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# The effects of lycopene on bone, kidney and pancreatic health of growing Wistar rats fed an obesogenic diet

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University of the Witwatersrand, Johannesburg

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# EFFECTS OF LYCOPENE ON BONE, KIDNEY AND PANCREATIC HEALTH OF GROWING WISTAR RATS FED A HIGH FRUCTOSE DIET

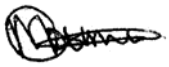
**Motlhale Gomotsegang**

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

Johannesburg, May 2024

## DECLARATION

I **Motlhale Gomotsegang** declare that this dissertation is my own. It is being submitted for the degree of Master of Science in Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University. I certify that all the experimental procedures used in this dissertation were approved by the Animal Ethics Screening Committee of the University of Witwatersrand (AESC number: 2022/03/02C)



**Motlhale Gomotsegang**

Signed on the 15th day May of 2024

# DEDICATION

In loving memory of my late father

Gabadise John Motlhale

1958 – 2013

## PRENTATIONS ARISING FROM THIS STUDY

**Gomotsegang Motlhale,** Nontobeko Gumede and Eliton Chivandi (2023). The Effects of lycopene on femora and tibia indices on Wistar rats fed a high fructose diet. The 14th Wits Postgraduate Cross-faculty Symposium, University of Witwatersrand, Johannesburg, South Africa, 6-8th September 2023.

**Gomotsegang Motlhale,** Nontobeko Gumede and Eliton Chivandi (2023). The effects of lycopene on femora and tibia and pancreatic health of growing Wistar rats fed a high-fructose diet. School of Physiology Research Day. University of Witwatersrand, Johannesburg, South Africa, 13th September 2023.

## PUBLICATIONS ARISING FROM THIS RESEARCH PROJECT

Currently none.

## ABSTRACT

Dietary fructose causes obesity, oxidative stress, and inflammation that compromise bone, kidney, and pancreatic health. Lycopene, a phytochemical, has antioxidant and anti-inflammatory properties. Its potential prophylactic effects on diet-induced bone, kidney and pancreatic derangements were evaluated in growing Wistar rats fed a high-fructose diet. Ninety-six 21-day old Wistar rat pups (48 females, 48 males) randomly allocated to treatment groups: I - standard rat chow (SRC) + plain drinking water (PDW) + plain gelatine cube (PGC), II - SRC + 20% fructose as drinking water (FDW) + PGC, III - SRC + FDW + 100mg/kg body mass fenofibrate per day (FF), IV - SRC + FDW + 30mg/kg body mass lycopene per day and V - SRC + FDW + 60mg/kg body mass lycopene per day and VI - SRC + FDW + 100mg/kg body mass lycopene per day were fed for 84 days. Body, kidneys, pancreata, femora and tibiae masses were determined. Kidney and pancreatic lipid content, pancreatic TBARS concentration, SOD and GPX1 activities and plasma CTX-1 and osteocalcin concentrations were measured. Femora and tibia, length, mid-shaft, sub-trochanteric medio-lateral, head and neck transverse, medio-lateral, and epicondylar diameters, maximum distal epiphyseal and proximal epiphyseal breadths and breaking strength were measured. Treatments had no effect on rats' pancreata and males' kidney masses ($P>0.05$). Fenofibrate increased the females' kidney masses ($P = 0.0056$). Rats' tibiae and females' femora masses were similar ($P>0.05$). CTX-1, osteocalcin and TBARS concentrations and SOD and GPX1 activities were similar ($P>0.05$). Treatments did not affect the rats' kidney and males' pancreatic lipid contents ($P>0.05$). Dietary fructose increased females' pancreatic lipid content ($P = 0.0048$). Lycopene prevented the fructose-induced increased pancreatic lipid content ($P = 0.0048$). Rats' tibiae and femora breaking strength and femora mid-shaft diameter (MIDD) of males were similar ($P>0.05$). Dietary fructose reduced the females' MIDD and males' sub-trochanteric medio-lateral diameter (STMLD) ($P>0.05$). Lycopene prevented the dietary fructose-induced MIDD decrease in females ($P = 0.0003$). Low dose lycopene decreased ($P = 0.0003$) the females' STMLD. Dietary fructose mediated sexually dimorphic effects and supplemental lycopene can protect females against fructose-induced pancreatic lipid accretion and MIDD reduction and males against STMLD reduction. In females low dose lycopene may increase the risk of femora fracturing through reduced STMLD.

## ACKNOWLEDGEMENTS

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**LIST OF ABBREVIATIONS**

| | |
|---|---|
| Acetyl-CoA | Acetyl coenzyme A |
| ANOVA | Analysis of variance |
| ATP | Adenosine triphosphate |
| ATP III | Adult treatment panel III |
| BMD | Bone mineral density |
| CHD | Coronary heart diseases |
| CKD | Chronic kidney disease |
| CTX-1 | C-telopeptide of type-1 collagen |
| DALY | Disability-adjusted life year |
| DNA | Deoxyribonucleic acid |
| DNL | *de novo* lipogenesis |
| EPB | Epiphyseal breadth |
| ER | Endoplasmic Reticulum |
| FDW | Fructose drinking water |
| FF | Fenofibrate per day |
| FFA | Free fatty acids |
| GLUT | Glucose transporters |
| GPX1 | Glutathione peroxidase |
| HTD | Head transverse diameter |
| ICR | Institute of cancer research |
| IR | Insulin resistance |
| ISN | International society of nephrology |

| | |
|---|---|
| LDL | Low density lipoprotein |
| MDA | Malondialdehyde |
| MDEB | Maximum distal epiphyseal breadth |
| MetS | Metabolic syndrome |
| MIDD | Mid-shaft diameter |
| MLD | Medio-lateral diameter |
| MPEB | Maximum proximal epiphyseal breadth |
| NAFLD | Non-alcoholic fatty liver diseases |
| NAFPD | Non-alcoholic fatty pancreatic disease |
| NCDs | Non-communicable diseases |
| NCEP | National cholesterol education programme |
| NTD | Neck transverse diameter |
| PBS | Phosphate buffered saline |
| PGC | Plain gelatine cube |
| PND | Post-natal day |
| ROS | Reactive oxygen species |
| SOD | Superoxide dismutase |
| SREBP-1c | Sterol regulatory element-binding protein 1 |
| STMLD | Sub-trochanteric medio-lateral diameter |
| T2DM | Type II diabetes mellitus |
| TBARS | Thiobarbituric Acid Reactants |
| TNF-α | Tumour necrosis alpha |
| UPR | Unfolded protein response |
| WHF | World heart federation |

| | |
|---|---|
| WHO | World health organisation |
| WOF | World obesity federation |
| XBP1 | X-box binding protein 1 |
| μCT | Micro-CT |

# CHAPTER 1: INTRODUCTION AND JUSTIFICATION

## 1.1 Introduction and justification

The consumption of unhealthy diets is one of the main causes of the global increase in metabolic diseases burden. According to the World Heart Federation, (WHF, 2008) unhealthy diets are characterised by diets low in fibre and high saturated and trans fatty acids content and carbonated and beverages sweetened with fructose. Such diets contribute to the development and increasing prevalence of non-communicable diseases (NCDs). These NCDs are lifestyle diseases that adversely affect and compromise human health globally. Such as metabolic diseases, which are highly associated with obesity, include coronary heart disease, metabolic syndrome, type 2 diabetes mellitus, chronic pancreas and renal disease, osteoporosis, and obesity (Baird *et al*., 2017). Obesity, a condition characterised by abnormal or excessive fat accumulation, predisposes individuals to an increased risk for the development of metabolic diseases resulting in ill-health (WHO, 2020). Visceral obesity is associated with high triglycerides level, insulin resistance (IR), microalbuminuria, low density lipoprotein (LDL), dyslipidaemia, type II diabetes and hyperglycaemia; all risk factors for metabolic syndrome (Alberti, 2005; Soiza *et al.*, 2018; Nilsson *et al.,* 2019; Macías *et al.,* 2021; Al Shehri *et al.*, 2022). Children are more likely to opt for snacks and fast food than choosing a balanced diet (Roblin, 2007). This unhealthy diet can impair the production and activity of immune cells and antibodies (Christ et al., 2019). According to the WHO approximately 2 million children under the age of five are overweight and 600 million adults are obese (WHO, 2020). The increased number of overweight children predisposes them to obesity. Obesity is a global pandemic causing an accumulation of an excess amount of body fats that induces a constellation of metabolic abnormalities that causes about 4 million deaths per annum (Otitoola *et al.,* 2021). Obesogenic diets containing fructose coupled with sedentary lifestyle are major drivers of obesity (Faruque *et al*., 2019). In addition to containing high levels of saturated and trans fats, the obesogenic diets are also characterised by fructose sweetened beverages.

Fructose, used as a sweetener, is a natural sugar found in fruits and honey but industrially produced from the hydrolysis of corn starch and used as a sweetening agent in the food industry (Tappy & Rosset, 2019). The consumption of fructose containing confectionery products and beverages is popular in developing countries, including Africa (Maarman & Madlala, 2016). South African children residing in urban settlements were reported to be the third most popular with the consumption of fructose-sweetened carbonated beverages (Audain *et al.,* 2019). The ingested fructose is metabolised in the liver but in a different way to glucose metabolism

(Taskinen *et al.*, 2019). According to Tappy and Rosset (2017), during fructolysis the majority of fructose is converted to glucose by gluconeogenesis, and little fructose is utilised for the synthesis of new fatty acids and triglycerides. However, with chronic consumption of a high-fructose diet, the metabolism of large amounts of dietary fructose favours *de novo* lipogenesis resulting in increased plasma triglycerides and marginal increase in plasma insulin and glucose (Ter Horst & Serlie, 2017). In female Sprague-Dawley rats, chronic consumption (12 weeks) of 20% fructose as drinking fluid induced the metabolic dysfunction characterised by visceral adiposity and hypertriglyceridemia (Gumede *et al*., 2020). Metabolic regulation and homeostasis involve the synergistic actions of several organs.

The pancreas controls metabolic homeostasis by secreting a variety of pancreatic hormones and digestive enzymes (Röder *et al.*, 2016). The abnormal deposition of fat in the pancreas as a result of dietary fructose in the absence of significant alcohol consumption can trigger the development of non-alcoholic fatty pancreatic disease (NAFPD) (Lesmana *et al*., 2015; Catanzaro *et al.*, 2016). Non-alcoholic fatty pancreatic disease is associated with increased incidence of obesity, a pandemic of global concern (Lazarus *et al*., 2022; Shah *et al*., 2019). *In vivo* studies have demonstrated that dietary fructose not only increases the production of free radicals but also reduces the activities of superoxide dismutase and glutathione peroxidase (Bratoeva *et al*., 2017; Dziadek *et al*., 2019). The excess reactive oxygen species (ROS) induced oxidative stress causes cellular apoptosis and increased lipid peroxidation in the pancreas and the kidneys (Topsakal *et al.*, 2016).

The kidneys eliminate metabolic wastes and preserve normal fluid and electrolyte balance (Silva & Mohebbi, 2022). Obesity and metabolic syndrome (MetS) are some potent risk factors for the development of chronic kidney disease (Kovesdy, 2022; Prasad *et al.*, 2022; Xiao *et al.*, 2022). The consumption of fructose-laden diets causes glomerulosclerosis, focal tubulointerstitial damage and kidney enlargement that can further deteriorate to chronic kidney diseases (Chapman *et al*., 2020; Pessoa *et al.*, 2020). Kidney diseases disrupt the normal balance of calcium and phosphorus important for bone function (Keung & Perwad, 2018).

Dietary fructose stimulates the absorption of calcium, magnesium, and phosphorus deposition in kidneys which disrupts bone mineral homeostasis (Bass *et al*., 2013). The high-fructose diet induced ROS mediates osteoblast and osteocyte apoptosis resulting in reduced osteogenesis and poor bone mineralisation (Agidigbi & Kim, 2019). Bacanli et al. (2017) have reported that

oxidative stress is associated with change in bone remodelling process increasing the fracture risk. In their study, Han et al. (2022) observed that chronic consumption of high-fructose caused a reduction in the thickness of the trabecular bones adversely affecting bone strength. Lifestyle induced metabolic derangements and diseases have to be managed to improve quality of life.

Various synthetic pharmaceutical agents are employed to manage the components of MetS, obesity, NAFPD, kidney and bone diseases (Kushner, 2018; Zhang *et al.*, 2021). However, such synthetic medications are generally inaccessible, mono-therapeutic, expensive and associated with increased side effects (Strang *et al.*, 2012). As a result, approximately 80% of the world's population prefers and depends on ethnomedicines for primary health care and to manage the multifaceted systemic ailments such as obesity and metabolic diseases (WHO, 2019). Medicinal plants are a source of plant-derived ethnomedicines that are either used as crude and/or purified extracts. These extracts contain phytochemicals that have been shown to possess health beneficial biological activities (Altemimi *et al*., 2017; Katiyar *et al.*, 2012; Nyakudya *et al.*, 2020) and lycopene is one such phytochemical.

Lycopene is a lipophilic carotenoid commonly present in significant concentrations in tomatoes and tomato based products, watermelons, apricot, cranberries and papaya (Cooperstone *et al.*, 2015; Imran *et al*., 2020). Studies have reported that lycopene has anti-hyperlipidaemia, anti-inflammatory and antioxidants effects (Caseiro *et al.*, 2020; Imran *et al.*, 2020; Mozos *et al.*, 2018; Salehi *et al.*, 2020). Lycopene has shown to exhibit reno-protective effects through its stimulation of increased GPX1 and SOD activities (Albrahim & Robert, 2022). In another study this phytochemical was reported to increase bone mineral density, downregulate the osteoclast differentiation and upregulate the osteoclast activity (Ardawi *et al*., 2016; Imran *et al.*, 2020). Furthermore, supplemental lycopene decreased diabetes caused pancreatic damage, and urine and blood glucose levels in streptozotocin-induced diabetic rats (Ozmen *et al*., 2016). Most studies that evaluated lycopene's effects on bone, kidney as well as pancreatic health used adult male rats and the ovariectomized female rats (Ardawi *et al.*, 2016; Mohammad and Amirhossein, 2018; Zheng *et al.*, 2019). The majority of these studies induced MetS using streptozotocin injections (Ozmen *et al.*, 2016; Mohammad & Amirhossein, 2018), a model that does not adequately mimic diet-induced metabolic derangements and disease. In addition, other previous studies evaluated the therapeutic potential and not its prophylactic potential. A comprehensive literature review has shown that no studies have assessed the potential

protective effects of lycopene using a rodent model that mimics children fed a calorie diet that specifically examined the benefits of the carotenoid on diet-induced metabolic derangement in the kidneys, pancreas, and bone.

## 1.2 Study aim and objectives

The study determined, in growing Wistar rats fed dietary fructose, the potential protective effects of lycopene given *per os* against possible bone, kidney, and pancreatic derangements. The study specifically evaluated the high-fructose diet and lycopene's effects on:

a) growth performance by measuring:
   - body mass
   - femora and tibia mas, length, and mass to length ratio.

b) bone health by specifically determining effects on:
   - femora mid-shaft diameter (MIDD), sub-trochanteric medio-lateral diameter (STMLD), head transverse diameter (HTD), neck transverse diameter (NTD) and epiphyseal breadth (EPB).
   - Tibia maximum distal epiphyseal breadth (MDEB), maximum proximal epiphyseal breadth (MPEB), and medio-lateral diameter (MLD).
   - tibia and femora breaking strength.
   - serum C-telopeptide of type-1 collagen (CTX-1) and osteocalcin concentration.

c) the pancreatic mass, fat content, oxidative stress and superoxide dismutase (SOD) and glutathione peroxidase (GPX1) activity

d) kidney mass and fat content.

## 1.3 Hypotheses

$H_0$: Orally administered lycopene as a dietary supplement does not affect the growth performance, femora and tibia, kidney, and pancreatic health of growing Wistar rats fed a high- fructose diet.

