Does Curcumin administered in the early post weaning period have long lasting beneficial effects in rats on a high fructose diet?

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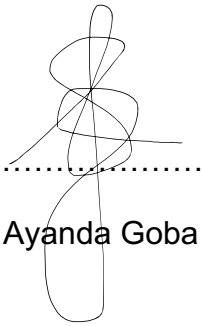
A dissertation submitted to the Faculty of Health Sciences, University of Witwatersrand, School of Physiology, in fulfilment of the requirements for the degree of Master of Science in Medicine.

Johannesburg, 2020

Declaration

I, **Lihle Ayanda Goba**, declare that this dissertation is my own work. 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.



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Lihle Ayanda Goba

Signed on the 15th day of June 2020

Dedication

This dissertation is dedicated to my late sister, Ntokozo Goba (1989-2012)

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I would like to thank my supervisors, Professor Kennedy H. Erlwanger and Dr. Janine Donaldson for their continued support, guidance and leadership throughout my studies.

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Table of Contents

Declaration ii

Dedication iii

Acknowledgements iv

List of abbreviations viii

List of figures xiii

List of tables xiv

Chapter 1: Introduction 1

1.1 Literature review 2

1.1.1 Obesity 2

1.1.2 Nonalcoholic fatty liver disease 3

1.1.3 Developmental programming for metabolic health 6

1.1.4 The early post weaning period– a critical period of developmental plasticity 6

1.1.5 Sex differences in response to dietary interventions 9

1.1.6 Management of obesity and NAFLD 9

1.1.7 Curcumin 10

1.1.8 Toxicity of curcumin 14

1.2 Justification for the study 14

1.3 Aim and study objectives 15

1.4 Hypotheses 16

Chapter 2: Materials and Method 17

2.1 List of reagents and consumables 18

2.2 Ethical clearance 18

2.3 Animals, housing and feeding 19

2.3.1 Preparation of gelatine cubes for administration of treatment 19

2.3.2 Rationale for dose for Curcumin and Fenofibrate used in the study 20

2.4 Experimental design 20

2.5 Assessment of Growth Performance 22

2.5.1 Determination of body mass 22

2.6 Terminal Procedures 22

2.6.1 Determination of linear growth 23

2.6.2 Determination of visceral organ morphometry 23

2.6.3 Circulating metabolic substrates 23
2.6.4 Plasma hormones associated with obesity 24
2.6.5 Measurement of red blood cell indices 25
2.6.6 Determination of kidney histopathology and hepatic histology scores for NAFLD 25
2.7 Statistical analyses 26
Chapter 3: Results 27
3.1 The effects of curcumin on growth performance 28
3.1.1 Body mass 28
3.1.2 Linear growth 34
3.1.3 Radiograph images of tibias and femurs 37
Subjective visualisation showed no differences in the radio density of the long bones from the males and the females (figures 3.5 to 3.8). 37
3.2 Circulating metabolic substrates 41
3.2.1 Triacylglycerol and cholesterol concentrations 41
3.3 Plasma hormones associated with obesity 43
3.3.1 Male rats 43
3.3.2 Female rats 44
3.4 The effect of orally administered curcumin on visceral organ morphometry 45
3.4.1 Male rats 45
3.4.2 Female rats 49
3.5 Hepatic steatosis scoring and histology 52
3.5.1 Nonalcoholic fatty liver disease score and representative liver histology photo sections (H and E staining, 40X magnification) 52
3.6 Red blood cell indices 56
3.6.1 Male rats 56
3.6.2 Female rats 57
Chapter 4: Discussion 58
4.1 Growth performance 59
4.2 Metabolic dysfunction parameters 62
4.2.1 Plasma cholesterol and triglyceride concentrations 62
4.2.2 Plasma hormones 64
4.2.3 Visceral organ morphometry 65
4.2.4 Liver histomorphometry and NAFLD scores 67
4.2.5 Red blood cell indices 69

