Tissue and Systemic Dipeptidyl Peptidase 4 and Glucose Homeostatic Responses in a Diet-induced Prediabetic Rat Model.

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Dissertation submitted in fulfillment of the requirements for the degree of Master of Medical Science in the School of Laboratory Medicine and Medical Sciences, University of KwaZulu-Natal

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PREFACE

Dipeptidyl peptidase-4(DPP-4) is a serine protease enzyme that is found in various tissues such as liver, lungs, kidney and small intestines as well as in a soluble form in plasma (sDPP-4). It is primarily responsible for inactivating incretins such as glucagon-like peptide-1 (GLP-1) resulting in impaired glucose regulation. Furthermore, DPP-4 activity has been found to be drastically elevated in individuals with type-2-diabetes mellitus (T2DM). T2DM is often preceded by prediabetes which is a condition characterized by elevated blood glucose levels but not high enough to be diagnosed as T2DM. However, the changes in plasma and tissue DPP-4 in prediabetes are still not understood. A high-fat high-carbohydrate (HFHC) diet is used in our laboratory to induce prediabetes induction and several studies have shown this model to closely mimic the human condition both in development and progression. Using this animal model, this study sought to investigate changes in plasma sDPP-4 and tissue DPP-4 expression in diet-induced prediabetes. The experimental work described in this dissertation was conducted at University of Kwa-Zulu natal, Westville campus, Durban, South Africa. It was conducted under the supervision of Prof Andile Khathi and co-supervised by Dr Nomusa Mzimela.

DECLARATION

I, Anele Shange hereby declare that the dissertation entitled:

"Tissue and Systemic Dipeptidyl Peptidase 4 and Glucose Homeostatic Responses in a Diet-induced Prediabetic Rat Model.."is the result of my own investigation and research and that it has not been submitted in part or in full for any other degree or to any other university. Where use of the work of others was made, it is duly acknowledged in the text.

Student: Anele Shange **Signature** [redacted] **Date:** 26 January 2026

Supervisor: Prof. A. Khathi **Signature** [redacted] **Date:** 26 January 2026

Co-supervisor: Dr NC Mzimela **Signatur** [redacted] **Date:** 26 January 2026

PLAGIARISM DECLARATION

School of Laboratory Medicine and Medical Sciences, College of Health Sciences MASTER'S DEGREE IN MEDICAL SCIENCES 2025

I know that plagiarism is wrong. Plagiarism is to use another's work and pretend that it is one's.

I have used the Vancouver convention for citation and referencing. Each contribution to, and quotation in, this dissertation from the works of other people has been attributed and has been cited and referenced.

This dissertation is my own work.

I have not allowed and will not allow anyone to copy my work with the intention of passing it off as his or her own work.

Signature: 

DEDICATION

I dedicate this work to God, my late son (Solwazi Akwande Zungu), my family and my partner.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my supervisor, Prof. Andile Khathi, for the unwavering support, patience, and guidance throughout my research journey. His expertise and experience were instrumental in the successful completion of this thesis, and his encouragement and constructive feedback have been invaluable to me. I thank him for making this work possible.

I am also profoundly grateful to my co-supervisor, Dr Nomusa Mzimela, for her insightful suggestions and constant encouragement. Her contributions have played a key role in the progress of this research.

I am truly thankful to my entire family, as well as my partner, Mthobisi Zungu, for their unwavering support and reassurance during this challenging journey. Despite the many obstacles, their prayers and belief in me have given me the strength to persevere and stay focused on my dreams.

To my friend, Desiree Thibela, thank you for the continuous support and encouragement throughout this journey. I am truly grateful.

I would also like to extend my appreciation to my fellow postgraduate students in the Department of Physiology. Their assistance in accessing resources and their collective efforts were essential in enabling me to conduct my research efficiently. I am also deeply grateful to the National Research Foundation for funding my studies, which made this research possible.

Finally, I thank God for carrying me through all the difficulties I have faced, the greatest of which was the heartbreaking loss of my son during the course of my studies. God strength and guidance have sustained me through the toughest moments.

Table of Contents

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ADA	American Diabetes Association
AREC	Animal Research Ethics Committee
CD26	Cluster of differentiation 26
DPP-4	Dipeptidyl peptidase-4
DM	Diabetes Mellitus
EASD	European Association for the study of Diabetes
ELISA	Enzyme-linked Immunosorbent Assay
FBG	Fasting blood glucose
RevMan	Review Manager
FPGT	Fasting Plasma Glucose Test
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GD	Gestational diabetes
GIP	Gastric Inhibitory Polypeptide
GLP-1	Glucagon-like-peptide-1
GRADE	Grading of Recommendations Assessment, Development, and Evaluation
HbA1c	Glycated haemoglobin
HNF-1a	Hepatocyte Nuclear Factor-1a
HPA	Hypothalamic Pituitary Adrenal
HRP	Horseradish Peroxidase
IDF	International Diabetes Federation
IGT	Impaired Glucose Tolerance
IL-12	Interleukin-12
MeSHs	Medical Subject Headings
NAFDL	Non-Alcoholic fatty Liver Disease
NPD	Non-prediabetic
OGTT	Oral Glucose Tolerance test
PRISMA	Preferred Reporting Items for Systematic Review and Meta-Analysis
PD	Prediabetes
RevMan	Review manager
RNA	Ribonucleic Acid
sDPP-4	Soluble Dipeptidyl peptidase-4
SAT	Subcutaneous adipose tissue

T2DM	Type 2 Diabetes Mellitus
UKZN	University of KwaZulu Natal
VAT	Visceral Adipose Tissue
WHO	World Health Organization

LIST OF APPENDICES

Ethical clearance

The study was conducted upon obtaining approval of ethical clearance from Animal Research Ethics Committee (AREC) of University of Kwa-Zulu Natal, Durban, South Africa (Ethics number: AREC/039/018M).

STUDY OUTLINE

The current dissertation is comprised of five chapters. Chapter 1 presents the introduction, including the aim of the study, the research question, objectives, and hypothesis. Chapter 2 provides a detailed literature review, reporting on the available information and highlighting the existing research gap. Chapter 3 is a systematic review that evaluates studies reporting on changes in tissue DPP-4 expression in individuals with type 2 diabetes and prediabetes. This work is authored by A. Shange and supervised by Prof. A. Khathi, with co-supervision by Dr. N.C. Mzimela. Chapter 4 presents the main experimental manuscript, which investigates changes in plasma and tissue DPP-4 expression in a diet-induced prediabetic rat model. This study is also authored by A. Shange, under the supervision of Prof. A. Khathi and co-supervision of Dr. N.C. Mzimela. Finally, Chapter 5 provides a synthesis of the findings and the overall conclusion of the dissertation.

ABSTRACT

Background

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycaemia, resulting from impaired insulin action, insulin secretion, or both. Type-2-diabetes mellitus (T2DM) is the most common occurring form of DM accounting for 90% of all cases. Various enzymes including dipeptidyl peptidase-4 (DPP-4), an enzyme found in various tissues including plasma (sDPP-4) that primarily degrades incretin hormones such as GLP-1 and GIP that stimulate insulin secretion and inhibit glucagon release and are dysregulated during T2DM. The onset of T2DM is often preceded by a condition called prediabetes whereby blood glucose levels are elevated but not high enough to be diagnosed as T2DM. However, there is a lack of studies that have documented DPP-4 expression in tissues as well as sDPP-4 concentrations during the prediabetic state and how these may influence glucose homeostasis. Therefore, this study investigated the changes in tissue and plasma DPP-4 expression in a diet-induced prediabetic rat model and examined how these changes influence glucose homeostasis.

Materials and Methods

Twelve male Sprague Dawley rats were randomly allocated into two equal groups (n=6 each). Experimental prediabetes was induced in the animals using a previously reported protocol. The first group (A) was fed a standard rat chow and supplied with tap water. The second group (B), group was fed high-fat high-carbohydrate (HFHC) diet supplemented with 15% fructose for 20 weeks to induce prediabetes. At the end of the 20-week induction period, the American Diabetes Association (ADA) criteria were used to confirm prediabetes. At the end of the induction period, the animals were then sacrificed, and ELISA was used to measure plasma sDPP-4, insulin, GLP-1, ghrelin and leptin in both groups. Furthermore, quantitative PCR was used to measure DPP-4 gene expression in tissues including the kidney, lungs and liver. Fold change in gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

Results and Discussion

The induction of prediabetes in the HFHC-diet fed group resulted in increased blood glucose and HbA1c levels by comparison to the non-prediabetic group. This was accompanied by increased plasma sDPP-4, insulin, ghrelin and leptin levels in the PD group by comparison to the NPD group. There was also decreased plasma GLP-1 in the PD group by comparison to the NPD group. The increased insulin, glucose and HbA1c levels reflect insulin resistance and disrupted glucose regulation. The elevated sDPP-4 and decreased plasma GLP-1 levels indicate impaired incretin signalling. Additionally, the rise in ghrelin and leptin suggest hormonal imbalance contributing to metabolic dysregulation. Furthermore,

the results showed increased DPP-4 expression in the kidney, whereas reduced expression was observed in the liver and lungs. These findings suggest that DPP-4 is regulated in a tissue-specific manner during the prediabetic state. The upregulation in the kidney may indicate a compensatory or pathological role in renal glucose handling or inflammation, while the downregulation in the liver and lungs could reflect altered metabolic or immune responses. Overall, this differential expression pattern points to a complex, tissue-dependent role of DPP-4 in the early stages of metabolic dysregulation.

Conclusion

Taken together, the results of the present study suggest a complex disruption of glucose homeostasis and hormonal signalling. The reduced plasma GLP-1 levels in the presence of elevated sDPP-4 suggest enhanced incretin degradation, which may impair insulin secretion and contribute to hyperglycaemia. Furthermore, the results suggest that DPP-4 expression varies between tissues and is differently regulated during the prediabetic state. Increased expression in the kidney, along with decreased levels in the liver and lungs, indicates that DPP-4 may have distinct, tissue-dependent roles in metabolic regulation and disease development. The observed metabolic and hormonal disturbances highlight the role of DPP-4 in impairing glucose homeostasis during the prediabetic state.

Chapter 1: Introduction

1. Background

Following a meal, the small intestines release incretins such as glucagon-like-peptide-1(GLP-1) which leads to surges in insulin secretion (1). This secretion of GLP-1 also leads to slower gastric emptying and suppression of appetite which are, in part, mediated via suppression of ghrelin secretion. However, chronic consumption of high calorie diets has been shown to leads to a dysregulation in GLP-1 activity and which is largely attributed to increased dipeptidyl peptidase-4 (DPP-4) activity (2). DPP-4 is an integral membrane protein found in various organs, including the brush border membranes of tissues that include, but not limited to, the small intestines, kidneys, lungs and liver (3). Although it is expressed on all body cells as an essential membrane protein, it is also released from the membrane and circulates in the plasma as a soluble protein (sDPP-4) (3,4). It is well known that DPP-4 and a few of its substrates interact with adipokines, which is crucial for energy metabolism (5). High levels of DPP-4 inhibit GLP-1 activity leading to impaired insulin secretion and activity as well as hyperglycaemia (6).

Type-2-diabetes mellitus (T2DM) is a metabolic disorder characterized by chronic hyperglycaemia and insulin resistance (7). It has been noted that T2DM is associated with drastically increased DPP-4 enzyme activity (8,9). DPP-4 has been proposed to be involved in the pathophysiology of T2DM by acting as a local mediator of inflammation and insulin resistance in adipose and hepatic tissue and this is because altered DPP-4 expression and activity are linked to increased body mass index and hyperglycaemia (10). The onset of T2DM is, however, often preceded by prediabetes which is a state of intermediate hyperglycaemia where the blood glucose levels are above the homeostatic range but not high enough for a diagnosis of T2DM (11,12). According to the American Diabetes Association, prediabetes is characterized by moderately elevated levels of glycated haemoglobin (HbA1c) as well as impaired fasting blood glucose (IFG) and impaired glucose tolerance (IGT) (13).

A recent study showed that prediabetes affected 7.5% of the world's population (374 million) in 2019, and that by 2030, that number is expected to rise to 8.0% (454 million)(14). Several studies have shown that many of the complications often associated with type 2 diabetes begin during prediabetes(15). While there is a lot of information regarding the changes in DPP-4 expression during type 2 diabetes mellitus, there is a paucity in literature regarding the changes in DPP-4 expression during prediabetes and the impact that this may have on the function of GLP-1. Animal models are commonly used in diabetes mellitus research to investigate tissue and molecular changes that would not be easy to investigate in humans(16). In our laboratory, we use a high fat high carbohydrate diet-induced animal model of prediabetes, which has been shown to mimic the human condition (17). Using this animal model, this study sought to investigate the changes of DPP-4 expression in selected tissues in a diet-induced animal model of prediabetes.

2.Aim

To investigate the changes of DPP-4 expression in selected tissues in a diet-induced animal model of prediabetes.

3.Research questions

- What are the changes in DPP-4 tissue expression in a diet-induced prediabetes animal model?
- Are there any associations of DPP-4 expression with markers of glucose homeostasis during a diet-induced prediabetic state?

4.Objectives

- To diagnose pre-diabetes using fasting blood glucose (FBG), oral glucose tolerance test and blood glycated haemoglobin concentrations (HbA1c) on diet-induced rat model
- To determine expression of DPP-4 in plasma during a prediabetic state.
- To determine DPP-4 activity in plasma and its tissue (liver, kidney, small intestine and lung) expression during a prediabetic state
- To investigate changes to DPP-4 in relation to glucose homeostasis during a diet-induced prediabetic state.
- To determine correlations of tissue plasma DPP-4 with markers of glucose homeostasis in diet-induced prediabetic rats

5. Hypothesis

There will be an increased plasma activity and expression of DPP-4 in tissues such as the lungs, kidney and liver during diet-induced prediabetes. There will be a correlation between DPP-4 and other biomarkers which are GLP-1, insulin, ghrelin and HbA1C

6. Null hypothesis

There will be no significant change in the DPP-4 expression levels in tissues such as the lungs, kidney and liver during diet-induced prediabetes. There will be no correlation between DPP-4 and other biomarkers which are GLP-1, insulin, ghrelin and HbA1C.

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Chapter 2: Literature review

2.1 Glucose homeostasis

Glucose homeostasis refers to a process of maintaining blood glucose levels within a narrow range of approximately 3.9-5.6mmol/L to maintain energy balance and ensure continued optimal bodily function (1). This process involves numerous hormones, principally insulin and glucagon, which are both produced by the pancreas. Insulin, produced by pancreatic beta cells plays a significant role in glucose homeostasis and metabolism (1,2) . It is primarily responsible for lowering blood glucose levels and preventing hyperglycaemia in the body by facilitating the entry of glucose into the cells (2). Glucagon, on the other hand, is produced by pancreatic alpha cells and is responsible for preventing hypoglycaemia during fasting periods by stimulating hepatic glucose production (3). In addition to these hormones, there is a specific group of hormones known as incretins and they are primarily responsible for stimulating insulin secretion in response to elevated blood glucose levels, thus helping to maintain glucose homeostasis (4,5).