$H_1$: Orally administered lycopene as a dietary supplement affects the growth performance, femora and tibia, kidney and pancreatic health of growing Wistar rats fed a high- fructose diet.

Having outlined the background, problem statement and justification of the study and listed the study aim, objectives and hypothesis in this Chapter, the next chapter reviews literature pertinent to this study.

# CHAPTER 2: LITERATURE REVIEW

## 2.1 Introduction

Obesity, the excessive accumulation of body fat, imposes serious risks to the health status of an individual and is associated with reduced life expectancy (WHO, 2022; Dhurandhar, 2022; WOF, 2022). Obesity affects more than 1 billion people among them 39 million, 340 million and 650 million children, teenagers and adults, respectively (WHO, 2022). The World Obesity Federation (WOF) predicts an additional 10% rise in adult obesity by 2030 (WOF, 2022). Each year obesity or overweight cause about 2.8 million deaths and 35.8 million disability-adjusted life year (DALY) (WHO, 2022). The increase in obesity is attributed to unhealthy diets that contain high levels of saturated fats and sugars, especially fructose (Faruque *et al*., 2019; Maejima *et al*., 2020; Mai & Yan, 2019). When the dietary energy intake exceeds energy expenditure, an individual then has a chronic positive energy balance resulting in the excess energy being converted into triglycerides that are stored in the adipose tissues causing adipocyte hyperplasia which manifests in obesity (Chooi *et al.,* 2019). Visceral adipocytes release pro-inflammatory cytokines tumour necrosis factor alpha (TNF-α) (Coppack, 2001) and interleukin-6 (Rakotoarivelo *et al*., 2018). These inflammatory cytokines impair tissues, sensitivity to insulin which results in the expansion of the fat deposit depot (Morris *et al*., 2015). Obesity has been associated with altered bone-regulating hormones, bone cell metabolism and oxidative stress that adversely affect bone health (Shapses *et al*., 2017; Khanna *et al.,* 2022; Naomi *et al.,* 2023). Cellular apoptosis, collagen formation as well as fibrosis caused by oxidative stress result in pancreas and kidney damage (Topsakal *et al*., 2016). Catanzaro et al. (2016) reported obesity to be an important risk factor for the development of NAFPD that compromises the pancreatic health. Furthermore, obesity increases the risk of developing MetS (Fahed *et al*., 2022). A study by Al Shehri (2022) has reported that overweight and obese individuals tend to have a high incidence of MetS.

## 2.2 Metabolic dysfunction

The idea of "syndrome X", later called MetS, which was speculated to be a key factor in the development of coronary heart diseases (CHD) and type II diabetes mellitus (T2DM) mediated through target tissue resistance to insulin action was first proposed by Reaven (Reaven, 1988). The risk factors for MetS include high plasma triglycerides and LDL-cholesterol concentrations, T2DM, dyslipidaemia, hyperglycaemia microalbuminuria and IR that are commonly associated with abdominal obesity (Alberti, 2005; Soiza *et al.,* 2018; Nilsson *et al.,* 2019; Macías *et al.*, 2021; Al Shehri *et al.,* 2022). According to Adult Treatment Panel III (ATP

III) and the National Cholesterol Education Programme (NCEP) guidelines MetS is diagnosed when the patient has any three to five clinical identifiable risk factors (Alberti, 2005; Huang, 2009). In children and adolescents, MetS epidemic is driven by the increase in childhood obesity (Ludwig & Ebbeling, 2018; Powell *et al.*, 2018). Previous studies reported that MetS, which is driven by obesity, to also be associated with the development of chronic kidney disease (Lin *et al.*, 2015; G. R. Prasad, 2014; R. Prasad *et al.*, 2022). In Africa the prevalence of MetS is reported to be at 16.04% and this is expected to rise (Roomi & Sheikhupura-Pakistan, 2019). In addition to genetic predisposition, the main cause of increased prevalence of MetS including living sedentary lifestyles and the consumption of processed foods (Er *et al*., 2022). The metabolism of dietary fructose increases the risk of developing MetS (Aoun *et al*., 2022). It has now been demonstrated that increased intake of fructose, be it in beverages and or confectionery products, is one of the major underlying causes of chronic metabolic diseases, (Tappy, 2018b). Indeed Sánchez-Lozada et al. (2007) contend that high fructose diets induce MetS.

## 2.3 Fructose

Fructose is a monosaccharide that is used as a sweetening agent in the food industry (Zargaraan *et al.*, 2016). In nature it is found mainly in the honey, fruits, and vegetables (Sollid, 2022). In Western diets sugar-sweetened beverage which contain fructose constitute 15–17% of the daily energy intake (Taskinen *et al.*, 2019; Herman and Birnbaum, 2021). The per capita fructose consumpton globally has been estimated at 2kg (Kmietowicz, 2012). However, this has now increased to 25kg per person per year (Muriel *et al.*, 2021). In South Africa's urban settlements, fructose-sweetened carbonated soft drinks rank among those highly preferred by children (Audain *et al*., 2019) which exposes them to becoming overwight and to developing metabolic derangements including obesity.

### 2.3.1 Fructose metabolism

Approximately 60 to 70% of fructose is absorbed by glucose transporter 2 (GLUT2) in the liver and while 30-40% by the kidneys, adipose tissue and other organs (Gonçalves *et al.*, 2020). Following ingestion, fructose is transported through the gastrointestinal tract to the small intestines where it is absorbed by glucose transporter 5 (GLUT5) transporters in the absorptive enterocytes (Hannou *et al.*, 2018b; Taskinen *et al.*, 2019; Herman and Birnbaum, 2021). Fructose is transported by GLUT-2 from enterocytes through the portal vein to the liver (Ferraris *et al.*, 2018; Koepsell, 2020; Khan *et al.*, 2021; Muriel *et al.*, 2021), where it is

metabolized differently than glucose (Taskinen *et al.,* 2019). In the liver, fructose-1-phosphatase catalyses fructose conversion to two three-carbon phosphorylated molecules (Hannou *et al.,* 2018b; Taskinen *et al.,* 2019). Glyceraldehyde-3-phosphate and other triose phosphate compounds derived from fructolysis are resynthesised into glucose via gluconeogenesis or metabolised into lactate or CKD coenzyme A (acetyl-CoA), which are oxidised or used for lipogenesis (Taskinen *et al.,* 2019; Shi *et al.,* 2021). Therefore, depending on the cellular requirements, fructose has an effect on both glucose homeostasis and lipogenesis (Tappy, 2018a).

### 2.3.2 Fructose and metabolic dysfunction

#### *2.3.2.1 Fructose and pro-inflammatory and pro-oxidative state*

*In vivo* fructose has been shown to suppress hepatic β-fatty acid oxidation and in the process to induce endoplasmic reticulum (ER) stress and uric acid formation (Softic *et al.,* 2016; Jensen *et al.,* 2018). The increased uric acid synthesis is one of the mechanisms by which fructose increases ROS generation (Madlala *et al.,* 2016). Fructose is rapidly phosphorylated by ketohexokinase to fructose-1-phosphate increasing the flux of trioses towards lipogenesis and depleting adenosine triphosphate (ATP) stores (Nakagawa *et al*., 2020; Jensen *et al.*, 2018; Shi *et al.,* 2021). Cellular ATP depletion results in mitochondrial dysfunction and increased ER stress (Malhotra and Kaufman, 2011; Taskinen *et al.,* 2019). Endoplasmic reticulum stress stimulates unfolded protein response (UPR) which in turn mediates the adaptative signalling pathway to restore normal ER function (Almanza *et al.,* 2019; Malhotra & Kaufman, 2011). Apoptosis sets in when UPR fails to restore normal ER function (Malhotra & Kaufman, 2011). However, a persisted chronic UPR response exacerbates the pathophysiological situation by mediating inflammation, apoptosis, insulin resistance and lipotoxicity (Ghemrawi *et al.,* 2018; Taskinen *et al.,* 2019). The transcription factor X-box binding protein 1 (XBP1) is a key regulator of the UPR and it (XBP1) is activated by the ER stress (Huang *et al*., 2022). Furthermore, fructose stimulates a pro-oxidative and pro-inflammatory state by mediating increased free radicals synthesis which is coupled to decreased systematic antioxidant protection against oxidative stress (Dziadek *et al.,* 2019). Increased oxidative stress is a marker of an imbalance between the production of free radicals and systematic antioxidants' ability to quench ROS (Pizzino *et al.,* 2017a). High-fat diets have shown to promote oxidative stress and to lower plasma levels of antioxidants glutathione peroxidase (GPX1) and plasma superoxide dismutase (SOD), respectively (Lasker *et al.,* 2019).

*2.3.2.2 Fructose and lipid synthesis*

In contrast to glucose metabolism, fructose metabolism leads to the production of *de novo* lipogenesis (DNL) metabolites (Softic *et al.*, 2016). Fructose metabolism directly stimulates sterol regulatory element-binding protein 1 (SREBP-1c) a major transcriptional regulator of insulin resistance (Softic *et al.*, 2020). This membrane-bound transcription factor family is responsible for activating genes that code for the enzymes needed for the synthesis of unsaturated fatty acids and cholesterol (Sattari, 2013). In addition, the high-fructose diet induced oxidative stress of mitochondrion enhances generation of acetyl-CoA and citrate that are crucial substrates for some reactions such as fatty acids and cholesterol synthesis (Felix *et al.*, 2021). Ter Horst and Serlie (2017) stated that excessive dietary fructose intake results in minor increases in plasma glucose and insulin concentrations but mediates a major increase in plasma triglycerides concentration. Furthermore, dietary fructose suppresses hepatic β-fatty acid oxidation thus promoting lipid accumulation and DNL (Softic *et al.*, 2016; Jensen *et al.*, 2018). Wistar rats consuming a standard rat chow and 20% fructose solution as drinking fluid for 8 weeks had significantly increased abdominal adiposity and plasma triglyceride concentration (Ramos *et al.*, 2017). Gumede et al. (2020) also observed that consumption of a 20% fructose solution as drinking fluid induced MetS characterised by hypoadiponectinemia, hypertriglyceridemia and visceral adiposity in female Sprague Dawley rats. The increased lipids resulting from dietary fructose cause accumulation of fat around kidneys, liver and pancreas resulting in organ damage. Further to impacting lipid metabolism, dietary fructose also affects glucose homeostasis by impacting cellular sensitivity to insulin.

*2.3.2.3 Fructose and insulin resistance*

As previously mentioned, chronic intake of dietary fructose increases lipogenesis and impairs insulin-regulated suppression of glucose production in the liver (Herman & Samuel, 2016). Therefore, insulin resistance develops when fructose is enough to accelerate lipogenesis. Fructose has been associated with increased synthesis of mediators for insulin resistance that includes diacylglycerol, ceramides, acyl-carnitines, and free fatty acids (Delarue & Magnan, 2007; Sokolowska & Blachnio-Zabielska, 2019; Yang *et al*., 2019). Furthermore, development of insulin resistance can also be influenced by increasing free fatty acids synthesis (Softic *et al.*, 2020 ). Insulin resistance is linked to metabolic syndrome whose incidence and prevalence is increased in overweight and obese individuals (Wondmkun, 2020). Individuals suffering with MetS have increased risk of developing kidney diseases (Srikanthan *et al*., 2014). Insulin resistance has been shown to compromise bone strength and to increase the risk to fracture

(Srikanthan *et al*., 2014). Poor dietary choices do not only impact lipid metabolism, kidney and bone health. It also impacts the architecture and function of the pancreas.

## 2.4 Non-alcoholic fatty pancreatic diseases

The pancreas facilitates the regulation of metabolic homeostasis by secreting a range of hormones and digestive enzymes (Röder *et al*., 2016). The exocrine pancreas is vital for nutrient digestion and absorption. It secretes a battery of digestive enzymes including amylases and lipases for carbohydrate and lipid digestion. The endocrine pancreas consists of islets of Langerhans that release insulin and glucagon which regulate blood glucose level. Consumption of obesogenic diets may trigger the development of NAFPD, a condition associated with obesity and MetS wherein fat accumulates in the pancreas in the absence of significant alcohol consumption (Lesmana *et al*.,2015; Catanzaro *et al.*, 2016). Compared to non-alcoholic fatty liver disease (NAFLD), NAFPD has received little attention from the global public health community. The prevalence of NAFPD is increasing due to increased incidence of obesity that has become a serious public health concern (Lazarus *et al.,* 2022; Shah *et al.,* 2019). In adults the prevalence of NAFPD been reported to be approximately 27% and is expected to rise due to the increasing prevalence of obesity caused by the consumption of unhealthy diets (Lesmana *et al.,* 2015)

The first description and comparison of pancreatic fat was done in 1933 where it was observed that pancreatic fat was doubled in obese compared to lean individuals (Ogilvie, 1933). Excessive fat deposition in the pancreas has been shown to be associated with increased visceral adiposity and it in turn has been demonstrated to induce NAFPD (Shah *et al.,* 2019). Obesity can induce pancreatic fat accumulation through two mechanisms: first by fat replacement where adipocytes replace the dead pancreatic acinar cells (Zhang *et al.*, 2021) and secondly by fat infiltration wherein fat is deposited in pancreatic acinar cells (Dite *et al.,* 2020) resulting in fat accumulation. While a correlation has been established between NAFPD and non-alcoholic fatty liver diseases (Wu & Wang, 2013), other studies report that pancreas is more susceptible to fat accumulation compared to the liver (Chang, 2022; E Silva *et al*., 2021). Obesity is associated with increased pancreatic fat and the localised secretion and release of TNF-α and interleukin-6 cause inflammation and associated organ dysfunction (Rakotoarivelo *et al*., 2018). Long term intake of dietary fructose increases TNF-α, a marker of pancreatic damage (Yeşilot *et al.,* 2022). Zucker rats fed high-fat diet developed pancreatic fibrosis due to excessive fat accumulation in acinar cells (Matsuda *et al.,* 2014) showing the negative effects

of obesogenic diets on pancreatic health. Consumption of such obesogenic diets also impact kidney health.

## 2.5 Chronic kidney disease

Obesity and MetS increase susceptibility to developing chronic kidney diseases (CKD) (Prasad *et al.*, 2022; Xiao *et al.*, 2022). Chronic kidney diseases are a cluster of kidney disorders that negatively affect kidney function manifest with reduced functional efficiency resulting in compromised health. CKD is a recognised global health problem that affects about 844 million people (Jager *et al.*, 2019; Kovesdy, 2022b). The International Society of Nephrology (ISN) global health survey estimates CKD prevalence to be 10.7% in South Africa (Ma et al., 2019). In addition to other organs, kidneys are reported as target organs that are affected by high fructose diet induced inflammation (Sweatt *et al*., 2016). Dietary fructose increases the risk of developing CKD (Chapman *et al.,* 2020). Its consumption causes increased secretion of the pro-inflammation mediators: uric acid and glycated products that cause kidney damage (Bratoeva *et al.*, 2017; Pessoa *et al.*, 2020). Kidney diseases have been shown to disrupt the normal metabolism of calcium, phosphorus, vitamin D and parathyroid hormone; all required for bone homeostasis (Keung & Perwad, 2018) resulting in increased bone derangements.