4.2.6 Limitations and recommendations **70**
Chapter 5: Conclusions 72
Chapter 6: References 75
Chapter 7: Appendices 91

List of abbreviations

ALP - Alkaline phosphatase

ALT - Alanine aminotransferase

ALX4 - Homeobox protein aristaless-like 4

AMPK - Adenosine monophosphate activated protein kinase

AREC - Animal research ethics committee

BMI - Body mass index

CAT - Catalase

ChREBP - Carbohydrate - responsive element binding protein

COD - PAP - Cholesterol oxidase phenol 4- aminoantipyrine peroxidase

CUR – Curcumin

DMSO - Dimethyl sulfoxide

DNL - De novo lipogenesis

ELISA - Enzyme linked immune - sorbent assay

FAS - Fatty acid synthase

FCUR - Fructose and curcumin

FEN - Fenofibrate

FIAF - Fasting - induced adipocyte factor

FS - Fructose solution

GC - Gelatine cube

GH - Growth hormone

GIT - Gastrointestinal tract

GPO - PAP - Glycerine phosphate oxidase peroxidase

Hb - Age - Haemoglobin advanced glycation end products

HOMA - IR - Homeostatic model assessment of insulin resistance

IGF - 1 - Insulin - like growth factor 1

IL - 1 - Interleukin 1

IRS - Insulin receptor substrate

JNK - c - Jun N - terminal kinases

LDL - Low - density lipoproteins

L.I - Large intestine

MAPK - Mitogen activated protein kinases

MCHC - Mean corpuscular haemoglobin concentration

MDA - Malondialdehyde

mRNA - Messenger RNA

NAFLD - Nonalcoholic fatty liver disease

NAS - Nonalcoholic fatty liver disease score

NASH - Nonalcoholic steatohepatitis

PPAR - y - Peroxisome proliferator- activated receptor

PDW - Plain drinking water

PGC - Plain gelatine cube

ROS - Reactive oxygen species

SEPT9 - Septin 9

SOD - Superoxide dismutase

S.I - Small intestine

SRC - Standard rat chow

SREBP - 1 - Sterol regulatory element - binding protein 1

TAG - Triacylglycerol

TNF - α - Tumour necrosis factor alpha

VLDL - Very low - density lipoproteins

Abstract

Increased intake of fructose is associated with metabolic disorders such as obesity and other obesity-related complications. These metabolic disorders also affect children and it is essential to find strategies to prevent the adverse effects. The early post weaning period is a window of developmental plasticity which can be targeted for long lasting beneficial health effects. The use of phytomedicines has increased as they are inexpensive, safe and potentially have positive metabolic health benefits. This study investigated the potential of a phytomedicine, curcumin, in the early post weaning phase to induce long term positive metabolic health benefits in male and female rats on a high fructose diet. Eighty, 21 - day old rat pups (n = 40 males and n = 40 females) were randomly allocated into five treatment groups (n = 8 males and n = 8 females per group); Group I was fed standard rat chow (SRC), plain drinking water (PDW) and plain gelatine cubes (PGC), II was fed SRC, 20% FS as drinking fluid and PGC from postnatal day 21 to 35 and then SRC and 20 % FS for the rest of the study, III was fed SRC, 20% FS and curcumin (500mg/kg body weight) in gelatine cubes (GC) from postnatal day 21 to day 35 and then SRC and 20% FS, IV was fed SRC, PDW and curcumin at 500mg/kg body weight in GC from postnatal day 21 to 35 and then SRC and PDW, while V was fed SRC, PDW and fenofibrate (100mg/kg body weight) in GC from postnatal day 21 to 35 and then SRC and 20% FS for the remainder of the study. Male rats fed fructose and curcumin were significantly ($p<0.05$) lower in body mass compared to the controls. The body mass of female rats showed no significant differences across treatment groups. No significant differences ($P>0.05$, ANOVA) were observed in the triaglycerol and cholesterol concentrations as well as the insulin and adiponectin concentrations between groups. In the visceral organs of the males, the fructose fed rats had smaller small intestines compared to controls, as well as lower absolute large intestine masses and relative stomach masses ($P<0.05$). In the male rats fed fructose, a microsteatosis score of 1 was observed. In the female rats fed curcumin and fenofibrate alone, a microsteatosis score of 1 was observed. In male and female rats fed fructose alone, a score of 1 for microsteatosis and 0 for macrosteatosis was observed. The negative effects of fructose in the liver were attenuated by curcumin. Thus, strategic

administration of curcumin during the early postweaning period should be further explored for the prophylaxis of diet induced NAFLD.

List of figures

Figure 1.1 Chemical structure of curcumin 11
Figure 1.2 Schematic showing the biological activities of curcumin 12
Figure 2.1 Illustration of the study design of male and female Sprague Dawley rats fed a high fructose diet over six weeks 21
Figure 3.1 Effects of orally administered curcumin on the initial and terminal body masses of forty male Sprague Dawley rats fed high fructose diet over six weeks. 29
Figure 3.2 Effects of orally administered curcumin on the percentage body mass gain of forty male Sprague Dawley rats fed a high fructose diet over six weeks. 30
Figure 3.3 Effects of orally administered curcumin on the initial and terminal body masses of forty female Sprague Dawley rats fed a high fructose diet over six weeks… 32
Figure 3.4 Effects of orally administered curcumin on the percentage body masses of forty female Sprague Dawley rats fed a high fructose diet over six weeks. 33
Figure 3.5 Radiograpth images representative tibiae of forty male Sprague Dawley rats in the different study groups. 37
Figure 3.6 Radiograph images of representative femura of forty male Sprague Dawley rats in the different study. 38
Figure 3.7 Radiograph images representative tibiae of forty female Sprague Dawley rats in the different study groups. 39
Figure 3.8 Radiograph images of representative femura of forty female Sprague Dawley rats in the different study groups. 40
Figure 3.9 Photo sections showing the effects of orally administered curcumin on the liver histology (H and E staining, 40X magnification) of forty male Sprague Dawley rats fed a high fructose diet over six weeks. 53
Figure 3.10 Photo sections showing the effects of orally administered curcumin on the liver histology (H and E staining, 40X magnification) of female Sprague Dawley rats fed a high fructose diet over six weeks. 55

List of tables

Table 3.1 Effect of orally administered curcumin on tibia and femur masses, lengths and mass to length ratios of forty male rats fed a high fructose diet over six weeks 35
Table 3.2 Effect of orally administered curcumin on tibia and femur masses, lengths and mass to length ratios of forty female Sprague Dawley rats fed a high fructose diet over six weeks 36
Table 3.3 Effects of orally administered curcumin on triaglycerol and cholesterol concentrations of forty male Sprague Dawley rats fed a high fructose diet over six weeks 41
Table 3.4 Effects of orally administered curcumin on triaglycerol and cholesterol concentrations of forty female Sprague Dawley rats fed a high fructose diet over six weeks 42
Table 3.5 Effects of orally administered curcumin on insulin and adiponectin concentrations and HOMA-IR of forty male Sprague Dawley rats fed a high fructose diet over six weeks 43
Table 3.6 Effects of orally administered curcumin on insulin and adiponectin concentrations and HOMA-IR of forty female Sprague Dawley rats fed a high fructose diet over six weeks 44
Table 3.7 Effects of orally administered curcumin on the gastro-intestinal tract, accessory organs, heart, kidneys, epididymal and visceral fat mass (absolute and relative to body mass) of forty male Sprague Dawley rats fed a high fructose diet over six weeks 47
Table 3.8 Effects of orally administered curcumin on the gastro-intestinal tract, accessory organs, heart, kidneys and visceral fat mass (absolute and relative to body mass) of forty female Sprague Dawley rats fed a high fructose diet over six weeks 50
Table 3.9 Effects of orally administered curcumin on hepatic steatosis scores of forty male Sprague Dawley rats fed a high fructose diet for six weeks 52
Table 3.10 Effects of orally administered curcumin in the overall hepatic steatosis scores of forty female Sprague Dawley rats fed a high fructose diet for six weeks 54
Table 3.11 Effect of orally administered curcumin on red blood cell indices of forty male Sprague Dawley rats fed a high fructose diet for six weeks 56

Table 3.12 Effects of orally administered curcumin on red blood cell indices of forty female Sprague Dawley rats fed a high fructose diet for six weeks 57

Chapter 1: Introduction

1.1 Literature review

1.1.1 Obesity

In 2016, more than 1.9 billion adults (18 years and older) were overweight. Of these, 650 million were obese, where obesity is defined as individuals with a body mass index (BMI) of over 30 kg/m^2 (Penfold & Ozanne, 2017). The world health organization (WHO) has reported that the global prevalence of obesity has tripled since 1975 (Abarca-Gómez et al., 2017). Currently, South Africa has the highest obesity prevalence in southern Africa at 28.3 %. Furthermore 18 percent of children and adolescents living in South Africa, aged five to nineteen, were overweight in 2016 (Ludwig, 2018).

Obesity generally occurs due to an imbalance between energy consumption and energy expenditure (Goldstein, 2002). Poor dietary choices, including the consumption of diets rich in fructose, and living sedentary lifestyles have been reported to be the major causes of obesity (Karise et al., 2017). Other causes of obesity include sleep deprivation, perinatal factors, environmental endocrine disrupting chemicals, hormones such as leptin, insulin, oestrogens and androgens and genetics (DNA variations affecting expression of function). The abovementioned factors influence appetite, metabolism and body fat distribution (Montalvany-Antonucci et al., 2018; Panzhinskiy et al., 2019; Porte, 2006), all of which are linked to obesity related diseases. Obesity is associated with an increased risk of developing more than twenty medical conditions that contribute to significant morbidity and mortality. The diseases include: insulin resistance, hyperlipidaemia, cardiovascular, psychological and musculoskeletal diseases and nonalcoholic fatty liver disease (NAFLD) (Rupérez et al., 2018). The risks of these diseases increase with increasing body mass index (Reference). A major focus of this dissertation is nonalcoholic fatty liver disease.

