods in all pregnant women.

The analysis of biomarkers utilising a single ethnic group was one of the strengths of the study. Ethnicity is a known risk factor for diabetes and related illnesses, hence it may be a confounding factor in a multi-ethnic cohort study. In addition, the study adjusted regression models for possible confounders. The study investigated an under-studied population with high risk for GDM due to the high prevalence of obesity, and thus the data produced is important for understanding the pathophysiology of GDM in such populations. The pregnant women used in the biomarker studies were recruited from multiple sites across urban Johannesburg and thus are more representative of the local population than would be the case if women were only recruited from a single site. Also, the study used targeted 'omics' to investigate a broad array of candidate biomarkers including both protein- and metabolite-based biomarkers. There are only a few studies of that nature, and none in sub-Saharan Africa.

Other strengths and limitations were discussed in each results chapter.

6.3.2 Future studies and recommendations

To increase GDM testing coverage the use of POC glucometers in the place of laboratory-based glucose measurement would be ideal. However, the current study found that the 7 glucometers tested were not suitable. Therefore, more glucose meters should be tested, and one would hope that current meters are improved by the manufacturers. In future studies should determine cut-off levels for GDM diagnosis specific to glucose measured in capillary blood. This study offers a thorough examination of the current GDM testing procedures used in SA, emphasising both successful strategies and ongoing difficulties. Despite its potential,

POC testing remains underutilised due to a lack of established guidelines and procedures for end users. Proper adoption would include healthcare worker training, regular maintenance, troubleshooting, and stringent quality control protocols which would necessitate a partnership with trained technicians. While government priorities in maternal and reproductive health coincide with SDG 3, the emphasis is on lowering morbidity and mortality like T2D and CVD, rather than addressing underlying risk factors [231]. As a result, more data is required to show how preventive methods like the use of POC for screening might enhance mother and child health outcomes.

The present study showed that the use of FPG in women at high risk for GDM would reduce the number of OGTTs that need to be performed. This study needs to be repeated and extended to all pregnant women as opposed to a high-risk population to determine if FPG can be used for both screening and diagnosis of GDM. Furthermore, this work provides a mirror for future research into what the GDM diagnosis approach might look like and how FPG could be used to reduce the amount of OGTTs performed routinely. Consequently, future studies may concentrate on that aspect. Caution is warranted when comparing findings across South African studies due to variability in pre-analytical procedures. For example, Dickson et al. [8,139] performed the study in local clinics without on-site laboratories, whereas our study sites were near laboratories. Thus, potentially complicating direct comparisons. In contrast, studies such as Adam and Rheeder [141] and Macaulay [9] had on-site laboratory support, aligning more closely with the current study's design. Notably, Macaulay reported that FPG alone identified 83.3% of GDM cases, consistent with the findings of the present study.

Cost-effectiveness studies which evaluated the use of FPG vs OGTT have not been performed. Future studies should evaluate the cost-effectiveness of using FPG as a screening and diagnostic test. In the current study we were able to show that more than half of the glucose tests would be reduced if the two-tiered approach for GDM diagnosis was used. The costs associated with OGTT apart from glucose analysis was not analysed, such as the cost of glucose solutions, blood collection tubes, etc.

The data on valine correlating with glycaemia in early pregnancy suggests that a longitudinal study should be undertaken to determine if BCAAs can be used to detect women early in pregnancy for future risk of GDM.

The association of BCAAs, As and FFAs with GDM points to associated metabolic pathways being dysfunctional in this disease. Therefore, future studies should use a broad panel of metabolites related to FFA and BCAA catabolism to determine where within these pathways the dysfunction lies.

The lack of glucose cut points for diagnosing GDM in early pregnancy was a drawback for the current study. More work should be undertaken to define such cut-points.

The prevalence of HIV is high in South African women of childbearing-age; in the present study it was just over 30%. There are contradictory views around the association of glucose metabolism with HIV. Thus, literature shows that glucose metabolism disorders such as GDM were more prevalent in HIV-positive pregnant women and in women using protease inhibitors as therapy [232,233], whilst a systematic review has found no relationship between being HIV positive and GDM [234]. In this study, we did not have information on the type of ART used by the HIV-positive women, therefore we could not explore the possible impact of ART therapy on GDM. In the current study, being HIV positive was not linked to GDM and there were significantly more HIV positive women who were non-GDM than those diagnosed with GDM.

6.3.3 Conclusion

The study found that the current risk factors for GDM cannot distinguish between women with and without GDM, with the exception of a history of GDM and glycosuria among the black African community. The glucometers evaluated in the current study are not effective for diagnosing GDM and the manufacturers of these instruments need to ensure that they are functional within this population. This study added to the existing literature supporting the use of FPG for GDM diagnosis by demonstrating its usage as a two-tiered strategy. This strategy would reduce the number of OGTTs required, which is particularly useful for places with limited resources. Furthermore, this investigation demonstrated that of the selected biomarkers used for GDM screening in early pregnancy only valine showed an association with glycaemia, whilst isoleucine and FFAs were higher and CAs were lower in GDM cases later in pregnancy. This suggests a disruption in the pathways related to FFA and BCAA metabolism in GDM.

REFERENCES

[1] International Association of Diabetes and Pregnancy Study Groups Consensus Panel, Metzger BE, Gabbe SG, Persson B, Buchanan TA, Catalano PA, et al. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care 2010;33:676–82. https://doi.org/10.2337/dc09-1848.

[2] Ferrari AJ, Santomauro DF, Aali A, Abate YH, Abbafati C, Abbastabar H, et al. Global incidence, prevalence, years lived with disability (YLDs), disability-adjusted life-years (DALYs), and healthy life expectancy (HALE) for 371 diseases and injuries in 204 countries and territories and 811 subnational locations, 1990–2021: a systematic analysis for the Global Burden of Disease Study 2021. The Lancet 2024;403:2133–61. https://doi.org/10.1016/S0140-6736(24)00757-8.

[3] IDF Diabetes Atlas 2025. Diabetes Atlas n.d. https://diabetesatlas.org/resources/idf-diabetes-atlas-2025/ (accessed September 26, 2025).

[4] Guariguata L, Linnenkamp U, Beagley J, Whiting DR, Cho NH. Global estimates of the prevalence of hyperglycaemia in pregnancy. Diabetes Res Clin Pract 2014;103:176–85. https://doi.org/10.1016/j.diabres.2013.11.003.

[5] McIntyre HD, Catalano P, Zhang C, Desoye G, Mathiesen ER, Damm P. Gestational diabetes mellitus. Nat Rev Dis Primer 2019;5:47. https://doi.org/10.1038/s41572-019-0098-8.

[6] Macaulay S, Dunger DB, Norris SA. Gestational Diabetes Mellitus in Africa: A Systematic Review. PLOS ONE 2014;9:e97871. https://doi.org/10.1371/journal.pone.0097871.

[7] Mwanri dickson, Kinabo J, Ramaiya K, Feskens EJM. Gestational diabetes mellitus in sub-Saharan Africa: systematic review and metaregression on prevalence and risk factors. Trop Med Int Health TM IH 2015;20:983–1002. https://doi.org/10.1111/tmi.12521.

[8] Dickson LM, Buchmann EJ, Janse van Rensburg C, Norris SA. Fasting plasma glucose and risk factor assessment: Comparing sensitivity and specificity in identifying gestational diabetes in urban black African women. South Afr Med J Suid-Afr Tydskr Vir Geneeskd 2019;110:21–6. https://doi.org/10.7196/SAMJ.2019.v110i1.14089.

[9] Macaulay S, Ngobeni M, Dunger DB, Norris SA. The prevalence of gestational diabetes mellitus amongst black South African women is a public health concern. Diabetes Res Clin Pract 2018;139:278–87. https://doi.org/10.1016/j.diabres.2018.03.012.

[10] Adam S, Rheeder P. Screening for gestational diabetes mellitus in a South African population: Prevalence, comparison of diagnostic criteria and the role of risk factors. South Afr Med J Suid-Afr Tydskr Vir Geneeskd 2017;107:523–7. https://doi.org/10.7196/SAMJ.2017.v107i6.12043.

[11] World Health Organization. Diagnostic criteria and classification of hyperglycaemia first detected in pregnancy. World Health Organization; 2013.

[12] American Diabetes Association. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes—2021. Diabetes Care 2020;44:S15–33. https://doi.org/10.2337/dc21-S002.

[13] Hod M, Kapur A, Sacks DA, Hadar E, Agarwal M, Di Renzo GC, et al. The International Federation of Gynecology and Obstetrics (FIGO) Initiative on gestational diabetes mellitus: A pragmatic guide for diagnosis, management, and care. Int J Gynaecol Obstet Off Organ Int Fed Gynaecol Obstet 2015;131 Suppl 3:S173-211. https://doi.org/10.1016/S0020-7292(15)30033-3.

[14] Ferrara A. Increasing Prevalence of Gestational Diabetes Mellitus: A public health perspective. Diabetes Care 2007;30:S141–6. https://doi.org/10.2337/dc07-s206.

[15] Popkin BM. The shift in stages of the nutrition transition in the developing world differs from past experiences! Public Health Nutr 2002;5:205–14. https://doi.org/10.1079/PHN2001295.

[16] Mthiyane DB, Wissink H, Chiwawa N. The impact of rural–urban migration in South Africa: A case of KwaDukuza municipality. J Local Gov Res Innov 2022;3:9. https://doi.org/10.4102/jolgri.v3i0.56.

[17] Popkin BM, Gordon-Larsen P. The nutrition transition: worldwide obesity dynamics and their determinants. Int J Obes Relat Metab Disord J Int Assoc Study Obes 2004;28 Suppl 3:S2-9. https://doi.org/10.1038/sj.ijo.0802804.
[18] Simelane T. National Food and Nutrition: South Africa. 2024.
[19] Ngubane MZ, Mndebele S, Kaseeram I. Economic growth, unemployment and poverty: Linear and non-linear evidence from South Africa. Heliyon 2023;9:e20267. https://doi.org/10.1016/j.heliyon.2023.e20267.
[20] Marmot M. The health gap: the challenge of an unequal world. The Lancet 2015;386:2442–4. https://doi.org/10.1016/S0140-6736(15)00150-6.
[21] Marmot M. Social determinants of health inequalities. Lancet Lond Engl 2005;365:1099–104. https://doi.org/10.1016/S0140-6736(05)71146-6.
[22] Nicolaou V, Levitt N, Huddle K, Norris SA. Perspectives on gestational diabetes mellitus in South Africa. S Afr Med J 2022;112:196–200.
[23] Maphumulo WT, Bhengu BR. Challenges of quality improvement in the healthcare of South Africa post-apartheid: A critical review. Curationis 2019;42:1901. https://doi.org/10.4102/curationis.v42i1.1901.
[24] Coetzee A, Hall DR, Conradie M. Hyperglycemia First Detected in Pregnancy in South Africa: Facts, Gaps, and Opportunities. Front Clin Diabetes Healthc 2022;3:895743. https://doi.org/10.3389/fcdhc.2022.895743.
[25] Kim C, Newton KM, Knopp RH. Gestational diabetes and the incidence of type 2 diabetes: a systematic review. Diabetes Care 2002;25:1862–8.
[26] Damm P. Future risk of diabetes in mother and child after gestational diabetes mellitus. Int J Gynecol Obstet 2009;104:S25–6. https://doi.org/10.1016/j.ijgo.2008.11.025.
[27] Group THSCR. Hyperglycemia and Adverse Pregnancy Outcomes. N Engl J Med 2008;358:1991–2002. https://doi.org/10.1056/NEJMoa0707943.
[28] Sweeting A, Wong J, Murphy HR, Ross GP. A Clinical Update on Gestational Diabetes Mellitus. Endocr Rev 2022;43:763–93. https://doi.org/10.1210/endrev/bnac003.
[29] Zhang C, Rawal S, Chong YS. Risk factors for gestational diabetes: is prevention possible? Diabetologia 2016;59:1385–90. https://doi.org/10.1007/s00125-016-3979-3.
[30] Baxley EG, Gobbo RW. Shoulder dystocia. Am Fam Physician 2004;69:1707–14.
[31] Weissgerber TL, Mudd LM. Preeclampsia and Diabetes. Curr Diab Rep 2015;15:579. https://doi.org/10.1007/s11892-015-0579-4.
[32] Yogev Y, Xenakis EMJ, Langer O. The association between preeclampsia and the severity of gestational diabetes: the impact of glycemic control. Am J Obstet Gynecol 2004;191:1655–60. https://doi.org/10.1016/j.ajog.2004.03.074.
[33] Getahun D, Fassett MJ, Jacobsen SJ. Gestational diabetes: risk of recurrence in subsequent pregnancies. Am J Obstet Gynecol 2010;203:467.e1-6. https://doi.org/10.1016/j.ajog.2010.05.032.
[34] England LJ, Dietz PM, Njoroge T, Callaghan WM, Bruce C, Buus RM, et al. Preventing type 2 diabetes: public health implications for women with a history of gestational diabetes mellitus. Am J Obstet Gynecol 2009;200:365.e1-8. https://doi.org/10.1016/j.ajog.2008.06.031.
[35] O'Sullivan JB. Body weight and subsequent diabetes mellitus. JAMA 1982;248:949–52.
[36] England LJ, Dietz PM, Njoroge T, Callaghan WM, Bruce C, Buus RM, et al. Preventing type 2 diabetes: public health implications for women with a history of gestational diabetes mellitus. Am J Obstet Gynecol 2009;200:365.e1-8. https://doi.org/10.1016/j.ajog.2008.06.031.
[37] Lauenborg J, Hansen T, Jensen DM, Vestergaard H, Mølsted-Pedersen L, Hornnes P, et al. Increasing incidence of diabetes after gestational diabetes: a long-term follow-up in a Danish population. Diabetes Care 2004;27:1194–9. https://doi.org/10.2337/diacare.27.5.1194.
[38] Catalano PM. Trying to understand gestational diabetes. Diabet Med J Br Diabet Assoc 2014;31:273–81. https://doi.org/10.1111/dme.12381.
[39] Marshall WJ, Lapsley M, Day A, Shipman K. Clinical Chemistry. Elsevier Health Sciences; 2020.

[40] Pei J, Wang B, Wang D. Current Studies on Molecular Mechanisms of Insulin Resistance. J Diabetes Res 2022;2022:1863429. https://doi.org/10.1155/2022/1863429.
[41] Pantham P, Aye ILMH, Powell TL. Inflammation in maternal obesity and gestational diabetes mellitus. Placenta 2015;36:709–15. https://doi.org/10.1016/j.placenta.2015.04.006.
[42] White MF, Shoelson SE, Keutmann H, Kahn CR. A cascade of tyrosine autophosphorylation in the beta-subunit activates the phosphotransferase of the insulin receptor. J Biol Chem 1988;263:2969–80.
[43] Kasuga M, Karlsson FA, Kahn CR. Insulin stimulates the phosphorylation of the 95,000-dalton subunit of its own receptor. Science 1982;215:185–7. https://doi.org/10.1126/science.7031900.
[44] Keshet Y, Seger R. The MAP kinase signaling cascades: a system of hundreds of components regulates a diverse array of physiological functions. Methods Mol Biol Clifton NJ 2010;661:3–38. https://doi.org/10.1007/978-1-60761-795-2_1.
[45] Abu Aqel Y, Alnesf A, Aigha II, Islam Z, Kolatkar PR, Teo A, et al. Glucokinase (GCK) in diabetes: from molecular mechanisms to disease pathogenesis. Cell Mol Biol Lett 2024;29:120. https://doi.org/10.1186/s11658-024-00640-3.
[46] Sonagra AD, Biradar SM, K. D, Murthy D.S. J. Normal Pregnancy- A State of Insulin Resistance. J Clin Diagn Res JCDR 2014;8:CC01–3. https://doi.org/10.7860/JCDR/2014/10068.5081.
[47] Newgard CB. Interplay between lipids and branched-chain amino acids in development of insulin resistance. Cell Metab 2012;15:606–14. https://doi.org/10.1016/j.cmet.2012.01.024.
[48] Edelstein RS, Wardecker BM, Chopik WJ, Moors AC, Shipman EL, Lin NJ. Prenatal hormones in first-time expectant parents: Longitudinal changes and within-couple correlations. Am J Hum Biol 2015;27:317–25. https://doi.org/10.1002/ajhb.22670.
[49] Kampmann U, Knorr S, Fuglsang J, Ovesen P. Determinants of Maternal Insulin Resistance during Pregnancy: An Updated Overview. J Diabetes Res 2019;2019:5320156. https://doi.org/10.1155/2019/5320156.
[50] Reynolds RM, Walker BR. Human insulin resistance: the role of glucocorticoids. Diabetes Obes Metab 2003;5:5–12. https://doi.org/10.1046/j.1463-1326.2003.00221.x.
[51] Berberoglu Z. Pathophysiology of Gestational Diabetes Mellitus. EMJ Diabet Diabetes 71 2019 2019;7:97–106. https://doi.org/10.33590/emjdiabet/10313616.
[52] Barbour LA, Shao J, Qiao L, Pulawa LK, Jensen DR, Bartke A, et al. Human placental growth hormone causes severe insulin resistance in transgenic mice. Am J Obstet Gynecol 2002;186:512–7. https://doi.org/10.1067/mob.2002.121256.
[53] RYAN EA, ENNS L. Role of Gestational Hormones in the Induction of Insulin Resistance*. J Clin Endocrinol Metab 1988;67:341–7. https://doi.org/10.1210/jcem-67-2-341.
[54] Metzger BE, Buchanan TA, Coustan DR, de Leiva A, Dunger DB, Hadden DR, et al. Summary and Recommendations of the Fifth International Workshop-Conference on Gestational Diabetes Mellitus. Diabetes Care 2007;30:S251–60. https://doi.org/10.2337/dc07-s225.
[55] Fuglsang J, Sandager P, Møller N, Fisker S, Orskov H, Ovesen P. Kinetics and secretion of placental growth hormone around parturition. Eur J Endocrinol 2006;154:449–57. https://doi.org/10.1530/eje.1.02109.
[56] Lønberg U, Damm P, Andersson A-M, Main KM, Chellakooty M, Lauenborg J, et al. Increase in maternal placental growth hormone during pregnancy and disappearance during parturition in normal and growth hormone-deficient pregnancies. Am J Obstet Gynecol 2003;188:247–51. https://doi.org/10.1067/mob.2003.82.
[57] F D, A K, A C, B D, Zc L, A A, et al. Pancreatic beta cells are important targets for the diabetogenic effects of glucocorticoids. J Clin Invest 1997;100. https://doi.org/10.1172/JCI119743.
[58] Delaunay F, Khan A, Cintra A, Davani B, Ling ZC, Andersson A, et al. Pancreatic beta cells are important targets for the diabetogenic effects of glucocorticoids. J Clin Invest 1997;100:2094–8. https://doi.org/10.1172/JCI119743.

[59] Mor G, Cardenas I, Abrahams V, Guller S. Inflammation and pregnancy: the role of the immune system at the implantation site. Ann N Y Acad Sci 2011;1221:80–7. https://doi.org/10.1111/j.1749-6632.2010.05938.x.
[60] Hauguel-de Mouzon S, Guerre-Millo M. The placenta cytokine network and inflammatory signals. Placenta 2006;27:794–8. https://doi.org/10.1016/j.placenta.2005.08.009.
[61] Esser N, Legrand-Poels S, Piette J, Scheen AJ, Paquot N. Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. Diabetes Res Clin Pract 2014;105:141–50. https://doi.org/10.1016/j.diabres.2014.04.006.
[62] Gregor MF, Hotamisligil GS. Inflammatory mechanisms in obesity. Annu Rev Immunol 2011;29:415–45. https://doi.org/10.1146/annurev-immunol-031210-101322.
[63] Tyagi S, Gupta P, Saini AS, Kaushal C, Sharma S. The peroxisome proliferator-activated receptor: A family of nuclear receptors role in various diseases. J Adv Pharm Technol Res 2011;2:236–40. https://doi.org/10.4103/2231-4040.90879.
[64] Zhang B, Berger J, Hu E, Szalkowski D, White-Carrington S, Spiegelman BM, et al. Negative regulation of peroxisome proliferator-activated receptor-gamma gene expression contributes to the antiadipogenic effects of tumor necrosis factor-alpha. Mol Endocrinol Baltim Md 1996;10:1457–66. https://doi.org/10.1210/mend.10.11.8923470.
[65] Kwok TC, Ojha S, Symonds ME. Chapter 50 - Obesity/Perinatal Origins of Obesity. In: Kovacs CS, Deal CL, editors. Matern.-Fetal Neonatal Endocrinol., Academic Press; 2020, p. 891–911. https://doi.org/10.1016/B978-0-12-814823-5.00051-9.
[66] Kim SY, England L, Wilson HG, Bish C, Satten GA, Dietz P. Percentage of gestational diabetes mellitus attributable to overweight and obesity. Am J Public Health 2010;100:1047–52. https://doi.org/10.2105/AJPH.2009.172890.
[67] Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-α in human obesity and insulin resistance. J Clin Invest 1995;95:2409–15. https://doi.org/10.1172/JCI117936.
[68] Kirwan JP, Hauguel-De Mouzon S, Lepercq J, Challier J-C, Huston-Presley L, Friedman JE, et al. TNF-alpha is a predictor of insulin resistance in human pregnancy. Diabetes 2002;51:2207–13. https://doi.org/10.2337/diabetes.51.7.2207.
[69] Xu J, Zhao YH, Chen YP, Yuan XL, Wang J, Zhu H, et al. Maternal circulating concentrations of tumor necrosis factor-alpha, leptin, and adiponectin in gestational diabetes mellitus: a systematic review and meta-analysis. ScientificWorldJournal 2014;2014:926932. https://doi.org/10.1155/2014/926932.
[70] Kuzmicki M, Telejko B, Szamatowicz J, Zonenberg A, Nikolajuk A, Kretowski A, et al. High resistin and interleukin-6 levels are associated with gestational diabetes mellitus. Gynecol Endocrinol Off J Int Soc Gynecol Endocrinol 2009;25:258–63. https://doi.org/10.1080/09513590802653825.
[71] Morisset A-S, Dubé M-C, Côté JA, Robitaille J, Weisnagel SJ, Tchernof A. Circulating interleukin-6 concentrations during and after gestational diabetes mellitus. Acta Obstet Gynecol Scand 2011;90:524–30. https://doi.org/10.1111/j.1600-0412.2011.01094.x.
[72] SIDDIQUI S, WAGHDHARE S, PANDA M, DUBEY S, JHA S. Association of IL-6 and CRP Levels with Gestational Diabetes Mellitus. Diabetes 2018;67:2417-PUB. https://doi.org/10.2337/db18-2417-PUB.
[73] Vozarova B, Weyer C, Hanson K, Tataranni PA, Bogardus C, Pratley RE. Circulating interleukin-6 in relation to adiposity, insulin action, and insulin secretion. Obes Res 2001;9:414–7. https://doi.org/10.1038/oby.2001.54.
[74] López-Tinoco C, Roca M, Fernández-Deudero A, García-Valero A, Bugatto F, Aguilar-Diosdado M, et al. Cytokine profile, metabolic syndrome and cardiovascular disease risk in women with late-onset gestational diabetes mellitus. Cytokine 2012;58:14–9. https://doi.org/10.1016/j.cyto.2011.12.004.

[75] Winkler G, Cseh K, Baranyi E, Melczer Z, Speer G, Hajós P, et al. Tumor necrosis factor system in insulin resistance in gestational diabetes. Diabetes Res Clin Pract 2002;56:93–9. https://doi.org/10.1016/s0168-8227(01)00355-2.
[76] Furman D, Campisi J, Verdin E, Carrera-Bastos P, Targ S, Franceschi C, et al. Chronic inflammation in the etiology of disease across the life span. Nat Med 2019;25:1822–32. https://doi.org/10.1038/s41591-019-0675-0.
[77] Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF, et al. A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. Cell Metab 2009;9:311–26. https://doi.org/10.1016/j.cmet.2009.02.002.
[78] Lackey DE, Lynch CJ, Olson KC, Mostaedi R, Ali M, Smith WH, et al. Regulation of adipose branched-chain amino acid catabolism enzyme expression and cross-adipose amino acid flux in human obesity. Am J Physiol Endocrinol Metab 2013;304:E1175-1187. https://doi.org/10.1152/ajpendo.00630.2012.
[79] Tremblay F, Marette A. Amino acid and insulin signaling via the mTOR/p70 S6 kinase pathway. A negative feedback mechanism leading to insulin resistance in skeletal muscle cells. J Biol Chem 2001;276:38052–60. https://doi.org/10.1074/jbc.M106703200.
[80] Khamzina L, Veilleux A, Bergeron S, Marette A. Increased activation of the mammalian target of rapamycin pathway in liver and skeletal muscle of obese rats: possible involvement in obesity-linked insulin resistance. Endocrinology 2005;146:1473–81. https://doi.org/10.1210/en.2004-0921.
[81] Tai ES, Tan MLS, Stevens RD, Low YL, Muehlbauer MJ, Goh DLM, et al. Insulin resistance is associated with a metabolic profile of altered protein metabolism in Chinese and Asian-Indian men. Diabetologia 2010;53:757–67. https://doi.org/10.1007/s00125-009-1637-8.
[82] Huffman KM, Shah SH, Stevens RD, Bain JR, Muehlbauer M, Slentz CA, et al. Relationships between circulating metabolic intermediates and insulin action in overweight to obese, inactive men and women. Diabetes Care 2009;32:1678–83. https://doi.org/10.2337/dc08-2075.
[83] Khambule L, George JA. The Role of Inflammation in the Development of GDM and the Use of Markers of Inflammation in GDM Screening. Adv Exp Med Biol 2019;1134:217–42. https://doi.org/10.1007/978-3-030-12668-1_12.
[84] She P, Van Horn C, Reid T, Hutson SM, Cooney RN, Lynch CJ. Obesity-related elevations in plasma leucine are associated with alterations in enzymes involved in branched chain amino acid (BCAA) metabolism. Am J Physiol Endocrinol Metab 2007;293:E1552–63. https://doi.org/10.1152/ajpendo.00134.2007.
[85] Herman MA, She P, Peroni OD, Lynch CJ, Kahn BB. Adipose Tissue Branched Chain Amino Acid (BCAA) Metabolism Modulates Circulating BCAA Levels. J Biol Chem 2010;285:11348–56. https://doi.org/10.1074/jbc.M109.075184.
[86] Koves TR, Li P, An J, Akimoto T, Slentz D, Ilkayeva O, et al. Peroxisome proliferator-activated receptor-gamma co-activator 1alpha-mediated metabolic remodeling of skeletal myocytes mimics exercise training and reverses lipid-induced mitochondrial inefficiency. J Biol Chem 2005;280:33588–98. https://doi.org/10.1074/jbc.M507621200.
[87] Muoio DM, Newgard CB. Molecular and metabolic mechanisms of insulin resistance and β-cell failure in type 2 diabetes. Nat Rev Mol Cell Biol 2008;9:193–205. https://doi.org/10.1038/nrm2327.
[88] Xiao Liang K. Interplay of mitochondria and diabetes: Unveiling novel therapeutic strategies. Mitochondrion 2024;75:101850. https://doi.org/10.1016/j.mito.2024.101850.
[89] Carl A. Burtis, David E. Bruns. Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics - E-Book : Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics - E-Book. vol. Seventh edition. St. Louis, Mo: Saunders; 2015.
[90] Knopp RH, Warth MR, Charles D, Childs M, Li JR, Mabuchi H, et al. Lipoprotein Metabolism in Pregnancy, Fat Transport to the Fetus, and the Effects of Diabetes. Biol Neonate 2009;50:297–317. https://doi.org/10.1159/000242614.

