

https://dspace.org/

The effect of a high fructose diet and glucocorticoids intervention on cardiac structure and function in male Sprague Dawley rats

Mokoena, Thobekile Sibonelo

2024
https://hdl.handle.net/10539/37707

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

THE EFFECT OF A HIGH FRUCTOSE DIET AND GLUCOCORTICOIDS INTERVENTION ON CARDIAC STRUCTURE AND FUNCTION IN MALE SPRAGUE DAWLEY RATS

Thobekile Sibonelo Mokoena

A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfilment of the requirements for the degree of Master of Science in Medicine

Johannesburg, South Africa

2023

Declaration

I Thobekile Sibonelo Mokoena, declare that this work is my own, except for the work contributions stated in the acknowledgements sections. It is being submitted for the Degree of Master of Science in Medicine in the School of Physiology, Faculty of Health Sciences, at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

I hereby certify that the work contained in this dissertation has been approved by the Animal Research Ethics Committee of the University of the Witwatersrand (AREC approval number: 2021/04/06C).



Thobekile Sibonelo Mokoena

Signed on ....15…..day of.....June..... 2023.





Dr Lebogang Mokotedi

Dr Sulè Gunter

Dedication

In memory of my grandmother

Popi Johanna Mokwena

1937-2017

Abstract

The impact of glucocorticoids on left ventricular (LV) morphology and function in animal models and their interaction with metabolic syndrome (MetS) remain unclear. This study aimed to determine the effects of glucocorticoids on LV structure and function and whether MetS exacerbates these effects. Male Sprague Dawley rats were assigned to control, glucocorticoids (GC), high fructose (HF) and glucocorticoids + high fructose (GC+HF) groups (n=10each). HF and GC+HF groups received a 20% fructose solution, while GC and GC+HF groups received 10mg/kg intraperitoneal injections of methylprednisolone daily for 10 weeks. After 10 weeks, LV function was assessed, and cardiac collagen content was determined. Relative wall thickness was greater in the GC group compared to the control ($p=0.01$). The heart weight indexed to body mass, LV weight indexed to body mass and the relative wall thickness were greater in the GC+HF compared to the control ($p=0.04$; $p=0.009$; and $p=0.01$ respectively). The LV end-diastolic volume was lower in the GC+HF group compared to the control ($p=0.007$) and the HF($p=0.01$). The lateral e' was lower in the GC and GC+HF groups compared to the control ($p=0.001$ and $p=0.005$ respectively) and HF ($p<0001$ and $p=0.0001$). The E/e' was greater in GC and GC+HF rats compared to control ($p<0.0001$ and $p=0.004$, respectively) and HF ($p<0.0001$ and $p=0.02$, respectively). Cardiac collagen content was greater in GC and GC+HF groups compared to control ($p=0.001$ and $p<0.0001$ respectively).

In conclusion, glucocorticoid administration induced cardiac remodelling, impaired LV relaxation and increased LV diastolic filling pressures. The presence of MetS resulted in concentric hypertrophy, but did not worsen LV diastolic dysfunction.

Presentations arising from this thesis

Oral presentation

T Mokoena, S Gunter, AME Millen, and L Mokotedi. The effects of a high fructose diet and glucocorticoid intervention on cardiac function in male Sprague Dawley rats. 49th Annual Conference of the Physiological Society of Southern Africa, Stellenbosch, South Africa, September 2022.

Acknowledgments

Foremost, I would like to express my sincere gratitude to my supervisors Dr Lebogang Mokotedi and Dr Sulè Gunter. Thank you for guiding me and teaching me all that you have taught me throughout my master's degree journey. Thank you for your continuous support, patience, and motivation. This has helped me tremendously in doing research and writing my dissertation. It is an honour to have you as my supervisors and being an important part of my growth both professionally and personally, I could not have asked for better supervisors. To Associate Professor Aletta Millen, thank you for assisting with performing the echocardiography. I would also like to thank Monica Gomes for assisting, me with histology and performing Elisa's.

To the personnel at the Wits Research Animal Facility (WRAF), thank you for letting me use the lab and for your hard work in helping with animal care and carrying out the procedures necessary for my research. Additionally, I want to thank you for your constant generosity, support, and encouragement.

To my parents, Thabisile and Maurice Mokoena, I am extremely grateful for your love, continuous support and encouragement not only throughout my research but throughout my entire academic journey. This means a lot to me, and it has helped me get through the obstacles I faced throughout my studies. To my twin sister and brother, Siphelele and Siphesihle Mokoena, thank you for always being there for me. Lastly to the rest of my family, thank you for your endless support.

Table of contents

Declaration ii

Dedication iii

Abstract iv

Presentations arising from this thesis v

Acknowledgments vi

Table of contents vii

List of figures x

List of tables xi

List of abbreviations xii

Chapter 1: Introduction 1

Chapter 2: Literature review 5

2.1. Metabolic syndrome as a major health concern 6

2.2. Pathogenesis of metabolic syndrome 7

2.3. Aetiology of metabolic syndrome 10

2.4. Metabolic syndrome and cardiovascular disease 11

2.5. Phenotypes of heart failure 11

2.6. Diastolic dysfunction as a preclinical measure of heart failure with a preserved ejection fraction 13

2.6.1. Pathophysiology of diastolic dysfunction 13

2.6.2. Assessment of diastolic function using echocardiography 14

2.7. Pathogenesis of heart failure with preserved ejection fraction in metabolic syndrome 16

2.8. Basic glucocorticoid physiology 18

2.9. Glucocorticoids' effect on cardiac function 21

2.10. Problem Statement 23

2.11. Aim and objectives 25

2.12. Hypotheses 25

Chapter 3: Methods 27

3.1. Animals and ethical clearance 28

3.2. Experimental design 28

3.2.1. 20% fructose solution preparation 31

3.2.2. Drug intervention 31

3.3. Measurement of body mass, food intake and water intake 31

3.4. Measurement of blood pressure 33

3.5. Fasting glucose and triglyceride concentrations 33

3.6. Oral glucose tolerance test 32

3.7. Echocardiography 32

3.8. Terminal procedures 37

3.8.1. Circulating insulin concentrations 37

3.8.2. Circulating cholesterol concentrations 37

3.8.3. Circulating high-sensitivity C-reactive protein concentrations 37

3.8.4. Cardiac tissue total collagen content 38

3.9. Statistical analysis 38

Chapter 4: Results 39

4.1. Food intake, water intake, body mass, visceral fat mass and blood pressures 40

4.2. Triglyceride and cholesterol concentrations 42

4.3. Blood glucose concentrations 44

4.4. Insulin and HOMA-IR 46

4.5. High-sensitivity C-reactive protein concentrations 48

4.6. Cardiac geometry 50

4.7. Left ventricular systolic function 50

4.8. Left ventricular diastolic function 51

4.9. Left ventricular total collagen content 54

Chapter 5: Discussion 56

5.1. The effects of glucocorticoids on cardiac remodelling 57

5.2. The effects of glucocorticoids on diastolic function 60

5.3. The high fructose diet as a model of metabolic syndrome 62

5.4. The effects of glucocorticoids on cardiac remodelling and diastolic function in a model of MetS 65

5.5. Limitations 68

5.6. Conclusion 70

References 71

Appendices 1155

Appendix A: Animal ethics certificate 116

Appendix B: Animal ethics modifications and extensions of experiments 117

List of figures

Figure 3. 1 Experimental design to address the effects of a high fructose diet and glucocorticoid intervention on cardiac morphology and function in male Sprague Dawley rats 30

Figure 3. 2 Example of a 2-D M mode in the parasternal long-axis view indicating left ventricular dimensions.....34

Figure 3. 3 Example of an echocardiographic image showing trans-mitral inflow velocity in early and late diastole using pulse wave Doppler. 35

Figure 3. 4 Example of an echocardiographic image showing the rate of tissue relaxation at the mitral annulus using tissue Doppler imaging. 36

Figure 4. 1 Blood triglyceride (A) and cholesterol (B) concentrations in the control, GC, HF and GC+HF groups.....43

Figure 4. 2 Fasting glucose concentrations at baseline and at termination (A) and the glucose concentrations following the oral glucose tolerance test (B) in the control, GC, HF and GC+HF groups.....45

Figure 4. 3 Fasting insulin concentrations (A) and the HOMA-IR (B) in the control, GC, HF and GC+HF groups.....47

Figure 4. 4 Circulating high sensitivity C-reactive protein (hs-CRP) concentrations in the control, GC, HF and GC+HF groups.....49

Figure 4.5 Total collagen content in left ventricular tissue of the control, GC, HF and GC+HF groups. Representative picrosirius red stained micrographs imaged at x100 magnification viewed in (A) bright-field and under (B) polarised light. (C) Total collagen content (% area fraction) calculated from picrosirius red stained LV sections (n=10 per group) ……..55

List of tables

Table 4. 1 Food intake, water intake, body mass, visceral fat mass and blood pressure in the control, GC, HF and GC+HF groups. 41

Table 4. 2 Left ventricular geometry, diastolic and systolic function of the control, GC, HF and GC+HF groups.....52

List of abbreviations

A	Trans-mitral blood flow velocity during late diastole
a’	Peak tissue lengthening velocity of the lateral mitral annulus during late diastole
ACTH	Adrenocorticotropic hormones
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
11β-HSD1	11β-hydroxysteroid dehydrogenase 1
11β-HSD2	11β-hydroxysteroid dehydrogenase 2
COX	Cyclooxygenase
CRH	Corticotropin-releasing hormone
CVD	Cardiovascular disease
DNA	Deoxyribonucleic acid
E	Trans-mitral blood flow velocity during early diastole
e’	Peak tissue lengthening velocity of the lateral mitral annulus during early diastole
e’/a’	Ratio of early to late mitral annular diastolic tissue lengthening velocity
E/e’	Left ventricular filling pressure
E/A	Ratio of early to late diastolic filling velocity
ELAM-1	Endothelial leukocyte adhesion molecule 1

FFA	Free fatty acids
F6P	Fructose-6-phosphate
G-CSF	Granulocyte-macrophage colony-stimulating factor
G6P	Glucose-6-phosphate
GREs	Glucocorticoid response elements
HDL	High-density lipoproteins
HFpEF	Heart failure with a preserved ejection fraction
HFrEF	Heart failure with a reduced ejection fraction
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
HPA	Hypothalamic-pituitary-adrenal (HPA) axis
hs-CRP	High-sensitivity C-reactive protein
ICAM-1	Intercellular adhesion molecule 1
IL-6	Interleukin-6
IDF	International Diabetes Federation
iNOS	Inducible nitric oxide synthase
LV	Left ventricle or Left ventricular
LVEDD	Left ventricular internal chamber diameter at end diastole
LVESD	Left ventricular internal chamber diameter at end systole
MCP	Monocyte chemotactic protein
MetS	Metabolic syndrome

MMPs	Matrix metalloproteases
NCEP ATP-III	National Cholesterol Education Program Adult Treatment Panel – III
NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B cells
PVN	Paraventricular nucleus
PWTd	Posterior wall thickness at end diastole
PWTs	Posterior wall thickness at end systole
ROS	Reactive oxygen species
SERCA	Sarcoplasmic reticulum calcium ATPase
SNS	Sympathetic nervous system
TNF-α	Tumour necrosis factor-alpha
VCAM-1	Vascular cell adhesion molecule-1
VLDL	Very low-density lipoproteins

Chapter 1: Introduction

Cardiovascular disease (CVD) is a major cause of morbidity and mortality worldwide, accounting for 17 million deaths annually (WHO, 2019). In South Africa, 1 in 6 deaths, or 17.3% of the total annual deaths are attributable to CVD (Statistics South Africa, 2015). Traditional risk factors for the development of CVD include obesity, hypertension, dyslipidaemia and diabetes (Tune *et al*., 2017). Although much research has been conducted on the mechanisms underlying the pathogenesis of CVD from these classical risk factors (Kannel, 2009; Liu and Li, 2015), recent evidence proposes that metabolic abnormalities may play a particularly crucial role in the development of heart disease (Lesnefsky *et al.,* 2001; Tune *et al*., 2017). Central obesity, dyslipidaemia, hypertension and dysglycaemia form a cluster of conditions, known as metabolic syndrome (MetS) that infers a cumulative risk of CVD development (Dekker *et al.*, 2005; Galassi *et al*., 2006). The rapid increase in MetS is a major global concern, with the prevalence estimated to be between 20 and 25% in the adult population worldwide (do Vale Moreira *et al.*, 2020; Belete *et al.*, 2021).

Metabolic alterations associated with MetS also impact on substrate utilisation in cardiomyocytes (Lopaschuk *et al.*, 2021). Under physiological conditions, ongoing energy production in the heart relies on glucose and fatty acids as fuel sources, with the latter considered to be the major substrate consumed by cardiac tissue (Lopaschuk *et al.*, 2021). With the onset of insulin resistance and dysglycaemia, glucose uptake by cardiomyocytes is diminished and fatty acids become an exclusive fuel source (Burkart *et al.*, 2007). Excessive fatty acid oxidation induces lipotoxicity, causing accumulation of fatty acids in the myocardial tissue and pronounced mitochondrial dysfunction that is associated with oxidative damage (Lopaschuk *et al.*, 2021). Oxidative damage, in turn, activates various pro-inflammatory and pro-fibrotic pathways in cardiac tissue (Codoñer-Franch *et al.*, 2011). Elevated myocardial fatty acid oxidation has therefore been implicated in several metabolic, morphological and functional changes in the heart (Borghetti *et al.*, 2018).

Glucocorticoids represent both the endogenous secreted hormone and exogenous therapies commonly administered (Timmermans *et al*., 2019). As a class of therapeutic drugs, glucocorticoids are widely utilised in non-endocrine and endocrine diseases (Schäcke *et al*., 2002). Glucocorticoids are used in a variety of anti-inflammatory and immunosuppressive therapies, including allergic and haematological disorders, as well as kidney, intestinal, liver, eye, and skin diseases (Timmermans *et al*., 2019). The main indications for long-term glucocorticoid therapy are autoimmune and rheumatic diseases, bronchial asthma and suppression of host-versus-graft reactions following organ transplantation surgery (Selyatitskaya *et al*., 2002).

Glucocorticoids were initially thought to have beneficial effects on heart disease through their effects on inflammation and cellular proliferation (Thiemermann, 2002). However, the administration of exogenous glucocorticoids deteriorates whole body energy metabolism and induces metabolic disorders, including obesity, dyslipidaemia and insulin resistance (Jin *et al.*, 2021). As a result of alterations in cardiomyocyte substrate utilisation, excess exogenous glucocorticoids may therefore exert unfavourable effects on cardiac metabolism. Recent evidence suggests that glucocorticoids are highly involved in the pathogenesis of CVD (Jin *et al.*, 2021). Chronic glucocorticoid therapy is reported to be involved in cardiac hypertrophy and pathological remodelling, which later impairs cardiac function (de Salvi Guimarães *et al.*, 2017).

The mechanisms whereby glucocorticoids induce cardiac remodelling are currently under investigation. However, given the particularly high prevalence of MetS currently reported and the potent effect that glucocorticoids have on whole body energy metabolism, further research should be performed to determine the specific cardiac effects of glucocorticoid therapy in a population with pre-established metabolic alterations. It is currently unclear whether adverse cardiac effects are expected from glucocorticoid therapy, in a population with pre-existing metabolic conditions. Therefore, further research is required to determine whether glucocorticoid therapy can accelerate the development of cardiac dysfunction in the presence of MetS. The effects of

glucocorticoids on cardiac function, therefore, warrant further investigation, particularly in the context of metabolic derangements associated with MetS.

This dissertation begins with a literature review in chapter 2, which highlights the current knowledge in the literature on the effects of MetS and glucocorticoid therapies on cardiac function. Chapter 3 outlines the methodological procedures used and Chapter 4 presents the results obtained in the current study. Chapter 5 discusses the findings in the context of the current literature and highlights the conclusion and limitations of the current study.

Chapter 2: Literature review

2.1. Metabolic syndrome as a major health concern

Since the first description of metabolic syndrome (MetS) by Gerald M. Raevan in the 1980s, the definition and diagnostic criteria have been continuously amended (Sarafidis and Nilsson, 2006). MetS has been defined as a cluster of interconnected metabolic abnormalities involving glucose metabolism (diabetes mellitus), lipid metabolism (hypercholesterolaemia and dyslipidaemia), elevated blood pressure, and central obesity by the International Diabetes Federation (IDF), National Cholesterol Education Program Adult Treatment Panel - III (NCEP ATP-III) guidelines, and many others (Amihăesei and Chelaru, 2014; Dobrowolski *et al.,* 2022). The most recent guidelines define MetS as the presence of central obesity (≥102 cm in males and ≥88cm in females of sub-Saharan ethnicity) (Kim *et al*., 2011; Dobrowolski *et al*., 2022), with the presence of two or more of the other components, including high blood glucose (≥5.6 mmoL/L; ≥100 mg/dL), hypertriglyceridaemia (≥1.7 mmoL/L; ≥150 mg/dL), low high-density lipoproteins (HDL)-cholesterol levels (≤1.03 mmoL/L; ≤40 mg/dL) or high blood pressure (≥130 mmHg systolic or ≥85 mmHg diastolic) (Dobrowolski *et al*., 2022).

While the global estimated prevalence of MetS is approximately 20-25%, there is limited available literature on the prevalence of MetS in the South African context. Disparate prevalence rates of between 5% and 62% have been reported (Mabetwa *et al.*, 2022), with differences owing to the diversity of the population and complications involving different MetS definitions being used. Nevertheless, an increased MetS prevalence rate has been confirmed in South Africa, compared to other areas of the world (Cameron *et al.*, 2007; Erasmus *et al.*, 2012; Beltrán-Sánchez *et al.*, 2013; Bhanushali *et al.*, 2013; Peer *et al*., 2016) as well as other African countries (Tran *et al.*, 2011; Kunbuma Tachang *et al.*, 2012; Belfki *et al.*, 2013; Magalhães *et al*., 2014). Within the South African population, the MetS prevalence rate is also greater amongst African individuals compared with Caucasians (Hoebel *et al*., 2014). Additionally, according to numerous studies, the prevalence of MetS among adults ranges from 20% to 60%, with females experiencing a

higher prevalence than males (Lee *et al*., 2015). MetS is clinically significant not only because of its high prevalence, but also because of its ability to predict the development of type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) (Haffner, 2007; Bodhini and Mohan, 2018). T2DM is a clinical disorder that is caused by the combination of inadequate response to insulin by insulin-sensitive tissue and the faulty functioning of beta cells(β-cell) resulting in reduced insulin secretion (Galicia-Garcia *et al.*, 2020) which is also considered as a metabolic disorder. Beta cells are pancreatic cell responsible for the synthesis of pre-proinsulin which is activated into insulin (Galicia-Garcia et al., 2020). Indeed, MetS significantly increases the risk of type-2 diabetes mellitus, CVD and all-cause mortality (Cornier *et al.*, 2008). It is also related to other co-morbidities including, non-alcoholic steatohepatitis, pro-thrombotic and pro-inflammatory complications, and reproductive disorders (O'Neill and O'Driscoll, 2015). In light of the growing prevalence of MetS, and the socioeconomic burden that it places on health systems (Bodhini and Mohan, 2018), much research has focused on identifying potential risk factors for the development of the syndrome.

2.2. Pathogenesis of metabolic syndrome

Clinical and epidemiological studies have shown that visceral obesity plays a critical role in the development of MetS (Kahn *et al.*, 2005; Genser *et al.*, 2016). As a result, the presence of central obesity is a necessary condition in the diagnosis of MetS (Reinehr *et al.*, 2007). With obesity, excessive adipose tissue synthesis is paralleled with inappropriate adipokine production and adipose dysfunction (Longo *et al.*, 2019). Aberrant adipokine concentrations results in the dysregulation of a wide range of physiological processes (Longo *et al.*, 2019) causing further metabolic dysfunction, increased production of pro-inflammatory cytokines, and obesity (Leon-Cabrera *et al.*, 2013). Visceral obesity is therefore a primary risk factor for the development of MetS.

Insulin resistance comprises the second important risk factor for the development of MetS (Gallagher *et al*., 2010). Insulin resistance is strongly associated with obesity, and it predicts the development of CVD and T2DM (Brown and Walker, 2016). Insulin promotes the uptake of glucose by splanchnic and peripheral tissues (muscles) and suppresses hepatic glucose production (Wilcox, 2005). Most of the insulin-dependent glucose removal occurs in skeletal muscle (~75%), while adipose tissue contributes to a lesser extent (Saltiel and Kahn, 2001).

The binding of insulin to plasma membrane-bound receptors in peripheral target cells initiates a coordinated anabolic response to nutrient availability. Upon binding of insulin to its receptor, glucose transporter type 4 (GLUT-4) is stimulated to translocate to the cell membrane, via the phosphatidylinositol 3-kinase (PI3K/Akt) pathway, to allow for the uptake of glucose into cells and lowering of circulating plasma glucose concentrations (Petersen and Shulman, 2018). When increased circulating insulin levels are necessary to achieve the integrated glucose-lowering response, insulin resistance is present.

Insulin secretion in insulin-resistant, non-diabetic individuals will increase in proportion to the severity of the insulin resistance, to maintain glucose within a normal range (Mezza *et al.*, 2019). Approximately one-fourth of adults with normal glucose tolerance show marked resistance to the uptake of glucose upon stimulation by insulin (DeFronzo, 2004). Pancreatic β-cells are therefore able to compensate for peripheral insulin resistance in the initial stages of the disease. However, β-cell decompensation later leads to the development of T2DM (Rachdaoui, 2020).

In addition to the effect that insulin resistance has on whole-body glucose metabolism, insulin also powerfully controls lipid metabolism (DeBose-Boyd and Ye, 2018). Insulin inhibits lipolysis in individuals who are not insulin resistant and encourages the conversion of glucose that has been absorbed by the cell into fatty acids for adipose tissue storage. (Chakrabarti *et al.*, 2013). This pro-lipogenic effect of insulin is largely mediated through the increased activity of endothelial lipoprotein-lipase, which functions to take up lipoproteins in adipose tissue (Chakrabarti *et al.*,

2013). Conversely, insulin resistance leads to increased lipolysis (Zhao *et al.*, 2020), whereby triglycerides are hydrolysed into glycerol and free fatty acids (FFA), for use as energy substrates. The high concentration of plasma FFA noted in insulin resistance further impedes glucose utilisation in muscle and the effect of insulin thereon. Therefore, a simultaneous increase in lipolysis and a decrease in glucose uptake necessitates exclusive use of mitochondrial fatty acid β-oxidation processes for energy production in tissues (Petersen and Shulman, 2018).

Owing to significantly increased rates of β-oxidation, a cumulative increase in toxic lipid intermediates occurs within the cells (Nishikawa and Araki, 2007). Excessive fatty acid oxidation within the mitochondria results in uncontrolled production of reactive oxygen species (ROS) production (Nishikawa and Araki, 2007). Increased production of ROS has been reported in diabetic patients and animal models of diabetes (Asmat *et al*., 2016). ROS induces endoplasmic reticulum stress and stimulates pro-inflammatory and pro-fibrotic responses (Farrell and O'Keane, 2016). Indeed, mitochondrial ROS production has been linked to the progression of several diabetic complications (Farrell and O'Keane, 2016).

Besides significant increases in oxidative stress, MetS also alters lipid metabolism (Xu *et al.*, 2019). Insulin resistance induced by MetS also hinders the clearance of very-low-density lipoprotein (VLDL) by inhibiting lipoprotein lipase activity (Heindel *et al.*, 2017; Xu *et al.*, 2019). As triglyceride concentrations are increased, they are transferred to HDL to form triglyceride-rich HDL (Heindel *et al.*, 2017). Triglyceride-rich HDL particles are easily cleared by hepatic lipase, which result in decreased circulating concentrations of HDL. The association between insulin resistance and elevated triglycerides and low HDL concentrations has consistently been reported in population-based studies, independent of sex and ethnicity (Howard, 1999).

Excessive visceral adiposity, insulin resistance and mitochondrial ROS production result in chronic activation of the innate immune system, whereby macrophages are recruited to adipocytes (Weisberg *et al.*, 2003; Pop-Busui and Pietropaolo, 2011). The infiltrating

macrophages secrete additional cytokines that serve to maintain the chronic, low-grade inflammatory state characteristic of obesity and MetS (Villarroya *et al.*, 2018; Reddy *et al.*, 2019). The chronic low-grade inflammatory state, in conjunction with obesity, hyperinsulinaemia and insulin resistance, hyperleptinaemia and sympathetic nervous system (SNS activation) further contribute to increases in blood pressure (Satou *et al*., 2018; da Silva *et al.*, 2020). Indeed, a positive correlation exists between MetS and excessive sympathetic and renin-angiotensin-aldosterone system (RAAS) activation (McHugh *et al.*, 2019). Ongoing RAAS activation causes vasoconstriction and fluid retention that leads to an increased workload on the heart and contributes to CVD risk (Seravalle and Grassi, 2016).

Taken together, a specific pattern of metabolic alterations is commonly seen in individuals with MetS. Recent research has focussed on gaining a better understanding of the aetiology of MetS. Although not fully understood, the root causes of the syndrome are most likely to be energy-dense, nutrient-poor diets, insufficient physical activity and circadian dysfunction (Standl, 2005).

2.3. Aetiology of metabolic syndrome

Epidemiological studies have reported significant increases in fructose consumption over the last two decades, which parallel the noteworthy increases in MetS prevalence during the same period (Taskinen *et al*., 2019; Semnani-Azad *et al.,* 2020). Fructose consumption has increased significantly, due to unchecked intake of sucrose and its use in the food industry as a sweetener (Cozma and Sievenpiper, 2014). Although fructose is slightly sweeter than glucose, it has a lower glycaemic index that elicits a blunted insulin response (Bantle, 2006). However, fructose has potent effects on lipogenesis and adipogenesis (Hernández-Díazcouder *et al.*, 2019).

Upon intestinal absorption, fructose is metabolised by hepatic fructokinase to produce glyceraldehyde 3-phosphate (G3P), which can act as a substrate for glycolysis, lipogenesis, gluconeogenesis and/or glycogenesis (Mayes, 1993). Conversion of fructose to G3P is unregulated in the liver, as the reaction occurs independent of insulin and does not receive

negative feedback regulation from phosphofructokinase in the glycolytic pathway (Taskinen *et al*., 2019). Excessive fructose consumption challenges the capacity of the liver, whereby circulating fructose concentrations remain increased and peripheral tissues can absorb it. Adipocytes lack fructokinase but will phosphorylate fructose to fructose-6-phosphate (F6P) (Froesch, 1972), which is easily converted to glucose-6-phosphate (G6P) (Senesi *et al.*, 2010). This reaction triggers increased pyruvate conversion to acetyl-CoA, which further results in greater acetyl-CoA and citrate cycling into fatty acid synthesis (Varma *et al.*, 2015). Increased acetyl-CoA cycling explains the lipogenic properties of fructose. High consumption of fructose is therefore a key risk factor for the development of MetS and associated obesity (Lustig *et al*., 2012; Chung *et al.*, 2014). These metabolic impairments lead to a wide range of complications and increase the risk for the development of T2DM and CVD.

2.4. Metabolic syndrome and cardiovascular disease

MetS is associated with a two-fold increase in CVD outcomes and a 1.5-fold increase in overall mortality (Li *et al.*, 2021). The association between MetS and CVD is largely attributed to accelerated atherosclerosis, as a function of a low-grade inflammatory state paired with ongoing dyslipidaemia (Reddy *et al.*, 2019). The risk of acute myocardial infarction or stroke is, therefore, 3-fold increase in individuals with MetS, compared with those who do not have the syndrome (Pop-Busui and Pietropaolo, 2011). Nevertheless, people with MetS also have a 2-fold risk of developing heart failure (Ingelsson *et al.*, 2006; Mottillo *et al.*, 2010). The association between MetS and heart failure has not been as well studied as with ischaemic heart disease, and further research is ongoing. Recent reports suggest that heart failure is becoming a serious public health concern, given the high risk of mortality among patients with MetS (Sharma *et al.*, 2022).

2.5. Phenotypes of heart failure

Heart failure, in broader terms, typically presents with signs and symptoms such as fatigue, oedema and dyspnoea. However, approximately half of patients with these symptoms do not

exhibit reductions in left ventricular (LV) ejection fraction. Owing to distinct differences in aetiology, heart failure commonly occurs in two phenotypes, namely heart failure with a reduced ejection fraction (HFrEF; EF < 50%) and heart failure with preserved ejection fraction (HFpEF, EF ≥ 50%) (ElGuindy and Yacoub, 2012). Importantly, the pattern of ventricular filling and remodelling differs substantially between these phenotypes (Fukuta and Little, 2007).