1.1.2 Nonalcoholic fatty liver disease

1.1.2.1 Definition

NAFLD is a term used to define a specific type of liver pathology with the presence of ≥ 5% lipid accumulation (Dong, 2017). NAFLD results from the accumulation of triglycerides in the hepatocytes of individuals who consume little to no alcohol (Santiago-Rolón et al., 2015).

It describes liver damage ranging from simple steatosis to steatohepatitis (nonalcoholic steatohepatitis (NASH)), which is marked by lobular inflammation (Pierce & Koppe, 2018).

1.1.2.2 Epidemiology

Ten to twenty-four percent of the general population are affected by NAFLD (Pappachan et al., 2017), with its prevalence increasing to between 57.5-74% in obese individuals (Cheng et al., 2017). The regional prevalence of NAFLD in the world is estimated to be 23.4 % in Asia, 23.7 % in Europe, 31.8% in Middle-East, 64.6 % in America and 13 % in Africa (Perumpail et al., 2017). In women, the prevalence of NAFLD increases with age, especially after menopause. The prevalence between females ages 30 to 40 years old is estimated to be 12.5%, 16.1% in ages 41 to 50 years old, 21.6% in ages 51 to 60 years old and 25.4% over 60 years old (Golabi et al., 2019; Perumpail et al., 2017). In males, the prevalence between ages 30-40 years old is estimated to be 16.1%, 22.3% in ages 41 to 50 years old, 29.3% in ages 51 to 60 years old, and 27.6% in over 60 years of age (Golabi et al., 2019; Perumpail et al., 2017).

1.1.2.3 Aetiology

Several factors are causal in the development of NAFLD, including epigenetic changes, genetics, alterations in gut microbiome composition, the environment and nutrition. Over consumption of western diets with high refined sugar (fructose) and fat content, is a major contributor to the global epidemic of metabolic diseases (Alexander-Aguilera et al., 2017).

Fructose is a monosaccharide naturally present in fruits and vegetables. Consumption of fructose, as early as in childhood, has been shown to negatively affect appetite regulation, increase risk for obesity, and may set the foundation for the development of metabolic diseases in adulthood (Clements et al., 2018). In addition, the retention of triglycerides is also a risk factor for the progression of NAFLD (Alexander-Aguilera et al., 2017).

The metabolic abnormalities leading to the accumulation of lipids are not well documented, but they could consist of changes in hepatic fat metabolism due to insulin resistance (Pappachan et al., 2017). Insulin maintains energy stores and regulates the uptake of glucose into tissues (Porte, 2006). Insulin resistance is the impairment of target cells to utilise insulin (Reaven, 2015). Insulin resistance can occur in the hepatocytes, kidney cells, pancreatic β-cells, muscle cells, adipocytes and endothelial cells (Goldstein, 2002). Insulin resistance, being the most common factor in the progression of NAFLD, alongside oxidative stress, plays a critical role in the pathogenesis of NAFLD. NAFLD is commonly associated with components of the metabolic syndrome including central obesity, insulin resistance, hypertension, hypertriglyceridemia as well as type 2 diabetes (Ohashi et al., 2018), however it can occur in the absence of overt metabolic disorders and is multifactorial in causes.

1.1.2.4 Pathogenesis

The pathogenesis of NAFLD involves a complex interaction between diet, hormones, obesity, changes in gut microbiota, an excessive accumulation of triglycerides and other lipid species in the hepatocytes. While it is well established that NAFLD is commonly observed in obese individuals with metabolic syndrome and in patients with type 2 diabetes, the pathophysiology of NAFLD is complex and consists of changes in multiple pathways and mechanisms. Excessive fructose consumption leads to increased gluconeogenesis, lactate, glycogen synthesis and de novo lipid synthesis (Zhang et al., 2017).

Insulin resistance is the central mechanism that leads to lipotoxicity that triggers hepatic inflammation and finally disease progression (Muller et al., 2019; Stefan et al., 2019). In

hepatocytes, insulin has three main functions: first, insulin promotes glycogen storage (Horton et al., 2017), second, it inhibits gluconeogenesis (Woods et al., 2017) and third, it activates de novo lipogenesis (Horst, 2017; Jegatheesan & De Bandt, 2017). In healthy individuals, pancreatic beta cells secrete insulin mainly in response to circulating glucose levels. Insulin acts on adipose tissue to promote esterification of fatty acids and storage into lipid droplets while inhibiting lipolysis (Chalasani et al., 2018; Chen et al., 2017). In individuals with NAFLD, insulin resistance results in increased adipocyte lipolysis and high circulating free fatty acids available for hepatic uptake (Wan et al., 2017). It also results in reduced hepatic glycogen storage and increased gluconeogenesis (Fortino et al., 2017). Hyperinsulinemia develops, disrupting hepatic de novo lipogenesis pathways. This leads to increased intrahepatic lipid accumulation and excessive triglyceride secretion in the form of very low density lipoprotein (VLDL) (Inzaugarat et al., 2017). The increased lipid load expands into the adipose tissue, resulting in reduced ability of the adipocytes to store these lipids in lipid droplets (Dongiovanni et al, 2015) Enlarged and impaired adipose tissue function leads to systemic inflammation and insulin resistance which expands to the liver thus promoting metabolic stress in the hepatocytes (Montalvany-Antonucci et al., 2018; Zhang et al., 2017). The hepatocytes are therefore not able to accommodate neutral lipids in the lipid droplets thus exposing cells to lipotoxic bioactive lipids (Muller et al., 2019). Lipotoxicity impairs insulin signalling, causing oxidative damage and promoting inflammation (Muller et al., 2019). Inflammation in the liver leads to secretion of the pro-inflammatory cytokine, tumour necrosis factor alpha (TNF-α) (Spahis et al., 2018).

In addition, high fructose diets also disrupt gut microbiota composition and diversity (Alwahsh & Gebhardt, 2017). Microbiota are primary nutrient sensors located within the gastrointestinal tract (Gresse et al., 2017). Diet alters gut bacterial composition in NAFLD individuals. NAFLD individuals have dysfunctional gut epithelial tight junctions, allowing the bacteria access to the systemic circulation, resulting in the release of pro-inflammatory cytokines which in turn promote hepatic steatosis and inflammation (Shojaei Zarghani et al., 2016).