## 2.6 Bone derangements

Hip fracture, whose incidence in increasing on an annual basis, is common in the elderly. Globally, about 1.6 million hip fractures occur and this number is estimated to reach 4.5 million by 2050 (Rapp *et al.*, 2019; Veronese & Maggi, 2018). This type of fracture imposes a heavy economic impact, considerable morbidity and mortality (Veronese & Maggi, 2018). Extracapsular fractures that can be intertrochanteric, trochanteric and subtrochanteric and intracapsular fractures that occur on the femoral head and femoral neck are the most common types of hip fractures (Keung, 2022). Deranged bone remodelling induces bone structural change, low bone mineral density, decreased bone breaking strength and ultimately fracture (Dai *et al*., 2016; Ivaska *et al*., 2022). The relationship between bone metabolism and overweight is controversial. It has been reported by one study that individuals with low body weight had an increased risk of fracture even after regaining normal weight (Kim *et al.*, 2023). Kim et al. (2017) observed a positive correlation between bone mineral density and obesity indicating that fat accumulation increased bone density while Patrick et al. (2000) observed decreased biochemical markers in obese individuals but increased bone formation markers and

a decline in bone resorption markers. In their study Irving et al. (2019) compared to the non-obese, unstable type intertrochanteric fractures were more common in the obese individuals. Excess body fat has been shown to mediate production and release of inflammatory cytokines that can promote bone resorption and reduce bone strength (Hou *et al.*, 2020) and increased risk of hip fracture in android obesity has been associated with low bone mineral density (Ma *et al.*, 2022).

Different models have and continue to be used in the study of metabolic derangements.

## 2.7 Rodent models in metabolic derangements studies

Several animal models can and are used to investigate the pathophysiology and aetiology of metabolic disorders inclusive of MetS. Rats and mice are the most popular rodent species used to imitate the phenotype and pathogenesis of human diseases, such as obesity and diabetes mellitus (Raut & Bandawane, 2018). Some of the animal models used to study metabolic disorders include chemically induced, genetically induced, metabolic programming, and diet induced models (Bellamkonda *et al*., 2018).

### 2.7.1 Genetically induced models

The primary purpose of genetically produced metabolic disorders models is to study the aetiology and pathophysiology of metabolic disorders caused by single-gene mutations (Good, 2005). Mice with single autosomal recessive mutations in the leptin receptor gene or the leptin gene are used as models for leptin receptor-deficient (db/db) and leptin-deficient (ob/ob) disorders, respectively (Ohno *et al.*, 2022; Wong *et al.*, 2016). The monogenic rat model used for studying the pathogenesis of genetically-induced MetS saves time (Wong *et al*., 2016). However, single gene MetS illnesses are relatively uncommon in humans, and they do not accurately reflect the enormously complex genetic, epigenetic, and environmental factors such as dietary effects that contribute to MetS (Micha, 2017).

### 2.7.2 Chemical-induced models

In chemical-induced models MetS is induced through injections of pharmacological agents or chemical substances such as glucocorticoids (Raut & Bandawane, 2018; Wong *et al.*, 2016). Glucocorticoids promote lipolysis that increase free fatty acids and stimulate differentiation of pre-adipocytes into mature adipocytes thus inducing MetS (Wong *et al*., 2016). Unfortunately, the therapeutic advantages of glucocorticoids when used frequently and in high doses are

restricted by the side effects that they elicit, for example, hypertension, diabetes mellitus, growth retardation, skin atrophy, osteoporosis and abdominal obesity (Ramamoorthy & Cidlowski, 2016; Gunawan *et al.*, 2021). Importantly, the pathophysiology of chemically-induced MetS is different to diet-induced MetS. Such chemically-induced models are suitable to mimic drug-related MetS, however they require long duration to meet the criteria for MetS (Eren *et al*., 2019).

### 2.7.3 Diet-induced models

Diet can affect metabolism through hormones, lipid and glucose metabolic pathways (Gunawan *et al.,* 2021). Therefore, diet plays an important role in the development of clinical manifestations of MetS. Animal studies have demonstrated that long term consumption of high-fat diet induced complete cocktail MetS phenotype inclusive of increased fat mass and body weight, hypertriglyceridemia, hepatic steatosis and hypertension in Sprague Dawley rats (Cheng *et al*., 2017). Sprague Dawley and Wistar outbred rats are mostly used in the induction of MetS as they are more prone to diet-induced insulin resistance and obesity with distinct traits (Marques *et al.*, 2016; Micha, 2017). In these rodent models of MetS high-carbohydrate, high-fat and high-fructose diets are used (Wong *et al.*, 2016; Raut & Bandawane, 2018; Gunawan *et al.*, 2021)

## 2.8 Interventions against metabolic syndrome

### 2.8.1 Lifestyle modifications

Sedentary lifestyles and intake of unhealthy diets are some of the key drivers of obesity and MetS. A study by Angelico et al. (2023) reported that physical activity, diet composition, and energy intake may strongly interact in promoting the development of MetS. Nilsson et al. (2019) contend that the risk of developing MetS is increased in individuals who make unhealthy lifestyles choices. The restriction of calorie intake in combination with increased physical activity through exercise are an efficient management strategy for MetS (van Namen *et al.,* 2019) hence the recommendation for individuals with MetS to engage and benefit from basic lifestyle intervention programs (Nilsson *et al.,* 2019). Sprague-Dawley rats with MetS resulting from long term feeding on a high fructose diet benefited from aerobic exercises as shown by Er et al. (2022). Although lifestyle modifications are having beneficial effects, they are also associated with poor patient compliance. Patients therefore depend on conventional

medicine to manage metabolic diseases caused by poor lifestyle choices (Parajuli *et al*., 2014). Several conventional pharmaceutical agents are used in the management of metabolic diseases.

### 2.8.2 Conventional pharmaceuticals

Fenofibrate is a peroxisome proliferator receptor agonist that is used to treat hypertriglyceridemia and hyperlipidemia thus managing dyslipidaemia (Kim *et al.*, 2019; Emami *et al.*, 2020; Mahmoudi *et al.*, 2022). While fenofibrate largely improves cardiovascular health, it may have some indirect effects on the bone kidney and pancreatic health. In male Wistar rats orally administered fenofibrate for 8 weeks restored intravenous injection streptozotocin induced oxidative stress in the pancreatic cells (Mohammad & Amirhossein, 2018). The effects of fenofibrate on the bone health is not clear. Some research suggest that it may not affect bone health while other studies reported an increased bone mineral density and reduced the risk of fracture (Kim *et al.*, 2019). However, fenofibrate is associated with poor patient compliance due to its increased adverse effects that includes constipation, asthenia, joint pain, headaches, nausea, back pain, diarrhoea, cough, asthenia, wheezing and nasopharyngitis (Micromedex, 2023). Metformin is used to reduce hepatic glucose production thus can be used to improve NAFLD and diabetes mellitus (Rena *et al.*, 2017; Zhou *et al.*, 2018). However, there are some factors that affect patients ability to adhere to metformin treatment such as affordability, and side effects that include memory loss in adults (Christofides, 2019). Majority of these conventional pharmacological agents, in addition to eliciting deleterious side effects and being relatively expensive, they are also inaccessible to most population hence the increasing dependence on plant-derived ethnomedicine.

### 2.8.3 Medicinal plants

Despite the availability of conventional medicines used to treat diabetes mellitus, osteoporosis, kidney diseases and obesity, there is a significant increase in the usage of herbal medicine and supplements. About 80% of the world's population prefer and relies on them for some aspects of primary healthcare (Ekor, 2014 ;WHO Report, 2019). Plant-based therapies are considered the first line of defence in the fight against illnesses and their related problems (Karri *et al*., 2019). The heavy dependency on plant-based therapies is due to the perception that they are natural and hence deemed to be safe, relatively more accessible and less costly compared to contemporary medications (Okoye *et al.*, 2014; van Wyk & Prinsloo, 2018; Wulandari & Sholihin, 2019). As a result of the increased disadvantages associated with conventional pharmaceuticals usage interest in exploring ethnomedicine has increased (Aziz *et al.*, 2018),

especially determining efficacy and possible toxicities. Plant-derived ethnomedicines contain secondary plant metabolites that exhibit biological activities that can be exploited to manage diseases (Altemimi *et al*., 2017). One such secondary plant metabolite is lycopene.

## 2.9 Lycopene

Lycopene, a lipophilic carotenoid, is highly concentrated in red fruits including apricots, grapes, cranberries, watermelons and tomatoes (Imran *et al.*, 2020; Sen, 2019). The carotenoid's bioavailability is increased in processed tomato products such as tomato soup, ketchup, pasteurised tomato juice and sauces (Cooperstone *et al*., 2015). The more thermodynamically stable all-trans lycopene is red in colour and its less stable tetra cis-isomer is which is more bioactive and better absorbed has a reddish tone (Moran *et al*., 2015;Caseiro *et al*., 2020). Cooking temperature, presence of lipids and the breakup of the food matrix are key determinants of the absorption of lycopene from the (Kalbhor *et al*., 2019). Lycopene is transported in the blood stream by lipoproteins and is easily and efficiently absorbed by adipocytes (McEneny *et al*., 2013).

### 2.9.1 Lycopene: antioxidant effects

In living entities antioxidants help protect cell and cell organelles during nutrient (carbohydrates, lipids and protein) metabolism by lowering ROS production which concomitantly reduces inflammation (Hussein *et al.,* 2022). Lycopene is a powerful antioxidant (Caseiro *et al.*, 2020). It has been demonstrated to protect deoxyribonucleic acid (DNA), proteins and lipids against oxidation (Caseiro *et al*., 2020; Imran *et al.*, 2020). Compared to other carotenoids, lycopene has the highest singlet oxygen free radical scavenging ability due to its multiple double bonds that donate many electrons to free radicals (Bin-Jumah *et al.*, 2022). Through its ability to scavenge oxygen radicals when there is little oxygen present, lycopene inhibits the lipid peroxidation process's propagation step (dos Santos *et al.*, 2015; Bacanli *et al.*, 2017). Furthermore, lycopene has been shown to mediate significant increases of the antioxidants enzymatical activity which aids to reduce oxidative stress (Bacanli *et al.*, 2017; Imran *et al.*, 2020). *In vivo* oxidative status is determined by measuring the activities of SOD and GPx (Albrahim & Robert, 2022) which constitute a greater component of the systemic antioxidant system. In their study with male Wistar rats, Albrahim and Robert (2022) observed that when administered at 25 and 50 mg/kg body weight lycopene mitigated against kidney dysfunction by increasing SOD and GPx activities hence the natural antioxidant defence system.

### 2.9.2 Lycopene: anti-lipidaemic effects

Lycopene lowers plasma lipid concentration by lowering LDL oxidation, triglycerides levels and reducing the total cholesterol (Mozos *et al*., 2018). Intracellularly, lycopene inhibits HMA-CoA reductase, a key regulatory enzyme in *de novo* cholesterol synthesis, thus reduces intracellular cholesterol concentration (Palozza *et al.,* 2012). Further to inhibiting HMG-CoA reductase activity, it modifies the LDL receptor, and suppresses the acyl-coenzyme A:cholesterol acyltransferase activity (Palozza *et al.*, 2012). Lowering the production of lipids reduces the risk of developing obesity and its associated metabolic repercussions including MetS. In Wistar rats fed a basal diet fortified with cholesterolat 1% (w/w), lycopene administered at 50 mg/kg body weight attenuated the high-cholesterol diet increased serum plasma lipid content (Albrahim, 2022) demonstration its lipid lowering activity.

The antioxidant and anti-lipidaemic properties of lycopene has shown to protects against the pancreatic kidney and bone diseases. Literature has reported that administration of lycopene for 10 weeks can reverse obesity-induced alteration in the bone microarchitecture in male rats administered high-fat diet (Xia *et al.*, 2022). Furthermore, it was demonstrated that lycopene enhances bone metabolism in obese mice by preventing the overexpression of certain antibodies in their femurs and tibias, including cathepsin K, NF-κBp65, p-NF-κBp65/NF-κBp65, and advanced glycation end products (Xia *et al.*, 2022). The study by Lee et al. (2021) reported that the lycopene inhibits NADPH oxidase-mediated ROS production and oxidative stress-induced inflammatory responses, including mitochondrial dysfunction, zymogen activation, and IL-6 thus delaying the development of alcoholic acute pancreatitis. An animal study of 6 weeks old male mice has reported that the administration of lycopene suppresses the high-fat diet induced renal inflammation by inhibiting the TLR4/MyD88 inflammatory pathway and blocking inflammation in mice kidneys, including NF-kB, TNF-a, and IL-6 (Liu *et al*., 2022).

The discourse in this chapter clearly demonstrate that obesogenic diets can compromised key organ health and function. A narrative of the potential of lycopene to mitigate diet-induced metabolic derangements that can negatively affect bone, kidney and pancreatic function was given. Thus having reviewed the literature pertinent to this study in the current chapter, the next Chapter focuses of the materials and methods used to execute the various aspects of the

experiment in the process of evaluating the potential of lycopene to mitigate diet-induced metabolic derangements in growing Wistar rats mimicking children fed an obesogenic diet.

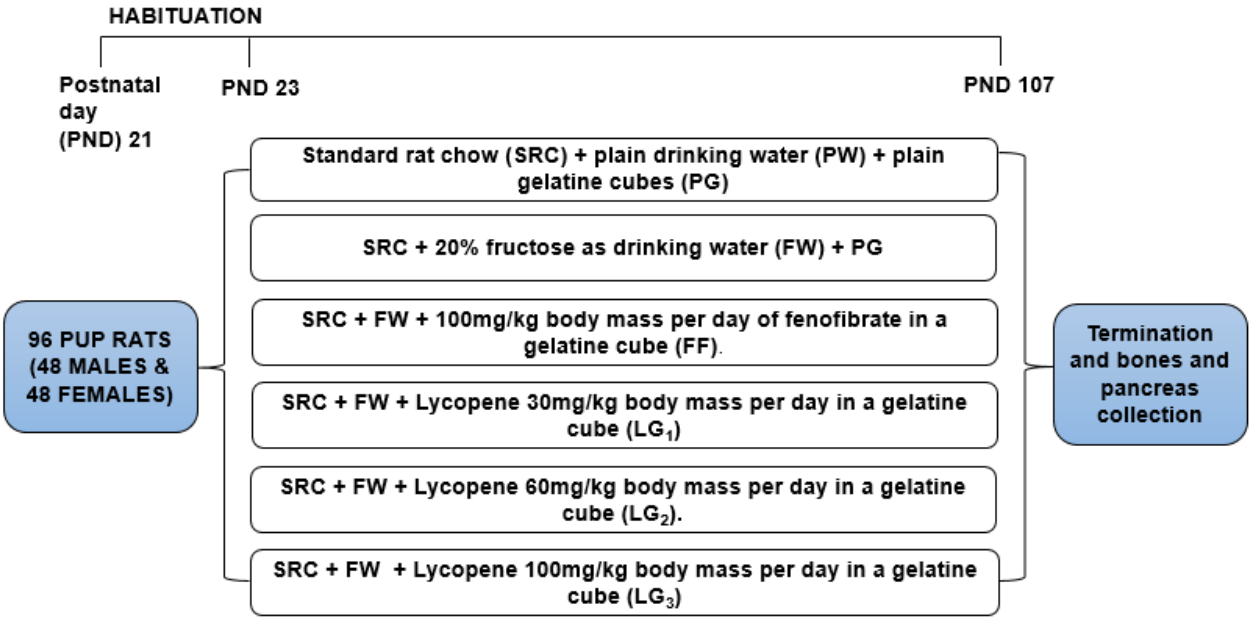
# CHAPTER 3: MATERIALS AND METHODS

## 3.1 Study site and animal use ethical clearance

The study, conducted from July 2022 to February 2023, was executed at the University of the Witwatersrand, Faculty of Health Sciences' Wits Research Animal Facility, and the Wits School of Physiology laboratories. The study received ethical clearance from the Animal Ethics Screening Committee (Ethical clearance certificate number: 2022/03/02B) for the handling and management of experimental animals.

In total ninety-six (48 females and 48 males) 21-day old Wistar rat pups were utilised in this study. The rats were housed individually in Perspex cages with clean wood shaving used as bedding. Each cage had a card that listed the ethical clearance number, treatment group and rat number which served to identify the rats. Bedding was changed twice in a week and the room where the feeding trial was executed was well ventilated. The rats had *ad libitum* access to standard rat chow and drinking (tap water and 20% fructose solution) fluid depending on treatment regimen. Nutrition hub (PTY) LTD (Stellenbosch, South Africa) supplied the commercial rat chow whose nutrient content is as shown in appendix III. The standard rat chow had a calcium:phosphorus ratio of 1.1:2.1. A 12-hour light dark cycle was maintained with light on from 07:00 to 19:00 hrs. Room temperature was kept at 25±2 °C and the rats were habituated to handling and experimental environment for 2 days before the start of data collection.