HFrEF is characterised by systolic dysfunction due to impaired ATP production, myocyte slippage or myocyte loss (Pinto *et al.*, 2016). The primary drivers of HFrEF are neurohormonal activation and eccentric cardiac remodelling (Yancy *et al.*, 2013; Reddy and Borlaug, 2020).

In contrast to HFrEF, progression of HFpEF is not well defined and phenotypic expression can differ significantly between individuals (Shah *et al.*, 2015). However, diastolic dysfunction is characteristic of HFpEF and is commonly present prior to the onset of clinical symptoms associated with HFpEF (Zile and Brutsaert, 2002). Individuals with HFpEF demonstrate significant impairments in myocardial relaxation that leads to increases in LV filling pressures (LeWinter and Meyer, 2013). The reduction in myocardial relaxation is largely attributed to decreased LV compliance, asynchronous relaxation, and increased passive stiffness (Pfeffer *et al*, 2019). Moreover, HFpEF is commonly accompanied by impaired atrial function, dysregulated autonomic tone, and endothelial dysfunction (Parikh *et al.*, 2018). Importantly, although ejection fraction is preserved in HFpEF, LV contractility may still be impaired (Tan *et al.*, 2009).

To date, the underlying mechanisms of HFrEF have been extensively studied and treatment strategies for HFrEF have proven effective to reduce the risk of hospitalisation and death in patients with HFrEF (Haydock and Flett, 2022). However, the pathophysiology of HFpEF is still poorly understood and current treatment strategies have failed to benefit patients with HFpEF (Borlaug and Paulus, 2011; Shah *et al.*, 2020). Studies have reported a significantly increased prevalence of HFpEF in patients with MetS compared to those without it (Fuentes *et al.*, 2007; Čelutkienė *et al.*, 2018). Furthermore, impairments in myocardial relaxation worsen with the

increasing burden of MetS (Fuentes *et al.*, 2007). In this regard, the pathophysiology of HFpEF, specifically in patients with MetS, warrants further investigation.

Before describing the pathogenesis of HFpEF in MetS, the section below briefly explains the pathophysiology of diastolic dysfunction and describes how diastolic function is measured using echocardiography.

2.6. Diastolic dysfunction as a preclinical measure of heart failure with a preserved ejection fraction

2.6.1. Pathophysiology of diastolic dysfunction

Diastolic dysfunction is a complex combination of impaired active relaxation (requiring ATP) and increased passive stiffness (Borlaug and Kass, 2006; Borlaug and Paulus, 2011; Sharma and Kass, 2014). For effective active relaxation, calcium needs to be taken up from troponin C to the sarcoplasmic reticulum by the sarcoplasmic reticulum ATPase (SERCA) pump, to allow for the termination of cross-bridges through the release of myosin from actin (Bers, 2008). Phospholamban is a protein that regulates the SERCA pump. The phosphorylation of phospholamban increases SERCA pump activity, which in turn, increases calcium uptake into the sarcoplasmic reticulum (Biesiadecki *et al.*, 2014). In addition, calcium is removed by various ATP-dependent calcium transporters from the cytosol to the extracellular space (Lewis *et al.*, 2017). Decreased availability of ATP, decreased phospholamban phosphorylation or irregularities in the ion channels responsible for the uptake of calcium, increases cytosolic calcium concentrations, which increases diastolic tension and impairs active relaxation (Brower *et al.*, 2006; Lewis *et al.*, 2017).

Ventricular filling during diastole is also influenced by chamber stiffness, which represents the passive elastic capacity of the ventricle (Brower *et al.*, 2006; Frangogiannis, 2017). The extracellular matrix and cardiomyocytes are the two main compartments that regulate myocardial stiffness (Brower *et al.*, 2006; Frangogiannis, 2017). The extracellular matrix is largely composed

of collagen, which is important for the structural integrity of the heart. Small changes in the synthesis and degradation of collagen leads to alterations in the passive mechanical properties of the heart because collagen is a relatively stiff material (Frangogiannis, 2017). Passive stiffness occurs due to alterations in collagen I to collagen III ratios within the extracellular matrix, which confers profibrotic changes (Spinale, 2002). Collagen I fibres increase the tensile stiffness of the extracellular matrix in tissues, compared to the more flexible properties of the thinner collagen III fibres (Spinale, 2002). A greater disposition of collagen I compared to collagen III fibres increases the stiffness of the ventricles (Spinale, 2002). Matrix metalloproteases (MMPs) are predominantly responsible for the degradation of extracellular matrix components such as collagen I fibres, hence impairments in the synthesis or degradation of MMPs result in a further accumulation of collagen I fibres in the heart with subsequent increased passive stiffness (Spinale, 2002).

In addition to changes in the extracellular matrix, intrinsic cardiomyocyte stiffness also contributes to myocardial passive stiffness. During normal relaxation of the ventricles, titin acts as a viscoelastic spring that is responsible for myocardial compliance (Zile and Brutsaert, 2002; Fukuda *et al.*, 2005). Titin has two isoforms namely, N2B which is the more rigid and N2BA which is more compliant (Zile and Brutsaert, 2002; Fukuda *et al.*, 2005). Increased expression of the more rigid isoform (N2B) prevents myocardial recoil reducing cardiomyocyte compliance (Zile and Brutsaert, 2002; Fukuda *et al.*, 2005). Hence changes in titin isotypes affect myocardial stiffness and contribute to diastolic dysfunction.

2.6.2. Assessment of diastolic function using echocardiography

Echocardiography is used to measure the velocity of blood flow across the mitral valve, using pulse wave Doppler, to visualise the phases of LV filling (Mottram and Marwick, 2005; Henein and Lindqvist, 2015; Mitter *et al*., 2017). From the mitral valve inflow velocity, the early (E) and late (A) periods of the diastolic inflow can be obtained (Mottram and Marwick, 2005; Henein and Lindqvist, 2015; Mitter *et al*., 2017). Since 70% of blood fills the ventricles during the rapid filling

phase, the E wave represents the rapid early phase (Mottram and Marwick, 2005; Henein and Lindqvist, 2015; Mitter *et al*., 2017). The remaining 30% of the blood filling the ventricles is due to atrial contraction, which is represented by the A wave. The E/A ratio provides a clinically useful index of myocardial filling (Mottram and Marwick, 2005; Henein and Lindqvist, 2015; Mitter *et al*., 2017). At the early stage of diastolic dysfunction (mild diastolic dysfunction), LV relaxation becomes impaired. In this instance, transmitral blood flow in the early period (E) is reduced (Mottram and Marwick, 2005; Henein and Lindqvist, 2015; Mitter *et al*., 2017). When LV relaxation is impaired, the heart relies more heavily on atrial contraction to compensate for the impaired left ventricular relaxation (Mottram and Marwick, 2005; Henein and Lindqvist, 2015; Mitter *et al*., 2017), which will produce a increased A wave compared to the E wave (Mottram and Marwick, 2005; Henein and Lindqvist, 2015; Mitter *et al*., 2017). As the diastolic function worsens, the left ventricle becomes less compliant, and LV filling pressures increase (Mottram and Marwick, 2005; Henein and Lindqvist, 2015; Mitter *et al*., 2017). Left atrial pressure, in turn, increases to compensate for the increased LV filling pressures (Mottram and Marwick, 2005; Henein and Lindqvist, 2015; Mitter *et al*., 2017). Increased left atrial filling pressures increase early ventricular filling, whereby the E wave will again increase in comparison to the A wave (Mottram and Marwick, 2005; Henein and Lindqvist, 2015; Mitter *et al*., 2017). Thus, moderate to severe diastolic dysfunction and severe diastolic dysfunction cause a pseudonormal pattern, when viewed with pulsed Doppler echocardiography (Mottram and Marwick, 2005; Henein and Lindqvist, 2015; Mitter *et al*., 2017). Because the E/A ratio may appear normal in moderate to severe diastolic dysfunction, further echocardiographic measures need to be considered.

A more sensitive indicator of LV relaxation may be obtained by measuring tissue lengthening at the mitral annulus, using tissue Doppler imaging in the apical 4 chamber view (Mitter *et al*., 2017). Similar to pulsed wave Doppler, the peak relaxation velocities during early (e') and late (a') diastole are obtained. Peak relaxation during early diastole (e') is less dependent on pre-load and

heart rate, and reductions in e' may therefore be more sensitive in detecting diastolic dysfunction, compared to trans-mitral flow (Mitter *et al*., 2017). Since the E wave is reliant on ventricular relaxation and left atrial driving forces, whereas the e' is determined by relaxation alone, the E/e' ratio is considered an index of LV filling pressures or an index of LV stiffness (Mitter *et al*., 2017). Therefore, the E/A and E/e' are used to diagnose diastolic dysfunction, as these measurements predict adverse cardiovascular outcomes and are associated with the development of heart failure.

2.7. Pathogenesis of heart failure with preserved ejection fraction in metabolic syndrome

The pathogenic mechanisms underlying HFpEF in MetS are not well characterised. One hypothesis proposes that exposure to comorbidities (such as obesity, hypertension and T2DM) in MetS induces a systemic inflammatory state, which leads to excessive production of ROS that ultimately causes hypertrophy, LV stiffness and diastolic dysfunction (Takimoto, 2012; van Heerebeek *et al.*, 2012; Paulus and Tschöpe, 2013).

Although MetS is a condition associated with low-grade systemic inflammation (Monteiro and Azevedo, 2010; Thomas *et al.*, 2018), it is also important to consider that MetS is characterised by significant dysregulation in whole-body lipid metabolism. In MetS, adipose tissue stores increase in size to store excess energy (Elks and Francis, 2010). With excessive adipose expansion, lipids "spill over" into the circulation, which results in increased ectopic lipid deposition and lipid uptake by non-adipose organs (Unger *et al.*, 2010). When lipids accumulate in non-adipose organs, cellular and tissue dysfunction (lipotoxicity) is induced (Leggat *et al*, 2021). Lipotoxicity drives cellular dysfunction mainly through oxidative stress, mitochondrial dysfunction and apoptosis (Leggat *et al*., 2021). The accumulated lipids serve as a substrate for energy metabolism through the process of fatty acid oxidation (Leggat *et al*., 2021). Increased substrate availability in MetS results in increased FFA in the circulation which significantly increases myocardial FFA uptake and myocardial lipid deposition (Wu *et al.*, 2020). Myocardial fatty acid

oxidation subsequently increases, while glucose oxidation decreases. This shift in substrate utilisation diminishes cardiac efficiency as more oxygen is required for the oxidation of fatty acids (Lopaschuk, 1996; Boudina *et al.*, 2007). Myocardial fatty acid oxidation is known to enhance ROS production as fatty acid oxidation is less oxygen efficient than glucose oxidation which results in the production of ROS by mitochondria (Lopaschuk *et al.*, 2010). Production of ROS can lead to reduced Nitric Oxide (NO) bioavailability by converting NO to peroxynitrite which could contribute to the pathogenesis of HFpEF through reducing protein kinase G activity in the cardiomyocytes, as discussed above. Furthermore, peroxynitrite reduces the phosphorylation of phospholambam which in turn decreases SERCA pump activity and reduces calcium uptake into the sarcoplasmic reticulum (Kohr *et al*., 2009). MetS modifies the availability of metabolic substrates, such that myocardial FFA uptake and myocardial lipid deposition increases (Wu *et al.*, 2020).

Lipotoxicity in MetS further induces myocardial mitochondrial dysfunction and reduces ATP availability, which may impair diastolic function (Leggat *et al*., 2021). Reduced ATP availability may lead to impaired relaxation through reduced ATP-dependent calcium channel activity, which may result in an inability to terminate cross-bridge activation during diastole, and subsequent increases in diastolic pressure (Leggat *et al*., 2021). Finally, ROS-induced mitochondrial dysfunction can lead to hypophosphorylation of titin and subsequent stiffening of cardiomyocytes (Leggat *et al*., 2021).

It is evident that the presence of MetS may impair cardiac function through a variety of mechanisms. While excess adiposity and dysregulated adipocytokine production significantly impacts on metabolic homeostasis, certain hormones have also been implicated in cardiac remodelling and dysfunction. Glucocorticoids regulate numerous important physiological processes, including, water and electrolyte balance (Hawkins *et al.*, 2012), the immune response (de Bosscher and Haegeman, 2009; Cruz-Topete *et al.*, 2016), growth (Donatti *et al.*, 2011),

reproduction (Whirledge and Cidlowski, 2017), mood and cognitive functions (Joëls, 2011; Tatomir *et al*., 2014; Farrell and O'Keane, 2016), metabolism (Vegiopoulos and Herzig, 2007), and development (Fowden and Forhead, 2015). Glucocorticoids are further intricately involved in cardiovascular function (Nussinovitch *et al.*, 2010; Cruz-Topete *et al.*, 2016). A robust discussion on the effect of glucocorticoid treatment on metabolism and cardiac function requires an understanding of the function and signalling pathways of endogenous glucocorticoids, which will be provided next.

2.8. Basic glucocorticoid physiology

Endogenous glucocorticoids are produced locally by the adrenal gland, along with mineralocorticoids and androgenic sex hormones (Yasir *et al*., 2022). Circulating glucocorticoid concentrations are closely controlled by the hypothalamic-pituitary-adrenal (HPA) axis, which is the major neuroendocrine regulator (Vale et al., 1981; Antoni, 1986). Under basal unstressed conditions, the adrenal glands release glucocorticoids in a circadian and ultradian rhythm, where levels peak in the mornings (in diurnal animals). The activity of the HPA axis is further increased upon physiological and emotional stress, to prepare the body to deal with and recover from stressors (Vale et al., 1981; Antoni, 1986). Neuropeptide corticotropin-releasing-hormone (CRH) which is produced in the hypothalamic paraventricular nucleus (PVN) is the main activator of the HPA axis. The release of CRH, in turn, stimulates the corticotroph cells to synthesise adrenocorticotropic hormone (ACTH) (Vale *et al.*, 1981; Antoni, 1986). Circulating ACTH travels to the adrenal cortex to stimulate the synthesis and secretion of cortisol in humans, and corticosterone in rats (Dallman *et al*., 1987).

Circulating glucocorticoids terminates HPA activation through negative feedback and exerts the intended physiological effects only once it binds to its receptor. In the bloodstream, glucocorticoids are kept inactive by binding them to either corticosteroid-binding globulin or to a lesser extent, albumin (Hammond, 2016). The inactive lipophilic glucocorticoid easily diffuses through the cell

membrane, awaiting activation. The intracellular enzyme, 11β-hydroxysteroid dehydrogenase 1(11β-HSD 1) is responsible for activating cortisone to active cortisol, while hydroxysteroid dehydrogenase 2 (11β-HSD2) reverses the reaction. Importantly, tissue-specific expression of 11β-HSD1 and 11β-HSD2 confers reliable spatial regulation by balancing the active and inactive forms of glucocorticoid (Seckl *et al*., 2004).

Once activated, glucocorticoids bind their receptor, a member of the nuclear receptor family of intracellular receptors, which also contain the androgen receptor, oestrogen receptor, progesterone receptor and mineralocorticoid receptor (Germain *et al.*, 2006). There is some cross-reactivity between the glucocorticoid and mineralocorticoid receptors, as the mineralocorticoid receptor can be activated by its own ligand and by glucocorticoids. However, the glucocorticoid receptor is only activated by cortisol (Reul and de Kloet, 1985).

Upon binding of a glucocorticoid to its receptor, the ligand-receptor complex translocates to the nucleus via a microtubule network (Galigniana *et al.*, 1998). In the nucleus, the ligand-receptor complex recognises and associates with specific deoxyribonucleic acid (DNA) target sites that are linked to hormone-regulated genes (Heitzer *et al.*, 2007). Glucocorticoids can be recruited to target genes either through interaction with other DNA bound transcription factors or through direct DNA binding, at elements termed glucocorticoid response elements (GREs). Once bound to positive GREs, a macromolecular complex is quickly assembled that initiates the functioning of the transcriptional machinery (Newton, 2000; Gjerstad *et al*., 2018). Mounting evidence also suggests that cortisol is involved in non-genomic signalling (Tasker *et al*., 2005; Groeneweg *et al.*, 2012).

Glucocorticoids regulate a wide range of physiological processes, namely stress, inflammatory and immune responses, with principal involvement in metabolism (Oakley and Cidlowski, 2013). Although a complete discussion of the physiological function of glucocorticoids is beyond the scope of this dissertation, a brief summary of the main functions will be provided next.

Firstly, cortisol suppresses certain functions of the immune system, by promoting apoptosis of proinflammatory T-cells (Thau *et al*., 2022), suppressing B-cell antibody production, and inhibiting neutrophil recruitment during inflammation (Kadmiel and Cidlowski, 2013). The anti-inflammatory properties of glucocorticoids are indeed well described. Secondly, cortisol mediates the stress response (Thau *et al*., 2022). SNS activation results in the release of catecholamines from the adrenal gland, which produce the fight or flight response (Thau *et al*., 2022). The hypothalamus also subsequently activates the HPA axis, where the acute release of cortisol activates catabolic reactions to provide energy to the body (Lee *et al*., 2015). In the presence of cortisol, pancreatic β-cells increase glucagon and decrease insulin production. Cortisol stimulates gluconeogenesis in liver cells, to increase blood glucose concentrations and availability of glucose to the brain (Thau *et al*., 2022). To supply gluconeogenesis, muscle cells increase protein degradation while simultaneously decreasing glucose uptake and consumption (Thau *et al*., 2022) Importantly, in adipose tissue, cortisol increases lipolysis (Legeza *et al.*, 2017).

Excess circulating glucocorticoid concentrations, therefore, has several adverse effects on metabolism, as glucocorticoids play an important role in adipogenesis and adipokine production (Legeza *et al.*, 2017). Patients with excess circulating glucocorticoid concentrations, as in Cushing syndrome, typically exhibit excess visceral adiposity, hypertension, dyslipidaemia, as well as insulin resistance and/or T2DM (Stout *et al*., 2019). Similarly, selective inhibition of cortisol activation, through 11β-HSD1 activity, improved various MetS parameters and suppressed adipocytokine production in obese patients (Wang *et al.*, 2012).

The anti-inflammatory, yet hypoinsulinaemic response to glucocorticoid signalling presents an interesting conundrum when patients with insulin resistance require anti-inflammatory treatment for systemic conditions. In this regard, several adverse effects have been reported following employment of exogenous (pharmacological) glucocorticoids (Oray *et al.*, 2016). While glucocorticoid treatment may confer beneficial anti-inflammatory effects, they also induce

hyperglycaemic responses that may contribute to the development of MetS, particularly if administered on a chronic basis (Coutinho and Chapman, 2011; Geer *et al*., 2014). Individuals consuming a high-fructose diet may therefore also be at an increased risk for developing MetS, when receiving glucocorticoid treatment simultaneously. However, the exact effects of pharmacological glucocorticoid treatment may not be identical to those of endogenous glucocorticoid signalling (Borges *et al*., 1997; Croxtall *et al.*, 2002; Elmore *et al.*, 2004) and the pharmacological industry has put much effort into separating the anti-inflammatory effects from the unwanted metabolic effects. In this regard, further research is required to determine the effects of glucocorticoid treatment, on metabolic parameters in individuals consuming a high fructose diet. The effects of exogenous glucocorticoids on the development of cardiovascular complications, particularly heart failure, in a population consuming a high fructose diet, therefore, warrants further investigation.

2.9. Glucocorticoids' effect on cardiac function

Glucocorticoids are common anti-inflammatory and immunosuppressive agents that are analogous to endogenous glucocorticoids (Ren et al., 2012; van Looveren et al., 2020). Various synthetic glucocorticoids (e.g., Prednisolone, Methylprednisolone, Dexamethasone, Budesonide, and Fluticasone) have been developed to serve as treatment for a variety of inflammatory and autoimmune diseases (Timmermans et al., 2019). The structure of endogenous glucocorticoids is preserved in the majority of synthetic glucocorticoids, with some modifications to side chains to selectively optimise specific characteristics for therapeutic use (pharmacokinetics, bioavailability and concurrent mineralocorticoid receptor activation (Timmermans *et al*., 2019). As such, synthetic glucocorticoids are more potent, as they bind to the glucocorticoid receptor to a greater extent and show minimal cross-reactivity with the mineralocorticoid receptor (Jung *et al.*, 2006). Importantly, some glucocorticoids such as dexamethasone do not require activation

by11βHSD1/2, while others (prednisolone) do, which may significantly impact on bioavailability (König *et al.*, 2007).

Synthetic glucocorticoids exert potent anti-inflammatory effects by decreasing the release of arachidonic acid from phospholipids and by inhibiting the production of cyclooxygenase (COX) (Rook, 1999; Giugliano *et al.*, 2003; Mallioris and Kounis, 2004). The inhibition of these two enzymes strongly inhibits the production of pro-inflammatory mediators, including prostaglandins, leukotrienes, eicosanoids, prostaglandin endoperoxides and thromboxane (Chaney, 2002; Koutsojannis *et al.*, 2004; Mallioris and Kounis, 2004). However, the main mechanism whereby glucocorticoids reduce inflammation is by inhibiting nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) transcription, causing reduced expression of inducible nitric oxide synthase (iNOS) and subsequently reducing cardiotoxic peroxynitrite levels (Ferdinandy and Schulz, 2001; Mungrue *et al.*, 2002). NF-kB is a transcription factor that regulates genes involved in several immune and inflammatory response processes (Liu *et al*., 2017). Decreased NF-kB activation further reduces expression of monocyte chemotactic protein (MCP), endothelial leukocyte adhesion molecule 1 (ELAM-1), intercellular adhesion molecule 1 (ICAM-1), and granulocyte-macrophage colony-stimulating factor (G-CSF) vascular cell adhesion molecule-1 (VCAM-1), resulting in decreased immune and endothelial cell activation (Thiemermann, 2002).

In addition to the anti-inflammatory effect of glucocorticoids, favourable immunomodulatory functions have also been reported. Glucocorticoids exert an immunomodulatory effect on the heart by upregulating CD95 and CD95 ligand expression, to induce apoptosis of monocytes, macrophages and T-lymphocytes (Koutsojannis *et al.*, 2004; Fan *et al.*, 2009). However, some exogenous glucocorticoids seem to protect the myocardium from apoptosis (Fan *et al.*, 2009), by blocking pro-apoptotic signals induced by cytokines, cyclic adenosine monophosphate (cAMP), and tumour suppressors (Viegas *et al.*, 2008). cAMP is an ATP derivative which plays an important role of a second messenger in signal transduction (Serezani *et al*., 2008).

Despite their widespread clinical use to suppress inflammation, little is known about the effects of exogenous glucocorticoids on cardiac physiology and pathology (Oakley and Cidlowski, 2015). Several molecular pathways involved in glucocorticoid-mediated cardioprotection and immune modulation have been reported (Ren *et al.*, 2012; van Looveren *et al*., 2020). However, long-term administration of exogenous glucocorticoids associated with glucocorticoid excess results mostly in cardiovascular complications (Hattori *et al.*, 2013). Indeed, exogenous glucocorticoid administration was found to be associated with heart failure, in a large population-based study (Souverein *et al.*, 2004). While most studies report an acute cardioprotective effect of exogenous glucocorticoids in ischaemia reperfusion injury (Enc *et al.*, 2006; Fan *et al.*, 2009; Tokudome *et al.*, 2009), recent reports suggest that chronic use of exogenous glucocorticoids elicits cardiac hypertrophy and pathological remodelling (Jin *et al*., 2021; Tanaka *et al*., 2021). Exogenous glucocorticoids also induce LV diastolic dysfunction, LV atrophy and fibrosis (Young *et al*., 2007; Mihailidou *et al.*, 2009; Yiu *et al.*, 2012; Hattori *et al.*, 2013).

The mechanisms whereby exogenous glucocorticoids induce negative effects on cardiac function are currently under investigation. However, further research is required to determine whether the adverse effects of glucocorticoids on cardiac structure and function are exacerbated in MetS associated with a high fructose diet. Given the significant increase in fructose consumption and the rising prevalence of undiagnosed insulin resistance, further research is required to determine whether MetS individuals are at an increased risk for glucocorticoid-induced cardiac dysfunction, particularly diastolic dysfunction and HFpEF. This study, therefore, aimed to determine whether glucocorticoid therapy exacerbates cardiac remodelling and dysfunction when administered to Sprague Dawley rats undergoing a high fructose diet to induce MetS.

2.10. Problem Statement

Cardiometabolic disease is the principal cause of death worldwide and is a major cause of mortality in South Africa. Excess adiposity, insulin resistance, and dyslipidaemia converge to form

metabolic syndrome (MetS), which significantly increases the risk of developing T2DM and CVD. The majority of research on MetS has focussed on understanding the mechanisms underlying the development of atherosclerosis and ischaemic heart disease. However, persons with MetS are also at an increased risk of developing heart failure, particularly heart failure with a preserved ejection fraction (HFpEF). The mechanisms underlying the development of heart failure with a preserved ejection fraction (HFpEF) are not fully understood and require further elucidation.

Individuals with MetS typically demonstrate a specific metabolic phenotype with significantly increased circulating insulin and triglyceride concentrations despite normal fasting blood glucose concentrations. Alterations in substrate utilisation place individuals who have MetS at increased risk of dysglycaemia, particularly in the context of pharmacological treatment practices. One class of drugs commonly prescribed for a variety of autoimmune and inflammatory conditions, is glucocorticoids. Although glucocorticoids confer anti-inflammatory and anti-proliferative benefits, they may also promote hyperglycaemic responses. The negative metabolic effects reported following glucocorticoid treatment may be enhanced in patients with MetS. Further investigation is required to determine whether glucocorticoid treatment may adversely affect the metabolic profile of individuals with MetS.

In addition, glucocorticoids have been implicated in the development of cardiac remodelling and dysfunction. However, direct evidence to support these effects in animal models remains limited. While several non-invasive methods can be used to assess cardiac morphology and function in humans, direct assessments of end-organ damage, such as cardiac fibrosis, typically require tissue samples, which cannot be obtained in human studies for ethical reasons. Consequently, animal studies provide a valuable platform for investigating the mechanisms underlying the effects of glucocorticoid treatment on left ventricular (LV) morphology and function, as well as for assessing long-term outcomes. By conducting this investigation in rodents, specific research questions related to glucocorticoid-induced LV morphology and function can be addressed, and

valuable insights can be gained that can further inform clinical studies and contribute to a better understanding of the effects of glucocorticoids on cardiovascular health. Moreover, it is not currently known whether the presence of MetS may exacerbate adverse cardiac remodelling and dysfunction, following glucocorticoid treatment. Due to the confounding effects present in human studies, it becomes difficult to accurately assess this relationship. Therefore, further research in animals is necessary to determine whether pre-existing MetS may accelerate cardiac remodelling and dysfunction with glucocorticoid treatment, without any confounding effects encountered in human studies.

2.11. Aim and objectives

This study aimed to determine the effects of glucocorticoids on LV structure and function in male Sprague Dawley rats. In addition, the present study determined whether the consumption of a high fructose diet (as a model of MetS) exacerbated the glucocorticoid-induced changes in LV morphology and function in male Sprague Dawley rats.

The specific objectives of the current study are to determine

1. the effects of glucocorticoids on cardiac morphology and function in male Sprague Dawley rats.
2. whether a high-fructose diet induced MetS in male Sprague Dawley rats.
3. whether the effects of glucocorticoids on LV structure and function are exacerbated by the consumption of a high fructose diet (as a model of MetS) in male Sprague Dawley rats.

2.12. Hypotheses

H_1: Glucocorticoid administration induces concentric remodelling, impairs LV relaxation, and increases LV diastolic filling pressures. In addition, the presence of MetS results in concentric hypertrophy which worsens LV diastolic dysfunction.

H_0: Glucocorticoid administration does not induce concentric remodelling, impair LV relaxation, or increase LV diastolic filling pressures. In addition, the presence of MetS does not result in concentric hypertrophy and does not worsen LV diastolic dysfunction.

Chapter 3: Methods

3.1. Animals and ethical clearance

Forty, three-month-old, male Sprague-Dawley rats were obtained from the Wits Research Animal Facility (WRAF) at the University of the Witwatersrand. Sprague Dawley rats were chosen due to their common use in biomedical research, including cardiovascular studies, ensuring comparability and drawing upon existing knowledge. The selection of 3-month-old rats aligns with the practice of studying disease progression and intervention effects. At this age, rats exhibit stable cardiovascular physiology, enabling reliable comparisons and investigating long-term effects of glucocorticoid treatment on cardiac health. In addition, male rats are often preferred in cardiovascular research due to their hormonal profile, which provides a more consistent baseline compared to female rats, whose oestrous cycle can introduce additional variability in experimental outcomes. Hence, we used male rats to minimise the potential confounding factors related to hormonal fluctuations, leading to more robust and interpretable results.

Rats were housed individually in temperature-controlled rooms (23 ± 2°C) with a 12-hour light-dark cycle and allowed free access to commercial rat chow, plain drinking water or a 20% fructose solution, depending on the treatment group. All experimental procedures were approved by the Animal Research Ethics Committee of the University of the Witwatersrand (AREC approval number: 2021/04/06/C).