2.2 Incretins

Incretin hormones, also referred to as incretins, are gastrointestinal peptides that are produced following dietary intake and promote insulin secretion (6). Incretin hormones have gained great interest because of their important involvement in the physiology of glucose homeostasis as well as the pathophysiology of type 2 diabetes and other metabolic disorders. Incretins play a role in glucose homeostasis as they slow down gastric emptying, which helps to moderate the rate of absorption of glucose into the bloodstream (7,8). Studies have shown that hormones such as leptin and ghrelin interact with incretins, and they play a huge role in glucose metabolism and energy balance (9,10). Leptin enhances insulin sensitivity and induces the feeling of satiety while ghrelin inhibits insulin secretion and induces the feeling of hunger (11). One of the most important incretin hormones is glucagon-like-peptide-1 (GLP-1) (6,7). Studies have shown that leptin and ghrelin interact with GLP-1 to modulate glucose metabolism (12,13).

2.3 Physiological roles of GLP-1

Glucagon-like peptide-1 (GLP-1) is a signal peptide postprandially secreted from L-cells of the intestinal mucosa and plays a crucial role in glucose homeostasis and overall physiology (14). It is primarily responsible for stimulating insulin release from the pancreatic beta cells and inhibiting glucagon release in the pancreas (13,14). GLP-1 also increases satiety which enhances feelings of fullness (15,16) GLP-1 also contributes to the regulation of postprandial blood glucose levels by

promoting insulin secretion and inhibiting glucagon release, which prevents excessive glucose production by the liver (17). It has also been found that this incretin slows down gastric emptying and by doing so, GLP-1 helps ensure a gradual release of nutrients into the bloodstream thus preventing rapid spikes in blood glucose (13,15,16). GLP-1 signals the brain to promote feelings of fullness, helping to regulate food intake and prevent overeating (18). Figure 1 below shows how GLP-1 enhances insulin secretion and inhibit glucagon release. There is an enzyme called dipeptidyl peptidase-4 (DPP-4) that has been shown by several studies to affects functions of GLP-1.

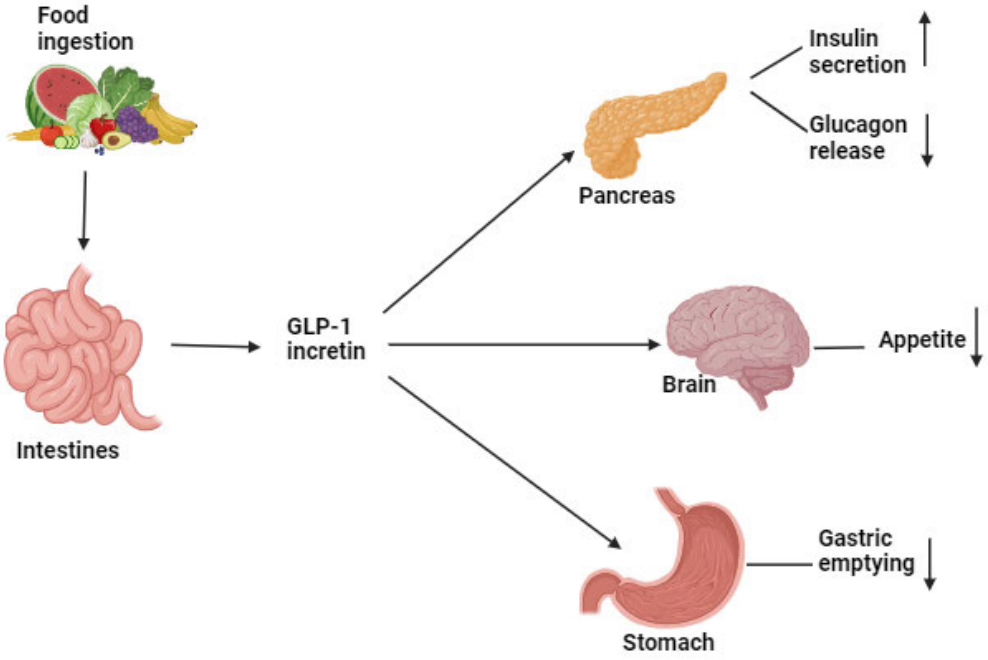

Figure 1: The schematic representation of GLP-1 physiological processes. Adapted from Gazzo et al., 2016

2.4 Physiological roles of DPP-4

Dipeptidyl peptidase 4 (DPP-4) is a serine protease that is expressed widely in many tissues (19). It is found in two forms; soluble DPP-4 also known as sDPP-4 which circulates in plasma and membrane-bond DPP-4, which is tethered to the cell membrane and expressed on various cell types (19). DPP-4 is involved in a number of physiological activities (19,20). It primarily inactivates peptides such as glucagon-like-peptide (GLP-1) and gastric inhibitory polypeptide (GIP)reducing their glucose-lowering effects (20,21). Through its action on incretins, DPP-4 indirectly affects appetite regulation, breaks down incretin hormones like GLP-1 and GIP leading to the alteration of the activity of the various hormones which are responsible for insulin secretion and suppression of appetite (21–23). High levels of DPP-4 lead to leptin resistance, which leads to increased appetite and food intake (24). Figure 2

shows how dipeptidyl-peptidase hinders the functions of incretins (GLP-1 and GIP) and the resulting physiological consequences.

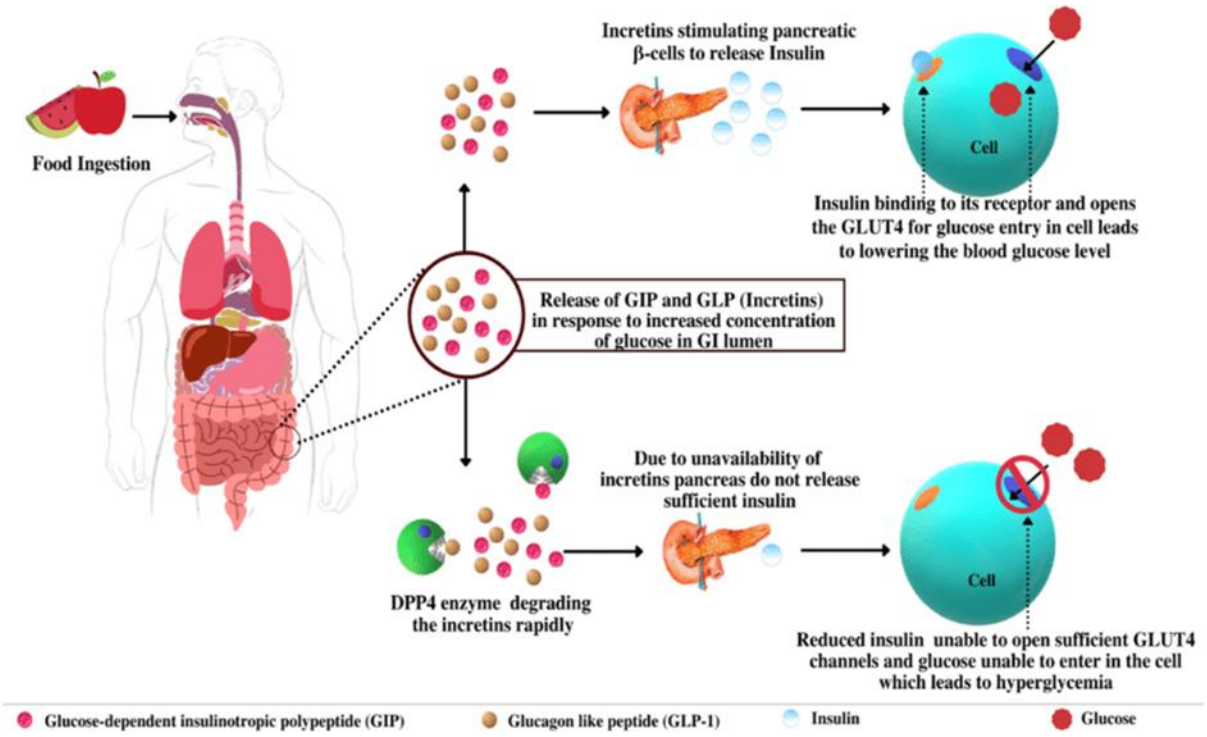

Figure 2: This diagram shows replace how DPP-4 degrades incretins (GLP-1 and GIP) in the body and the physiological consequences. Adapted from Sharma et al., 2022

2.5 Diabetes mellitus

Diabetes Mellitus (DM) refers to a group of diseases that are characterized by hyperglycaemia that occurs as a results of insulin resistance or insufficient insulin production (25). There are three types of diabetes mellitus, and type 1 diabetes (T1DM) occurs when the pancreas produces little or no insulin due to autoimmune-mediated destruction of pancreatic β-cells. Type 2 diabetes (T2DM) arises from insulin resistance, often linked to excess fat and physiological stress. Lastly, there is gestational diabetes (GDM) which develops during pregnancy when placental hormones interfere with the body's ability to use or produce insulin (26–29). The diagnostic criteria for DM in accordance with the recommendations of international associations such as the International Diabetes Federation (IDF), European Association for the Study of Diabetes (EASD), and the World Health Organization (WHO) are as follows, 2-hour postprandial blood glucose values of greater or equal to 11.1 mmol/L, Fasting plasma glucose of greater or equal to 7.0 mmol/L (fasting time 8–12h)(29,30). Further to these values the American Diabetes Association (ADA) has an additional diagnostic criterion for diabetes mellitus of glycated haemoglobin (HbA1c) 48 mmol/mol in the blood (30,31). According to the latest global estimate from the

International Diabetes Federation (IDF), there were 415 million persons with diabetes mellitus in 2015, with a projected 642 million by 2040 (32). The NCD Risk Factor Collaboration and the WHO provided a comparable estimate of 422 million in 2014 (33,34). Type 2 diabetes is the most common type of diabetes mellitus ,accounting for approximately 90% of all cases (35,36).

Type 2 diabetes mellitus affects the regulation and functioning of hormones such as ghrelin, leptin and GLP-1 (37). It leads to decreased ghrelin levels which results in decreased appetite and delayed or slow gastric emptying (38). It also leads to decreased GLP-1 secretion and increased leptin levels which both result in accelerated gastric emptying (38,39). High leptin levels also result in decreased appetite whereas decreased GLP-1secretion leads to increased appetite and increased food intake (15–17). As reduced GLP-1 secretion is mostly observed in individuals with insulin resistance, this leads to the disturbance of many hormones in the body [17,40]. It causes reduced leptin sensitivity and alters ghrelin secretion (40). Figure 3 summarises the effects of T2DM on ghrelin, leptin and GLP-1 and the resulting physiological consequences.

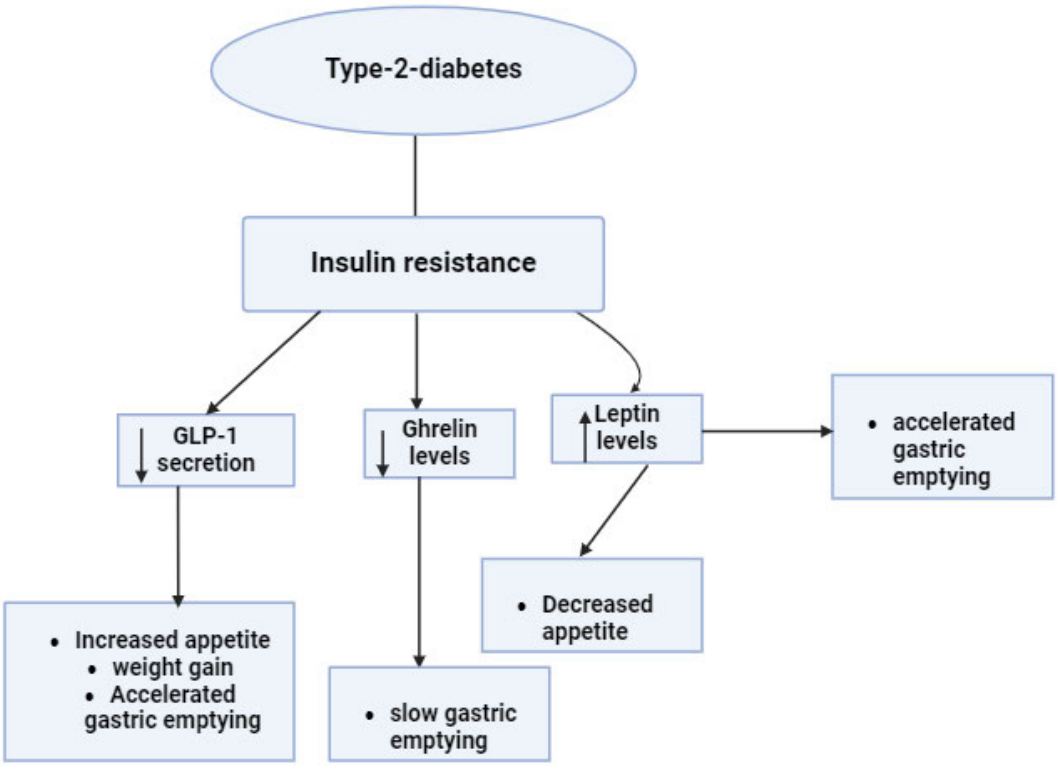

Figure 3: The effects of T2DM on ghrelin, leptin and GLP-1 and the resulting physiological consequences. (created by author)

In T2DM, there are elevated DPP-4 levels and activity which results in enhanced incretins (GLP-1and GIP) degradation which subsequently reduces the beneficial effects of these incretins and results in impaired insulin and glucose regulation (41). Increased DPP-4 levels contribute to increased insulin resistance in T2DM (42). However, the onset of T2DM is often preceded by a long-lasting condition known as prediabetes (PD) (43).

2.6 Prediabetes

Prediabetes, also known as intermediate hyperglycaemia, is a condition where the blood glucose levels are raised above the homeostatic range but not high enough for a diagnosis of T2DM (43). According to the World Health Organization (WHO), prediabetes is defined as a state of intermediate hyperglycaemia based on two specific parameters: impaired glucose tolerance (IGT), which is defined as plasma glucose levels of 7.8-11.0 mmol/L based on a 2-hour oral glucose tolerance test (OGTT), and impaired fasting glucose (IFG), which is defined as fasting plasma glucose (FPG) of 6.1-6.9 mmol/L (44,45). On the other hand, the American Diabetes Association (ADA) has a lower cut-off value for FPG of 5.6-6.9 mmol/L and the same cut-off value for IGT 7.8-11.0mmol/L (46). American Diabetes Association (ADA) also has an additional haemoglobin A1c (HbA1c)-based criteria for the definition of prediabetes, which is a level of 39-46mmol/mol (46). The global prevalence of prediabetes tolerance was estimated to be 7.5% (374 million) in 2019 and is projected to reach 8.0% (454 million) by 2030 (47). Without intervention, prediabetes can progress to type 2 diabetes(48). The body begins to lose its ability to regulate blood sugar effectively, which can result in higher levels of glucose circulating in the bloodstream over time (48). If untreated, prediabetes can result in long-term health problems known as microvascular and macrovascular consequences, which are mostly brought on by the impacts of insulin resistance and persistently elevated blood sugar (49,50). Even before a diagnosis of type 2 diabetes, prediabetes can raise the risk of serious complications because the underlying metabolic inefficiency can gradually harm organs and blood vessels over time (51).