1.1.2.5 Diagnosis and staging of NAFLD

NAFLD can be diagnosed with the evidence of hepatic steatosis on imaging or histology, together with the lack of a secondary cause of hepatic fat accumulation (Barrière et al., 2018). Currently, liver biopsy is the gold standard test for the diagnosis of NAFLD and for staging of the liver disease (Anavi et al., 2017; Pierce & Koppe, 2018) .

Diseases such as obesity and NAFLD may be prevented by strategic interventions during critical developmentally plastic life stages through a process of developmental programming.

1.1.3 Developmental programming for metabolic health

The Barker hypothesis states that unfavourable nutrition in early life, including prenatally, increases susceptibility to the development of metabolic syndrome, including obesity, diabetes, insulin sensitivity and hyperlipidaemia (Barker et al., 1993). Studies have shown that it is possible to prevent this early developmental programming using nutritional, hormonal or pharmacological interventions in the period of developmental plasticity. The main critical periods of developmental plasticity include gestation (Zeltser, 2015), suckling (Ajibola, 2018) and weaning (Gresse et al., 2017; Luo et al., 2016). The suckling period has been targeted for preventive interventions against future development of metabolic syndrome as it serves as a critical window for epigenetic modification. Interventions during critical periods (mainly early postweaning) of developmental plasticity can programme not only for disease but good health outcomes later in life (Reynolds and Caton, 2012).

1.1.4 The early post weaning period– a critical period of developmental plasticity

During critical periods of development, nutritional, hormonal and metabolic environment permanently change the body's structure, physiology and metabolism and leads to disease later in life. The suckling-weaning period is a period of developmental plasticity which is characterized by a profound change in nutrition (Wankhade et al., 2016). Studies

have shown that increased nutrition and growth during the suckling period is associated with increased obesity later in life (Aparecida et al., 2016), whereas reduced nutrition and growth during this time window permanently reduced weight gain (Cripps et al., 2009) and conferred resistance to diet-induced obesity (Ozanne et al., 2004). For this reason, it has been targeted for prophylactic intervention against future development of metabolic syndrome as it serves as a critical window for epigenetic modification. Various lifestyle factors influence epigenetic modification such as diet, physical activity and obesity. Weaning is associated with an increase in stress hormones (Septin-9 (SEPT-9) and Homeobox protein aristaless-like 4 (ALX4) (hormones that direct the formation of body structures during early embryonic development) that influence epigenetic modification via DNA methylation and histone modification (Neal-Kluever et al., 2019). These result in changes in enzyme function and development of disease.

There are marked changes in the activity of rate limiting enzymes of metabolic pathways in the liver in early post weaning: Increases are observed in ATP-citrate lyase, acetyl-coA carboxylase and fatty acid synthase (major lipogenic enzymes) and lipogenesis (Rodrigues et al., 2011). The change in lipogenic activity, when offspring change from milk to solid food, is due to an increase in the rate of transcription of genes encoding for lipogenic enzymes, as mentioned above (Freudenberg et al., 2012), in the liver and white adipose tissue, which is preceded by similar changes in the levels of their corresponding mRNA (MacDonald et al., 2017). Evidence from several studies suggests that weaning onto a high carbohydrate diet results in a marked increase in plasma glucagon and insulin and also causes a decrease in plasma glucagon after meals (Prabhakar et al., 2015). The decrease in plasma glucagon can facilitate the insulin-induced expression of acetyl-coA carboxylase and fatty acid synthase (FAS) mRNA, as glucagon usually restricts the expression of lipogenic enzyme mRNA in white adipose tissue and the liver (Reynolds and Caton, 2012; Zeltser, 2015). Glucagon acts by increasing hepatic glucose production, stimulating de novo lipogenesis and inhibiting glucose breakdown and glycogen formation, thus affecting energy homeostasis (Cheng et al., 2017).

Studies have observed that weaning onto an obesogenic diet interrupts the development of the gut (Neal-Kluever et al., 2019), altering gut morphology and function, thereby

resulting in a decline in nutrition in neonates. Neonates undergo several morphological and functional changes in digestive function (Ishikawa et al., 2018). For example, the oesophagus shows an accelerated cell proliferation in the epithelium and an increased production and accumulation of mucus in the glands (Pierzynowski et al., 2016). The stomach shows rapid growth and a marked increase in acid secretion capacity.

Weaning also promotes dysbiosis of the gut microbiota (Abarca-Gómez et al., 2017). The gut is populated by a variety of indigenous microbes that influence both gut and liver function. Alterations in gut microbiota composition have been associated with the presence of obesity, which is accompanied by a low-grade inflammatory state (increases in tumour necrosis factor alpha (TNF-α) and interleukin 1 (IL-1)) in the intestines and increases the risk of several diseases including type 2 diabetes mellitus (Upadhyay et al., 2018). Recently, gut microbiota are recognized to alter production or bioavailability of small molecules, proteins and nutrients to contribute to inflammation in the host (Neal-Kluever et al., 2019). The diversity of the microbiome is different in adults and children. Infant microbiota composition is prone to environment changes (Laursen et al., 2017). Intestinal microbiota varies in children. Microbiota may suppress fasting induced adipocyte factor (fiaf) and lipoprotein lipase inhibitor, increasing lipid storage in adipocytes (Stefan et al., 2019). Adenosine monophosphate-activated protein kinase (AMPK), an important enzyme involved in energy homeostasis, may be suppressed by microbiota resulting in alterations in energy metabolism and hormone function (Abarca-Gómez et al., 2017). Hormones such as cortisol, insulin, epidermal growth factor (EGF) and insulin-like growth factor 1 (IGF-1) are found at high concentrations in maternal colostrum ingestion (Gresse et al., 2017).

Diet plays an important role in gut microbiota composition. Consumption of high sugar in the diet typically starts early in life. High fat and sugar diet habits in childhood, can predispose the development of several diseases in adulthood such as obesity, NAFLD and metabolic syndrome (Ishikawa et al., 2018; King et al., 2014). Data from animal studies suggests that post-natal environments are important in determining good metabolic health in adulthood (Goncalves et al., 2019). Thus, the early post weaning

period represents an important potential target for dietary manipulations with long lasting health benefits.

1.1.5 Sex differences in response to dietary interventions

Sexual dimorphism in disease susceptibility and metabolism have been well recognized. For example weaning of offspring onto a high fat or a high fructose diet results in obesity development with hyperinsulinemia and hyperglycaemia in males and obesity with hyperinsulinemia in females (King et al., 2014).