3.2. Experimental design

The current study was a randomised controlled experimental intervention study. The rats underwent a two-week habituation period before undergoing experimental procedures. During this period, body mass, water intake, and food intake were measured once a week and blood pressure was measured twice a week. After the two-week habituation period, rats were randomly divided into the control (n=10), glucocorticoids (GC; n=10), high fructose (HF; n=10) and glucocorticoids + high fructose (GC+HF; n=10) groups. Previous studies of a similar nature have shown that a sample size of n=10 is adequate to provide statistical power for analysis. All

experimental groups had *ad libitum* access to food and drinking liquid throughout the study. Rats in the HF and GC+HF groups received a 20% fructose solution in drinking water while rats in the control and GC groups received tap water. Rats in the GC and GC+HF groups received 10mg/kg daily injections of methylprednisolone for ten weeks.

Body mass, water intake, food intake, blood glucose, blood triglyceride concentrations, and blood pressure measurements were taken at baseline and following the 10-week intervention period. Before termination, rats were fasted overnight, and an oral glucose tolerance test (OGTT) was conducted. Thereafter, rats were anaesthetised, and cardiac function was assessed using echocardiography. Following echocardiography, rats were terminated, whereafter blood and tissue samples were collected and stored at -80°C for further analysis. A summary of the experimental design is outlined in Figure 3.1 below.

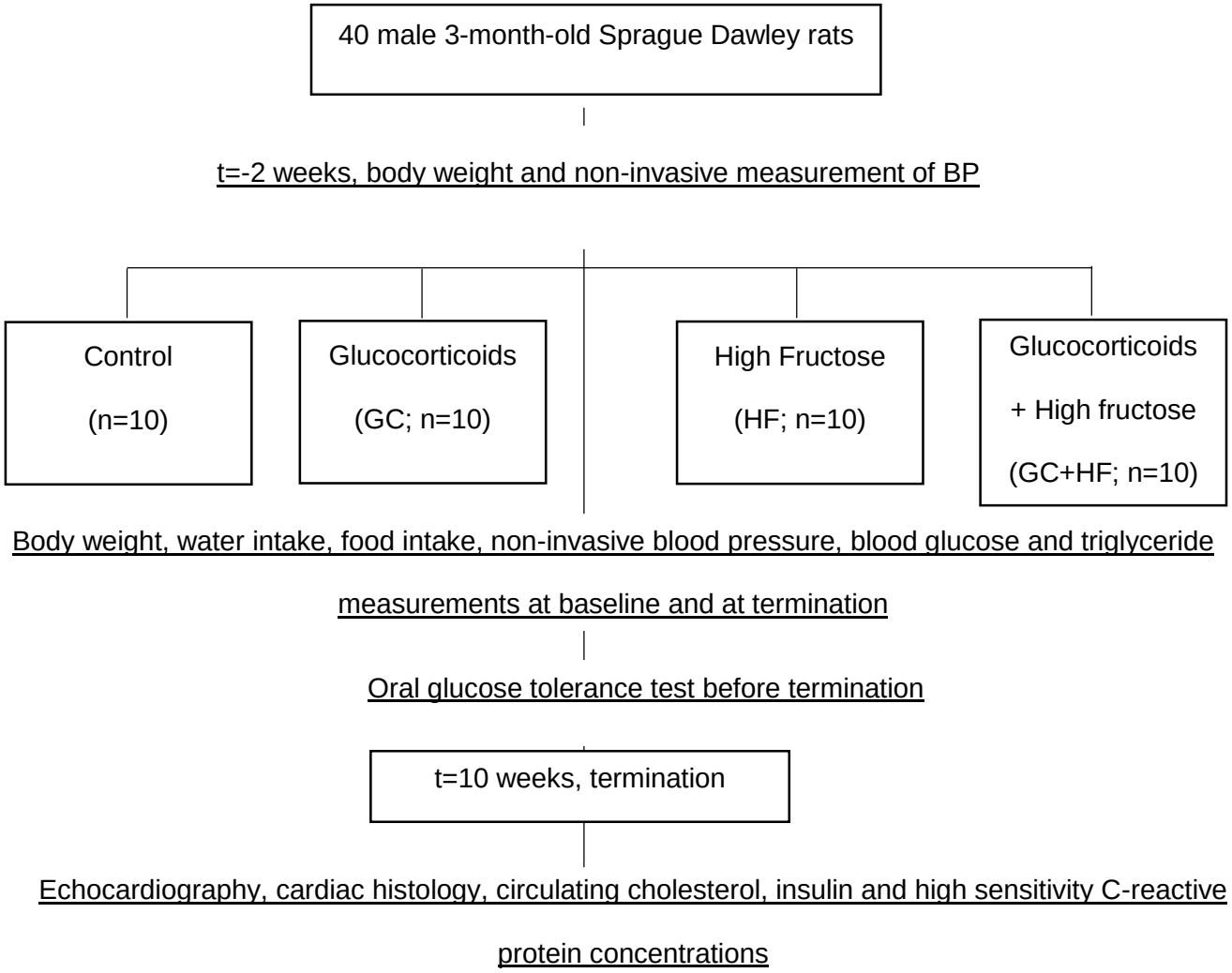

Figure 3. 1 Experimental design to address the effects of a high fructose diet and glucocorticoid intervention on cardiac morphology and function in male Sprague Dawley rats

3.2.1. 20% fructose solution preparation

The rats in the HF and GC+HF groups received a 20% fructose solution after the habituation period. The fructose solution was prepared by mixing warm water and fructose in a 1:5 ratio. Briefly, 200g of fructose (Granulated fruit sugar, Nature's Choice, South Africa) was weighed using a digital scale (Snowrex Electronic Scale, Clover Scales, Johannesburg) and mixed in 1 litre of warm water. Fresh fructose solution was prepared every week and was allowed to cool for at least 1 hour before administration. To distinguish the normal tap water from the fructose solution, a blue food dye was used to colour the fructose solution and a red food dye was used to colour the tap water.

3.2.2. Drug intervention

Following the two-week habituation period, the rats in the GC and GC+HF groups received intraperitoneal injections of methylprednisolone, a glucocorticoid (Methylprednisolone, Xixia Pharmaceuticals, Gauteng) at a dosage of 10 mg/kg daily for ten weeks.

3.3. Measurement of body mass, food intake and water intake

To assess body mass, food intake and water intake, the rats were kept in individual cages. The rats were weighed at baseline and termination using a digital scale (Snowrex Electronic Scale, Clover Scales, Johannesburg). The weekly baseline and terminal food intake for each rat was determined by adding 500g of food at the beginning of the week and subtracting the remaining amount of food left in the cage from the total amounts supplied at the beginning of the week. The weekly baseline and terminal water intake for each rat were determined by filling the water bottles with either fructose water or plain water. The water bottles were weighed at the beginning of the week using a digital scale (Snowrex Electronic Scale, Clover Scales, Johannesburg), and the amount of remaining water in the bottles was subtracted from the total amount supplied at the start of the week.

3.4. Measurement of blood pressure

Blood pressure was measured at baseline and at termination using a non-invasive tail-cuff machine (Biopac Systems, Santa Barbara, CA, USA). To measure the blood pressure, rats were placed in restrainers, and their tails were heated using a heating pad to ensure maximum blood flow to their tails. After the rat tails were heated, the cuff of the blood pressure machine was positioned at the base of the tail and blood pressure measurements were taken. To avoid diurnal variation, all blood pressure measurements were taken at midday.

3.5. Fasting glucose and triglyceride concentrations

Rats were fasted overnight, and blood glucose and triglyceride concentrations were determined from a drop of blood obtained from the tail vein using a pinprick. Blood glucose concentrations were measured using a calibrated glucometer (Contour Plus Meter, Bayer, Isando-Johannesburg, South Africa). Blood triglyceride concentrations were determined using a calibrated Accutrend GCT meter (Roche, Mannheim, Germany).

3.6. Oral glucose tolerance test

Before termination, rats were fasted overnight for 12 hours whereafter a drop of blood was obtained from the tail vein using a pinprick. Fasting blood glucose concentrations were determined using a calibrated glucometer (Contour Plus Meter, Bayer, Isando-Johannesburg, South Africa). Immediately thereafter, the rats were gavaged with 2 g.kg-1 glucose and blood glucose measurements were repeated at 15, 30, 60, 120 and 180 minutes.

3.7. Echocardiography

Echocardiography was performed by an experienced observer, according to the American Society of Echocardiography conventions (Sahn *et al*.,1979). Before performing echocardiography, the rats were anaesthetised with oxygen (3-1% inhalation) and isoflurane. The rats were placed in the left lateral decubitus position with their anterior chest shaved. An ultrasound linked to a high-resolution probe(10MHz) was used to perform the echocardiography (Affiniti CVx, Philips

Healthcare, Andover, Massachusetts). Two-dimensional directed M-mode echocardiography in the parasternal long-axis view was used to determine the dimension of the left ventricle. Posterior wall thickness (PWT), internal diameter, septal wall thickness, LV end-systolic diameter (LVESD), and the LV end-diastolic diameter (LVEDD) were measured during diastole and systole (Figure 3.2). Relative wall thickness (RWT) was calculated as ((PWT in diastole x 2)/LVEDD) (Ganau *et al.*, 1992).

LV systolic function was determined by calculating LV ejection fraction (EF) using the Teichholz method (Teichholz *et al.*, 1976) and LV endocardial fractional shortening (FSend) using the equation LV (EDD-ESD)/EDD (Teichholz *et al*.,1976), respectively.

LV diastolic function was determined from the mitral valve inflow patterns using pulsed Doppler imaging. In the apical 4-chamber view, the early (E) and late (A) diastolic inflow velocity were obtained with the sample volume placed at the mitral valve leaflet tip. The ratio of early to late diastolic inflow (E/A), was recorded as a marker of relaxation (Figure 3.3). To determine diastolic function using tissue Doppler imaging (TDI), peak myocardial tissue lengthening velocities during early (e') and late (a') diastole were recorded at the lateral mitral annulus in the apical four-chamber view (Figure 3.4). Data were expressed as e' (an index of myocardial relaxation), e'/a' (an index of myocardial stiffness) and E/e' (an index of LV filling pressure).

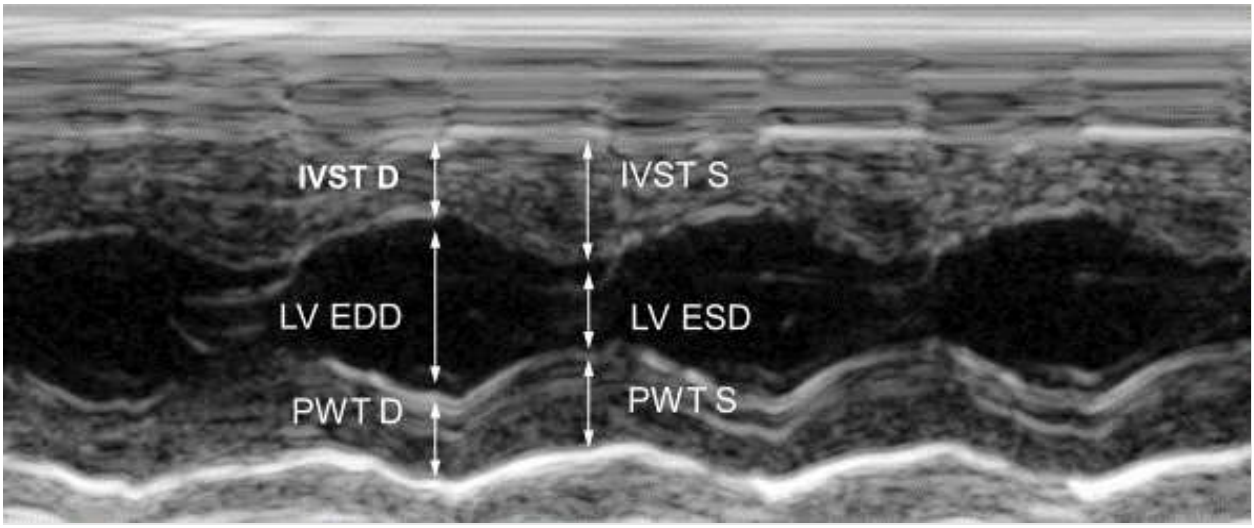

Figure 3. 2 Example of a 2-D M mode in the parasternal long-axis view indicating left ventricular dimensions.

Intraventricular septal wall thickness during diastole (IVST D); Left ventricular end-diastolic internal diameter (LV EDD); Posterior wall thickness during diastole (PWT D); Intraventricular septal wall thickness during systole (IVST S); Left ventricular end-systolic internal diameter (LV ESD); Posterior wall thickness during systole (PWT S).

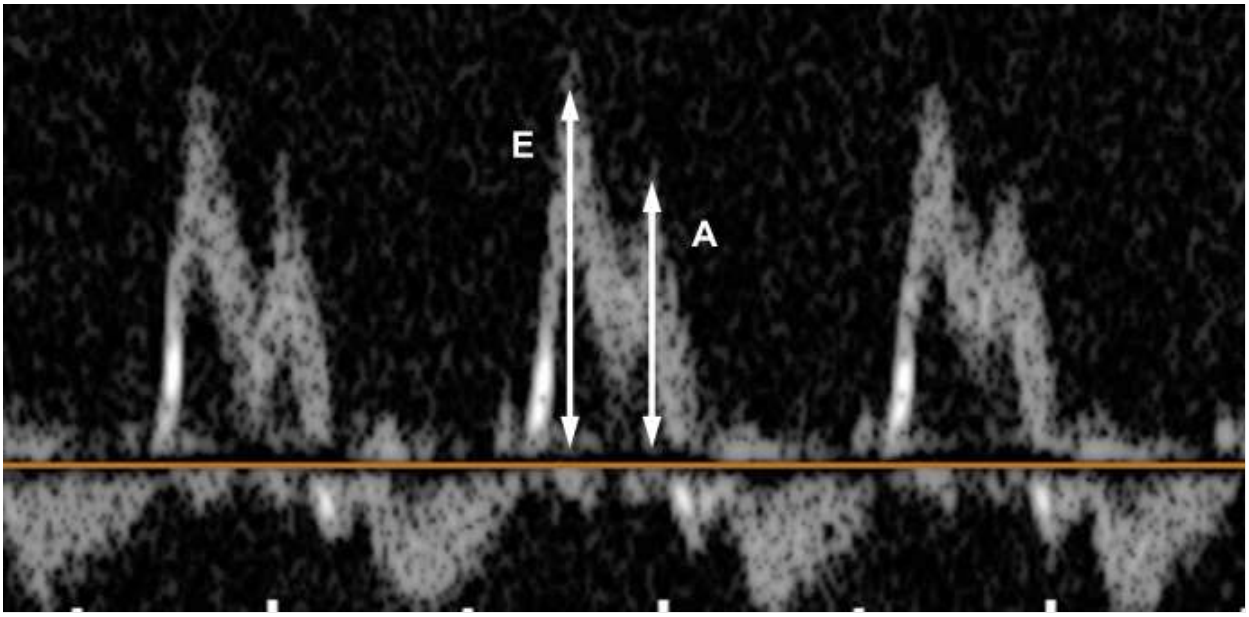

Figure 3. 3 Example of an echocardiographic image showing trans-mitral inflow velocity in early and late diastole using pulse wave Doppler.

Early diastolic filling velocity (E); Late diastolic filling velocity (A).

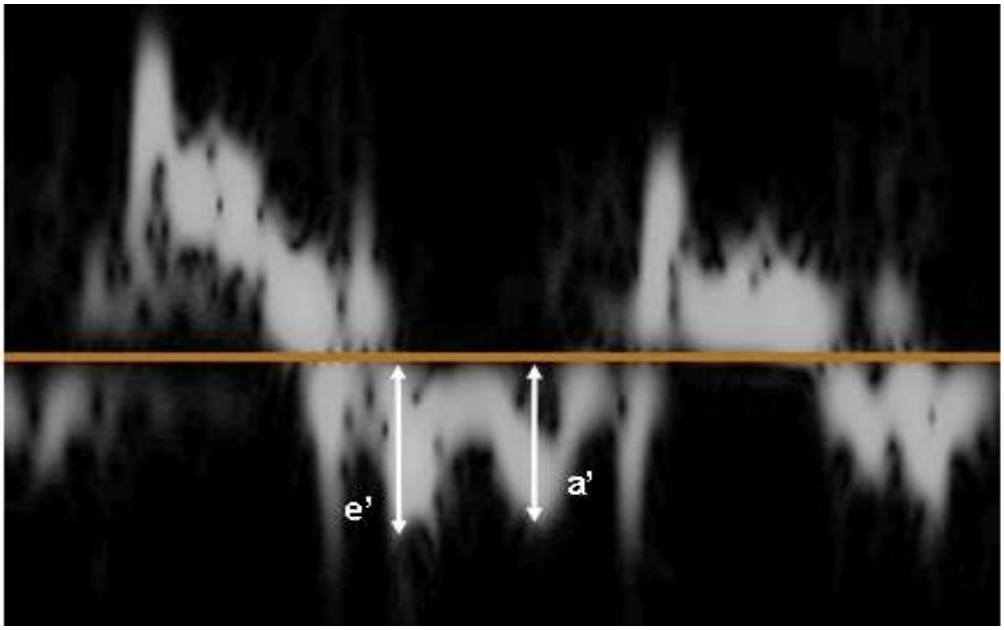

Figure 3. 4 Example of an echocardiographic image showing the rate of tissue relaxation at the mitral annulus using tissue Doppler imaging.

Early diastolic mitral annulus velocity (e'); Late diastolic mitral annulus velocity (a').

3.8. Terminal procedures

After echocardiography, a thoracotomy was performed, and blood was drawn directly from the heart. Thereafter, blood was collected into serum tubes for serum and ethylenediaminetetraacetic acid (EDTA) tubes for plasma. The blood in serum tubes was allowed to clot for 2 hours at room temperature. Blood was then centrifuged at a speed of 3400rpm for 15 minutes, and serum and plasma were collected and stored at -80°C until assayed. The hearts and visceral fat of the rats were sampled, weighed, and stored at -80°C until assayed. The visceral fat mass relative to body mass (%BM) was calculated using the formula: Relative visceral fat mass = [visceral fat mass (g) /terminal body mass (g)] x 100.

3.8.1. Circulating insulin concentrations

The concentration of insulin in plasma was measured using a rat insulin enzyme-linked immunosorbent assay (ELISA) kit (Elabscience ®, Rat INS(Insulin) ELISA kit, Wuhan, Hubei Province, China) according to the instructions provided by the manufacturer. The lower detection limit for insulin was 6.25 pg/ml with a coefficient of variation of <10%. Insulin resistance was estimated by computing the homeostatic model assessment index (HOMA-IR) according to the equation HOMA-IR= fasting insulin (ng/mL) x fasting blood glucose (mmol/L)/22.5 (Divi *et al*., 2012).

3.8.2. Circulating cholesterol concentrations

Plasma cholesterol concentrations were determined using a colorimetric-based clinical chemistry analyser (IDEXX VetTestR Clinical Chemistry Analyser, IDEXX Laboratories Inc., USA) as per the manufacturer's instructions**.**

3.8.3. Circulating high-sensitivity C-reactive protein concentrations

High-sensitivity C-reactive protein (hs-CRP) serum concentrations were measured using ELISA kits (Elabscience Biotechnology Co. Ltd, Wuhan, China) according to the instructions provided by

the manufacturer. The lower detection limit for hs-CRP was 7.81 pg/ml with a coefficient of variation of <10%.

3.8.4. Cardiac tissue total collagen content

To quantify the degree of fibrosis in left ventricular tissue samples, tissue samples were fixed in 10% buffered formalin and routinely processed for paraffin embedding. Five µm thick tissue sections were deparaffinised, rehydrated, and stained with a 0.1% Sirius Red solution dissolved in aqueous saturated picric acid for 60 min at room temperature. After washing in two changes of acidified water, slides were dehydrated in 100% ethanol, cleared with xylene, and mounted with DPX mounting medium. The samples were analysed using a Zeiss Axioskop 2 Plus microscope equipped with a Zeiss AxioCam (Zeiss, Peabody, MA, USA). Tissue sections viewed under bright-field and polarized light were obtained with a 10x objective lens (x100 magnification). Using ImageJ software, the collagen area fraction was calculated for each tissue section by dividing the collagen area by the total tissue area.

3.9. Statistical analysis

Data are expressed as means ± SEM. Data analysis was performed using SAS software, version 9.4 (SAS Institute Inc., USA). A repeated-measures analysis of variance (ANOVA) followed by a Tukey post hoc test was used to determine the differences in body mass, food intake, water intake, blood pressure, blood glucose and triglyceride concentrations between the groups. A one-way analysis of variance (ANOVA) followed by a Tukey post hoc test was used to determine differences in echocardiographic measures, circulating hs-CRP and insulin concentrations and the total collagen content between the groups. Differences with a p-value smaller than 0.05 was considered statistically significant.

Chapter 4: Results

4.1. Food intake, water intake, body mass, visceral fat mass and blood pressures

Table 4.1 shows the food intake, water intake, body mass, visceral fat mass and blood pressures (mean ± SEM) of the control, GC, HF and GC+HF groups. The water intake was significantly higher in the HF and GC+HF groups at termination compared to baseline (both $p<0.0001$). At termination, the water intake was significantly higher in the HF and GC+HF compared to the control and GC groups (both $p<0.0001$).

The body mass was significantly higher in all the groups at termination compared to baseline (all $p<0.001$). At termination, the body mass was significantly higher in the HF group compared to the control, GC and GC+HF groups ($p=0.0049$, $p<0.0001$ and $p=0.04$ respectively). At termination, the body mass was significantly lower in the GC group compared to the GC+HF group ($p=0.02$).

At termination, the visceral fat mass was significantly higher in the HF and GC+HF groups compared to the control group ($p=0.002$ and $p=0.03$ respectively). The visceral fat mass was significantly lower in the GC group compared to the HF group ($p=0.03$). There were no significant differences in the visceral fat mass between the control and GC groups ($p=0.73$). The visceral fat mass relative to body mass (%BM) was significantly higher in the HF and GC+HF groups compared to the control group (both $p=0.04$). The %BM in the GC group was numerically higher than that of the control group but was not statistically significant ($p=0.06$).

Food intake and blood pressure were similar between the groups at baseline and at termination (all $p>0.05$).

Table 4. 1 Food intake, water intake, body mass, visceral fat mass and blood pressure in the control, GC, HF and GC+HF groups.

	Control (n=10)	GC (n=10)	HF (n=10)	GC+HF (n=10)
Baseline				
Food intake (g, for 7 days)	162 ± 8	155 ± 8	164 ± 8	157 ± 8
Water intake (ml, for 7 days)	318 ± 13	299 ± 13	325 ± 13	327 ± 13
Body mass (g)	467 ± 7	473 ± 7	477± 7	464 ± 7
Systolic blood pressure (mm Hg)	127 ± 1	127 ± 1	127± 1	127 ± 1
Diastolic blood pressure (mm Hg)	89 ± 2	89 ± 2	88± 2	87 ± 1
Termination				
Food intake (g, for 7 days)	173 ± 8	156 ± 8	177 ± 8	178 ± 8
Water intake (ml, for 7 days)	320 ± 14	296 ± 13†	445 ± 13 ^{a*}	446 ± 14 $^{a*\#}$
Body mass (g)	539 ± 7 ^{a}	510 ± 7 ^{a†}	577± 7 ^{a*}	545 ± 7 $^{a\dagger\#}$
Visceral fat mass (g)	9.34 ± 0.56	10.16 ± 0.56†	12.50 ± 0.56*	11.75 ± 0.59*
Visceral fat mass (% BM)	1.73 ± 0.11	2.01 ± 0.11	2.16 ± 0.11*	2.17 ± 0.11*
Systolic blood pressure (mm Hg)	127 ± 1	127 ± 1	126± 1	126 ± 1
Diastolic blood pressure (mm Hg)	88 ± 2	87 ± 2	87± 2	88 ± 2

Data expressed as means ± SEM. $^{a}p<0.05$ at termination vs baseline.

*$p<0.05$ vs control; $^{\#}p<0.05$ vs GC; $^{\dagger}p<0.05$ vs HF. GC: glucocorticoids group; HF: high fructose group; GC+HF: glucocorticoids and high fructose

4.2. Triglyceride and cholesterol concentrations

Figure 4.1 shows the blood triglyceride and cholesterol concentrations (mean ± SEM) of the control, GC, HF and GC+HF groups. The blood triglyceride concentrations were significantly higher at termination compared to baseline in the GC (2.13 ± 0.11 mmol/L vs 1.71 ± 0.11 mmol/L; p=0.0001), HF (2.95 ± 0.11 mmol/L vs 1.64 ± 0.11 mmol/L; p=0.0001), and GC+HF groups (2.55 ± 0.11 mmol/L vs 1.41 ± 0.01 mmol/L; p=0.02). At termination, the blood triglyceride concentrations were significantly higher in the GC (2.13 ± 0.11 mmol/L), HF (2.95 ± 0.11 mmol/L) and GC+HF (2.55 ± 0.11 mmol/L) groups compared to the control group (1.59 ± 0.11 mmol/L) (p=0.02, p=0.0001 and p=0.0001 respectively). At termination, the blood triglyceride concentrations were significantly lower in the GC group (2.13±0.11 mmol/L) compared to the HF group (2.95±0.11 mmol/L; p<0.0001).

The cholesterol concentrations were significantly higher in the GC (63.89±2.58 mg/d; p=0.02) and GC +HF (63.11±2.58 mg/dL; p=0.03) groups compared to the control group (52.22±2.58 mg/dL). There were no significant differences in the cholesterol concentrations between the control and HF groups and between the GC and GC+HF groups (p>0.05).

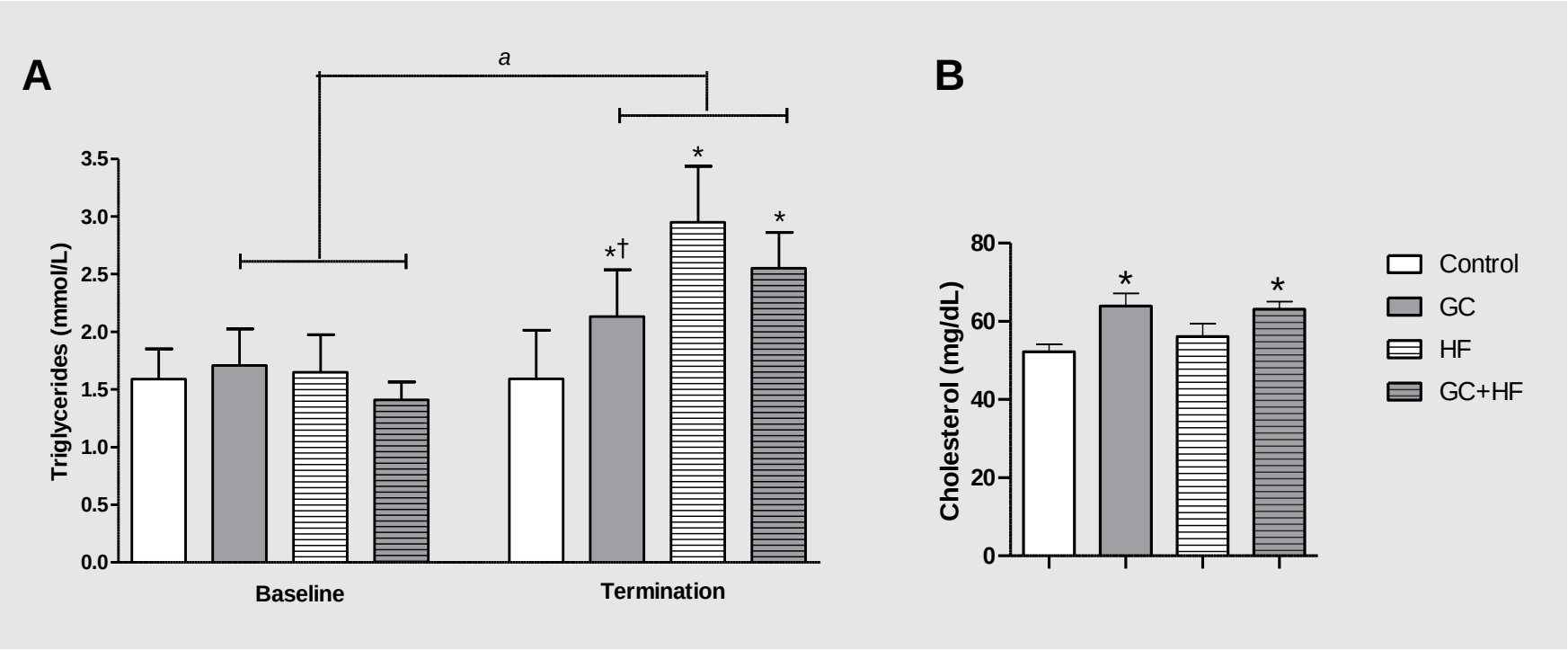

Figure 4. 1 Blood triglyceride (A) and cholesterol (B) concentrations in the control, GC, HF and GC+HF groups.