According to a study by Naidoo et al., impaired renal function during the prediabetic stage causes abnormalities in calcium homeostasis (52). The study further demonstrated that the purpose of calcium-regulating organs is to maintain normocalcaemia by compensating for renal calcium waste (52). Increased intestinal calcium absorption, increased renal calcium reabsorption, increased renal vitamin D activation, and decreased bone resorption followed by increased bone production are the main impacts of prediabetes on the calcium-regulating organs (52). Along with higher osteocalcin and lower deoxypyridinoline levels, this was demonstrated by enhanced expression of intestinal and renal calcium transport markers and another study on the subject by Liu et al. provided support for this (53). Prediabetes also affects different hormones such as thyroid hormones, cortisol and also sex hormones (54). There are high levels of cortisol during the prediabetic state which contribute to insulin resistance

(54,55). PD results in low levels of thyroid hormones and decreased sex hormones, which are decreased testosterone levels in males and decreased oestrogen hormone in females (56). While the effects of prediabetes on various hormones are known, its effects on incretins such as GLP-1 as well as on tissue and systemic expression of DPP-4 during the PD state are hitherto not known

2.7 Use of animal models in the study of prediabetes

Animal models have made great contributions to the study of diabetes mellitus (57,58). They allow researchers to manipulate *in vivo* genetic and environmental factors that may influence the development of the disease and the emergence of its consequences, gaining new insights into its management and treatment in people (59,60). The main advantages of using animal models are that there is an improved understanding of disease mechanisms, and it is easy to identify potential therapeutic targets, and the study can be easily translated to clinical practice (61). The use of animal models is now very common in prediabetes research (62,63). One of the most common animal models for studying prediabetes is the C57BL/6 mouse model which is fed a high-fat diet over a 16-week period in several studies (64,65). In our laboratory, prediabetes is induced in Sprague Dawley rats where they are fed a high fat high carbohydrate diet *ad libitum* for 20 weeks. This animal model has been shown, through various studies, to mimic the human condition (66). There are several metabolic aberrations that have been seen in this model including elevated blood glucose levels, elevated body weight, insulin resistance and the development of hormonal abnormalities (66,67). However, no studies have been done using this model to investigate changes in GLP-1 and DPP4 concentrations.

2.8 Justification of the study

Glucose homeostasis is a process of maintaining blood glucose levels which involves insulin and glucagon hormones (1,68). There are hormones known as incretins which are responsible for stimulating insulin secretion in response to elevated blood glucose levels (4,6,7). One of the incretin hormones is GLP-1, which stimulates insulin release (7). The enzyme dipeptidyl peptidase-4 (DPP-4) has been shown by several studies to affect functions of GLP-1 (2,21,69). Studies have shown that there are high levels of DPP-4 in T2DM particularly in tissues such as the liver, kidney, lungs and small intestines (69,70). We know that the onset of T2DM is preceded by prediabetes which is a condition where there are high blood glucose levels but not high enough to be classified as T2DM (43,51,71). Measuring tissue gene expression of DPP-4 is essential because as mentioned that DPP-4 is the primary enzyme responsible for degrading the incretin hormones GLP-1 and GIP, therefore, changes in its expression may directly influence incretin availability and contribute to early disturbances in glucose regulation. Furthermore, correlating tissue DPP-4 expression with circulating incretins and energy-metabolic hormones such as leptin and ghrelin will help determine whether increased DPP-4 activity

and expression is associated with impaired incretin action, disrupted appetite regulation, and altered energy balance metabolic pathways known to deteriorate during the transition from normoglycaemia to prediabetic stage. However, there have been no studies to investigate the changes that occur in tissue DPP-4 expression during the prediabetic state. In our laboratory, we use a HFHC diet-induced animal model of prediabetes that has been shown to mimic the human condition (72,73). Therefore, using this animal model, this study sought to investigate changes in DPP-4 expression in tissues such as the liver, kidney and lungs in diet-induced prediabetes.

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Bridge

The literature review documented that DPP-4, and incretin hormones play a crucial role in glucose homeostasis. The presence of sDPP-4 reduces the levels of incretin hormones such as GLP-1 and GIP resulting in impaired insulin secretion. These incretin hormones are responsible for stimulating insulin secretion and inhibit glucagon secretion. Furthermore, the literature review highlighted that during T2DM, there are drastically elevated levels of sDPP-4, resulting in reduced levels of GLP-1 and ultimately affecting blood glucose regulation in the body. T2DM is often preceded by a condition called prediabetes. However, the literature review identified a critical knowledge gap regarding changes in DPP-4 expression in tissues and levels in plasma during the prediabetic state. This observation led to the objective of the systematic review that aimed to investigate changes in tissue DPP-4 expression as well as changes in plasma levels of sDPP-4 during T2DM and PD.

DETAILS OF THE NEXT MANUSCRIPT

The next manuscript is titled "**Dipeptidyl Peptidase 4(DPP-4) Expression changes in Tissues During Type-2-Diabetes and Prediabetic States: A Systematic Review and Meta-analysis**" and is authored by A. Shange, NC Mzimela and A. Khathi.

Author Contribution: A. Shange was responsible for study conceptualization, study design, first draft writing and manuscript editing.

The Dipeptidyl Peptidase 4(DPP-4) Expression changes in Tissues During Type-2-Diabetes and Prediabetic States: A Systematic Review and Meta-analysis

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Abstract

Background

Dipeptidyl peptidase-4 (DPP-4) is a multifunctional protein that can bind to a range of extracellular ligands in addition to its enzymatic activity. It exists as a transmembrane and soluble protein. Since the introduction of DPP-4 inhibitors for the treatment of type 2 diabetes mellitus (T2DM), the significance of DPP-4 for the scientific and medical communities has increased significantly. T2D is often preceded by prediabetes (PD). Prediabetes is a condition where the body has high blood glucose levels but not high enough to be classified as type 2 diabetes. This systematic review aimed to evaluate studies that investigated the changes in DPP-4 expression in tissues in individuals with PD and T2DM and changes to DPP-4 in relation to glucose homeostasis in T2DM.

Methods

This systemic review and meta-analysis were prepared following the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) Checklist 2020 guidelines. MEDLINE, COCHRANE library, EMBASE, Google scholar and African Journal Online were the search engines used. All the obtained articles were screened and relevant studies that met the inclusion criteria were selected for the study. The risk of bias was assessed using the Downs and Black Checklist, and the Forest plot in Review Manager v5.4 was utilised for the meta-analysis. The Grading of Recommendations Assessment, Development, and Evaluation approach was used to determine the strength of the evidence.

Results

A total four studies met the inclusion criteria and were considered for this systematic review. The studies focused on patients with T2DM and on patients with PD. However, the changes in DPP-4 tissue expression in tissues during the prediabetic state and how the changes affect glucose homeostasis were unclear due to limited studies.

Conclusion

A total of four articles were considered in the systematic review. Further research is needed to fully elucidate the mechanisms underlying the relationship between DPP-4 activity and T2DM onset and DPP-4 expression in tissues in the prediabetic state.

Key words

Dipeptidyl peptidase-4, Type 2 diabetes Mellitus, Glucose Homoeostasis, Prediabetes, Tissue expression

Introduction

1.1 Background

Type 2 diabetes mellitus(T2DM) is a metabolic disorder characterized by insulin resistance(1). It accounts for 90% of diabetes cases (2). It is often preceded by prediabetes (PD) (3).It is a state of intermediate hyperglycaemia where the blood glucose levels are above the homeostatic range but not high enough to be classified a s type 2 diabetes (3,4). In 2019, the global prevalence of prediabetes was estimated to be 7.5%, affecting approximately 374 million people, and expected to rise to 8.0%, or 454 million individuals, by 2030 (5,6). The World Health Organization (WHO) defines prediabetes based on impaired fasting glucose (IFG) levels between 6.1 and 6.9 mmol/L, and impaired glucose tolerance (IGT), identified by a 2-hour plasma glucose level ranging from 7.8 to 11.0 mmol/L (7,8). In comparison, the American Diabetes Association (ADA) uses the same IGT range but applies a lower IFG threshold of 5.6 to 6.9 mmol/L (9). Furthermore, the ADA also considers a glycated haemoglobin (HbA1c) level of 5.7% to 6.4% as part of the diagnostic criteria for prediabetes (9,10). Interestingly, previous studies have shown that type 2 diabetes is associated with drastically altered DPP-4 enzyme activity, specifically indicated that individuals with type 2 diabetes often exhibit elevated DPP-4 activity in their blood plasma (11). This increased activity can influence the levels of incretin hormones such as glucagon-like-peptide (GLP-1) and glucose-dependant insulinotropic polypeptide (GIP), which are involved in glucose homeostasis and controlling appetite and maintaining body weight (11,12).

Dipeptidyl peptidase 4 (DPP-4) is a serine protease that exists as either a membrane-localized protein or as a soluble form (11,13). It is found in various organs, including the brush border membranes of the intestine, kidneys, the liver, pancreas, lungs, glandular epithelial cells, and immune system cells (11,13,14). DPP-4 plays a crucial role in glucose homeostasis as it inactivates incretin hormones such as GLP-1 and GIP which play key roles in glucose homeostasis by stimulating insulin secretion (15). Due to its altered expression and activity being linked with elevated body mass index and hyperglycaemia, DPP-4 is believed to contribute to the development of type 2 diabetes by acting as a local mediator of inflammation and insulin resistance in adipose and liver tissues, thereby potentially connecting obesity with T2DM pathogenesis (16,17). Additionally, a published previous study has shown that high levels of DPP-4 in the body potentially result in elevated levels of blood glucose in the body (18).

Although DPP-4 has been extensively studied in the context of metabolic diseases, there is a lack of research specifically investigating its expression in tissues during the prediabetic state. Therefore, this systematic review aimed to evaluate studies that have investigated changes in tissue-specific DPP-4

expression during prediabetes and type 2 diabetes. Additionally, the review will explore evidence linking alterations in DPP-4 expression to disruptions in glucose homeostasis associated with PD and T2DM.

1.2 Research question(s)

1. Are there differences in DPP-4 expressions in various tissues during the prediabetic state?
2. Are there correlations in DPP-4 expression in various tissues with glucose homeostatic markers in prediabetes and type-2 diabetes?

1.3 Objectives

1. To investigate studies that reported changes in DPP-4 expression in various tissues during prediabetes and type 2 diabetes.
2. Rephrase to "To investigate studies that reported correlations of DPP-4 expression with glucose homeostatic biomarkers in prediabetes and type-2-diabetes.

2.Materials and Methods

2.1 Design

This systemic review protocol was prepared following the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) Checklist 2020 guidelines. The protocol for this systematic review was registered in the PROSPERO International Prospective Register of systematic reviews, under the following registration number **CRD42023459958**.

2.2 Eligibility criteria for studies

The studies were included in the systematic review and meta-analysis if they met the following inclusion criteria: Eligible clinical studies were observational in design, including cross-sectional, comparative cross-sectional, case-control, or cohort studies, and involved participants who were non-diabetic, prediabetic, or had type 2 diabetes. Studies with the minimum of 100 participants that reported community-based clinical cross-sectional investigations were eligible. Cross-sectional and prospective and retrospective observational studies that investigated the changes in DPP-4 expression and activity in the prediabetic and T2D states were included. Lastly, all prediabetic and T2DM patients, aged above 18 years of all ethnicities were included. Patients previously or currently diagnosed with type 1 diabetes

and gestational diabetes were excluded in this review. Also, the studies involving people with a history of liver disease, kidney disease, heart disease, and depression were excluded. Additionally, reports from pregnant women and professional sports athletes were not used.

2.3 Criteria for diagnosis of T2DM and PD

Diagnostic criteria for T2DM and PD were as follows: fasting plasma glucose (FPG) level of 126mg/dL (7.0 mmol/L), 2 hours postprandial blood glucose (2 h- OGTT), 200mg/dL (11,2mmol/L) or higher during a 75g glucose tolerance test (OGII) with glycated haemoglobin (HbA1c) ranging from 5,7% to 6.4% as per the American Diabetes Association (ADA).

2.4 Search Strategy/Data sources

The research databases used were MEDLINE (From 1963-2023), EMBASE, and ICTRP (1963-2023) and African Journal Online (from 1998-2023). Medical subject headings (MeSHs) and keyword terms are ("Dipeptidyl peptidase-4" OR "DPP-4") AND ("Type 2 Diabetes Mellitus" OR "T2D" OR "prediabetes") AND ("glucose homeostasis" OR "tissue expression").

2.4 Selection of included study

Two independent reviewers (AS & NCM) conducted the validity assessment for each included study. Reviewers independently screened the titles, abstracts, and full texts to acquire relevant studies.

2.5 Analysis

MicroSoft Excel file was used to record the extracted data of study records selected as eligible reports. The pre-defined list of variables was considered in each report used as categories in an Excel file. Therefore, the baseline characteristics of eligible research reports obtained was the author, year of publication, country, and study setting. The methodology of the study reported was considered with the categories (design, period, sampling strategy, and whether participants are normal or pre-diabetic population) considered. Finally, the outcomes from different gender, ages, ethnicity, DPP4 treated changes /markers were then extracted.

2.6 Risk of bias

To measure the potential risk of bias in individual studies, the Downs and Black Checklist was used(19). For clarity, the scores were rated as follows; excellent (25–26), good (20–24), moderate (14–19), poor (11–13), and very poor (< 10).Two reviewers (AS, and NCM) were responsible for the independent judgments which were based on the four domains of the Downs and Black checklist tool which is reporting bias (10 items), external validity (3 items), internal validity (6 items), and selection bias (7 items). In a situation where there was a difference in opinions between AS and NCM. AK was then responsible for adjudication.

2.7 Strategy for data synthesis

For the meta-analysis of reported data, a Review Manager version 5.4 software Forest plot was used(20). Using this RevMan Forest plot, eligible data from all reported studies was meta-analysed depending on their sample size and the odds ratio in both prediabetic and control groups. Additionally, an odd ratio and confidence interval was used to plot the forest plot where the solid lines will represent the 95% confidence interval. Each reported study was represented as a horizontal line on the y-axis to list the primary author and year of study. The forest plot also included the weight of the study results that was automatically obtained using RevMan software.

2.8 Data synthesis

A larger overlap between the confidence intervals suggested better homogeneity, which was tested for using the RevMan forest plot (20). $I^2$was then computed using the forest plot, yielding a result between 0% and 100%. A score of more than 75% was regarded as indicative of considerable heterogeneity, whereas a value of less than 25% was thought to be a signal of strong homogeneity. Nonetheless, a 50% value was regarded as the average.

2.9 Assessment of strength of evidence

AS, NCM and AK were in charge of determining the evidence's strength. The Grading of Recommendations Assessment, Development, and Evaluation technique (GRADE) was used to assess the studies that were part of the review (21). Moreover, a GRADE pro tool was used to construct the summary of findings table.