[91] Butte NF. Carbohydrate and lipid metabolism in pregnancy: normal compared with gestational diabetes mellitus. Am J Clin Nutr 2000;71:1256S-61S. https://doi.org/10.1093/ajcn/71.5.1256s.
[92] Boden G, Jadali F, White J, Liang Y, Mozzoli M, Chen X, et al. Effects of fat on insulin-stimulated carbohydrate metabolism in normal men. J Clin Invest 1991;88:960–6. https://doi.org/10.1172/JCI115399.
[93] Boden G, Chen X, Ruiz J, White JV, Rossetti L. Mechanisms of fatty acid-induced inhibition of glucose uptake. J Clin Invest 1994;93:2438–46. https://doi.org/10.1172/JCI117252.
[94] Roden M, Price TB, Perseghin G, Petersen KF, Rothman DL, Cline GW, et al. Mechanism of free fatty acid-induced insulin resistance in humans. J Clin Invest 1996;97:2859–65. https://doi.org/10.1172/JCI118742.
[95] Sivan E, Homko CJ, Whittaker PG, Reece EA, Chen X, Boden G. Free fatty acids and insulin resistance during pregnancy. J Clin Endocrinol Metab 1998;83:2338–42. https://doi.org/10.1210/jcem.83.7.4927.
[96] Schooneman MG, Vaz FM, Houten SM, Soeters MR. Acylcarnitines: reflecting or inflicting insulin resistance? Diabetes 2013;62:1–8. https://doi.org/10.2337/db12-0466.
[97] Newgard CB. Interplay between lipids and branched-chain amino acids in development of insulin resistance. Cell Metab 2012;15:606–14. https://doi.org/10.1016/j.cmet.2012.01.024.
[98] Schleh MW, Ryan BJ, Ahn C, Ludzki AC, Van Pelt DW, Pitchford LM, et al. Impaired suppression of fatty acid release by insulin is a strong predictor of reduced whole-body insulin-mediated glucose uptake and skeletal muscle insulin receptor activation. Acta Physiol 2025;241:e14249. https://doi.org/10.1111/apha.14249.
[99] Scholtens DM, Muehlbauer MJ, Daya NR, Stevens RD, Dyer AR, Lowe LP, et al. Metabolomics Reveals Broad-Scale Metabolic Perturbations in Hyperglycemic Mothers During Pregnancy. Diabetes Care 2014;37:158–66. https://doi.org/10.2337/dc13-0989.
[100] Enquobahrie DA, Denis M, Tadesse MG, Gelaye B, Ressom HW, Williams MA. Maternal Early Pregnancy Serum Metabolites and Risk of Gestational Diabetes Mellitus. J Clin Endocrinol Metab 2015;100:4348–56. https://doi.org/10.1210/jc.2015-2862.
[101] Ryckman KK, Spracklen CN, Smith CJ, Robinson JG, Saftlas AF. Maternal lipid levels during pregnancy and gestational diabetes: a systematic review and meta-analysis. BJOG Int J Obstet Gynaecol 2015;122:643–51. https://doi.org/10.1111/1471-0528.13261.
[102] Metzger BE, Phelps RL, Freinkel N, Navickas IA. Effects of gestational diabetes on diurnal profiles of plasma glucose, lipids, and individual amino acids. Diabetes Care 1980;3:402–9. https://doi.org/10.2337/diacare.3.3.402.
[103] de Seymour JV, Conlon CA, Sulek K, Villas Bôas SG, McCowan LME, Kenny LC, et al. Early pregnancy metabolite profiling discovers a potential biomarker for the subsequent development of gestational diabetes mellitus. Acta Diabetol 2014;51:887–90. https://doi.org/10.1007/s00592-014-0626-7.
[104] KIM K-S, KIM S-K, CHO Y-W, PARK SW. The Association between Free Fatty Acid Level at Midpregnancy and Postpartum Glucose Intolerance in Women with Gestational Diabetes Mellitus. Diabetes 2018;67:1419-P. https://doi.org/10.2337/db18-1419-P.
[105] Ellard S, Bellanné-Chantelot C, Hattersley AT, European Molecular Genetics Quality Network (EMQN) MODY group. Best practice guidelines for the molecular genetic diagnosis of maturity-onset diabetes of the young. Diabetologia 2008;51:546–53. https://doi.org/10.1007/s00125-008-0942-y.
[106] Petersen JS, Dyrberg T, Damm P, Kühl C, Mølsted-Pedersen L, Buschard K. GAD65 autoantibodies in women with gestational or insulin dependent diabetes mellitus diagnosed during pregnancy. Diabetologia 1996;39:1329–33. https://doi.org/10.1007/s001250050578.
[107] Füchtenbusch M, Ferber K, Standl E, Ziegler AG. Prediction of type 1 diabetes postpartum in patients with gestational diabetes mellitus by combined islet cell autoantibody screening: a prospective multicenter study. Diabetes 1997;46:1459–67. https://doi.org/10.2337/diab.46.9.1459.

[108] Damm P, Kühl C, Buschard K, Jakobsen BK, Svejgaard A, Sodoyez-Goffaux F, et al. Prevalence and predictive value of islet cell antibodies and insulin autoantibodies in women with gestational diabetes. Diabet Med J Br Diabet Assoc 1994;11:558–63. https://doi.org/10.1111/j.1464-5491.1994.tb02035.x.
[109] Jha S, Siddiqui S, Waghdhare S, Dubey S, Krishna S, Subramanian K, et al. Identification of a novel glucokinase mutation in an Indian woman with GCK-MODY. Lancet Diabetes Endocrinol 2016;4:302. https://doi.org/10.1016/S2213-8587(16)00040-1.
[110] Zhang C, Bao W, Rong Y, Yang H, Bowers K, Yeung E, et al. Genetic variants and the risk of gestational diabetes mellitus: a systematic review. Hum Reprod Update 2013;19:376–90. https://doi.org/10.1093/humupd/dmt013.
[111] Le Fur S, Le Stunff C, Bougnères P. Increased insulin resistance in obese children who have both 972 IRS-1 and 1057 IRS-2 polymorphisms. Diabetes 2002;51 Suppl 3:S304-307. https://doi.org/10.2337/diabetes.51.2007.s304.
[112] Bhatt SP, Guleria R. Association of IRS1 (Gly972Arg) and IRS2 (Gly1057Asp) genes polymorphisms with OSA and NAFLD in Asian Indians. PLOS ONE 2021;16:e0245408. https://doi.org/10.1371/journal.pone.0245408.
[113] Ayaz L, Karakaş Çelik S, Cayan F. The G1057D polymorphism of insulin receptor substrate-2 associated with gestational diabetes mellitus. Gynecol Endocrinol Off J Int Soc Gynecol Endocrinol 2014;30:165–8. https://doi.org/10.3109/09513590.2013.871516.
[114] Zeng Q, Tan B, Han F, Huang X, Huang J, Wei Y, et al. Association of solute carrier family 30 A8 zinc transporter gene variations with gestational diabetes mellitus risk in a Chinese population. Front Endocrinol 2023;14:1159714. https://doi.org/10.3389/fendo.2023.1159714.
[115] Steyn A, Crowther NJ, Norris SA, Rabionet R, Estivill X, Ramsay M. Epigenetic modification of the pentose phosphate pathway and the IGF-axis in women with gestational diabetes mellitus. Epigenomics 2019;11:1371–85. https://doi.org/10.2217/epi-2018-0206.
[116] Catalano PM, Tyzbir ED, Roman NM, Amini SB, Sims EA. Longitudinal changes in insulin release and insulin resistance in nonobese pregnant women. Am J Obstet Gynecol 1991;165:1667–72.
[117] Catalano PM, Tyzbir ED, Roman NM, Amini SB, Sims EAH. Longitudinal changes in insulin release and insulin resistance in nonobese pregnant women. Am J Obstet Gynecol 1991;165:1667–72. https://doi.org/10.1016/0002-9378(91)90012-G.
[118] Hiéronimus S, Le Meaux J-P. Relevance of gestational diabetes mellitus screening and comparison of selective with universal strategies. Diabetes Metab 2010;36:575–86. https://doi.org/10.1016/j.diabet.2010.11.010.
[119] Africa SS. sdg | Search Results | Statistics South Africa 2015. https://www.statssa.gov.za/?s=sdg&sitem=statistics%20by%20theme (accessed March 4, 2025).
[120] THE 17 GOALS | Sustainable Development n.d. https://sdgs.un.org/goals (accessed March 24, 2025).
[121] Lowe LP, Metzger BE, Dyer AR, Lowe J, McCance DR, Lappin TRJ, et al. Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study. Diabetes Care 2012;35:574–80. https://doi.org/10.2337/dc11-1687.
[122] Blumer I, Hadar E, Hadden DR, Jovanovič L, Mestman JH, Murad MH, et al. Diabetes and Pregnancy: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 2013;98:4227–49. https://doi.org/10.1210/jc.2013-2465.
[123] SEMDSA 2017 guidelines for management of Type 2 Diabetes Mellitus n.d.
[124] Vandorsten JP, Dodson WC, Espeland MA, Grobman WA, Guise JM, Mercer BM, et al. NIH consensus development conference: diagnosing gestational diabetes mellitus. NIH Consens State Sci Statements 2013;29:1–31.
[125] ACOG Practice Bulletin No. 190: Gestational Diabetes Mellitus. Obstet Gynecol 2018;131:e49. https://doi.org/10.1097/AOG.0000000000002501.

[126] Walker JD. NICE guidance on diabetes in pregnancy: management of diabetes and its complications from preconception to the postnatal period. NICE clinical guideline 63. London, March 2008. Diabet Med 2008;25:1025–7. https://doi.org/10.1111/j.1464-5491.2008.02532.x.
[127] Coustan DR, Dyer AR, Metzger BE. One-step or 2-step testing for gestational diabetes: which is better? Am J Obstet Gynecol 2021;225:634–44. https://doi.org/10.1016/j.ajog.2021.05.009.
[128] Simmons D, Moses RG. Gestational Diabetes Mellitus: To Screen or Not to Screen? Diabetes Care 2013;36:2877–8. https://doi.org/10.2337/dc13-0833.
[129] Chan AY, Cockram CS, Swaminathan R. Effect of delay in separating plasma for glucose measurement upon the interpretation of oral glucose tolerance tests. Ann Clin Biochem 1990;27 (Pt 1):73–4. https://doi.org/10.1177/000456329002700115.
[130] Mattock M, Phillips R, Keen H. Effect of Delay in Separating Plasma for Glucose Measurement upon the Interpretation of Oral Glucose Tolerance Tests. Ann Clin Biochem 1990;27:604–5. https://doi.org/10.1177/000456329002700618.
[131] Jones KL, Horowitz M, Wishart MJ, Maddox AF, Harding PE, Chatterton BE. Relationships between gastric emptying, intragastric meal distribution and blood glucose concentrations in diabetes mellitus. J Nucl Med Off Publ Soc Nucl Med 1995;36:2220–8.
[132] Reutrakul S, Zaidi N, Wroblewski K, Kay HH, Ismail M, Ehrmann DA, et al. Sleep Disturbances and Their Relationship to Glucose Tolerance in Pregnancy. Diabetes Care 2011;34:2454–7. https://doi.org/10.2337/dc11-0780.
[133] Pintaudi B, Di Vieste G, D'anna R, Chiereghin F, Biamonte E, Corrado F, et al. The Analytical Reliability of the Oral Glucose Tolerance Test for the Diagnosis of Gestational Diabetes: An Observational, Retrospective Study in a Caucasian Population. J Clin Med 2022;11. https://doi.org/10.3390/jcm11030564.
[134] Agarwal MM, Dhatt GS, Othman Y. Gestational diabetes mellitus prevalence: Effect of the laboratory analytical variation. Diabetes Res Clin Pract 2015;109:493–9. https://doi.org/10.1016/j.diabres.2015.06.001.
[135] Nwali SA, Onoh RC, Dimejesi IB, Obi VO, Jombo SE, Edenya OO. Universal versus selective screening for gestational diabetes mellitus among antenatal clinic attendees in Abakaliki: using the one-step 75 gram oral glucose tolerance test. BMC Pregnancy Childbirth 2021;21:735. https://doi.org/10.1186/s12884-021-04168-8.
[136] Abindu V, Hope D, Aleni M, Andru M, Ayiasi RM, Afayo V, et al. Missed Diagnosis of Gestational Diabetes Mellitus Due to Selective Screening: Evidence from a Cross-Sectional Study in the West Nile Sub-Region, Uganda. Diabetes Metab Syndr Obes 2024;17:1309–19. https://doi.org/10.2147/DMSO.S447855.
[137] Adam S, Rheeder P. Screening for gestational diabetes mellitus in a South African population: Prevalence, comparison of diagnostic criteria and the role of risk factors. S Afr Med J 2017;107:523–7. https://doi.org/10.7196/SAMJ.2017.v107i6.12043.
[138] Daly N, Carroll C, Flynn I, Harley R, Maguire PJ, Turner MJ. Evaluation of point-of-care maternal glucose measurements for the diagnosis of gestational diabetes mellitus. BJOG Int J Obstet Gynaecol 2017;124:1746–52. https://doi.org/10.1111/1471-0528.14255.
[139] Dickson LM, Buchmann EJ, Janse van Rensburg C, Norris SA. Accuracy of five plasma calibrated glucometers to screen for and diagnose gestational diabetes mellitus in a low resource clinic setting. J Clin Transl Endocrinol 2018;16:100174. https://doi.org/10.1016/j.jcte.2018.12.003.
[140] García-Claver A, Ramos-Corral R, Laviña-Fañanás C, Solans-Blecua I, Puzo-Foncillas J. Capillary glucose concentration during oral glucose tolerance test for the diagnosis of gestational diabetes. Int J Gynecol Obstet 2020;150:234–40. https://doi.org/10.1002/ijgo.13178.
[141] Adam S, Rheeder P. Evaluating the utility of a point-of-care glucometer for the diagnosis of gestational diabetes. Int J Gynaecol Obstet Off Organ Int Fed Gynaecol Obstet 2018;141:91–6. https://doi.org/10.1002/ijgo.12399.
[142] Anguizola J, Matsuda R, Barnaby OS, Hoy KS, Wa C, DeBolt E, et al. Review: Glycation of human serum albumin. Clin Chim Acta 2013;425:64–76. https://doi.org/10.1016/j.cca.2013.07.013.

[143] Kim KJ, Lee B-W. The Roles of Glycated Albumin as Intermediate Glycation Index and Pathogenic Protein. Diabetes Metab J 2012;36:98–107. https://doi.org/10.4093/dmj.2012.36.2.98.
[144] Rescalli A, Varoni EM, Cellesi F, Cerveri P. Analytical Challenges in Diabetes Management: Towards Glycated Albumin Point-of-Care Detection. Biosensors 2022;12:687. https://doi.org/10.3390/bios12090687.
[145] Sacks DB, Arnold M, Bakris GL, Bruns DE, Horvath AR, Kirkman MS, et al. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Clin Chem 2011;57:e1–47. https://doi.org/10.1373/clinchem.2010.161596.
[146] Gowda GN, Raftery D. Biomarker Discovery and Translation in Metabolomics. Curr Metabolomics 2013;1:227. https://doi.org/10.2174/2213235X113019990005.
[147] Dudzik D, Zorawski M, Skotnicki M, Zarzycki W, Kozlowska G, Bibik-Malinowska K, et al. Metabolic fingerprint of Gestational Diabetes Mellitus. J Proteomics 2014;103:57–71. https://doi.org/10.1016/j.jprot.2014.03.025.
[148] Chorell E, Hall UA, Gustavsson C, Berntorp K, Puhkala J, Luoto R, et al. Pregnancy to postpartum transition of serum metabolites in women with gestational diabetes. Metabolism 2017;72:27–36. https://doi.org/10.1016/j.metabol.2016.12.018.
[149] Bentley-Lewis R, Huynh J, Xiong G, Lee H, Wenger J, Clish C, et al. Metabolomic profiling in the prediction of gestational diabetes mellitus. Diabetologia 2015;58:1329–32. https://doi.org/10.1007/s00125-015-3553-4.
[150] Park S, Park JY, Lee JH, Kim S-H. Plasma levels of lysine, tyrosine, and valine during pregnancy are independent risk factors of insulin resistance and gestational diabetes. Metab Syndr Relat Disord 2015;13:64–70. https://doi.org/10.1089/met.2014.0113.
[151] Rahimi N, Razi F, Nasli-Esfahani E, Qorbani M, Shirzad N, Larijani B. Amino acid profiling in the gestational diabetes mellitus. J Diabetes Metab Disord 2017;16:13. https://doi.org/10.1186/s40200-016-0283-1.
[152] Cetin I, de Santis MSN, Taricco E, Radaelli T, Teng C, Ronzoni S, et al. Maternal and fetal amino acid concentrations in normal pregnancies and in pregnancies with gestational diabetes mellitus. Am J Obstet Gynecol 2005;192:610–7. https://doi.org/10.1016/j.ajog.2004.08.011.
[153] Mao X, Chen X, Chen C, Zhang H, Law KP. Metabolomics in gestational diabetes. Clin Chim Acta Int J Clin Chem 2017;475:116–27. https://doi.org/10.1016/j.cca.2017.10.019.
[154] Bujang MA, Adnan TH. Requirements for Minimum Sample Size for Sensitivity and Specificity Analysis. J Clin Diagn Res JCDR 2016;10:YE01–6. https://doi.org/10.7860/JCDR/2016/18129.8744.
[155] Chen X, Stein TP, Steer RA, Scholl TO. Individual free fatty acids have unique associations with inflammatory biomarkers, insulin resistance and insulin secretion in healthy and gestational diabetic pregnant women. BMJ Open Diabetes Res Care 2019;7:e000632. https://doi.org/10.1136/bmjdrc-2018-000632.
[156] Association AD. Gestational Diabetes Mellitus. Diabetes Care 2004;27:S88-90. https://doi.org/10.2337/diacare.27.2007.S88.
[157] Recommendations | Diabetes in pregnancy: management from preconception to the postnatal period | Guidance | NICE 2015. https://www.nice.org.uk/guidance/ng3/chapter/Recommendations#gestational-diabetes (accessed August 23, 2024).
[158] South African Doctors Hospitals Medical - State Hospitals - Charlotte Maxeke Johannesburg Academic Hospital - Parktown, Gauteng, South Africa n.d. https://doctors-hospitals-medical-cape-town-south-africa.blaauwberg.net/hospitals_clinics_state_hospitals/state_public_hospitals_clinics_gauteng_south_africa/charlotte_maxeke_johannesburg_academic_hospital_parktown_gauteng_south_africa (accessed January 4, 2023).

[159] The Chris Hani Baragwanath Hospital, South Africa | The World's 3rd Biggest Hospital, in South Africa - Contact Details (Address, Phone Numbers, Email Address) and Map n.d. https://www.chrishanibaragwanathhospital.co.za/ (accessed January 4, 2023).
[160] Rahima Moosa Mother and Child Hospital - Wits University n.d. https://www.wits.ac.za/clinicalmed/departments/paediatrics-and-child-health/hospital-services/rahima-moosa-mother-and-child-hospital/ (accessed April 23, 2024).
[161] Rispel L. Rahima Moosa: South Africa's only mother and child hospital is falling apart - a veteran doctor reflects on why. The Conversation 2023. http://theconversation.com/rahima-moosa-south-africas-only-mother-and-child-hospital-is-falling-apart-a-veteran-doctor-reflects-on-why-202977 (accessed April 23, 2024).
[162] Teboho. Hillbrow Health Precinct - Johannesburg Development Agency 2018. https://www.jda.org.za/hillbrow-health-precinct/ (accessed April 23, 2024).
[163] Harrison A, Colvin CJ, Kuo C, Swartz A, Lurie M. Sustained High HIV Incidence in Young Women in Southern Africa: Social, Behavioral, and Structural Factors and Emerging Intervention Approaches. Curr HIV/AIDS Rep 2015;12:207–15. https://doi.org/10.1007/s11904-015-0261-0.
[164] Kouzuma T, Uemastu Y, Usami T, Imamura S. Study of glycated amino acid elimination reaction for an improved enzymatic glycated albumin measurement method. Clin Chim Acta Int J Clin Chem 2004;346:135–43. https://doi.org/10.1016/j.cccn.2004.02.019.
[165] Wang H, Li N, Chivese T, Werfalli M, Sun H, Yuen L, et al. IDF Diabetes Atlas: Estimation of Global and Regional Gestational Diabetes Mellitus Prevalence for 2021 by International Association of Diabetes in Pregnancy Study Group's Criteria. Diabetes Res Clin Pract 2022;183:109050. https://doi.org/10.1016/j.diabres.2021.109050.
[166] Ogurtsova K, da Rocha Fernandes JD, Huang Y, Linnenkamp U, Guariguata L, Cho NH, et al. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. Diabetes Res Clin Pract 2017;128:40–50. https://doi.org/10.1016/j.diabres.2017.03.024.
[167] Hinneh T, Jahn A, Agbozo F. Barriers to screening, diagnosis and management of hyperglycaemia in pregnancy in Africa: a systematic review. Int Health 2022;14:211–21. https://doi.org/10.1093/inthealth/ihab054.
[168] Macleod K, Katz LB, Cameron H. Capillary and Venous Blood Glucose Accuracy in Blood Glucose Meters Versus Reference Standards: The Impact of Study Design on Accuracy Evaluations. J Diabetes Sci Technol 2019;13:546–52. https://doi.org/10.1177/1932296818790228.
[169] Voglová Hagerf B, Protus M, Nemetova L, Mraz M, Kieslichova E, Uchytilova E, et al. Accuracy and Feasibility of Real-time Continuous Glucose Monitoring in Critically Ill Patients After Abdominal Surgery and Solid Organ Transplantation. Diabetes Care 2024;47:956–63. https://doi.org/10.2337/dc23-1663.
[170] Abu-Arafeh A, Jordan H, Drummond G. Reporting of method comparison studies: a review of advice, an assessment of current practice, and specific suggestions for future reports. Br J Anaesth 2016;117:569–75. https://doi.org/10.1093/bja/aew320.
[171] EFLM Biological Variation n.d. https://biologicalvariation.eu/ (accessed December 7, 2023).
[172] Al-Hasani W, Ranasinghe R, Rogers H, Spanier W, Spears K, Gayle C, et al. Clinical utility of point of care glucose in the assessment of gestational diabetes: Prospective cohort study. n.d.
[173] O'Malley EG, Reynolds CME, O'Kelly R, Killalea A, Sheehan SR, Turner MJ. A Prospective Evaluation of Point-of-Care Measurements of Maternal Glucose for the Diagnosis of Gestational Diabetes Mellitus. Clin Chem 2020;66:316–23. https://doi.org/10.1093/clinchem/hvz005.
[174] Gallardo-Rincón H, Lomelin-Gascon J, Martinez-Juarez LA, Montoya A, Ortega-Montiel J, Galicia-Hernandez V, et al. Diagnostic Accuracy of Capillary Blood Glucometer Testing for Gestational Diabetes. Diabetes Metab Syndr Obes Targets Ther 2022;15:3855–70. https://doi.org/10.2147/DMSO.S389420.