Negative dietary manipulations such as high fructose have been demonstrated to elicit detrimental effects in the liver of both males and females. It has been reported that men have higher postprandial hepatic de novo lipogenesis (DNL) when compared to age and BMI matched women (Ohashi et al., 2018). This observation is in line with findings from animal work which have suggested a role for oestrogen attenuating hepatic DNL, with acetyl-coA carboxylase activity being reduced with oestrogen treatment in female rats (Roberts et al., 2017). Oophorectomy has been reported to increase the expression of SREBP-1c and liver triaglycerols (TAG) content (Horton et al., 2017). Hussain et al. (2019) reported that after a high oral fructose load, markers of DNL were significantly higher in men compared to women. A study by Anavi et al. (2017) observed that the hepatic protein level of acetyl – coA carboxylase and FAS was significantly upregulated in female rats on a high fat high fructose diet for 12 weeks compared to male rats who showed no change (Anavi et al., 2017).

Thus, it is clear that the effects of weaning onto a specific diet can be sex specific. It is therefore important to note that studies should consider both males and females to investigate how they respond to dietary manipulations and treatments.

1.1.6 Management of obesity and NAFLD

Metabolic diseases continue to pose a threat to human health. Invasive and noninvasive approaches have been used in the management of obesity. The noninvasive methods include weight loss through anti-obesity medication (drugs targeting insulin resistance)

and lifestyle changes (change in diet and increases in physical activity). The invasive methods include weight loss through anti-obesity surgery (Black & Gupta, 2018).

Several commercially supplied drugs (Pierce & Koppe, 2018) are currently used in managing obesity and metabolic diseases associated with it. These include metformin, orlistat (Arzola-Paniagua et al., 2016) and lorcaserin (Tronieri et al., 2017) For the purposes of this dissertation, the focus will be on fenofibrate which was used as a positive control pharmacological intervention.

1.1.6.1 Fenofibrate

Fenofibrate is a drug from the group of fibrates which have been used to treat hypertriglyceridaemia and combined hyperlipidaemia patients. It acts by binding and activating its agonist, peroxisome proliferator-activated receptor alpha (PPAR-α). PPAR-α regulates enzymes that are important for lipoprotein and fat metabolism (Wahba et al., 2016). This results in the breakdown of fat and a decrease in circulating free fatty acids and triglycerides. It has also been reported to decrease total cholesterol concentrations and LDL concentrations whilst increasing HDL concentrations (Oidor-Chan et al., 2016).

In rodents, fenofibrate has been shown to reduce liver fat and reduce body weight. In previous studies where 100mg/kg fenofibrate was administered in rodents, it markedly improved NAFLD, ameliorating dyslipidaemia and fat accumulation in the liver, improved insulin resistance and ameliorate oxidative stress (Soria et al., 2002). Hepatic steatosis was reduced in fenofibrate treated rodents (Oidor-Chan et al., 2016).

Though commercially drugs are used to treat obesity and metabolic diseases, there is however, a growing use of medicinal plants and their biologically active phytochemicals (Chen et al., 2017; Mukonowenzou, 2016) for the holistic management of diseases. For the current study we used curcumin.

1.1.7 Curcumin

Curcumin [1, 7-bis (4-hydroxy-3-methoxyphenol)-1, 6-heptadiene-3, 5 Dione] is the active compound of Turmeric, extracted from the rhizome of *Curcuma longa* (Gupta et al., 2013).

1.1.7.1 Curcumin mechanisms of action

Curcumin (figure 1.1) has been reported to prevent lipid accumulation, regulate energy metabolism, and decrease levels of intracellular lipids (Maithilikarpagaselvi et al., 2016). Curcumin regulates transcription factors peroxisome proliferator-activated receptor y (PPAR- y), sterol regulatory element-binding protein 1 (SREBP-1), and carbohydrate-responsive element-binding protein (ChREBP). that play important roles in adipogenesis and lipogenesis (Farzaei et al., 2018). Previous studies indicate that curcumin restricts a number of signalling pathways known to be involved in inflammation and metabolic diseases (Shehzad, Ha, Subhan, and Lee, 2011). Curcumin treatment resulted in elevated phosphor- adenosine monophosphate-activated protein kinase levels, reduced glycerol-3-phosphate acyl-transferase, and increased carnitine palmitoyltransferase 1 expression which led to fatty acid oxidation and decreased fatty acid esterification (Adibian, 2019).

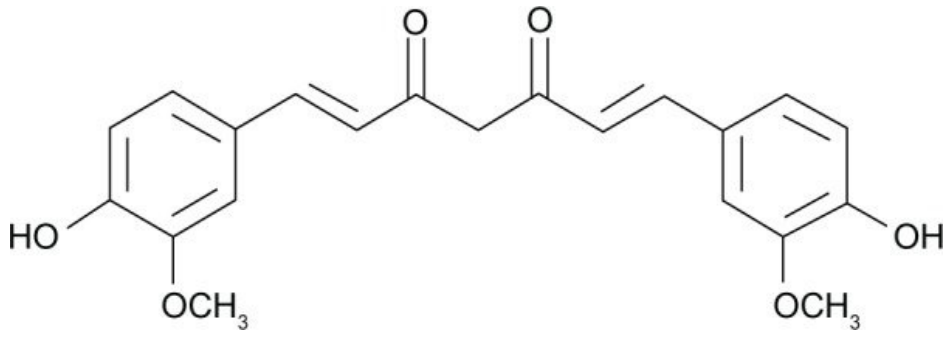

Figure 1.1 Chemical structure of curcumin.

1.1.7.2 Bioavailability of curcumin

Gupta et al. (2013) showed that curcumin has poor absorption, metabolism and bioavailability resulting in low plasma and tissue levels (Black & Gupta, 2018; Gupta et al., 2013). Numerous approaches have been introduced to improve the bioavailability of curcumin. These include: the use of piperine, liposomal curcumin, curcumin nanoparticles and the use of structural analogues of curcumin (demethoxycurcumin and bisdemethoxycurcumin (EF-24). The above mentioned have been reported to have rapid absorption with a peak plasma half-life 2014).

1.1.7.3 Curcumin bioactivity

Previous findings have proven that curcumin has several biological activities (see figure 1.2). These include therapeutic potential as anti-obesity, anti-diabetic, antioxidant and anti-inflammatory, all of which are hepatoprotective .(Inzaugarat et al., 2017; Kimura et al., 2018; Li et al., 2009).