3.Results

3.1Study selection

The initial search resulted in a total of articles 2703. The titles and abstracts were screened for eligibility. A total of 64 articles remained and 60 full- text articles were then assessed for eligibility. A total of four articles met the eligibility criteria as shown in Figure 1.

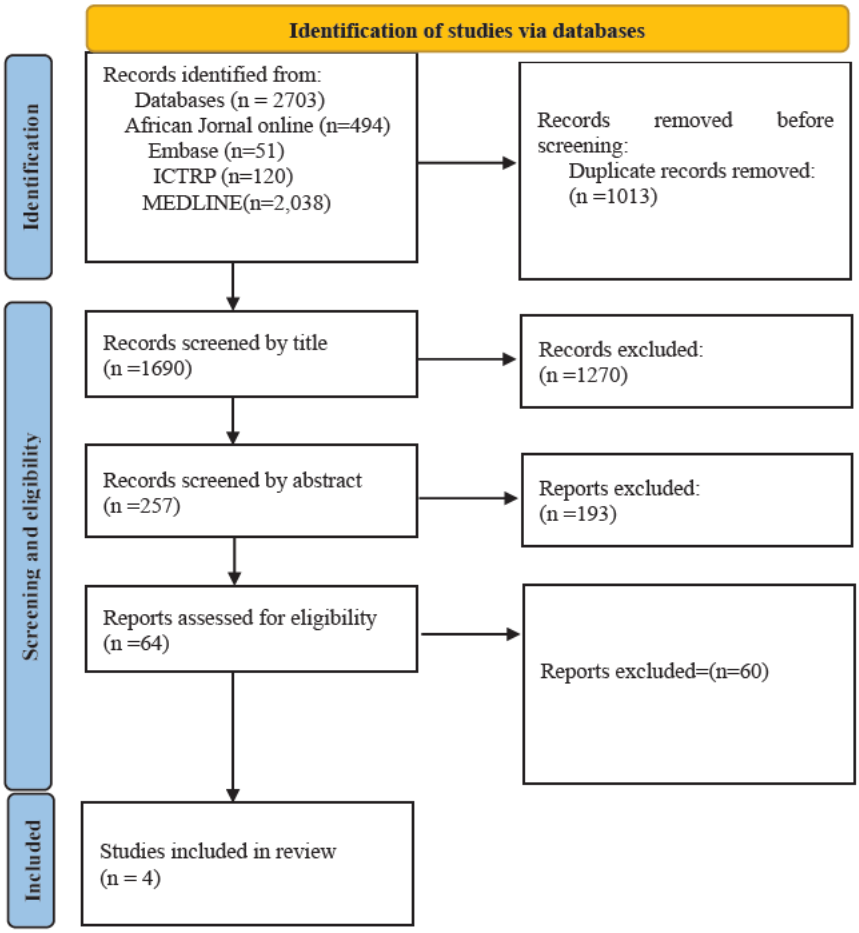

Figure 1: A Prisma flow diagram representing the procedure for identifying and selecting studies included in the review.

3.2 Summary of the subgroups

Human studies or non-diabetic and pre-diabetic individual were included (e.g., all clinical studies such as randomised/ non-randomised trial, high dose/low dose of DPP-4, all settings such as hospital/ home care were included, participant characteristics such as male/female, all ethnicities, reports that reported for population aged from 25 to 45 years).

Table 1: Summary of the subgroups of the included studies

Study	Design	Sample Size	Age Range	Sex	Ethnicity	Setting
Zheng et al	Prospective community-based cohort	474	18–70 years	Male and female	Chinese (Asian)	Community/outpatient
Sarker et al	Prospective observational cohort	123	30–50 years	Male and female	Indian	Outpatient clinic, Kolkata
Sell et al	Cross-sectional analysis	196	24–86 years	Male and female	Mixed (not specified)	Hospital (metabolic clinic)
Bugliani et al	Comparative observational (post-mortem)	43	25–45 years	Male and female	Mostly Caucasian	Tissue bank

Table 1: Cont.

Study	Tissue/Measurement	Prediabetic Stage	Key Outcome
Zheng et al	Plasma DPP-4 activity	Combined IFG/IGT	Elevated DPP-4 predicted prediabetes/T2DM
Sarker et al	Plasma DPP-4 activity and cytokines	Combined IFG/IGT	High DPP-4 linked to hyperglycaemia and inflammation
Sell et al	DPP-4 mRNA/protein in VAT and SAT	IGT (via OGTT)	Higher VAT DPP-4 in IGT/T2DM; linked to insulin resistance(T2DM)
Bugliani et al	DPP-4 expression in pancreatic α- and β-cells	(T2DM vs non-DM)	Lower pancreatic DPP-4 in T2DM vs non-diabetic donors

3.3Appraisal of the quality of included studies

To measure the potential risk of bias in individual studies, the Downs and Black Checklist was used. For clarity, the scores were rated as follows; excellent (25–26), good (20–24), moderate (14–19), poor (11–13), and very poor (< 10). Two studies were rated excellent, one study was rated good, and one study was rated moderate as shown in Table 2. Downs and Black answers for all questions per report for all reports has been attached.

Table 2: Appraisal of the quality of included studies

Study	Design	Downs & Black Rating	Score
Zheng et al	Prospective cohort	Excellent	25
Sarker et al	Prospective cohort	Excellent	26
Sell et al	Cross-sectional	Good	21
Bugliani et al	Comparative observational	Moderate	18

3.4 Data synthesis for eligible reports

The forest plot was used to synthesise data for eligible studies as shown in figure 2. After the meta-analysis was done, it showed the heterogeneity of 98%. The experimental group represent the prediabetic group and the control group represent the non-diabetic.

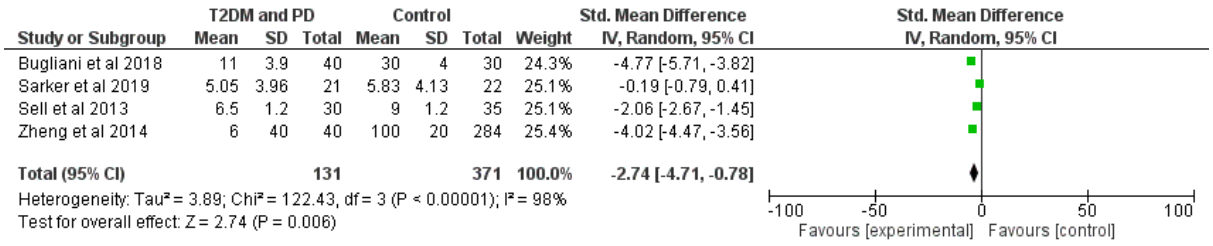

Figure 2: Forest plot representing included studies

3.5 Summary of findings (SoF) Table

The table below (table 3)summarizes the expression levels or changes in DPP-4 expression (Increased, decreased or no changes), specify the tissue(s) that was examined, defined the prediabetic stage studied (IFG, IGT or combined IFG/IGT).

Table 3: Summary of the findings

Study	Tissue Examined	Prediabetic Stage (IFG/IGT)	DPP-4 Expression	Risk of Bias	Study Conclusion
Zheng et al.	Plasma	Combined IFG/IGT	Increased	Excellent	Elevated plasma DPP-4 at baseline predicted progression to prediabetes and T2DM over 4 years.
Sarker et al	Plasma	Combined IFG/IGT	Increased	Excellent	High baseline DPP-4 activity associated with future hyperglycaemia and inflammation.
Sell et al	Visceral and Subcutaneous Adipose Tissue	IGT and T2DM	Increased in VAT	Good	DPP-4 expression significantly higher in VAT in IGT/T2DM groups; associated with insulin resistance.
Bugliani et al	Pancreatic α- and β-cells	(T2DM focused)	Decreased	Moderate	Reduced DPP-4 expression in islets of T2DM donors. Included for tissue relevance despite smaller sample size.

4. Discussion

The search for relevant studies on changes in tissue expression of DPP-4 during the T2DM and PD was extremely challenging due to the limited availability of related studies. Moreover, the exact mechanisms underlying changes in DPP-4 expression and activity in prediabetes remain poorly understood. Several studies have shown that there are elevated DPP-4 levels in individuals with T2DM (11,22). However, T2DM is often preceded by prediabetes (3,23). Therefore, this systematic review aimed to summarize the current information on changes in DPP-4 expression in tissues during T2DM and PD.

A study conducted by Yang and Zheng et al. confirmed that in individuals with prediabetes or type 2 diabetes, DPP-4 activity and active GLP-1 levels were significantly associated with certain metabolic

parameters, aligning with their previous research findings (24). Furthermore, their study revealed a strong correlation between age and DPP-4 activity in individuals who developed prediabetes and type 2 diabetes (24). In particular, the liver and small intestine are primary sources of circulating DPP-4, yet no study has measured DPP-4 expression in these tissues among prediabetic humans (25,26).

A study by Sarker et al, documented that DPP-4 activity in individuals with T2DM and PD is both elevated. Plasma DPP-4 activity is significantly elevated in individuals with type 2 diabetes mellitus (T2DM), regardless of obesity status (27). This suggests that adipose-derived DPP-4 may not be the primary contributor to plasma DPP-4 activity (27). However, plasma DPP-4 levels were higher in obese T2DM individuals compared to non-obese T2DM individuals, potentially due to adipose tissue's contribution to plasma DPP-4 levels (27). Notably, this increase in plasma DPP-4 levels in obese T2DM individuals did not translate to increased plasma DPP-4 activity, as activity levels were comparable between obese and non-obese T2DM groups. These findings support the notion that DPP-4, as an adipokine, may serve as a link between adiposity and T2DM (25,28).

Adipose tissue has emerged as one of the most metabolically active sources of DPP-4, particularly in obese and insulin-resistant individuals (28,29). A study by Sell et al 2014, a well-designed cross-sectional study involving over 100 adults (including individuals with normal glucose tolerance, impaired glucose tolerance [IGT], and T2DM), directly measured DPP-4 expression in visceral (VAT) and subcutaneous adipose tissue (SAT) (29). This study excluded individuals with confounding conditions such as liver, kidney, or heart disease, thereby increasing the validity of its findings regarding adipose-specific expression (29). The results revealed significantly higher DPP-4 expression in visceral adipose tissue compared to subcutaneous fat, with the highest levels observed in participants with IGT and T2DM (29). This suggests a potential depot-specific role of DPP-4 in mediating insulin resistance and chronic inflammation.

According to a previous study, DPP-4 is modulated in a tissue-specific manner, meaning its expression largely depends on underlying physiological conditions (11,25,30). Factors such as insulin resistance and hyperglycemia play a significant role in metabolic disorders like prediabetes (2). One possible explanation for this variability is that different tissues respond uniquely to metabolic stress, leading to either upregulation or downregulation of DPP-4 expression based on local demands and signalling environments. The reviewed studies provide converging evidence that DPP-4 expression and activity are differentially regulated across tissues during the progression from normoglycemia to T2DM (29). Elevated sDPP-4 levels are consistently associated with prediabetes and diabetes, with adipose tissue

particularly visceral fat emerging as a major contributor (29). Meanwhile, islet expression appears to decline in diabetes, indicating a possible loss of regulatory balance in endocrine pancreas function.

A study by Bugliani et al provided histological and molecular insight into DPP-4 expression within the pancreas, specifically in α- and β-cells (31). While this study included fewer than 100 donors and therefore fell below the standard inclusion threshold for this systematic review, it was retained due to its relevance and unique tissue-specific focus. Importantly, no other large-scale human study was identified that directly assessed islet DPP-4 expression in both T2DM and non-diabetic donors. Moreover, it revealed a significant reduction in DPP-4 expression in the islets of individuals with T2DM compared to non-diabetic controls (31). This finding challenges the general trend of increased DPP-4 activity observed in plasma and adipose tissue and may indicate a tissue-specific downregulation of DPP-4 in response to hyperglycemia, oxidative stress, or β-cell dysfunction (31). The decrease in pancreatic DPP-4 could also reflect the loss of functional β-cell mass, which is a known feature of progressive T2DM.

The decrease in pancreatic DPP-4 could also reflect the loss of functional β-cell mass, which is a known feature of progressive T2DM. The available evidence affirms that DPP-4 is differentially expressed in key metabolic tissues and that these patterns are modulated during the transition from prediabetes to T2DM. While plasma and adipose DPP-4 levels increase with disease progression, pancreatic islet expression appears to decline, underscoring the tissue-specific complexity of DPP-4. Despite strong evidence linking DPP-4 to T2DM, none of the reviewed studies directly assessed DPP-4 expression specifically in individuals with prediabetes, nor did they investigate its expression in key target tissues such as liver, kidneys, lungs, or small intestine organs that are of particular interest in our upcoming study. The literature currently focuses on plasma measurements and a few specific tissues (e.g., adipose and pancreas), indicating a gap in comprehensive tissue-level exploration in prediabetic cohorts.

Although the included studies primarily evaluated plasma, adipose, and pancreatic tissues, prior literature confirms that DPP-4 is also highly expressed in the liver, kidney, small intestine, and lungs tissues that play key roles in glucose homeostasis and metabolic regulation (11,30). Therefore, expanding investigations to these organs is important. For instance, hepatic DPP-4 has been shown to be upregulated in individuals with non-alcoholic fatty liver disease (NAFLD), which frequently coexists with T2DM (32). Similarly, renal DPP-4 activity has been implicated in diabetic nephropathy, while intestinal DPP-4 is responsible for the degradation of incretins at the mucosal surface (33). While some

of these studies included individuals who developed prediabetes during follow-up, neither reported tissue-specific DPP-4 expression in these individuals.

Limitations

The main limitation is that only few articles had an excellent risk of bias which is clear indication that more studies are still needed that will outline expression of DPP-4 in various tissues during the prediabetic state. Furthermore, some studies did not consider the role of DPP-4 in other metabolic pathways and diseases and measurement of DPP-4 expression in different tissues was not the same. These limitations highlight the need for further research to address these gaps and provide a more comprehensive understanding of the DPP-4 expression in various tissues in the body during a prediabetic stage.

Conclusion

DPP-4 expression is altered in various tissues in individuals with T2DM. However, the lack of studies on DPP-4 expression in prediabetes highlights the need for further research in this area. Some of the evaluated studies employed outdated measurements, and others did not follow the same criteria for identifying prediabetes and type 2 diabetes. Additionally, While the studies have certain limitations, the consistency of the findings across different tissues and study designs strengthen the evidence of DPP4 expression in the tissues of these organs, small intestines, kidneys, lungs, and liver. Further research is required to investigate the expression of dipeptidyl peptidase-4 (DPP-4) in tissues during the prediabetic state.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding this systematic review.

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PROLOGUE

The systematic review revealed a paucity of studies regarding changes in DPP-4 expression in tissues and sDPP-4 in plasma during prediabetes. Despite the well-established role of DPP-4 in glucose homeostasis, no studies to date have specifically investigated tissue-specific DPP-4 expression in individuals with prediabetes. Most existing studies have focused on comparisons between individuals with normal glucose tolerance and those with type 2 diabetes mellitus (T2DM). This gap led to the development of the original research manuscript, which aimed to investigate changes in DPP-4 expression in tissues as well as concentrations of sDPP-4 in plasma of diet-induced prediabetic rats, thereby providing new insights into the role of DPP-4 in glucose metabolism.