[175] Lv H, Zhang G, Kang X, Yuan H, Lv Y, Wang W, et al. Factors Interfering With the Accuracy of Five Blood Glucose Meters Used in Chinese Hospitals. J Clin Lab Anal 2013;27:354–66. https://doi.org/10.1002/jcla.21611.
[176] EP15 A3 User Verification of Precision & Bias Estimation. Clin Lab Stand Inst n.d. https://clsi.org/standards/products/method-evaluation/documents/ep15/ (accessed July 19, 2024).
[177] VivaChek Ino Blood Glucose Meter. Practical and easy to use, with test strips on NHS prescription. VivaChek Blood Glucose Meter n.d. https://www.vivachekdiabetes.co.uk/vivachek (accessed August 11, 2024).
[178] SD BIOSENSOR | PRODUCTS n.d. https://www.sdbiosensor.com/product/product_view?product_no=246 (accessed August 11, 2024).
[179] O'Sullivan JB, Mahan CM, Charles D, Dandrow RV. Screening criteria for high-risk gestational diabetic patients. Am J Obstet Gynecol 1973;116:895–900. https://doi.org/10.1016/S0002-9378(16)33833-9.
[180] Dahanayaka NJ, Agampodi SB, Ranasinghe OR, Jayaweera PM, Wickramasinghe WA, Adhikari AN, et al. Inadequacy of the risk factor based approach to detect gestational diabetes mellitus. Ceylon Med J 2012;57:5–9. https://doi.org/10.4038/cmj.v57i1.4193.
[181] Simmons D, Devers MC, Wolmarans L, Johnson E. Difficulties in the Use of Risk Factors to Screen for Gestational Diabetes Mellitus. Diabetes Care 2009;32:e8–e8. https://doi.org/10.2337/dc08-1313.
[182] Huang Y, Hu Y, Ma YU, Ye G. Glycated albumin is an optimal biomarker for gestational diabetes mellitus. Exp Ther Med 2015;10:2145–9. https://doi.org/10.3892/etm.2015.2808.
[183] Hashimoto K, Koga M. Indicators of glycemic control in patients with gestational diabetes mellitus and pregnant women with diabetes mellitus. World J Diabetes 2015;6:1045. https://doi.org/10.4239/wjd.v6.i8.1045.
[184] Gan T, Liu X, Xu G. Glycated Albumin Versus HbA1c in the Evaluation of Glycemic Control in Patients With Diabetes and CKD. Kidney Int Rep 2018;3:542–54. https://doi.org/10.1016/j.ekir.2017.11.009.
[185] Freedman BI, Shenoy RN, Planer JA, Clay KD, Shihabi ZK, Burkart JM, et al. Comparison of Glycated Albumin and Hemoglobin A1c Concentrations in Diabetic Subjects on Peritoneal and Hemodialysis. Perit Dial Int 2010;30:72–9. https://doi.org/10.3747/pdi.2008.00243.
[186] Sugawara D, Sato H, Ichihashi K, Nagai K, Kawano A. Glycated albumin level during late pregnancy as a predictive factor for neonatal outcomes of women with diabetes. J Matern-Fetal Neonatal Med Off J Eur Assoc Perinat Med Fed Asia Ocean Perinat Soc Int Soc Perinat Obstet 2018;31:2007–12. https://doi.org/10.1080/14767058.2017.1333103.
[187] International Expert Committee Report on the Role of the A1C Assay in the Diagnosis of Diabetes. Diabetes Care 2009;32:1327–34. https://doi.org/10.2337/dc09-9033.
[188] HAPO Study Cooperative Research Group, Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, et al. Hyperglycemia and adverse pregnancy outcomes. N Engl J Med 2008;358:1991–2002. https://doi.org/10.1056/NEJMoa0707943.
[189] Youden WJ. Index for rating diagnostic tests. Cancer 1950;3:32–5. https://doi.org/10.1002/1097-0142(1950)3:1%253C32::aid-cncr2820030106%253E3.0.co;2-3.
[190] Sacks DA, Hadden DR, Maresh M, Deerochanawong C, Dyer AR, Metzger BE, et al. Frequency of gestational diabetes mellitus at collaborating centers based on IADPSG consensus panel-recommended criteria: the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study. Diabetes Care 2012;35:526–8. https://doi.org/10.2337/dc11-1641.
[191] Agarwal MM, Weigl B, Hod M. Gestational diabetes screening: The low-cost algorithm. Int J Gynecol Obstet 2011;115:S30–3. https://doi.org/10.1016/S0020-7292(11)60009-X.
[192] Deitch JM, Lee I-L, Yates CJ, Kathpal E, Lawton P, Shahid I, et al. Simplified gestational diabetes screening with a triaging fasting plasma glucose reduces the burden of oral glucose tolerance

tests during pregnancy – A large tertiary comparative cohort study. Diabetes Res Clin Pract 2024;209:111120. https://doi.org/10.1016/j.diabres.2024.111120.
[193] Grunnet LG, Hjort L, Minja DT, Msemo OA, Møller SL, Prasad RB, et al. High Prevalence of Gestational Diabetes Mellitus in Rural Tanzania—Diagnosis Mainly Based on Fasting Blood Glucose from Oral Glucose Tolerance Test. Int J Environ Res Public Health 2020;17:3109. https://doi.org/10.3390/ijerph17093109.
[194] Ryser Rüetschi J, Jornayvaz F, Rivest R, Huhn E, Irion O, Boulvain M. Fasting glycaemia to simplify screening for gestational diabetes. BJOG Int J Obstet Gynaecol 2016;123:2219–22. https://doi.org/10.1111/1471-0528.13857.
[195] Chan ES, Chadha R, Koning L de. Maternal Hemoglobin A1c in the Third-Trimester May Underestimate Maternal Hyperglycemia and Its Impact on Offspring in Perinatal Demise Associated With Gestational Diabetes Mellitus: An Autopsy Case Series. Pediatr Dev Pathol Off J Soc Pediatr Pathol Paediatr Pathol Soc 2023;26:472–81. https://doi.org/10.1177/10935266231194697.
[196] Ho Y-R, Wang P, Lu M-C, Tseng S-T, Yang C-P, Yan Y-H. Associations of mid-pregnancy HbA1c with gestational diabetes and risk of adverse pregnancy outcomes in high-risk Taiwanese women. PLoS ONE 2017;12:e0177563. https://doi.org/10.1371/journal.pone.0177563.
[197] Negrea M-C, Oriot P, Courcelles A, Gruson D, Alexopoulou O. Performance of glycated hemoglobin A1c for the diagnosis of gestational diabetes mellitus during the SARS-CoV-2 pandemic in Belgium (2020-2021). Eur J Obstet Gynecol Reprod Biol 2023;289:36–41. https://doi.org/10.1016/j.ejogrb.2023.08.368.
[198] Chume FC, Renz PB, Hernandez MK, Freitas PAC, Camargo JL. Is there a role for glycated albumin in the diagnosis of gestational diabetes mellitus? Endocrine 2021;72:681–7. https://doi.org/10.1007/s12020-021-02673-6.
[199] Zhu J, Chen Y, Li C, Tao M, Teng Y. The diagnostic value of glycated albumin in gestational diabetes mellitus. J Endocrinol Invest 2018;41:121–8. https://doi.org/10.1007/s40618-016-0605-7.
[200] Jiang R, Wu S, Fang C, Wang C, Yang Y, Liu C, et al. Amino acids levels in early pregnancy predict subsequent gestational diabetes. J Diabetes 2020;12:503–11. https://doi.org/10.1111/1753-0407.13018.
[201] Furse S, White SL, Meek CL, Jenkins B, Petry CJ, Vieira MC, et al. Altered triglyceride and phospholipid metabolism predates the diagnosis of gestational diabetes in obese pregnancy. Mol Omics 2019;15:420–30. https://doi.org/10.1039/C9MO00117D.
[202] Roy C, Tremblay P-Y, Anassour-Laouan-Sidi E, Lucas M, Forest J-C, Giguère Y, et al. Risk of gestational diabetes mellitus in relation to plasma concentrations of amino acids and acylcarnitines: A nested case-control study. Diabetes Res Clin Pract 2018;140:183–90. https://doi.org/10.1016/j.diabres.2018.03.058.
[203] Koos BJ, Gornbein JA. Early pregnancy metabolites predict gestational diabetes mellitus: implications for fetal programming. Am J Obstet Gynecol 2021;224:215.e1-215.e7. https://doi.org/10.1016/j.ajog.2020.07.050.
[204] Lin J, Zhao D, Liang Y, Liang Z, Wang M, Tang X, et al. Proteomic analysis of plasma total exosomes and placenta-derived exosomes in patients with gestational diabetes mellitus in the first and second trimesters. BMC Pregnancy Childbirth 2024;24:713. https://doi.org/10.1186/s12884-024-06919-9.
[205] Korkmazer E, Solak N. Correlation between inflammatory markers and insulin resistance in pregnancy. J Obstet Gynaecol J Inst Obstet Gynaecol 2015;35:142–5. https://doi.org/10.3109/01443615.2014.948408.
[206] Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E, et al. Metabolite profiles and the risk of developing diabetes. Nat Med 2011;17:448–53. https://doi.org/10.1038/nm.2307.
[207] Tillin T, Hughes AD, Wang Q, Würtz P, Ala-Korpela M, Sattar N, et al. Diabetes risk and amino acid profiles: cross-sectional and prospective analyses of ethnicity, amino acids and diabetes in

a South Asian and European cohort from the SABRE (Southall And Brent REvisited) Study. Diabetologia 2015;58:968–79. https://doi.org/10.1007/s00125-015-3517-8.
[208] Batch BC, Shah SH, Newgard CB, Turer CB, Haynes C, Bain JR, et al. Branched chain amino acids are novel biomarkers for discrimination of metabolic wellness. Metabolism 2013;62:961–9. https://doi.org/10.1016/j.metabol.2013.01.007.
[209] Ruiz-Canela M, Toledo E, Clish CB, Hruby A, Liang L, Salas-Salvadó J, et al. Plasma branched-chain amino acids and incident cardiovascular disease in the PREDIMED trial. Clin Chem 2016;62:582–92. https://doi.org/10.1373/clinchem.2015.251710.
[210] Park S, Park JY, Lee JH, Kim S-H. Plasma levels of lysine, tyrosine, and valine during pregnancy are independent risk factors of insulin resistance and gestational diabetes. Metab Syndr Relat Disord 2015;13:64–70. https://doi.org/10.1089/met.2014.0113.
[211] Sandler V, Reisetter AC, Bain JR, Muehlbauer MJ, Nodzenski M, Stevens RD, et al. Associations of maternal BMI and insulin resistance with the maternal metabolome and newborn outcomes. Diabetologia 2017;60:518–30. https://doi.org/10.1007/s00125-016-4182-2.
[212] Pinto J, Almeida LM, Martins AS, Duarte D, Barros AS, Galhano E, et al. Prediction of Gestational Diabetes through NMR Metabolomics of Maternal Blood. J Proteome Res 2015;14:2696–706. https://doi.org/10.1021/acs.jproteome.5b00260.
[213] Heath H, Rosario R, McMichael LE, Fanter R, Alarcon N, Quintana-Diaz A, et al. Gestational Diabetes Is Characterized by Decreased Medium-Chain Acylcarnitines and Elevated Purine Degradation Metabolites across Pregnancy: A Case–Control Time-Course Analysis. J Proteome Res 2023;22:1603–13. https://doi.org/10.1021/acs.jproteome.2c00430.
[214] Dudzik D, Zorawski M, Skotnicki M, Zarzycki W, Kozlowska G, Bibik-Malinowska K, et al. Metabolic fingerprint of Gestational Diabetes Mellitus. J Proteomics 2014;103:57–71. https://doi.org/10.1016/j.jprot.2014.03.025.
[215] Macaulay S, Munthali RJ, Dunger DB, Norris SA. The effects of gestational diabetes mellitus on fetal growth and neonatal birth measures in an African cohort. Diabet Med J Br Diabet Assoc 2018;35:1425–33. https://doi.org/10.1111/dme.13668.
[216] FDA: Guidance for industry: bioanalytical method... - Google Scholar n.d. https://scholar.google.com/scholar_lookup?title=Guidance%20for%20Industry%3A%20Bioanalytical%20Method%20Validation&author=FDA&publication_year=2013 (accessed January 9, 2025).
[217] Sivan E, Boden G. Free fatty acids, insulin resistance, and pregnancy. Curr Diab Rep 2003;3:319–22. https://doi.org/10.1007/s11892-003-0024-y.
[218] Ravichandran LV, Esposito DL, Chen J, Quon MJ. Protein kinase C-zeta phosphorylates insulin receptor substrate-1 and impairs its ability to activate phosphatidylinositol 3-kinase in response to insulin. J Biol Chem 2001;276:3543–9. https://doi.org/10.1074/jbc.M007231200.
[219] Lin Y, Wu J, Zhu Y, Hinkle SN, Rawal S, Liang L, et al. A longitudinal study of plasma acylcarnitines throughout pregnancy and associations with risk of gestational diabetes mellitus. Clin Nutr Edinb Scotl 2021;40:4863–70. https://doi.org/10.1016/j.clnu.2021.07.008.
[220] Chen Q, Francis E, Hu G, Chen L. Metabolomic profiling of women with gestational diabetes mellitus and their offspring: Review of metabolomics studies. J Diabetes Complications 2018;32:512–23. https://doi.org/10.1016/j.jdiacomp.2018.01.007.
[221] Wang Q-Y, You L-H, Xiang L-L, Zhu Y-T, Zeng Y. Current progress in metabolomics of gestational diabetes mellitus. World J Diabetes 2021;12:1164–86. https://doi.org/10.4239/wjd.v12.i8.1164.
[222] Ramzan I, Ardavani A, Vanweert F, Mellett A, Atherton PJ, Idris I. The Association between Circulating Branched Chain Amino Acids and the Temporal Risk of Developing Type 2 Diabetes Mellitus: A Systematic Review & Meta-Analysis. Nutrients 2022;14:4411. https://doi.org/10.3390/nu14204411.
[223] Chevalier S, Marliss EB, Morais JA, Lamarche M, Gougeon R. Whole-body protein anabolic response is resistant to the action of insulin in obese women. Am J Clin Nutr 2005;82:355–65. https://doi.org/10.1093/ajcn.82.2.355.

[224] Africa SS. Living condition | Statistics South Africa 2025. https://www.statssa.gov.za/?page_id=739 (accessed March 4, 2025).
[225] Weile LKK, Kahn JG, Marseille E, Jensen DM, Damm P, Lohse N. Global cost-effectiveness of GDM screening and management: current knowledge and future needs. Best Pract Res Clin Obstet Gynaecol 2015;29:206–24. https://doi.org/10.1016/j.bpobgyn.2014.06.009.
[226] Coetzee A, van de Vyver M, Hoffmann M, Hall DR, Mason D, Conradie M. A comparison between point-of-care testing and venous glucose determination for the diagnosis of diabetes mellitus 6-12 weeks after gestational diabetes. Diabet Med J Br Diabet Assoc 2019;36:591–9. https://doi.org/10.1111/dme.13903.
[227] Coetzee A, Sadhai N, Mason D, Hall DR, Conradie M. Evidence to support the classification of hyperglycemia first detected in pregnancy to predict diabetes 6-12 weeks postpartum: A single center cohort study. Diabetes Res Clin Pract 2020;169:108421. https://doi.org/10.1016/j.diabres.2020.108421.
[228] Daly N, Flynn I, Carroll C, Farren M, McKeating A, Turner MJ. Impact of Implementing Preanalytical Laboratory Standards on the Diagnosis of Gestational Diabetes Mellitus: A Prospective Observational Study. Clin Chem 2016;62:387–91. https://doi.org/10.1373/clinchem.2015.247478.
[229] Sacks DB, Arnold M, Bakris GL, Bruns DE, Horvath AR, Lernmark Å, et al. Guidelines and Recommendations for Laboratory Analysis in the Diagnosis and Management of Diabetes Mellitus. Diabetes Care 2023;46:e151–99. https://doi.org/10.2337/dci23-0036.
[230] Catalano PM, Nizielski SE, Shao J, Preston L, Qiao L, Friedman JE. Downregulated IRS-1 and PPARgamma in obese women with gestational diabetes: relationship to FFA during pregnancy. Am J Physiol Endocrinol Metab 2002;282:E522-533. https://doi.org/10.1152/ajpendo.00124.2001.
[231] Veeraswamy S, Vijayam B, Gupta VK, Kapur A. Gestational diabetes: The public health relevance and approach. Diabetes Res Clin Pract 2012;97:350–8. https://doi.org/10.1016/j.diabres.2012.04.024.
[232] Pinnetti C, Floridia M, Cingolani A, Visconti E, Cavaliere AF, Celentano and Lucia Pastore, et al. Effect of HCV Infection on Glucose Metabolism in Pregnant Women with HIV Receiving HAART. HIV Clin Trials 2009;10:403–12. https://doi.org/10.1310/hct1006-403.
[233] Biadgo B, Ambachew S, Abebe M, Melku M. Gestational diabetes mellitus in HIV-infected pregnant women: A systematic review and meta-analysis. Diabetes Res Clin Pract 2019;155:107800. https://doi.org/10.1016/j.diabres.2019.107800.
[234] Soepnel LM, Norris SA, Schrier VJMM, Browne JL, Rijken MJ, Gray G, et al. The association between HIV, antiretroviral therapy, and gestational diabetes mellitus. AIDS Lond Engl 2017;31:113–25. https://doi.org/10.1097/QAD.0000000000001277.

APPENDIX A: PLAGIARISM DECLARATION

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I Lungile Khambule (Student number: 458524) am a student registered for the degree of Doctor of Philosophy in the academic year 2025.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature:  Date: 29 September 2025

APPENDIX B: CO-AUTHOR DECLARATION

Declaration: Student's contribution to article(s) and agreement of co-author(s)

I, Lungile Khambule, student number 458524, declare that this Thesis is my own work and that I contributed adequately towards research findings published in the article(s) stated below which are included in my Thesis.

Signature of Student ..... **Date** 20 March 2025

Name of Primary Supervisor: Jaya George

Signature of Primary Supervisor ..... **Date** 20 March 2025

Agreement by co-authors: By signing this declaration, the co-authors listed below agree to the use of the article(s) by the student as part of his/her Thesis/Dissertation/Research Report. In cases where the student is not the 1st author of a published article, the primary supervisor must explain (under comments) why the student is entitled to use the paper for his/her degree purposes.

Article 1: Title: Performance of POC glucose testing for the diagnosis of gestational diabetes in South Africa.

Journal name, year, volume and page numbers: *Int J Gynecol Obstet*, 2024;00:1-10

Authors	Name	Signature	Date
1st author	Chemedzai Chikomba		27-Mar-2025
2nd author	Yasmin Adam		26 March 2025
3rd author	Lubayna Khan		21st March 2025
4th author	Cathy Haldane	Cathy Haldane	26 March 2025
5th author	Beatrice Vetter		27-Mar-2025
6th author	Jaya George	JGeorge	27th March 2025

Comments by primary supervisor:

.....

.....

Article 2: Title: Fasting plasma glucose performs better than glycated haemoglobin and glycated albumin for diagnosing gestational diabetes.

Journal name, year, volume and page numbers: *Int J Gynecol Obstet*, submitted and waiting for response

Authors	Name	Signature	Date
1st author	Nigel Crowther		27th March 2025
2nd author	Lawrence Chauke		27th March 2025
3rd author	Jaya George	JGeorge	27th March 2025

Comments by primary supervisor:

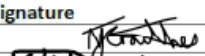
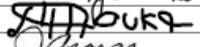
.....

.....

Article 3: Title: The use of novel biomarkers for the diagnosis of gestational diabetes mellitus in black African women.....

Journal name, year, volume and page numbers: Written in the format of the journal BJOG

1

Authors	Name	Signature	Date
1st author	Nigel Crowther		27th March 2025
2nd author	Lawrence Chauke		27th March 2025
3rd author	Jaya George		27th March 2025

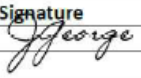
Comments by primary supervisor:

.....

.....

Article 4: Title: The Role of Inflammation in the Development of GDM and the Use of Markers of Inflammation in GDM Screening

Journal name, year, volume and page numbers: *Adv Exp Med Biol,* 1134:217-242

Authors	Name	Signature	Date
1st author	Jaya George		27th March 2025
2nd author			
3rd author			

Comments by primary supervisor:

.....

.....

APPENDIX C: QUESTIONNAIRE

Study no:

GDM STUDY QUESTIONNAIRE

All questions contained in this questionnaire are strictly confidential

PERSONAL DETAILS

Education:	☐ Did not matriculate ☐ Matriculated ☐ Tertiary education	
Marital status:	☐ Single ☐ Partnered ☐ Married ☐ Separated ☐ Divorced ☐ Widowed	
Employment status:	☐ Unemployed	Last employment (year):
	☐ Student	
	☐ Employed	

Health History

☐ Hypertension ☐ Diabetes ☐ TB ☐ HIV? ☐Polycystic ovarian syndrome

Do you drink alcohol? ☐Daily ☐Occasionally ☐Never ☐Stopped

Do you smoke snuff/cigarettes/cigars? ☐Daily ☐Occasionally ☐Never ☐Stopped

Family History

Diabetes ☐Mother ☐Father☐ Siblings
Hypertension ☐Mother ☐Father ☐Siblings

ANTHROPOMETRIC DATA

Weight (kg):	
Height (m):	
BMI (kg/m^2):	
Systolic BP (mmHg):	
Diastolic BP (mmHg):	
blood glucose:	

Any comments:

APPENDIX D: INFORMATION SHEET AND CONSENT FORM

Information sheet for women in early pregnancy

Markers for gestational diabetes in Black Africans

Department of Chemical Pathology, Faculty of Health Science, University of Witwatersrand

Dear expecting mother,

As you have been informed in our poster, we are doing a study on gestational diabetes mellitus (GDM). This is a disease where the blood sugar levels are higher than normal during pregnancy. These blood sugar levels during pregnancy can be bad for both mother and child. The infant is at risk of having low blood sugar, difficulty breathing and, difficult birth and high birthweight which may require a caesarian delivery. The mother is also at risk of developing diabetes (sugar disease) later on in life.

In this study we want to set up a test that will be able to find women who are at risk of having GDM early in pregnancy and based on this study stop the disease and linked complications from developing.

If you are 20 weeks pregnant or less, we are inviting you to take part in our study. During your clinic visit, we will ask you to donate five teaspoons of your blood (25mL) that will be collected by a qualified nurse. We will use the blood to measure things that may help us to see if you have GDM. This is not a painful procedure, only slight discomfort from the needle and finger prick should be anticipated. The nurse will take your body weight, height, blood pressure, blood glucose measurements. We will also ask you to answer a short questionnaire on personal details, health history and family health history. Thereafter, light refreshments will be served.

The potential benefits of taking part in our study are that your blood sugar levels will be measured and if they are high we will refer you to a clinician for a check up. Most importantly, all volunteers that participate in this study will give us information that will be used to help us find women who at risk of having GDM and thus make sure that all women who are at risk will have a chance to stop the disease from happening.

All blood results and personal information will be kept confidential. You will receive a study number and only the private investigator and supervisors will have access to the file that has both your name and the study number. Blood sample results will be given to you on your request after the study.

Participation in this study is voluntary and you are free to refuse to participate or withdraw your consent and discontinue participation at any time. Such refusal or discontinuance will not affect you or your baby regular treatments or medical care in any way. A signed copy of this consent form will be made available to you

Markers for gestational diabetes in Black Africans

Department of Chemical Pathology, Faculty of Health Science, University of Witwatersrand

Dear expecting mother,

As you have been informed in our poster we are doing a study on gestational diabetes mellitus (GDM), a disease where the blood sugar levels are higher than normal during pregnancy. The high blood sugar levels during pregnancy can be detrimental for both mother and child. The infant is at risk of having low blood sugar, high birthweight, difficulty breathing and birth trauma. The mother is at risk of developing diabetes later on in life.

The methods we use for finding women who may have GDM are not very good. Therefore, in this study we are trying to find a blood test that will improve these methods. To find such a blood test we need to test blood taken from women who we know have GDM, and from women who we know do not have GDM. We are therefore asking you if you want to take part in this study because we know that you have been tested for GDM.

You can contribute to the study by giving us three teaspoons of blood (15mL) that will be collected by a qualified nurse. The nurse will also take your body weight, height, blood pressure, blood glucose measurements. We will also ask you to answer a short questionnaire on personal details, health history and family health history. Thereafter, a light breakfast will be served.

This is not a painful procedure, only slight discomfort from the needle and finger prick should be anticipated.

Most importantly, all volunteers that participate in this study will give us information that will be used to help us more easily diagnose GDM and make sure that all women with this disease have a healthy pregnancy and a healthy baby.

Efforts will be made to keep personal information confidential. Absolute confidentiality cannot be guaranteed. Personal information may be disclosed if required by law.

Participation in this study is voluntary and you are free to refuse to participate or withdraw your consent and discontinue participation at any time. Such refusal or discontinuance will not affect you or your baby regular treatments or medical care in any way. A signed copy of this consent form will be made available to you

Consent form for women in early pregnancy

I have been fully informed as to the procedures to be followed, the purpose, possible risk and benefits of the study.

In signing this consent form, I agree to participate in this study and undergo the above mentioned procedures.

I also understand that I can ask any questions about the study at any time.

Participant signature: Date:

Principal Investigator: Lungile Khambule, Room 3Q07, Department of Chemical Pathology, Wits Medical School, Tel: 011-489-8831, Cell: 0610039983

Supervisor: Prof. Jaya George Tel: 011-489-8499

APPENDIX E: ETHIC CERTIFICATES

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG	HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research and Innovation)

TO: Ms L Khambule
School of Pathology
Department of Chemical Pathology
Charlotte Maxeke Johannesburg Academic Hospital

E-mail: Lungile.Khambule@wits.ac.za

CC: Supervisor: Professor J George and Ms C Chikomba
<Jaya.George@wits.ac.za>
and <HREC-Medical Research Office@wits.ac.za>

FROM: Mr Iain Burns
Human Research Ethics Committee (Medical)
Tel: 011 717 1252

E-mail: Iain.Burns@wits.ac.za

DATE: 2022/05/19

REF: R14/49

PROTOCOL NO: **M220219** (This is your ethics application reference number. Please quote it in all enquiries, oral or written, relating to this study.)

PROJECT TITLE: *A comparison between whole blood point of care testing and plasma glucose determination for the diagnosis of gestational diabetes mellitus*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to Government funding of the University.

MSWorks2000/Iain0007/Clearscan.wps

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research and Innovation)

TO:	Ms L Khambule School of Pathology Department of Chemical Pathology Medical School University E-mail: Lungile.Khambule@wits.ac.za
CC:	Supervisor: Professors NJ Crowther and J George <Nigel.Crowther@nhls.ac.za> and <HREC-Medical Research Office@wits.ac.za>
FROM:	Mr Iain Burns Human Research Ethics Committee (Medical) Tel: 011 717 1252 E-mail: Iain.Burns@wits.ac.za
DATE:	2023/07/05
REF:	R14/49
PROTOCOL NO:	**M230675** (This is your ethics application reference number. Please quote it in all enquiries, oral or written, relating to this study.)
PROJECT TITLE:	*The use of novel biomarkers to improve screening for gestational diabetes in a Black Africal population*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to Government funding of the University.