## 3.2 Study design



On post-natal day (PND) 23-day old Wistar rat pups were randomly allocated to treatment groups: control (plain drinking water), 20% fructose water (FW) + plain gelatine (PG), FW + 100 mg/kg body mass fenofibrate (FF) (Sigma-Aldrich Co., St Louis, MO USA), FW + 30mg/kg body mass lycopene per day ($LG_1$) (Changsha Staherb Natural Ingredient Co., Ltd, Hunan, China), FW + 60mg/kg body mass lycopene per day ($LG_2$) and FW + 100mg/kg body mass lycopene per day ($LG_3$) and fed for 84 days. Fenofibrate and lycopene were administered *per os* in gelatine cubes. Fenofibrate and lycopene doses were as recommended by Salehi et al. (2012) and Zhang et al. (2020), respectively. The three different doses of lycopene were used to provide a comprehensive understanding of their effects, helps establish dose-response relationship and enhances the credibility of the study. The fenofibrate is used as a lipid lowering agent therefore in this study it was used as positive control to compare and validate the effects of lycopene (Pan et al., 2012). In order to monitor growth and ensure constant dosage of the interventions, body mass was measured weekly. The rats were fasted overnight on PND 106 and then weighed on PND 107. Thereafter they were euthanised and tissue samples collected.

### 3.3 Terminal procedures, sample collection and storage

On PND 107 the rats were euthanised by an overdose of sodium pentobarbitone (Eutha-naze, Bayer, Johannesburg, South Africa) administered intraperitoneally. The thorax was then opened, and blood was collected by cardiac puncture using 20G needles and 10 ml syringes into SST gel blood collection tubes. Immediately thereafter the blood was cetrifuged at 3000 × g for 12 minutes the serum was then decanted into 1.5 ml microtubes and stored in a freezer at -80˚C before measurement of oxidative stress (TBARS assays) and CTX-1 and osteocalcin assays. Pancreata and kidneys were excised and weighed. Each pancreas was cut into two parts and fixed in the liquid nitrogen and later frozen-stored at -20°C. One part of the pancreas and the left kidney from each rat were used for the determination of lipid content. The other part of the pancreas was used for determination of oxidative stress (TBARS assay) and natural antioxidant enzymes, SOD and GPX1 activities. The femora and tibia from the right hand side of each rat's carcass were extracted, wrapped in cheese cloth soaked in 0.9% saline fluid and frozen-stored at -20°C pending measurements of mass, length, femora MIDD, STMLD, HTD, NTD, and tibia EPB, MDEB, MPEB, and MLD as well as determination bone breaking strength.

### 3.4 Determination of kidneys and pancreata fat content

The kidney and pancreatic samples preserved at -20ºC were thawed at room temperature for 30 minutes prior to the determination. Samples from male and female rats in each treatment group were pooled into a composite sample. The composite sample was then homogenised using the mortar and pestle. Kidney and or pancreatic fat content was determined by solvent extraction (petroleum ether) using Soxhlet apparatus (Gebr.mbH, 37079 Gottingen, Germany) as described by the Association of Analytical Chemists (AOAC, 2006; method number 920.39). Briefly, about 0.3-0.6 g from each composite sample was weighed and placed inside an extraction thimble soaked with petroleum ether but containing fat-free cotton wool. The extractor containing the weighed sample was loaded onto the extraction chamber. The distillation flask with 200ml of petroleum ether was placed on the heating pad, and the Soxhlet extract on top of the flask. The reflux condenser was then placed on top of the Soxhlet extractor. Cooling water to the condenser was turned on and the heating mantle switched on. Heat was controlled at 40±2 ºC using a thermostat. Each sub sample was extracted for 4 hours. After extraction, petroleum ether was blown off using a rota-evaporator (Buchi Rotavapor-R, Buchi Laboratoriums. Technik AG, CH.9230 Flawl/Schweiz) leaving the extracted fat in the flask. The distillation flask with the fat was placed in an oven set at 50 ºC for an hour to remove residual petroleum ether from the fat. Following cooling in a desiccator, the flask containing the fat was weighed. The percentage fat from each sub-sample was computed using the formula: $\text{mass of the flask with oil (g)} - \text{mass of the flask (g)} \div \text{mass of the sample (g)} \times 100$. Each assay was done in triplicate.

### 3.5 Determination of pancreatic oxidant and antioxidant status

#### 3.5.1 Preparation of the pancreas homogenates

Each pancreas sample was rinsed thrice in a cold phosphate buffered saline (PBS) to ensure through removal of excess blood. Thereafter, each of the rinsed pancreas sample was dried on filter paper and put in a pre-weighed microtube. The microtube containing pancreas sample was then weighed and then stored in the ice box. The required PBS was calculated in a ratio of 1 average pancreatic weight: 9 PBS. The pancreas samples immersed in the PBS were then homogenised using a sonicator for about 10 seconds and placed back into the ice box. This process was repeated twice to thoroughly homogenise each sample without denaturing proteins. Each sample homogenate in the microtube was then centrifuged at 5000 ×g at 2-8 ºC

for 10 minutes and the supernatant from each centrifuges sample homogenate was decanted into a 1.5 ml microtube and stored at -80 ºC pending the determination of pancreatic thiobarbituric acid reactive species (TBARS) concentration and GPX1 and SOD activities.

### 3.5.2 Determination of TBARS

The pancreatic TBARS concentration was estimated as described by Niehaus and Samuelsson, 1968. Firstly, protein concentration of the samples was measured as described by Bradford (1976). Briefly, 1 µL of the supernatant from the pancreatic sample's homogenates were diluted with distilled water at the ratio at 100 folds. 0.1 mL and 0.4ml of the working reagent and chromogenic agent, respectively were then added to the 10 mL glass test tube with 0.1 µL of diluted supernatant. The mixture was incubated at 100 ºC in a water bath for 60 minutes. Each glass test tube with its contents was cooled to room temperature using running water. Thereafter the contents were centrifuged at 1600 × g for 10 minutes and supernatant absorbance read at 540nm with a microplate reader (Bio-Tek Instruments, Vermont, USA). TBARS concentration of each sample was computed using the formula: $(\Delta A - b) \div axf \div Cpr$. Where:

y = The absolute OD value of standard ($OD_{Standard} - OD_{Blank}$)

x = the concentration of standard

a = the slope of standard curve

b = the intercept of standard curve

f = dilution factor of sample before test

$C_{pr}$ = Concentration of protein in samples (gprot/L)

$\Delta A$ = Absolute OD ($OD_{Sample}$ - $OD_{Blank}$)

### 3.5.3 Determination of glutathione peroxidase 1

The pancreatic GPX1 activity was determined using the rat specific biochemical assay kit (Elabscience®b, Rat ELISA kit, Wuhan, Hubei Province, China) following the manufacturer's instructions (Winterbourn *et al.,* 1975). Glutathione peroxidase activity is measured by monitoring the constant decrease in NADPH concentration with hydrogen peroxide ($H_2O_2$), as stated by Flohé & Günzler (1984). The sample glutathione peroxidase was measured by a microplate reader (Multiskan Ascent, Lab system, model number 354, Helsinki, Finland) at 450nm.

### 3.5.4 Determination of superoxide dismutase

The pancreatic SOD was determined using the rat specific biochemical assay kit (Elabscience®b, Rat ELISA kit, Wuhan, Hubei Province, China) following the manufacturer's

instructions. The pancreatic superoxide dismutase (SOD) activity was measured by the water-olubletetrazoliumsalt (WST-1) method. Xanthine oxidase (XO) catalyses WST-1 and reacts with $O^{2-}$ to generate water-soluble formazan dye. Pancreatic SOD inhibits the disproportionation of superoxide anions; thus, there is a negative correlation with the amount of formazan dye formed. SOD activity was determined by the colorimetric analysis of WST-1 products developed at 450nm measured using a Multiscan Ascent microplate reader (Lab system, model 354, Helsinki, Finland).

### 3.6 Determination of femora and tibiae morphometry

The femora and tibia mass was meausred using the weighing scale (Snowrex Electronic Scale, Clover Scales, Johannesburg). Micro-CT (μCT) is used to image and quantify bone in three dimentions (Valverde-Franco et al., 2004). The μCT scanner was set according to the manufacturer's instructions. Prior to scanning, the femora and tibiae that were frozen-stored preserved in physiological saline, were first thawed at room temperature for about an hour and then defleshed. Thereafter each fermur and tibia was scanned using the μCT system (SkyScan Bruker, Banner Lane, United Kingdom) and the NRecon software (Banner Lane, United Kingdom) coupled to the scanner reconstructed images into a three-dimensional structure. The ruler in μCT scan software was used to take the measurements for the femora mid-shaft diameter (MIDD), sub-trochanteric medio-lateral diameter (STMLD), head transverse diameter (HTD), neck transverse diameter (NTD), epiphyseal breadth (EPB) as well as maximum distal epiphyseal breadth (MDEB), maximum proximal epiphyseal breadth (MPEB) and medio-lateral diameter (MLD).

### 3.7 Determination of femora and tibia breaking strength

Immediately following the determination of femora and tibiae morphometry, the bones were returned to frozen-storage wrapped in a cheese cloth soaked in physiological saline before determining breaking strength of the femora and tibiae. The bones were thawed at room temperature for an hour. The 50 kg load cell TA.XT Plus Texture Analyser machine (Stable Micro Systems Ltd., Vienna court, Godalming, England) interfaced with exponent stable micro system software for Windows was used to measure femora and tibia breaking strength. Prior to determining bone breaking strength the texture analyser machine was calibrated and the test mode was set to compression with the test speed of 5mm/min and distance of 5 mm. Breaking

strength was determined from each bone's mid-point which (mid-point) was determined using an electronic calliper (Hi-impact, Dejuca, South Africa) as described by Medeiros et al. (2002).

## 3.8 Determination of serum concentration of hormones regulating bone metabolism

### 3.8.1 Determination of osteocalcin.

The serum osteocalcin was determined using the rat specific biochemical assay kit (Elabscience®b, Rat ELISA kit, Wuhan, Hubei Province, China) according to guidelines from the manufacturer. In summary, the serum samples were diluted with Phosphate buffered Saline at ratio 1:4. 100 µL of biotinylated working solution was added to each well in the plate with 100 µL of diluted supernatants of serum and incubated at 37 ºC for 60 min and washed 3 times. Following the addition of 100 µL of concentrated HRP conjugate in each well, the plate was incubated at 37 ºC for 30 minutes. Thereafter, 90 µL of the substrate reagent was added to each well and re-incubated at 37 ºC for 15 minutes in the dark. Lastly 50 µL of the stopping solution was used to stop the reaction. The absorbance of each serum sample was then measured using a microplate reader (Multiskan Ascent, Lab system, model number 354, Helsinki, Finland) at 450nm.

### 3.8.2 Determination of cross-linked C-telopeptide of type I collagen

The serum CTX-1 was determined using the rat specific biochemical assay kit (Elabscience®b, Rat ELISA kit, Wuhan, Hubei Province, China) following the manufacturer's instructions. Briefly, 100 µL of biotinylated working solution was added to each well in the plate with 100 µL serum and incubated at 37 ºC for 60 min and washed 3 times. Following the addition of 100 µL of concentrated HRP conjugate in each well, the plate was incubated at 37 ºC for 30 minutes. Thereafter, 90 µL of the substrate reagent was added to each well and re-incubated at 37 ºC for 15 minutes in the dark. Lastly 50 µL of the stopping solution was used to stop the reaction. All the incubations were at 37 ºC. Finally, the absorbance was measured spectrophotometrically with the microplate reader (Multiskan Ascent, Lab system, model number 354, Helsinki, Finland) at wavelength of 450nm.

## 3.9 Statistical analyses

Parametric and non-parametric data are presented as mean ±SD and median and range, respectively. Data was analysed using GraphPad Prism statistical software (version 8) (GraphPad Inc. San Diego, USA). A one-way ANOVA was used to analyse multiple-group

parametric data followed by a Tukey's post hoc test to compare means. Group non-parametric data was analysed using the Kruskal-Wallis test followed by a multiple-comparisons Dunn's post hoc test. Significance was set at $P < 0.05$.

Having outlined the material and methods used to collect data in the current chapter, the next chapter focuses on a narrative that speaks to and gives results of the current study.

# CHAPTER 4: RESULTS

## 4.1 Mortality and morbidity

Throughout the *in vivo* trial, no iatrogenic or incidental morbidities or fatalities were recorded.

## 4.2 Growth performance

Figure 4.1a and figure 4.1b shows the weaning, induction, and termination of female and male Wistar rats, respectively. Female rats had similar (P>0.05) weaning body masses but significantly gained (P = 0.001) body mass during the 2-day habituation period and grew (P<0.000) during the trial. The weaning and induction body masses of the male rats were similar (P>0.05) but rats grew significantly (P = 0.001) during the course of the trial.

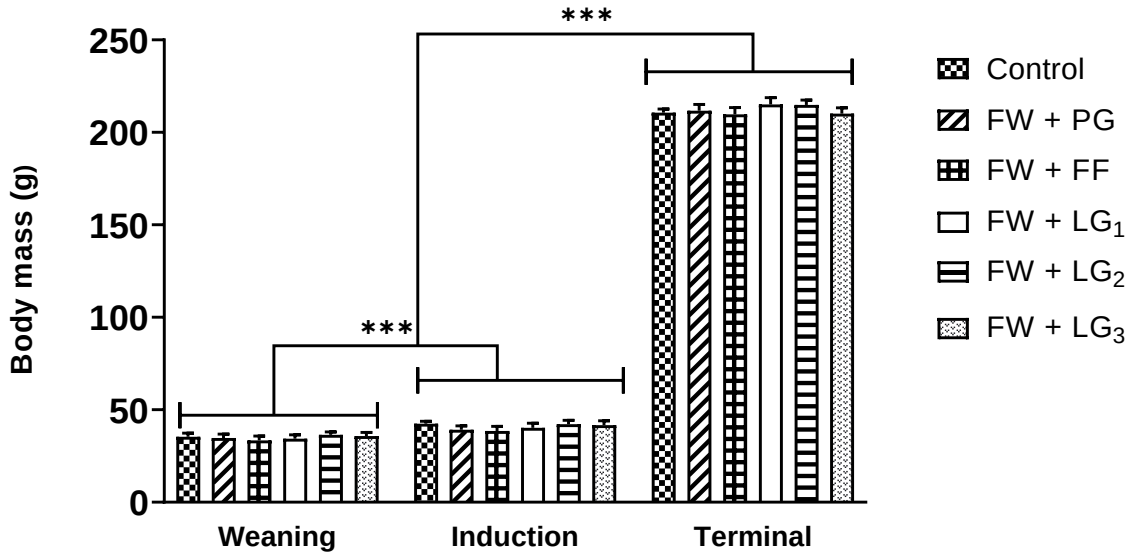



**Figure 4.1a: Effects of lycopene on the growth of the female Wistar rats**

*** = significantly different at P < 0.0001. Control = plain drinking water and plain gelatine cubes, FW + PG = 20% fructose as a drinking fluid and plain gelatine cubes, FW + FF = 20% fructose as drinking fluid and 100mg/kg body mass per day of fenofibrate in gelatine cubes, FW + $LG_1$ = 20% fructose as a drinking fluid and 30mg/kg of body mass of lycopene per day in gelatine cubes, FW + $LG_2$ = 20% fructose as a drinking fluid and 60 mg/kg of body mass of lycopene per day in gelatine cubes, and FW + $LG_3$ = 20% fructose as a drinking fluid and 100mg/kg of body mass of lycopene per day in gelatine cubes. Data expressed as mean ± SD, n = 8 per group.