DETAILS OF THE NEXT MANUSCRIPT

The next manuscript is titled "**Tissue and Systemic Dipeptidyl Peptidase 4 and Glucose Homeostatic Responses in a Diet-induced Prediabetic Rat Model.**" and is authored by A. Shange, N.C Mzimela and A. Khathi.

Author Contribution: A. Shange was responsible for study conceptualization, study design, first draft writing and manuscript editing.

Tissue and Systemic Dipeptidyl Peptidase 4 and Glucose Homeostatic Responses in a Diet-induced Prediabetic Rat Model.

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Abstract

Background

Type-2-diabetes mellitus (T2DM) is characterized by drastically elevated levels of dipeptidyl peptidase (DPP-4), a serine protease that degrades incretin hormones such as GLP-1 that stimulate insulin secretion and inhibit glucagon release. It is often preceded by a condition known as prediabetes. However, changes in tissue and plasma DPP-4 levels during the prediabetic state remain poorly understood. Therefore, this study investigated changes in tissue expression and plasma concentration of DPP-4 in a diet-induced prediabetic rat model and examined how these changes influence glucose homeostasis.

Materials and methods

Twelve male Sprague Dawley rats were randomly allocated into two equal groups (n=6 each). Experimental prediabetes was induced in the animals using a previously reported protocol. The first group (A) was fed a standard rat chow and supplied with tap water. The second group (B), group was fed high-fat high-carbohydrate (HFHC) diet supplemented with 15% fructose for 20 weeks to induce prediabetes. At the end of the 20-week induction period, the American Diabetes Association (ADA) criteria were used to confirm prediabetes. At the end of the induction period, the animals were sacrificed, and ELISA was used to measure plasma sDPP-4, insulin, GLP-1, ghrelin and leptin in both groups. Furthermore, quantitative PCR was used to measure DPP-4 gene expression in tissues such as the kidney, lungs and liver.

Results and Discussion

The plasma sDPP-4 levels were significantly increased, alongside elevated blood glucose and glycated haemoglobin (HbA1c) levels, indicating impaired glucose regulation. Plasma insulin, ghrelin, and leptin were also elevated, suggesting disrupted incretin signalling and altered hormonal regulation of appetite and metabolism. The reduction in GLP-1, despite hyperinsulinemia, suggests enhanced incretin degradation due to elevated DPP-4 activity, potentially contributing to impaired insulin secretion. Tissue-specific analysis revealed an upregulation of DPP-4 expression in the kidney, while a downregulation was observed in the liver and lungs. These findings suggest that DPP-4 is both systemically and tissue-specifically dysregulated across tissues in the prediabetic state. Furthermore, the tissue-specific changes in DPP-4 expression further support its complex role in glucose homeostasis and early metabolic dysfunction.

Introduction

1.1 Background

Dipeptidyl peptidase 4 (DPP-4), also known as a cluster of differentiation 26 (CD26), is a serine protease that exists as either a membrane-localized or as a soluble protein (1). As an integral membrane protein, it is found in various organs, including the brush border membranes of the intestines, kidneys, vascular endothelium, the liver, lungs, pancreas, glandular epithelial and immune system cells (1). Soluble DPP-4 (sDPP-4) that circulates in plasma (2) is released from adipose tissue as a pro-inflammatory adipokine linking insulin resistance to low-grade inflammation (1,2). DPP-4 plays a role in glucose homeostasis by inactivating GLP-1 which stimulates insulin secretion (3,4). Elevated levels of sDPP-4 are associated with elevated levels of blood glucose in the body (5,6). DPP-4 has been shown to affect insulin sensitivity, possibly through non-enzymatic interactions with other membrane proteins (7). It also affects metabolic control through its proteolytic effect on other regulatory peptides (7,8). The degradation of incretins by DPP-4 decreases their insulinotropic effect and leads to higher blood glucose levels (9).

Since DPP-4 is involved in the breakdown of circulating active GLP-1 to biologically inactive fragments, the plasma active glucagon-like peptide-1 (GLP-1) level may also be related to the emergence of insulin resistance and type 2 diabetes (9). GLP-1 is involved in insulin biosynthesis, insulin secretion and cell proliferation (10,11). Some studies have documented an increase in DPP-4 expression in the livers, small intestines and kidneys of rats fed a high fat diet (12,13). Additionally, there are studies that previously looked at the changes in plasma DPP-4 and tissue DPP-4 expression during type-2-diabetes (14). These findings suggest that the changes n sDPP-4 concentration and tissue DPP-4 expression may be implicated in the development of metabolic conditions such as T2DM (14). However, the onset of T2DM is often preceded by prediabetes (15).

Prediabetes is a condition where the blood glucose levels are above the normal range but not high enough for a diagnosis of T2DM (16,17) . The global prevalence rate of prediabetes population in 2017 was estimated to be 352 million and also estimated to be 470 million by 2030 (18). Studies show that it may take between 10-20 years for this often-asymptomatic condition to develop into overt T2DM (18). A number of studies have recently shown that individuals with early forms of dysglycemia, or prediabetes, are clearly at a higher risk of vascular problems that are often linked with type 2 diabetes (19).

While there are several studies that describe changes in sDPP-4 concentrations and DPP-4 expression in both the non-diabetic and T2D states, there have been no studies that look at these changes during

prediabetes (12,20,21). Animal models have been used in the diabetes research and one of the models is the high fat high carbohydrate (HFHC) diet-induced animal model to mimic the human condition of prediabetes (22,23) . However, no study has used this animal model to investigate changes in DPP-4 expression in tissues during the prediabetic state. Therefore, the aim of this study was to investigate the changes in plasma sDPP-4 concentration and DPP-4 tissue expression in a high fat high carbohydrate diet induced rat model of prediabetes.

2. Material and methods

2.1 Animals

Male Sprague-Dawley rats (120-160 g) were bred in the Biomedical Research Unit (BRU) of the University of KwaZulu-Natal. The animals were kept under standard laboratory conditions of constant temperature of 22 ± 2 °C ◦C, carbon dioxide (CO_2) content of <5000 p.m., a relative humidity of 55 ± 5%, illumination (12 h light/dark cycle, lights on at 07h00) and the noise level was maintained at less than 65 decibels. The animals were allowed access to food and water *ad libitum*. Before being exposed to a well-established experimental diet (HFHC) to induce pre-diabetes, they animals were allowed to acclimatize to their new surroundings for a week while eating regular rat chow and were drinking tap water. All experimental procedures were conducted according to the animal ethics guidelines of the Animal Research Ethics Committee (AREC) of UKZN, Durban, South Africa and this study was a sub-study under the ethical clearance of ethics number: AREC/039/018M).

2.2 Experimental design and Induction of prediabetes

Rats were randomly allocated into two equal groups (n=6, each). Experimental prediabetes was induced in the animals using a previously reported protocol(22). For the experimental period of 20 weeks, group 1 was fed high-fat high-carbohydrate supplemented with 15% fructose to induce prediabetes, while group 2 was fed the standard rat chow and supplied with tap water. The HFHC diet was composed of carbohydrates (55%), fats (30%), and proteins (15%kcal/g) while the standard diet was composed of carbohydrates (35%), fats (15%), proteins (30%) as well as fibre and vitamins (20%). The American Diabetes Association (ADA) criteria were used to confirm prediabetes at the end of the 20 weeks experimental period (16). Briefly, this consisted of measuring the fasting blood glucose (FBG) concentrations to determine if they were in the range of 5.6 to 6.9 mmol/L for a diagnosis of prediabetes. This also consisted of checking for impaired glucose tolerance using the oral glucose tolerance test. Lastly, this was also confirmed by measuring the blood HbA1c concentrations to determine if they were in the range of 5.7 to 6.4% for a diagnosis of prediabetes.

2.3 Oral Glucose Tolerance Test responses

In order to evaluate each animal's glucose tolerance response, an oral glucose tolerance test was administered. In accordance with a recognized laboratory procedure, this test was carried out subsequent to carbohydrate loading (22,24,25). At time 0, glucose levels were measured following a 12-hour fast. An 18-gauge gavage needle, which is 38 mm long, curved, and has a 21/4 mm ball end (Able Scientific, Canning Vale, Australia), was then used to administer a monosaccharide syrup orally. The tail-prick method was used to draw blood, and a OneTouch select glucometer (Lifescan, Mosta, Malta, United Kingdom) was used to measure the glucose levels (26). After carbohydrate loading, glucose levels were then measured 15, 30, 60, and 120 minutes later. Rats with impaired glucose tolerance (IGT) with plasma glucose levels of 7.8 to 11.0 mmol/L 2 h postprandial were diagnosed as prediabetic.

2.4. Experimental design

Following induction of prediabetes, the animals were classified into the non-prediabetic (NPD) and prediabetic group (PD) (n=6 each). Body weight measured in all animals after the induction period. Additionally, 24h caloric intake was determined in all animals at the end of the induction period to note differences between the groups. Animals were exposed to anaesthesia for surgical procedures. Blood collected using EDTA tubes.

2.5 Collection and processing of plasma and tissues from normal and prediabetic rats

The study was terminated after feeding animals the experimental diets for 20 weeks. After sacrifice, blood was collected. Blood was collected as per experimental requirement and used to determine concentrations of HbA1c while the remainder of the blood was centrifuged (Eppendorf centrifuge 5403, LGBW, Germany) for 15 min at 4 ◦C (503 g) to collect plasma. Additionally, liver, kidney and lung tissue were collected after sacrifice. The plasma and tissues samples were then stored in a Bio Ultra Freezer (Labotec, Umhlanga, South Africa) at 80 ◦C until biochemical analysis was performed.

2.6 Biochemical Analysis

Glycated haemoglobin was measured using the respective sandwich and rat competitive-ELISA kits (Elabscience Biotechnology Co., Ltd, Wuhan, China) as per the manufacturer's instructions. As part of the standard experimental procedure in the ELISA kits, micro-ELISA plates were coated with antibodies. The plasma samples were pipetted into the corresponding wells, and the appropriate biotinylated detection antibody (50 µL) was immediately added. The samples were then incubated for

45 minutes at 37 ◦C, and the unbound components were washed away with the provided wash buffer. The wells were then filled with 100 µL of avidin–horseradish peroxidase (HRP), which was incubated for 30 minutes at 37 ◦C. Following a second wash to remove the unattached components, the substrate reagent (90 µL) was added to the wells, and the samples were then incubated for 15 minutes at 37 ◦C. Finally, to halt the reaction and enable the necessary measurements, a stop solution (50 µL) was lastly added to the micro-wells. Duplicates were conducted for these assays Using a nano-spectrophotometer (BMG Labtech, Ortenburg, Germany), the optical density at 450 nm was measured. Plasma insulin, sDPP-4, ghrelin and GLP-1 were measured using insulin enzyme-linked immunosorbent assay (ELISA) kits (Elabscience and Biotechnology, Wuhan, China), per manufacturer's instructions. The concentrations of plasma insulin and glycated haemoglobin in the samples were calculated using their corresponding standard curves.

2.7 Q-Polymerase Chain Reaction (PCR) for DPP-4 mRNA quantification

The kidney, liver and lung tissues were collected and homogenized and total RNA was isolated from the kidney, liver and lungs frozen tissue samples using the Maxwell® RSC simplyRNA Tissue Kit(a) (Elabscience and Biotechnology, Wuhan, China). A Nanodrop 2000 (Thermo Scientific, Roche, South Africa) was used to determine the purity and concentration of RNA and purity ratio (A260/A280) of 1.7–2.1 was considered acceptable for conversion of RNA to cDNA. The first strand cDNA was synthesised using RNA template and GoScript™ Reverse transcriptase (Invitrogen, Paisley, UK), nucleus free water, GoScript™ 5X Reaction Buffer, MgCl+2 and gene specific primers. The final reaction volume in this protocol was 20µ for cDNA. The 40µl of GoTaq® qPCR Reaction Mix was mixed with each 10µl of cDNA template and reference standards or water (no-template control) in multiwell plates at room temperature or on ice. The Go Taq® qPCR Master Mix was used to mix with the final reaction mixture before being run on PCR. Final qPCR volume was 50µl. PCR was performed using Applied Biosystem Quantstudio®5 RT PCR system (Thermo-Scientific, Applied Biosystem Quantstudio®5, South Africa). To analyse the expression of DPP-4 gene, we performed the PCR using gene specific primers. DPP-4 primers (forward: 5'-AGTGGCACGGCAACACATT-3', reverse:5'-AGAGCTTCTATCCCGATGACTT-3'). GAPDH primers, (forward:5'-GGAGCGAGATCCCTCCAA AT, reverse- GGCTGTTGT CATACTTCTCATGG-3') synthesised by Promega Anatech. The denaturing step was carried out at 95°C in the thermal cycler for 2 minutes. DNA amplification then started for 40 cycles with 95 °C denaturation for 30 s, 60 °C annealing for 60 s. Triplicates were conducted for these assays. Fold gene expression was calculated using the formula $2^{-\Delta\Delta Ct}$.

2.8 Statistical analysis

All data were expressed as means ± standard deviation. For statistical analysis, GraphPadInStat Software (version 8.00, GraphPad Software, San Diego, California, USA) was utilized. A normality test was used to test if data was normally distributed rat population. An unpaired t-test was used to analyse data. The value of $p < 0.05$ was considered statistically significant between the groups compared.

3. Results

3.1 Fasting Blood glucose and OGTT response

The fasting blood glucose levels were measured in both experimental groups after the prediabetes induction. The results showed that the PD group had significantly higher fasting blood glucose concentration when compared to the NPD group (PD vs. NPD) ($p < 0.0001$) (Figure 1A). An OGTT was also conducted at 20 weeks which was at the end of the period of induction of prediabetes and blood glucose concentrations in the PD group were significantly higher compared to the NPD group. The same trend was observed for the duration of the prediabetes induction period (PD vs. NPD) ($p < 0.0001$) (Figure 1B). Glycated haemoglobin levels were measured in the blood of both experimental groups after the prediabetes induction. The results showed that the PD group had significantly higher fasting blood glucose concentration when compared to the NPD group (PD vs. NPD) ($p < 0.0001$) (Figure 1C).