MSWorks2000/Iain0007/Clearscan.wps

Preprint

Chapter 14

The role of inflammation in the development of GDM and the use of markers of inflammation in GDM screening

Lungile Khambule*, Jaya Anna George
Department of Chemical Pathology, National Health Laboratory Services and Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

***Correspondence** lungile.khambule@wits.ac.za

Running title Markers of inflammation as biomarkers of GDM

Abstract

Gestational diabetes mellitus is a hyperglycaemic state first recognised in pregnancy. GDM affects both mother and child. Women with GDM and their new-borns are at risk of developing type 2 diabetes in the future. The screening and diagnostic criteria for GDM are inconsistent and thus novel biomarkers of GDM are required to strengthen the screening and diagnostic processes in GDM. Chronic low-grade inflammation is linked to the majority of the well-established risk factors of GDM such as old age, obesity and PCOS.This review provides and overview of the present knowledge on the pathology of GDM, the screening criteria applied, the role of inflammation in the development of GDM and the use of markers of inflammation namely cytokines, oxidative stress markers, lipids, amino acids and iron markers in screening and diagnosis of GDM.

Key words GDM, inflammation, biomarkers, cytokines, pregnancy, metabolites

1 Introduction

Gestational diabetes mellitus is defined as high blood glucose first discovered in pregnancy [1]. The lack of consistency in the testing protocols and diagnostic criteria affects prevalence rates. GDM is said to be steadily increasing with the rising prevalence of obesity and type 2 diabetes (T2D). The global prevalence ranges between 1-14% with higher rates reported from Asian, Latin American and Middle Eastern populations [2, 3]. A meta-analysis from India noted prevalence estimates ranging from 0-41.9%, with International Association of the Diabetes and Pregnancy Study Groups (IADPSG) criteria picking up significantly more GDM (19.19% [CI: 15.5, 23.6]) compared to the World Health Association (WHO) criteria (10.13% [CI: 8.17, 12.50]) [3]. They also noted a higher prevalence in urban compared to rural areas. Data from Mexico showed that the prevalence of GDM was higher in the north in keeping with a higher prevalence of obesity in the north [4]. Little is known about GDM in Africa. A recent meta-analysis noted that the prevalence varied from 0% in Tanzania to as high as 13.9% in Nigeria [5]. There has been an increase in the reported prevalence of diabetes in Africa, which is expected to affect 41.5 million people by 2035, because of lifestyle changes [6].

Short term complications of GDM include infants who are large for their gestational age, preeclampsia, polyhydramnios, stillbirth, hypoglycaemia and hyperbilirubinemia, and respiratory distress syndrome [7]. The long term complications are: neonates born from mothers with GDM have increased risk of being overweight in childhood with impaired pancreatic beta-cell function as an adult, which may lead to T2D [8, 9]. Women with GDM are at increased risk of developing T2D, with high cardiovascular risk and early atherosclerosis after pregnancy [10]. A recent meta-analysis showed that treating GDM results in less preeclampsia, shoulder dystocia, and macrosomia but has no effect on neonatal hypoglycaemia or future poor metabolic outcomes [11].

The determination of risk factors for GDM is complicated by inconsistencies in diagnostic factors referred to above as well as by inconsistencies in measurement of risk factors across studies. Despite these issues, a number of risk factors have emerged consistently including advanced maternal age, overweight/obesity, excessive gestational weight gain, ethnicity, genetic polymorphisms, low or high birthweight, family or past history of GDM, and other insulin resistant states, such as polycystic ovarian syndrome (PCOS) [12-14]. Ethnicity and obesity are two strong independent risk factors for GDM [15]. In a cohort of 123,040 women without recognized pre-gravid diabetes, Asian and Filipino women had an increased risk of

GDM at a lower Body Mass Index (BMI) cut point, particularly as compared with non-Hispanic white and African American women [16]. These risk factors are either directly or indirectly associated with impaired β-cell function and insulin sensitivity.

Considering that obesity is a major risk factor for GDM, it is possible that underlying biological functions that are affected by high body fat mass could be detected in patients who are at risk of GDM. Inflammation, particularly chronic low-grade inflammation is associated with obesity [17]. Although normal pregnancy is also associated with inflammation, mostly due to innate immune response to the foetal allograft [18], women with GDM have been shown to have even higher inflammatory markers when compared to women with a normal pregnancy [19]. Therefore, this review provides an overview of the present knowledge on the pathology of GDM, the screening criteria applied, the role of inflammation in the development of GDM and the use of markers of inflammation such as cytokines, lipids, amino acids, oxidative stress markers and iron markers in screening and diagnosis of GDM. The review will only cover markers that are directly or indirectly linked to inflammation, upregulated or downregulated in GDM patients and linked to the features of GDM such as obesity and insulin resistance. Inflammatory markers, oxidative stress markers, lipids and amino acids that can be detected in blood will be discussed as these are more feasible and applicable for screening and diagnosing GDM.

2 Pathophysiology of GDM

As pregnancy advances the demand for insulin increases and, in most cases, the pregnant women can meet the demand. Placental secretion of diabetogenic hormones, such as growth hormone, corticotropin releasing hormone, placental lactogen, and progesterone, leads to increasing insulin resistance. The inability to overcome the insulin resistance of pregnancy despite β-cell hyperplasia leads to GDM.

β-cell dysfunction occurs when the β-cells lose the ability to adequately sense blood glucose levels or to release sufficient insulin in response to a glucose load. β-cell dysfunction is thought to result from prolonged excessive insulin production in response to a chronic fuel load. Reduced insulin-stimulated glucose uptake is damaging to β-cells, leading to a vicious cycle of hyperglycaemia, increased insulin resistance and worsening β-cell dysfunction [20].

The cause of the insulin resistance on GDM is multifactorial and arises because of hormones released from the fetal-placental unit and maternal fat accretion, excess lipolysis and release of

free fatty acids (FFA) and of inflammatory mediators and adipokines [21]. The ongoing low-grade inflammation in adipose tissue impairs insulin signalling, which further stimulates production of proteins implicated in insulin resistance. Adipocytes synthesize substances with chemotactic and adhesive properties such as macrophage chemotactic protein-1 (MCP-1) and vascular and intracellular adhesion molecules (VCAM and ICAM), which enhance influx of lymphocytes and monocytes. These then interact with adipocytes to initiate a perpetuating cycle of macrophage recruitment, production of inflammatory cytokines and impairment of adipocyte function, with adverse effects including insulin resistance and endothelial dysfunction [21]. This is summarized in **Fig. 1**.

The placenta contributes to insulin resistance during pregnancy via its secretion of hormones and cytokines as well as via the transport of glucose, amino acids, and lipids across the placenta. Glucose transport occurs via GLUT1, by carrier-mediated sodium-independent diffusion and maternal hyperglycaemia contributes to foetal macrosomia [22]. In addition to the effects that are seen in the fetus because of transport of nutrients across the placenta, recent studies have reported that GDM is associated with placental DNA hypermethylation as well as release of miRNAs [23, 24].

3 Screening and diagnosis of GDM

While the evidence shows that treatment of GDM is of benefit to both mother and child [11], there is a lack of consensus in the approach to the screening and diagnosis for GDM. The initial criteria for the diagnosis of GDM were chosen to identify women at high risk of developing diabetes after pregnancy [25]. Data that has emerged since then has shown that the risk of adverse perinatal outcomes is associated in a continuous and graded fashion with maternal hyperglycaemia [1]. It is because of this that the International Association of Diabetes Study Groups (IADPSG) recommends universal or selective screening for high risk women to detect overt diabetes mellitus at the first antenatal visit. They recommend universal screening at 24-28 weeks using 75g of glucose in a 2-hour oral glucose tolerance test (OGTT). On the other hand the NICE guidelines recommend either self-monitoring of blood glucose or a 75g OGTT as soon as possible after booking for women who have had GDM in previous pregnancies and risk factor based screening at 24-28 weeks for others [26]. There is no agreement about which factors best define GDM risk. For example the NICE guidelines classify a family history of diabetes as a risk factor while the Australian Diabetes in Pregnancy Society (ADIPS) does not [15, 26]. Several groups recognize increased BMI as a risk factor but the definition of an

increased BMI varies with NICE defining it as BMI ≥ 30kg/m^2 and ADIPS defining it as ≥ 25kg/m^2 [9, 10].

Another area of controversy is with the diagnostic criteria. Some of these are summarized in **Table 1**.

4 The role of inflammation in the development of GDM

Inflammation is a complex mechanism that occurs in response to the invasion of foreign matter or in the case of injury. This process is characterized by redness, heat, swelling, pain and loss of function in the affected area which soon disappears after the infected area has been cleared. However, in a case of chronic inflammation, which is characterized by persistent secretion of cytokines and chemokines, it is more likely to cause more damage. The role of inflammation in the development of GDM is described below and the use of inflammatory markers, lipids, amino acids, oxidative stress markers and iron markers in the screening and diagnosis of GDM are discussed.

4.1 Cytokines

Cytokines are signalling proteins that bind onto their receptors which are present in various cells and they induce inflammation through the activation of the of the c-JUN N-terminal kinase (JNK) and nuclear factor-kappa B (NF-κB) pathways. The JNK activation signals for phosphorylation of the serine residues in the insulin receptor substrate-1 (IRS-1) which prevents insulin signalling that would normally occur through the tyrosine kinase cascade [27]. Like, the JNK pathway, NF-κB activation has been implicated in various chronic inflammatory diseases such as rheumatoid disease, atherosclerosis, asthma and chronic obstructive pulmonary disease [28]. The activation of the NF-κB by cytokines and chemokines leads to the production of pro-inflammatory cytokines and recruits leukocytes and monocytes to the site of injury or infection [28]. It is important to note that cytokines could function in both ways as they could collaborate to propagate inflammation or work antagonistically depending on the type of cytokine. In most cases, the anti-inflammatory cytokines inhibit the pro-inflammatory cytokines and vice versa.

Obesity, a known risk factor for GDM, is a chronic and heterogenous disease disproportionately affecting women, with up to 30% of women in general and over half of the pregnant women in

some populations considered overweight or obese [29]. Adipose tissue which was originally believed to be a passive depot of energy is now considered an endocrine organ that secretes adipokines incuding adiponectin and leptin as well as numerous cytokines (TNF-α, IL-6 and IL-1) which have wide ranging metabolic effects [30, 31]. In addition to adipocytes, macrophages from adipose tissue also secrete pro-inflammatory adipokines [32]. There is a positive loop between pro-inflammatory cytokines, macrophages and monocytes in the presence of enlarged adipocytes (**Fig. 2**). Adipocytes are not the only source of cytokine secretion in pregnancy. The placenta contributes to inflammation and insulin resistance by also secreting pro-inflammatory cytokines, as mentioned above.

Adipokines contribute to insulin resistance in obese individuals and may be implicated in the insulin resistance seen in GDM, directly through regulation of insulin secretion and indirectly through inflammatory mediators and their effects on glucose metabolism [33].

The proportion of studies aimed at investigating the role of cytokines in GDM are far less than those involved in investigations of T2D and cardiovascular diseases (CVS) even though GDM shares similar characteristics with these diseases. The sections below discuss various cytokines including adipokines which are in keeping with the criteria mentioned in the introduction above.

4.1.1 Adiponectin

Adiponectin is an anti-inflammatory protein and is capable of amplifying insulin secretion by signalling for the expression of insulin gene and exocytosis of insulin granules [34]. It has been shown to induce insulin secretion and fatty acid oxidation in part by 5' adenosine monophosphate-activated protein kinase (AMPK) mediation. AMPK is an enzyme that plays a role in muscle fatty acid oxidation and insulin sensitivity [35-37]. The AMPK activity is reduced by the recruitment of pro-inflammatory cytokines and also feeds into the dysfunctional lipid metabolism and inflammatory pathways seen in individuals with elevated adipose tissue. Adiponectin is also known to supress pro-inflammatory cytokine production, seen in mice and in humans [38, 39]. Pro-inflammatory cytokines like TNF-α indirectly terminate the activation of AMPK to reduce fatty acid oxidation and insulin sensitivity [40] (**Fig. 3**). In GDM studies, adiponectin has been investigated more than all other adipokines [34]. The majority of these studies, including prospective ones, have led to agreement that adiponectin is inversely related to GDM [30, 34, 41-49] (**Table 2**). In the few studies that reported no differences in adiponectin levels between women with GDM and those without [50-52], the sample size was low and the

lack of adjustment for co-factors such as BMI could have affected the results. López-Tinoco et al showed that in their population, low adiponectin levels were seen in obese women who later developed GDM related to pregravid BMI [30]. In accordance with López-Tinocoet al, other studies showed an association of obesity with low adiponectin levels in non-pregnant individuals [53, 54]. In contrast, low adiponectin levels independent of pregravid BMI were observed in GDM cases in women with normal glucose tolerance [49]. However, gestational weight gain was not discussed despite its role in low adiponectin levels. Park et al found that women with GDM gained more weight in pregnancy and had low adiponectin levels when compared to women who were not diagnosed with GDM [43]. These findings suggest that body weight gain does not favour secretion of adiponectin and therefore tilts the balance between pro-inflammatory and anti-inflammatory cytokines towards the pro-inflammatory side.

4.1.2 Tumor-necrosis factor-α (TNF-α)

Tumor-necrosis factor-α (TNF-α) is a pro-inflammatory cytokine that is predominantly activated by macrophages and T lymphocytes. TNF-α can be expressed in placenta, adipose tissue, immune cells such as mast cells, B lymphocytes, natural killer (NK) cells, neutrophils and endothelial cells [55]. The binding of TNF-α onto either TNF-receptor type I (TNF-RI) or -receptor type II (TNF-RII) mainly activates the NF-κB pathways which leads to a further pro-inflammatory cytokine and immune cell secretion [56] (**Fig. 3**). Apart from inducing inflammation, TNF-α reduces insulin sensitivity by disrupting the translocation of glucose transporter GLUT-4 and insulin signal transduction [57, 58]. The expression of TNF-α in placenta is thought to contribute to the insulin resistance associated with pregnancy. However, in normal pregnancy, β-cells can compensate for the low insulin sensitivity by producing and secreting more insulin. TNF-α expression may come from different sources and, when combined, contribute to the development of GDM as seen in **Fig. 3**. For example, in addition to TNF-α expression in the placenta, it is also activated by the increase in adipose tissue, hyperglycaemia and chronic inflammation.

The majority of the studies have shown that TNF-α levels are elevated in women with GDM compared to healthy pregnant women [59-64] (**Table 2**). However, other studies did not show a significant difference in TNF-α levels between GDM cases and controls [65, 66]. There are only a few prospective studies on TNF-α and out of four prospective studies included in the current review, one study showed a non-significant difference in TNF-α levels between GDM cases and healthy controls [66]. Furthermore, a systematic review and meta-analysis on

cytokines showed that TNF-α levels are higher in GDM cases than in controls [67]. Multivariate linear regression analysis reported that pregravid BMI was the most predictive indicator of TNF-α levels in women with GDM [49]. Similarly, Winkler et al suggested that the elevated TNF-α levels seen in women with GDM is strongly linked to the increase in body fat mass [64]. Moreover, TNF-α correlated inversely to insulin sensitivity and correlated with c-peptide concentrations in a BMI matched population [49, 68]. These studies suggest that there is a correlation of body weight with TNF-α in pregnancy, although there are other factors in play such as insulin resistance that contribute to the development of GDM.

4.1.3 Interleukin-6

Interleukin-6 (IL-6) operates similarly to TNF-α to induce inflammatory responses in non-pregnant individuals with obesity and /or T2D [19, 69]. Many of the studies performed in GDM are cross sectional but the majority of the studies have indicated a positive correlation of IL6 with GDM [70-73] (**Table 2**). Serum IL-6 levels were significantly high in GDM females as compared controls and IL-6 levels correlated with pregravid BMI, fasting blood sugar (FBS) levels and postprandial sugar levels [70]. Similarly, women with GDM had significantly higher IL-6 and resistin levels compared to pregnant women with normal glucose tolerance [71]. In Pima Indians, a population with high rates of obesity and T2D, IL-6 levels correlated positively with adiposity and negatively with insulin action in women with GDM [72]. Furthermore, Morisset et al suggested that IL-6 is a strong biomarker for GDM independent of BMI in women with GDM [73]. This is consistent with the possibility that adipose tissue plays a role in the development of GDM by inducing inflammatory responses and activating pro-inflammatory cytokines. In contrast, IL6 showed no significant difference between women with late-onset GDM and controls although TNF-α, leptin and adiponectin levels were significantly different between the groups [30]. A recent study showed that high molecular weight and fasting glucose are more robust risk factors for GDM compared to adiponectin, omentin-1 and IL-6 when using the IADPSG criteria [74]. It is possible that the inconsistency in results of the studies mentioned above is caused by the differences in methodology, sample size and adjusted variables.

4.1.4 Adipocyte fatty acid-binding protein

Adipocyte fatty acid-binding protein (AFABP) functions as a pro-inflammatory cytokine and is highly expressed in macrophages and endothelial cells [75]. Previous studies of non-pregnant individuals have shown a link between the AFABP, obesity and associated complications such as metabolic syndrome [76-78]. In GDM, AFABP is a promising marker as it has been seen to

be elevated in GDM cases compared to non-GDM women independent of their insulin sensitivity state [76, 79] (**Table 2**). Furthermore, this binding protein has been linked to adiposity markers in fetuses of women with GDM [79] which may connect GDM with macrosomia. One prospective study performed in an Indian population indicated that elevated AFABP levels in the first trimester of pregnancy may be useful in identifying women who are at risk of GDM [80]. Further studies, particularly, prospective ones are still required to investigate the significance of AFABP in GDM.

4.1.5 Cytokine/adipokines with contradictory results

There are other promising cytokines that are either not extensively studied or have shown contradictory results in GDM. These cytokines have been implicated in inflammation in animal models and in non-pregnant individuals. Therefore, these could play a role in the development of GDM. **Table 2** summarizes the relevant studies performed in GDM.

Leptin is a cytokine active in adipocytes but also found in various body organs such as the brain, placenta, gastro-intestinal tract (GIT), skeletal muscle and immune cells [81]. The binding of leptin to leptin receptors induces the Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway which mainly regulates energy homeostasis, including insulin sensitivity regulation and appetite [82-85]. Leptin can pass through the blood brain barrier (BBB) and acts on the hypothalamus to promote satiety and regulate energy expenditure [82]. Apart from its actions in the central nervous system, leptin can act on peripheral organs such as adipose tissue, muscles, pancreas and liver to improve insulin sensitivity, reduce lipolysis, reduce amino acid absorption in intestines, increase glucose uptake in muscles and reduce gluconeogenesis in the liver [82, 86]. This effect appears to be attenuated in obesity [87]. An abnormal increase in leptin is observed in obese individuals and is indicative of leptin resistance that may be caused in part by a negative feedback loop between leptin and suppressor of cytokine signalling (SOCS)-3 transcription factor which in turn inhibits the action of leptin [88, 89].

During acute infection, leptin activates dendritic cells, monocytes, macrophages, neutrophils and natural killer cells [90, 91]. Moreover, leptin stimulates the production of other pro-inflammatory cytokines namely, IL-6 and TNF-α [90]. In the state of prolonged activation, leptin can contribute to the manifestation of chronic inflammation as seen in obesity and T2D. However, the function of leptin in pregnancy is not clearly understood. A systematic review in pregnant women has indicated that first and second trimester leptin levels are higher in women

who later develop GDM compared to those who do not, although there has been significant study heterogeneity [30, 41, 62, 67, 92-94]. Other studies have indicated that leptin is not significantly different among women with GDM and those without [42, 50, 52, 95]. Similar to studies performed in non-pregnant individuals, leptin increases with the increase in body weight in pregnancy [30, 93].

Retinol binding protein 4 (RBP-4) is a member of the lipocalin family of proteins and is predominantly synthesized in the liver and partly in adipocytes [96]. As mentioned above, this pro-inflammatory cytokine is suggested to play a role in the development of GDM. However, more studies, particularly prospective ones, are required to gain more conclusive information. In multi-marker diagnostic models, a model that consisted of BMI, RBP-4, n-acetylaspartic acid and C16:1 (cis-7) could identify women with GDM better than other models including those using other cytokines and lipids [97]. RBP-4 correlated with fetal birth weight of neonates born from women with GDM [98]. The plasma RBP-4 concentration was significantly higher in women with GDM and correlated positively with transthyretin, fasting plasma glucose, insulin and triglyceride concentrations, as well as blood pressure, abdominal fat area, and homeostasis model assessment of insulin resistance (HOMA-IR) [99]. In one prospective study there was evidence of a positive association of early pregnancy elevated RBP-4 concentrations with increased GDM risk, particularly among women with advanced age and obesity [100]. In contrast, Krzyzanowska and associates reported low levels of RBP-4 in women with GDM and RBP-4 was not significantly associated with glucose or HOMA-IR [101]. Although RBP-4 levels were higher in women with GDM than in controls, there was no significant correlation between RBP-4 and age, BMI, insulin and HOMA-IR [102, 103]. A prospective study indicated that RBP-4 was not significantly higher altered in pathological pregnancies including GDM [104].

Resistin is a hormone that belongs to a family of proteins generically known as resistin-like molecules (RELM), which are characterized by the consistent presence of a segment rich in cysteine at the C-terminal end [105]. This hormone contributes to inflammation by inducing the production of monocytes and macrophages and correlates with other pro-inflammatory markers such as TNF-α and IL-6 [75, 105]. Despite its association with adipose hypertrophy [19, 106, 107], previous studies have shown that resistin is not associated with insulin resistance and its indices [71, 106]. Furthermore, other studies reviewed in a meta-analysis have shown that resistin levels are not significantly different between women with GDM and women without

GDM [108]. Four prospective studies have indicated that resistin levels cannot predict women who are at risk of GDM [46, 104, 109, 110].

Visfatin is a cytokine which is mostly expressed in visceral adipose tissue (VAT) and is thought to improve insulin sensitivity by mimicking insulin [19]. However, this effect could be explained by its ability to up-regulate cytokine secretion in monocytes [111]. Despite its conflicting effects, visfatin appears to be elevated in women with GDM. In case control studies, visfatin levels were found to be higher in women with GDM despite non-significant correlation with insulin, BMI, glucose [112, 113]. However, Park et al reported low visfatin levels in GDM cases when compared to controls [43].

4.2 Lipids and their metabolites

Fatty acids are the building blocks of lipids and are stored in adipose tissue. Increase in adipose tissue means that the storage of free fatty acids and lipids is limited and thus lipids leak from adipose tissue and into the bloodstream which ultimately elevates circulatory free fatty acids, cholesterol, lipoproteins and phospholipids. Normal pregnancy is associated with an increase in free fatty acids in the first and second trimester which normalizes in the third trimester of pregnancy [114]. This could be a result of hyperphagia and lipid synthesis associated with normal pregnancy in order to ensure sufficient energy for the growing foetus. In general, postprandial insulin secretion aims to supress lipolysis to sustain energy levels and the reverse occurs during fasting state [115, 116]. Low insulin sensitivity associated with GDM indicates that the suppressing role of insulin in the postprandial state is compromised and therefore lipolysis is not regulated which further explains the increase in free fatty acids in women with GDM compared to controls. Furthermore, elevated free fatty acids are connected to fetal complications such as macrosomia because of elevated fatty acid transportation to the fetus in GDM cases [117].

Elevated circulatory free fatty acids have been shown to induce the innate immune response by binding to toll-like receptors (TLRs). TLR activation induces the secretion of pro-inflammatory cytokines [118]. In addition, free fatty acids have been shown to play a role in β-cell inflammation by inducing IL-1β, IL-6, and IL-8 secretion in human islets and IL-1β secretion in mouse islets via the IL-1 receptor [119]. (**Fig. 3**).

A case control study reported that pregravid BMI is a significant contributor to the increase in serum fatty acids at 28 weeks of pregnancy and serum fatty acids were higher in GDM cases when compared to controls [120]. In addition, serum triglycerides and pregravid BMI significantly correlated with fetal birth weight [117]. The Hyperglycaemia and Adverse Pregnancy Outcome (HAPO) study showed that pregnant women with high fasting plasma glucose had higher triglyceride levels and lower insulin sensitivity in the third trimester of pregnancy [121]. Similarly, free fatty acids were associated with insulin resistance in early pregnancy [122]. Furthermore, women with GDM and high pregravid BMI had altered mRNA expression levels of genes involved in fatty acid uptake and metabolism [123]. A systematic review of 60 studies indicated that triglyceride levels were significantly elevated in women with GDM compared with those without GDM and HDL-C levels were significantly lower in women with GDM compared with those without GDM in the second and third trimester [124]. In summary, lipids and free fatty acids levels are associated with GDM and GDM-associated risk factors, thus promising potential biomarkers of GDM **(Table 2).**

4.3 Amino acids and their metabolites

The demand for amino acids is higher in pregnancy because of their crucial role in protein synthesis, gluconeogenesis and ketogenesis. In addition to protein synthesis and fuel generation, some amino acids are regulators of biological processes. For example, branched chain amino acids (BCAAs) play a role in regulating the immune system and insulin sensitivity. Alanine can inhibit cell apoptosis. Arginine can be broken down into nitric acid and used to regulate inflammation. Tyrosine, produced from phenylalanine, is a precursor for the synthesis of catecholamines which acts as a messenger during the immune response [125].

Elevated circulatory BCAAs have been linked to insulin related diseases such as the metabolic syndrome and T2D [126-128]. The proposed mechanism used by the BCAAs, particularly leucine, involves the amino acids induction of the mammalian target of rapamycin (mTOR) pathway [129-132]. The mTOR pathway is a serine-threonine kinase that behaves as a nutrient sensor. The function of the mTOR signalling pathway is to relay signals related to nutrition, cell growth and cell survival. BCAAs, particularly leucine, activate the mTOR pathway through the activation of the class III phosphoinositide 3-kinase (PI3K) and consequently mTOR phosphorylation (**Fig. 3**). The activation of mTOR leads to the phosphorylation of two main downstream effectors, namely, ribosomal substrate 6 kinase 1 (S6K1) and eukaryotic initiation

factor 4E-binding protein 1 (4E-BP1) [133]. The activation of mTOR signalling pathway has been linked with secretion of pro-inflammatory cytokines in cancerous cells through the activation of IL-1A [134] and in peripheral blood mononuclear cells (PBMCs) [135].

Investigations have shown a link between elevated circulatory amino acids with GDM and T2D [43, 121, 136-138]. The majority of these studies indicate that BCAAs are elevated in women with GDM. Alanine has also been identified as a predictor of GDM in metabolomic studies [121, 137, 139, 140] (**Table 2**).