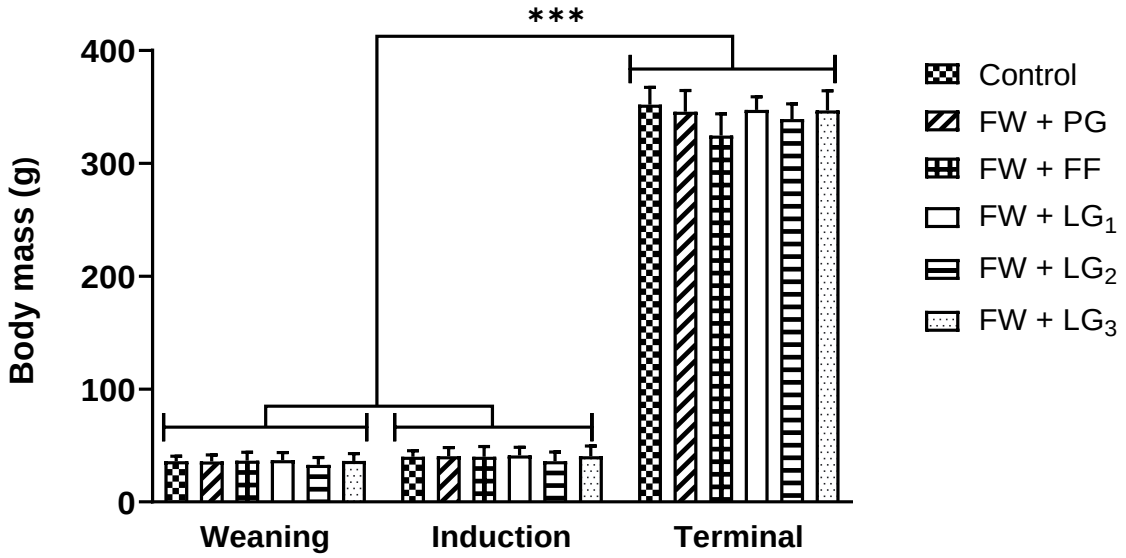



**Figure 4.1b: Effects of lycopene on the growth of the male Wistar rats**

*** = significantly different at $P < 0.0001$. Control = plain drinking water and plain gelatine cubes, FW + PG = 20% fructose as a drinking fluid and plain gelatine cubes, FW + FF = 20% fructose as drinking fluid and 100mg/kg body mass per day of fenofibrate in gelatine cubes, FW + $LG_1$ = 20% fructose as a drinking fluid and 30mg/kg of body mass of lycopene per day in gelatine cubes, FW + $LG_2$ = 20% fructose as a drinking fluid and 60 mg/kg of body mass of lycopene per day in gelatine cubes, and FW + $LG_3$ = 20% fructose as a drinking fluid and 100mg/kg of body mass of lycopene per day in gelatine cubes. Data expressed as mean ± SD, n = 8 per treatment.

## 4.3 Abdominal fat, kidney and pancreatic weight

Table 4.1 shows the effects of lycopene on the abdominal fat, kidney and pancreatic masses of female and male rats fed high-fructose diet. The treatments had no effect ($P > 0.05$) on the mean abdominal fat and pancreatic masses of the rats. The orally administered fenofibrate significantly increased ($P = 0.0056$) absolute kidney mass compared to control ($P = 0.0371$) and high dose lycopene ($P = 0.0021$). The female rats administered high fructose ($P = 0.0490$), control ($P = 0018$), low ($P = 0.0015$), medium ($P = 0.0252$) and high ($P <0.0001$) doses of lycopene had similar ($P > 0.05$) and significantly lower ($P = 0.0018$) relative kidney mass than the fenofibrate. The male rats administered low, medium and high lycopene had similar relative kidney mass with control and high fructose diet but significantly lower ($P = 0.0023$) relative kidney mass on the rats administered fenofibrate.

**Table 4.1: Effects of lycopene on the kidney and pancreatic masses of female and male rats fed a high-fructose diet**

| Parameters | Control | FW + PG | FW + FF | FW + $LG_1$ | FW + $LG_2$ | FW + $LG_3$ | Significance level |
|---|---|---|---|---|---|---|---|
| ***Females*** | | | | | | | |
| Pancreas (g) | 0.74±0.25 | 1.02±0.39 | 0.89±0.18 | 1.06±0.27 | 0.99±0.16 | 0.99±0.28 | ns |
| Pancreas (%) | 0.35±0.11 | 0.48±0.18 | 0.42±0.09 | 0.49±0.12 | 0.46±0.06 | 0.47±0.13 | ns |
| Kidney (g) | $1.50±0.13^{a}$ | $1.56±0.09^{ab}$ | $1.66±0.12^{b}$ | $1.53±0.07^{ab}$ | $1.57±0.10^{ab}$ | $1.45±0.09^{a}$ | ** |
| Kidney (%) | $0.71±0.05^{b}$ | $0.74±0.03^{b}$ | $0.79±0.04^{a}$ | $0.71±0.04^{b}$ | $0.73±0.04^{b}$ | $0.69±0.02^{b}$ | *** |
| Abdominal fat (g) | 6.20±1.25 | 7.14±2.78 | 7.49±1.63 | 8.33±1.44 | 8.27±1.23 | 6.75±1.61 | ns |
| Abdominal fat (%) | 2.94±0.56 | 3.34±1.18 | 3.55±0.67 | 3.86±0.51 | 3.84±0.49 | 3.20±0.71 | ns |
| ***Males*** | | | | | | | |
| Pancreas (g) | 1.26±0.20 | 1.18±0.15 | 1.40±0.31 | 1.30±0.44 | 1.40±0.27 | 1.33±0.34 | ns |
| Pancreas (%) | 0.36±0.05 | 0.34±0.03 | 0.44±0.12 | 0.37±0.14 | 0.41±0.09 | 0.39±0.10 | ns |
| Kidney (g) | 2.32±0.53 | 2.43±0.15 | 2.58±0.20 | 2.36±0.12 | 2.33±0.09 | 2.41±0.13 | ns |
| Kidney (%) | $0.66±0.14^{b}$ | $0.70±0.04^{ab}$ | $0.79±0.03^{a}$ | $0.68±0.02^{b}$ | $0.69±0.02^{b}$ | $0.70±0.02^{b}$ | ** |
| Abdominal fat (g) | 9.21±1.66 | 8.45±1.77 | 9.11±2.20 | 8.50±2.33 | 8.31±1.89 | 9.09±1.34 | ns |
| Abdominal fat (%) | 2.62±0.47 | 2.43±0.38 | 2.79±0.59 | 2.45±0.67 | 2.44±0.52 | 2.62±0.38 | ns |

ns = not significant, $P > 0.05$, **$P < 0.01$, *** $P < 0.0001$ $^{ab}$Within a row means with different superscript are significantly different at $P < 0.05$. Control = plain drinking water and plain gelatine cubes, FW + PG = 20% fructose as a drinking fluid and plain gelatine cubes, FW + FF = 20% fructose as drinking fluid and 100mg/kg body mass per day of fenofibrate in gelatine cubes, FW + $LG_1$ = 20% fructose as a drinking fluid and 30mg/kg of body mass of lycopene per day in gelatine cubes, FW + $LG_2$ = 20% fructose as a drinking fluid and 60 mg/kg of body mass of lycopene per day in gelatine cubes, and FW + $LG_3$ = 20% fructose as a drinking fluid and 100mg/kg of body mass of lycopene per day in gelatine cubes. Data presented as mean ± SD, n = 8.

## 4.4 Pancreatic lipid content

Figures 4.2a and 4.2b show the effects of lycopene on pancreatic lipid content in female and male rats fed a high fructose diet.

The high-fructose diet, FW + PG, significantly increased (P = 0.0048) females' pancreatic lipid content compared to female counterparts administered the low (P = 0,0294), medium (P = 0.0086) and high (P = 0.0121) dose lycopene as well as control (P = 0.0066) counterparts. While female rats administered fenofibrate had similar (P>0.05) pancreatic lipid content compared to counterparts that were fed the high fructose diet, though statistically not significant, the fenofibrate decreased the fructose-induced increase in pancreatic lipid content by 69%. In male rats, treatment regimens (control, fructose, fenofibrate and lycopene doses) did not affect (P > 0.05**)** pancreatic lipid content. Despite the similarity in the pancreatic lipid content of male rats across treatment regimens, it is important to note that there is upwards trend from high fructose diet by 100% to control counterparts and the downward trend from high fructose diet to low, medium, and high lycopene doses by 17, 8% and 56%, respectively.

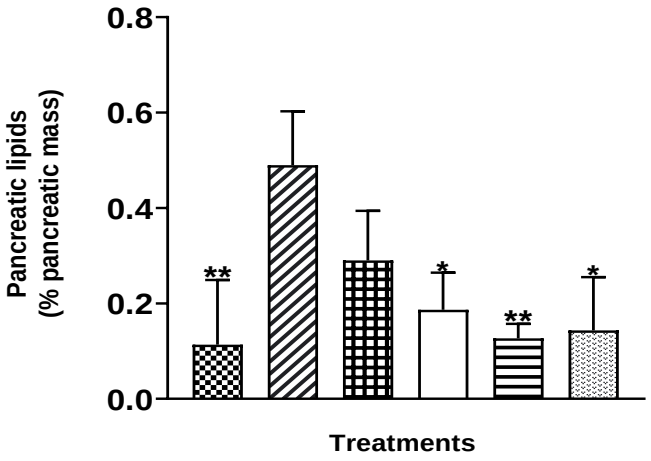



**Figure 4.2a: Effects of lycopene on pancreatic lipid content of female rats fed high-fructose diet**

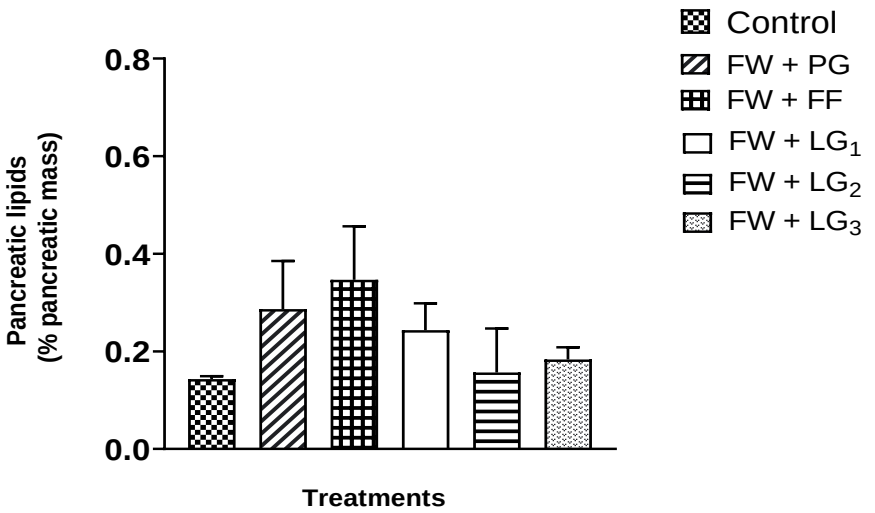



**Figure 4.2b: Effects of lycopene on pancreatic lipid content of male rats fed high-fructose diet**

* = significantly different at $P < 0.05$ when compared to FW + PG, ** = significant at $P < 0.01$ when compared to FW + PG. Control = plain drinking water and plain gelatine cubes, FW + PG = 20% fructose as a drinking fluid and plain gelatine cubes, FW + FF = 20% fructose as drinking fluid and 100mg/kg body mass per day of fenofibrate in gelatine cubes, FW + $LG_1$ = 20% fructose as a drinking fluid and 30mg/kg of body mass of lycopene per day in gelatine cubes, FW + $LG_2$ = 20% fructose as a drinking fluid and 60 mg/kg of body mass of lycopene per day in gelatine cubes, and FW + $LG_3$ = 20% fructose as a drinking fluid and 100mg/kg of body mass of lycopene per day in gelatine cubes. Data presented as mean ± SD, n = 3.

## 4.5 Kidney lipid content

Table 4.2 below shows the effects of lycopene on the lipid content of the kidneys of the rats fed dietary fructose. Treatment regimens had no significant effect ($P > 0.05$) on the rats' kidney lipid content.

**Table 4.2: Effects of lycopene on the kidney lipid content of female and male rats fed a high-fructose diet**

| Parameters | Control | FW + PG | FW + FF | FW + $LG_1$ | FW + $LG_2$ | FW + $LG_3$ | Significance level |
|---|---|---|---|---|---|---|---|
| **Females** | | | | | | | |
| Kidney lipid content (%) | 0.12±0.01 | 0.16±0.13 | 0.23±0.14 | 0.20±0.09 | 0.32±0.28 | 0.15±0.03 | ns |
| **Males** | | | | | | | |
| Kidney lipid content (%) | 0.12±0.03 | 0.30±0.35 | 0.11±0.02 | 0.07±0.02 | 0.15±0.12 | 0.10±0.07 | ns |

ns = not significant, P > 0.05. Control = plain drinking water and plain gelatine cubes, FW + PG = 20% fructose as a drinking fluid and plain gelatine cubes, FW + FF = 20% fructose as drinking fluid and 100mg/kg body mass per day of fenofibrate in gelatine cubes, FW + $LG_1$ = 20% fructose as a drinking fluid and 30mg/kg of body mass of lycopene per day in gelatine cubes, FW + $LG_2$ = 20% fructose as a drinking fluid and 60 mg/kg of body mass of lycopene per day in gelatine cubes, and FW + $LG_3$ = 20% fructose as a drinking fluid and 100mg/kg of body mass of lycopene per day in gelatine cubes. Data presented as mean ± SD, n = 3.

## 4.6 Pancreatic oxidant and antioxidant status

Table 4.3 below shows the effects of lycopene on pancreatic homogenate malondialdehyde (MDA) concentration, a marker of oxidative stress and pancreatic homogenate GPX1 and SOD activities, markers of antioxidant response, of female and male rats fed high-fructose diet. Although treatment regimens had no significant ($P>0.05$) effect on the pancreatic homogenate MDA concentration and GPX-1 and SOD activities of the rats, in males, there is an upward trend of MDA concentration from control counterparts to high fructose diet by 75% and there is a downward trend of MDA concentration from high fructose diet to three administered lycopene doses by 100%.

**Table 4.3: The effects of lycopene on the pancreatic oxidants and antioxidants status of female and male rats fed a high-fructose diet**

| Parameter | Control | FW + PG | FW + FF | FW + $LG_1$ | FW + $LG_2$ | FW + $LG_3$ | Significance level |
|---|---|---|---|---|---|---|---|
| ***Females*** | | | | | | | |
| MDA (µmol/gprot) | 0.10±0.06 | 0.07±0.05 | 0.07±0.04 | 0.08±0.04 | 0.05±0.02 | 0.06±0.02 | ns |
| GPX1 (pg/mL) | 3397±3013 | 2798±929.9 | 2980±882.4 | 2477±1090 | 2547±1363 | 3024±1540 | ns |
| SOD (ng/mL) | 1632±1303 | 2396±2475 | 1860±1196 | 1498±933.4 | 2529±1599 | 1722±1026 | ns |
| ***Males*** | | | | | | | |
| MDA (µmol/gprot) | 0.08±0.03 | 0.14±0.17 | 0.11±0.09 | 0.07±0.03 | 0.06±0.02 | 0.06±0.03 | ns |
| GPX1 (pg/mL) | 2719±1297 | 2490±1250 | 2091±713.5 | 2397±1285 | 2008±1159 | 2701±1120 | ns |
| SOD (ng/mL) | 1829±976.7 | 3400±2454 | 2191±2395 | 2961±1934 | 4331±1782 | 4679±2102 | ns |

ns = not significant, P > 0.05. Control = plain drinking water and plain gelatine cubes, FW + PG = 20% fructose as a drinking fluid and plain gelatine cubes, FW + FF = 20% fructose as drinking fluid and 100mg/kg body mass per day of fenofibrate in gelatine cubes, FW + $LG_1$ = 20% fructose as a drinking fluid and 30mg/kg of body mass of lycopene per day in gelatine cubes, FW + $LG_2$ = 20% fructose as a drinking fluid and 60 mg/kg of body mass of lycopene per day in gelatine cubes, and FW + $LG_3$ = 20% fructose as a drinking fluid and 100mg/kg of body mass of lycopene per day in gelatine cubes. MDA = malondialdehyde, GPX1 = glutathione peroxidase and SOD = superoxide peroxidase. Data presented as mean ± SD, n = 6-7.