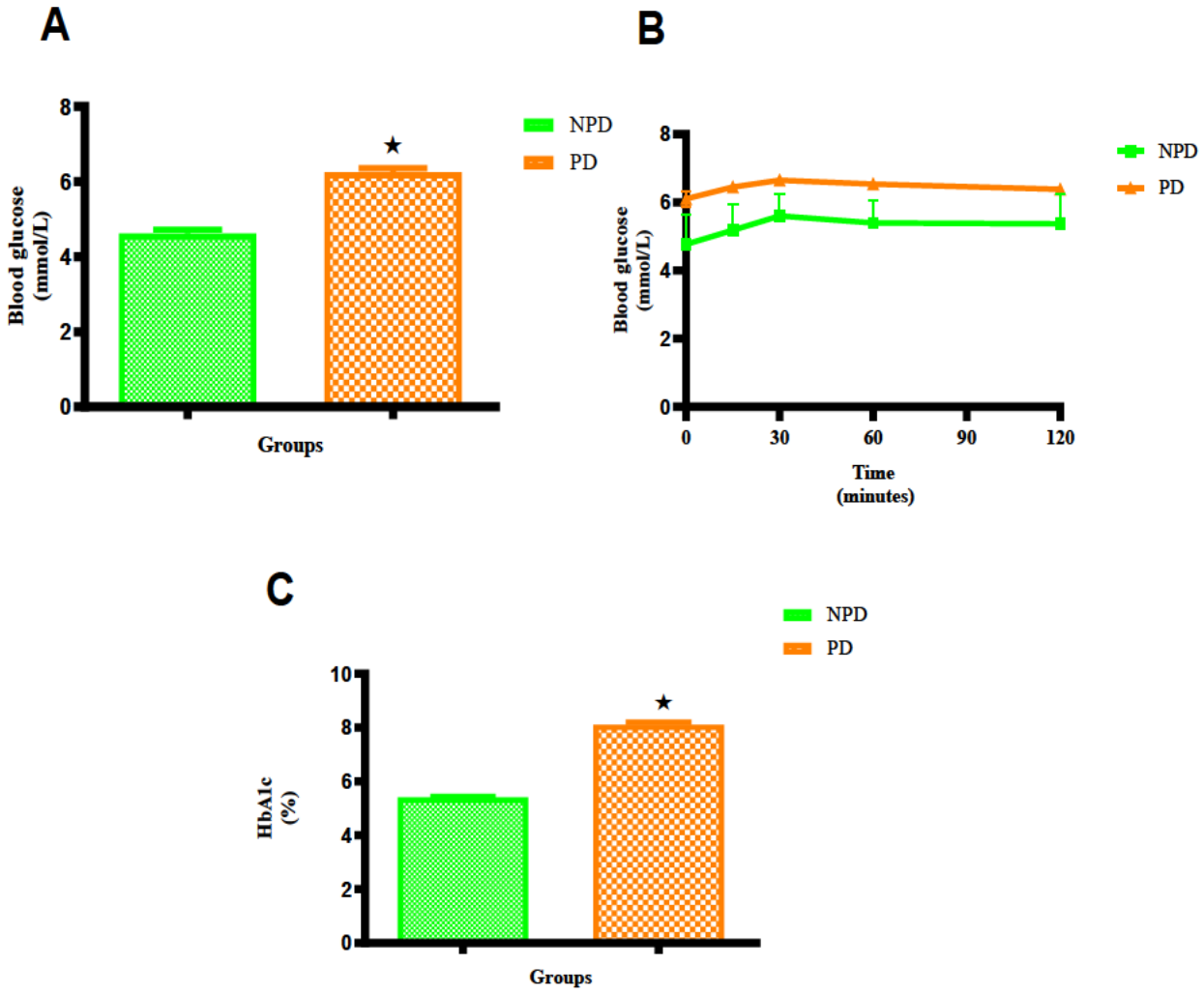


Figure 1: Comparison of fasting blood glucose levels, OGTT and glycated haemoglobin responses in non-prediabetic and diet-induced prediabetic rats. The values are presented as the standard deviation of mean ± SD. ⋆=$p<0.05$ denotes comparison with NPD.

3.2 Body weight

The body weight of all the animals in both experimental groups was measured after the prediabetes induction period. The results showed that at the end of prediabetes induction, the PD group had significantly higher body weights in comparison to the NPD group (PD vs. NPD) ($p < 0.0001$).

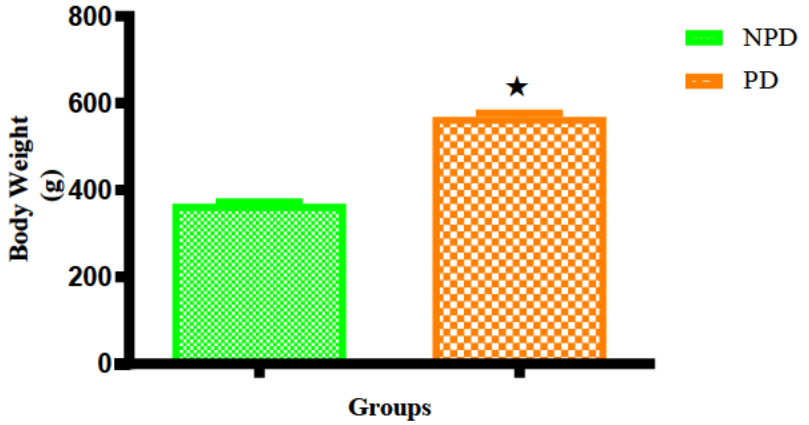


Figure 2: Comparison of body weight in non-prediabetic and diet-induced prediabetic rats. The values are presented as the standard deviation of mean ± SD. ⋆=p<0.05 denotes comparison with NPD.

3.3 Plasma sDPP-4

Plasma DPP-4 levels were measured in all the animals of both experimental groups after the prediabetes induction period. The results showed that at the end of prediabetes induction, the PD group had a significantly higher plasma sDPP-4 levels in comparison to the NPD group (PD vs. NPD) (p <0.0851).

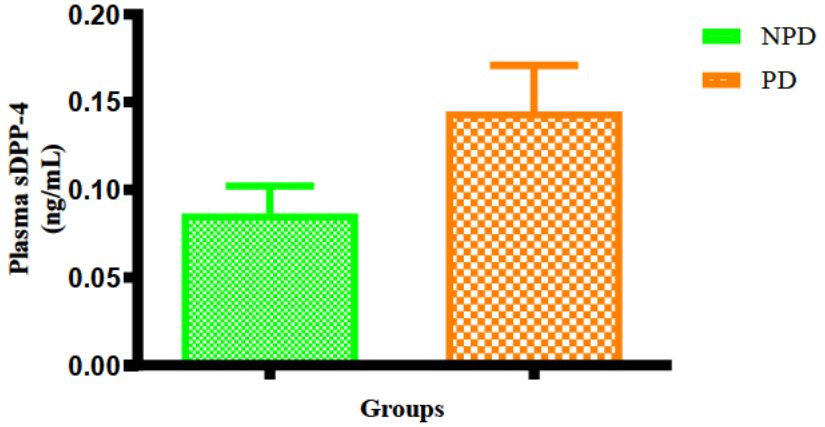


Figure 3: Comparison of plasma levels of sDPP-4 in non-prediabetic and diet-induced prediabetic rats. The values are presented as the standard deviation of mean ± SD.

3.4 Plasma insulin

Plasma insulin concentrations were measured in both experimental groups at the end of the prediabetes induction period. The results showed that at the end of prediabetes induction, the PD group had significantly higher plasma insulin concentration when compared to the NPD group (PD vs. NPD) ($p < 0.0001$).

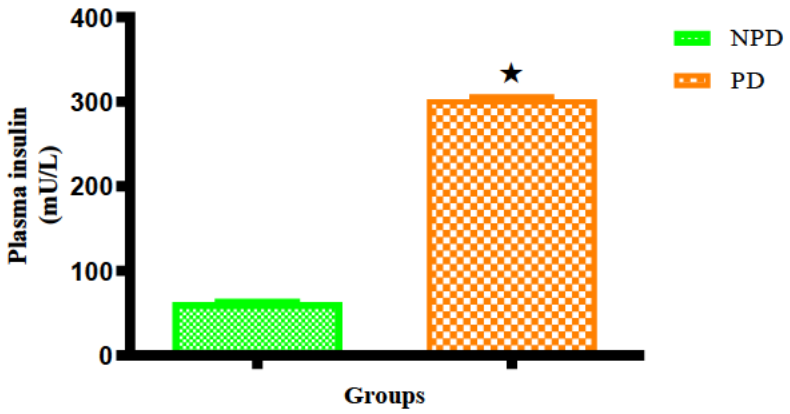


Figure 4: Comparison of plasma insulin in non-prediabetic and diet-induced prediabetic rats. The values are presented as the standard deviation of mean ± SD. ⋆=$p<0.05$ denotes comparison with NPD.

3.5 Plasma GLP-1

Plasma GLP-1 was measured in both experimental groups after the prediabetes induction period. The results showed that at the end of prediabetes induction, the PD group had significantly lower plasma GLP-1 concentration when compared to the NPD group (PD vs. NPD) ($p < 0.0001$).

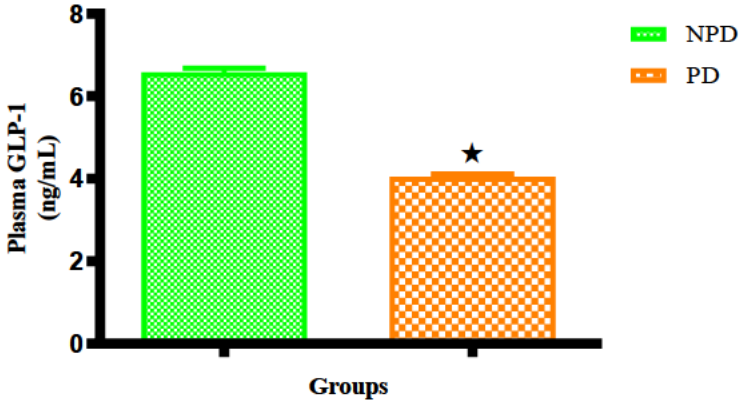


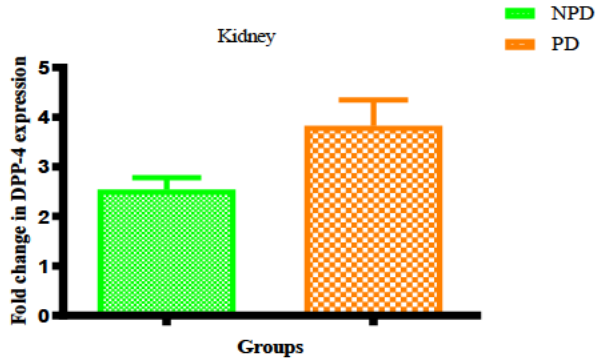
Figure 5: Comparison of plasma GLP-1 in non-prediabetic and diet-induced prediabetic rats. The values are presented as the standard deviation of mean ± SD. ⋆=p<0.05 denotes comparison with NPD.

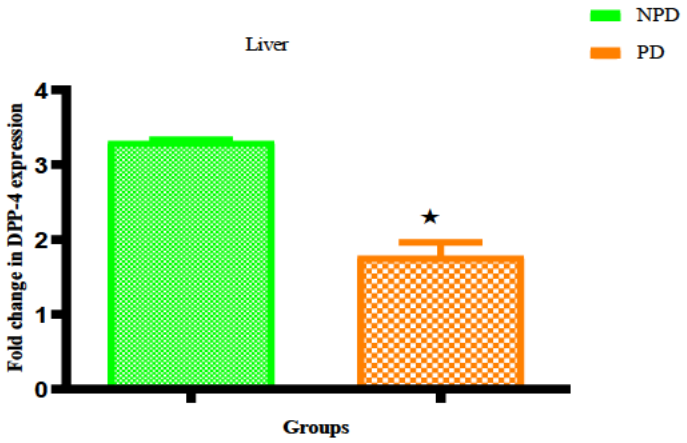
3.6 Correlation analysis between HbA1c and sDPP4, GLP-1 and also between sDPP4 and GLP-1

The correlation between HbA1c and sDPP4, GLP-1 and also between sDPP4 and GLP-1 was calculated between the NPD and PD groups. There was a non-significant negative correlation between HbA1c and sDPP4 of the NPD group ($r = -0.6$, $p = 0.19$) and also non-significant negative correlation between HbA1c and sDPP4 of the PD group ($r = -0.12$, $p = 0.80$). In contrast the association between HbA1c and GLP-1 concentration was positive and non-significant in the NPD ($r = 0.5$, $p = 0.3$) and PD groups ($r = 0.2$, $p = 0.6$). The was a non-significant negative correlation between sDPP4 and GLP-1 of the NPD group ($r = -0.6$, $p = 0.14$) and also non-significant positive correlation between sDPP4 and GLP-1 of the PD group ($r = 0.6$, $p = 0.20$).

3.7 DPP-4 Gene expression in kidney, liver and lungs of normal and prediabetic rats.

DPP-4 expression was measured in the kidneys, liver and lungs of all animals in both the experimental groups after the prediabetes induction period. The results showed that at the end of prediabetes induction, the PD group expressed significantly higher levels of kidney DPP-4 expression when compared to the NPD group. However, the PD group exhibited significantly lower levels of DPP-4 expression in both the liver and lungs when compared the NPD group (PD vs. NPD) ($p < 0.05$).





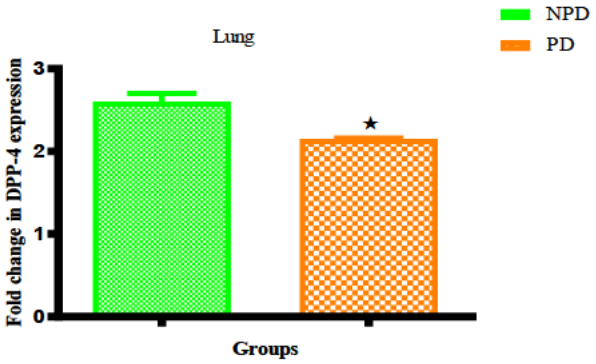


Figure 6: Comparison of expression DPP-4 in kidney, liver, and lungs in non-prediabetic and diet-induced prediabetic rats. The values are presented as the standard deviation of mean ± SD. ⋆=$p<0.05$ denotes comparison with NPD.

3.8 Plasma Ghrelin

Plasma ghrelin was measured in both experimental groups after the prediabetes induction period. The results showed that at the end of prediabetes induction, the PD group had significantly higher ghrelin concentration when compared to the NPD group (PD vs. NPD) ($p < 0.0001$).

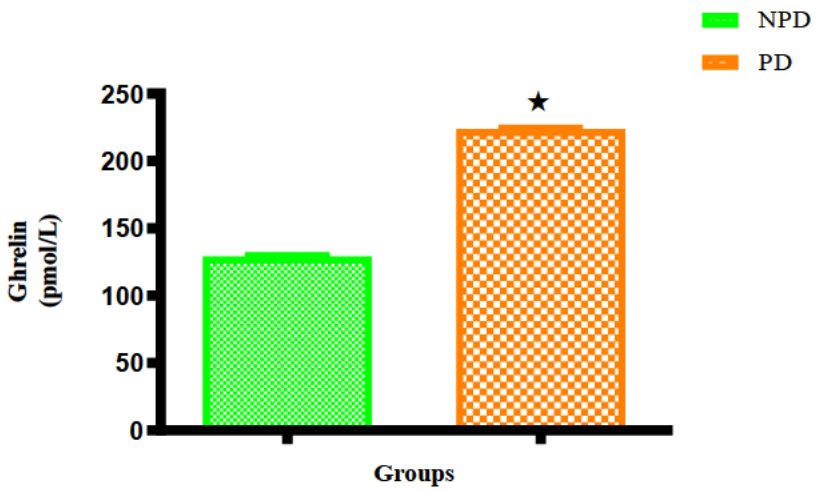


Figure 7: Comparison of ghrelin in non-prediabetic and diet-induced prediabetic rats. The values are presented as the standard deviation of mean ± SD. ⋆=$p<0.05$ denotes comparison with NPD.