[a] $p<0.05$ at termination vs baseline. *$p<0.05$ vs control; [†]$p<0.05$ vs HF. GC: glucocorticoids group; HF: high fructose group; GC+HF: glucocorticoids and high fructose group

4.3. Blood glucose concentrations

Figure 4.2 shows the fasting glucose concentrations at baseline and at termination, and the glucose concentrations from the oral glucose tolerance test (OGTT) (mean ± SEM) in the control, GC, HF and GC+HF groups. Fasting glucose concentrations were similar between the groups at baseline and at termination (all $p>0.05$; Figure 4.2A).

During administration of the OGTT (Figure 4.2B), the blood glucose concentrations were significantly higher in the control group after 30 minutes (5.96 ± 0.44 mmol/L) compared to baseline (0 minutes) (3.40 ± 0.44 mmol/L; $p=0.04$). The blood glucose concentrations were significantly higher in the GC group (7.13±0.43 mmol/L) after 30 minutes compared to baseline (3.60±0.43 mmol/L; $p<0.0001$). The blood glucose concentrations were significantly higher in the HF group after 30 (7.26 ± 0.43 mmol/L; $p<0.0001$) and 60 minutes (6.13 ± 0.44 mmol/L; $p=0.04$) compared to baseline (0 minutes: 3.64 ± 0.44 mmol/L). The blood glucose concentrations were significantly higher in the GC+HF group after 15 (8.19 ± 0.47 mmol/L; $p<0.0001$), 30 (10.47 ± 0.46 mmol/L; $p<0.0001$) and 60 minutes (6.13 ± 0.44 mmol/L; $p=0.04$) compared to baseline (0 minutes: 3.59 ± 0.46 mmol/L) There were no significant differences in the glucose concentrations between the control, GC and HF groups throughout the different time points (all $p>0.05$).

The blood glucose concentrations were significantly higher in the GC+HF group compared to the control group after 15 (8.19 ± 0.48 mmol/L vs 5.48 ± 0.43 mmol/L; $p=0.02$) and 30 minutes (10.48 ± 0.46 mmol/L vs 5.96 ± 0.43 mmol/L; $p<0.0001$). The glucose concentrations were significantly higher in the GC+HF group compared to the GC group after 15 (4.99±0.43 mmol/L vs 8.19±0.46 mmol/L; $p=0.0002$) and 30 minutes (7.13±0.43 mmol/L vs 10.48±0.46 mmol/L; $p<0.0001$). The glucose concentrations were significantly higher in the GC+HF group compared to the HF group after 15 (8.19 ± 0.48 mmol/L vs 5.56 ± 0.43 mmol/L; $p=0.03$) and 30 minutes (10.48 ± 0.46 mmol/L vs 7.26 ± 0.43 mmol/; $p=0.001$).

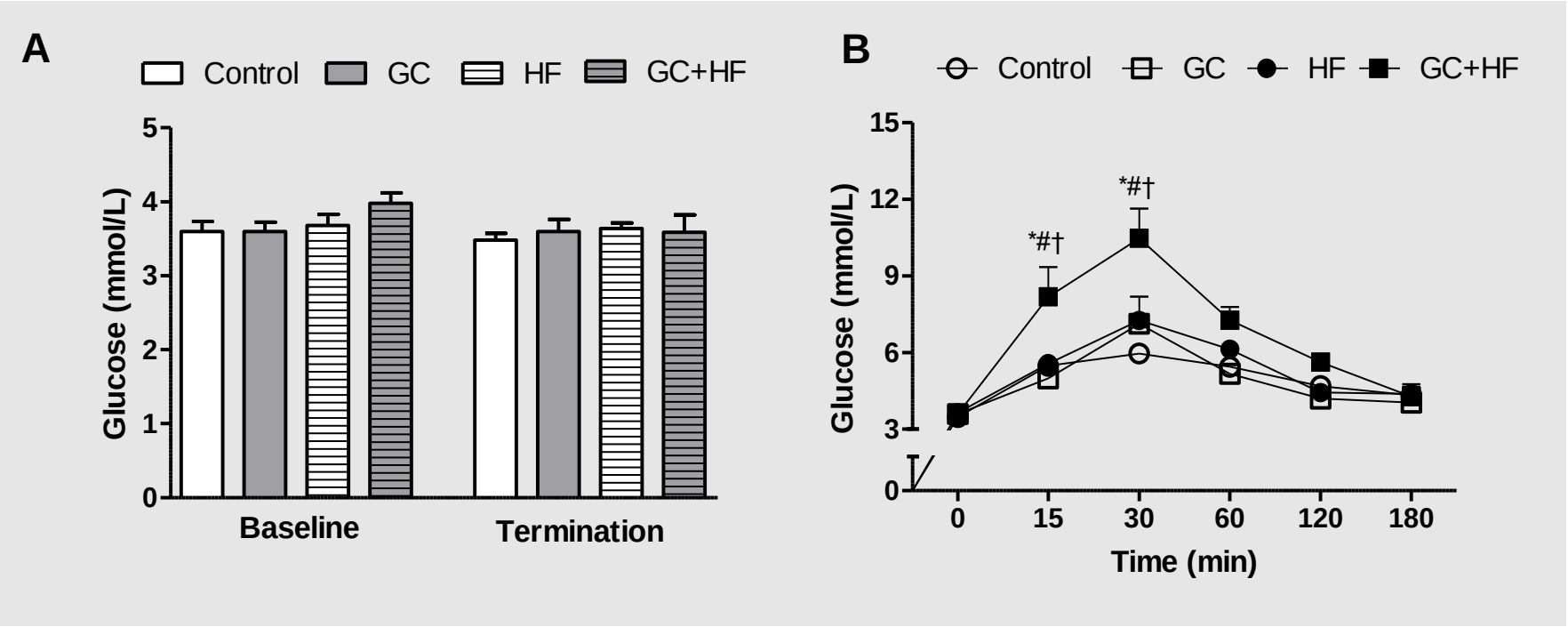

Figure 4. 2 Fasting glucose concentrations at baseline and at termination (A) and the glucose concentrations following the oral glucose tolerance test (B) in the control, GC, HF and GC+HF groups. *p<0.05 GC+HF vs control; #p<0.05 GC+HF vs HF †p<0.05 GC+HF vs GC.GC: glucocorticoids group; HF: high fructose group; GC+HF: glucocorticoids and high fructose group.

4.4. Insulin and HOMA-IR

Figure 4.3 show the plasma insulin concentrations and the HOMA-IR (mean ± SEM) in the control, GC, HF and GC+HF groups. The plasma insulin concentrations were significantly higher in the HF (275.50 ± 9.03 pg/ml; p=0.01) and GC+HF (268.35 ± 9.03 pg/ml; p=0.05) groups compared to the control group (233.99 ± 9.03 pg/ml; Figure 4.3A). The plasma insulin concentrations were significantly higher in the HF (275.50 ± 9.03 pg/ml; p=0.01) and GC+HF (268.35 ± 9.03 pg/ml; p=0.05) groups compared to the GC group (227.48 ± 9.03 pg/ml; Figure 4.3A).

The HOMA-IR was significantly higher in the HF group (0.4 ± 0.02) compared to the control group (0.3 ± 0.02; p=0.04; Figure 4.3B).

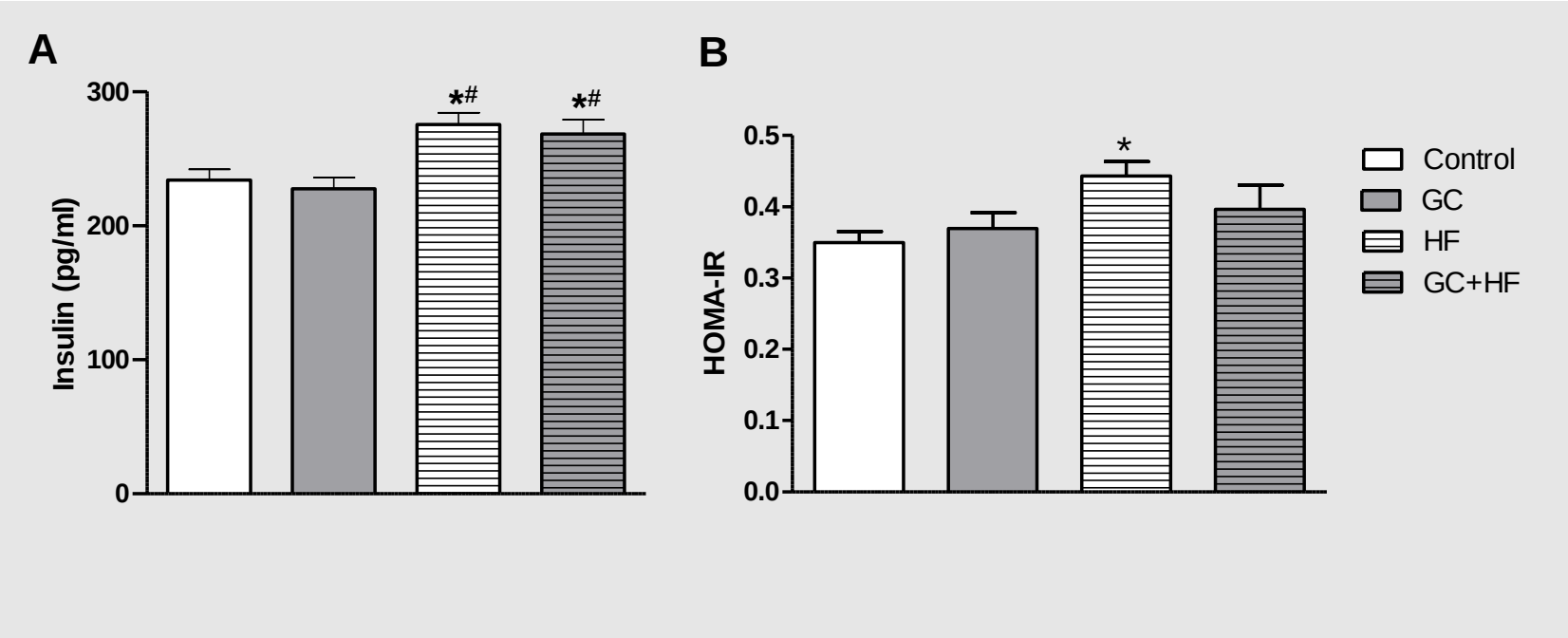

Figure 4. 3 Fasting insulin concentrations (A) and the HOMA-IR (B) in the control, GC, HF and GC+HF groups.

*p<0.05 vs control; #p<0.05 vs GC. GC: glucocorticoids group; high fructose group; GC+HF: glucocorticoids and high fructose group.

4.5. High-sensitivity C-reactive protein concentrations

Figure 4.4 shows the high sensitivity C-reactive protein (hs-CRP) concentrations (mean ± SEM) of the control, GC, HF and the GC+HF group. The hs-CRP concentrations were significantly higher in the HF group (165.96 ± 8.01 pg/ml) compared to the control and GC group (131.13 ± 8.01 pg/ml; p=0.02 and 127.46 ± 8.01 pg/ml; p=0.01 respectively). There were no significant differences in the hs-CRP concentrations between the control, GC and GC+HF groups (p>0.05).

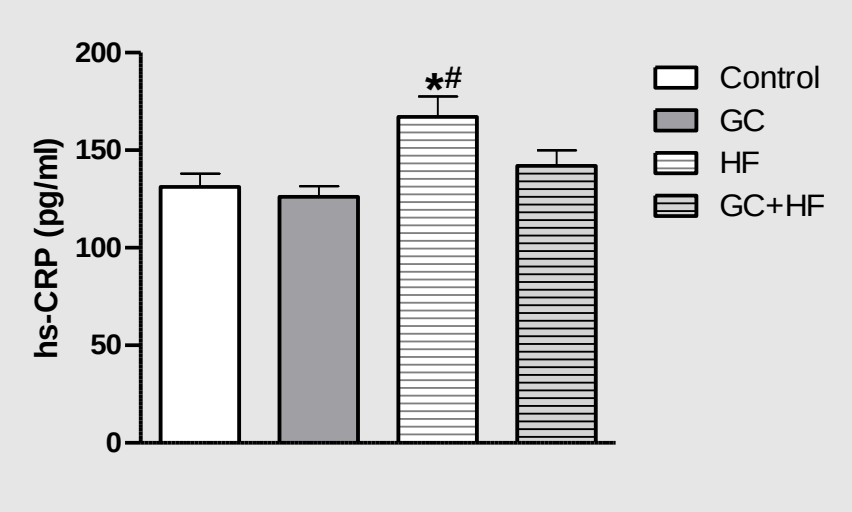

Figure 4. 4 Circulating high sensitivity C-reactive protein (hs-CRP) concentrations in the control, GC, HF and GC+HF groups.

*p<0.05 vs control #p<0.05 vs GC. GC: glucocorticoids group; HF: high fructose group; GC+HF: glucocorticoids and high fructose group.

4.6. Cardiac geometry

Table 4.2 shows the cardiac geometry of the control, GC, HF and GC+HF groups. The heart weight was significantly higher in the HF and GC+HF groups compared to the control group ($p=0.002$ and $p=0.02$ respectively). The heart weight was significantly lower in the GC group compared to the HF and GC+HF groups ($p<0.0001$ and $p=0.0006$ respectively). There were no significant differences in the heart weights between the HF and GC+HF groups ($p>0.05$). The heart weight indexed to body mass was significantly higher in the GC+HF group compared to the control ($p=0.04$) and the GC ($p=0.047$) groups.

The LV weight was significantly higher in the GC+HF group compared to the control ($p=0.009$) and the GC ($p=0.007$) groups. The LV weight was significantly higher in the HF group compared to the GC group ($p=0.04$). There were no significant differences in the LV weight between the control and HF groups and between the HF and GC+HF groups (all $p>0.05$). The LV weight indexed to body mass was significantly higher in the GC+HF group compared to the control group ($p=0.03$). There were no further differences between the groups.

There were no significant differences in the LV posterior wall thickness in diastole and systole between the control, GC, HF and GC+HF groups (all $p>0.05$). The relative wall thickness was significantly higher in the GC and GC+HF groups compared to the control group ($p=0.001$ and $p=0.008$ respectively). There were no significant differences in the relative wall thickness between the GC, HF and GC+HF groups (all $p>0.05$). The LV end-diastolic volume was significantly lower in the GC+HF group compared to the control ($p=0.007$) and the HF group ($p=0.01$). There were no significant differences in the LV end-diastolic volume between the control and GC groups (all $p>0.05$).

4.7. Left ventricular systolic function

There were no significant differences in LV ejection fraction or endocardial fractional shortening between the groups (all $p>0.05$).

4.8. Left ventricular diastolic function

The early diastolic filling velocity (E) was significantly higher in the GC and HF groups compared to control ($p<0.0001$ and $p=0.009$ respectively). The E was also significantly higher in the GC group when compared to HF and GC+HF groups (both $p<0.0001$). The ratio of early to late diastolic filling velocity (E/A) was significantly higher in the HF group compared to the control group ($p=0.03$). There were no further significant differences in the E/A between the groups (all $p>0.05$).

The peak velocity during early diastole at the mitral annulus (e') was significantly lower in the GC ($p=0.0013$) and GC+HF ($p=0.0050$) groups compared to the control group. The e' was significantly lower in the GC ($p<0001$) and GC+HF ($p=0.0001$) groups compared to the HF group. There were no significant differences in the e' between the GC and GC+HF groups ($p>0.05$).

The ratio of early to late mitral annulus flow velocity (e'/a') was significantly lower in the GC and GC+HF groups compared to the control ($p=0.0005$ and $p=0.02$ respectively) and the HF ($p<0.0001$ and $p=0.0005$ respectively) groups. There were no significant differences in the e'/a' between the control and HF groups ($p>0.05$). The index of LV filling pressures (E/e') was significantly higher in the GC and GC+HF groups compared to the control ($p<0.0001$ and $p=0.004$ respectively) and the HF ($p<0.0001$ and $p=0.02$ respectively) groups. There were no significant differences in the E/e' between the control and HF groups ($p>0.05$). There were no significant differences in late diastolic filling velocity (A) and the peak velocity during late diastole (a') between the groups (all $p>0.05$).

Table 4. 2 Left ventricular geometry, diastolic and systolic function of the control, GC, HF and GC+HF groups

	Control (n=10)	**GC (n=10)**	**HF (n=10)**	**GC+HF (n=10)**
Cardiac geometry				
Heart weight	1.47 ± 0.05	1.40 ± 0.04†	1.70 ± 0.05*	1.65 ± 0.05*#
Heart weight/body mass ×10^3	2.74 ± 0.08	2.73 ± 0.08	2.94 ± 0.08	3.04 ± 0.08 *#
LV weight	0.85 ± 0.03	0.85 ± 0.03†	0.98 ± 0.03	1.01± 0.03*#
LV weight/body mass ×10^3	1.58± 0.06	1.67 ± 0.06	1.69± 0.07	1.86 ± 0.07*
LV end-diastolic diameter (cm)	0.76± 0.02	0.72± 0.02	0.76± 0.02	0.68± 0.02*†
LV end-diastolic posterior wall thickness (cm)	0.18± 0.006	0.20±0.006	0.22±0.006	0.20±0.006
LV end-systolic diameter (cm)	0.41±0.02	0.34±0.02	0.41±0.02	0.39±0.02
LV end-systolic posterior wall thickness (cm)	0.31±0.01	0.34±0.01	0.31±0.01	0.31±0.01
Relative wall thickness	0.47± 0.02	0.59± 0.02*	0.54± 0.02	0.56± 0.02*
LV end-diastolic volume (ml)	0.98±0.05	0.83±0.05	0.97±0.05	0.71±0.05*†
LV end-systolic volume (ml)	0.18±0.03	0.16±0.03	0.18±0.03	0.18±0.03
Left ventricular systolic function				
Ejection fraction (%)	81.86±3.62	87.47±3.44	81.72±3.62	75.28±3.62
Endocardial fractional shortening (%)	45.94±2.01	45.79±2.01	45.81±2.01	52.80±1.91
Left ventricular diastolic function				
E (cm/s)	90.96±1.86	111.89±1.76*	100.32±1.86*#	93.95±1.86#
A (cm/s)	85.56±4.38	90.89±4.38	75.58±4.38	81.93±4.38
E/A	1.08±0.07	1.28±0.07	1.36±0.07*	1.16±0.07
e’ (cm/s)	5.99±0.17	5.04±0.16*†	6.29±0.17	5.13±0.17*†
a’ (cm/s)	5.70±0.28	6.49±0.26	5.48±0.28	5.86±0.28
e’/a’	1.07±0.05	0.78±0.05*†	1.16±0.05	0.86±0.05*†
E/e’	15.24±0.62	20.29±0.59*†	16.15±0.62	18.36±0.62*†

Data expressed as means ± SEM. *p<0.05 vs control; #p<0.05 vs GC; †p<0.05 vs HF. GC: glucocorticoids group; HF: high fructose group; GC+HF: glucocorticoids and high fructose group; LV: left ventricle.

4.9. Left ventricular total collagen content

Figure 4.5A shows significant collagen accumulation in the GC and the GC+HF groups compared to the control group as shown by the higher red staining of cardiac tissue sections visualised under bright-field microscopy. Figure 4.5B shows cardiac tissue sections under polarised light, where thin fibres appear green and thick collagen fibres appear red or orange. Figure 4.5C shows the total collagen content of the groups (mean ± SEM). The total collagen content was significantly higher in the GC (16.67 ± 1.81%; $p=0.001$) and GC+HF (21.50 ± 1.81%; $p<0.0001$) groups compared to the control group (6.05±1.81%). The total collagen content was significantly lower in the HF group (11.89 ± 1.81 %) compared to the GC+HF group (21.50 ± 1.81%; $p=0.003$). There were no significant differences in the total collagen content between the HF and control groups and between the GC and GC+HF groups (both $p>0.05$).

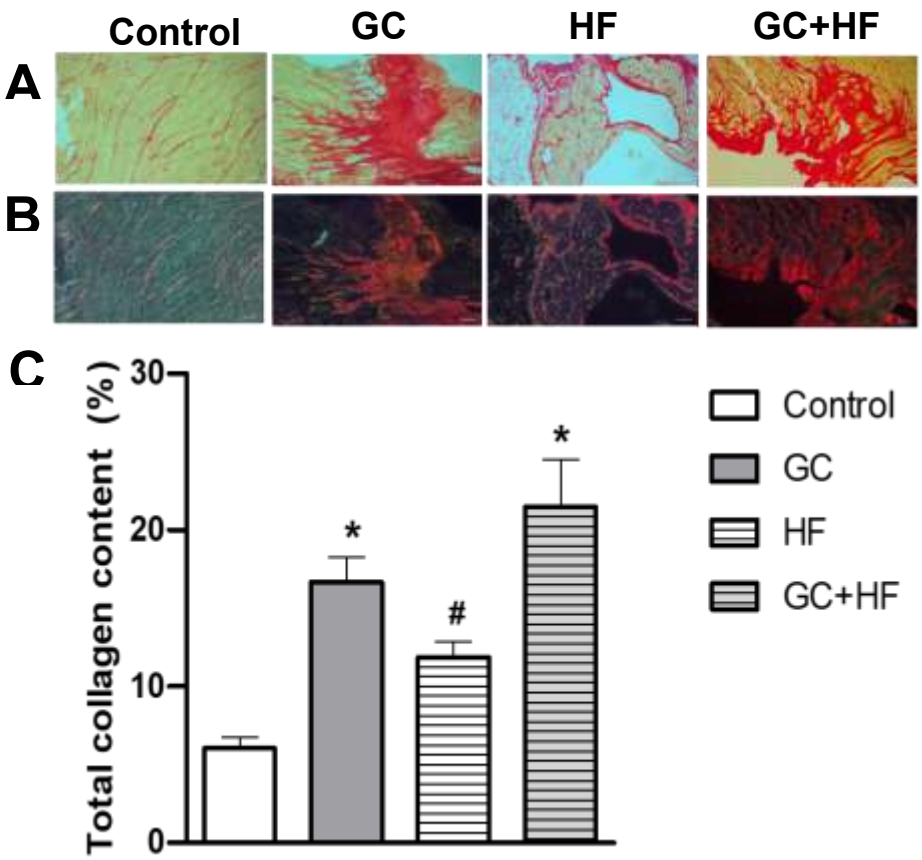

Figure 4.5 Total collagen content in left ventricular tissue of the control, GC, HF and GC+HF groups. Representative picrosirius red stained micrographs imaged at x100 magnification viewed in (A) bright-field and under (B) polarised light. (C) Total collagen content (% area fraction) calculated from picrosirius red stained LV sections (n=10 per group). *p<0.05 vs control; #p<0.05 vs GC+HF

Chapter 5: Discussion

In the present study, we investigated the effects of glucocorticoids on cardiac structure and function and determined whether the consumption of a high fructose diet (as a model of MetS) exacerbated the glucocorticoid-induced changes in LV morphology and function in male Sprague Dawley rats. The main findings of the study are that administration of glucocorticoids may have caused cardiac remodelling, impaired LV relaxation, and increased LV filling pressures. Glucocorticoid-induced diastolic dysfunction occurred independent of changes in blood pressure. The presence of MetS was confirmed in rats consuming a high fructose diet as indicated by the higher body mass, visceral fat mass, circulating triglycerides, hs-CRP and insulin concentrations, and HOMA-IR scores. Glucocorticoid-induced cardiac remodelling was enhanced in the presence of MetS. However, glucocorticoid-induced diastolic dysfunction was not augmented in rats with MetS.

5.1. The effects of glucocorticoids on cardiac remodelling

In the present study, the administration of glucocorticoids resulted in significant alterations to cardiac geometry. Glucocorticoid treatment resulted in a higher the relative wall thickness compared to the control group. However, the heart and LV weights were similar between the control and the GC groups. These results suggest that the administration of glucocorticoids may have caused concentric remodelling. Our results are consistent with previous studies reporting that glucocorticoid treatment increased the relative wall thickness without increasing the LV mass index in normotensive patients (Muiesan *et al.,* 2003; Toja *et al.,* 2012). However, in other studies, increases in relative wall thickness were accompanied by greater LV mass indexed to body mass (Oui *et al.*, 2015; Tanaka *et al.*, 2021). During the concentric remodelling process, relative wall thickness increases prior to detectable increases in LV mass (Gaasch and Zile, 2011). It is therefore likely that administration of glucocorticoids for a longer period may have led to more significant changes in the LV mass and that long term glucocorticoid treatment may result in the development of concentric hypertrophy (Tanaka *et al.*, 2021).

The molecular mechanisms whereby glucocorticoids promote cardiac remodelling have not been completely elucidated. However, studies have reported that glucocorticoid-induced hypertension may gradually cause pressure overload leading to heart failure through LV remodelling rather than the attenuation of myocardial function (Tanaka *et al.,* 2021; Kamenický *et al.,* 2014). Kamenický *et al.,* (2014) demonstrated that relative wall thickness was associated with increased systolic blood pressure in patients with Cushing's syndrome compared to controls (Kamenický *et al.*, 2014). However, other studies have shown that relative wall thickness, LV mass index or geometry are not related to blood pressure in patients with Cushing's syndrome (Fallo *et al.*, 1994; Avenatti *et al.*, 2017) or with the administration of exogenous glucocorticoids (Smith *et al.*, 2004; Oui *et al.*, 2015; Takano *et al.*, 2015; Tanaka *et al.*, 2021). Endogenous glucocorticoids are well-accepted to cause increases in systolic blood pressure, mainly through the co-activation of the mineralocorticoid receptor in the kidney (Whitworth *et al*., 2000; Isidori *et al.*, 2015). However, the hypertensive response to synthetic exogenous glucocorticoids is considerably decreased, through modification of certain functional groups to reduce mineralocorticoid co-activation (Grossmann *et al.*, 2004). Therefore, recent studies suggest that glucocorticoids have a limited potential to induce hypertension directly (Panoulas *et al.*, 2008; Yasir *et al*., 2022). Individuals prescribed long term glucocorticoids are suggested to be at an increased risk of hypertension, largely through glucocorticoid-induced weight gain, visceral adiposity and lipodystrophy (Fardet *et al.*, 2007; Fardet *et al*., 2012). Moreover, threshold dose-response patterns have been reported following glucocorticoid administration, where hypertensive responses were noted only at increased dosages (Panoulas *et al.*, 2008; Yasir *et al*., 2022). In the present study, blood pressures were similar in the control and GC groups, therefore our results suggest that concentric remodelling may have been caused by mechanisms other than pressure overload in the heart. Taken together, these findings indicate that the effects of hypertension on concentric remodelling with the administration of glucocorticoids remain to be fully elucidated and might depend, at least in part, on the dose or duration of glucocorticoid administration (Tanaka *et al.*, 2021).

In the present study, the administration of glucocorticoids resulted in a higher LV total collagen content (a marker of cardiac fibrosis) in addition to the increased relative wall thickness. Indeed, increased myocardial fibrosis has been linked to LV concentric remodeling (Gaasch and Zile, 2011; Konstam *et al.*, 2011). In clinical studies, Yui *et al.,* (2012) showed increased collagen deposition in normotensive patients with Cushing's syndrome (Yiu *et al.*, 2012). The authors reported that LV mass and blood pressure were not correlated to the increased myocardial fibrosis in these patients, which suggests that glucocorticoid-induced myocardial fibrosis significantly contributes to the development of cardiac concentric remodelling, independent of LV hypertrophy (Yiu *et al.*, 2012).

The mechanism whereby exogenous glucocorticoid treatment promotes fibrosis is specific to heart tissue (Oakley and Cidlowski, 2015). As the circulating concentration of glucocorticoids is considerably increased than aldosterone, mineralocorticoid receptors in cardiomyocytes are primarily activated by glucocorticoids (Jia *et al*., 2017). In mineralocorticoid target tissues, 11β-HSD2 inactivates glucocorticoids, permitting aldosterone to compete for binding of the mineralocorticoid receptor and exert its effect (Chapman *et al*., 2013). However, in the heart, the expression of 11β-HSD1 is particularly low (Gray *et al.*, 2017) and consequently, the active glucocorticoid can co-activate the mineralocorticoid receptor (Whitworth *et al*., 2000; Isidori *et al.*, 2015). Under pathological conditions (excess glucocorticoid levels), experimental studies have shown that glucocorticoids occupy mineralocorticoid receptors in cardiomyocytes (Young *et al*., 2007; Mihailidou *et al.*, 2009), resulting in the accumulation of ROS (Funder, 2007; Mihailidou *et al.*, 2009), which can, in turn, induce fibrosis in cardiac tissue (Young *et al*., 2007; Mihailidou *et al.*, 2009; Omori *et al.*, 2014; Jia *et al*., 2017). In the present study, relative wall thickness was associated with increased LV total collagen content (r=0.54; p=0.0004; data not shown). This association remained materially unaltered even after adjusting for systolic and diastolic blood pressure (partial r=0.54; p=0.0005: data not shown). Therefore, taken together, our results show

that the administration of glucocorticoids increased myocardial fibrosis, which may have affected the myocardial remodelling processes, independent of elevated blood pressure. Future molecular studies should investigate whether the administration of glucocorticoids can promote myocardial fibrosis and remodelling through the activation of mineralocorticoid receptors in cardiomyocytes.