4.4 Oxidative stress markers

Oxidative stress is a state when there is an imbalance between reactive oxidative species (ROS) and anti-oxidants with the ROS being higher than the anti-oxidants. ROS are charged species and can bind with other compounds in the body changing their natural structural morphology and possibly their function. It is believed that elevated ROS play a role in inducing insulin resistance by interacting with the insulin receptor and the JNK signalling pathway [141]. In non-pregnant individuals, oxidative stress activates the NF-κB and JNK pathways which further feeds into inflammation [142].

Elevated free fatty acids have been shown to cause oxidative stress through mitochondria uncoupling which give rise to ROS [141, 143]. The combination of ROS and lipids could lead to the formation of oxidized lipids [122]. Oxidized lipids can attenuate lipid metabolism, lipid transport and placental development [115]. Another mechanism of how enlarged adipocytes can cause oxidative stress is by over-consumption of oxygen which generates free radicals in the mitochondrial respiratory chain [54].

Oxidative stress is a feature in normal pregnancy and is partly generated in the placenta but is heightened in pathological pregnancies such as GDM [144, 145]. It is possible that an increase in adipose tissue common in women who develop GDM exacerbate oxidative stress by further elevated free fatty acids. Evidently, GDM is associated with elevated ROS and defects in antioxidant defences [75].

Oxidative stress markers such as malondialdehyde (MDA), oxidized low density lipoproteins (Ox-LDL), nitrotyrosine, advanced glycated end products (AGEs) and anti-oxidants, glutathione (GSH)/glutathione peroxidase (GPx) are promising biomarkers for GDM [146] (**Table 2**). However, there have been only a few studies on oxidative stress in GDM.

MDA is formed by lipid peroxidation of polyunsaturated fatty acids [146] and has been shown to be associated with intrauterine growth restriction (IUGR) [147]. In a Turkish population, elevated MDA and ox-LDL levels were evident in all pregnancies but higher in women with GDM [148]. Similarly, MDA levels were found to be higher in GDM cases when compared to healthy controls and MDA levels correlated positively to leptin and resistin and were negatively correlated with HOMA-IR [149]. In two longitudinal studies, MDA levels were found to be significantly higher in obese women later diagnosed with GDM [150, 151]. In a GDM and preeclampsia study, MDA was the only oxidative stress marker elevated in GDM cases when compared to controls among other oxidative stress markers such as protein oxidation markers (AOPPs), myeloperoxidase (MPO) and lipid hydroperoxide (LHP) [152].

Oxidized low density lipoproteins are one of many oxidized lipid markers and are linked to diseases associated with dyslipidaemia, such as atherosclerosis [153]. In GDM, Ox-LDL has been shown to be elevated when compared to healthy controls [148, 154]. The study further proposed that Ox-LDL is linked to macrosomia [148].

Nitrotyrosine is formed through the binding of the highly reactive ROS species peroxynitrite (ONOO-) with serine, tyrosine and threonine hydroxyl groups [155]. Lyall and colleagues have indicated that nitrotyrosine oxidative stress occurs in the placenta of women with diabetes in pregnancy (GDM and overt diabetes), which is in agreement with previous studies showing that the placenta induces oxidative stress [156, 157]. Georgiou and associates have shown that nitrotyrosine is higher in GDM cases at the first trimester (11 weeks) but declines after 28 weeks of pregnancy [109].

AGES are formed by glycation and oxidative reactions and can alter signalling pathways inside cells, the conformation of molecules and the expression of genes. In addition, this attenuation could ultimately cause inflammation by inducing pro-inflammatory markers through NF-κB dependent pathways [146, 158, 159]. As expected, AGEs have been shown to be associated with hyperglycaemia-related illnesses such as retinopathy and nephropathy and thus is a potential marker of GDM [158]. In GDM, AGEs were found to be higher in women with GDM [160]. However, it is more likely to be used for diagnostic processes than early screening because of its dependency on hyperglycaemia which is only present later in pregnancy in most cases of GDM.

GSH is an antioxidant which reduces oxidative stress by donating electrons to unstable ROS resulting in the formation of ox-GSH [146, 161]. Alternately, GSH functions as a substrate for the antioxidant GPx. GPx requires GSH to neutralize reactive peroxides. It is unclear at what

concentration GSH and GPx are present in women with GDM. Peuchant and colleagues have shown that GPx levels are significantly lower in GDM cases and in pregnant women with T2D [162]. A longitudinal study by Arribas et al showed that GPx was significantly lower in early pregnancy when compared to healthy controls but increased later in pregnancy [151].

4.5 Iron markers

Iron is a transitional element used in various biological systems such as oxygen transportation, cellular proliferation, protein synthesis, antimicrobial effects and energy production [163]. However, due to the reactive nature of iron, too much iron could produce ROS through a process known as Fenton reactions [164]. Elevated iron could also alter insulin signalling in the liver and adipocytes and increase non-esterified fatty acid oxidation in muscles [165, 166]. Elevated serum ferritin levels have been observed in numerous chronic inflammatory diseases such as lupus and diabetes [167-170] including GDM [171-173]. The high circulatory iron concentration activates hepcidin thus increasing hepcidin levels as seen in women with GDM and women with impaired glucose tolerance (IGT) [174]. In contrast, Akhlaghi and colleagues have reported low serum iron concentrations in women with GDM as compared to healthy controls [175].

The mechanisms involved in the increase of iron levels are not clearly understood although chronic inflammation could be an important piece of the puzzle [171]. One other way that iron levels in pregnancy are elevated is through iron supplement consumption. Although the majority of the studies mentioned above support the case that circulating iron levels are higher in GDM cases, more studies aimed at investigating the mechanisms involved in iron increase in pregnancy are required (**Table 2**).

5 Conclusions

It is likely that the development of GDM is caused by different factors which synergistically collaborate to suppress the production of insulin, in part via secretion of pro-inflammatory cytokines and mediation of pro-inflammatory signalling pathways. The balance between the pro-inflammatory cytokines and anti-inflammatory cytokines is what distinguishes the disease state from the healthy state. The disease state favours pro-inflammatory cytokine secretion.

Furthermore, the activation of pro-inflammatory signalling pathways through fatty acids, amino acids, ROS and markers of oxidative stress and iron contributes to the development of GDM.

In summary, there is strong possibility that adiponectin, TNF-α, IL-6, AFABP-4, lipids, BCAAs, alanine, oxidative stress markers and iron markers are biomarkers of GDM and could be used for screening in early pregnancy or late pregnancy. However, more prospective studies are required to confirm this. The conflicting reports in RBP-4, resistin, visfatin and leptin levels may indicate that these markers are not involved in the development of GDM. However, it is important to consider the heterogeneity between studies which could have led to the conflict in results. Future studies should assess the diagnostic ability of the markers reviewed in the current review as a combined set considering the substantial knowledge that these markers are interlinked and are all implicated in the development of GDM.

Acknowledgements

Lungile Khambule is supported by National Research Foundation (NRF) and the University of the Witwatersrand

References

1. Metzger BE (2016) Diabetes Mellitus and Pregnancy. In: Endocrinology: Adult and Pediatric. 7th ed. Philadelphia, USA Elsevier/Saunders; 2016. pp. 788-804. https://www.clinicalkey.com/#!/content/book/3-s2.0-B9780323189071000457?scrollTo=%23hl0000856
2. Wild S, Roglic G, Green A, Sicree R, King H (2004) Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 27:1047-1053
3. Li KT, Naik S, Alexander M, Mathad JS (2018) Screening and diagnosis of gestational diabetes in India: a systematic review and meta-analysis. Acta Diabetol 55:613-625
4. Dainelli L, Prieto-Patron A, Silva-Zolezzi I, Sosa-Rubi SG, Espino y Sosa S, Reyes-Muñoz E et al (2018) Screening and management of gestational diabetes in Mexico:

results from a survey of multilocation, multi-health care institution practitioners. Diabetes MetabSyndrObes Targets Ther 11:105-116

5. Macaulay S, Dunger DB, Norris SA (2014) Gestational Diabetes Mellitus in Africa: A Systematic Review. PLoS One 9:e97871. doi:10.1371/journal.pone.0097871
6. IDF Diabetes Atlas (accessed 5 Dec 2018). https://www.idf.org/e-library/epidemiology-research/diabetes-atlas/19-atlas-6th-edition.html
7. Mohammadbeigi A, Farhadifar F, Soufizadeh N, Mohammadsalehi N, Rezaiee M, Aghaei M (2013) Fetal Macrosomia: Risk Factors, Maternal, and Perinatal Outcome. Ann Med Health Sci Res 3:546-550
8. Nehring I, Chmitorz A, Reulen H, Kries R von, Ensenauer R (2013) Gestational diabetes predicts the risk of childhood overweight and abdominal circumference independent of maternal obesity. Diabet Med 30:1449-1456
9. Kelstrup L, Damm P, Mathiesen ER, Hansen T, Vaag AA, Pedersen O et al (2013) Insulin resistance and impaired pancreatic β-cell function in adult offspring of women with diabetes in pregnancy. J Clin Endocrinol Metab 98:3793-3801.
10. West NA, Kechris K, Dabelea D (2013) Exposure to Maternal Diabetes in Utero and DNA Methylation Patterns in the Offspring. Immunometabolism 1:1–9
11. Hartling L, Dryden DM, Guthrie A, Muise M, Vandermeer B, Donovan L (2013) Benefits and harms of treating gestational diabetes mellitus: a systematic review and meta-analysis for the U.S. Preventive Services Task Force and the National Institutes of Health Office of Medical Applications of Research. Ann Intern Med 159:123-129
12. Durnwald C (2015) Gestational diabetes: Linking epidemiology, excessive gestational weight gain, adverse pregnancy outcomes, and future metabolic syndrome. Semin Perinatol 39:254-258
13. Jenum AK, Mørkrid K, Sletner L, Vangen S, Vange S, Torper JL et al (2012) Impact of ethnicity on gestational diabetes identified with the WHO and the modified International Association of Diabetes and Pregnancy Study Groups criteria: a population-based cohort study. Eur J Endocrinol 166:317-324
14. Anghebem-Oliveira MI, Martins BR, Alberton D, Ramos EA de S, Picheth G, Rego FG de M (2017) Type 2 diabetes-associated genetic variants of FTO, LEPR, PPARg, and TCF7L2 in gestational diabetes in a Brazilian population. Arch Endocrinol Metab 61:238-248

15. Hedderson MM, Williams MA, Holt VL, Weiss NS, Ferrara A (2008) Body mass index and weight gain prior to pregnancy and risk of gestational diabetes mellitus. Am J Obstet Gynecol 198:409.e1-7
16. Hedderson M, Ehrlich S, Sridhar S, Darbinian J, Moore S, Ferrara A (2012) Racial/ethnic disparities in the prevalence of gestational diabetes mellitus by BMI. Diabetes Care 35:1492-1498
17. Makki K, Froguel P, Wolowczuk I (2013) Adipose tissue in obesity-related inflammation and insulin resistance: cells, cytokines, and chemokines. ISRN Inflamm 2013: 139239. doi:10.1155/2013/139239
18. Challis JR, Lockwood CJ, Myatt L, Norman JE, Strauss JF, Petraglia F (2009) Inflammation and Pregnancy. Reprod Sci 16:206-215
19. Briana DD, Malamitsi-Puchner A. Reviews: adipocytokines in normal and complicated pregnancies. ReprodSci Thousand Oaks Calif. 2009;16: 921–937. doi:10.1177/1933719109336614
20. Ashcroft FM, Rohm M, Clark A, Brereton MF (2017) Is Type 2 Diabetes a Glycogen Storage Disease of Pancreatic β Cells? Cell Metab 26:17-23
21. Bergmann K, Sypniewska G (2013) Diabetes as a complication of adipose tissue dysfunction. Is there a role for potential new biomarkers? Clin Chem Lab Med 51:177-185
22. Augustin R (2010) The protein family of glucose transport facilitators: It's not only about glucose after all. IUBMB Life 62:315-333
23. Lesseur C, Chen J (2018) Adverse Maternal Metabolic Intrauterine Environment and Placental Epigenetics: Implications for Fetal Metabolic Programming. Curr Environ Health Rep Sep 28. doi: 10.1007/s40572-018-0217-9. [Epub ahead of print]
24. Li J, Song L, Zhou L, Wu J, Sheng C, Chen H et al (2015) A MicroRNA Signature in Gestational Diabetes Mellitus Associated with Risk of Macrosomia. Cell PhysiolBiochemInt J Exp Cell Physiol Biochem Pharmacol 37:243-252
25. O'sullivan JB, Mahan CM (1954) CRITERIA FOR THE ORAL GLUCOSE TOLERANCE TEST IN PREGNANCY. Diabetes 13:278-285
26. Diabetes in pregnancy: management from preconception to the postnatal period | Guidance and guidelines | NICE (Accessed 5 Dec 2018). https://www.nice.org.uk/guidance/ng3

27. Esser N, Legrand-Poels S, Piette J, Scheen AJ, Paquot N (2014) Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. Diabetes Res Clin Pract 105:141-150
28. Lawrence T (2009) The Nuclear Factor NF-κB Pathway in Inflammation. Cold Spring Harb Perspect Biol 1(6):a001651. doi: 10.1101/cshperspect.a001651
29. Charo L, Lacoursiere DY (2014) Introduction: obesity and lifestyle issues in women. ClinObstet Gynecol 57:433-445
30. López-Tinoco C, Roca M, Fernández-Deudero A, García-Valero A, Bugatto F, Aguilar-Diosdado M et al (2012) Cytokine profile, metabolic syndrome and cardiovascular disease risk in women with late-onset gestational diabetes mellitus. Cytokine 58:14-19
31. Richardson AC, Carpenter MW (2007) Inflammatory mediators in gestational diabetes mellitus. Obstet Gynecol Clin North Am 34:213-224, viii
32. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW (2003) Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 112:1796-1808
33. Bellos I, Fitrou G, Pergialiotis V, Perrea DN, Daskalakis G (2018) Serum levels of adipokines in gestational diabetes: a systematic review. J Endocrinol Invest Nov 3. doi: 10.1007/s40618-018-0973-2. [Epub ahead of print]
34. Fasshauer M, Blüher M, Stumvoll M (2014) Adipokines in gestational diabetes. Lancet Diabetes Endocrinol 2:488-499
35. Tomas E, Tsao T-S, Saha AK, Murrey HE, Zhang C cheng, Itani SI et al (2002) Enhanced muscle fat oxidation and glucose transport by ACRP30 globular domain: Acetyl–CoA carboxylase inhibition and AMP-activated protein kinase activation. Proc Natl Acad Sci USA 99:16309-16313
36. Wu X, Motoshima H, Mahadev K, Stalker TJ, Scalia R, Goldstein BJ (2003) Involvement of AMP-activated protein kinase in glucose uptake stimulated by the globular domain of adiponectin in primary rat adipocytes. Diabetes 52:1355-1363
37. Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S et al (2002) Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med 8:1288-1295
38. Maeda N, Shimomura I, Kishida K, Nishizawa H, Matsuda M, Nagaretani H et al (2002) Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. Nat Med 8:731-737

39. Maeda N, Takahashi M, Funahashi T, Kihara S, Nishizawa H, Kishida K et al PPARgamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. Diabetes 50:2094-2099
40. Ruderman NB, Shulman GI (2016) Metabolic Syndrome. Endocrinology: Adult and Pediatric. 7th ed. Philadelphia, Elsevier/Saunders; 2016. pp. 752-769.e7. https://www.clinicalkey.com/#!/content/book/3-s2.0-B9780323189071000433?scrollTo=%23hl0000833
41. Bao W, Baecker A, Song Y, Kiely M, Liu S, Zhang C (2015) Adipokine levels during the first or early second trimester of pregnancy and subsequent risk of gestational diabetes mellitus: A systematic review. Metabolism 64:756-764
42. Horosz E, Bomba-Opon DA, Szymanska M, Wielgos M (2011) Third trimester plasma adiponectin and leptin in gestational diabetes and normal pregnancies. Diabetes Res Clin Pract 93:350-356
43. Park S, Kim M-Y, Baik SH, Woo J-T, Kwon YJ, Daily JW et al (2013) Gestational diabetes is associated with high energy and saturated fat intakes and with low plasma visfatin and adiponectin levels independent of prepregnancy BMI. Eur J Clin Nutr 67:196-201
44. Retnakaran R, Hanley AJG, Raif N, Connelly PW, Sermer M, Zinman B (2004) Reduced Adiponectin Concentration in Women With Gestational Diabetes: A potential factor in progression to type 2 diabetes. Diabetes Care 27:799-800
45. Retnakaran R, Hanley AJG, Raif N, Hirning CR, Connelly PW, Sermer M et al (2005) Adiponectin and beta cell dysfunction in gestational diabetes: pathophysiological implications. Diabetologia 48:993-1001
46. Lain KY, Daftary AR, Ness RB, Roberts JM (2008) First trimester adipocytokine concentrations and risk of developing gestational diabetes later in pregnancy. Clin Endocrinol (Oxf) 69:407-411
47. Williams MA, Qiu C, Muy-Rivera M, Vadachkoria S, Song T, Luthy DA (2004) Plasma adiponectin concentrations in early pregnancy and subsequent risk of gestational diabetes mellitus. J Clin Endocrinol Metab 89:2306-2311
48. Lacroix M, Battista M-C, Doyon M, Ménard J, Ardilouze J-L, Perron P et al (2013) Lower Adiponectin Levels at First Trimester of Pregnancy Are Associated With Increased Insulin Resistance and Higher Risk of Developing Gestational Diabetes Mellitus. Diabetes Care 36:1577-1583

49. Kinalski M, Telejko B, Kuźmicki M, Krętowski A, Kinalska I (2005) Tumor Necrosis Factor Alpha System and Plasma Adiponectin Concentration in Women with Gestational Diabetes. Horm Metab Res 37:450-454
50. Skvarca A, Tomazic M, Krhin B, Blagus R, Janez A (2012) Adipocytokines and Insulin Resistance across Various Degrees of Glucose Tolerance in Pregnancy. J Int Med Res 40:583-589
51. Lappas M, Yee K, Permezel M, Rice GE (2018) Release and regulation of leptin, resistin and adiponectin from human placenta, fetal membranes, and maternal adipose tissue and skeletal muscle from normal and gestational diabetes mellitus-complicated pregnancies.J Endocrinol 186:457-465
52. Saucedo R, Zarate A, Basurto L, Hernandez M, Puello E, Galvan R et al (2011) Relationship between circulating adipokines and insulin resistance during pregnancy and postpartum in women with gestational diabetes. Arch Med Res 42:318-323
53. Kishida K, Shimomura TF and I. Molecular Mechanisms of Diabetes and Atherosclerosis: Role of Adiponectin. In: Endocrine, Metabolic & Immune Disorders - Drug Targets 31 May 2012 (accessed 20 Nov 2018). http://www.eurekaselect.com/97769/article
54. Fernández-Sánchez A, Madrigal-Santillán E, Bautista M, Esquivel-Soto J, Morales-González Á, Esquivel-Chirino C et al (2011) Inflammation, Oxidative Stress, and Obesity. Int J Mol Sci 12:3117-3132
55. Bradley JR (2008) TNF-mediated inflammatory disease. J Pathol 214:149-160
56. Bouwmeester T, Bauch A, Ruffner H, Angrand P-O, Bergamini G, Croughton K et al (2004) A physical and functional map of the human TNF-alpha/NF-kappa B signal transduction pathway. Nat Cell Biol 6: 97-105
57. Stephens JM, Lee J, Pilch PF (1997) Tumor necrosis factor-alpha-induced insulin resistance in 3T3-L1 adipocytes is accompanied by a loss of insulin receptor substrate-1 and GLUT4 expression without a loss of insulin receptor-mediated signal transduction. J Biol Chem 272:971-976
58. Hotamisligil GS, Murray DL, Choy LN, Spiegelman BM (1994) Tumor necrosis factor alpha inhibits signaling from the insulin receptor. Proc Natl Acad Sci USA 91:4854-4858
59. Altinova AE, Toruner F, Bozkurt N, Bukan N, Karakoc A, Yetkin I et al (2007) Circulating concentrations of adiponectin and tumor necrosis factor-α in gestational diabetes mellitus. Gynecol Endocrinol 23:161-165

60. Salmi A, Zaki M n., Zakaria, Nor Aliza G, Rasool H (2012) Arterial stiffness, inflammatory and pro-atherogenic markers in gestational diabetes mellitus. Vasa 41:96-104
61. Kirwan JP, Mouzon SH-D, Lepercq J, Challier J-C, Huston-Presley L, Friedman JE et al (2002) TNF-α Is a Predictor of Insulin Resistance in Human Pregnancy. Diabetes 51:2207-2213
62. Gao X, Yang H, Zhao Y (2008) Variations of tumor necrosis factor-alpha, leptin and adiponectin in mid-trimester of gestational diabetes mellitus. Chin Med J (Engl) 121:701-705
63. Guillemette L, Lacroix M, Battista M-C, Doyon M, Moreau J, Ménard J et al (2014) TNFα dynamics during the oral glucose tolerance test vary according to the level of insulin resistance in pregnant women. J Clin Endocrinol Metab 99:1862-1869
64. Winkler G, Cseh K, Baranyi É, Melczer Z, Speer G, Hajós P et al (2002) Tumor necrosis factor system in insulin resistance in gestational diabetes. Diabetes Res Clin Pract 56:93-99
65. Gueuvoghlanian-Silva BY, Torloni MR, Mattar R, Oliveira LS de, Scomparini FB, Nakamura MU et al (2012) Profile of Inflammatory Mediators in Gestational Diabetes Mellitus: Phenotype and Genotype. Am J Reprod Immunol 67:241-250
66. Georgiou HM, Lappas M, Georgiou GM, Marita A, Bryant VJ, Hiscock R et al (2008) Screening for biomarkers predictive of gestational diabetes mellitus. Acta Diabetol 45:157-165
67. Xu J, Zhao YH, Chen YP, Yuan XL, Wang J, Zhu H et al (2014) Maternal circulating concentrations of tumor necrosis factor-alpha, leptin, and adiponectin in gestational diabetes mellitus: a systematic review and meta-analysis. Scientific World Journal 2014:926932. doi:10.1155/2014/926932
68. McLachlan KA, O'Neal D, Jenkins A, Alford FP (2006) Do adiponectin, TNFα, leptin and CRP relate to insulin resistance in pregnancy? Studies in women with and without gestational diabetes, during and after pregnancy. Diabetes Metab Res Rev 22:131-138
69. Maury E, Brichard SM (2010) Adipokine dysregulation, adipose tissue inflammation and metabolic syndrome. Mol Cell Endocrinol 314:1-16
70. Siddiqui S, Waghdhare S, Panda M, Dubey S, Jha S (2018) Association of IL-6 and CRP Levels with Gestational Diabetes Mellitus. Diabetes 67:2417-PUB. doi:10.2337/db18-2417-PUB. http://diabetes.diabetesjournals.org/content/67/Supplement_1/2417-PUB

71. Kuzmicki M, Telejko B, Szamatowicz J, Zonenberg A, Nikolajuk A, Kretowski A et al (2009) High resistin and interleukin-6 levels are associated with gestational diabetes mellitus. Gynecol Endocrinol 25:258-263
72. Vozarova B, Weyer C, Hanson K, Tataranni PA, Bogardus C, Pratley RE (2001) Circulating interleukin-6 in relation to adiposity, insulin action, and insulin secretion. Obes Res 9:414-417
73. Morisset A-S, Dubé M-C, Côté JA, Robitaille J, Weisnagel SJ, Tchernof A (2011) Circulating interleukin-6 concentrations during and after gestational diabetes mellitus. ActaObstetGynecol Scand 90:524-530
74. Abell SK, Shorakae S, Boyle JA, De Courten B, Stepto NK, Teede HJ et al (2018) Role of serum biomarkers to optimise a validated clinical risk prediction tool for gestational diabetes. Aust NZ J Obstet Gynaecol Jun 14. doi: 10.1111/ajo.12833. [Epub ahead of print]
75. Abell SK, De Courten B, Boyle JA, Teede HJ (2015) Inflammatory and Other Biomarkers: Role in Pathophysiology and Prediction of Gestational Diabetes Mellitus. Int J Mol Sci 16:13442-13473
76. Kralisch S, Fasshauer M (2013) Adipocyte fatty acid binding protein: a novel adipokine involved in the pathogenesis of metabolic and vascular disease? Diabetologia 56:10-21
77. Xu A, Wang Y, Xu JY, Stejskal D, Tam S, Zhang J et al (2006) Adipocyte Fatty Acid–Binding Protein Is a Plasma Biomarker Closely Associated with Obesity and Metabolic Syndrome. Clin Chem. 52:405-413
78. Stejskal D, Karpisek M (2006) Adipocyte fatty acid binding protein in a Caucasian population: a new marker of metabolic syndrome? Eur J Clin Invest 36:621-625
79. Ortega-Senovilla H, Schaefer-Graf U, Meitzner K, Abou-Dakn M, Graf K, Kintscher U et al (2011) Gestational Diabetes Mellitus Causes Changes in the Concentrations of Adipocyte Fatty Acid–Binding Protein and Other Adipocytokines in Cord Blood. Diabetes Care 34:2061-2066
80. Tu W-J, Guo M, Shi X-D, Cai Y, Liu Q, Fu C-W (2017) First-Trimester Serum Fatty Acid-Binding Protein 4 and Subsequent Gestational Diabetes Mellitus.Obstet Gynecol 130:1011. doi:10.1097/AOG.0000000000002310
81. Iikuni N, Lam QLK, Lu L, Matarese G, La Cava A (2008) Leptin and Inflammation. CurrImmunol Rev 4:70-79
82. Triantafyllou GA, Paschou SA, Mantzoros CS (2016) Leptin and Hormones. Endocrinol Metab Clin North Am 45:633-645