3.9 Plasma Leptin

Plasma leptin was measured in both experimental groups after the prediabetes induction period. The results showed that at the end of prediabetes induction, the PD group had significantly higher leptin concentration when compared to the NPD group (PD vs. NPD) ($p < 0.0001$).

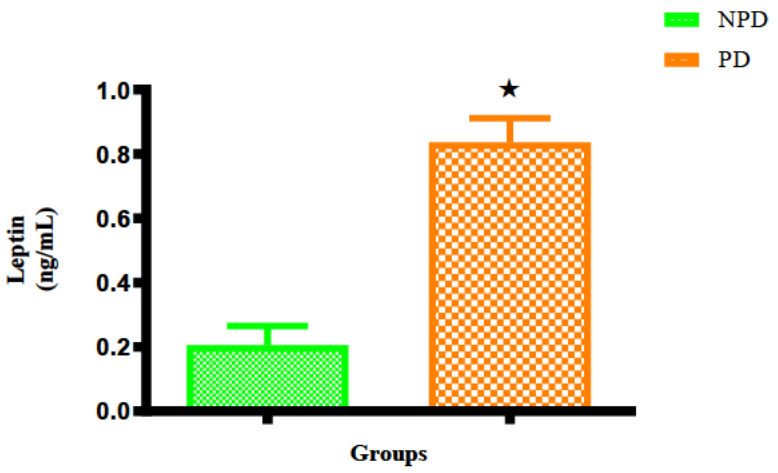


Figure 8: Comparison of leptin in non-prediabetic and diet-induced prediabetic rats. The values are presented as the standard deviation of mean ± SD. ⋆=$p<0.05$ denotes comparison with NPD.

4. Discussion

DPP-4 is a multifunctional protein that has a variety of functions in addition to its main function as a proteolytic enzyme (1,2,27). DPP-4 has been identified as a receptor or ligand for a variety of different molecules, allowing it to alter physiological processes such as cell-extracellular matrix contact, cell migration and proliferation, either alone or in combination with its enzymatic activity (27,28). DPP-4 plays a pivotal role in glucose homeostasis as it inactivates incretins like GLP-1 and GIP (9,29,30). This results in reduced insulin secretion, increased glucagon secretion and elevated blood glucose levels (29,30). Previous studies have shown that in T2DM, there is increased DPP-4 expression in tissues such as small intestines, liver, kidneys and lungs (13,31). Elevated DPP-4 levels resulted high inactivating of incretins hormones like GLP-1 and GIP which led to in high blood glucose levels during T2DM (13). The onset of T2DM, however, is often preceded by a condition known as prediabetes, a condition where there are high blood glucose levels but not high enough to be classified as T2DM (16,32). While changes in insulin have been investigated during PD, the tissue expression of DPP-4 in relation to plasma incretin levels during prediabetes is not well understood. Hence, this study investigated the changes of DPP-4 expression in lungs, kidney and liver during a diet-induced prediabetes. Moreover, the study investigated the associations of DPP-4 expression with markers of glucose homeostasis during a diet-induced prediabetic state.

Body weight is a measurement of the total body mass (33). Under normal conditions, body weight has been shown to be a factor in blood glucose handling (33,34). Elevated body weight is often linked to increased likelihood of developing insulin resistance as the cells are not responding to insulin as they

should leading to elevated blood glucose levels (34–36). Previous studies have shown that due to the moderate insulin resistance seen during the prediabetic state, existing adipocytes can grow larger due to various metabolic changes associated with the onset of insulin resistance (34,35,37). Furthermore, insulin resistance is known to affect energy expenditure by disrupting energy metabolism potentially leading to decreased energy expenditure ultimately leading to weight gain (37). The results of the present study show that the PD group had significantly higher body weights compared to the NPD group. These results correspond with the previous studies which showed that chronic consumption of a diet high in carbohydrates and saturated fats leads to increased body weights which is associated with the prediabetic state (22). A recent study by Msane et al showed that body weight is increased in the prediabetic state, and this is caused by the energy imbalance where energy intake exceeds energy expenditure (33). Furthermore, the increased body weight in prediabetes is a result of the failure of the skeletal muscle cells to respond properly to insulin which promotes fat storage resulting in the weight gain (38,39).

Blood glucose plays a pivotal role in energy production, metabolic health and organ function (40) . Managing and monitoring blood glucose levels is important for overall health and preventing chronic conditions like type 2 diabetes mellitus (40,41). Previous studies have documented that during prediabetes, blood glucose levels are elevated above the normal range but below the threshold for a diagnosis of T2DM when using fasting blood glucose levels as well as the oral glucose tolerance test (42,43). In this study, we observed higher fasting blood glucose levels in the PD group compared to the NPD group. We also observed that the blood glucose concentrations in the PD group were significantly higher compared to the NPD group during the duration of the OGT test conducted at the end of the prediabetes induction period. Our findings agree with previous studies, showing elevated blood glucose levels during prediabetic state (22,33) .These results suggest the onset of insulin resistance as it has been shown that when cells become resistant to insulin, they do not respond effectively to the hormone's signal resulting in elevated fasting blood glucose levels and impairment in glucose tolerance (33,44,45) . Furthermore, these results align with our observed results on body weight as it has been shown that increased body weight is associated with increased body fat which has been shown to lead to impaired glucose regulation (33,35). Additionally, the ADA recommends the use of glycated hemoglobin to diagnose T2DM and prediabetes (46). Furthermore, HbA1c levels between 5.7% and 6.4% are often considered diagnostic of prediabetes (47,48). In this study, PD had significantly higher glycated hemoglobin levels compared to the NPD group.

Insulin plays a crucial role in glucose homeostasis (33,49). Under normal conditions insulin is secreted from the pancreas in response to elevated blood glucose levels and promotes the entry of glucose into the cells of tissues such as skeletal muscle and adipose tissue (49,50). Previous studies have shown that

during prediabetes, the cells become less sensitive to insulin resulting in the pancreas secreting higher levels of insulin to compensate for the resistance (22,50). In the present study, we observed that the PD group had significantly higher plasma insulin levels compared to the NPD group. These results correspond with the previous studies which have shown that both insulin and glucose concentrations are elevated above the normal range but below those of T2DM (32,51,52). In the prediabetic state, insulin levels are moderate, and this is due to cells particularly in the liver, muscles and liver in the body becoming less responsive to insulin and potentially leading to increased insulin production (16,53) Additionally, these results align with our results that showed elevated blood glucose levels as it has been shown that high blood glucose in the presence of elevated body weight is associated with insulin resistance (33,35). Moreover, when blood glucose levels are elevated, the body attempts to compensate by producing more insulin resulting in elevated insulin levels (54).

GLP-1 is an incretin hormone that promotes insulin secretion which helps in lowering blood glucose levels in the body (55,56). Under normal conditions the primary function of GLP-1 is to stimulate insulin secretion from the pancreas when blood glucose levels are elevated thus help lower blood glucose levels (56). Previous studies have documented that in the prediabetic state there is reduced insulin sensitivity that affect GLP-1 secretion hence there are reduced GLP-1 levels in the prediabetic group compared to the non-prediabetic group (11,19). The present study shows that PD group had significantly lower plasma GLP-1 levels compared to the NPD group. These results align with the previous studies which showed that low plasma GLP-1 results are associated with impaired glucose regulation (13,55,56). Additionally, these results align with our data on plasma insulin levels as it has been shown that elevated insulin levels reduce the demand for incretin GLP-1 to enhance insulin secretion as a compensatory response, which contribute to the observed reduction in plasma GLP-1 levels in prediabetes (56). Furthermore, GLP-1 levels are influenced by the dysregulation of several hormones involved in glucose homeostasis, including insulin, glucagon and leptin (57–59). These effects are mediated through multiple pathways that impact nutrient sensing, inflammatory signalling and gut hormone secretion (59) .

DPP-4 is an enzyme that exists in two forms, as a soluble (sDPP-4) or membrane bound (29). It is primarily responsible for inactivating incretins like GLP-1 and GIP, consequently, reducing insulin secretion and elevating blood glucose levels (8,9,12). There are previous studies that looked at plasma DPP-4 activity and levels in T2DM and concluded that increased sDPP-4 activity and levels are associated with hyperglycaemia and obesity caused by high body weight (13,60). While the changes in DPP-4 levels in T2DM are well established, there was a lack of studies that specifically look at the plasma DPP-4 activity and expression in the prediabetic state. In the present study, we found that plasma

DPP-4 was elevated in the PD group compared to NPD group. Indeed, these findings align with our results for elevated body weight and impaired glucose tolerance in the PD group compared to the NPD group. Furthermore, elevated blood glucose levels affect endothelial function, which potentially lead to more DPP-4 being released into the bloodstream (60,61). Moreover, hyperglycaemia can cause oxidative stress and inflammation in T2DM, which raises the expression and activity of DPP-4 (62,63). We speculate that this might be the case for prediabetes due to the moderate hyperglycaemia observed in this condition. Furthermore, these results align with our observed results on GLP-1 levels as it has been shown that when sDPP-4 levels are high, GLP-1 is degraded more rapidly, leading to lower plasma GLP-1 levels and this potentially contribute to impaired glucose regulation and insulin secretion (64,65). This is further evidenced by the presence of a strongly negative correlation between sDPP-4 and plasma GLP-1 with a correlation coefficient of -1. As sDPP-4 levels increase, the concentration of plasma GP-1 decreases.

As previously mentioned, DPP-4 exists as a soluble (sDPP-4) and membrane bound enzyme(5,29). A study by McKillop et al., reported that that the highest levels of DPP-4 expression and activity were found in the lung, small intestine, with moderate levels in tissues including the pancreas, liver and kidney during high fat diet which is an indication of T2DM (13). The increased expression and activity of DPP-4 seen in the mentioned organs compared to the normal control may be a compensatory response to the increased availability of specific substrates such as incretins (GLP-1 and GIP) secreted from the intestine and pancreas and high blood glucose levels (12,13). In the present study DPP-4 expression was investigated in liver, kidney and lungs to investigate and assess the changes of this enzyme in tissues during diet-induced prediabetes.

DPP-4 expression in the lung was found to be reduced in the prediabetic group comparing to a non-diabetic group. The change in DPP-4 expression in the lung may have been influenced by factors such as metabolic diseases, inflammatory responses and hormonal regulation. Changes in cytokine levels like TNF-a and IL-6 may affect DPP-4 expression in the lung. Notably, this study is showing repeatedly that there is tissue specific regulation of DPP-4 expression which corresponds with the information demonstrated by previous studies that DPP-4 plays distinct roles in tissues hence the differences in its regulation in prediabetic state and is easily affected by different factors (13,14,31,65). DPP-4 is regulated in a tissue specific approach and plays a significant role in stimulating in incretins (GLP-1 and GIP) secretion and improving glucose tolerance (12,13). Our results are broadly corresponding with previous studies that have demonstrated that apart from the GLP-1 pathway, little is understood about other mechanisms by which DPP-4 plays in the prediabetic state.

DPP-4 expression was downregulated in the liver in this present study, an intriguing finding in this study is that DPP-4 expression in the liver during prediabetic state decreased comparing to non-prediabetic group which is interesting because previous studies of high fat diet or T2DM demonstrated an increase in the DPP-4 expression in the liver which shows that the regulation of DPP-4 expression in the liver is complex and influenced by different factors such as hepatic inflammation and oxidative stress (13). A (HFHC diet may induce hepatic inflammation, leading to elevated expression of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β (66–68). We postulate that this inflammatory environment may also contribute to the decreased expression of DPP-4 in the liver.

In the present study, DPP-4 expression was upregulated in the kidney suggesting that DPP-4 plays various roles in different physiological context, and its dysregulation may contribute to other various diseases other than metabolic diseases. However, we have no knowledge about the exact mechanisms that cause alterations in DPP-4 activity and expression in prediabetes. We found that DPP-4 expression is downregulated in the liver and kidney, a study by McKillop et al documented that DPP-4 expression was upregulated via an IL-12 dependent mechanism by antigenic and mitogenic stimulus in T2DM and we believe it might also be the cause in prediabetes. Our data correlated with the findings from other studies that the upregulation of DPP-4 caused by high fat diet was not uniform (13). Additionally, a study by Yang et al demonstrated that the elevated DPP-4 levels may contribute to insulin deficiency in many organs such as liver and kidney, and as the present study has showed that insulin deficiency is associated with T2DM which often progressed from prediabetes (12). The insulin levels during the prediabetic state were elevated comparing to the non-diabetic group, this led to elevated DPP-4 expression. DPP-4 degrades incretins which stimulates insulin secretion and on the other hand elevated insulin levels increase DPP-4 expression (12,13).

Leptin is a hormone primarily responsible for regulating energy balance by inhibiting hunger, thereby helping to regulate body weight (45,69,70). Under normal conditions, leptin signals the body to reduce appetite and increase energy expenditure, promoting a balance between food intake and energy use (33,45,70). Previous studies have shown that during the prediabetic state, the brain becomes less sensitive to leptin's signals, leading to impaired appetite regulation and increased food intake (33). The results of the present study show that the PD group had significantly higher leptin levels compared to the NPD group. These results correspond with previous studies which have shown elevated leptin levels in the prediabetic group (33,71). These results suggest leptin resistance as it has been shown that the body become less sensitivity to leptin’s signals thus resulting in high leptin levels. Interestingly previous studies have shown that DPP-4 also cleaves other peptides and affects other hormones in the body and consequently affecting feeding through actions on gastric emptying, insulin secretion and satiety

ultimately affecting hormones such as ghrelin and leptin in the body (72,73). Furthermore, these results align with our observed results on insulin as it has been shown that increased leptin is associated with insulin resistance which has been shown to affect the body's ability to regulate hunger, energy balance and metabolism effectively (73).

Ghrelin is a hunger hormone (74,75). Under normal conditions, ghrelin signals the body increase hunger and balance energy intake and expenditure (76,77). Previous studies have shown that in the prediabetic state, ghrelin levels are slightly higher in prediabetes, and the slightly higher levels are associated with high insulin levels and insulin resistance (78). The result of the present study shows that the PD group had higher ghrelin levels compared to the NPD group. These results correspond with previous studies which have shown slightly high ghrelin levels in the prediabetic group (78). Additionally, elevated ghrelin levels stimulate appetite leading to more food intake and potentially contributing to weight gain and several metabolic disorders (77). Furthermore, these results align with our observed results on glycated hemoglobin as it has been shown that increased HbA1c is associated with increased ghrelin levels which have been shown to compensate for glucose metabolism and insulin sensitivity (78,79). Corresponding to our findings that resulted in high levels of DPP-4 expression in the kidney, this results in more degradation of incretins GLP-1 and GIP. These incretin hormones also play a role in gastric emptying, so elevated DPP-4 expression in the kidney lead to increased ghrelin levels (80,81). These results strongly suggest that DPP-4 in different organs and cells may indeed have different specific roles and mechanisms that are not fully understood thus far in prediabetes.