5.2. The effects of glucocorticoids on diastolic function

In the present study, the administration of glucocorticoids impaired diastolic function, as measured by pulse Doppler and Tissue Doppler Imaging. The administration of glucocorticoids impaired LV relaxation as indexed by increased E, reduced lateral e' and e'/a'. Glucocorticoid therapy further increased LV filling pressures as indexed by the increased E/e', compared to the control group. In addition, lateral e', e'/a', and the E/e' were all associated with increased total collagen content ($r=-0.44$ $p=0.0058$, $r=-0.41$ $p=0.01$ and $r=0.45$ $p=0.0045$ respectively: data not shown). While the measurement of trans-mitral inflow velocity is corroborated using pulsed wave Doppler, Tissue Doppler Imaging measures are more sensitive (Mitter *et al*., 2017).

Our results are in agreement with other clinical and experimental studies that reported diastolic dysfunction following the administration of glucocorticoids (Toja *et al*., 2012; Hattori *et al*., 2013; Ohtake *et al*., 2014; de Salvi Guimarães *et al*., 2017; Tanaka *et al*., 2021). Administration of glucocorticoids may impair the active processes of LV relaxation by decreasing the phosphorylation of phospholamban and by reducing Na^{+}/Ca^{2+} exchanger protein levels (de Salvi Guimarães *et al.*, 2017), which impairs SERCA activity and leads to abnormal active LV relaxation. In addition, glucocorticoid administration impairs passive LV relaxation by promoting excessive collagen production and deposition through alterations in MMP activity, that in turn, increases myocardial fibrosis (Bal *et al.,* 2009; Roy *et al.,* 2009; Tanaka *et al*., 2021). Glucocorticoid-induced impaired LV active and passive processes may therefore lead to increased LV filling pressures.

In the current study, the E/A ratio in the GC group was similar to the control group despite increased LV filling pressures. This suggests that a pseudonormal filling pattern may have been present, as increased filling pressures may have resulted in a compensatory increase in early diastolic filling (E). Therefore, in the present study the administration of glucocorticoids could have caused moderate to severe diastolic dysfunction.

The mechanisms by which glucocorticoid administration leads to impaired LV relaxation and increased filling pressures are currently not well elucidated. However, in addition to activating mineralocorticoid receptors within the myocardium, which may lead to myocardial stiffness and diastolic dysfunction, excess glucocorticoid administration may impair diastolic function through increased lipogenesis. In the present study, the administration of glucocorticoids increased circulating triglyceride and cholesterol levels, despite having no effect on insulin signalling. Indeed, lipid accumulation is now recognised as one of the most important metabolic abnormalities that may contribute to the development of diastolic dysfunction (DeMarco *et al.*, 2013). Increased than normal circulating triglyceride and cholesterol levels may result in dyslipidaemia and ectopic deposition of lipids in the myocardium, which may increase cardiac steatosis (Lopaschuk *et al*., 2007; Zhang and Ren, 2011). Cardiac steatosis may alter substrate metabolism, characterised by greater fatty acid β-oxidation, enhanced ROS production, and decreased oxygen efficiency (Lopaschuk *et al.,* 2010). This may lead to reduced NO bioavailability, decreased SERCA-mediated calcium uptake into the sarcoplasmic reticulum, and stiffening of cardiomyocytes (Kohr *et al.,* 2009). Therefore, it is likely that the impaired LV relaxation and increased filling pressures in the current study may also be due to increased lipogenesis with the administration of glucocorticoids. Future molecular studies will need to determine whether the administration of glucocorticoids cause lipid over storage (steatosis), ROS generation in cardiomyocytes, and subsequent development of diastolic dysfunction.

5.3. The high fructose diet as a model of metabolic syndrome

In the present study, a ten-week high fructose diet increased the body mass, visceral fat mass, and visceral fat mass relative to body mass, compared to the control group. In addition, the high fructose diet increased circulating triglyceride and insulin concentrations, and caused insulin resistance, as measured by the HOMA-IR score. These results suggest that the consumption of the high fructose diet promotes MetS through the development of three pathological characteristics, namely: increased visceral adiposity, dyslipidaemia, and insulin resistance. Our results confirm numerous previous studies that have reported increased visceral adiposity, dyslipidaemia and insulin resistance following the consumption of a high-fructose diet (Jurgens *et al.,* 2005; Bocarsley *et al.,* 2010; Sadowska and Rygielska, 2019; Fakhoury-Sayegh, 2019; Kitagawa *et al.,* 2020).

Growing evidence suggests that a high fructose diet induces MetS by causing rapid *de novo* lipogenesis, hepatic lipid accumulation, and visceral obesity, which all contribute to reduced sensitivity to insulin (Lanaspa *et al.*, 2012; Crescenzo *et al.*, 2013; Sangüesa *et al.*, 2017). Fructose is absorbed from the intestines into the portal blood through facilitated diffusion by the glucose transporter 5 (GLUT5) on the luminal side and by glucose transporter 2 (GLUT2) on the basolateral side (Douard and Ferraris, 2008; Tappy and Le, 2010). This route of absorption causes increased fructose uptake by the liver via specific hexose transporters (Rutledge and Adeli, 2007; Softic *et al.*, 2020). Fructose is phosphorylated by fructokinase to form fructose 1-phosphate which then undergoes hydrolysis to form three-carbon molecules, including dihydroxyacetone phosphate, glyceraldehyde and glycerol 3-phosphate (Rutledge and Adeli, 2007). These three-carbon molecules can then be converted to glucose through gluconeogenesis, but more commonly they are used for the synthesis of glycerol and fatty acids, which generate triglycerides through esterification (Rutledge and Adeli, 2007; Taskinen *et al*., 2019; Softic *et al.,* 2020). As the pathways involved in the conversion of fructose to triglycerides

are largely unregulated, the consumption of a high fructose diet commonly results in excessive triglyceride synthesis and adiposity (Rutledge and Adeli, 2007; Taskinen *et al*., 2019; Softic *et al.,* 2020). The mechanisms whereby fructose consumption alters adipose content have been described in the literature.

Fructose consumption also increases hepatic *de novo* lipogenesis, which in turn upregulates the production and secretion of very-low-density lipoproteins (VLDLs) (Rutledge and Adeli, 2007; Taskinen *et al*., 2019; Softic *et al.,* 2020). Circulating VLDLs are then hydrolysed and resynthesised as triglycerides within adipose tissue (Rutledge and Adeli, 2007; Taskinen *et al*., 2019; Softic *et al.,* 2020). Increased consumption of fructose not only increases triglyceride and VLDL production, but also increases peroxisome proliferator-activated receptor γ (PPARγ) expression, which induces adipogenesis (Zhang *et al.*, 2017). Furthermore, high fructose consumption stimulates glucocorticoid activation by increasing 11β-HSD1 expression in adipocytes (London and Castonguay, 2011). Increased, 11β-HSD1 activity not only converts 11-dehydrocorticosterone to the active corticosterone form, but also promotes white adipose tissue expansion and accumulation of fat in visceral adipose tissue (London and Castonguay, 2011; Prince *et al.*, 2017). High-fructose consumption-induced glucocorticoid activation further stimulates pro-adipogenic and pro-lipogenic factors such as PPARy and sterol response element binding proteins (SREBP-1) (Galitzky and Bouloumié, 2013) which augments fat accumulation in visceral adipose tissue.

With increasing visceral adiposity, high levels of non-esterified fatty acids may be taken up by the liver through the portal system, where they may be stored as triglycerides (Stanhope and Havel, 2008). Accumulation of triglycerides in the liver interferes with the insulin signalling pathway and may cause hepatic insulin resistance (Seppälä-Lindroos *et al.*, 2002; Morino *et al*., 2006). Hepatic lipid accumulation stimulates the production of diacylglycerol (DAG), which activates novel protein kinase C (nPKC) (Newton, 2003). Activation of nPKC is associated with decreased insulin

receptor substrate 1 (IRS-1) tyrosine phosphorylation (Itani *et al.*, 2002; Yu *et al.*, 2002; Dey *et al.*, 2006), which impairs insulin signalling. In addition, activation of nPKC can cause serine phosphorylation of IRS-1 and IRS-2 via the phosphorylation of downstream signalling molecules, extracellular signal-regulated kinase (ERK)1/2 and c-JUN NH_2-terminal kinase (JNK), which may also impair insulin signalling and cause insulin resistance (Strack *et al.*, 1997; Gao *et al.*, 2004). The impaired insulin signalling in the liver leads to decreased glycogenolysis, glycogen synthesis and increased gluconeogenesis (Stanhope and Havel, 2008), which in turn results in insulin secretion as a compensatory response (Stanhope and Havel, 2008). Furthermore, increased circulating FFA and triglycerides can cause DAG production, nPKC activation and impaire insulin signalling in skeletal muscle (Stanhope and Havel, 2008). This results in whole-body insulin resistance which may lead to the development of T2DM.

In the present study, the consumption of a high fructose di*et al*so increased the circulating hs-CRP concentrations compared to the control group. Indeed, a high-fructose diet leads to hypertriglyceridaemia and obesity which can cause oxidative stress and the production of proinflammatory cytokines including TNF-α, IL-6 and IL-1β (Wei and Pagliassotti, 2004). These proinflammatory cytokines, in turn, stimulate the production of CRP in the liver, a sensitive and reproducible marker of inflammation (Halcox *et al.*, 2014). The increased production of CRP stimulates JNK and ERK1/2 activity which increases the serine phosphorylation of IRS-1 leading to impaired insulin signalling and consequently insulin resistance (D'Alessandro's *et al.,* 2007; Rutledge and Adeli, 2007; Softic *et al.,* 2020). A chronic inflammatory state is indeed characteristic of insulin resistance. Therefore, taken together, our results show that consumption of a high fructose diet induces MetS by increasing visceral adiposity and by causing hypertriglyceridaemia, and insulin resistance. Future studies should identify the exact molecular mechanisms whereby a high fructose diet induces MetS.

5.4. The effects of glucocorticoids on cardiac remodelling and diastolic function in a model of MetS

In the present study, the administration of glucocorticoids in a model of MetS increased the heart and LV weights indexed to body mass, compared to the control and the GC groups. The increased relative heart weights in the GC+HF group was accompanied by an increased relative wall thickness that was similar to the GC group. However, the administration of glucocorticoids in a model of MetS reduced LV end-diastolic volume and the end-diastolic diameter compared to the control group. These results suggest that the administration of glucocorticoids in a model of MetS may have caused concentric hypertrophy. While the administration of glucocorticoids may have caused increased collagen deposition and fibrosis in rats fed a normal diet, the concomitant presence of MetS certainly amplified cardiac hypertrophic responses. Therefore, it is likely that the cumulative effects of glucocorticoids and MetS largely drive cardiac remodelling through compounding metabolic derangements (Lelis *et al.*, 2020). Indeed, in the present study glucose tolerance was further impaired in the GC+HF group. Studies conducted on the effects of glucocorticoids on cardiac structure and function in models of MetS are very limited. Nonetheless, clinical studies have reported concentric hypertrophy in patients with Cushing's syndrome who have adverse metabolic outcomes (increased visceral adiposity, dyslipidaemia and impaired glucose tolerance) (Takagi *et al.,* 2009; Toja *et al.,* 2012; Avenatti *et al.,* 2017). In addition, these studies reported that remission of hypercortisolism reversed abnormalities in LV mass measurements and resolved concentric hypertrophy in a proportion of patients (Pereira *et al.,* 2010; Toja *et al.,* 2012). The administration of glucocorticoids in dietary animal models of MetS has been shown to reduce inflammation, but also to accelerate metabolic abnormalities, whereby visceral adiposity, hypertriglyceridaemia and insulin resistance occurred within a shorter time compared to the dietary models alone (Giddings *et al.*,1985; Gounarides *et al.*, 2008; Huang *et al.*, 2016; Tsai *et al.*, 2019). However, to the best of our knowledge, this is the first study to report that glucocorticoids induce concentric hypertrophy in an *in vivo* dietary model of MetS.

The mechanisms whereby administration of glucocorticoids cause concentric hypertrophy in models of MetS are yet to be investigated. However, clinical studies have reported associations between increased cardiac fibrosis, abnormal glucose metabolism and lipogenesis with concentric hypertrophy (Pereira *et al.*, 2010). This, therefore, suggests that metabolic alterations that may have been caused by glucocorticoids, including hyperglycaemia and steatosis within the heart, induces structural and fibrotic modifications that may also impair cardiac function (Takagi *et al.*, 2009; Pereira *et al.*, 2010). In addition, glucocorticoids not only bring about adverse metabolic effects, but they may directly impact cardiac signalling negatively. Takagi *et al.,* (2009) reported that serum cortisol was independently associated with LV mass index in patients with Cushing's syndrome (Takagi *et al.*, 2009). Indeed, studies have reported that endogenous cortisol may be involved in the development of LV hypertrophy by increasing the entry of cardiomyocytes into the cell cycle thereby leading to cardiomyocyte proliferation (Giraud *et al.*, 2006) and by increasing the expression of angiotensinogen mRNA to promote cardiomyocyte hypertrophy (Lumbers *et al.*, 2005). The findings of the study cited above suggest that abnormal cortisol regulation may be an important risk factor for cardiac hypertrophy in patients with Cushing's syndrome. Future molecular studies need to investigate the molecular mechanisms associated with glucocorticoid-induced concentric hypertrophy in a population with established metabolic alterations. In addition, future studies should further investigate whether employment of exogenous glucocorticoids exert the same effects.

In the present study, although the administration of glucocorticoids in a model of MetS may have caused concentric hypertrophy, glucocorticoid-induced diastolic dysfunction was not exacerbated by exposure to MetS, as there were no significant differences in the lateral e', e'/a' and E/e' between the GC and GC+HF groups. Clinical studies have reported that glucocorticoids impair LV relaxation and increase LV filling pressures in patients with Cushing's syndrome and that the effects are reversed following Cushing's syndrome treatment (Pereira *et al.,* 2010; Toja *et al.,*

2012). However, these glucocorticoid-induced diastolic impairments have not been shown in pre-clinical models of MetS. Therefore, to the best of our knowledge, this is the first study to report that glucocorticoid-induced diastolic dysfunction is not worsened in a diet-induced *in vivo* model of MetS.

This study has important implications. We found that glucocorticoids induced moderate to severe diastolic dysfunction, independent of the metabolic profile. Although no further impairments in diastolic function were noted in the presence of MetS, moderate to severe diastolic dysfunction was already present following employment of glucocorticoids. However, the presence of MetS components significantly worsened cardiac remodelling. While glucocorticoid administration may have caused diastolic dysfunction, MetS may exert severe negative effects on the extracellular matrix that, when combined with glucocorticoids, may result in LV hypertrophy. It is widely accepted that the presence of LV hypertrophy predicts cardiovascular morbidity and mortality, even after adjustment for cardiovascular risk factors including age, obesity, smoking, dyslipidemia, diabetes and blood pressure (Bornstein *et al*., 2022). While the adverse effects of glucocorticoids have been extensively reported, the cumulatively increased risk for CVD in patients with MetS receiving glucocorticoid treatment, may not be as apparent.

We show that rats receiving glucocorticoid treatment in the presence of MetS developed significant reductions in LV end-diastolic volume. These changes in LV end-diastolic volume are consistent with impaired diastolic filling and a fibrotic, hypertrophic LV response. The LV hypertrophy noted in GC+HF rats increased the myocardial oxygen demand, in hearts that were already metabolically compromised. It is therefore likely that in this study, although the diastolic dysfunction was not further impaired, the risk for systolic dysfunction associated with overt heart failure, was significantly increased. Although LV ejection fraction is one the most commonly used indices of systolic function in clinical practice, it is dependent on chamber size and pressure (Borlaug *et al.*, 2009). LV ejection fraction is therefore a better indicator of ventricular-arterial

coupling, rather than contractility (Borlaug *et al.*, 2009). Thus, the use of more sensitive echocardiographic parameters (Speckle Tracking Imaging (STE)) in this study, may have detected early LV systolic dysfunction (Dandel *et al.*, 2009), which can yield important information for clinical risk stratification and management. In this regard, studies have reported that subclinical LV systolic dysfunction is present in patients with Cushing's syndrome (Pereira *et al.*, 2010; Yiu *et al.*, 2012). Compared to controls, patients with Cushing's syndrome showed concentric LV remodelling, while myocardial deformation properties (LV circumferential and longitudinal strain and strain rate) where significantly impaired, indicating the presence of subclinical LV systolic dysfunction (Pereira *et al.*, 2010; Yiu *et al.*, 2012).

Previous research has suggested that myocardial systolic function may be compromised before any noticeable changes in systolic chamber function (ejection fraction), as the presence of concentric remodelling may aid in maintaining chamber function (Shah, 2013). In this regard, studies have reported that ejection fraction is preserved by the hypertrophied myocardial wall in HFpEF, while concentric cardiac remodelling increases myocardial tissue stress and strain (Yiu *et al.*, 2012; Adeniran *et al.*, 2015). Therefore, in the present study, the concentric hypertrophy may have maintained the ejection fraction within adequate ranges. However, it is unknown whether myocardial strain was affected which may have caused a reduction in systolic performance. Future studies should therefore include more sensitive measures of systolic function to elucidate the role of glucocorticoids on early systolic performance in a model of MetS.

5.5. Limitations

The present study has further limitations. The current study made use of methylprednisolone which exhibits stronger glucocorticoid anti-inflammatory activity (5x more potent) than cortisol (Sardana and Sachdeva, 2022). While modifications to the synthetic methylprednisolone induces greater beneficial anti-inflammatory effects, it is not certain to which extent it affects cardiac structure and function, when compared to endogenous cortisol in patients with Cushing's

syndrome (who have high circulating cortisol levels). However, several studies have reported myocardial remodelling and diastolic dysfunction with similar dosages of methylprednisolone in other experimental models (Oui *et al.,* 2015; Tanaka *et al*., 2021). In addition, other studies have reported myocardial remodelling and diastolic dysfunction independent of cortisol levels in patients with Cushing's syndrome, which suggests that the duration of exposure to high levels of glucocorticoids might be more significant than the magnitude of circulating hormone concentrations (Muiesan *et al.*, 2003). While intraperitoneal injections have been shown to cause less stress compared to other routes of administration, there is still a possibility that it could have influenced the results, particularly considering that the control group did not receive any injections. To address this concern and maintain consistency in the experimental procedures, future experiments will involve administering saline intraperitoneal injections to the control group. This approach will minimise the potential confounding effects of the injection procedure itself. Furthermore, to evaluate the potential impact of stress on the results, corticosterone concentrations will be measured in all groups to ensure that any observed effects are indeed attributable to the specific treatment conditions rather than stress-related factors.

In the current study, we used a non-invasive approach rather than catheter-based systems to measure blood pressure. Future studies should assess blood pressure using invasive catheter-based methods as these methods are more sensitive and provide direct and continuous measurements of arterial pressure. In addition, echocardiography was only performed once at termination. Future studies should conduct longitudinal echocardiographic assessments with multiple time points to provide a more comprehensive understanding of the temporal changes in echocardiographic parameters. Furthermore, although the present study did not show any changes in LV systolic function using LV ejection phase indices (LV fractional shortening and ejection fraction), there may have been pre-clinical systolic function impairments (myocardial deformation and motion) with the administration of glucocorticoids. Future studies should use two-dimensional Speckle Tracking Echocardiography, since it is considered to be a more sensitive

index of myocardial systolic performance and may provide additional information on LV myocardial deformation and motion (Dandel *et al.*, 2009). Moreover,the effects of glucocorticoids on cardiac morphology and function were assessed in male rats. Future studies should determine whether the administration of glucocorticoids impair cardiac structure and function in female rats.

5.6. Conclusion

In conclusion, the evidence presented in this dissertation adds to our understanding of the role of glucocorticoids on cardiac structure and function in a model of MetS. The present study showed that the administration of glucocorticoids may have caused concentric remodelling and impaired LV diastolic function, independent of hypertension and LV hypertrophy. These findings add to the evidence that glucocorticoids can mediate myocardial remodelling and diastolic dysfunction, which may lead to HFpEF. Furthermore, we show that glucocorticoid-induced diastolic dysfunction was not exacerbated by exposure to MetS, however cardiac remodelling did progress to concentric hypertrophy. Taken together, patients with MetS receiving glucocorticoid therapy may be at an increased risk for developing HFpEF. Given the high prevalence of obesity, insulin resistance, dyslipidaemia, T2DM and MetS, regular echocardiographic screening is recommended following the administration of glucocorticoids, particularly in patients with potential metabolic alterations. Future studies should elucidate the molecular mechanisms whereby glucocorticoids impair cardiac function in patients with MetS.

References

Adeniran, I., MacIver, D.H., Hancox, J.C. and Zhang, H. (2015) 'Abnormal calcium homeostasis in heart failure with preserved ejection fraction is related to both reduced contractile function and incomplete relaxation: an electromechanically detailed biophysical modeling study', *Frontiers in physiology*, 6(MAR). Available at: https://doi.org/10.3389/FPHYS.2015.00078.

Amihăesei, I.C. and Chelaru, L. (2014) 'Metabolic syndrome a widespread threatening condition; risk factors, diagnostic criteria, therapeutic options, prevention and controversies: an overview.', *Revista medico-chirurgicala a Societatii de Medici si Naturalisti din Iasi* [Preprint].

Antoni, F.A. (1986) 'Hypothalamic control of adrenocorticotropin secretion: advances since the discovery of 41-residue corticotropin-releasing factor.', *Endocrine Reviews*, 7(4), pp. 351–378. Available at: https://doi.org/10.1210/EDRV-7-4-351.

Asmat, U., Abad, K. and Ismail, K. (2016) 'Diabetes mellitus and oxidative stress-A concise review', *Saudi pharmaceutical journal : SPJ : the official publication of the Saudi Pharmaceutical Society*, 24(5), pp. 547–553. Available at: https://doi.org/10.1016/J.JSPS.2015.03.013.

Avenatti, E., Rebellato, A., Iannaccone, A., Battocchio, M., Dassie, F., Veglio, F., Milan, A. and Fallo, F. (2017) 'Left ventricular geometry and 24-h blood pressure profile in Cushing's syndrome', *Endocrine*, 55(2), pp. 547–554. Available at: https://doi.org/10.1007/s12020-016-0986-6.

Bal, M.P., de Vries, W.B., Steendijk, P., Homoet-Van Der Kraak, P., van der Leij, F.R., Baan, J. and van Oosterhout, M.F.M. (2009) *Histopathological Changes of the Heart After Neonatal Dexamethasone Treatment: Studies in 4-, 8-, and 50-Week-Old Rats*, *Pediatr Res*.

Bantle, J.P. (2006) 'Is fructose the optimal low glycemic index sweetener?', *Nestle Nutrition workshop series. Clinical & performance programme*, 11. Available at: https://doi.org/10.1159/000094427.

Belete, R., Ataro, Z., Abdu, A. and Sheleme, M. (2021) 'Global prevalence of metabolic syndrome among patients with type I diabetes mellitus: a systematic review and meta-analysis', *Diabetology and Metabolic Syndrome*. BioMed Central Ltd. Available at: https://doi.org/10.1186/s13098-021-00641-8.

Belfki, H., Ali, S. ben, Aounallah-Skhiri, H., Traissac, P., Bougatef, S., Maire, B., Delpeuch, F., Achour, N. and ben Romdhane, H. (2013) 'Prevalence and determinants of the metabolic syndrome among Tunisian adults: Results of the Transition and Health Impact in North Africa (TAHINA) project', *Public Health Nutrition*, 16(4), pp. 582–590. Available at: https://doi.org/10.1017/S1368980012003291.

Beltrán-Sánchez, H., Harhay, M.O., Harhay, M.M. and McElligott, S. (2013) 'Prevalence and trends of metabolic syndrome in the adult U.S. population, 1999-2010', *Journal of the American College of Cardiology*, 62(8), pp. 697–703. Available at: https://doi.org/10.1016/J.JACC.2013.05.064.

Bers, D.M. (2008) 'Calcium cycling and signaling in cardiac myocytes', *Annual review of physiology*, 70, pp. 23–49. Available at: https://doi.org/10.1146/ANNUREV.PHYSIOL.70.113006.100455.

Bhanushali, C.J., Kumar, K., Wutoh, A.K., Karavatas, S., Habib, M.J., Daniel, M. and Lee, E. (2013) 'Association between Lifestyle Factors and Metabolic Syndrome among African Americans in the United States', *Journal of nutrition and metabolism*, 2013. Available at: https://doi.org/10.1155/2013/516475.

Biesiadecki, B.J., Davis, J.P., Ziolo, M.T. and Janssen, P.M.L. (2014) 'Tri-modal regulation of cardiac muscle relaxation; intracellular calcium decline, thin filament deactivation, and cross-bridge cycling kinetics', *Biophysical Reviews*, 6(3–4), pp. 273–289. Available at: https://doi.org/10.1007/S12551-014-0143-5.

Bocarsly, M.E., Powell, E.S., Avena, N.M. and Hoebel, B.G. (2010) 'High-fructose corn syrup causes characteristics of obesity in rats: Increased body weight, body fat and triglyceride levels', *Pharmacology Biochemistry and Behavior*, 97(1), pp. 101–106. Available at: https://doi.org/10.1016/j.pbb.2010.02.012.

Bodhini, D. and Mohan, V. (2018) 'Mediators of insulin resistance & cardiometabolic risk: Newer insights', *The Indian journal of medical research*, 148(2), pp. 127–129. Available at: https://doi.org/10.4103/IJMR.IJMR_969_18.

Borges, M.H.S., DiNinno, F.B. and Lengyel, A.M.J. (1997) 'Different effects of growth hormone releasing peptide (GHRP-6) and GH-releasing hormone on GH release in endogenous and exogenous hypercortisolism', *Clinical Endocrinology*, 46(6), pp. 713–718. Available at: https://doi.org/10.1046/J.1365-2265.1997.1981008.X.

Borghetti, G., von Lewinski, D., Eaton, D.M., Sourij, H., Houser, S.R. and Wallner, M. (2018) 'Diabetic cardiomyopathy: Current and future therapies. Beyond glycemic control', *Frontiers in Physiology*. Frontiers Media S.A. Available at: https://doi.org/10.3389/fphys.2018.01514.

Borlaug, B.A. and Kass, D.A. (2006) 'Mechanisms of diastolic dysfunction in heart failure', *Trends in cardiovascular medicine*, 16(8), pp. 273–279. Available at: https://doi.org/10.1016/J.TCM.2006.05.003.

Borlaug, B.A., Lam, C.S.P., Roger, V.L., Rodeheffer, R.J. and Redfield, M.M. (2009) 'Contractility and ventricular systolic stiffening in hypertensive heart disease insights into the

pathogenesis of heart failure with preserved ejection fraction', *Journal of the American College of Cardiology*, 54(5), pp. 410–418. Available at: https://doi.org/10.1016/J.JACC.2009.05.013.

Borlaug, B.A. and Paulus, W.J. (2011) 'Heart failure with preserved ejection fraction: pathophysiology, diagnosis, and treatment', *European heart journal*, 32(6), pp. 670–679. Available at: https://doi.org/10.1093/EURHEARTJ/EHQ426.

Bornstein, A.B., Rao, S.S. and Marwaha, K. (2022) 'Left Ventricular Hypertrophy', *StatPearls [Internet]. Treasure Island (FL)* [Preprint]. Available at: https://www.ncbi.nlm.nih.gov/books/NBK557534/ (Accessed: 17 January 2023).

de Bosscher, K. and Haegeman, G. (2009) 'Minireview: Latest Perspectives on Antiinflammatory Actions of Glucocorticoids', *Molecular Endocrinology*, 23(3), p. 281. Available at: https://doi.org/10.1210/ME.2008-0283.

Boudina, S., Sena, S., Theobald, H., Sheng, X., Wright, J.J., Xia, X.H., Aziz, S., Johnson, J.I., Bugger, H., Zaha, V.G. and Abel, E.D. (2007) 'Mitochondrial energetics in the heart in obesity-related diabetes: direct evidence for increased uncoupled respiration and activation of uncoupling proteins', *Diabetes*, 56(10), pp. 2457–2466. Available at: https://doi.org/10.2337/DB07-0481.