## 4.7 Femora and tibiae indices and breaking strength

### 4.7.1 Femora and tibiae indices

The effects of lycopene on femora and tibiae of female and male rats fed dietary fructose are shown on Tables 4.4 and 4.5, respectively. In females, treatment regimens had no effect ($P>0.05$) on femora and tibiae lengths, masses and mass to length ratio. The intake of the high-fructose diet significantly reduced ($P = 0.0003$) the mean femora mid-shaft diameter (MIDD) compared to control in female rats. However, orally administered lycopene at 60 and 100 mg/kg body mass, respectively, resulted in significantly wider ($P<0.05$) femora MIDD compared to counterparts that had the high fructose diet. In female rats orally administered lycopene at 30 mg/kg body mass for 12 weeks reduced ($P = 0.0003$) their femora sub-trochanteric medio-lateral diameter (STMLD) compared to that of control, fructose-only, and fructose with 60 mg/kg body mass lycopene fed counterparts.

In males, treatment regimens had no effect femora length, mass to length ratio, HTD, NTD and EPB as well as tibiae mass, mass to length ratio, MDEB, MPED and MLD ($P>0.05$). However orally administered fenofibrate in males fed a high fructose diet significantly decreased (0.0427) femora mass compared to counterparts administered a low (30 mg/kg body mass) lycopene dose. The administration of fenofibrate reduced (0.0315) femora MIDD compared to control counterparts in male rats. Chronic intake of a high fructose diet significantly ($P = 0.0360$) reduced males' femora STMLD compared to that of control counterparts and fenofibrate decreased ($P = 0.0430$) males' tibiae lengths compared to the fructose-only fed group.

**Table 4.4: Effects of lycopene on femora and tibiae indices of female rats fed high-fructose diet**

| Parameters | Control | FW + PG | FW + FF | FW + $LG_1$ | FW + $LG_2$ | FW + $LG_3$ | Significance level |
|---|---|---|---|---|---|---|---|
| **Femora** | | | | | | | |
| Mass (mg) | 663.9±29.59 | 638.1±33.37 | 706.0±121.4 | 655.4±61.48 | 699.0±107.4 | 634.5±24.81 | ns |
| Length (mm) | 31.40±0.39 | 30.38±0.21 | 31.75±2.18 | 30.70±0.45 | 30.98±1.30 | 30.30±0.42 | ns |
| Seedor index | 21.14±0.84 | 21.01±1.16 | 22.13±2.18 | 21.37±2.19 | 22.49±2.46 | 20.94±0.73 | ns |
| MIDD (mm) | $3.33±0.25^{a}$ | $2.78±0.10^{b}$ | $2.85±0.33^{bc}$ | $3.13±0.10^{ab}$ | $3.53±0.21^{a}$ | $3.23±0.05^{ac}$ | *** |
| STMLD (mm) | $2.95±0.28^{ac}$ | $3.23±0.32^{a}$ | $2.55±0.31^{bc}$ | $2.40±0.27^{b}$ | $3.28±0.32^{a}$ | $2.88±0.17^{abc}$ | *** |
| HTD (mm) | 3.45±0.21 | 3.53±0.13 | 3.28±0.26 | 3.50±0.29 | 3.50±0.22 | 3.40±0.24 | ns |
| NTD (mm) | 2.23±0.25 | 2.43±0.15 | 2.10±0.29 | 2.15±0.13 | 2.30±0.22 | 2.25±0.10 | ns |
| EPB (mm) | 6.45±0.31 | 5.45±0.87 | 5.80±0.29 | 5.60±0.36 | 5.65±0.17 | 5.73±0.33 | ns |
| **Tibia** | | | | | | | |
| Mass (mg) | 580.6±47.03 | 543.3±24.32 | 607.3±94.80 | 558.2±20.19 | 599.3±75.88 | 544.3±35.3 | ns |
| Length (mm) | 34.80±0.46 | 35.15±0.57 | 35.40±2.06 | 35.18±0.39 | 35.20±0.90 | 33.98±0.49 | ns |
| Seedor index | 16.69±1.49 | 15.45±0.56 | 17.09±1.62 | 15.87±0.47 | 17.00±1.71 | 16.03±1.11 | ns |
| MDEB (mm) | 3.33±0.15 | 5.13±0.32 | 2.93±0.17 | 3.10±0.55 | 3.15±0.13 | 3.33±0.26 | ns |
| MPEB (mm) | 6.65±0.17 | 6.58±0.33 | 6.30±0.36 | 5.95±0.42 | 6.33±0.43 | 6.60±0.24 | ns |
| MLD (mm) | 2.08±0.24 | 2.08±0.29 | 2.35±0.25 | 2.00±0.16 | 2.25±0.17 | 2.28±0.21 | ns |

ns = not significant, $P > 0.05$, ***$P < 0.001$. $^{abc}$Within row means with different superscript are significantly different at $P < 0.05$. Control = plain drinking water and plain gelatine cubes, FW + PG = 20% fructose as a drinking fluid and plain gelatine cubes, FW + FF = 20% fructose as drinking

fluid and 100mg/kg body mass per day of fenofibrate in gelatine cubes, FW + $LG_1$ = 20% fructose as a drinking fluid and 30mg/kg of body mass of lycopene per day in gelatine cubes, FW + $LG_2$ = 20% fructose as a drinking fluid and 60 mg/kg of body mass of lycopene per day in gelatine cubes, and FW + $LG_3$ = 20% fructose as a drinking fluid and 100mg/kg of body mass of lycopene per day in gelatine cubes. MIDD = mid-shaft circumference, STMLD = sub-trochanteric medio-lateral diameter, HTD = head transverse diameter, NTD = neck transverse diameter, EPB = epiphyseal breath, MDEB = maximum distal epiphyseal breadth, MPEB = maximum proximal epiphyseal breadth, and MLD Medio-lateral diameter. Data presented as mean ± SD, n = 4.

**Table 4.5: Effects of lycopene on femora and tibiae indices tibia and femora of male rats fed high-fructose diet**

| Parameters | Control | FW + PG | FW + FF | FW + $LG_1$ | FW + $LG_2$ | FW + $LG_3$ | Significance level |
|---|---|---|---|---|---|---|---|
| **Femora** | | | | | | | |
| Mass (mg) | 871.6±70.21$^{ab}$ | 831.7±39.47$^{ab}$ | 770.9±64.63$^{a}$ | 914.8±75.87$^{b}$ | 862.9±23.2$^{ab}$ | 855.7±44.07$^{ab}$ | * |
| Length (mm) | 34.45±1.56 | 33.35±0.79 | 32.60±0.63 | 34.15±0.52 | 33.75±0.31 | 34.00±0.52 | ns |
| Seedor index | 25.40±3.02 | 24.93±0.65 | 23.63±1.52 | 26.78±2.11 | 25.57±0.57 | 25.17±1.21 | ns |
| MIDD (mm) | 4.23±0.90$^{a}$ | 3.23±0.43$^{ab}$ | 3.00±0.47$^{b}$ | 3.48±0.22$^{ab}$ | 3.60±0.32$^{ab}$ | 3.73±0.10$^{ab}$ | * |
| STMLD (mm) | 3.98±1.17$^{a}$ | 2.65±0.35$^{b}$ | 2.90±0.14$^{ab}$ | 3.25±0.19$^{ab}$ | 3.45±0.48$^{ab}$ | 2.85±0.26$^{ab}$ | * |
| HTD (mm) | 4.40±1.36 | 3.73±0.10 | 3.60±0.34 | 3.68±0.33 | 3.70±0.16 | 3.65±0.06 | ns |
| NTD (mm) | 2.70±1.02 | 2.23±0.21 | 2.23±0.28 | 2.35±0.13 | 2.40±0.12 | 2.43±0.29 | ns |
| EPB (mm) | 5.85±1.57 | 6.13±0.25 | 5.78±0.41 | 5.90±0.37 | 5.83±0.29 | 6.33±0.39 | ns |
| **Tibia** | | | | | | | |
| Mass (mg) | 744.3±38.12 | 736.2±24.18 | 691.0±46.65 | 760.9±49.31 | 751.9±17.30 | 773.8±27.10 | ns |
| Length (mm) | 37.88±0.63$^{ab}$ | 38.50±0.88$^{a}$ | 37.00±0.75$^{b}$ | 38.28±0.62$^{ab}$ | 37.73±0.10$^{ab}$ | 37.90±0.29$^{ab}$ | * |
| Seedor index | 19.66±1.07 | 19.12±0.34 | 18.67±1.01 | 19.87±1.04 | 19.94±0.46 | 20.42±0.60 | ns |
| MDEB (mm) | 3,75±0.70 | 3.33±0.39 | 2.98±0.17 | 3.33±0.10 | 3.25±0.24 | 3.43±0.33 | ns |
| MPEB (mm) | 6.65±0.90 | 6.98±0.21 | 6.48±0.45 | 6.70±0.60 | 6.88±0.22 | 6.98±0.19 | ns |
| MLD (mm) | 2.43±0.25 | 2.45±0.31 | 2.20±0.27 | 2.43±0.22 | 2.53±0.25 | 2.83±0.38 | ns |

ns = not significant, P > 0.05, *P < 0.05. [ab] within row means with different superscript are significantly different at P < 0.05. Control = plain drinking water and plain gelatine cubes, FW + PG = 20% fructose as a drinking fluid and plain gelatine cubes, FW + FF = 20% fructose as drinking fluid and 100mg/kg body mass per day of fenofibrate in gelatine cubes, FW + $LG_1$ = 20% fructose as a drinking fluid and 30mg/kg of body mass of lycopene per day in gelatine cubes, FW + $LG_2$ = 20% fructose as a drinking fluid and 60 mg/kg of body mass of lycopene per day in gelatine cubes, and FW + $LG_3$ = 20% fructose as a drinking fluid and 100mg/kg of body mass of lycopene per day in gelatine cubes. MIDD = mid-shaft diameter, STMLD = sub-trochanteric medio-lateral diameter, HTD = head transverse diameter, NTD = neck transverse diameter, EPB = epiphyseal breath, MDEB = maximum distal epiphyseal breadth, MPEB = maximum proximal epiphyseal breadth, and MLD = Medio-lateral diameter. Data presented as mean ± SD, n = 4.

### 4.7.2 Femora and tibia breaking strength

The effects of lycopene on femora and tibia breaking strength of female and male Wistar rats fed a high fructose diet are shown in Table 4.6 below.

Treatment regimens had no effect on the females' tibiae and males' femora and tibia breaking strengths ($P>0.05$) however orally administered fenofibrate significantly reduced ($P = 0.0265$) the females' femora breaking strength compared to that of counterparts fed the high fructose diet.

**Table 4.6: Effects of lycopene on femora and tibiae breaking strength of female and male rats fed a high fructose diet**

| Breaking strength | Control | FW + PG | FW + FF | FW + $LG_1$ | FW + $LG_2$ | FW + $LG_3$ | Significance level |
|---|---|---|---|---|---|---|---|
| ***Females*** | | | | | | | |
| Femora (N) | $87.97\pm4.27^{ab}$ | $89.57\pm2.84^{a}$ | $61.07\pm20.9^{b}$ | $66.53\pm17.31^{ab}$ | $75.70\pm2.58^{ab}$ | $79.36\pm10.01^{ab}$ | * |
| Tibia (N) | 49.99±3.65 | 51.03±1.06 | 41.31±13.75 | 42.48±5.52 | 47.25±8.03 | 33.65±16.27 | ns |
| ***Males*** | | | | | | | |
| Femora (N) | 115.0±5.59 | 93.45±16.19 | 76.23±17.45 | 101.0±14.24 | 85.82±33.19 | 103.0±20.17 | ns |
| Tibia (N) | 58.36±8.74 | 63.65±23.72 | 51.00±6.18 | 56.77±4.57 | 52.28±8.27 | 55.66±3.93 | ns |

ns = not significant $P > 0.05$, * = significantly different at $P < 0.05$. Control = plain drinking water and plain gelatine cubes, FW + PG = 20% fructose as a drinking fluid and plain gelatine cubes, FW + FF = 20% fructose as drinking fluid and 100mg/kg body mass per day of fenofibrate in gelatine cubes, FW + $LG_1$ = 20% fructose as a drinking fluid and 30mg/kg of body mass of lycopene per day in gelatine cubes, FW + $LG_2$ = 20% fructose as a drinking fluid and 60 mg/kg of body mass of lycopene per day in gelatine cubes, and FW + $LG_3$ = 20% fructose as a drinking fluid and 100mg/kg of body mass of lycopene per day in gelatine cubes. Data presented as mean ± SD, n = 4.

## 4.8 Hormones regulating bone metabolism

The effect of lycopene on plasma CTX-1 and osteocalcin concentration of female and male Wistar rats fed high-fructose diet are shown in Table 4.7 below. Treatment regimens had no significant effect ($P>0.05$) on the rats' plasma CTX-1 and osteocalcin concentration.

**Table 4.7: Effects of lycopene on plasma C-telopeptide of type-I collagen and osteoccalcin concentration of female and male rats fed a high-fructose diet**

| Parameter | Control | FW + PG | FW + FF | FW + $LG_1$ | FW + $LG_2$ | FW + $LG_3$ | Significance level |
|---|---|---|---|---|---|---|---|
| ***Females*** | | | | | | | |
| Osteocalcin (ng/mL) | 11.65±4.97 | 17.77±3.44 | 18.88±6.13 | 16.20±5.77 | 18.10±3.55 | 18.10±5.92 | ns |
| CTX-1 (ng/mL) | 21.25±3.96 | 22.80±3.80 | 24.35±5.23 | 24.37±5.89 | 20.71±5.02 | 19.84±3.79 | ns |
| ***Males*** | | | | | | | |
| Osteocalcin (ng/mL) | 15.25±5.29 | 18.85±4.94 | 17.63±5.96 | 13.96±6.61 | 16.25±4.53 | 17.66±5.71 | ns |
| CTX-1 (ng/mL) | 25.46±2.01 | 25.30±6.14 | 25.76±4.49 | 25.26±6.10 | 25.02±4.30 | 28.80±6.45 | ns |

ns = not significant, P > 0.05. Control = plain drinking water and plain gelatine cubes, FW + PG = 20% fructose as a drinking fluid and plain gelatine cubes, FW + FF = 20% fructose as drinking fluid and 100mg/kg body mass per day of fenofibrate in gelatine cubes, FW + $LG_1$ = 20% fructose as a drinking fluid and 30mg/kg of body mass of lycopene per day in gelatine cubes, FW + $LG_2$ = 20% fructose as a drinking fluid and 60 mg/kg of body mass of lycopene per day in gelatine cubes, and FW + $LG_3$ = 20% fructose as a drinking fluid and 100mg/kg of body mass of lycopene per day in gelatine cubes. CTX-1= C-telopeptide of type I collagen. Data presented as mean ± SD, n = 6-7.

Having outlined the result section of this study in this chapter, the next chapter will focus will discuss the outcomes of this study.