83. Moon H-S, Huh JY, Dincer F, Schneider BE, Hasselgren P-O, Mantzoros CS (2015) Identification and Saturable Nature of Signaling Pathways Induced by Metreleptin in Humans: Comparative Evaluation of In Vivo, Ex Vivo, and In Vitro Administration. Diabetes 64:828-839
84. Chan JL, Moschos SJ, Bullen J, Heist K, Li X, Kim Y-B et al (2005) Recombinant Methionyl Human Leptin Administration Activates Signal Transducer and Activator of Transcription 3 Signaling in Peripheral Blood Mononuclear Cells in Vivo and Regulates Soluble Tumor Necrosis Factor-α Receptor Levels in Humans with Relative Leptin Deficiency. J Clin Endocrinol Metab 90:1625-1631
85. Chen P-C, Kryukova YN, Shyng S-L (2013) Leptin Regulates KATP Channel Trafficking in Pancreatic β-Cells by a Signaling Mechanism Involving AMP-activated Protein Kinase (AMPK) and cAMP-dependent Protein Kinase (PKA). J Biol Chem 288:34098-34109
86. Sáinz N, Barrenetxe J, Moreno-Aliaga MJ, Martínez JA (2015) Leptin resistance and diet-induced obesity: central and peripheral actions of leptin. Metabolism 64:35-46
87. Heymsfield SB, Greenberg AS, Fujioka K, Dixon RM, Kushner R, Hunt T et al (1999) Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. JAMA 282:1568-1575
88. Abel ED, Litwin SE, Sweeney G (2008) Cardiac remodeling in obesity. Physiol Rev 88:389-419
89. Bjørbæk C, El-Haschimi K, Frantz JD, Flier JS (1999) The Role of SOCS-3 in Leptin Signaling and Leptin Resistance. J Biol Chem 274:30059-30065
90. Santos-Alvarez J, Goberna R, Sánchez-Margalet V (1999) Human leptin stimulates proliferation and activation of human circulating monocytes. Cell Immunol 194:6-11
91. Gabay C, Dreyer M, Pellegrinelli N, Chicheportiche R, Meier CA (2001) Leptin directly induces the secretion of interleukin 1 receptor antagonist in human monocytes. J Clin Endocrinol Metab86:783-791
92. Cseh K, Baranyi E, Melczer Z, Csákány GM, Speer G, Kovács M et al (2002) The pathophysiological influence of leptin and the tumor necrosis factor system on maternal insulin resistance: negative correlation with anthropometric parameters of neonates in gestational diabetes. Gynecol Endocrinol Off J Int Soc Gynecol Endocrinol 16:453-460
93. Kautzky-Willer A, Pacini G, Tura A, Bieglmayer C, Schneider B, Ludvik B et al (2001) Increased plasma leptin in gestational diabetes. Diabetologia 44:164-172

94. Chen D, Xia G, Xu P, Dong M (2010) Peripartum serum leptin and soluble leptin receptor levels in women with gestational diabetes. Acta Obstet Gynecol Scand 89:1595-1599
95. Maple-Brown L, Ye C, Hanley AJ, Connelly PW, Sermer M, Zinman B et al (2012) Maternal Pregravid Weight Is the Primary Determinant of Serum Leptin and Its Metabolic Associations in Pregnancy, Irrespective of Gestational Glucose Tolerance Status. J Clin Endocrinol Metab 97:4148-4155
96. Fasshauer M, Blüher M, Stumvoll M (2014) Adipokines in gestational diabetes. Lancet Diabetes Endocrinol 2:488-499
97. Hou W, Meng X, Zhao A, Zhao W, Pan J, Tang J et al (2018) Development of Multimarker Diagnostic Models from Metabolomics Analysis for Gestational Diabetes Mellitus (GDM). Mol Cell Proteomics 17:431-441
98. Chan T-F, Chen H-S, Chen Y-C, Lee C-H, Chou F-H, Chen I-J et al (2007) Increased Serum Retinol-Binding Protein 4 Concentrations in Women With Gestational Diabetes Mellitus. Reprod Sci 14:169-174
99. Choi SH, Kwak SH, Youn B-S, Lim S, Park YJ, Lee H et al (2008) High Plasma Retinol Binding Protein-4 and Low Plasma Adiponectin Concentrations Are Associated with Severity of Glucose Intolerance in Women with Previous Gestational Diabetes Mellitus. J Clin Endocrinol Metab 93:3142-3148
100. Du C, Kong F (2019) A Prospective Study of Maternal Plasma Concentrations of Retinol-Binding Protein 4 and Risk of Gestational Diabetes Mellitus. Ann Nutr Metab 74:1-8
101. Krzyzanowska K, Zemany L, Krugluger W, Schernthaner GH, Mittermayer F, Schnack C et al (2008) Serum concentrations of retinol-binding protein 4 in women with and without gestational diabetes. Diabetologia 51:1115-1122
102. Lewandowski KC, Stojanovic N, Bienkiewicz M, Tan BK, Prelevic GM, Press M et al (2008) Elevated concentrations of retinol-binding protein-4 (RBP-4) in gestational diabetes mellitus: Negative correlation with soluble vascular cell adhesion molecule-1 (sVCAM-1). Gynecol Endocrinol 24:300-305
103. Su Y-X, Hong J, Yan Q, Xu C, Gu W-Q, Zhang Y-F et al (2010) Increased serum retinol-binding protein-4 levels in pregnant women with and without gestational diabetes mellitus. Diabetes Metab 36:470-475

104. Nanda S, Nikoletakis G, Markova D, Poon LCY, Nicolaides KH (2013) Maternal serum retinol-binding protein-4 at 11–13weeks' gestation in normal and pathological pregnancies. Metabolism 62:814-819

105. Fonseca-Alaniz MH, Takada J, Alonso-Vale MIC, Lima FB (2007) Adipose tissue as an endocrine organ: from theory to practice. J Pediatr (Rio J) 83:S192-S203

106. Courten BV de, Degawa-Yamauchi M, Considine RV, Tataranni PA (2004) High Serum Resistin Is Associated With an Increase in Adiposity But Not a Worsening of Insulin Resistance in Pima Indians. Diabetes 53:1279-1284

107. Jamaluddin MS, Weakley SM, Yao Q, Chen C (2012 Resistin: functional roles and therapeutic considerations for cardiovascular disease. Br J Pharmacol 165: 622-632

108. Lobo TF, Torloni MR, Gueuvoghlanian-Silva BY, Mattar R, Daher S (2013) Resistin concentration and gestational diabetes: a systematic review of the literature. J ReprodImmunol 97:120-127

109. Georgiou HM, Lappas M, Georgiou GM, Marita A, Bryant VJ, Hiscock R et al (2008) Screening for biomarkers predictive of gestational diabetes mellitus. Acta Diabetol 45:157-165

110. Lowe LP, Metzger BE, Lowe WL, Dyer AR, McDade TW, McIntyre HD. (2010) Inflammatory Mediators and Glucose in Pregnancy: Results from a Subset of the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study. J Clin Endocrinol Metab 95:5427-5434

111. Moschen AR, Kaser A, Enrich B, Mosheimer B, Theurl M, Niederegger H et al (2007) Visfatin, an Adipocytokine with Proinflammatory and Immunomodulating Properties. J Immunol 178:1748-1758

112. Krzyzanowska K, Krugluger W, Mittermayer F, Rahman R, Haider D, Shnawa N et al (2006) Increased visfatin concentrations in women with gestational diabetes mellitus. Clin Sci Lond Engl 1979 110:605-609

113. Ferreira AFA, Rezende JC, Vaikousi E, Akolekar R, Nicolaides KH (2011) Maternal Serum Visfatin at 11–13 Weeks of Gestation in Gestational Diabetes Mellitus. Clin Chem 57:609-613

114. Herrera E, Lasunción MA, Gomez-Coronado D, Aranda P, López-Luna P, Maier I (1988) Role of lipoprotein lipase activity on lipoprotein metabolism and the fate of circulating triglycerides in pregnancy. Am J Obstet Gynecol 158:1575-1583

115. Salzer L, Tenenbaum-Gavish K, Hod M (2015) Metabolic disorder of pregnancy (understanding pathophysiology of diabetes and preeclampsia). Best Pract Res Clin Obstet Gynaecol 29:328-338

116. Butte NF (2000) Carbohydrate and lipid metabolism in pregnancy: normal compared with gestational diabetes mellitus. Am J Clin Nutr 71:1256s-1261s

117. Di Cianni G, Miccoli R, Volpe L, Lencioni C, Ghio A, Giovannitti MG et al (2005) Maternal triglyceride levels and newborn weight in pregnant women with normal glucose tolerance. Diabet Med J Br Diabet Assoc 22:21-25

118. Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS (2006) TLR4 links innate immunity and fatty acid–induced insulin resistance. J Clin Invest 116:3015-3025

119. Böni-Schnetzler M, Boller S, Debray S, Bouzakri K, Meier DT, Prazak R et al (2009) Free Fatty Acids Induce a Proinflammatory Response in Islets via the Abundantly Expressed Interleukin-1 Receptor I. Endocrinology 150:5218-5229

120. Chen X, Scholl TO, Leskiw M, Savaille J, Stein TP (2010) Differences in maternal circulating fatty acid composition and dietary fat intake in women with gestational diabetes mellitus or mild gestational hyperglycemia. Diabetes Care 33:2049-2054

121. Scholtens DM, Muehlbauer MJ, Daya NR, Stevens RD, Dyer AR, Lowe LP et al (2014) Metabolomics Reveals Broad-Scale Metabolic Perturbations in Hyperglycemic Mothers During Pregnancy. Diabetes Care 37:158-166

122. Sivan E, Homko CJ, Whittaker PG, Reece EA, Chen X, Boden G (1998) Free fatty acids and insulin resistance during pregnancy. J Clin Endocrinol Metab 83: 2338-2342

123. Segura MT, Demmelmair H, Krauss-Etschmann S, Nathan P, Dehmel S, Padilla MC et al (2017) Maternal BMI and gestational diabetes alter placental lipid transporters and fatty acid composition. Placenta 57:144-151

124. Ryckman KK, Spracklen CN, Smith CJ, Robinson JG, Saftlas AF (2015) Maternal lipid levels during pregnancy and gestational diabetes: a systematic review and meta-analysis. BJOG Int J Obstet Gynaecol 122:643-651

125. Li P, Yin Y-L, Li D, Kim SW, Wu G (2007) Amino acids and immune function. Br J Nutr 98:237-252

126. Fiehn O, Garvey WT, Newman JW, Lok KH, Hoppel CL, Adams SH (2010) Plasma metabolomic profiles reflective of glucose homeostasis in non-diabetic and type

2 diabetic obese African-American women. PloS One 5:e15234. doi:10.1371/journal.pone.0015234

127. Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E et al (2011) Metabolite profiles and the risk of developing diabetes. Nat Med 17:448-453

128. Tillin T, Hughes AD, Wang Q, Würtz P, Ala-Korpela M, Sattar N et al (2015) Diabetes risk and amino acid profiles: cross-sectional and prospective analyses of ethnicity, amino acids and diabetes in a South Asian and European cohort from the SABRE (Southall And Brent REvisited) Study. Diabetologia 58:968-979

129. Tremblay F, Marette A (2001) Amino acid and insulin signaling via the mTOR/p70 S6 kinase pathway. A negative feedback mechanism leading to insulin resistance in skeletal muscle cells. J Biol Chem 276:38052-38060

130. Drummond MJ, Bell JA, Fujita S, Dreyer HC, Glynn EL, Volpi E et al (2008) Amino Acids are Necessary for the Insulin-Induced Activation of mTOR/S6K1 Signaling and Protein Synthesis in Healthy and Insulin Resistant Human Skeletal Muscle. Clin Nutr Edinb Scotl 27:447-456

131. Leibowitz G, Cerasi E, Ketzinel-Gilad M (2008) The role of mTOR in the adaptation and failure of beta-cells in type 2 diabetes. Diabetes Obes Metab 10 Suppl 4:157-169

132. Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF et al (2009) A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. Cell Metab 9:311-326

133. Hay N, Sonenberg N (2004) Upstream and downstream of mTOR. Genes Dev 18:1926-1945

134. Laberge R-M, Sun Y, Orjalo AV, Patil CK, Freund A, Zhou L et al (2015) MTOR regulates the pro-tumorigenic senescence-associated secretory phenotype by promoting IL1A translation. Nat Cell Biol 17:1049-1061

135. Zhenyukh O, Civantos E, Ruiz-Ortega M, Sánchez MS, Vázquez C, Peiró C et al (2017) High concentration of branched-chain amino acids promotes oxidative stress, inflammation and migration of human peripheral blood mononuclear cells via mTORC1 activation. Free Radic Biol Med 104:165-177

136. Cetin I, de Santis MSN, Taricco E, Radaelli T, Teng C, Ronzoni S et al (2005) Maternal and fetal amino acid concentrations in normal pregnancies and in pregnancies with gestational diabetes mellitus. Am J Obstet Gynecol 192 610-617

137. Enquobahrie DA, Denis M, Tadesse MG, Gelaye B, Ressom HW, Williams MA (2015) Maternal Early Pregnancy Serum Metabolites and Risk of Gestational Diabetes Mellitus. J Clin Endocrinol Metab 100:4348-4356

138. Rahimi N, Razi F, Nasli-Esfahani E, Qorbani M, Shirzad N, Larijani B (2017) Amino acid profiling in the gestational diabetes mellitus. J Diabetes Metab Disord 16:13. doi:10.1186/s40200-016-0283-1

139. Bentley-Lewis R, Huynh J, Xiong G, Lee H, Wenger J, Clish C et al (2015) Metabolomic profiling in the prediction of gestational diabetes mellitus. Diabetologia 58:1329-1332

140. de Seymour JV, Conlon CA, Sulek K, Villas Bôas SG, McCowan LME, Kenny LC et al (2014) Early pregnancy metabolite profiling discovers a potential biomarker for the subsequent development of gestational diabetes mellitus. Acta Diabetol 51:887-890

141. Nakamura S, Takamura T, Matsuzawa-Nagata N, Takayama H, Misu H, Noda H et al (2009) Palmitate induces insulin resistance in H4IIEC3 hepatocytes through reactive oxygen species produced by mitochondria. J Biol Chem 284:14809-14818

142. Keaney JF, Larson MG, Vasan RS, Wilson PWF, Lipinska I, Corey D et al (2003) Obesity and Systemic Oxidative Stress. Arterioscler Thromb Vasc Biol 23:434-439

143. Herrera E, Ortega-Senovilla H (2010) Disturbances in lipid metabolism in diabetic pregnancy – Are these the cause of the problem? Best Pract Res Clin Endocrinol Metab 24:515-525

144. Carone D, Loverro G, Greco P, Capuano F, Selvaggi L (1993) Lipid peroxidation products and antioxidant enzymes in red blood cells during normal and diabetic pregnancy. Eur J Obstet Gynecol Reprod Biol 51:103-109

145. Kinalski M, Śledziewski A, Telejko B, Kowalska I, Krętowski A, Zarzycki W et al (2001) Lipid Peroxidation, Antioxidant Defence and Acid-Base Status in Cord Blood at Birth: The Influence of Diabetes. Horm Metab Res 33:227-231

146. Cuffe JS, Xu ZC, Perkins AV (2017) Biomarkers of oxidative stress in pregnancy complications. Biomark Med 11:295-306

147. Karowicz-Bilinska A, Kędziora-Kornatowska K, Bartosz G (2007) Indices of oxidative stress in pregnancy with fetal growth restriction. Free Radic Res 41:870-873

148. Aydemir B, Baykara O, Cinemre FBS, Cinemre H, Tuten A, Kiziler AR et al (2016) LOX-1 gene variants and maternal levels of plasma oxidized LDL and

malondialdehyde in patients with gestational diabetes mellitus. Arch Gynecol Obstet 293:517-527

149. Shang M, Dong X, Hou L (2018) Correlation of adipokines and markers of oxidative stress in women with gestational diabetes mellitus and their newborns. J Obstet Gynaecol Res 44:637-646

150. León-Reyes G, Guzmán-Grenfell AM, Medina-Navarro R, Montoya-Estrada A, Moreno-Eutimio MA, Fuentes-García S et al (2018) Is gestational diabetes mellitus in obese women predicted by oxidative damage in red blood cells? Gynecol Endocrinol 34:995-1000

151. Arribas L, Almansa I, Miranda M, Muriach M, Romero FJ, Villar VM (2016) Serum Malondialdehyde Concentration and Glutathione Peroxidase Activity in a Longitudinal Study of Gestational Diabetes. PLoS One 11:e0155353. doi:10.1371/journal.pone.0155353

152. Karacay Ö, Sepici-Dincel A, Karcaaltincaba D, Sahin D, Yalvaç S, Akyol M et al (2010) A quantitative evaluation of total antioxidant status and oxidative stress markers in preeclampsia and gestational diabetic patients in 24–36 weeks of gestation. Diabetes Res Clin Pract 89:231-238

153. Parthasarathy S, Raghavamenon A, Garelnabi MO, Santanam N (2010) Oxidized Low-Density Lipoprotein. Methods Mol Biol 610:403-417

154. Ghaneei A, Yassini S, Ghanei ME, Shojaoddiny-Ardekani A (2015) Increased serum oxidized low-density lipoprotein levels in pregnancies complicated by gestational diabetes mellitus. Iran J Reprod Med 13:421-424

155. Pacher P, Beckman JS, Liaudet L (2007) Nitric oxide and peroxynitrite in health and disease. Physiol Rev 87:315-424

156. Lyall F, Gibson JL, Greer IA, Brockman DE, Eis AL, Myatt L (1998) Increased Nitrotyrosine in the Diabetic Placenta: Evidence for oxidative stress. Diabetes Care 21:1753-1758

157. Myatt L, Cui X (2004) Oxidative stress in the placenta. Histochem Cell Biol 122:369-382

158. Singh VP, Bali A, Singh N, Jaggi AS (2014) Advanced Glycation End Products and Diabetic Complications. Korean J PhysiolPharmacolOff J Korean Physiol Soc Korean Soc Pharmacol 18:1-14

159. Lappas M, Permezel M, Rice GE (2008) Advanced glycation endproducts mediate pro-inflammatory actions in human gestational tissues via nuclear factor-κB and extracellular signal-regulated kinase 1/2. J Endocrinol 193:269-277

160. Harsem NK, Braekke K, Torjussen T, Hanssen K, Staff AC (2008) Advanced glycation end products in pregnancies complicated with diabetes mellitus or preeclampsia. Hypertens Pregnancy 27:374-386

161. Tietze F (1969) Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: Applications to mammalian blood and other tissues. Anal Biochem 27:502-522

162. Peuchant E, Brun J-L, Rigalleau V, Dubourg L, Thomas M-J, Daniel J-Y et al (2004) Oxidative and antioxidative status in pregnant women with either gestational or type 1 diabetes. Clin Biochem. 37:293-298

163. Abbaspour N, Hurrell R, Kelishadi R (2014) Review on iron and its importance for human health. J Res Med Sci Off J Isfahan Univ Med Sci 19:164-174

164. Fenton HJH (1894) LXXIII.—Oxidation of tartaric acid in presence of iron. J Chem Soc Trans 65:899-910

165. Zhuang T, Han H, Yang Z (2014) Iron, Oxidative Stress and Gestational Diabetes. Nutrients 6:3968-3980

166. Zein S, Rachidi S, Hininger-Favier I (2014) Is oxidative stress induced by iron status associated with gestational diabetes mellitus? J Trace Elem Med Biol Organ Soc Miner Trace Elem GMS 28:65-69

167. Kalantar-Zadeh K, Rodriguez RA, Humphreys MH (2004) Association between serum ferritin and measures of inflammation, nutrition and iron in haemodialysis patients. Nephrol Dial Transplant 19:141-149

168. Lim M-K, Lee C-K, Ju YS, Cho YS, Lee M-S, Yoo B et al (2001) Serum ferritin as a serologic marker of activity in systemic lupus erythematosus. Rheumatol Int 20:89-93

169. Ford ES, Cogswell ME (1999) Diabetes and serum ferritin concentration among U.S. adults. Diabetes Care 22:1978-1983

170. Fumeron F, Péan F, Driss F, Balkau B, Tichet J, Marre M et al (2006) Ferritin and transferrin are both predictive of the onset of hyperglycemia in men and women over 3 years: the data from an epidemiological study on the Insulin Resistance Syndrome (DESIR) study. Diabetes Care 29:2090-2094

171. Chen J, Tan B, Karteris E, Zervou S, Digby J, Hillhouse EW et al (2006) Secretion of adiponectin by human placenta: differential modulation of adiponectin and its receptors by cytokines. Diabetologia 49:1292. doi:10.1007/s00125-006-0194-7

172. Khambalia AZ, Aimone A, Nagubandi P, Roberts CL, McElduff A, Morris JM et al (2016) High maternal iron status, dietary iron intake and iron supplement use in pregnancy and risk of gestational diabetes mellitus: a prospective study and systematic review. Diabet Med 33:1211-1221

173. Amiri FN, Basirat Z, Omidvar S, Sharbatdaran M, Tilaki KH, Pouramir M (2013) Comparison of the serum iron, ferritin levels and total iron-binding capacity between pregnant women with and without gestational diabetes. J Nat SciBiol Med 4:302-305

174. Derbent AU, Simavli SA, Kaygusuz I, Gumus II, Yılmaz S, Yildirim M et al (2013) Serum hepcidin is associated with parameters of glucose metabolism in women with gestational diabetes mellitus. J Matern-Fetal Neonatal Med Off J Eur Assoc Perinat Med Fed Asia Ocean Perinat Soc Int Soc Perinat Obstet. 2013;26:1112-1115

175. Akhlaghi F, Bagheri SM, Rajabi O (2012) A Comparative Study of Relationship between Micronutrients and Gestational Diabetes. ISRN Obstet Gynecol 2012:470419. doi: 10.5402/2012/470419

176. Park S, Park JY, Lee JH, Kim S-H (2015) Plasma levels of lysine, tyrosine, and valine during pregnancy are independent risk factors of insulin resistance and gestational diabetes. Metab Syndr Relat Disord 13:64-70

177. Chen X, Scholl TO, Stein TP (2006) Association of Elevated Serum Ferritin Levels and the Risk of Gestational Diabetes Mellitus in Pregnant Women: The Camden Study. Diabetes Care 29:1077-1082

Fig. 1 Women who are obese have insulin resistance, down-regulation of adiponectin and up-regulation of leptin, resistin and retinol-binding protein-4 (RBP4), with features of chronic inflammation characterised by increased tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and interleukin-12 (IL-12). Fetal and placental hormones may contribute to inflammation and increase insulin resistance

Fig. 2 Schematic diagram indicating the positive loop between pro-inflammatory cytokines macrophages and monocytes in the presence of enlarged adipocytes

Fig. 3 Schematic diagram summarising the role of inflammation in target tissues. Elevated pro-inflammatory cytokine mediate JNK and NF-κB which inhibit the AMPK pathway and induce production of pro-inflammatory cytokines. JNK and NF-κB are also activated by TLR-4 and IL-1R in response to free fatty acids. NF-κB and BCAAs activate the mTOR pathway which in-turn inhibits the function of IRS and thus reduces insulin signalling. Anti-inflammatory markers induce the AMPK pathway and thus increase expression of insulin genes. Oxidative stress occurs in mitochondria due to elevated ROS

Table 1 Diagnostic criteria for gestational diabetes

Glucose (mM)	**IADPSG**	**WHO**	**NICE**	**NDDG**	**ADA 2 steps**	**ADA 1 step**
Test	75 g OGTT	75 g OGTT	75 g OGTT	100 g OGTT	50 g non-fasting OGTT	75 g OGTT
0 hours	5.1	7.0	5.6	5.8		≥ 5.1 mM
1 hour	10.0	-	-	10.6	≥ 10 mM	≥ 10 mM
2 hours	8.5	7.8	7.8	9.2		≥ 8.5 mM
3 hours				8.0		
Number of abnormal values needed for the diagnosis of GDM	≥ 1	≥ 1	≥ 1	≥ 2	If 1 hour ≥ 10 mM go on to 3 hr. 100g OGTT	≥ 1

Values are presented in mmol/L. NDDG: National Diabetes Data Group; OGTT: oral glucose tolerance test; IADPSG: The International Association of Diabetes and Pregnancy Study Groups. WHO: World Health Organization, NICE: National Institute for Health Care Excellence, NDDG: National Diabetes Data Group

Received: 27 March 2024 | Revised: 14 August 2024 | Accepted: 3 September 2024

DOI: 10.1002/ijgo.15914

CLINICAL ARTICLE

Obstetrics

Performance of point-of-care glucose testing for the diagnosis of gestational diabetes in South Africa

L. Khambule[1] | C. Chikomba[2,1] | Y. Adam[3] | L. Khan[1] | C. Haldane[4] | B. Vetter[4] | J. George[5]

[1]Department of Chemical Pathology, University of the Witwatersrand, Johannesburg, South Africa

[2]Department of Chemical Pathology, National Health Laboratory Service, Johannesburg, South Africa

[3]Department of Obstetrics and Gynaecology, Chris Hani Baragwanath Academic Hospital, University of the Witwatersrand, Johannesburg, South Africa

[4]FIND, Geneva, Switzerland

[5]Wits Diagnostic and Innovation Hub, National Health Laboratory Service, Johannesburg, South Africa

Correspondence
J. George, Wits Diagnostic and Innovation Hub, Wits Health Consortium and University of Witwatersrand, 31 Princess of Wales Terrace, Parktown, Johannesburg 2193, South Africa.
Email: jaya.george@nhls.ac.za

Funding information
Swiss Agency for Development and Cooperation

Abstract

Objective: Lack of accessibility to oral glucose tolerance tests (OGTTs) in South Africa means many pregnant women go without testing for gestational diabetes mellitus (GDM). This study evaluated point-of-care (POC) glucometers against the laboratory-based glucose method in pregnant women.

Methods: This was a cross-sectional study on pregnant women attending the prenatal clinic in Johannesburg who were recommended for the OGTT. OGTTs were conducted as per International Association of Diabetes and Pregnancy Study Groups (IADPSG) guidelines. Women who consented to the study donated both venous and capillary blood for laboratory-based and POC glucose measurements using seven POC glucometers: I-STAT, Xpress, LDX, VivaChek-Ino, Accu-Chek Active, StatStrip, and Codefree. By assessing sensitivity, specificity, and area under the receiver operating characteristic curve (AUC) and comparing Bland–Altman plots, the diagnostic accuracy of each glucose meter was compared with the reference method, the laboratory-based glucose method.