Brower, G.L., Gardner, J.D., Forman, M.F., Murray, D.B., Voloshenyuk, T., Levick, S.P. and Janicki, J.S. (2006) 'The relationship between myocardial extracellular matrix remodeling and ventricular function', *European journal of cardio-thoracic surgery : official journal of the European Association for Cardio-thoracic Surgery*, 30(4), pp. 604–610. Available at: https://doi.org/10.1016/J.EJCTS.2006.07.006.

Brown, A.E. and Walker, M. (2016) ‘Genetics of Insulin Resistance and the Metabolic Syndrome’, *Current cardiology reports*, 18(8). Available at: https://doi.org/10.1007/S11886-016-0755-4.

Burkart, E.M., Sambandam, N., Han, X., Gross, R.W., Courtois, M., Gierasch, C.M., Shoghi, K., Welch, M.J. and Kelly, D.P. (2007) ‘Nuclear receptors PPARbeta/delta and PPARalpha direct distinct metabolic regulatory programs in the mouse heart’, *The Journal of clinical investigation*, 117(12), pp. 3930–3939. Available at: https://doi.org/10.1172/JCI32578.

Cameron, A.J., Magliano, D.J., Zimmet, P.Z., Welborn, T. and Shaw, J.E. (2007) ‘The Metabolic Syndrome in Australia: Prevalence using four definitions’, *Diabetes Research and Clinical Practice*, 77(3), pp. 471–478. Available at: https://doi.org/10.1016/J.DIABRES.2007.02.002.

Čelutkienė, J., Jakštaitė, A.M., Badarienė, J., Solovjova, S., Slivovskaja, I., Navickas, R., Kazėnaitė, E., Rinkūnienė, E., Čypienė, A., Misiūra, J., Ryliškytė, L., Laucevičius, A. and Coats, A. (2018) ‘Detection of Early Heart Failure with Preserved Ejection Fraction (HFpEF) in Metabolic Syndrome Patients Detected as Part of a National Screening Programme in Middle Aged Subjects’, *International Cardiovascular Forum Journal*, 13. Available at: https://doi.org/10.17987/ICFJ.V13I0.550.

Chakrabarti, P., Kim, J.Y., Singh, M., Shin, Y.-K., Kim, J., Kumbrink, J., Wu, Y., Lee, M.-J., Kirsch, K.H., Fried, S.K. and Kandror, K. v. (2013) ‘Insulin inhibits lipolysis in adipocytes via the evolutionarily conserved mTORC1-Egr1-ATGL-mediated pathway’, *Molecular and cellular biology*, 33(18), pp. 3659–3666. Available at: https://doi.org/10.1128/MCB.01584-12.

Chaney, M.A. (2002) ‘Corticosteroids and cardiopulmonary bypass : a review of clinical investigations’, *Chest*, 121(3), pp. 921–931. Available at: https://doi.org/10.1378/CHEST.121.3.921.

Chapman, K., Holmes, M. and Seckl, J. (2013) '11β-hydroxysteroid dehydrogenases: intracellular gate-keepers of tissue glucocorticoid action', *Physiological reviews*, 93(3), pp. 1139–1206. Available at: https://doi.org/10.1152/PHYSREV.00020.2012.

Chung, M., Ma, J., Patel, K., Berger, S., Lau, J. and Lichtenstein, A.H. (2014) 'Fructose, high-fructose corn syrup, sucrose, and nonalcoholic fatty liver disease or indexes of liver health: a systematic review and meta-analysis', *The American journal of clinical nutrition*, 100(3), pp. 833–849. Available at: https://doi.org/10.3945/AJCN.114.086314.

Codoñer-Franch, P., Valls-Bellés, V., Arilla-Codoñer, A. and Alonso-Iglesias, E. (2011) 'Oxidant mechanisms in childhood obesity: The link between inflammation and oxidative stress', *Translational Research*. Mosby Inc., pp. 369–384. Available at: https://doi.org/10.1016/j.trsl.2011.08.004.

Cornier, M.A., Dabelea, D., Hernandez, T.L., Lindstrom, R.C., Steig, A.J., Stob, N.R., van Pelt, R.E., Wang, H. and Eckel, R.H. (2008) 'The metabolic syndrome', *Endocrine reviews*, 29(7), pp. 777–822. Available at: https://doi.org/10.1210/ER.2008-0024.

Coutinho, A.E. and Chapman, K.E. (2011) 'The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights', *Molecular and cellular endocrinology*, 335(1), pp. 2–13. Available at: https://doi.org/10.1016/J.MCE.2010.04.005.

Cozma, A.I. and Sievenpiper, J.L. (2014) 'The role of fructose, sucrose and high-fructose corn syrup in diabetes', *European Endocrinology*, 10(1), pp. 51–60. Available at: https://doi.org/10.17925/ee.2014.10.01.51.

Crescenzo, R., Bianco, F., Falcone, I., Coppola, P., Liverini, G. and Iossa, S. (2013) 'Increased hepatic de novo lipogenesis and mitochondrial efficiency in a model of obesity induced by

diets rich in fructose', *European journal of nutrition*, 52(2), pp. 537–545. Available at: https://doi.org/10.1007/S00394-012-0356-Y.

Croxtall, J.D., van Hal, P.T.W., Choudhury, Q., Gilroy, D.W. and Flower, R.J. (2002) 'Different glucocorticoids vary in their genomic and non-genomic mechanism of action in A549 cells', *British Journal of Pharmacology*, 135(2), pp. 511–519. Available at: https://doi.org/10.1038/SJ.BJP.0704474.

Cruz-Topete, D., Myers, P.H., Foley, J.F., Willis, M.S. and Cidlowski, J.A. (2016) 'Corticosteroids Are Essential for Maintaining Cardiovascular Function in Male Mice', *Endocrinology*, 157(7), pp. 2759–2771. Available at: https://doi.org/10.1210/EN.2015-1604.

D'Alessandris, C., Lauro, R., Presta, I. and Sesti, G. (2007) 'C-reactive protein induces phosphorylation of insulin receptor substrate-1 on Ser307 and Ser 612 in L6 myocytes, thereby impairing the insulin signalling pathway that promotes glucose transport', *Diabetologia*, 50(4), pp. 840–849. Available at: https://doi.org/10.1007/S00125-006-0522-Y.

Dallman, M.F., Akana, S.F., Jacobson, L., Levin, N., Cascio, C.S. And Shinsako, J. (1987) 'Characterization of corticosterone feedback regulation of ACTH secretion', *Annals of the New York Academy of Sciences*, 512(1), pp. 402–414. Available at: https://doi.org/10.1111/J.1749-6632.1987.TB24976.X.

Dandel, M., Lehmkuhl, H., Knosalla, C., Suramelashvili, N. and Hetzer, R. (2009) 'Strain and strain rate imaging by echocardiography - basic concepts and clinical applicability', *Current cardiology reviews*, 5(2), pp. 133–148. Available at: https://doi.org/10.2174/157340309788166642.

DeBose-Boyd, R.A. and Ye, J. (2018) 'SREBPs in Lipid Metabolism, Insulin Signaling, and Beyond', *Trends in biochemical sciences*, 43(5), pp. 358–368. Available at: https://doi.org/10.1016/J.TIBS.2018.01.005.

DeFronzo, R.A. (2004) 'Pathogenesis of type 2 diabetes mellitus', *Medical Clinics of North America*, 88(4), pp. 787–835. Available at: https://doi.org/10.1016/j.mcna.2004.04.013.

Dekker, J.M., Girman, C., Rhodes, T., Nijpels, G., Stehouwer, C.D.A., Bouter, L.M. and Heine, R.J. (2005) 'Metabolic syndrome and 10-year cardiovascular disease risk in the Hoorn Study', *Circulation*, 112(5), pp. 666–673. Available at: https://doi.org/10.1161/CIRCULATIONAHA.104.516948.

DeMarco, V.G., Ford, D.A., Henriksen, E.J., Aroor, A.R., Johnson, M.S., Habibi, J., Ma, L., Yang, M., Albert, C.J., Lally, J.W., Ford, C.A., Prasannarong, M., Hayden, M.R., Whaley-Connell, A.T. and Sowers, J.R. (2013) 'Obesity-related alterations in cardiac lipid profile and nondipping blood pressure pattern during transition to diastolic dysfunction in male db/db mice', *Endocrinology*, 154(1), pp. 159–171. Available at: https://doi.org/10.1210/EN.2012-1835.

Dey, D., Basu, D., Roy, S.S., Bandyopadhyay, A. and Bhattacharya, S. (2006) 'Involvement of novel PKC isoforms in FFA induced defects in insulin signaling', *Molecular and Cellular Endocrinology*, 246(1–2), pp. 60–64. Available at: https://doi.org/10.1016/j.mce.2005.12.014.

Divi, M.S., Bellamkonda, R. and Dasireddy, K. (2012) 'Evaluation of Antidiabetic and Antihyperlipedemic Pontential of Aqueous Extract of Moringa Oleifera in Fructose Fed Insulin Resistant and Stz Induced Diabetic Wister Rats: A comparative Study'. Asian Journal of Pharmaceutical and Clinical Research, 5(1), pp 67-72.

Dobrowolski, P., Prejbisz, A., Kuryłowicz, A., Baska, A., Burchardt, P., Chlebus, K., Dzida, G., Jankowski, P., Jaroszewicz, J., Jaworski, P., Kamiński, K., Kapłon-Cieślicka, A., Klocek, M., Kukla, M., Mamcarz, A., Mastalerz-Migas, A., Narkiewicz, K., Ostrowska, L., Śliż, D., Tarnowski, W., Wolf, J., Wyleżoł, M., Zdrojewski, T., Banach, M., Januszewicz, A. and Bogdański, P. (2022) 'Metabolic syndrome - a new definition and management guidelines: A joint position paper by the Polish Society of Hypertension, Polish Society for the Treatment of Obesity, Polish Lipid Association, Polish Association for Study of Liver, Polish Society of Family Medicine, Polish Society of Lifestyle Medicine, Division of Prevention and Epidemiology Polish Cardiac Society, "Club 30" Polish Cardiac Society, and Division of Metabolic and Bariatric Surgery Society of Polish Surgeons', *Archives of medical science : AMS*, 18(5), pp. 1133–1156. Available at: https://doi.org/10.5114/AOMS/152921.

Donatti, T.L., Koch, V.H.K., Takayama, L. and Pereira, R.M.R. (2011) 'Effects of glucocorticoids on growth and bone mineralization', *Jornal de pediatria*, 87(1), pp. 4–12. Available at: https://doi.org/10.2223/JPED.2052.

Douard, V. and Ferraris, R.P. (2008) 'Regulation of the fructose transporter GLUT5 in health and disease', *American journal of physiology. Endocrinology and metabolism*, 295(2). Available at: https://doi.org/10.1152/AJPENDO.90245.2008.

ElGuindy, A. and Yacoub, M.H. (2012) 'Heart failure with preserved ejection fraction', *Global Cardiology Science and Practice*, 2012(1), p. 10. Available at: https://doi.org/10.5339/gcsp.2012.10.

Elks, C.M. and Francis, J. (2010) 'Central adiposity, systemic inflammation, and the metabolic syndrome', *Current Hypertension Reports*, pp. 99–104. Available at: https://doi.org/10.1007/s11906-010-0096-4.

Elmore, S.W., Pratt, J.K., Coghlan, M.J., Mao, Y., Green, B.E., Anderson, D.D., Stashko, M.A., Lin, C.W., Falls, D., Nakane, M., Miller, L., Tyree, C.M., Miner, J.N. and Lane, B. (2004) 'Differentiation of in vitro transcriptional repression and activation profiles of selective glucocorticoid modulators', *Bioorganic and Medicinal Chemistry Letters*, 14(7), pp. 1721–1727. Available at: https://doi.org/10.1016/j.bmcl.2004.01.044.

Enc, Y., Karaca, P., Ayoglu, U., Camur, G., Kurc, E. and Cicek, S. (2006) 'The acute cardioprotective effect of glucocorticoid in myocardial ischemia-reperfusion injury occurring during cardiopulmonary bypass.', *Heart and Vessels*, 21(3), pp. 152–156. Available at: https://doi.org/10.1007/S00380-005-0887-8.

Erasmus, R.T., Soita, D.J., Hassan, M.S., Blanco-Blanco, E., Vergotine, Z., Kengne, A.P. and Matsha, T.E. (2012) 'High prevalence of diabetes mellitus and metabolic syndrome in a South African coloured population: baseline data of a study in Bellville, Cape Town', *South African medical journal = Suid-Afrikaanse tydskrif vir geneeskunde*, 102(11 Pt 1), pp. 841–844. Available at: https://doi.org/10.7196/SAMJ.5670.

Fakhoury-Sayegh, N., Trak-Smayra, V., Sayegh, R., Haidar, F., Obeid, O., Asmar, S. and Khazzaka, A. (2019) 'Fructose threshold for inducing organ damage in a rat model of nonalcoholic fatty liver disease', *Nutrition Research*, 62, pp. 101–112. Available at: https://doi.org/10.1016/j.nutres.2018.11.003.

Fallo, F., Budano, S., Sonino, N., Muiesan, M.L., Agabiti-Rosei, E. and Boscaro, M. (1994) 'Left ventricular structural characteristics in Cushing's syndrome.', *Journal of Human Hypertension*, 8(7), pp. 509–513. Available at: https://europepmc.org/article/med/7932514 (Accessed: 17 January 2023).

Fan, W.J., Genade, S., Genis, A., Huisamen, B. and Lochner, A. (2009) 'Dexamethasone-induced cardioprotection: a role for the phosphatase MKP-1?', *Life sciences*, 84(23–24), pp. 838–846. Available at: https://doi.org/10.1016/J.LFS.2009.03.014.

Fardet, L., Flahault, A., Kettaneh, A., Tiev, K.P., Généreau, T., Tolédano, C., Lebbé, C. and Cabane, J. (2007) 'Corticosteroid-induced clinical adverse events: frequency, risk factors and patient's opinion', *The British journal of dermatology*, 157(1), pp. 142–148. Available at: https://doi.org/10.1111/J.1365-2133.2007.07950.X.

Fardet, L., Petersen, I. and Nazareth, I. (2012) 'Risk of cardiovascular events in people prescribed glucocorticoids with iatrogenic Cushing's syndrome: cohort study', *BMJ (Clinical research ed.)*, 345(7871). Available at: https://doi.org/10.1136/BMJ.E4928.

Farrell, C. and O'Keane, V. (2016) 'Epigenetics and the glucocorticoid receptor: A review of the implications in depression', *Psychiatry research*, 242, pp. 349–356. Available at: https://doi.org/10.1016/J.PSYCHRES.2016.06.022.

Ferdinandy, P. and Schulz, R. (2001) 'Peroxynitrite: toxic or protective in the heart?', *Circulation research*, 88(2). Available at: https://doi.org/10.1161/01.RES.88.2.E12.

Fowden, A.L. and Forhead, A.J. (2015) 'Glucocorticoids as regulatory signals during intrauterine development', *Experimental Physiology*, 100(12), pp. 1477–1487. Available at: https://doi.org/10.1113/EP085212.

Frangogiannis, N.G. (2017) 'The extracellular matrix in myocardial injury, repair, and remodeling', *The Journal of clinical investigation*, 127(5), pp. 1600–1612. Available at: https://doi.org/10.1172/JCI87491.

Froesch, E.R. (1972) ‘Fructose metabolism in adipose tissue’, *Acta medica Scandinavica. Supplementum*, 542(542 S), pp. 37–46. Available at: https://doi.org/10.1111/J.0954-6820.1972.TB05317.X.

Fuentes, L.D. las, Brown, A.L., Mathews, S.J., Waggoner, A.D., Soto, P.F., Gropler, R.J. and Dávila-Román, V.G. (2007) ‘Metabolic syndrome is associated with abnormal left ventricular diastolic function independent of left ventricular mass’, *European heart journal*, 28(5), pp. 553–559. Available at: https://doi.org/10.1093/EURHEARTJ/EHL526.

Fukuda, N., Wu, Y., Nair, P. and Granzier, H.L. (2005) ‘Phosphorylation of titin modulates passive stiffness of cardiac muscle in a titin isoform-dependent manner’, *The Journal of general physiology*, 125(3), pp. 257–271. Available at: https://doi.org/10.1085/JGP.200409177.

Fukuta, H. and Little, W.C. (2007) ‘Contribution of systolic and diastolic abnormalities to heart failure with a normal and a reduced ejection fraction.’, *undefined*, 49(4), pp. 229–240. Available at: https://doi.org/10.1016/J.PCAD.2006.08.009.

Funder, J.W. (2007) ‘Mineralocorticoid receptor activation and oxidative stress’, *Hypertension (Dallas, Tex.: 1979)*, 50(5), pp. 840–841. Available at: https://doi.org/10.1161/HYPERTENSIONAHA.107.098012.

Gaasch, W.H. and Zile, M.R. (2011) ‘Left ventricular structural remodeling in health and disease: with special emphasis on volume, mass, and geometry’, *Journal of the American College of Cardiology*, 58(17), pp. 1733–1740. Available at: https://doi.org/10.1016/J.JACC.2011.07.022.

Galassi, A., Reynolds, K. and He, J. (2006) ‘Metabolic syndrome and risk of cardiovascular disease: a meta-analysis’, *The American journal of medicine*, 119(10), pp. 812–819. Available at: https://doi.org/10.1016/J.AMJMED.2006.02.031.

Galicia-Garcia, U., Benito-Vicente, A., Jebari, S., Larrea-Sebal, A., Siddiqi, H., Uribe, K.B., Ostolaza, H. and Martín, C. (2020) 'Pathophysiology of type 2 diabetes mellitus', *International Journal of Molecular Sciences*. MDPI AG, pp. 1–34. Available at: https://doi.org/10.3390/ijms21176275.

Galigniana, M.D., Scruggs, J.L., Herrington, J., Welsh, M.J., Carter-Su, C., Housley, P.R. and Pratt, W.B. (1998) 'Heat shock protein 90-dependent (geldanamycin-inhibited) movement of the glucocorticoid receptor through the cytoplasm to the nucleus requires intact cytoskeleton', *Molecular endocrinology (Baltimore, Md.)*, 12(12), pp. 1903–1913. Available at: https://doi.org/10.1210/MEND.12.12.0204.

Galitzky, J. and Bouloumié, A. (2013) 'Human visceral-fat-specific glucocorticoid tuning of adipogenesis', *Cell Metabolism*. Cell Press, pp. 3–5. Available at: https://doi.org/10.1016/j.cmet.2013.06.008.

Gallagher, E.J., Leroith, D. and Karnieli, E. (2010) 'Insulin resistance in obesity as the underlying cause for the metabolic syndrome', *The Mount Sinai journal of medicine, New York*, 77(5), pp. 511–523. Available at: https://doi.org/10.1002/MSJ.20212.

Ganau, A., Devereux, R.B., Roman, M.J., de Simone, G., Pickering, T.G., Saba, P.S., Vargiu, P., Simongini, I. and Laragh, J.H. (1992) 'Patterns of left ventricular hypertrophy and geometric remodeling in essential hypertension', *Journal of the American College of Cardiology*, 19(7), pp. 1550–1558. Available at: https://doi.org/10.1016/0735-1097(92)90617-V.

Gao, Z., Zhang, X., Zuberi, A., Hwang, D., Quon, M.J., Lefevre, M. and Ye, J. (2004) 'Inhibition of insulin sensitivity by free fatty acids requires activation of multiple serine kinases in 3T3-L1 adipocytes', *Molecular endocrinology (Baltimore, Md.)*, 18(8), pp. 2024–2034. Available at: https://doi.org/10.1210/ME.2003-0383.

Geer, E.B., Islam, J. and Buettner, C. (2014) ‘Mechanisms of glucocorticoid-induced insulin resistance: focus on adipose tissue function and lipid metabolism’, *Endocrinology and metabolism clinics of North America*, 43(1), pp. 75–102. Available at: https://doi.org/10.1016/J.ECL.2013.10.005.

Genser, L., Casella Mariolo, J.R., Castagneto-Gissey, L., Panagiotopoulos, S. and Rubino, F. (2016) ‘Obesity, Type 2 Diabetes, and the Metabolic Syndrome: Pathophysiologic Relationships and Guidelines for Surgical Intervention’, *The Surgical clinics of North America*, 96(4), pp. 681–701. Available at: https://doi.org/10.1016/J.SUC.2016.03.013.

Germain, P., Staels, B., Dacquet, C., Spedding, M. and Laudet, V. (2006) ‘Overview of nomenclature of nuclear receptors’, *Pharmacological reviews*, 58(4), pp. 685–704. Available at: https://doi.org/10.1124/PR.58.4.2.

Giddings, S.J., Orland, M.J., Weir, G.C., Bonner-Weir, S. and Permutt, M.A. (1985) *Impaired jnsulin Biosynthetic Capacity in a Rat Model for Non-insulin-dependent Diabetes Studies with Dexamethasone*. Available at: http://diabetesjournals.org/diabetes/article-pdf/34/3/235/353754/34-3-235.pdf.

Giraud, G.D., Louey, S., Jonker, S., Schultz, J. and Thornburg, K.L. (2006) ‘Cortisol stimulates cell cycle activity in the cardiomyocyte of the sheep fetus’, *Endocrinology*, 147(8), pp. 3643–3649. Available at: https://doi.org/10.1210/en.2006-0061.

Giugliano, G.R., Giugliano, R.P., Gibson, C.M. and Kuntz, R.E. (2003) ‘Meta-analysis of corticosteroid treatment in acute myocardial infarction’, *American Journal of Cardiology*, 91(9), pp. 1055–1059. Available at: https://doi.org/10.1016/S0002-9149(03)00148-6.

Gjerstad, J.K., Lightman, S.L. and Spiga, F. (2018) ‘Role of glucocorticoid negative feedback in the regulation of HPA axis pulsatility’, *https://doi.org/10.1080/10253890.2018.1470238*, 21(5), pp. 403–416. Available at: https://doi.org/10.1080/10253890.2018.1470238.

Gounarides, J.S., Korach-André, M., Killary, K., Argentieri, G., Turner, O. and Laurent, D. (2008) 'Effect of dexamethasone on glucose tolerance and fat metabolism in a diet-induced obesity mouse model', *Endocrinology*, 149(2), pp. 758–766. Available at: https://doi.org/10.1210/en.2007-1214.

Gray, G.A., White, C.I., Castellan, R.F.P., McSweeney, S.J. and Chapman, K.E. (2017) 'Getting to the heart of intracellular glucocorticoid regeneration: 11β-HSD1 in the myocardium', *Journal of molecular endocrinology*, 58(1), pp. R1–R13. Available at: https://doi.org/10.1530/JME-16-0128.

Groeneweg, F.L., Karst, H., de Kloet, E.R. and Joëls, M. (2012) 'Mineralocorticoid and glucocorticoid receptors at the neuronal membrane, regulators of nongenomic corticosteroid signalling', *Molecular and Cellular Endocrinology*, 350(2), pp. 299–309. Available at: https://doi.org/10.1016/J.MCE.2011.06.020.

Grossmann, C., Scholz, T., Rochel, M., Bumke-Vogt, C., Oelkers, W., Pfeiffer, A.F.H., Diederich, S. and Bähr, V. (2004) 'Transactivation via the human glucocorticoid and mineralocorticoid receptor by therapeutically used steroids in CV-1 cells: a comparison of their glucocorticoid and mineralocorticoid properties', *European journal of endocrinology*, 151(3), pp. 397–406. Available at: https://doi.org/10.1530/EJE.0.1510397.

Haffner, S.M. (2007) 'Abdominal adiposity and cardiometabolic risk: do we have all the answers?', *The American journal of medicine*, 120(9 Suppl 1). Available at: https://doi.org/10.1016/J.AMJMED.2007.06.006.

Halcox, J.P.J., Roy, C., Tubach, F., Banegas, J.R., Dallongeville, J., de Backer, G., Guallar, E., Sazova, O., Medina, J., Perk, J., Steg, P.G., Rodríguez-Artalejo, F. and Borghi, C. (2014) 'C-reactive protein levels in patients at cardiovascular risk: EURIKA study', *BMC*

Cardiovascular Disorders, 14, p. 25. Available at: https://doi.org/10.1186/1471-2261-14-25.

Hammond, G.L. (2016) 'Plasma steroid-binding proteins: primary gatekeepers of steroid hormone action', *The Journal of endocrinology*, 230(1), pp. R13–R25. Available at: https://doi.org/10.1530/JOE-16-0070.

Hattori, T., Murase, T., Iwase, E., Takahashi, K., Ohtake, M., Tsuboi, K., Ohtake, M., Miyachi, M., Murohara, T. and Nagata, K. (2013) 'Glucocorticoid-Induced Hypertension And Cardiac Injury: Effects Of Mineralocorticoid And Glucocorticoid Receptor Antagonism', *Nagoya Journal of Medical Science* [Preprint]. Available at: https://doi.org/10.18999/NAGJMS.75.1-2.81.

Hawkins, U.A., Gomez-Sanchez, E.P., Gomez-Sanchez, C.M. and Gomez-Sanchez, C.E. (2012) 'The ubiquitous mineralocorticoid receptor: clinical implications', *Current hypertension reports*, 14(6), pp. 573–580. Available at: https://doi.org/10.1007/S11906-012-0297-0.

Haydock, P.M. and Flett, A.S. (2022) 'Management of heart failure with reduced ejection fraction', *Heart (British Cardiac Society)*, 108(19), pp. 1571–1579. Available at: https://doi.org/10.1136/heartjnl-2020-318811.

van Heerebeek, L., Hamdani, N., Falcão-Pires, I., Leite-Moreira, A.F., Begieneman, M.P.V., Bronzwaer, J.G.F., van der Velden, J., Stienen, G.J.M., Laarman, G.J., Somsen, A., Verheugt, F.W.A., Niessen, H.W.M. and Paulus, W.J. (2012) 'Low myocardial protein kinase G activity in heart failure with preserved ejection fraction', *Circulation*, 126(7), pp. 830–839. Available at: https://doi.org/10.1161/CIRCULATIONAHA.111.076075.

Heindel, J.J., Blumberg, B., Cave, M., Machtinger, R., Mantovani, A., Mendez, M.A., Nadal, A., Palanza, P., Panzica, G., Sargis, R., Vandenberg, L.N. and vom Saal, F. (2017)

‘Metabolism disrupting chemicals and metabolic disorders’, *Reproductive Toxicology*, 68, pp. 3–33. Available at: https://doi.org/10.1016/j.reprotox.2016.10.001.

Heitzer, M.D., Wolf, I.M., Sanchez, E.R., Witchel, S.F. and DeFranco, D.B. (2007) ‘Glucocorticoid receptor physiology’, *Reviews in Endocrine and Metabolic Disorders*, 8(4), pp. 321–330. Available at: https://doi.org/10.1007/S11154-007-9059-8/FIGURES/3.

Henein, M.Y. and Lindqvist, P. (2015) ‘Assessment of Left Ventricular Diastolic Function by Doppler Echocardiography’, *Cardiac failure review*, 1(2), p. 87. Available at: https://doi.org/10.15420/CFR.2015.1.2.87.

Hernández-Díazcouder, A., Romero-Nava, R., Carbó, R., Sánchez-Lozada, L.G. and Sánchez-Muñoz, F. (2019) ‘High Fructose Intake and Adipogenesis’, *International journal of molecular sciences*, 20(11). Available at: https://doi.org/10.3390/IJMS20112787.

Hoebel, S., Malan, L. and de Ridder, H. (2014) ‘Differences in MetS marker prevalence between black African and Caucasian teachers from the North West Province: Sympathetic Activity and Ambulatory Blood Pressure in Africans (SABPA) Study’, *https://doi.org/10.1080/22201009.2011.10872251*, 16(1), pp. 49–56. Available at: https://doi.org/10.1080/22201009.2011.10872251.

Howard, B. v. (1999) ‘Insulin resistance and lipid metabolism’, *The American journal of cardiology*, 84(1A), pp. 28–32. Available at: https://doi.org/10.1016/S0002-9149(99)00355-0.

Huang, Y.H., Chen, C.J., Tang, K.S., Sheen, J.M., Tiao, M.M., Tain, Y.L., Chen, C.C., Chu, E.W., Li, S.W., Yu, H.R. and Huang, L.T. (2016) ‘Postnatal high-fat diet increases liver steatosis and apoptosis threatened by prenatal dexamethasone through the oxidative effect’, *International Journal of Molecular Sciences*, 17(3). Available at: https://doi.org/10.3390/ijms17030369.

Ingelsson, E., Ärnlöv, J., Lind, L. and Sundström, J. (2006) 'Metabolic syndrome and risk for heart failure in middle-aged men', *Heart*, 92(10), p. 1409. Available at: https://doi.org/10.1136/HRT.2006.089011.