# CHAPTER 5: DISCUSSION

This study evaluated the potential prophylactic effects of lycopene on high-fructose diet induced femora and tibiae, kidney, and pancreatic metabolic derangements in growing Wistar rats mimicking children fed a high calorie obesogenic diet. Chronic consumption of the high-fructose diet reduced the female Wistar rats' femora mid-shaft diameter and males' sub-trochanteric medio-lateral diameter and mediated increased deposition of fat (lipids) in the pancreata of the female rats. The rats' growth performance as measured by body mass, abdominal fat yield, pancreata masses and oxidative stress and natural antioxidant enzymes activities, kidney masses and lipid content, femora mass, lengths, HTD, NTD and EPB, tibia mass, length, MDEB, MPEB, and MLD, femora and tibiae strength, and serum CTX-1 and osteocalcin concentrations were not affected by the administration of high-fructose diet. Treatment with lycopene prevented the high-fructose diet-induced increased pancreatic lipid accretion and reduced femora mid-shaft diameter in females and reduced femora sub-trochanteric medio-lateral diameters in males. However, in female Wistar rats fed the high fructose diet, orally administered low dose (30 mg/kg body mass) lycopene caused a reduction in their femora sub-trochanteric medio-lateral diameters. Furthermore, in females fed the high-fructose diet, intervening with orally administered fenofibrate increased kidney masses, reduced the femora mid-shaft diameters and reduced the strength of femora.

### 5.1 Effect on growth performance

Ramos et al. (2017) reported that the intake of a diet fortified with 20% fructose as a drinking fluid by Wistar rats for 8 weeks had no effect on the body mass but caused an increase in visceral adiposity. Similar findings were reported by Lembede et al. (2018). According to Ramos et al. (2017) the high adipogenic and low energetic nature of fructose was the reason for the observed increase in visceral adiposity without corresponding increase in body mass. In the current study, chronic intake of dietary fructose had no effect on the rats' body mass and visceral adiposity. These findings suggest dietary fructose neither compromised the rats' growth performance nor altered the deposition of visceral fat. It has to be noted that the current study used growing not adult rats. Young and growing rats have been shown to have greater resistance to the development of visceral adiposity induced by a high-fructose diet (Ibrahim *et al*., 2017). An interesting finding from the current study was that during the 2-day habituation period, females had a significant increase in body mass compared to their induction while their male counterparts' mean habituation body masses were similar to their mean induction body masses. These findings suggest that the stress associated with weaning and a change in the

environment affected the males more than females. The human study has reported that females have been reported to be more prone to stress effect than male (Coplan *et al*., 2018). Elevated stress levels trigger an increase in the secretion of the glucocorticoid cortisol, which can subsequently lead to heightened appetite, greater food intake, and weight gain (Rahati *et al*., 2022). Thus observations from the current study are at variance with those by Rahati et al., (2022). Body mass is an important indicator of animal's health status. Gut fill, hydration status and visceral organ size impact body mass making it an inaccurate measure of growth (Owens *et al*., 1995). Hence the current study also investigated femora and tibiae lengths and masses. These long bones respond to somatomedins in a dose-dependent manner (Giustina *et al*., 2008) thus are a more reliable indicator of the effects of interventions on growth performance. Previous literature reported that chronic consumption of high fat high fructose diet by growing male Sprague Dawley rats for 12 weeks had no effect on their femora and tibiae lengths and masses (Yarrow *et al.*, 2016). Similarly, the current study shows that chronic consumption of the high-fructose diet did not affect growing female and male Wistar rats' femora and tibiae lengths and masses. This finding further suggests that high-fructose did not compromise growth performance. In the current study it was observed that orally administered fenofibrate and lycopene neither increased nor decreased the female and male growing Wistar rats' femora and tibiae lengths and masses suggesting that none of them negatively affected growth performance. Nyakudya et al. (2019) also reported similarities in the femora and tibiae lengths of growing female and male growing Sprague-Dawley rats on chronic consumption of a high-fructose diet.

## 5.2 Effect on pancreatic and kidney health

The pancreas is a key organ in the maintenance of homeostasis. Its endocrine beta and alpha cells secrete insulin and glucagon, respectively which are critical to the maintenance of blood glucose homeostasis and energy balance from metabolism of fuel biomolecules. Exocrine pancreatic acinar cells secrete digestive enzymes necessary for digestion in the small intestines. The bicarbonate-rich fluid from exocrine pancreatic ductular cells neutralises acid chyme preventing duodenal ulceration and creates an alkaline environment for digestive enzymes' activities. Chronic consumption of obesogenic diets has been shown to impact pancreatic health and function (Papantoniou *et al.*, 2019; Yeşilot *et al.*, 2022). Using the Institute of Cancer Research (ICR) mice fed a high-fructose diet for eight weeks, Hattori et al. (2021) reported that pancreata masses of the fructose-diet fed mice were the same as control. Furthermore, Yin et

al. (2019) reported that in male Sprague-Dawley rats fed a high fat diet for four weeks and 1% streptozotocin injection, lycopene administered at 10 and 20 mg/kg body weight for ten weeks following exposure to the high calorie diet did not impact the pancreatic index. Findings from the current study also showed that consumption of high-fructose diet for 12 weeks neither increased nor reduced pancreata masses. Importantly, findings from the current study also showed that in the high fructose diet fed Wistar rats, orally administered lycopene did not alter pancreatic mass.

Interestingly, although consumption of the high-fructose diet had no effect on pancreatic mass, it however increased the pancreatic lipid content in female Wistar rats. Recent research reported of instances where instead of fat infiltrating the pancreas, there are cases where pancreatic parenchyma cells are replaced by adipocytes (Dite *et al*., 2020; Zhang *et al*., 2021). Based on the findings from the current study, it can be argued and perhaps speculated that although the pancreata masses were similar across treatment regimes, in female Wistar rats the high-fructose diet could have mediated replacement of pancreatic parenchyma cells with adipocytes resulting in higher fat yield per unit mass of pancreatic tissue. In their study Krisnamurti et al. (2022) observed that histological assays reported fatty pancreata in Sprague-Dawley rats fed a high fructose and high cholesterol diet. The high fructose diet induced increased pancreatic lipid yield in female Wistar in the current study point to possible development of fatty pancreata through replacement of pancreatic parenchyma tissue with adipocytes. Findings from the current study suggest that females are more susceptible to high-fructose diet induced increase in pancreatic lipid accretion and can possibly be more at risk to diet-induced pancreatic fatty diseases compared to males. The sex specific response to the high fructose diet might be linked to differences in androgen type(s) and concentration in females and males. Oestrogen, found in higher concentrations in females, has been shown to influence in lipid metabolism and storage in various tissues including the pancreas (Mauvais-Jarvis *et al*., 2013). It thus can be argued that observed increased pancreatic lipid yield in female Wistar rats fed the high-fructose diet when compared to male counterparts could have been oestrogen mediated. Male Wistar rats chronically fed laboratory chow supplemented with 2% cholesterol and 0.25% sodium-cholate-hydrate had significantly increased cholesterol and triglycerides in the liver but their pancreatic cholesterol and triglyceride content was similar to control counterparts (Csonka *et al*., 2017). Similarly, findings from the current study also show similarities in pancreatic lipid yield between that of male Wistar rats fed the high-fructose diet and control counterparts. Despite this observed similarity in pancreatic lipid content between

the two groups, male Wistar rats fed the high-fructose diet had double the amount of pancreatic lipid content compared to the control pointing to some degree of biological albeit not statistical significance. This suggests that with time consumption of the high-fructose diet could induce significant lipid accretion in the pancreata of male Wistar rats. Hepatic metabolism of fructose generates metabolic substrates that are used to fuel increased *de novo* lipogenesis and concomitant fat deposition in the liver (Johnson *et al*., 2020). Overtime this fat synthesised by the liver is redistributed to non-adipose tissue including the pancreas (E Silva *et al*., 2021). Pinte et al. (2019) and Krisnamurti et al. (2022) contend that increased fat deposition in the pancreas causes pancreatic cell hypertrophy and hyperplasia which leads to the development of NAFPD. Non-alcoholic fatty pancreatic disease can cause further pancreatic degeneration that results in insulin resistance, β cell function failure, acute pancreatitis, pancreaticoduodenal leakage and T2DM (Beeman and Garbow, 2017; Paul and Shihaz, 2020; Singh *et al.*, 2021) hence the need to evaluate the efficacy and safety of lycopene, a potential prophylactic and therapeutic agent. Lycopene's lipid lowering property is ascribed to its ability to inhibit *de novo* cholesterol synthesis which concomitantly lowers fat content (Mozos *et al*., 2018). Furthermore, it contributes to lower plasma circulating and stored cholesterol by inhibiting the absorption of dietary cholesterol from the small intestines (Palozza *et al*., 2012). Fenofibrate, a stimulant of lipoprotein lipase and a PPAR-α agonist, well known for mitigating diet-induced fatty liver disorders (Pan *et al*., 2012), is also used to treat hypercholesterolaemia. In male Wistar rats fed a high fat (obesogenic) diet lycopene administered at 25 and 50 mg/kg reduced lipid accumulation in the rats' kidneys (Albrahim & Robert, 2022). Although findings from the current point to similarities in the kidney fat content of the Wistar rats fed the high-fructose diet and their control counterparts, it is important to note in females the orally administered lycopene prevented the obesogenic (high fructose) diet-induced increased pancreatic lipid accretion which points to lycopene's lipid lowering activity in key organs. It is important to note that, findings from the current study shows the downwards trend of pancreatic lipid content from high fructose diet to fenofibrate in female rats. This suggests that unlike lycopene which prevented the high fructose diet-induced pancreatic lipid accretion, fenofibrate only managed to "dampen" the high calorie diet's effect on pancreatic lipid deposition in females.

Prolonged consumption of high calorie diets has been shown to increase fat deposition resulting in obesity and oxidative stress from excessive production of ROS due to increased lipid peroxidation (Nakagawa *et al*., 2020). Malondialdehyde (MDA), a highly reactive compound occurs naturally and is marker of oxidative stress. In their study Jarukamjorn et al. (2016) observed that chronic feeding ICR rats a high fat, high fructose diet increased MDA

concentration and heightened CAT, GPX-1 and SOD activities. In contrast, finding from the current study showed that long term (12 weeks) feeding of a high-fructose diet neither mediated increased pancreatic homogenate MDA concentration nor pancreatic homogenate GPX-1 and SOD activities in female and male Wistar rats. However, despite the lack of statistical significant differences in the MDA concentration and GPX-1 activities of the Wistar rats fed the high fructose diet and their control counterparts, the MDA concentration of males fed the high fructose diet was about 75% higher compared to that of control counterparts suggestive of a possible tendency towards increased risk to diet-induced oxidative stress than females. Increased MDA concentrations are associated with cellular, cell organelles and DNA damage (Engwa *et al.*, 2022). Such oxidative stress induced damage disrupts normal cellular function and contributes to genesis of metabolic diseases. As reported previously fructose and lipid accumulation can increase reactive oxygen species production, for example, superoxide anion and hydrogen peroxide (Madlala *et al.,* 2016; Colak and Pap, 2021). The increased ROS then causes oxidative stress because of imbalance between oxidants and antioxidants (Pizzino *et al*., 2017b). Lycopene is recognised as a powerful antioxidant (Caseiro *et al.,* 2020). A previous study showed that in male diabetic Sprague Dawley rats, administered lycopene mediated an elevation the CAT, GPX-1 and SOD activities (Zheng *et al*., 2019) indicating an enhanced antioxidant response. Tuberculosis therapeutic agents isoniazid and rifampicin are known to cause oxidative stress as a side effects (Zhuang *et al*., 2022). It has been demonstrated that orally administered lycopene at a dose of 5 mg/kg body mass mediates kidney protection against the isoniazid and rifampicin induced shift in oxidant and antioxidant balance that favours oxidative stress (Bedir *et al*., 2021). In this study while treatments did not affect female and male Wistar rats' pancreata homogenate MDA concentration and hence oxidative stress level, it is noteworthy to indicate that the MDA concentration of male rats that consumed the high fructose diet without either lycopene and or fenofibrate as an intervention was 75% higher than that of control counterparts. Though this difference was not statistically significant, it points to a "biological significance" which might mean that chronic consumption of dietary fructose in males may predispose then to potential oxidative stress. This assertion is supported by the observation that in male rats that had lycopene as an intervention the 75% non-statistically significant increase in MDA concentration was "attenuated by some 50% to 75% which points to and support the notion that lycopene has antioxidant activity and can protect against oxidative stress. The multiple double bonds in the chemical structure of lycopene allows it to be a potent donor of electrons to free radicals (Islamian & Mehrali, 2015) which explains its ability to quench increased ROS production thus preventing oxidative stress.

Chronic consumption of dietary fructose causes obesity and the development of metabolic complications and diseases such as metabolic syndrome (Bier *et al*., 2022). The metabolism of dietary fructose in the liver which promotes *de novo* lipogenesis has now also been demonstrated in kidneys. In the study Bier et al. (2022) observed the kidneys of mature male Sprague Dawley rats fed a high-fructose diet were heavier, had higher total adipophilin and triglyceride content and increased ectopic fat compared to those of control counterparts. In contrast, findings from the current study show that consumption of a high-fructose diet by growing male and female Wistar for 12 weeks did not impact their kidney and abdominal fat masses. While long term intake of fructose increases adiposity and fat deposition in key organs such as the liver and kidneys (Bier *et al*., 2022) this is usually the case in adult animals. It can, therefore, be argued that the observed lack of impact dietary fructose on the rats' kidney and abdominal (visceral) fat mass could be due to that fact that the current study used growing Wistar rats. Growing animals, rats included, use the "extra calories" from fructose to support growth and to fuel their relatively high metabolic needs compared to non-growing adult rats that put on weight from excess calories (de Moura *et al*., 2009). Furthermore, it has been established that young rats have greater resistance to the development of visceral adiposity induced by a high-fructose diet (Ibrahim *et al*., 2017) thus in the current study dietary fructose's lack of impact on the rats' kidney and visceral fat yield could be due to their younger age at study inception and during the course of the study. Similarly, the lack of impact by dietary fructose on the rats' kidney fat content observed in the current study is most likely due to the younger rats' resistance to development of visceral adiposity (Ibrahim *et al*., 2017) when compared to adult rats that are more prone to developing visceral adiposity including excessive fat deposition both in the liver and kidneys (Bier *et al*., 2022). Despite dietary fructose's lack of significant impact on the rats' kidney lipid content, it important to point out that the male Wistar rats fed the high fructose diet had 1.5 times more kidney fat content compared to that of control as well as fenofibrate and lycopene administered counterparts. This is suggestive of dietary fructose's propensity to mediate increased fat deposition in the kidneys and fenofibrate and lycopene anti-adiposity activity. Increased fat infiltration in kidneys predisposes them to oxidative stress, inflammation and subsequent fibrosis (Wang *et al*., 2022). Chronic kidney fat accumulation can lead to proteinuria, glomerular and tubular diseases (Wang *et al*., 2022) and this would compromise bone development, remodelling and repair since kidneys regulates the calcium and phosphate homeostasis (Wei *et al.*, 2016).

## 5.3 Effect on femora and tibiae health

Bone turnover markers increase in direct proportion to fracture risk regardless of bone mineral density (Coates, 2013). It has been reported that serum CTX-1 is a more sensitive and specific marker of bone resorption than other resorption indicators (Jain & Camacho, 2018). Previous study reported that male Wistar rats fed a high carbohydrate high fat diet for 16 week had significantly elevated CTX-1 but "normal" osteocalcin concentrations compared to control counterparts (Wong *et al*., 2018). In their study Dai et al. (2017) observed that chronic consumption of a high fructose diet did not impact the plasma osteocalcin concentration of male Wistar rats (Dai *et al*., 2017). The decrease in osteoblastic differentiation and proliferation was reported to be the reason for the osteocalcin observations (Wong *et al*., 2018). In the current study consumption of the high-fructose diet had no effect on the serum osteocalcin and CTX-1 concentrations which suggest dietary fructose may have had no effect on the bone turnover. The similarity in serum CTX-1 and osteocalcin concentration across treatments regimens further suggests that, like dietary fructose, both lycopene and fenofibrate may also not have affected bone homeostasis via stimulatory effects by these two hormones. However, further investigations on effects on other serum and molecular markers of bone turnover markers could help shed more light.