Results: Data were analyzed for 1076 pregnant women. Based on OGTT testing, 83 women had GDM (7.7%). Overall, the POC glucometers performed poorly, with sensitivity ranging from 17.6% to 87.18% and specificity ranging between 62.7% and 99.8%. The AUC ranged from 0.59 to 0.79. All POC glucometers showed moderate to poor reliability. Laboratory-based fasting plasma glucose (FPG) surpassed the POC glucometers in sensitivity, specificity, and AUC, with values of 94.0%, 100%, and 0.98, respectively.

Conclusion: We demonstrated that laboratory-based FPG has the potential to be used as a diagnostic test for GDM and that the POC glucometers cannot replace OGTT laboratory-based measurements.

KEYWORDS
diagnostic accuracy, fasting plasma glucose, gestational diabetes mellitus, point-of-care testing

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
© 2024 The Author(s). *International Journal of Gynecology & Obstetrics* published by John Wiley & Sons Ltd on behalf of International Federation of Gynecology and Obstetrics.

1 | INTRODUCTION

Gestational diabetes mellitus (GDM), defined as hyperglycemia first discovered in pregnancy, is one of the most common complications of pregnancy, affecting about 16% of pregnant women worldwide,[1] with the majority of cases (87.6%) occurring in low- and middle-income countries (LMICs).[2] In 2011, the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study showed that blood glucose thresholds below the conventional GDM diagnostic criteria were linked to adverse maternal and neonatal outcomes.[3] Since then, large-scale meta-analyses have demonstrated that women with GDM are more likely to develop type 2 diabetes mellitus (T2DM) and cardiovascular diseases (CVD).[4] Children born to women with GDM are at risk of macrosomia and hypoglycemia after birth, as well as long-term complications such as childhood obesity, early onset of T2DM and CVD.[5] The first step to optimum GDM care and T2DM prevention/delay is the diagnosis.[6] In 2015, the International Federation of Gynecology and Obstetrics (FIGO) published GDM consensus guidelines recommending a universal screening approach, the oral glucose tolerance test (OGTT) being the gold standard for screening and diagnosing GDM.[6] While these recommendations are widely recognized, LMICs like South Africa face many challenges to GDM screening, including individual, healthcare work-related, and health-system barriers.[7]

In South Africa, the prevalence of GDM is estimated to be about 10%.[8] However, this may be an underestimate because only women at high risk for GDM are screened.[8] Diagnosis of GDM in South Africa is made based on an OGTT conducted at 24–28 weeks of gestation.[8] However, the test involves laboratory-based glucose readings and there are only a few facilities that provide this service.[9] The point-of-care (POC) testing offers many operational advantages to the OGTT such as reduced turnaround time, eradication of pre-analytical sample handling, stability errors, and prompt health interventions.[10] In South Africa, strip-based glucometers are readily available for self-monitoring among diabetics; however, strips are affected by external variables such as humidity, temperature, hematocrit, medicines, and hypotension.[11] Cassette-based devices minimize the limitations of strips.[12] However, the evidence for the performance of the glucometers in the various clinical scenarios has been inconsistent.[13,14] Therefore, this study aims to determine the accuracy of seven POC glucometers in diagnosing GDM among pregnant women in South Africa. We anticipate that the study will identify POC glucometers that accurately detect GDM.

2 | MATERIALS AND METHODS

2.1 | Study design

This cross-sectional study was conducted between July 13, 2022 and February 28, 2023 among pregnant women who attend the antenatal clinic at Chris Hani Baragwanath Academic Hospital (CHBAH).

Institutional ethics approval was obtained at the University of Witwatersrand Human Research Ethics Committee (Medical) (clearance no: M220219). All women provided written informed consent (Figures S1 and S2). The study followed the Standards for Reporting Diagnostic accuracy studies (STARD) guidelines (see Table S1 for the checklist).

2.2 | Study population

Based on an estimated GDM prevalence of 10%,[8] the minimum sample size was 1070, to detect sensitivity and specificity of at least 0.80 with a power of 80% and alpha threshold of 0.05.[15] Eligible women were between 24 and 32 weeks of gestation and had one or more conventional risk factors for GDM as per the Society of Endocrinology, Metabolism and Diabetes of South Africa (SEMDSA) guidelines.[16] The women had to fast the night before blood withdrawal for at least 8 h.

Women who were <18 years old, non-fasted, acutely ill, or on high-dose vitamin C (>2000 mg/day) or corticosteroid therapy were excluded, as were those with previous bariatric surgery, prediabetics, and those who smoked or vomited during the OGTT. Prediabetics were defined as fasting plasma glucose (FPG) >7 mmol/L and/or glycated hemoglobin (HbA1c) >6.5% and/or glucose level >11.1 mmol/L at 2-h post-glucose load.[16]

2.3 | Medical history and sample collection

Data on women's age and gestational age by last date of menstrual cycle, confirmed by ultrasound scans, parity, gravidity, history of miscarriages, medication, and body weight were collected on the day of the OGTT test by L.K. and L.K.

2.4 | Sample collection

Capillary and venous plasma glucose samples were collected at three OGTT time points: fasting, and 1 and 2 h post-glucose load. Each time, a finger was pricked once, and the blood was collected using a capillary tube to analyze the glucometers except for I-STAT and LDX glucometers as these required a larger volume of blood. Venous blood was collected in sodium fluoride and sodium citrate tubes, immediately analyzed on I-STAT and LDX, and the rest of the blood was used for laboratory-based glucose measurement. Information on the POC glucometers is reported in Table S2.

2.5 | Oral glucose tolerance test

The OGTTs were conducted by qualified nurses and diagnosed as per International Association of Diabetes and Pregnancy Study Groups (IADPSG) guidelines.[17] However, in this study, samples were kept in an ice slurry and separated within 10 min after the end of the OGTT. Glucose was measured in the central laboratory using the Roche Cobas 8000/502 (Roche Diagnostics, Mannheim, Germany).

2.6 | Statistical analysis

Statistical analyses and graphical representations for method agreement and reliability were conducted using MedCalc software v. 22.021 (Belgium, Germany) and the rest was performed using STATA 18 software (StataCorp, College Station, TX, USA). A *P*-value <0.05 was assumed to be statistically significant. Shapiro–Wilk test was used to test for normality, normally distributed data was reported by means ± standard deviation, and non-normally distributed were reported as medians and interquartile range (IQR). Categorical variables were expressed as sample numbers and percentages. Demographic and clinical characteristics in the GDM and non-GDM groups were compared using Student *t*-test (for normally distributed data) or the Wilcoxon rank-sum test (for skewed data). Categorical data were analyzed using a chi-squared or Fisher exact test where expected frequencies were <5.

Methods were compared by Pearson correlation coefficient (PCC) and scatterplots with the least-squares best-fit line and 95% confidence interval (CI) band, intra-rater class coefficient (ICC), and Bland–Altman (BA) plots.[18] The ICC estimates and their 95% CIs were based on single-measurement, absolute-agreement, two-way random-effects model.

Sensitivity, specificity, negative predictive values (NPVs), positive predictive values (PPVs), and area under the receiver operating characteristic curve (AUC) were calculated to determine the diagnostic accuracy of each POC glucometer. The laboratory-based OGTT method and GDM diagnosis by IADPSG criteria were used as the reference method. Three criteria for GDM diagnosis were analyzed: (1) GDM diagnosis by OGTT (as per IADPSG criteria); (2) FPG at 5.1 mmol/L; and (3) FPG at 4.8 mmol/L. Both reference and classification methods were binary variables (classified as GDM yes/no). Criteria 2 and 3 were guided by reports in the literature indicating that FPG can potentially diagnose GDM.[9,10]

An ICC, PCC, and AUC near the value of 1.0 suggest a high degree of method reliability, correlation, or accuracy, respectively. For BA plots, POC glucometers with a mean difference of 1.3% or less and with narrow 95% limits of agreements were deemed optimum.[19]

3 | RESULTS

3.1 | Study population

In total, 1144 women were recruited, of whom 68 (5.94%) were excluded from the study. Reasons for exclusion were: incomplete OGTT (6, 0.52%), withdrew from the study (2, 0.17%), vomited (1, 0.09%), <18 years (1, 0.09%), and prediabetic (9, 0.79%). Among the 1125 women included in the study, 49 (4.36%) had incomplete laboratory-based glucose measurements for the following reasons: the specimen was insufficient, grossly hemolyzed, lipidemic, or clotted. Therefore, data were analyzed for 1082 women (Figure 1).

The mean age of this population was 35 years (IQR 29–39). Mean gestational age was 25 weeks (IQR 21–29). Based on OGTT testing using the IADPSG criteria, 83 (7.7%) women had GDM.

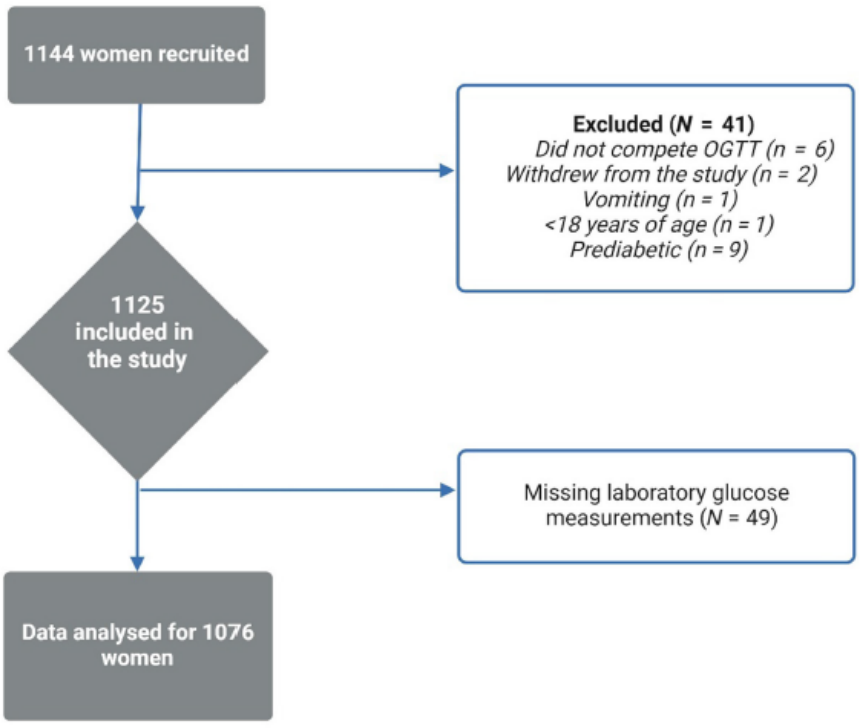


FIGURE 1 Diagram of participant recruitment. OGTT, oral glucose tolerance test.

Women with GDM had a significantly higher median (IQR) weight (89 [76–104] vs 80 [67–95] kg, $P=0.001$). Of note, there were no significant differences between groups in age, parity, and miscarriages ($P>0.05$) (Table 1).

3.2 | Method agreement and reliability analysis

Average glucose levels measured with the seven POC glucometers are shown in Table 2. Data were normally distributed, and the PCC

TABLE 1 Clinical characteristics, overall and by gestational diabetes mellitus (GDM) status.

Variable	Total observations	All	Non-GDM	GDM	*P*-value
Age (years)	1048	35 (29;39)	35 (29;39)	37 (31;39)	0.125
Gestational age (weeks)	1015	25 (21;29)	25 (21;29)	24 (20;28)	0.492
Miscarriages (*n*)	1034	0 (0;1)	0 (0;1)	0 (0;1)	0.889
Parity (*n*)	1034	2 (1;3)	2 (1;3)	2 (1;2)	0.198
Gravidity (*n*)	1034	4 (2;5)	4 (3;5)	3 (2;4)	0.255
Weight (kg)	1062	80 (68;95)	80 (67;95)	89 (73;101)	0.001
Fasting glucose from the laboratory (mmol/L)	1076	4.2 (3.9;4.5)	4.1 (3.9;4.4)	5.5 (5.2;5.8)	<0.0001
OGTT, 1-h glucose from the laboratory (mmol/L)	1076	5.7 (4.9;6.8)	5.6 (4.9;6.6)	8.2 (6.2;9.7)	<0.0001
OGTT, 2-h glucose from the laboratory (mmol/L)	1076	5.2 (4.5;6.0)	5.1 (4.5;5.8)	7.0 (5.8;8.6)	<0.0001

Note: Data presented are median (IQR) unless stated otherwise.
Abbreviation: OGTT, oral glucose tolerance test.

TABLE 2 Average glucose measurements of the point-of-care (POC) glucose testing at each oral glucose tolerance test (OGTT) time point.

		Median glucose level (mmol/L)		
Device	Time point	All participants	Non-GDM	GDM
Laboratory	Fasting	4.2 (3.9–4.5)	4.1 (3.9–4.4)	5.5 (5.2–6.0)
	OGTT, 1 h	5.8 (6.9–5.8)	5.7 (4.9–6.6)	8.2 (6.2–10.4)
	OGTT, 2 h	5.2 (4.5–6.0)	5.1 (4.5;5.8)	7.1 (5.9–8.8)
I-STAT	Fasting	3.7 (3.4–4.0)	3.6 (3.4–3.9)	4.5 (4.1–5.1)
	OGTT, 1 h	4.9 (4.1–5.9)	4.8 (4.1–5.7)	7.2 (5.6–8.8)
	OGTT, 2 h	4.4 (3.9–5.1)	4.3 (3.8–5.0)	5.9 (4.9–7.4)
Xpress	Fasting	4.5 (4.1;4.9)	4.4 (4.1–4.9)	5.7 (4.9–6.3)
	OGTT, 1 h	7.2 (6.3–8.3)	7.0 (6.2–8.0)	9.0 (7.7–11.2)
	OGTT, 2 h	6.4 (5.7–7.3)	6.3 (5.6–7.0)	8.4 (7.0–10.1)
LDX	Fasting	3.8 (3.5–4.1)	3.7 (3.5–4.0)	4.6 (4.2–5.2)
	OGTT, 1 h	5.1 (4.2–5.9)	4.9 (4.2–5.7)	7.2 (5.7–8.5)
	OGTT, 2 h	4.5 (3.9–5.1)	4.4 (3.9–5.0)	6.0 (5.1–7.3)
VivaChek Ino	Fasting	4.7 (4.3–5.1)	4.6 (4.3–5.0)	6.1 (5.2–6.5)
	OGTT, 1 h	7.5 (6.5–8.6)	7.3 (6.5–8.3)	9.7 (8.3–11.3)
	OGTT, 2 h	6.6 (5.9–7.5)	6.5 (5.8–7.3)	9.0 (7.3–10.6)
Accu-Chek Active	Fasting	4.5 (4.1–4.9)	4.4 (4.1–4.8)	5.5 (4.8–6.3)
	OGTT, 1 h	7.1 (6.1–8.2)	7.0 (6.0–8.0)	9.6 (8.1–10.9)
	OGTT, 2 h	6.2 (5.4–7.1)	6.2 (5.3–6.9)	8.1 (6.8–10.0)
StatStrip	Fasting	4.3 (3.9–4.8)	4.2 (3.8–4.7)	5.4 (4.9–5.9)
	OGTT, 1 h	6.9 (6.0–8.0)	6.3 (5.9–7.8)	9.0 (7.6–10.7)
	OGTT, 2 h	6.1 (5.4–7.0)	6.1 (5.3–6.8)	8.0 (6.8–9.5)
Codefree	Fasting	4.8 (4.5;5.3)	4.7 (4.4–5.2)	5.8 (5.3–6.6)
	OGTT, 1 h	7.6 (6.6, 8.9)	7.5 (6.5–8.6)	10.0 (8.0–11.7)
	OGTT, 2 h	6.7 (6.0–7.6)	6.6 (6.0–7.4)	8.7 (7.5–11.0)

Note: Data are presented as median (interquartile range).
Abbreviation: GDM, gestational diabetes.

ranged from 0.62 for Codefree at fasting to 0.87 for I-STAT and LDX at the 1-h time point ($P < 0.001$ for all; Figures 2–4).

According to the BA plots, StatStrip had the optimum mean differences (0.20%) at fasting and a desirable mean difference (−2.82%) at 1-h post-glucose load. Codefree had the highest mean difference (23.1%) at 1-h post-glucose load. All POC glucometers, including StatStrip, had wide 95% limits of agreement at all time points. The postprandial glucose readings showed the biggest mean percentage difference. According to the ICC estimates, all POC glucometers had moderate to poor reliability with a wide 95% CIs (Table 3; Figures 5–7).

3.3 | Accuracy of POC glucometers

For criterion '1', GDM diagnosis by OGTT (as per IADPSG criteria, sensitivity [95% CI] varied from 17.57 (15.17–19.96) for I-STAT to 87.18 (85.14–89.22) for Codefree) (Table 4). Specificity (95% CI) was poorest for Codefree at 62.71 (59.76–65.66) and was highest for I-STAT at 99.78 (99.48–100). The NPVs (95% CI) were good for all POC devices and ranged from 93.76 (92.24–95.15) for LDX to 98.35 (97.58–99.13) for the Codefree. PPVs (95% CI) ranged between 16.08 (13.83–18.32) for Codefree and 86.67 (84.53–88.81) for the I-STAT. The AUC (95% CI) ranged from 0.59 (0.54–0.63) for I-STAT to 0.79 (0.74–0.85) for Accu-Chek.

The accuracy of POC glucometers when using FPG glucose alone was lower than when all three time points were used (Tables S3 and S4). However, for laboratory-based FPG at 5.1 mmol/L threshold, sensitivity (95% CI) and specificity (95% CI) were 100 (100–100) and 99.50 (99.08–99.2), respectively (Table S4).

4 | DISCUSSION

This study, conducted among pregnant women in a tertiary care hospital in South Africa, investigated the accuracy of seven POC

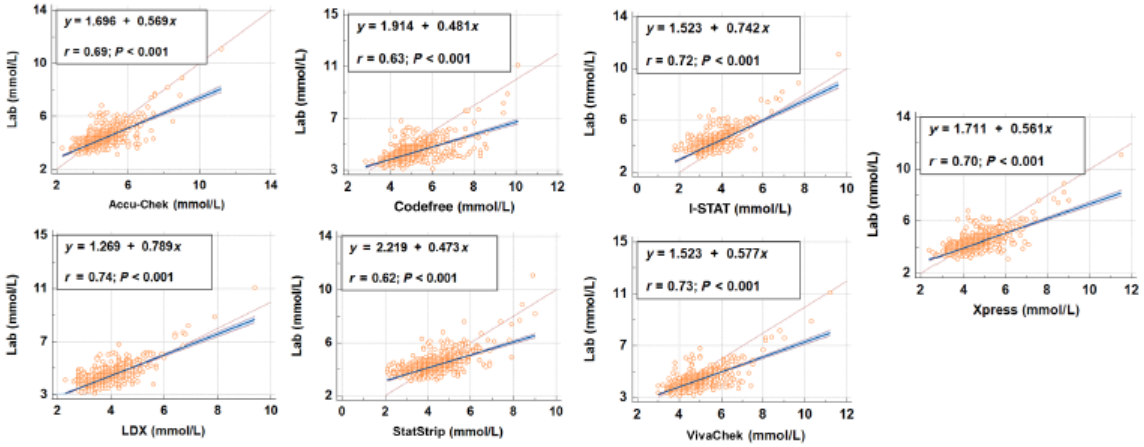


FIGURE 2 Scatterplots with the least-squares best-fit line +95% confidence band (blue) and line $y = x$ (orange) for each point-of-care glucometer against laboratory-based glucose measurements at fasting/0 h.

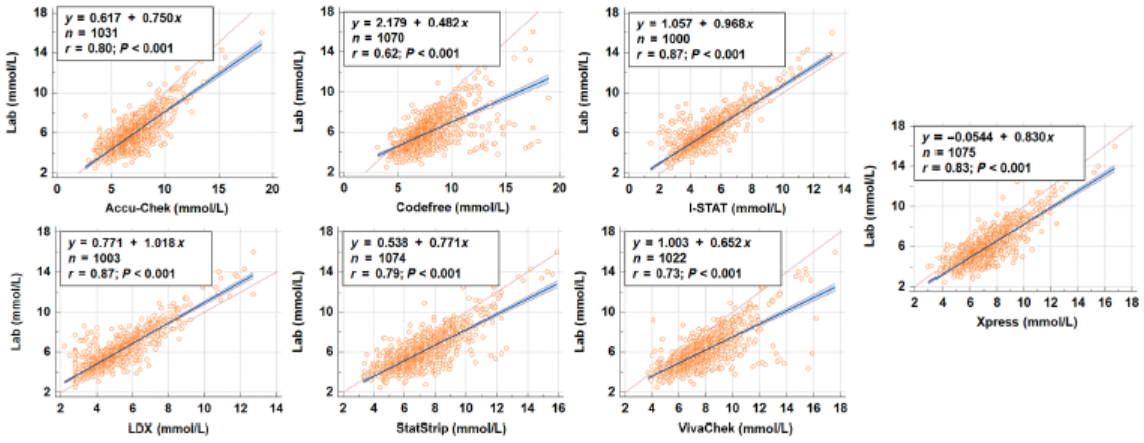


FIGURE 3 Scatterplots with the least-squares best-fit line +95% confidence band (blue) and line $y = x$ (orange) for each point-of-care glucometer against laboratory-based glucose measurements at 1-h post-glucose load.

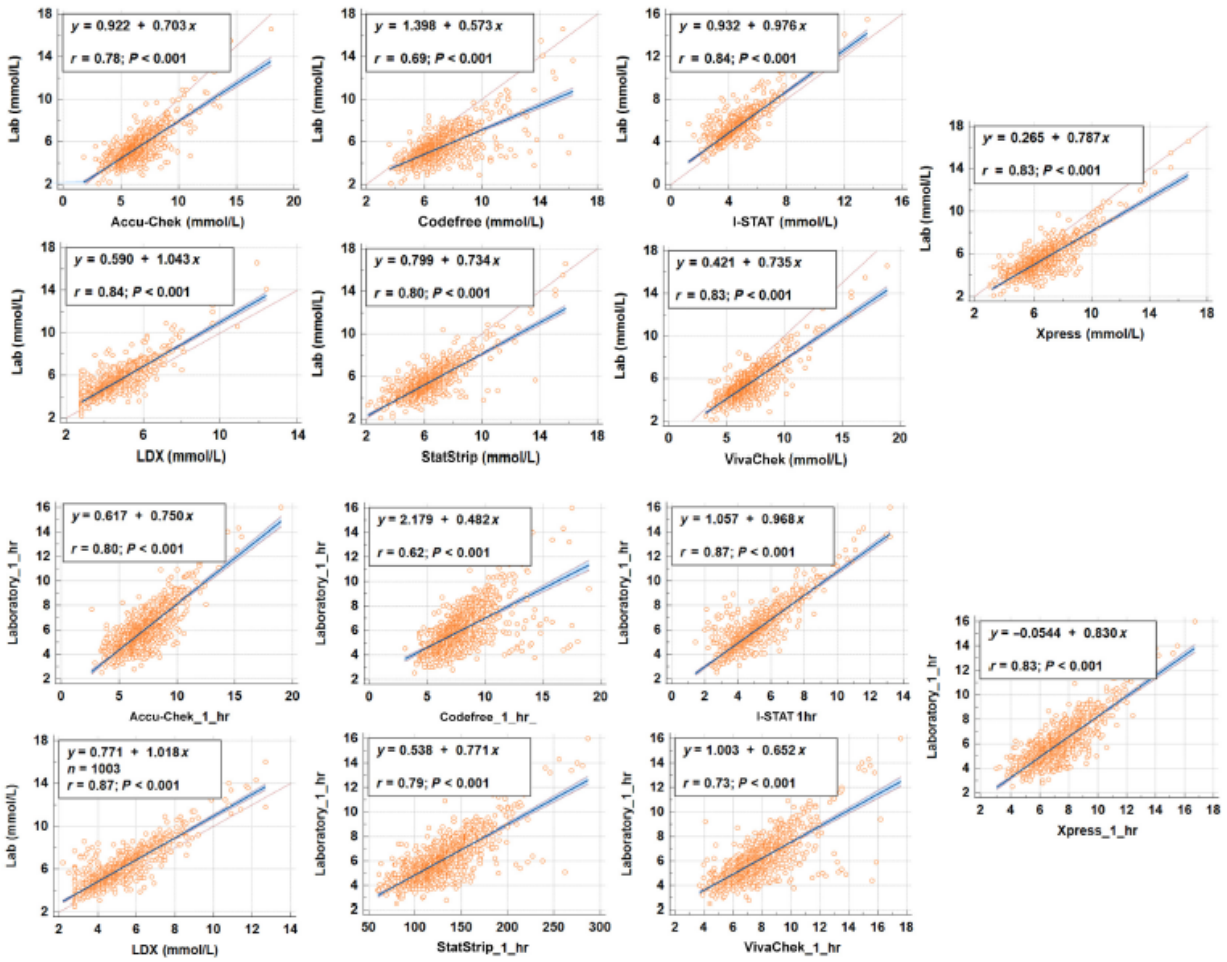


FIGURE 4 Scatterplots with the least-squares best-fit line +95% confidence band (blue) and line $y=x$ (orange) for each point-of-care glucometer against laboratory-based glucose measurements at 2-h post-glucose load.

glucometers in diagnosing GDM. Compared with laboratory diagnosis, the POC glucose testing performed with a sensitivity ranging between 18% and 87% and a specificity ranging between 63% and 99%. The number of diagnoses that would have been missed with the POC glucose testing ranged from 10 to 61.