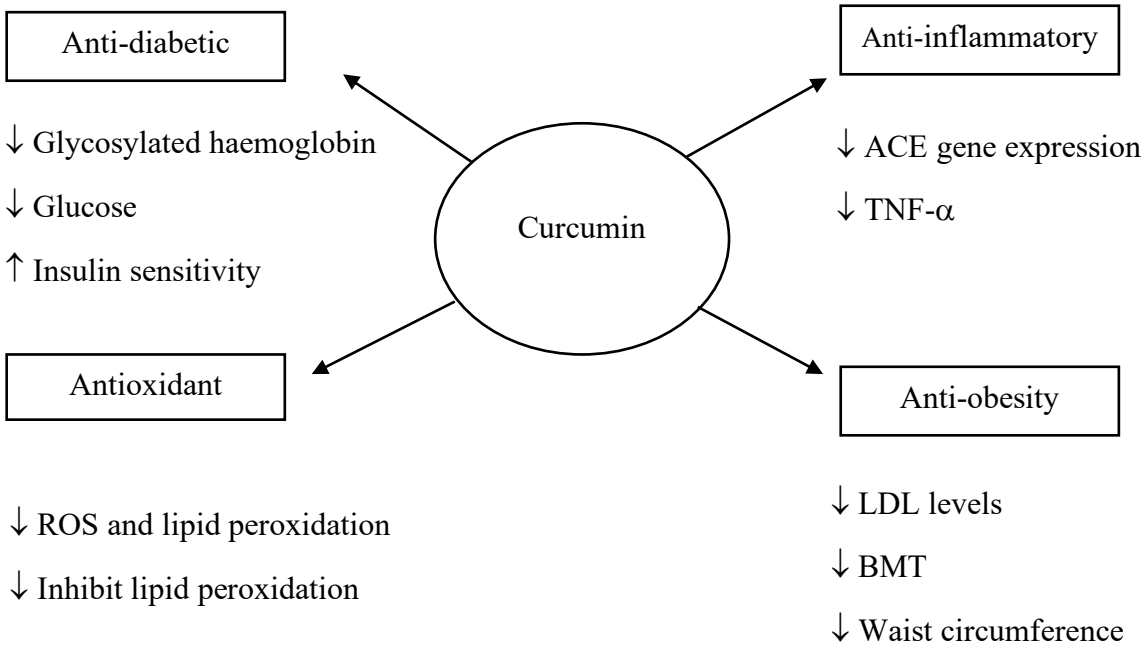

Figure 1.2 Schematic showing the biological activities of curcumin

1.1.7.4 Hypoglycaemic/antidiabetic effects of curcumin

According to a review article by Ejaz et al.(2009), the hypoglycaemic response of curcumin was initially documented in 1972 and obtained scientific recognition as a potential remedial agent for diabetes mellitus (Ejaz et al., 2009). Oral administration of curcumin restricted body weight gain and reduced the amounts of glucose and glycosylated haemoglobin (HBA1c) in the blood of two rat models for drug (alloxan and

streptozotocin) induced diabetes (Ghosh et al., 2015). Curcumin also reportedly improved insulin sensitivity (Gupta et al., 2013).

1.1.7.5 Anti-obesity effects of curcumin

In a study by Miyazawa et al. (2018), obese individuals received 750 mg of curcumin orally for 12 weeks (Miyazawa et al., 2018). Following the treatment period, the individuals showed decreased LDL levels at 6 and 12 weeks as well as increased HDL levels at 12 weeks when compared to baseline. In another study by Article et al. (2017) individuals diagnosed with NAFLD received 1g of curcumin orally for 8 weeks along with a diet (Article et al., 2017). Supplementation with curcumin significantly reduced BMI and waist circumference and AST and ALT serum levels.

1.1.7.6 Antioxidant effects of curcumin

Curcumin contains a variety of functional antioxidant groups, including the carbon to carbon double bonds and phenyl rings. Curcumin can thus eliminate lipid radicals in the cell membrane and become a phenoxy radical (Ghosh et al., 2015; Soleimani et al., 2018), therefore it is considered a very strong lipid antioxidant. Furthermore curcumin was found to inhibit lipid peroxidation and neutralize reactive oxygen species (ROS) such as superoxide, peroxyl and hydroxyl radicals (El-Moselhy et al., 2011).

1.1.7.7 Anti-inflammatory effects of curcumin

Tumour necrosis factor α (TNF-α) is a major mediator of inflammation in most diseases, and this effect is regulated by the activation of a transcription factor, nuclear factor (NF-$_k$B) (Maithilikarpagaselvi et al., 2016). NF-$_k$B is also activated by most inflammatory cytokines such as high glucose and fatty acids (Shakibaei & Mobasheri, 2010). Curcumin has been shown to block NF-$_k$B activation increased by several different

inflammatory stimuli. Curcumin has also been shown to suppress inflammation through many different mechanisms.

1.1.8 Toxicity of curcumin

Studies involving curcumin in humans did not observe any toxic effects, and curcumin was deemed safe at a high dose of 6g a day orally for 4-7 weeks (Soleimani et al., 2018). In another study by Prasad et al. (2014), curcumin administered orally in mice and rats at 5g/kg for 14 days also did not show any toxic effects (Prasad et al., 2014). Due to poor absorption, some studies administered curcumin in Nano formulations (Kimura et al., 2018; Soleimani et al., 2018). Gutierres et al. (2019) observed that a single dose of 90mg/kg curcumin in yoghurt in diabetic rats did not demonstrate toxicity or any kind of abnormality (Gutierres et al., 2019). Curcumin has also been administered in humans to observe if the nontoxic effects would be seen in humans too. Chuengsamarn et al. (2012) observed that 1.5g/kg curcumin administered orally for 6 months in type 2 diabetes mellitus patients did not show severe side effects (Chuengsamarn et al., 2012). However gastrointestinal effects such as nausea and constipation occurred in some patients. In another study, curcuminoids (38.06 % curcumin, 18.85 % demethoxycurcumin and 42.58 % bisdemethoxycurcumin) were administered. Na et al. (2013) observed no severe reactions in diabetic and obese patients who received 300mg a day of curcuminoids (Na et al., 2011). However, changes in biochemical parameters and liver enzymes were observed. These studies showed that curcumin in its various forms did not result in toxic effects. It is deemed nontoxic at high doses in both animals and humans.

1.2 Justification for the study

Curcumin has been utilized as a therapeutic agent for several diseases associated with inflammation, including obesity (Ghosh et al., 2015). Previous studies have shown that curcumin ameliorated hyperglycaemic and pro-inflammatory conditions in high fat diet induced obese adult rats (King et al., 2014). Our ongoing studies have shown that

phytochemicals such as S-allylcysteine, oleanolic acid and curcumin, when administered during the neonatal period, can have long lasting beneficial health effects in rats on a high fructose diet. The consumption of fructose by children has been shown to be culpable in the development of metabolic disorders later in life including obesity and NAFLD thus the early post weaning period represents a potential window of developmental plasticity which can be targeted for long lasting beneficial health effects. Previous studies have used curcumin as a remedy and not a preventive medicine thus this study will assess the effects of curcumin when orally administered during the early post weaning period and observe whether the administration of this phytochemical is protective against fructose-induced metabolic dysfunction in adulthood. Most studies tend to focus on a single sex; however, it is well recognized that there is sexual dimorphism in response to dietary and therapeutic interventions thus this study will assess the effects in both male and female rats.