Bass et al. (2013) report that in male Sprague-Dawley rats long term consumption of a high-fructose diet increased bone strength compared to the high glucose diet. This observation is in tandem with the observed obesity-induced increase in bone mineral density due to associated increased mechanical load (Piñar-Gutierrez *et al*., 2022; Stárka *et al*., 2020). However, findings from the current study show that the consumption of high-fructose diet had no statistically significant effect on the Wistar rats' femora and tibiae strength. Despite the observed similarity in bone breaking strength across treatment regimens in the current study, femora of males that consumed the high fructose diet had a breaking strength that was 23% lower compared to that of controls pointing to potential dietary-fructose induced weakening. Yang et al. (2021) reports that men are more susceptible to increased visceral adiposity and less susceptible to subcutaneous fat. Furthermore, it has been shown that feeding pathogen-free male BALB/c mice a high fat diet can lead to obesity-induced bone fragility and bone loss through homeostasis disequilibrium of the gut microbiota (Song *et al*., 2023) suggesting that men can be linked to obesity-induced increased risk of bone fragility. However, further investigations on effects on other serum and molecular markers of bone turnover markers could help shed

more light. Different factors, among them, bone mineral density (BMD) affect bone remodelling (Segheto *et al*., 2020). Lycopene, a lipophilic carotenoid, has been reported to have potential protective effects against bone loss (Walallawita *et al*., 2020). Findings from the current study show that intake of the high-fructose diet alone and or with lycopene as a dietary supplement had no effect on the Wistar rats' femora and tibiae strength compared to controls. However, females fed a high-fructose diet and administered fenofibrate as an intervention had decreased femora strength compared to counterparts that consumed the high fructose diet. These findings suggest while dietary fructose and supplemental lycopene did not compromise femora and tibiae strength, fenofibrate's use to mitigate high-calorie diet induced metabolic derangements and diseases could lead to weakened femora in females and predispose them to increased risk of fracturing. The fenofibrate-induced bone fracturing risk has also been reported by Shi et al. (2017) who observed that in mice of type-2 diabetes model induced by feeding a high-fat diet treatment with fenofibrate decreased bone quality compromising structure and strength. In vivo fenofibrate mediates decreased expression of collagen I and osteocalcin that results in reduced bone strength (Shi *et al*., 2017).

Han et al. (2022) reported that chronic consumption of a high fructose diet decreased the thickness of trabecular bones of male rats compared to control counterparts. Decreased bone thickness compromises bone health and thin and weak bones are associated with increased risk of fracturing (Stephens *et al.,* 2016). Bone health is critical especially in weight-bearing anti-gravity bones, for example, femora and tibiae. Femora bear weight and offer support and are the longest and strongest bones in the human body (Kiratli *et al.,* 2000; Jackson *et al.,* 2018). In their study Han and Hahn (2016) reported that intertrochanteric fractures are mostly a result of altered bone mineral content. Findings from the current study demonstrated that long term intake of the high-fructose diet reduced the male Wistar rats' sub-trochanteric medio-lateral diameters, which is in agreement with observed decreased trabecular bone thickness in male rats fed a high fructose diet (Han *et al*., 2022). Although the current study did not determine femora and tibiae mineral content, one can speculate that the observed decrease in the sub-trochanteric medio-lateral diameters in rats fed the high fructose diet could have been due to altered bone mineral homeostasis. This high fructose diet induced decreased diameters are likely to compromise femora strength increasing the risk of fracturing. Dietary fructose was observed to stimulate increased deposition of calcium, magnesium and phosphorus in kidneys disrupting bone mineral homeostasis in the process (Bass et al., 2013).The resultant bone mineral imbalance can affect the femora mid-shaft diameter. In a human study where femora

were evaluated using dual-energy x-ray absorptiometry scanning, it was observed that reduced femora BMD was associated with increased risk of fragility fracture (Khoury & Szalay, 2007). In the current study, consumption of the high-fructose diet significantly reduced the mid-shaft diameter in female Wistar rats pointing to possible dietary fructose induced weakening of femora strength and resistance to loading thus predisposing them to increased risk of fracturing. Metabolism of dietary fructose produces metabolic substrates that promote increased *de novo* synthesis of free fatty acids (FFAs) which (FFAs) on being metabolised result in excessive ROS production through mitochondrial fatty acids β-oxidation (Qiao *et al.,* 2021). The increased ROS stimulates signal transduction pathways that mediate apoptosis in bone marrow (Qiao *et al.,* 2021). In the Framingham Osteoporosis Study, Sahni et al. (2009) observed that in both men and women the consumption of carotenoid-based antioxidants as dietary supplements increased BMD in females' lumbar spine and males' trochanter; which indicates protective effects on bone health. Findings from the current study show that the administered lycopene prevented the dietary fructose induced reduced sub-trochanteric medio-lateral diameter and mid-shaft dimeters in males and females, respectively which points to its prophylactic effect against diet-induced bone structural alterations. However, it is important to point out that findings from the current study showed that intervening with a low dose of lycopene (30 mg/kg body mass daily) reduced the female Wistar rats' femora sub-trochanteric medio-lateral diameters which suggests that while lycopene showed protective effects on bone health, in female's low dose supplemental lycopene may compromise bone health. Findings from the current study show that unlike lycopene which protected against the high-fructose diet induced reduction in femora diameters, in females intervening with fenofibrate did not protect their femora MIDD from fructose induced decrease in diameter. Furthermore, in males fed the high fructose diet, administered fenofibrate decreased femora STMLD. Thus, it can be argued that use of fenofibrate in the management of diet-induced metabolic derangements and diseases should be done with caution as it may compromise femora health in males.

A cross sectional study instituted by the International Diabetes Federation in adult females and males suffering with metabolic syndrome found no association between metabolic syndrome and femora neck bone width (Muka *et al*., 2015). However, this study did not take the cause of metabolic syndrome into consideration. Current study findings treatments did not affect femora head and neck transverse diameter suggesting the dietary fructose had no detrimental effect on femora head and neck transverse diameters. Importantly, these findings also suggest that fenofibrate and lycopene can be used to mitigate the effects of diet-induced metabolic

derangements without risk of compromising femora head and neck transverse diameters. In the study on male Wistar rats fed a high fat/high sucrose diet, Gerbaix et al. (2012) observed that the diet improved bone mass and BMD but did not impact tibial bone volumes. In the current study, chronic consumption of the high-fructose diet did not affect tibial medio-lateral diameter, epiphyseal, maximum distal epiphyseal and maximum proximal epiphyseal breadth suggesting that it does not affect these tibiae anthropometric parameters.

Dietary fructose mediated sexual dimorphic responses in growing female and male Wistar rats. In females the consumption of high-fructose diet induced pancreatic lipid accretion and reduced femora mid-shaft diameters but in males it reduced the femora sub-trochanteric medio-lateral diameters. This effects suggests a potential link between diet and metabolic health in females highlighting the importance of dietary choices in maintaining overall health and density. In males the above findings indicate a specific impact on the bone structure, potentially affecting bone strength and overall skeletal health. The above findings highlight the importance of considering gender differences thus healthcare providers can tailor interventions to address specific needs for individuals of all genders. Lycopene protected against dietary fructose-induced increased pancreatic lipid accretion and reduced femora mid-shaft diameters in females. Furthermore, it protected males against high fructose diet-induced reduction of femora sub-trochanteric medio-lateral diameters. However, the low dose of lycopene reduced the females' femora sub-trochanteric medio-lateral diameters. Lycopene, therefore, could potentially be exploited as a prophylactic agent against obesogenic diet-induced non-alcoholic pancreatic fatty diseases in females. It can also be used as a dietary supplement to protect against diet-induced reduction in femora mid-shaft diameters and femora sub-trochanteric medio-lateral diameters in females and males, respectively. This is significant because it shows how lycopene may be used as a natural remedy to lessen the damaging effects of dietary intake on bone and metabolic health. By promoting the consumption of lycopene-rich foods, healthcare providers can potentially support better health outcomes and reduce the burden of metabolic disorders and bone-related conditions in female populations. However, caution is advised as administering lycopene at a low dose can compromise the femora sub-trochanteric medio-lateral diameters of females.

Having discussed the outcome of this study in this chapter, the next chapter will focus on the conclusion, limitations and recommendations pertinent to this study.

# CHAPTER 6: CONCLUSIONS, LIMITATIONS AND RECOMMENDATIONS

The current study evaluated the prophylactic effects of lycopene on growth performance, kidney and pancreatic mass and lipid content as well as the pancreatic oxidative and anti-oxidative status, abdominal fat and tibia and femora morphometry and breaking strength and serum concentration of osteocalcin and CTX-1 in growing Wistar rats fed a high-fructose diet.

The findings from this study showed that the chronic consumption of high-fructose diet for 12 weeks did not have a significant effect on the growth performance, abdominal fat, pancreatic mass, oxidative stress and natural antioxidant enzymes activities, kidney mass and lipid content, femora mass, lengths, head transverse diameter and neck transverse diameter, and tibia mass, length, maximum distal epiphyseal breadth, maximum proximal epiphyseal breadth, and medio-lateral diameter and serum osteocalcin and CTX-1. Dietary fructose mediated sexual dimorphic effects. In female rats, dietary fructose mediated increased the pancreatic lipid accretion and reduced femora mid-shaft diameter and in male rats, it reduced the femora sub-trochanteric medio-lateral diameter. Orally administered lycopene prevented the high-fructose diet induced increase in the pancreatic lipid accretion in females, and the reduction in the mid-shaft diameter and sub-trochanteric medio-lateral diameter in female and male rats, respectively. These findings demonstrated that when used as a dietary supplement, lycopene can potentially protect growing Wistar rats, and possibly growing children against high-fructose diet induced pancreatic lipid accretion which may increase risk for the development of on NAPFD and negative effects on femora diameters. While the lycopene showed positive effects on pancreatic lipid accretion and femora diameters, its low dose (30 mg/kg body weighed) caused a reduction in the femora sub-trochanteric medio-lateral diameter in females suggesting that its use as a dietary supplement requires caution as it might increase the risk of fracturing in females. This outcome requires further evaluation to determine the mechanism that underpins the low lycopene dose's negative effects on female femora diameter. The gene expression profiling with quantitative real-time polymerase chain reaction or ribonucleic acid sequencing to assess changes in gene expression level in the femur tissue exposed to low dose lycopene can help identify key gene or signalling pathways affected by the low dose of lycopene. Considering that not enough tests were conducted in the current study to assess bone health, histological analysis on the femur tissue sections using haematoxylin and eosin stain can help assess morphological changes such as alterations in the bone structure, or inflammations induced.

Long term consumption of the high-fructose diet increased the female femora mid-shaft diameter and as an intervention orally administered fenofibrate caused increased kidney masses, reduced tibia and femora strength and reduced the femora mid-shaft diameter thus compromising bone and kidney health. These findings suggest that use of fenofibrate to mitigate components of diet-induced MetS in growing children need caution as it elicits negative effects. In contemporary studies similar deleterious effects of fenofibrate have been reported in growing rats (Pan *et al.*, 2012; Lembede, 2014; Shi *et al*., 2017). Despite its mediation of deleterious effects, fenofibrate 'dampened' the high-fructose induced pancreatic lipid deposition potentially indicating some level of protection action against diet-induced NAPFD but lycopene demonstrated efficacy because it prevented diet-induced pancreatic lipid accretion.

Establishing mechanisms by which interventions such as lycopene prevent obesogenic diet induced metabolic derangements give better understanding. Histological analysis could have helped establish how the lycopene prevented pancreatic lipid accretion and narrowing of femora diameters in rats fed the obesogenic high-fructose diet. Therefore, future studies on the prophylactic effects of lycopene on the bone, kidney and the pancreas should include molecular assays in order elucidate potential mechanisms through which the lycopene exerts the prophylactic effects observed in the current study.

# CHAPTER 7: REFERENCES

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# APPENDICES

## Appendix I: Animal Ethics and Modifications and Extensions to Experiments

AESC 2012 M&E

**Please note that only type written applications will be accepted.**

**UNIVERSITY OF THE WITWATERSRAND**
**ANIMAL ETHICS SCREENING COMMITTEE**
**MODIFICATIONS AND EXTENSIONS TO EXPERIMENTS**

a. Name: Mercy Shafe (2404174)
b. School and email address: School of Physiology. 2404174@students.wits.ac.za
c. Experiment to be modified / extended **AESC NO**

| | | | |
|---|---|---|---|
| Original AESC number | **2022** | **03** | **02C** |
| Other M&Es: | | | nil |

d. Project Title: **Lycopene: Protective Potential Against Diet-Induced Metabolic Derangements in Wistar Rats.**

| | | No. | Species |
|---|---|---|---|
| e. | Number and species of animals originally approved: | 96 | Wistar Rats (Female: 48, Male: 48) |
| f. | Number of additional animals previously allocated on M&Es: | 0 | |
| g. | Total number of animals allocated to the experiment to date: | 10 | |
| h. | Number of animals used to date: | 10 | |

i. Specific modification / extension requested:

A new MSc Med student, Ms. Motlhale Gomotsegang (student no: 2643348; email address: 2643348@students.wits.ac.za) has registered with the School of Physiology, University of Witwatersrand, under the supervision of Prof. Eliton Chivandi (Wits) and Dr. Nontobeko Gumede (UL). Currently Ms. Gomotsegang is attached, as a co-worker, to aforementioned project. She has written and successfully presented her MSc. Medicine (Physiology) protocol with the study titled: **Effects of Lycopene on Bone, kidney, and Pancreatic Health of growing Wistar Rats fed an Obesogenic Diet.**

Ms. Gomotsegang Motlhale will conduct assays on the pancreata (fat content, lipid profile, histology, oxidant and antioxidant markers), kidneys (fat content, lipid profile, surrogate biomarkers, histology, oxidant and antioxidant markers) and bone (bone indices, bone breaking strength and histology) samples. Motlhale will also assay for serum surrogate markers (amylase and lipase) of pancreatic health and serum concentrations of hormones (osteocalcin, C-telopeptide of type I collagen and parathyroid hormone) that regulate bone health. Additionally, serum inflammatory and anti-oxidant biomarkers will also be analysed.

Motivation for modification / extension:

The animal study has just commenced and the tissues have not yet been collected. I am requesting your good office to allow Ms Motlhale Gomotsegang to use the stated tissues from the rats under my ethical clearance as this will be in conformity with the principle of the 3Rs and help reduce the number of animals (rats) by avoiding the need to repeat the study with new rats.

Date: 11/08/2022 Signature: 

Recommendations
Addition of a co-worker and addition of tissue samples as stated above.
Condition: the co-worker requires to assist to the WRAF induction training.

Signature: FMichael

Date: 12/08/2022

Chairman, AESC

## Appendix II: Approval of the research topic

UNIVERSITY OF THE
WITWATERSRAND
JOHANNESBURG

Private Bag 3 Wits, 2050
Fax: 027117172119
Tel: 02711 7172076

Reference: Mrs Sandra Benn
E-mail: sandra.benn@wits.ac.za

21 July 2022
Person No: 2643348
PAG

Miss G Motlhale
P.O.BOX 421
SETLAGOLE
Setlagole
2773
South Africa

Dear Miss Gomotsegang Motlhale

**Master of Science in Medicine: Approval of Title**

We have pleasure in advising that your proposal entitled *Effects of lycopene on bone, kidney, and pancreatic health in growing Wistar rats fed an obesogenic diet* has been approved. Please note that any amendments to this title have to be endorsed by the Faculty's higher degrees committee and formally approved.

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

**Appendix III: Commercial rat chow nutrient content.**