In vitro glycolysis is a key consideration with laboratory GDM diagnosis and pre-analytical protocols should be designed to minimize glycolysis. As a result, the HAPO research included strict pre-analytical procedures for sample collection and processing.[3] In South Africa, most OGTT testing during pregnancy is performed in tertiary academic or provincial tertiary hospitals with variable proximity to a laboratory. Nurses are unable to centrifuge samples immediately and transport on ice slurry rarely happens. Pre-analytical error is likely significant in these settings, underpinning the need for POC testing. Considering that POC glucometers are readily available and, in most cases, less expensive than conventional laboratory-based measurements, POC testing is an economically viable choice.[20] However, our study shows a lack of diagnostic accuracy among POC glucometers, with the number of GDM cases diagnosed based on glucose meter readings varying widely, which indicates that the POC glucose testing cannot be used interchangeably. Such variation has been reported previously in a comparative performance of five POC glucometers.[13] Our results are in line with several other studies which suggest that POC glucose testing compares poorly with laboratory measures for the diagnosis of GDM.[21,22] In a study among 529 pregnant women attending a primary healthcare clinic in Johannesburg, the Accu-Chek Active glucose meter diagnosed GDM with a sensitivity of 27.0% and a specificity of 89.4%, which is not very different from our PPV and NPV results.[23] In contrast to the above study, Gallardo-Rincón et al. reported a sensitivity and specificity of 89.5% and 66.6%, respectively, for Accu-Chek Instant.[24] According to Daly et al., lowering the diagnostic threshold for FPG from 5.1 to 4.8 mmol/L increased sensitivity from 51% to 92.5% but decreased specificity from 100% to 76.5%.[10] Interestingly, the same authors also used a regression equation to predict plasma glucose levels based on glucose meter readings, indicating that

TABLE 3 Average difference, Bland–Altman 95% limits of agreement, intraclass correlation coefficient (ICC, 95% confidence interval [CI]) of point-of-care glucometers at three time points.

	Observations (*n*)	Differences (% [SD])	95% Limits of agreement (%)	ICC (95% CI)
At fasting (0 h)				
Accu-Chek Active	1021	−4.86 (12.1)	−28.5–19.0	0.525 (0.364–0.639)
Codefree	1036	−12.2 (10.8)	−33.3–9.10	0.332 (−0.024–0.572)
I-STAT	976	16.6 (15.6)	−17.6–50.8	0.385 (−0.067–0.657)
LDX	987	13.0 (13.0)	−12.5–38.5	0.458 (0.013–0.703)
StatStrip	1062	0.20 (17.7)	−34.7–35.1	0.500 (0.452–0.544)
VivaChek	1001	−9.62 (10.3)	−29.9–10.6	0.450 (0.042–0.674)
Xpress	1071	−5.34 (12.2)	−29.2–18.5	0.521 (0.332–0.648)
At 1 h post-glucose load				
Accu-Chek Active	1008	−15.8 (16.2)	−47.5–16.0	0.592 (−0.011–0.811)
Codefree	1049	−23.1 (15.0)	−52.4–6.21	0.344 (−0.066–0.613)
I-STAT	978	20.0 (26.0)	−31.0–70.9	0.706 (0.098–0.875)
LDX	981	18.1 (21.2)	−23.5–59.7	0.708 (0.126–0.873)
StatStrip	1051	−2.82 (22.3)	−46.6–41.0	0.599 (0.058–0.806)
VivaChek	999	−21.2 (14.4)	−49.6–7.03	0.438 (−0.079–0.717)
Xpress	1052	−17.8 (12.9)	−43.1–7.57	0.587 (−0.068–0.830)
At 2 h post-glucose load				
Accu-Chek active	988	−14.1 (15.7)	−44.8–16.6	0.569 (0.049–0.784)
Codefree	1033	−21.7 (13.2)	−47.5–4.0	0.361 (−0.085–0.647)
I-STAT	980	20.0 (23.6)	−26.2–66.2	0.624 (0.015–0.834)
LDX	976	18.0 (18.3)	−17.9–54.0	0.631 (0.045–0.833)
StatStrip	1051	−12.9 (14.4)	−41.1–15.2	0.606 (0.096–0.804)
VivaChek	993	−19.8 (12.0)	−43.3–3.69	0.490 (−0.094–0.777)
Xpress	1057	−16.8 (12.2)	−40.7–7.01	0.559 (−0.073–0.812)

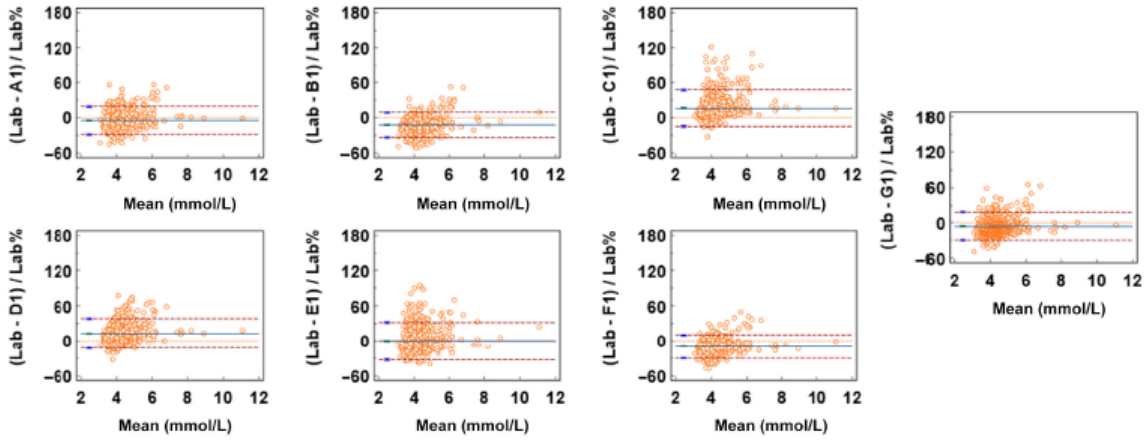


FIGURE 5 Bland–Altman plots (mean difference [blue] and 95% limit of agreement [red]) of each point-of-care glucometer against laboratory-based measurements at fasting. A1, Accu-Chek active; B1, Codefree; C1, I-STAT; D1, LDX; E1, StatStrip; F1, VivaChek; G1, Xpress.

such an approach may be acceptable in LMICs where plasma samples are subject to glycolysis.[10] In the present study, the greatest differences between POC-measured and laboratory-measured glucose levels were at the 1-hour time point which may be an indication of poor performance of the POC glucose testing at higher glucose concentrations.

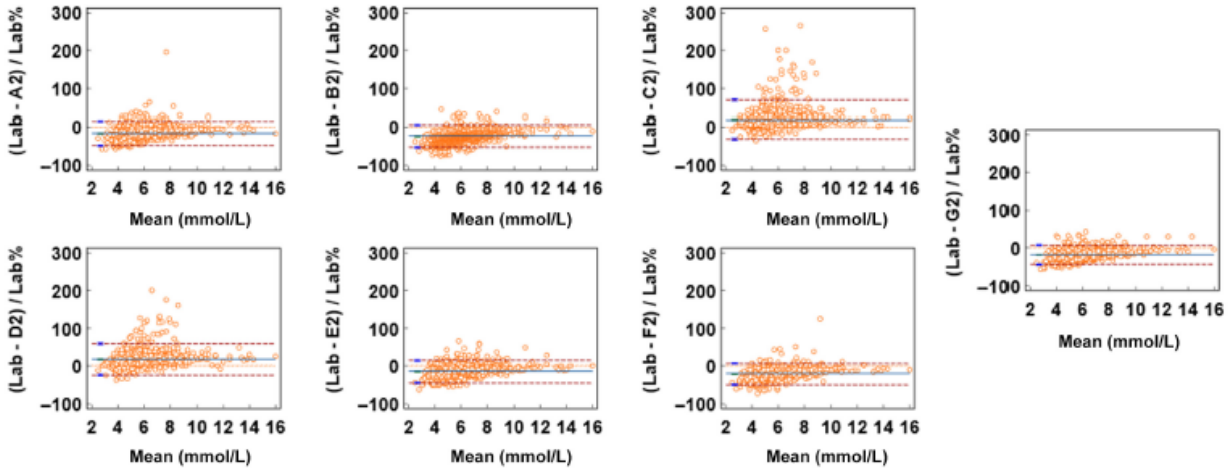


FIGURE 6 Bland–Altman plots (mean difference [blue] and 95% limit of agreement [red]) of each point-of-care glucometer against laboratory-based measurements at 1-h post-glucose load. A2, Accu-Chek active; B2, Codefree; C2, I-STAT; D2, LDX; E2, StatStrip; F2, VivaChek; G2, Xpress.

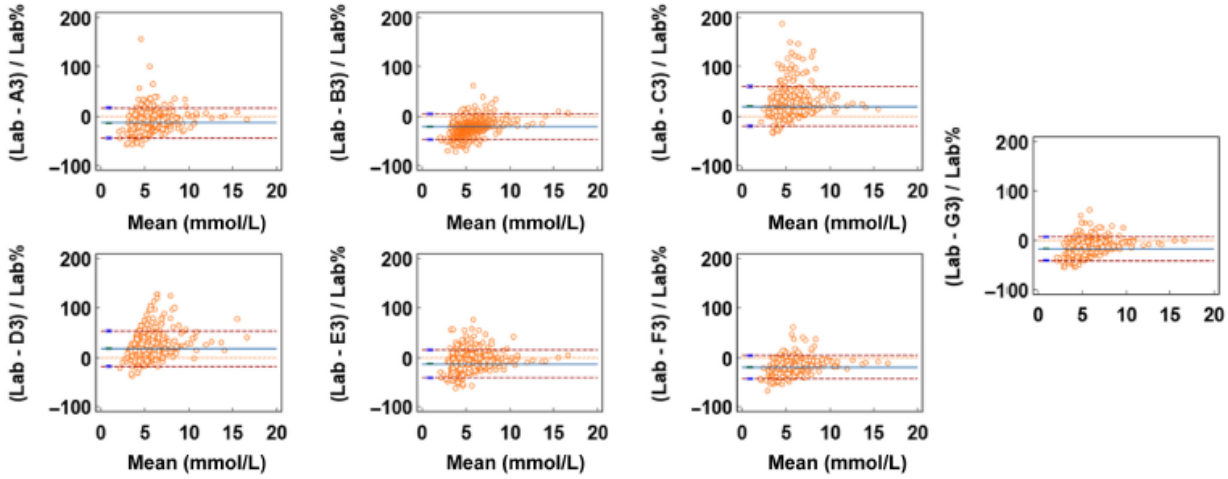


FIGURE 7 Bland–Altman plots (mean difference [blue] and 95% limit of agreement [red]) of each point-of-care glucometer against laboratory-based measurements at 1-h post-glucose load. A3, Accu-Chek active; B3, Codefree; C3, I-STAT; D3, LDX; E3, StatStrip; F3, VivaChek; and G3, Xpress.

Another option for GDM screening is the use of FPG measurements. In the current study, the measurement of FPG showed good sensitivity and specificity for diagnosing GDM when samples were analyzed in the laboratory. These findings are in line with those from several other studies conducted in South Africa which showed high accuracy of laboratory fasting glucose in diagnosing GDM.[13,23] In addition, the FPG may be a better GDM screening test than universal OGTT testing due to its quick turnaround and simplicity of use. However, it is important to determine the diagnostic performance and optimal cut-off of FPG in the screening of GDM. In addition, it must be noted that pre-analytical errors and glycolysis are also risks with laboratory analysis of FPG. In addition, the use of laboratory-based FPG would not address the geographical barriers in the country, as the women would still need to travel to the clinic to get tested.

Given the high NPV of the glucometers at fasting, which suggests the likelihood of not having GDM when tested negative, we recommend the use of POC for screening for GDM using FPG at 5.1 mmol/L and reserve OGTTs (by laboratory-based glucose measurements) for women who test positive. Furthermore, we recommend that future studies should perform modeling analyses to evaluate the impact of having access to POC GDM screening versus being undiagnosed/untreated for GDM entirely. Of note, if POC glucose testing is to be used for diagnosis, manufacturers should meet the performance specifications as recommended by the Milan hierarchy.[25]

Our study had several limitations. The first was the length of time between the collection of blood and analysis in the central laboratory. During this time, there was a risk of glycolysis. POC analysis was sometimes interrupted mid-OGTT due to POC glucometer malfunctions or

TABLE 4 The diagnostic accuracy of each point-of-care glucometer's oral glucose tolerance test (OGTT) results as compared with laboratory-based OGTT measurements.

	Accu-Chek active	Codefree	I-STAT	LDX	StatStrip	VivaChek	Xpress
Observations	982	1030	969	964	1076	983	1052
PPV (% [95% CI])	28.25 (25.44–31.07)	16.08 (13.83–18.32)	86.67 (84.53–88.81)	83.33 (80.98–85.69)	27.06 (24.41–29.72)	17.96 (15.56–20.36)	24.36 (21.77–26.96)
NPV (% [95% CI])	98.02 (97.15;98.89)	98.35 (97.58–99.13)	93.61 (92.07–95.15)	93.76 (92.24–95.29)	97.20 (96.22–98.19)	98.00 (97.12–98.87)	98.07 (97.24–98.90)
Sensitivity (% [95% CI])	80.77 (78.30–83.23)	87.18 (85.14–89.22)	17.57 (15.17–19.96)	20.27 (17.73–22.81)	71.08 (68.38–73.79)	82.19 (79.80–84.58)	81.71 (79.37–84.04)
Specificity (% [95% CI])	82.30 (79.91–84.69)	62.71 (59.76–65.66)	99.78 (99.48–100.00)	99.66 (99.30–100.00)	83.99 (81.80–86.18)	69.89 (67.02–72.76)	78.56 (76.08–81.04)
False-positive (*n*)	160	355	2	3	159	274	208
False-negative (*n*)	15	10	61	59	24	13	15
True-positive (*n*)	63	68	13	15	59	60	67
True-negative (*n*)	744	597	893	887	834	636	762
AUC	0.79 (0.74–0.85)	0.74 (0.69–0.79)	0.59 (0.54–0.63)	0.59 (0.55–0.64)	0.76 (0.69–0.82)	0.74 (0.68–0.80)	0.78 (0.73–0.84)

Abbreviations: AUC, area under the curve; CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.

insufficient cartridges/strips, especially for I-STAT and LDX, whose cartridges were stored far from the clinic. This contributed to the uneven observations across the POC glucometers. Patient information such as singleton or twin pregnancy, fetal weight, and oligo/polyhydramnios was not collected. Finally, this study was conducted in a high-risk group, which may have skewed the results for the study population compared with the healthy pregnant women. Our study also had several notable strengths, including the number of women participating in the study, as well as the wide range of POC glucometers. As such, these results are a valuable source of information on the accuracy of POC glucose testing in diagnosing GDM among women in South Africa.

5 | CONCLUSIONS

Based on the present study, the use of these POC glucometers for the diagnosis of GDM cannot be recommended. Nonetheless, the POC glucometers have the potential to screen for GDM when a two-step screening process is considered. This may be beneficial in resource-constrained settings where access to laboratory measurement of glucose may be difficult. In addition, laboratory-based FPG has the potential to be used as a diagnostic test for GDM.

AUTHOR CONTRIBUTIONS

JG, CC, and LK contributed to study design. JG, CC, YA, LK and LK contributed to the implementation and analysis plan. LK and LK collected blood samples and study variables. CH, BV, and JG contributed to the management of the project. LK drafted the manuscript, and all authors (LK, CC, YA, LK, CH, BV, and JG) revised and approved the final manuscript.

ACKNOWLEDGMENTS

We are grateful for the support of the patients, nurses, and National Health Laboratory Service staff members from CHBAH.

FUNDING INFORMATION

Funding for the study was provided by FIND through a grant from the Swiss Agency for Development and Cooperation.

CONFLICT OF INTEREST STATEMENT

CH and BV are employees of FIND. The remaining authors have no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

REFERENCES

1. Wang H, Li N, Chivese T, et al. IDF Diabetes Atlas: estimation of global and regional gestational diabetes mellitus prevalence for 2021 by international association of diabetes in pregnancy study group's criteria. *Diabetes Res Clin Pract*. 2022;183:109050. doi:10.1016/j.diabres.2021.109050

2. Ogurtsova K, da Rocha Fernandes JD, Huang Y, et al. IDF diabetes atlas: global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes Res Clin Pract*. 2017;128:40-50. doi:10.1016/j.diabres.2017.03.024
3. HAPO Study Cooperative Research Group, Metzger BE, Lowe LP, et al. Hyperglycemia and adverse pregnancy outcomes. *N Engl J Med*. 2008;358:1991-2002. doi:10.1056/NEJMoa0707943
4. Li Z, Cheng Y, Wang D, et al. Incidence rate of type 2 diabetes mellitus after gestational diabetes mellitus: a systematic review and meta-analysis of 170,139 women. *J Diabetes Res*. 2020;2020:3076463. doi:10.1155/2020/3076463
5. Plows JF, Stanley JL, Baker PN, Reynolds CM, Vickers MH. The pathophysiology of gestational diabetes mellitus. *Int J Mol Sci*. 2018;19:3342. doi:10.3390/ijms19113342
6. Hod M, Kapur A, Sacks DA, et al. The International Federation of Gynecology and Obstetrics (FIGO) initiative on gestational diabetes mellitus: a pragmatic guide for diagnosis, management, and care. *Int J Gynaecol Obstet*. 2015;131(Suppl 3):S173-S211. doi:10.1016/S0020-7292(15)30033-3
7. Hinneh T, Jahn A, Agbozo F. Barriers to screening, diagnosis and management of hyperglycaemia in pregnancy in Africa: a systematic review. *Int Health*. 2022;14:211-221. doi:10.1093/inthealth/ihab054
8. Macaulay S, Ngobeni M, Dunger DB, Norris SA. The prevalence of gestational diabetes mellitus amongst black south African women is a public health concern. *Diabetes Res Clin Pract*. 2018;139:278-287. doi:10.1016/j.diabres.2018.03.012
9. Dickson LM, Buchmann EJ, Janse van Rensburg C, Norris SA. Fasting plasma glucose and risk factor assessment: comparing sensitivity and specificity in identifying gestational diabetes in urban black African women. *S Afr Med J*. 2019;110:21-26. doi:10.7196/SAMJ.2019.v110i1.14089
10. Daly N, Carroll C, Flynn I, Harley R, Maguire PJ, Turner MJ. Evaluation of point-of-care maternal glucose measurements for the diagnosis of gestational diabetes mellitus. *BJOG*. 2017;124:1746-1752. doi:10.1111/1471-0528.14255
11. Macleod K, Katz LB, Cameron H. Capillary and venous blood glucose accuracy in blood glucose meters versus reference standards: the impact of study design on accuracy evaluations. *J Diabetes Sci Technol*. 2019;13:546-552. doi:10.1177/1932296818790228
12. Voglová Hagerf B, Protus M, Nemetova L, et al. Accuracy and feasibility of real-time continuous glucose monitoring in critically ill patients after abdominal surgery and solid organ transplantation. *Diabetes Care*. 2024;47:956-963. doi:10.2337/dc23-1663
13. Dickson LM, Buchmann EJ, Janse van Rensburg C, Norris SA. Accuracy of five plasma calibrated glucometers to screen for and diagnose gestational diabetes mellitus in a low resource clinic setting. *J Clin Transl Endocrinol*. 2018;16:100174. doi:10.1016/j.jcte.2018.12.003
14. García-Claver A, Ramos-Corral R, Laviña-Fañanás C, Solans-Blecua I, Puzo-Foncillas J. Capillary glucose concentration during oral glucose tolerance test for the diagnosis of gestational diabetes. *Int J Gynecol Obstet*. 2020;150:234-240. doi:10.1002/ijgo.13178
15. Bujang MA, Adnan TH. Requirements for minimum sample size for sensitivity and specificity analysis. *J Clin Diagn Res*. 2016;10:YE01-YE06. doi:10.7860/JCDR/2016/18129.8744
16. Veronique N and Kenneth RL Huddle. The 2017 SEMDSA Guidelines for the Management of Type 2 Diabetes. *Journal of Endocrinology, Metabolism and Diabetes of South Africa* 2017; 22(1): S103-S106. http://dx.doi.org/10.1080/16089677.2015.1056468
17. International Association of Diabetes and Pregnancy Study Groups Consensus Panel, Metzger BE, Gabbe SG, et al. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. *Diabetes Care*. 2010;33:676-682. doi:10.2337/dc09-1848
18. Abu-Arafeh A, Jordan H, Drummond G. Reporting of method comparison studies: a review of advice, an assessment of current practice, and specific suggestions for future reports. *Br J Anaesth*. 2016;117:569-575. doi:10.1093/bja/aew320
19. EFLM. Biological variation. Accessed December 7, 2023. https://biologicalvariation.eu/
20. Lingervelder D, Koffijberg H, Kusters R, IJzerman MJ. Health economic evidence of point-of-care testing: a systematic review. *Pharmacoecon Open*. 2021;5:157-173. doi:10.1007/s41669-020-00248-1
21. Al-Hasani W, Ranasinghe R, Rogers H, et al. Clinical utility of point of care glucose in the assessment of gestational diabetes: Prospective cohort study. *BJOG*. 2024;131(9):1270-1278.
22. O'Malley EG, Reynolds CME, O'Kelly R, Killalea A, Sheehan SR, Turner MJ. A prospective evaluation of point-of-care measurements of maternal glucose for the diagnosis of gestational diabetes mellitus. *Clin Chem*. 2020;66:316-323. doi:10.1093/clinchem/hvz005
23. Adam S, Rheeder P. Evaluating the utility of a point-of-care glucometer for the diagnosis of gestational diabetes. *Int J Gynaecol Obstet*. 2018;141:91-96. doi:10.1002/ijgo.12399
24. Gallardo-Rincón H, Lomelin-Gascon J, Martinez-Juarez LA, et al. Diagnostic accuracy of capillary blood glucometer testing for gestational diabetes. *Diabetes Metab Syndr Obes*. 2022;15:3855-3870. doi:10.2147/DMSO.S389420
25. Milan JG. The new hierarchy: update from the EFLM strategic conference on defining analytical performance goals. Quality specifications for QC and QAP. *Pathology*. 2016;48:S16. doi:10.1016/j.pathol.2015.12.044

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Khambule L, Chikomba C, Adam Y, et al. Performance of point-of-care glucose testing for the diagnosis of gestational diabetes in South Africa. *Int J Gynecol Obstet*. 2024;00:1-10. doi:10.1002/ijgo.15914

APPENDIX G: TURN-IT-IN REPORT

GDM PHD study 5th draft with ABSTRACT 20250325-3.docx

ORIGINALITY REPORT

11%	7%	10%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.ncbi.nlm.nih.gov Internet Source	1%
2	wiredspace.wits.ac.za Internet Source	1%
3	hdl.handle.net Internet Source	<1%
4	www.frontiersin.org Internet Source	<1%
5	listens.online Internet Source	<1%
6	Khambule, Lungile. "Association of Branched Chain Amino Acids and Aromatic Amino Acids with Cardiometabolic Risk Factors", University of the Witwatersrand, Johannesburg (South Africa), 2025 Publication	<1%
7	link.springer.com Internet Source	<1%

8	Benhalima, Katrien, Roland Devlieger, and André Van Assche. "Screening and management of gestational diabetes", Best Practice & Research Clinical Obstetrics & Gynaecology, 2015. Publication	<1 %
9	"Biomarkers in Diabetes", Springer Science and Business Media LLC, 2023 Publication	<1 %
10	L M Dickson, E J Buchmann, C Janse van Rensburg, S A Norris. "Fasting plasma glucose and risk factor assessment: Comparing sensitivity and specificity in identifying gestational diabetes in urban black African women", South African Medical Journal, 2019 Publication	<1 %
11	infospace.mrc.ac.za Internet Source	<1 %
12	mdpi-res.com Internet Source	<1 %
13	Moshe Hod, Lois G. Jovanovic, Gian Carlo Di Renzo, Alberto de Leiva, Oded Langer. "Textbook of Diabetes and Pregnancy", CRC Press, 2018 Publication	<1 %

14	Hodson, K., S. Robson, and R. Taylor. "Gestational diabetes: emerging concepts in pathophysiology", Obstetric Medicine, 2010. Publication	<1 %
15	www.springermedizin.de Internet Source	<1 %
16	Albishi, Sara Ali. "Consequences of the Metabolic Syndrome Among African Americans, Hispanics and Whites.", Howard University, 2020 Publication	<1 %
17	Gradidge, Philippe Jean-Luc. "Factors Associated With Obesity and Metabolic Syndrome in an Ageing Cohort of Black Women Living in Soweto, Johannesburg (Study of Women in and Entering Endocrine Transition [Sweet])", University of the Witwatersrand, Johannesburg (South Africa), 2025 Publication	<1 %
18	worldwidescience.org Internet Source	<1 %
19	Jaff, Nicole Gusti. "An Investigation of the Metabolic, Hormonal and Anthropometric Characteristics of the Menopausal Transition in Black Urban South African Women",	<1 %

	University of the Witwatersrand, Johannesburg (South Africa), 2025 Publication	
20	aran.library.nuigalway.ie Internet Source	<1 %
21	"Nutrition and Diet in Maternal Diabetes", Springer Science and Business Media LLC, 2018 Publication	<1 %
22	Hannah Heath, Rodrigo Rosario, Lauren E. McMichael, Rob Fanter et al. "Gestational Diabetes Is Characterized by Decreased Medium-Chain Acylcarnitines and Elevated Purine Degradation Metabolites across Pregnancy: A Case–Control Time-Course Analysis", Journal of Proteome Research, 2023 Publication	<1 %
23	Boutros, Paula Hage. "A National Prospective Cohort Study in Lebanon to Evaluate the Impact of the Lebanese Mediterranean Diet on Maternal and Infant Outcomes", McGill University (Canada), 2024 Publication	<1 %
24	Hobbs, Angela Wendy. "Genome-Wide Gene Expression Analysis in Black South African Women Who Develop Gestational Diabetes	<1 %

	Mellitus", University of the Witwatersrand, Johannesburg (South Africa), 2025 Publication	
25	"Biomarkers in Nutrition", Springer Science and Business Media LLC, 2022 Publication	<1 %
26	Dias, Stephanie Charmaine. "Investigating Molecular Biomarkers During Gestational Diabetes Mellitus", University of Pretoria (South Africa), 2023 Publication	<1 %
27	Li, Zhongbo. "Elucidating and Overcoming Anti-Peg Antibodies to PEGylated Therapeutics and Nanomedicine", The University of North Carolina at Chapel Hill, 2024 Publication	<1 %
28	H. David McIntyre, Patrick Catalano, Cuilin Zhang, Gernot Desoye, Elisabeth R. Mathiesen, Peter Damm. "Gestational diabetes mellitus", Nature Reviews Disease Primers, 2019 Publication	<1 %
29	Mukesh M Agarwal. "Gestational diabetes mellitus: Screening with fasting plasma glucose", World Journal of Diabetes, 2016 Publication	<1 %