Conclusion

The findings of this study suggest that during PD, there is moderate insulin resistance which results in increased blood glucose levels. The elevated blood glucose levels promote shedding of DPP-4 from tissues, leading to increased soluble DPP-4 levels in plasma. The increased sDPP-4 is correlated to the downregulation of GLP-1. This downregulation of GLP-1 could lead to the observed upregulation of ghrelin which may contribute to the development of obesity. Obesity has been linked to the development of leptin resistance which may cause further dysregulation in glucose homeostasis during prediabetes. the observed differences in the expression of DPP-4 in the various tissues selected in this study strongly suggest that there are several additional roles or mechanisms of DPP-4 that may play a role in the progression of prediabetes that need to be explored.

Limitations and Recommendations for future studies

Several studies have shown that the expression of DPP-4 is linked to the amount of inflammation in the body. Due to budget constraints, we could not measure the proinflammatory cytokines in the plasma and biomarkers of the oxidative stress. This is recommended for future studies to establish the link between inflammation and DPP-4 expression during prediabetes. Further investigation on the expression of DPP4-4 in the other tissues such as the small intestines, vascular endothelium and pancreas is recommended as several studies have shown changes in the expression in these tissues during T2DM. Additionally, more investigation of cytokines (TNF-a, IL-6 and IL-1b) involved or contributing to DPP-4 expression need to be explored.

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Chapter 5: Synthesis and conclusion

DPP-4 is an enzyme found in various organs and plays a crucial role in glucose homeostasis (1). It is mainly responsible for inactivating incretins hormones such as GLP-1 and GIP (1). These incretin hormones are released from the gut in response to meals, stimulating insulin secretion, inhibiting glucagon release and in doing so regulating glucose homeostasis (1–3) .Previous studies have shown that there are drastically elevated levels of DPP-4 in T2DM (4,5). T2DM is a chronic metabolic disorder characterized by insulin resistance (6). It is the most common form of diabetes mellitus, accounting for about 90% of all diabetes cases (6,7). It is often preceded by a condition known as prediabetes (8). Prediabetes is a metabolic condition of intermediate hyperglycaemia in which blood glucose levels are elevated above normal but not high enough to meet the diagnostic criteria for T2DM (9–11). Furthermore, the global prevalence of prediabetes was estimated at 7.5% (approximately 374 million individuals) in 2019 and is projected to increase to 8.0% (around 454 million individuals) by 2030 (12). Without timely intervention, prediabetes frequently progresses to type 2 diabetes mellitus (T2DM), increasing the risk of associated complications (9,13,14). Hence, the aim of the study was to investigate the tissue and plasma DPP-4 changes during the prediabetic state and associations of DPP-4 expression with markers of glucose homeostasis during a diet-induced prediabetic state.

Animal studies play a crucial role in advancing diabetes research, offering valuable insights into the pathophysiology, progression, and potential treatment strategies for the disease (15–17).The primary benefits of utilising animal models are that disease mechanisms are better understood, possible treatment targets are easily identified, and lastly, the study can be easily applied to clinical settings (15,17,18) . In our laboratory, Sprague Dawley rats were fed high carbohydrate high fat diet *ad libitum* for 20 weeks to induce prediabetes. This model has been shown to consistently demonstrate key features of prediabetes, including elevated blood glucose, increased body weight, insulin resistance, and hormonal imbalances (9). Consequently, a comprehensive literature review was undertaken to evaluate existing research on the role of DPP-4 in both type 2 diabetes mellitus (T2DM) and prediabetes, with a particular focus on its interaction with incretin hormones such as GLP-1 and GIP. The literature review documented current findings, highlighted the known physiological roles of DPP-4 and GLP-1 in glucose regulation. Through this process, the literature not only consolidated available evidence but also identified significant gaps in understanding, especially regarding the regulation and expression of DPP-4 during the prediabetic state. These insights established a strong foundation for the current investigation, aimed at further exploring these mechanisms using an established diet-induced animal model of prediabetes.

The literature review extensively documented the role of DPP-4 during T2DM and prediabetes, as well as how DPP-4 degrades incretin hormones resulting in impaired glucose regulation (2). The literature

review also showed that elevated DPP-4 activity has been well documented in T2DM and is associated with insulin resistance, increased appetite and disrupted hormonal signalling (1,19). Although much is known about GLP-1 and DPP-4 in T2D, the hormonal changes that occur during the prediabetic state particularly those involving incretins are not well understood. Furthermore, elevated DPP-4 activity has been linked to increased insulin resistance, impaired glucose regulation, and enhanced appetite (20,21). Additionally, in T2DM, DPP-4 levels are significantly upregulated in tissues like the liver, kidneys, and intestine (5). Moreover, dysregulation of GLP-1 signalling has been observed in T2DM, where reduced secretion and impaired sensitivity are common (3,22). Additionally, GLP-1's interaction with appetite-regulating hormones such as leptin and ghrelin suggests broader systemic implications beyond glucose control (23,24). Despite well-established GLP-1 physiology in diabetes mellitus, there is limited information detailing its function and how DPP-4 expression affects this incretin hormone during the prediabetic state. Elevated DPP-4 levels, especially in tissues such as the liver, kidneys, lungs and intestines, have been associated with increased insulin resistance and impaired glucose regulation in T2DM (4,21,25,26). Additionally, as DPP-4 exists in both membrane-bound and soluble forms, both have distinct roles in metabolic dysfunction (1) . This gap in knowledge led us to conduct a systematic review and meta-analysis to consolidate existing evidence and explore patterns of DPP-4 expression and activity in prediabetic populations.

To conduct the systematic review the search engines used were African online journal, MEDLINE or PubMed, African journals online, EMBASE and ICTRP. The key words for the search were “Dipeptidyl peptidase-4(DPP-4)”, “Tissue expression”, “Prediabetes” and “Type-2-diabetes mellitus. Downs and Black checklist were used for risk of bias, for clarity, the scores were rated as follows; excellent (25–26), good (20–24), moderate (14–19), poor (11–13), and very poor (< 10)(27). Review Manager v5.4 Forest plot was used for Meta analysis(28). This systematic review included all prediabetic and type 2 diabetes (T2D) patients aged over 18 years, regardless of ethnicity. The findings from this systematic review and meta-analysis revealed a significant gap in the literature, indicating that no study to date has specifically focused on tissue-specific changes in DPP-4 expression during the prediabetic stage. These results provided a critical foundation for the development of Chapter 4 which was the original research manuscript.

The findings of the manuscript confirm that during prediabetes, plasma DPP-4 levels increase significantly, correlating with elevated blood glucose and insulin levels, suggesting early metabolic dysregulation (29). This study found elevated body weight, fasting glucose, and HbA1c levels in the PD group compared to the NPD group. These results are consistent with previous studies that associate high fat high carbohydrate diets with increased body weight and impaired glucose regulation (9,10,30). Additionally, elevated insulin levels in the PD group support the concept of insulin resistance. GLP-1 levels were significantly lower in the PD group, consistent with the elevated DPP-4 activity observed,

confirming that GLP-1 degradation contributes to impaired glucose tolerance (31). Moreover, a negative correlation between plasma DPP-4 and GLP-1 was identified, suggesting that increased DPP-4 directly reduces GLP-1 availability.

Interestingly, tissue-specific regulation of DPP-4 was observed, with reduced expression in the lungs and liver and increased expression in the kidney, indicating that different tissues may respond uniquely to prediabetes. These observations illustrate the complicated, tissue-dependent regulation of DPP-4 and its potential impact on incretin function and glucose homeostasis. Furthermore, the study also revealed elevated levels of leptin and ghrelin in the PD group, indicating disturbances in energy balance and appetite regulation. Leptin resistance, from elevated leptin levels, likely contributes to ongoing weight gain and metabolic dysfunction (32). Elevated ghrelin levels may further worsen appetite and glucose imbalances (33). Furthermore, the connection between DPP-4 activity and other metabolic hormones like leptin and ghrelin suggests that DPP-4 has a broader role beyond incretin degradation. The findings reported in the literature are consistent with those observed in this manuscript.

Conclusion

The findings of this study suggest that hyperglycaemia induces tissue shedding of DPP-4, increasing plasma soluble DPP-4, which correlates with suppressed GLP-1 expression. Furthermore, this study indicates tissue-specific mechanisms influence the regulation of DPP-4 in the prediabetic state as there was upregulation and downregulation of DPP-4 in tissues. Both the insulin resistance and the consumption of the HFHC diet may independently play a role in the DPP-4 expression and therefore longitudinal studies may be needed to confirm their involvement. The varying changes observed in DPP-4 expression in various organs strongly imply additional pathophysiological roles or mechanisms of DPP-4 that still need to be explored in prediabetes.

Limitations and recommendations for future studies

The main limitation of the systematic review was the limitation of articles, there was no article that specifically documented the DPP-4 expression changes in prediabetes. The limited number of studies with a low risk of bias underscores the need for additional high-quality research to characterize DPP-4 expression in various tissues during the prediabetic state. It is recommended that more studies be done to further understand DPP-4 expression and its associations with glucose homeostasis in prediabetic state. The limitation for the original manuscript was the budget, we were unable to measure proinflammatory cytokines in the plasma. It is recommended that future studies include this analysis to

better establish the link between inflammation and DPP-4 expression during prediabetes. Additionally, further investigation into DPP-4 expression in other tissues such as the small intestine, vascular endothelium and pancreas is advised, as several studies have reported altered expression in these tissues during type 2 diabetes mellitus.

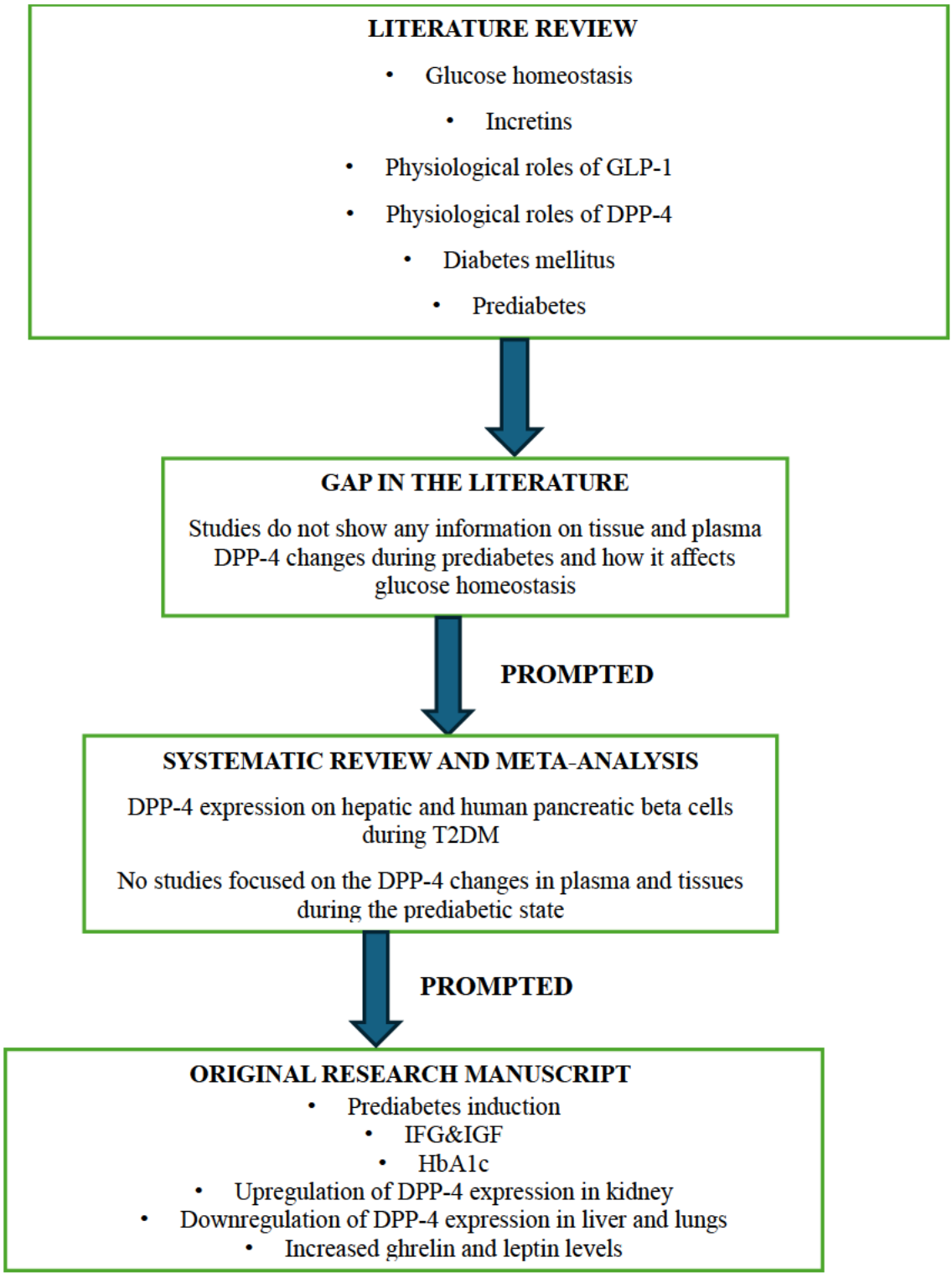

Figure 1: A diagram showing a summary of findings in the chapters of this dissertation

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Appendix

Ethical clearance certificate

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

23 June 2022

Ms Angezwa Siboto (212518628)
School of Laboratory Medicine & Medical Sciences
Westville Campus

Dear Ms Siboto,

Protocol reference number: AREC/00003221/2021 (previous AREC reference number: AREC/039/018M)
Project title: Evaluating the protective effects of rhenium (V) compound with uracil derived ligands in cardiovascular, hepatic and renal function in a high-fat high-carbohydrate diet-induced prediabetes rat model.

Full Approval – Research Application

With regard to your revised application received on 09 June 2022, the Animal Research Ethics Committee has accepted the documents submitted and **FULL APPROVAL** for the protocol has been granted.

Please note: There must be adherence to national and institutional COVID-19 regulations and guidelines at all times. Researchers will be personally responsible and liable for non-adherence to national regulations. If in doubt, please contact the Research Ethics Chair and/or the University Dean of Research for advice.

Please note: Any Veterinary and Para-Veterinary procedures must be conducted by a SAVC registered VET or SAVC authorized person.

Any alteration/s to the approved research protocol, i.e Title of Project, Location of the Study, Research Approach and Methods must be reviewed and approved through the amendment/modification prior to its implementation. In case you have further queries, please quote the above reference number.

Please note: Research data should be securely stored in the discipline/department for a period of 5 years.

The ethical clearance certificate is only valid for a period of one year from the date of issue. Renewal for the study must be applied for before 22 June 2023.

I take this opportunity of wishing you everything of the best with your study.

Yours faithfully [redacted]

Dr Sanil D Singh, BVSc, MS, PhD
Chair: Animal Research Ethics Committee

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cc Supervisor: Dr Andile Khathi; Dr Phikelelani Ngubane
cc BRU Manager: Dr Jaca

Animal Research Ethics Committee (AREC)
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