1.3 Aim and study objectives

This study aims to investigate whether curcumin administered during the early post weaning stage can prevent the development of high fructose diet-induced metabolic dysfunction in growing male and female Sprague Dawley rats.

The following objectives were investigated in male and female rats fed a high fructose diet, either with or without curcumin during the early post weaning period:

1. Growth performance by measuring body mass and linear growth.
2. Metabolic dysfunction parameters, by measuring visceral adiposity, circulating metabolic substrates (cholesterol, triglycerides and glucose), plasma hormones associated with obesity (adiponectin & insulin),
3. Markers of health by measuring red blood cell indices.
4. Development of NAFLD by determining the hepatic histology scores for NAFLD.

1.4 Hypotheses

H_0 Curcumin administered during the early post weaning period will have no effect in preventing the high-fructose diet-induced metabolic dysfunction in male and female Sprague Dawley rats.

H_1 Curcumin administered during the early post weaning period will prevent the high fructose diet-induced metabolic dysfunction in male and female Sprague Dawley rats.

Chapter 2: Materials and Method

2.1 List of reagents and consumables

Curcumin and Fenofibrate were sourced from Sigma Aldrich (Johannesburg, South Africa).

The following staining reagents were obtained from Merck, Johannesburg, South Africa: Gill's haematoxylin, Ponceau fuschin, Phosphomolybdic acid, light green, Scott's tap water substitute, DPX, xylene and stable iron haematoxylin.

List of other reagents and consumables: Commercially sourced standard rat chow (LabChef, Stellenbosch, South Africa), fructose (Nature's Choice, Randvaal, South Africa), electronic balances (Presica 310M, Presica Instruments AG, Switzerland), calibrated Contour plus glucometer (Bayer, Isando, South Africa), sodium pentobarbitone (Eutha-naze, Bayer Johannesburg, South Africa), centrifuge (Rotofix 32A, Hettich Zentrifugen, Germany), microtubes (Greiner bio-one Diagnostics GmbH, Austria), oven (Salvis ®, Salvis Lab, Switzerland), digital vernier callipers (Major Tech (Pty) Ltd, KTV 150 digital calliper, Elandsfontein, South Africa), spectrophotometrically (Multiskan Ascent, Lab system, Helsinki, Finland), Enzyme Linked Immuno-sorbent Assay (ELISA) kit (DRG®, Rat insulin, USA and DRG® Rat adiponectin, USA, respectively), plate reader (Multiskan Ascent, Lab system, Helsinki, Finland), haematocrit meter (URIT Digital HB meter, Accurex Biomedical Pty. Ltd), Microm STP 120 tissue processor (Thermo Fisher Scientific, Walldorf, Germany), Microtome (Leica Microsystems, Nussloch, Germany), light microscope (Reichert®, Austria), Graphpad Prism 7 (Graphpad Software Inc, San Diego, USA).

2.2 Ethical clearance

Ethical clearance for the study was obtained from the Animal Research Ethics Committee (AREC) of the University of the Witwatersrand, South Africa. The AREC

clearance number was 2018/05/22B. Copies of the certificate (and further modifications to the study) are included in Appendices 1 and 2.

2.3 Animals, housing and feeding

A total of eighty (40 males and 40 females), 21-day-old Sprague Dawley rat pups were used in the current study. The rats were housed individually in Perspex cages with dimensions compliant with the South African National Standard guidelines for the care and use of animals for scientific purposes (SANS document 10386:2008). Wood shavings were used as bedding. The study took place in the Central Animal Services Unit of the University of the Witwatersrand, Johannesburg, South Africa. Room temperature was maintained at 25± 2°C with a 12-h light/dark cycle (lights on from 07h00 - 19h00). Commercially sourced standard rat chow (LabChef, Stellenbosch, South Africa) was provided *ad libitum* together with either plain drinking water (PDW) or 20% Fructose Solution (20% FS where 400g of fructose (Nature's Choice, Randvaal, South Africa) was added to 2 litres of water) as drinking fluid. Gelatine was used as a stress free route of oral administration, thus treatments using curcumin and fenofibrate were prepared into gelatine cubes.

2.3.1 Preparation of gelatine cubes for administration of treatment

The gelatine cubes were made by mixing 8g gelatine, 8 g sugar, and 5ml of Bovril in 100 ml hot water. Curcumin and fenofibrate were first dissolved in 0, 5% Dimethyl sulfoxide (DMSO) solution and then mixed into the gelatine stock solution. Curcumin and fenofibrate were dissolved seperately. Gelatine cubes with curucmin and fenofibrate were prepared respectively. The gelatine solutions with the respective treatments were placed into 4 ml ice trays and allowed to set in a refrigerator after which they were cut into 1 ml cubes that were fed to the rats.

2.3.2 Rationale for dose for Curcumin and Fenofibrate used in the study

The dose for curcumin administered using the gelatine cubes at 500mg/kg body weight was based on doses used in previous metabolic studies (Shehzad et al., 2011; Ghosh, Banerjee, Sil, 2015). Fenofibrate was used as a positive control as it is known to improve metabolic disease traits such as insulin sensitivity and reduce hyperglycaemia. The dose for fenofibrate (100mg/kg) used in the current study was also based on doses used in previous metabolic studies (Oidor-Chan et al., 2016).

2.4 Experimental design

The rat pups were randomly allocated (on postnatal day 21) to one of the following experimental groups (8 of each sex per group): Group one, the negative control, was fed standard rat chow (SRC), plain drinking water (PDW) and plain gelatine cubes (PGC) from postnatal day 21 to 35 and then SRC and PDW for the rest of the study (day 36 to 63); group two, in which metabolic dysfunction was induced, was fed SRC, 20% FS as drinking fluid and PGC from postnatal day 21 to 35 and then SRC and 20 % FS for the remaining duration of the study; group 3, in which the potential preventive effects of curcumin were investigated, was fed SRC, 20% FS as drinking fluid and curcumin administered at 500mg/kg body weight in gelatine cubes (GC) from postnatal day 21 to day 35 and then SRC and 20% FS for the remainder of the study; group 4, in which the effects of curcumin alone were investigated, was fed SRC, PDW and curcumin at 500mg/kg body weight in GC from postnatal day 21 to 35 and then SRC and PDW for the remainder of the study, group 5, the positive control, was fed SRC, PDW and Fenofibrate in GC at 100mg/kg body weight from postnatal day 21 to 35 and then SRC and 20% FS for the remainder of the study. The total duration of the study was 6 weeks. Figure 2.1 shows an illustration of the experimental design.