Isidori, A.M., Graziadio, C., Paragliola, R.M., Cozzolino, A., Ambrogio, A.G., Colao, A., Corsello, S.M. and Pivonello, R. (2015) 'The hypertension of Cushing's syndrome: controversies in the pathophysiology and focus on cardiovascular complications', *Journal of hypertension*, 33(1), pp. 44–60. Available at: https://doi.org/10.1097/HJH.0000000000000415.

Itani, S.I., Ruderman, N.B., Schmieder, F. and Boden, G. (2002) 'Lipid-induced insulin resistance in human muscle is associated with changes in diacylglycerol, protein kinase C, and IkappaB-alpha', *Diabetes*, 51(7), pp. 2005–2011. Available at: https://doi.org/10.2337/DIABETES.51.7.2005.

Jia, G., Jia, Y. and Sowers, J.R. (2017) 'Role of mineralocorticoid receptor activation in cardiac diastolic dysfunction', *Biochimica et biophysica acta. Molecular basis of disease*, 1863(8), pp. 2012–2018. Available at: https://doi.org/10.1016/J.BBADIS.2016.10.025.

Jin, Z., Xia, F., Dong, J., Lin, T., Cai, Y., Chen, J., Chen, X., Huang, Z., Wang, Q., Chen, H. and Zhang, J. (2021) 'Omentin-1 attenuates glucocorticoid-induced cardiac injury by phosphorylating gsk3β', *Journal of Molecular Endocrinology*, 66(4), pp. 273–283. Available at: https://doi.org/10.1530/JME-20-0236.

Joëls, M. (2011) 'Impact of glucocorticoids on brain function: relevance for mood disorders', *Psychoneuroendocrinology*, 36(3), pp. 406–414. Available at: https://doi.org/10.1016/J.PSYNEUEN.2010.03.004.

Jung, J.H., Min, P.K., Kim, J.Y., Park, S., Choi, E.Y., Ko, Y.G., Choi, D., Jang, Y., Shim, W.H. and Cho, S.Y. (2006) 'Systemic immunosuppressive therapy inhibits in-stent restenosis in

patients with renal allograft', *Catheterization and Cardiovascular Interventions*, 68(4), pp. 567–573. Available at: https://doi.org/10.1002/CCD.20799.

Jürgens, H., Haass, W., Castañeda, T.R., Schürmann, A., Koebnick, C., Dombrowski, F., Otto, B., Nawrocki, A.R., Scherer, P.E., Spranger, J., Ristow, M., Joost, H.G., Havel, P.J. and Tschöp, M.H. (2005) 'Consuming fructose-sweetened beverages increases body adiposity in mice', *Obesity Research*, 13(7), pp. 1145–1156. Available at: https://doi.org/10.1038/oby.2005.136.

Kadmiel, M. and Cidlowski, J.A. (2013) 'Glucocorticoid receptor signaling in health and disease', *Trends in pharmacological sciences*, 34(9), pp. 518–530. Available at: https://doi.org/10.1016/J.TIPS.2013.07.003.

Kahn, R., Buse, J., Ferrannini, E. and Stern, M. (2005) 'The metabolic syndrome: time for a critical appraisal: joint statement from the American Diabetes Association and the European Association for the Study of Diabetes', *Diabetes care*, 28(9), pp. 2289–2304. Available at: https://doi.org/10.2337/DIACARE.28.9.2289.

Kamenický, P., Redheuil, A., Roux, C., Salenave, S., Kachenoura, N., Raissouni, Z., Macron, L., Guignat, L., Jublanc, C., Azarine, A., Brailly, S., Young, J., Mousseaux, E. and Chanson, P. (2014) 'Cardiac structure and function in Cushing's syndrome: a cardiac magnetic resonance imaging study', *The Journal of clinical endocrinology and metabolism*, 99(11), pp. E2144–E2153. Available at: https://doi.org/10.1210/JC.2014-1783.

Kannel, W.B. (2009) 'Hypertension: Reflections on Risks and Prognostication', *Med Clin North Am*, 93(3), p. 541. Available at: https://doi.org/10.1016/j.mcna.2009.02.006.

Kim, L.J., Nalls, M.A., Eiriksdottir, G., Sigurdsson, S., Launer, L.J., Koster, A., Chaves, P.H.M., Jonsdottir, B., Garcia, M., Gudnason, V. and Harris, T.B. (2011) 'Associations of Visceral and Liver Fat With the Metabolic Syndrome Across the Spectrum of Obesity: The AGES-

Reykjavik Study', *Obesity*, 19(6), pp. 1265–1271. Available at: https://doi.org/10.1038/OBY.2010.291.

Kitagawa, A., Ohta, Y., Ohashi, K., Yashiro, K. and Fukuzawa, K. (2020) *Effect of High Fructose-Induced Metabolic Syndrome on Tissue Vitamin E and Lipid Peroxide Levels in Rats*, *J Nutr Sci Vitaminol*.

Kohr, M.J., Davis, J.P. and Ziolo, M.T. (2009) 'Peroxynitrite Increases Protein Phosphatase Activity and Promotes the Interaction of Phospholamban with Protein Phosphatase 2a in the Myocardium', *Nitric oxide : biology and chemistry*, 20(3), pp. 217–221. Available at: https://doi.org/10.1016/J.NIOX.2009.01.003.

König, A., Leibig, M., Rieber, J., Schiele, T.M., Theisen, K., Siebert, U., Gothe, R.M. and Klauss, V. (2007) 'Randomized comparison of dexamethasone-eluting stents with bare metal stent implantation in patients with acute coronary syndrome: serial angiographic and sonographic analysis', *American heart journal*, 153(6). Available at: https://doi.org/10.1016/J.AHJ.2007.03.032.

Konstam, M.A., Kramer, D.G., Patel, A.R., Maron, M.S. and Udelson, J.E. (2011) 'Left ventricular remodeling in heart failure: current concepts in clinical significance and assessment', *JACC. Cardiovascular imaging*, 4(1), pp. 98–108. Available at: https://doi.org/10.1016/J.JCMG.2010.10.008.

Koutsojannis, C.M., Mallioris, C.N., Kounis, N.G., Goto, Y., Takagi, S., Miyazaki, S. and Nonogi, H. (2004) 'Corticosteroids, Kounis syndrome and the treatment of refractory vasospastic angina', *Circulation journal : official journal of the Japanese Circulation Society*, 68(8), pp. 806–807. Available at: https://doi.org/10.1253/CIRCJ.68.806.

Kunbuma Tachang, G., Choukem, S.-P., Ndjebet, J., Dzudie, A. and Titanji, V.P.K. (2012) 'Prevalence of hyperglycaemia, obesity and metabolic syndrome (a three component

study) among hospital personnel in the Littoral Region of Cameroon', *International Journal of Medicine and Medical Sciences*, 4(10), pp. 232–237. Available at: https://doi.org/10.5897/IJMMS12.104.

Lanaspa, M.A., Sanchez-Lozada, L.G., Cicerchi, C., Li, N., Roncal-Jimenez, C.A., Ishimoto, T., Le, M., Garcia, G.E., Thomas, J.B., Rivard, C.J., Andres-Hernando, A., Hunter, B., Schreiner, G., Rodriguez-Iturbe, B., Sautin, Y.Y. and Johnson, R.J. (2012) 'Uric acid stimulates fructokinase and accelerates fructose metabolism in the development of fatty liver', *PloS one*, 7(10). Available at: https://doi.org/10.1371/JOURNAL.PONE.0047948.

Lee, D.Y., Kim, E. and Choi, M.H. (2015) 'Technical and clinical aspects of cortisol as a biochemical marker of chronic stress', *BMB reports*, 48(4), pp. 209–216. Available at: https://doi.org/10.5483/BMBREP.2015.48.4.275.

Legeza, B., Marcolongo, P., Gamberucci, A., Varga, V., Bánhegyi, G., Benedetti, A. and Odermatt, A. (2017) 'Fructose, Glucocorticoids and Adipose Tissue: Implications for the Metabolic Syndrome', *Nutrients*, 9(5). Available at: https://doi.org/10.3390/NU9050426.

Leggat, J., Bidault, G. and Vidal-Puig, A. (2021) 'Lipotoxicity: a driver of heart failure with preserved ejection fraction?', *Clinical Science*, 135(19), pp. 2265–2283. Available at: https://doi.org/10.1042/CS20210127.

Lelis, D. de F., Andrade, J.M.O., Almenara, C.C.P., Broseguini-Filho, G.B., Mill, J.G. and Baldo, M.P. (2020) 'High fructose intake and the route towards cardiometabolic diseases', *Life Sciences*. Elsevier Inc. Available at: https://doi.org/10.1016/j.lfs.2020.118235.

Leon-Cabrera, S., Solís-Lozano, L., Suárez-Álvarez, K., González-Chávez, A., Béjar, Y.L., Robles-Díaz, G. and Escobedo, G. (2013) 'Hyperleptinemia is associated with parameters of low-grade systemic inflammation and metabolic dysfunction in obese human beings',

Frontiers in Integrative Neuroscience, 7(AUG). Available at: https://doi.org/10.3389/FNINT.2013.00062.

Lesnefsky, E.J., Moghaddas, S., Tandler, B., Kerner, J. and Hoppel, C.L. (2001) 'Mitochondrial dysfunction in cardiac disease: Ischemia - reperfusion, aging, and heart failure', *Journal of Molecular and Cellular Cardiology*, 33(6), pp. 1065–1089. Available at: https://doi.org/10.1006/JMCC.2001.1378.

LeWinter, M.M. and Meyer, M. (2013) 'Mechanisms of diastolic dysfunction in heart failure with a preserved ejection fraction if it's not one thing it's another', *Circulation: Heart Failure*. Lippincott Williams and Wilkins, pp. 1112–1115. Available at: https://doi.org/10.1161/CIRCHEARTFAILURE.113.000825.

Li, X., Zhai, Y., Zhao, J., He, H., Li, Y., Liu, Y., Feng, A., Li, L., Huang, T., Xu, A. and Lyu, J. (2021) 'Impact of Metabolic Syndrome and It's Components on Prognosis in Patients With Cardiovascular Diseases: A Meta-Analysis.', *Frontiers in cardiovascular medicine*, 8, p. 704145. Available at: https://doi.org/10.3389/fcvm.2021.704145.

Liu, H.H. and Li, J.J. (2015) 'Aging and dyslipidemia: A review of potential mechanisms', *Ageing Research Reviews*, 19, pp. 43–52. Available at: https://doi.org/10.1016/J.ARR.2014.12.001.

London, E. and Castonguay, T.W. (2011) 'High fructose diets increase 11β-hydroxysteroid dehydrogenase type 1 in liver and visceral adipose in rats within 24-h exposure', *Obesity (Silver Spring, Md.)*, 19(5), pp. 925–932. Available at: https://doi.org/10.1038/OBY.2010.284.

Longo, M., Zatterale, F., Naderi, J., Parrillo, L., Formisano, P., Raciti, G.A., Beguinot, F. and Miele, C. (2019) 'Adipose Tissue Dysfunction as Determinant of Obesity-Associated Metabolic

Complications', *International journal of molecular sciences*, 20(9). Available at: https://doi.org/10.3390/IJMS20092358.

van Looveren, K., Wallaeys, C. and Libert, C. (2020) 'Potential of glucocorticoids to treat intestinal inflammation during sepsis', *Current Opinion in Pharmacology*. Elsevier Ltd, pp. 1–7. Available at: https://doi.org/10.1016/j.coph.2019.12.005.

Lopaschuk, G.D. (1996) 'Abnormal mechanical function in diabetes: relationship to altered myocardial carbohydrate/lipid metabolism', *Coronary artery disease*, 7(2), pp. 116–123. Available at: https://doi.org/10.1097/00019501-199602000-00004.

Lopaschuk, G.D., Folmes, C.D.L. and Stanley, W.C. (2007) 'Cardiac energy metabolism in obesity', *Circulation research*, 101(4), pp. 335–347. Available at: https://doi.org/10.1161/CIRCRESAHA.107.150417.

Lopaschuk, G.D., Karwi, Q.G., Tian, R., Wende, A.R. and Abel, E.D. (2021) 'Cardiac Energy Metabolism in Heart Failure', *Circulation research*, 128(10), pp. 1487–1513. Available at: https://doi.org/10.1161/CIRCRESAHA.121.318241.

Lopaschuk, G.D., Ussher, J.R., Folmes, C.D.L., Jaswal, J.S. and Stanley, W.C. (2010) 'Myocardial fatty acid metabolism in health and disease', *Physiological reviews*, 90(1), pp. 207–258. Available at: https://doi.org/10.1152/PHYSREV.00015.2009.

López-Jaramillo, P., Gómez-Arbeláez, D., López-López, J., López-López, C., Martínez-Ortega, J., Gómez-Rodríguez, A. and Triana-Cubillos, S. (2014) 'The role of leptin/adiponectin ratio in metabolic syndrome and diabetes', *Hormone molecular biology and clinical investigation*, 18(1), pp. 37–45. Available at: https://doi.org/10.1515/HMBCI-2013-0053.

Lumbers, E.R., Boyce, A.C., Joulianos, G., Kumarasamy, V., Barner, E., Segar, J.L. and Burrell, J.H. (2005) 'Effects of cortisol on cardiac myocytes and on expression of cardiac genes in

fetal sheep', *American journal of physiology. Regulatory, integrative and comparative physiology*, 288(3). Available at: https://doi.org/10.1152/AJPREGU.00556.2004.

Lustig, R.H., Schmidt, L.A. and Brindis, C.D. (2012) 'Public health: The toxic truth about sugar', *Nature*, 482(7383), pp. 27–29. Available at: https://doi.org/10.1038/482027A.

Mabetwa, E.M., Mokwena, K.E., Mphekgwana, P.M. and Modjadji, P. (2022) 'Metabolic Syndrome and Its Components among Taxi Drivers in the City of Tshwane, South Africa', *Applied Sciences (Switzerland)*, 12(3), p. 1767. Available at: https://doi.org/10.3390/APP12031767.

Magalhães, P., Capingana, D.P. and Mill, J.G. (2014) 'Prevalence of the metabolic syndrome and determination of optimal cut-off values of waist circumference in university employees from Angola', *undefined*, 25(1). Available at: https://doi.org/10.5830/CVJA-2013-086.

Mallioris, C.N. and Kounis, N.G. (2004) 'Glucocorticoids, Kounis syndrome and the prevention of atrial fibrillation', *European heart journal*, 25(23), pp. 2175–2176. Available at: https://doi.org/10.1016/J.EHJ.2004.08.014.

Mayes, P.A. (1993) 'Intermediary metabolism of fructose', *The American Journal of Clinical Nutrition*, 58(5), pp. 754S-765S. Available at: https://doi.org/10.1093/AJCN/58.5.754S.

McHugh, K., DeVore, A.D., Wu, J., Matsouaka, R.A., Fonarow, G.C., Heidenreich, P.A., Yancy, C.W., Green, J.B., Altman, N. and Hernandez, A.F. (2019) 'Heart Failure With Preserved Ejection Fraction and Diabetes: JACC State-of-the-Art Review', *Journal of the American College of Cardiology*, 73(5), pp. 602–611. Available at: https://doi.org/10.1016/J.JACC.2018.11.033.

Mezza, T., Cinti, F., Cefalo, C.M.A., Pontecorvi, A., Kulkarni, R.N. and Giaccari, A. (2019) 'β-Cell Fate in Human Insulin Resistance and Type 2 Diabetes: A Perspective on Islet Plasticity', *Diabetes*, 68(6), pp. 1121–1129. Available at: https://doi.org/10.2337/DB18-0856.

Mihailidou, A.S., Le, T.Y.L., Mardini, M. and Funder, J.W. (2009) 'Glucocorticoids activate cardiac mineralocorticoid receptors during experimental myocardial infarction', *Hypertension (Dallas, Tex. : 1979)*, 54(6), pp. 1306–1312. Available at: https://doi.org/10.1161/HYPERTENSIONAHA.109.136242.

Mitter, S.S., Shah, S.J. and Thomas, J.D. (2017a) 'A Test in Context: E/A and E/e' to Assess Diastolic Dysfunction and LV Filling Pressure', *Journal of the American College of Cardiology*, 69(11), pp. 1451–1464. Available at: https://doi.org/10.1016/J.JACC.2016.12.037.

Mitter, S.S., Shah, S.J. and Thomas, J.D. (2017b) 'A Test in Context: E/A and E/e' to Assess Diastolic Dysfunction and LV Filling Pressure', *Journal of the American College of Cardiology*, 69(11), pp. 1451–1464. Available at: https://doi.org/10.1016/J.JACC.2016.12.037.

Monteiro, R. and Azevedo, I. (2010) 'Chronic inflammation in obesity and the metabolic syndrome', *Mediators of inflammation*, 2010. Available at: https://doi.org/10.1155/2010/289645.

Morino, K., Petersen, K.F. and Shulman, G.I. (2006) 'Molecular mechanisms of insulin resistance in humans and their potential links with mitochondrial dysfunction', *Diabetes*, 55 Suppl 2(Suppl 2). Available at: https://doi.org/10.2337/DB06-S002.

Mottillo, S., Filion, K.B., Genest, J., Joseph, L., Pilote, L., Poirier, P., Rinfret, S., Schiffrin, E.L. and Eisenberg, M.J. (2010) 'The metabolic syndrome and cardiovascular risk a systematic

review and meta-analysis', *Journal of the American College of Cardiology*, 56(14), pp. 1113–1132. Available at: https://doi.org/10.1016/J.JACC.2010.05.034.

Mottram, P.M. and Marwick, T.H. (2005) 'Assessment of diastolic function: what the general cardiologist needs to know', *Heart (British Cardiac Society)*, 91(5), pp. 681–695. Available at: https://doi.org/10.1136/HRT.2003.029413.

Muiesan, M.L., Lupia, M., Salvetti, M., Grigoletto, C., Sonino, N., Boscaro, M., Agabiti Rosei, E., Mantero, F. and Fallo, F. (2003) 'Left ventricular structural andnfunctional characteristics in Cushing's syndrome', *Journal of the American College of Cardiology*, 41(12), pp. 2275–2279. Available at: https://doi.org/10.1016/S0735-1097(03)00493-5.

Mungrue, I.N., Gros, R., You, X., Pirani, A., Azad, A., Csont, T., Schulz, R., Butany, J., Stewart, D.J. and Husain, M. (2002) 'Cardiomyocyte overexpression of iNOS in mice results in peroxynitrite generation, heart block, and sudden death', *The Journal of Clinical Investigation*, 109(6), p. 735. Available at: https://doi.org/10.1172/JCI13265.

Newton, A.C. (2003) 'Regulation of the ABC kinases by phosphorylation: protein kinase C as a paradigm', *The Biochemical journal*, 370(Pt 2), pp. 361–371. Available at: https://doi.org/10.1042/BJ20021626.

Newton, R. (2000) 'Molecular mechanisms of glucocorticoid action: what is important?', *Thorax*, 55(7), pp. 603–613. Available at: https://doi.org/10.1136/THORAX.55.7.603.

Nishikawa, T. and Araki, E. (2007) 'Impact of mitochondrial ROS production in the pathogenesis of diabetes mellitus and its complications', *Antioxidants & redox signaling*, 9(3), pp. 343–353. Available at: https://doi.org/10.1089/ARS.2006.1458.

Nussinovitch, U., Freire de Carvalho, J., Maria R. Pereira, R. and Shoenfeld, Y. (2010) 'Glucocorticoids and the cardiovascular system: state of the art', *Current pharmaceutical*

design, 16(32), pp. 3574–3585. Available at: https://doi.org/10.2174/138161210793797870.

Oakley, R.H. and Cidlowski, J.A. (2013) 'The biology of the glucocorticoid receptor: new signaling mechanisms in health and disease', *The Journal of allergy and clinical immunology*, 132(5), pp. 1033–1044. Available at: https://doi.org/10.1016/J.JACI.2013.09.007.

Oakley, R.H. and Cidlowski, J.A. (2015) 'Glucocorticoid signaling in the heart: A cardiomyocyte perspective', *The Journal of Steroid Biochemistry and Molecular Biology*, 153, pp. 27–34. Available at: https://doi.org/10.1016/J.JSBMB.2015.03.009.

Omori, Y., Mano, T., Ohtani, T., Sakata, Y., Takeda, Y., Tamaki, S., Tsukamoto, Y., Miwa, T., Yamamoto, K. and Komuro, I. (2014) 'Glucocorticoids Induce Cardiac Fibrosis via Mineralocorticoid Receptor in Oxidative Stress: Contribution of Elongation Factor Eleven-Nineteen Lysine-Rich Leukemia (ELL)', *Yonago Acta Medica*, 57(3), p. 109. Available at: /pmc/articles/PMC4209341/ (Accessed: 17 January 2023).

O'Neill, S. and O'Driscoll, L. (2015) 'Metabolic syndrome: A closer look at the growing epidemic and its associated pathologies', *Obesity Reviews*, 16(1), pp. 1–12. Available at: https://doi.org/10.1111/OBR.12229.

Oray, M., Abu Samra, K., Ebrahimiadib, N., Meese, H. and Foster, C.S. (2016) 'Long-term side effects of glucocorticoids', *Expert opinion on drug safety*, 15(4), pp. 457–465. Available at: https://doi.org/10.1517/14740338.2016.1140743.

Oui, H., Jeon, S., Lee, G., Park, S., Cho, K.O. and Choi, J. (2015) 'Tissue Doppler and strain imaging of left ventricle in Beagle dogs with iatrogenic hypercortisolism', *Journal of Veterinary Science*, 16(3), pp. 357–365. Available at: https://doi.org/10.4142/JVS.2015.16.3.357.

Panoulas, V.F., Douglas, K.M.J., Stavropoulos-Kalinoglou, A., Metsios, G.S., Nightingale, P., Kita, M.D., Elisaf, M.S. and Kitas, G.D. (2008) 'Long-term exposure to medium-dose glucocorticoid therapy associates with hypertension in patients with rheumatoid arthritis', *Rheumatology (Oxford, England)*, 47(1), pp. 72–75. Available at: https://doi.org/10.1093/RHEUMATOLOGY/KEM311.

Parikh, K.S., Sharma, K., Fiuzat, M., Surks, H.K., George, J.T., Honarpour, N., Depre, C., Desvigne-Nickens, P., Nkulikiyinka, R., Lewis, G.D., Gomberg-Maitland, M., O'Connor, C.M., Stockbridge, N., Califf, R.M., Konstam, M.A., Januzzi, J.L., Solomon, S.D., Borlaug, B.A., Shah, S.J., Redfield, M.M. and Felker, G.M. (2018) 'Heart Failure With Preserved Ejection Fraction Expert Panel Report: Current Controversies and Implications for Clinical Trials', *JACC: Heart Failure*, 6(8), pp. 619–632. Available at: https://doi.org/10.1016/J.JCHF.2018.06.008.

Paulus, W.J. and Tschöpe, C. (2013) 'A novel paradigm for heart failure with preserved ejection fraction: comorbidities drive myocardial dysfunction and remodeling through coronary microvascular endothelial inflammation', *Journal of the American College of Cardiology*, 62(4), pp. 263–271. Available at: https://doi.org/10.1016/J.JACC.2013.02.092.

Peer, N., Steyn, K. and Levitt, N. (2016) 'Differential obesity indices identify the metabolic syndrome in Black men and women in Cape Town: the CRIBSA study', *Journal of public health (Oxford, England)*, 38(1), pp. 175–182. Available at: https://doi.org/10.1093/PUBMED/FDU115.

Pereira, A.M., Delgado, V., Romijn, J.A., Smit, J.W.A., Bax, J.J. and Feelders, R.A. (2010) 'Cardiac dysfunction is reversed upon successful treatment of Cushing's syndrome', *European Journal of Endocrinology*, 162(2), pp. 331–340. Available at: https://doi.org/10.1530/EJE-09-0621.

Petersen, M.C. and Shulman, G.I. (2018) ‘Mechanisms of Insulin Action and Insulin Resistance’, *Physiological reviews*, 98(4), pp. 2133–2223. Available at: https://doi.org/10.1152/PHYSREV.00063.2017.

Pfeffer, M.A., Shah, A.M. and Borlaug, B.A. (2019) ‘Heart Failure With Preserved Ejection Fraction In Perspective’, *Circulation research*, 124(11), pp. 1598–1617. Available at: https://doi.org/10.1161/CIRCRESAHA.119.313572.

Pinto, Y.M., Elliott, P.M., Arbustini, E., Adler, Y., Anastasakis, A., Böhm, M., Duboc, D., Gimeno, J., de Groote, P., Imazio, M., Heymans, S., Klingel, K., Komajda, M., Limongelli, G., Linhart, A., Mogensen, J., Moon, J., Pieper, P.G., Seferovic, P.M., Schueler, S., Zamorano, J.L., Caforio, A.L.P. and Charron, P. (2016) ‘Proposal for a revised definition of dilated cardiomyopathy, hypokinetic non-dilated cardiomyopathy, and its implications for clinical practice: a position statement of the ESC working group on myocardial and pericardial diseases’, *European heart journal*, 37(23), pp. 1850–1858. Available at: https://doi.org/10.1093/EURHEARTJ/EHV727.

Pop-Busui, R. and Pietropaolo, M. (2011) ‘Metabolic Syndrome and Inflammation’, in *Immunoendocrinology: Scientific and Clinical Aspects*. Humana Press, pp. 69–92. Available at: https://doi.org/10.1007/978-1-60327-478-4_5.

Prince, P.D., Santander, Y.A., Gerez, E.M., Höcht, C., Polizio, A.H., Mayer, M.A., Taira, C.A., Fraga, C.G., Galleano, M. and Carranza, A. (2017) ‘Fructose increases corticosterone production in association with NADPH metabolism alterations in rat epididymal white adipose tissue’, *The Journal of Nutritional Biochemistry*, 46, pp. 109–116. Available at: https://doi.org/10.1016/J.JNUTBIO.2017.02.021.

Rachdaoui, N. (2020) ‘Insulin: The Friend and the Foe in the Development of Type 2 Diabetes Mellitus’, *International journal of molecular sciences*, 21(5), pp. 1–21. Available at: https://doi.org/10.3390/IJMS21051770.

Reaven, GM. (1988) ‘Banting lecture 1988. Role of insulin resistance in human disease’. *Diabetes*, 37(12), pp. 1595–1607.

Reddy, P., Lent-Schochet, D., Ramakrishnan, N., McLaughlin, M. and Jialal, I. (2019) ‘Metabolic syndrome is an inflammatory disorder: A conspiracy between adipose tissue and phagocytes’, *Clinica chimica acta; international journal of clinical chemistry*, 496, pp. 35–44. Available at: https://doi.org/10.1016/J.CCA.2019.06.019.

Reddy, Y.N.V. and Borlaug, B.A. (2020) ‘Heart Failure With Preserved Ejection Fraction: Where Do We Stand?’, *Mayo Clinic proceedings*, 95(4), pp. 629–631. Available at: https://doi.org/10.1016/J.MAYOCP.2020.02.015.

Reinehr, T., de Sousa, G., Toschke, A.M. and Andler, W. (2007) ‘Comparison of metabolic syndrome prevalence using eight different definitions: a critical approach’, *Archives of Disease in Childhood*, 92(12), p. 1067. Available at: https://doi.org/10.1136/ADC.2006.104588.

Ren, R., Oakley, R.H., Cruz-Topete, D. and Cidlowski, J.A. (2012) ‘Dual role for glucocorticoids in cardiomyocyte hypertrophy and apoptosis’, *Endocrinology*, 153(11), pp. 5346–5360. Available at: https://doi.org/10.1210/EN.2012-1563.

Reul, J.M.H.M. and de Kloet, E.R. (1985) ‘Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation’, *Endocrinology*, 117(6), pp. 2505–2511. Available at: https://doi.org/10.1210/ENDO-117-6-2505.

Rook, G.A.W. (1999) ‘Glucocorticoids and immune function’, *Bailliere’s best practice & research. Clinical endocrinology & metabolism*, 13(4), pp. 567–581. Available at: https://doi.org/10.1053/BEEM.1999.0044.

Roy, S.G., De, P., Mukherjee, D., Chander, V., Konar, A., Bandyopadhyay, D. and Bandyopadhyay, A. (2009) ‘Excess of glucocorticoid induces cardiac dysfunction via activating angiotensin II pathway’, *Cellular Physiology and Biochemistry*, 24(1–2), pp. 1–10. Available at: https://doi.org/10.1159/000227803.

Rutledge, A.C. and Adeli, K. (2007) ‘Fructose and the metabolic syndrome: pathophysiology and molecular mechanisms’, *Nutrition reviews*, 65(6 Pt 2). Available at: https://doi.org/10.1111/J.1753-4887.2007.TB00322.X.

Sadowska, J. and Rygielska, M. (2019) ‘The effect of high fructose corn syrup on the plasma insulin and leptin concentration, body weight gain and fat accumulation in rat’, *Advances in Clinical and Experimental Medicine*, 28(7), pp. 879–884. Available at: https://doi.org/10.17219/acem/94069.

Sahn, D.J., Demaria, A., Kisslo, J. and Weyman, A. (1979) *Recommendations Regarding Quantitation in M-Mode Echocardiography: Results of a Survey of Echocardiographic Measurements The Committee On M-Mode Standardization Of The American Society Of Echocardiography with the technical assistance of Melissa Lebedeck*. Available at: http://ahajournals.org.

Saltiel, A.R. and Kahn, C.R. (2001) ‘Insulin signalling and the regulation of glucose and lipid metabolism’, *Nature*, 414(6865), pp. 799–806. Available at: https://doi.org/10.1038/414799A.

de Salvi Guimarães, F., de Moraes, W.M.A.M., Bozi, L.H.M., Souza, P.R., Antonio, E.L., Bocalini, D.S., Tucci, P.J.F., Ribeiro, D.A., Brum, P.C. and Medeiros, A. (2017) ‘Dexamethasone-

induced cardiac deterioration is associated with both calcium handling abnormalities and calcineurin signaling pathway activation', *Molecular and Cellular Biochemistry*, 424(1–2), pp. 87–98. Available at: https://doi.org/10.1007/s11010-016-2846-3.

Sangüesa, G., Shaligram, S., Akther, F., Roglans, N., Laguna, J.C., Rahimian, R. and Alegret, M. (2017) 'Type of supplemented simple sugar, not merely calorie intake, determines adverse effects on metabolism and aortic function in female rats', *American journal of physiology. Heart and circulatory physiology*, 312(2), pp. H289–H304. Available at: https://doi.org/10.1152/AJPHEART.00339.2016.

Sarafidis, P.A. and Nilsson, P.M. (2006) 'The metabolic syndrome: a glance at its history', *Journal of hypertension*, 24(4), pp. 621–626. Available at: https://doi.org/10.1097/01.HJH.0000217840.26971.B6.

Sardana, K. and Sachdeva, S. (2022) 'Update on pharmacology, actions, dosimetry and regimens of oral glucocorticoids in dermatology', *Journal of cosmetic dermatology*, 21(11). Available at: https://doi.org/10.1111/JOCD.15108.

Satou, R., Penrose, H. and Navar, L.G. (2018) 'Inflammation as a Regulator of the Renin-Angiotensin System and Blood Pressure', *Current hypertension reports*, 20(12). Available at: https://doi.org/10.1007/S11906-018-0900-0.

Schäcke, H., Döcke, W.D. and Asadullah, K. (2002) 'Mechanisms involved in the side effects of glucocorticoids', *Pharmacology and Therapeutics*, 96(1), pp. 23–43. Available at: https://doi.org/10.1016/S0163-7258(02)00297-8.

Seckl, J.R. (2004) '11β-hydroxysteroid dehydrogenases: changing glucocorticoid action', *Current Opinion in Pharmacology*, 4(6), pp. 597–602. Available at: https://doi.org/10.1016/J.COPH.2004.09.001.

Selyatitskaya, V.G., Kuz'minova, O.I. and Odintsov, S. v. (2002) 'Development of insulin resistance in experimental animals during long-term glucocorticoid treatment', *Bulletin of experimental biology and medicine*, 133(4), pp. 339–341. Available at: https://doi.org/10.1023/A:1016281501423.

Semnani-Azad, Z., Khan, T.A., Blanco Mejia, S., de Souza, R.J., Leiter, L.A., Kendall, C.W.C., Hanley, A.J. and Sievenpiper, J.L. (2020) 'Association of Major Food Sources of Fructose-Containing Sugars With Incident Metabolic Syndrome: A Systematic Review and Meta-analysis', *JAMA network open*, 3(7). Available at: https://doi.org/10.1001/JAMANETWORKOPEN.2020.9993.

Senesi, S., Legeza, B., Balázs, Z., Csala, M., Marcolongo, P., Kereszturi, É., Szelényi, P., Egger, C., Fulceri, R., Mandl, J., Giunti, R., Odermatt, A., Bánhegyi, G. and Benedetti, A. (2010) 'Contribution of fructose-6-phosphate to glucocorticoid activation in the endoplasmic reticulum: possible implication in the metabolic syndrome', *Endocrinology*, 151(10), pp. 4830–4839. Available at: https://doi.org/10.1210/EN.2010-0614.

Seppälä-Lindroos, A., Vehkavaara, S., Häkkinen, A.-M., Goto, T., Westerbacka, J., Sovijärvi, A., Halavaara, J. and Yki-Järvinen, H. (2002) 'Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men', *The Journal of clinical endocrinology and metabolism*, 87(7), pp. 3023–3028. Available at: https://doi.org/10.1210/JCEM.87.7.8638.

Seravalle, G. and Grassi, G. (2016) 'Sympathetic Nervous System, Hypertension, Obesity and Metabolic Syndrome', *High blood pressure & cardiovascular prevention : the official journal of the Italian Society of Hypertension*, 23(3), pp. 175–179. Available at: https://doi.org/10.1007/S40292-016-0137-4.

Shah, A.M. (2013) ‘Ventricular remodeling in heart failure with preserved ejection fraction’, *Current Heart Failure Reports*, 10(4), pp. 341–349. Available at: https://doi.org/10.1007/s11897-013-0166-4.

Shah, S.J., Borlaug, B.A., Kitzman, D.W., McCulloch, A.D., Blaxall, B.C., Agarwal, R., Chirinos, J.A., Collins, S., Deo, R.C., Gladwin, M.T., Granzier, H., Hummel, S.L., Kass, D.A., Redfield, M.M., Sam, F., Wang, T.J., Desvigne-Nickens, P. and Adhikari, B.B. (2020) ‘Research Priorities for Heart Failure With Preserved Ejection Fraction: National Heart, Lung, and Blood Institute Working Group Summary’, *Circulation*, 141(12), pp. 1001–1026. Available at: https://doi.org/10.1161/CIRCULATIONAHA.119.041886.

Serezani, C.H., Ballinger, M.N., Aronoff, D.M. and Peters-Golden, M. (2008) ‘Cyclic AMP: Master Regulator of Innate Immune Cell Function’. *American Journal of Respiratory Cell and Molecular Biology*, 39, pp.127-132. Available at: https://doi.org/10.1165/rcmb.2008-0091TR

Shah, S.J., Katz, D.H., Selvaraj, S., Burke, M.A., Yancy, C.W., Gheorghiade, M., Bonow, R.O., Huang, C.C. and Deo, R.C. (2015) ‘Phenomapping for novel classification of heart failure with preserved ejection fraction’, *Circulation*, 131(3), pp. 269–279. Available at: https://doi.org/10.1161/CIRCULATIONAHA.114.010637.

Sharma, A., Razaghizad, A., Ferreira, J.P., Machu, J.L., Bozec, E., Girerd, N., Rossignol, P. and Zannad, F. (2022) ‘Metabolic Syndrome and the Risk of Preclinical Heart Failure: Insights after 17 Years of Follow-Up from the STANISLAS Cohort’, *Cardiology*, 147(3), pp. 281–287. Available at: https://doi.org/10.1159/000523717.

Sharma, K. and Kass, D.A. (2014) ‘Heart failure with preserved ejection fraction: mechanisms, clinical features, and therapies’, *Circulation research*, 115(1), pp. 79–96. Available at: https://doi.org/10.1161/CIRCRESAHA.115.302922.

da Silva, A.A., do Carmo, J.M., Li, X., Wang, Z., Mouton, A.J. and Hall, J.E. (2020) 'Role of Hyperinsulinemia and Insulin Resistance in Hypertension: Metabolic Syndrome Revisited', *The Canadian journal of cardiology*, 36(5), pp. 671–682. Available at: https://doi.org/10.1016/J.CJCA.2020.02.066.

Smith, S.A., Tobias, A., Fine, D.M., Jacob, K.A. and Ployngam, T. (2004) 'Corticosteroid-Associated Congestive Heart Failure in 12 Cats'.

Softic, S., Stanhope, K.L., Boucher, J., Divanovic, S., Lanaspa, M.A., Johnson, R.J. and Kahn, C.R. (2020) 'Fructose and hepatic insulin resistance', *Critical reviews in clinical laboratory sciences*, 57(5), pp. 308–322. Available at: https://doi.org/10.1080/10408363.2019.1711360.

Souverein, P.C., Berard, A., van Staa, T.P., Cooper, C., Egberts, A.C.G., Leufkens, H.G.M. and Walker, B.R. (2004) 'Use of oral glucocorticoids and risk of cardiovascular and cerebrovascular disease in a population based case-control study', *Heart (British Cardiac Society)*, 90(8), pp. 859–865. Available at: https://doi.org/10.1136/HRT.2003.020180.

Spinale, F.G. (2002) 'Matrix Metalloproteinases', *Circulation Research*, 90(5), pp. 520–530. Available at: https://doi.org/10.1161/01.RES.0000013290.12884.A3.

Standl, E. (2005) 'Aetiology and consequences of the metabolic syndrome', *European Heart Journal Supplements*, 7(suppl_D), pp. D10–D13. Available at: https://doi.org/10.1093/EURHEARTJ/SUI023.

Stanhope, K.L. and Havel, P.J. (2008) 'Fructose consumption: Potential mechanisms for its effects to increase visceral adiposity and induce dyslipidemia and insulin resistance', *Current opinion in lipidology*, 19(1), pp. 16–24. Available at: https://doi.org/10.1097/MOL.0B013E3282F2B24A.

Statistics South Africa (2015) *Statistical release Mortality and causes of death in South Africa, 2014: Findings from death notification*. Available at: www.statssa.gov.za.

Stout, A., Friedly, J. and Standaert, C.J. (2019) 'Systemic Absorption and Side Effects of Locally Injected Glucocorticoids.', *PM & R : the Journal of Injury, Function, and Rehabilitation*, 11(4), pp. 409–419. Available at: https://doi.org/10.1002/PMRJ.12042.

Strack, V., Stoyanov, B., Bossenmaier, B., Mosthaf, L., Kellerer, M. and Häring, H.U. (1997) 'Impact of mutations at different serine residues on the tyrosine kinase activity of the insulin receptor', *Biochemical and biophysical research communications*, 239(1), pp. 235–239. Available at: https://doi.org/10.1006/BBRC.1997.7457.

Takagi, S., Tanabe, A., Tsuiki, M., Naruse, M. and Takano, K. (2009) 'Hypokalemia, diabetes mellitus, and hypercortisolemia are the major contributing factors to cardiac dysfunction in adrenal Cushing's syndrome', *Endocrine journal*, 56(8), pp. 1009–1018. Available at: https://doi.org/10.1507/ENDOCRJ.K09E-198.

Takano, H., Kokubu, A., Sugimoto, K., Sunahara, H., Aoki, T. and Fijii, Y. (2015) 'Left ventricular structural and functional abnormalities in dogs with hyperadrenocorticism.', *Journal of Veterinary Cardiology : the Official Journal of the European Society of Veterinary Cardiology*, 17(3), pp. 173–181. Available at: https://doi.org/10.1016/J.JVC.2015.07.002.

Takimoto, E. (2012) 'Cyclic GMP-dependent signaling in cardiac myocytes', *Circulation journal : official journal of the Japanese Circulation Society*, 76(8), pp. 1819–1825. Available at: https://doi.org/10.1253/CIRCJ.CJ-12-0664.

Tan, Y.T., Wenzelburger, F., Lee, E., Heatlie, G., Leyva, F., Patel, K., Frenneaux, M. and Sanderson, J.E. (2009) 'The pathophysiology of heart failure with normal ejection fraction: exercise echocardiography reveals complex abnormalities of both systolic and diastolic ventricular function involving torsion, untwist, and longitudinal motion', *Journal of the*

American College of Cardiology, 54(1), pp. 36–46. Available at: https://doi.org/10.1016/J.JACC.2009.03.037.

Tanaka, S., Shibuya, H., Suzuki, S., Kanno, N., Harada, Y., Sato, A., Soeta, S. and Hara, Y. (2021) 'Long-term administration of prednisolone: Effects on the myocardial tissue of healthy beagle dogs', *The Journal of veterinary medical science*, 83(1), pp. 84–93. Available at: https://doi.org/10.1292/JVMS.20-0401.

Tappy, L. and Le, K.A. (2010) 'Metabolic effects of fructose and the worldwide increase in obesity', *Physiological reviews*, 90(1), pp. 23–46. Available at: https://doi.org/10.1152/PHYSREV.00019.2009.

Tasker, J.G., Di, S. and Malcher-Lopes, R. (2005) 'Rapid central corticosteroid effects: evidence for membrane glucocorticoid receptors in the brain', *Integrative and comparative biology*, 45(4), pp. 665–671. Available at: https://doi.org/10.1093/ICB/45.4.665.

Taskinen, M.R., Packard, C.J. and Borén, J. (2019) 'Dietary Fructose and the Metabolic Syndrome', *Nutrients*, 11(9). Available at: https://doi.org/10.3390/NU11091987.

Tatomir, A., Micu, C. and Crivii, C. (2014) 'The impact of stress and glucocorticoids on memory', *Clujul medical (1957)*, 87(1), pp. 3–6. Available at: https://doi.org/10.15386/CJM.2014.8872.871.AT1CM2.

Teichholz, L.E., Kreulen, T., Herman, M. v. and Gorlin, R. (1976) 'Problems in echocardiographic volume determinations: Echocardiographic-angiographic correlations in the presence or absence of asynergy', *The American Journal of Cardiology*, 37(1), pp. 7–11. Available at: https://doi.org/10.1016/0002-9149(76)90491-4.

Thau, L., Gandhi, J. and Sharma, S. (2022) *Physiology, Cortisol*, *StatPearls*. StatPearls Publishing. Available at: http://www.ncbi.nlm.nih.gov/pubmed/30855827 (Accessed: 8 November 2022).

Thiemermann, C. (2002) 'Corticosteroids and cardioprotection', *Nature medicine*, 8(5), pp. 453–455. Available at: https://doi.org/10.1038/NM0502-453.

Thomas, N.E., Rowe, D.A., Murtagh, E.M., Stephens, J.W. and Williams, R. (2018) 'Associations between metabolic syndrome components and markers of inflammation in Welsh school children', *European journal of pediatrics*, 177(3), pp. 409–417. Available at: https://doi.org/10.1007/S00431-017-3065-Y.

Timmermans, S., Souffriau, J. and Libert, C. (2019) 'A General Introduction to Glucocorticoid Biology', *Frontiers in immunology*, 10(JULY). Available at: https://doi.org/10.3389/FIMMU.2019.01545.

Toja, P.M., Branzi, G., Ciambellotti, F., Radaelli, P., de Martin, M., Lonati, L.M., Scacchi, M., Parati, G., Cavagnini, F. and Giraldi, F.P. (2012) 'Clinical relevance of cardiac structure and function abnormalities in patients with Cushing's syndrome before and after cure', *Clinical endocrinology*, 76(3), pp. 332–338. Available at: https://doi.org/10.1111/J.1365-2265.2011.04206.X.

Tokudome, S., Sano, M., Shinmura, K., Matsuhashi, T., Morizane, S., Moriyama, H., Tamaki, K., Hayashida, K., Nakanishi, H., Yoshikawa, N., Shimizu, N., Endo, J., Katayama, T., Murata, M., Yuasa, S., Kaneda, R., Tomita, K., Eguchi, N., Urade, Y., Asano, K., Utsunomiya, Y., Suzuki, T., Taguchi, R., Tanaka, H. and Fukuda, K. (2009) 'Glucocorticoid protects rodent hearts from ischemia/reperfusion injury by activating lipocalin-type prostaglandin D synthase-derived PGD2 biosynthesis', *Journal of Clinical Investigation*, 119(6), pp. 1477–1488. Available at: https://doi.org/10.1172/JCI37413.

Tran, A., Gelaye, B., Girma, B., Lemma, S., Berhane, Y., Bekele, T., Khali, A. and Williams, M.A. (2011) 'Prevalence of metabolic syndrome among working adults in Ethiopia', *International Journal of Hypertension*, 2011. Available at: https://doi.org/10.4061/2011/193719.

Tsai, C.C., Tiao, M.M., Sheen, J.M., Huang, L.T., Tain, Y.L., Lin, I.C., Lin, Y.J., Lai, Y.J., Chen, C.C., Chang, K.A. and Yu, H.R. (2019) 'Obesity programmed by prenatal dexamethasone and postnatal high-fat diet leads to distinct alterations in nutrition sensory signals and circadian-clock genes in visceral adipose tissue', *Lipids in Health and Disease*, 18(1). Available at: https://doi.org/10.1186/S12944-019-0963-1.

Tune, J.D., Goodwill, A.G., Sassoon, D.J. and Mather, K.J. (2017) 'Cardiovascular Consequences of Metabolic Syndrome'. Available at: https://doi.org/10.1016/j.trsl.2017.01.001.

Unger, R.H., Clark, G.O., Scherer, P.E. and Orci, L. (2010) 'Lipid homeostasis, lipotoxicity and the metabolic syndrome', *Biochimica et biophysica acta*, 1801(3), pp. 209–214. Available at: https://doi.org/10.1016/J.BBALIP.2009.10.006.

do Vale Moreira, N.C., Hussain, A., Bhowmik, B., Mdala, I., Siddiquee, T., Fernandes, V.O., Montenegro Júnior, R.M. and Meyer, H.E. (2020) 'Prevalence of Metabolic Syndrome by different definitions, and its association with type 2 diabetes, pre-diabetes, and cardiovascular disease risk in Brazil', *Diabetes and Metabolic Syndrome: Clinical Research and Reviews*, 14(5), pp. 1217–1224. Available at: https://doi.org/10.1016/j.dsx.2020.05.043.

Vale, W., Spiess, J., Rivier, C. and Rivier, J. (1981) 'Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin', *Science (New York, N.Y.)*, 213(4514), pp. 1394–1397. Available at: https://doi.org/10.1126/SCIENCE.6267699.

Varma, V., Boros, L.G., Nolen, G.T., Chang, C.W., Wabitsch, M., Beger, R.D. and Kaput, J. (2015) 'Metabolic fate of fructose in human adipocytes: a targeted 13C tracer fate association study', *Metabolomics*, 11(3), pp. 529–544. Available at: https://doi.org/10.1007/S11306-014-0716-0.

Vegiopoulos, A. and Herzig, S. (2007) 'Glucocorticoids, metabolism and metabolic diseases', *Molecular and cellular endocrinology*, 275(1–2), pp. 43–61. Available at: https://doi.org/10.1016/J.MCE.2007.05.015.

Viegas, L.R., Hoijman, E., Beato, M. and Pecci, A. (2008) 'Mechanisms involved in tissue-specific apopotosis regulated by glucocorticoids', *The Journal of steroid biochemistry and molecular biology*, 109(3–5), pp. 273–278. Available at: https://doi.org/10.1016/J.JSBMB.2008.03.007.

Villarroya, F., Cereijo, R., Gavaldà-Navarro, A., Villarroya, J. and Giralt, M. (2018) 'Inflammation of brown/beige adipose tissues in obesity and metabolic disease', *Journal of internal medicine*, 284(5), pp. 492–504. Available at: https://doi.org/10.1111/JOIM.12803.

Wang, L., Liu, J., Zhang, A., Cheng, P., Zhang, X., Lv, S., Wu, L., Yu, J., Di, W., Zha, J., Kong, X., Qi, H., Zhong, Y. and Ding, G. (2012) 'BVT.2733, a Selective 11β-Hydroxysteroid Dehydrogenase Type 1 Inhibitor, Attenuates Obesity and Inflammation in Diet-Induced Obese Mice', *PLOS ONE*, 7(7), p. e40056. Available at: https://doi.org/10.1371/JOURNAL.PONE.0040056.

Wei, Y. and Pagliassotti, M.J. (2004) 'Hepatospecific effects of fructose on c-jun NH2-terminal kinase: implications for hepatic insulin resistance', *American journal of physiology. Endocrinology and metabolism*, 287(5). Available at: https://doi.org/10.1152/AJPENDO.00185.2004.

Weisberg, S.P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R.L. and Ferrante, A.W. (2003) 'Obesity is associated with macrophage accumulation in adipose tissue', *The Journal of clinical investigation*, 112(12), pp. 1796–1808. Available at: https://doi.org/10.1172/JCI19246.

Whirledge, S. and Cidlowski, J.A. (2017) 'Glucocorticoids and Reproduction: Traffic Control on the Road to Reproduction', *Trends in endocrinology and metabolism: TEM*, 28(6), pp. 399–415. Available at: https://doi.org/10.1016/J.TEM.2017.02.005.

Whitworth, J.A., Mangos, G.J. and Kelly, J.J. (2000) 'Cushing, cortisol, and cardiovascular disease', *Hypertension (Dallas, Tex.: 1979)*, 36(5), pp. 912–916. Available at: https://doi.org/10.1161/01.HYP.36.5.912.

Wilcox, G. (2005) 'Insulin and Insulin Resistance', *Clinical Biochemist Reviews*, 26(2), p. 19. Available at: /pmc/articles/PMC1204764/ (Accessed: 8 November 2022).

World Health Organisation, 2019. World Health Statistics 2019. Luxemburg: World Health Organisation.

Wu, C.K., Lee, J.K., Hsu, J.C., Su, M.Y.M., Wu, Y.F., Lin, T.T., Lan, C.W., Hwang, J.J. and Lin, L.Y. (2020) 'Myocardial adipose deposition and the development of heart failure with preserved ejection fraction', *European Journal of Heart Failure*, 22(3), pp. 445–454. Available at: https://doi.org/10.1002/EJHF.1617.

Xu, H., Li, X., Adams, H., Kubena, K. and Guo, S. (2019) 'Etiology of metabolic syndrome and dietary intervention', *International Journal of Molecular Sciences*. MDPI AG. Available at: https://doi.org/10.3390/ijms20010128.

Yancy, C.W., Jessup, M., Bozkurt, B., Butler, J., Casey, D.E., Drazner, M.H., Fonarow, G.C., Geraci, S.A., Horwich, T., Januzzi, J.L., Johnson, M.R., Kasper, E.K., Levy, W.C.,

Masoudi, F.A., McBride, P.E., McMurray, J.J.V., Mitchell, J.E., Peterson, P.N., Riegel, B., Sam, F., Stevenson, L.W., Tang, W.H.W., Tsai, E.J. and Wilkoff, B.L. (2013) '2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines', *Journal of the American College of Cardiology*, 62(16). Available at: https://doi.org/10.1016/J.JACC.2013.05.019.

Yasir, M., Goyal, A. and Sonthalia, S. (2022) 'Corticosteroid Adverse Effects', *StatPearls* [Preprint]. Available at: https://www.ncbi.nlm.nih.gov/books/NBK531462/ (Accessed: 17 January 2023).

Yiu, K.H., Marsan, N.A., Delgado, V., Biermasz, N.R., Holman, E.R., Smit, J.W.A., Feelders, R.A., Bax, J.J. and Pereira, A.M. (2012) 'Increased myocardial fibrosis and left ventricular dysfunction in Cushing's syndrome', *European Journal of Endocrinology*, 166(1), pp. 27–34. Available at: https://doi.org/10.1530/EJE-11-0601.

Young, M.J., Lam, E.Y.M. and Rickard, A.J. (2007) 'Mineralocorticoid receptor activation and cardiac fibrosis', *Clinical science (London, England : 1979)*, 112(9), pp. 467–475. Available at: https://doi.org/10.1042/CS20060275.

Yu, C., Chen, Y., Cline, G.W., Zhang, D., Zong, H., Wang, Y., Bergeron, R., Kim, J.K., Cushman, S.W., Cooney, G.J., Atcheson, B., White, M.F., Kraegen, E.W. and Shulman, G.I. (2002) 'Mechanism by which fatty acids inhibit insulin activation of insulin receptor substrate-1 (IRS-1)-associated phosphatidylinositol 3-kinase activity in muscle', *The Journal of biological chemistry*, 277(52), pp. 50230–50236. Available at: https://doi.org/10.1074/JBC.M200958200.

Zhang, M., Li, Y., Wei, X., Tian, F., Ouyang, F., Zhao, S. and Liu, L. (2017) 'Indispensable role of lipoprotein bound-ApoE in adipogenesis and endocytosis induced by postprandial TRL',

Biochemical and Biophysical Research Communications, 493(1), pp. 298–305. Available at: https://doi.org/10.1016/j.bbrc.2017.09.027.

Zhang, Y. and Ren, J. (2011) 'Role of cardiac steatosis and lipotoxicity in obesity cardiomyopathy', *Hypertension (Dallas, Tex. : 1979)*, 57(2), pp. 148–150. Available at: https://doi.org/10.1161/HYPERTENSIONAHA.110.164178.

Zhao, J., Wu, Y., Rong, X., Zheng, C. and Guo, J. (2020) 'Anti-lipolysis induced by insulin in diverse pathophysiologic conditions of adipose tissue', *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, 13, pp. 1575–1585. Available at: https://doi.org/10.2147/DMSO.S250699.

Zile, M.R. and Brutsaert, D.L. (2002) 'New concepts in diastolic dysfunction and diastolic heart failure: Part I: Diagnosis, prognosis, and measurements of diastolic function', *Circulation*, 105(11), pp. 1387–1393. Available at: https://doi.org/10.1161/hc1102.105289.

Appendices

Appendix A: Animal ethics certificate

ANIMALS RESEARCH ETHICS COMMITTEE (AREC)

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

STRICTLY CONFIDENTIAL

CLEARANCE CERTIFICATE NUMBER: 2021/04/06/C

APPLICANT: Dr L Mokotedi
School: School of Physiology; Department: N/A; Location: WRAF

PROJECT TITLE: Effect of inflammation, a high-fructose diet and anti-inflammatory drugs on cardiometabolic function in male Sprague Dawley rats exposed to collagen-induced arthritis.
Category: C; Species and Numbers involved: *90X 3 month old, male Sprague Dawley Rats*

Approval is hereby given for the use of animals for the research project named above and described in the application reviewed by a quorate meeting of the AREC held on 28 Apr 2021. This approval remains valid until 12 May 2023 and is conditional to the following (if blank there are no special conditions):

Condition 1	Condition 2	Condition 3	Condition 4

All material changes to the approved research must be reported to the AREC before they are implemented. Failure to do so will invalidate this clearance certificate.

An annual progress report must be provided to the AREC.

The use of these animals is subject to AREC guidelines on the use and care of laboratory animals, is limited to the procedures described in the application and is subject to additional conditions listed below:

I, the Chair of the AREC (or my designated representative) am satisfied that the proposed research is ethical as judged by local law, international standards and University policy.

Signed: _____ Date: 13 May 2021

(Deputy Chairperson of the AREC)

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

Signed: _____ Date: 13 May 2021

(Registered Veterinarian)

CC: Student supervisor: «Title1» «Initials1» «Supervisor_surname»
Director Wits Research Animal Facility (WRAF): Dr Kim Jardine

Appendix B: Animal ethics modifications and extensions of experiments

AESC 2012 M&E

Please note that only typewritten applications will be accepted.

UNIVERSITY OF THE WITWATERSRAND

ANIMAL ETHICS SCREENING COMMITTEE
MODIFICATIONS AND EXTENSIONS TO EXPERIMENTS

a. Name: Lebogang Mokotedi

b. Department: School of Physiology

c. Experiment to be modified / extended

		AESC NO	
Original AESC number	**2021**	**04**	**06C**
Other M&Es :			4

d. Project Title: **Effect of inflammation, a high fructose diet and anti-inflammatory drugs on cardiometabolic function in male Sprague Dawley rats exposed to collagen induced arthritis.**

		No.	Species
e.	Number and species of animals originally approved:	90	SD
f.	Number of additional animals previously allocated on M&Es:	20	
g.	Total number of animals allocated to the experiment to date:	90	
h.	Number of animals used to date:	90	

i. Specific modification / extension requested:
- addition of a group of animals receiving Prednisolone alone (n=10)

j. Motivation for modification / extension:

In the study design, we had a group with fructose alone and a group with prednisolone & fructose. From preliminary analysis, we have seen that the fasting blood glucose concentration is greater in the rats receiving prednisolone & fructose (4.87 ± 0.38) compared to the rats receiving fructose alone (4.49 ±0.39; p=0.03). As a result, we would like to measure the glucose concentration in a group with prednisolone alone to attest the additive effects of both interventions. We would therefore like to request 10 more SD rats. The duration of the interventions as well as all other procedures will remain unchanged.

Date: 10/12/2021

Signature: 

RECOMMENDATIONS Approved: 10 additional Sprague Dawley rats to receive prednisolone at dose used in comparative group.

Date: 15 December 2021

Signature: 
pp. AREC Chairman