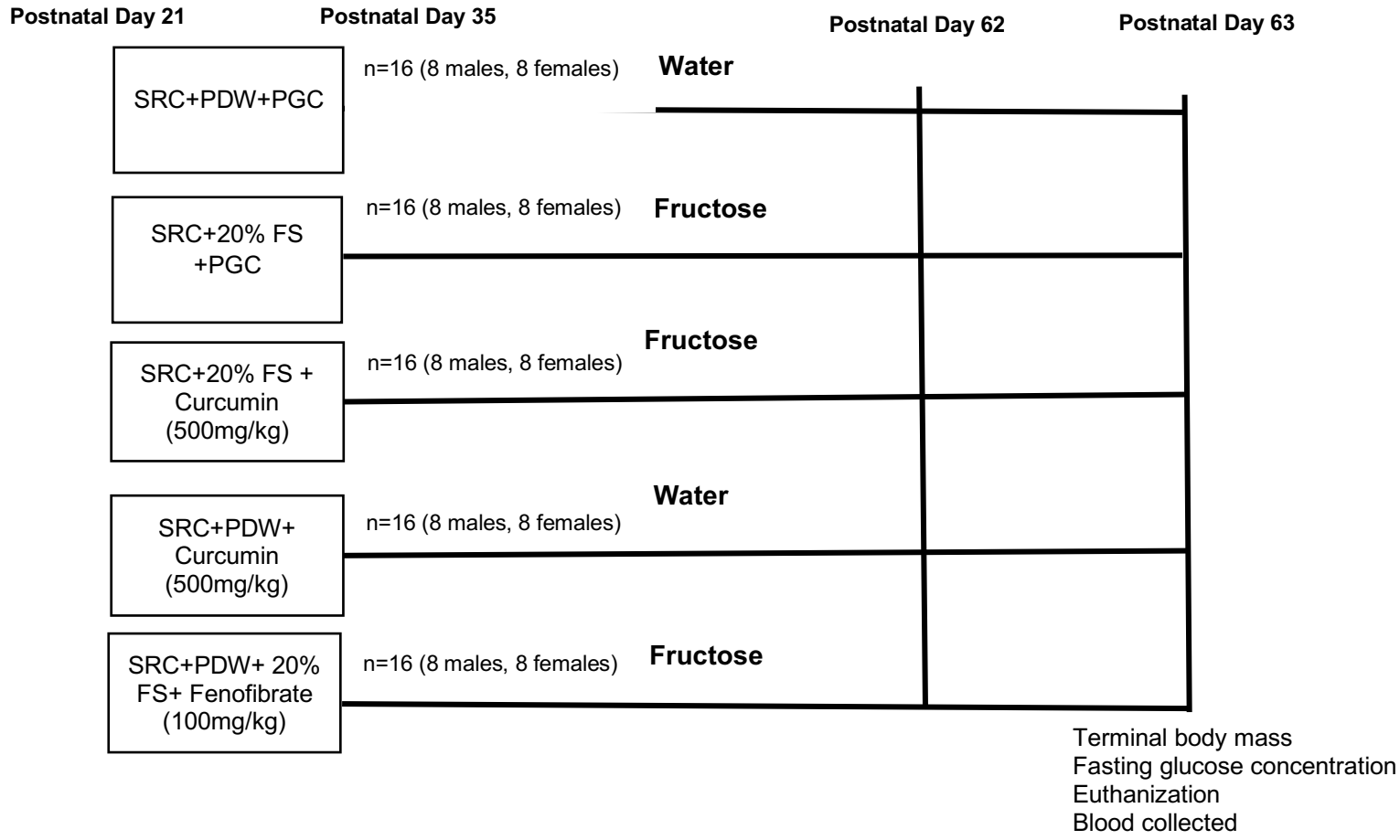

Figure 2.1 Illustration of the study design of forty male and female Sprague Dawley rats fed a high fructose diet for six weeks

Key: SRC-standard rat chow, PDW- plain drinking water, FS-fructose solution, PGC-plain gelatine solution, GC-gelatine cube

2.5 Assessment of Growth Performance

2.5.1 Determination of body mass

The rats were individually weighed daily, during the first two weeks where treatment was administered. The daily weighing allowed for adjustment of the amount of fenofibrate or curcumin in the cubes to maintain a constant required dose rate.

Thereafter the rats were weighed twice a week to monitor their growth. This was done by placing each rat in a pre-weighed container and then onto an electronic balance (Presica 310M, Presica Instruments AG, Switzerland).

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2.6 Terminal Procedures

On postnatal day 62 the rats were fasted overnight. On postnatal day 63, the rats were prepared for euthanization. Postnatal 63 is important as it represents the end of adolescence and the onset of adulthood. This was the targeted period for this study. Their terminal body masses were then measured using an electronic balance (Presica 310M, Presica Instruments AG, Switzerland). Thereafter a drop of blood was obtained from the tail vein via pinprick and used to determine blood glucose using a calibrated Contour plus glucometer (Bayer, Isando, South Africa). The rats were then euthanized with an overdose of sodium pentobarbitone (Eutha-naze, Bayer Johannesburg, South Africa) at 200mg/kg body weight. Following euthanasia, blood was drawn via cardiac puncture and collected into plain heparinized blood tubes (BD Vacutainer, Plymouth, UK). The blood was then centrifuged (Rotofix 32A, Hettich Zentrifugen, Germany) at 4000rpm for 15 minutes and the plasma was collected into microtubes (Greiner bio-one Diagnostics GmbH, Austria) and stored at -20°C for later determination of plasma insulin, adiponectin, triglycerides and cholesterol concentrations.

2.6.1 Determination of linear growth

The right hind leg was removed from each of the rat carcasses and the flesh was removed. Thereafter the femur and tibia were separated. The bones were then dried to a constant mass in an oven (Salvis ®, Salvis Lab, Switzerland) at 40°C for at least 7 days. The dry tibiae and femur masses were then determined using an electronic balance (Presica 310M, Presica Instruments AG, Switzerland). The lengths of the tibiae and femurs were measured using digital vernier calipers (Major Tech (Pty) Ltd, KTV 150 digital caliper, Elandsfontein, South Africa). The density of the tibiae and femurs were calculated using the mass to length ratio (Seedor *et al.,* 1991)

Mass to length ratio (mg/mm) = dry mass of bone (mg) /bone length (mm).

2.6.2 Determination of visceral organ morphometry

Following blood collection, the abdomen was cut open by a midline incision. The small and large intestines, stomach, caecum, liver, kidneys, heart and visceral and epididymal (males only) fat were carefully dissected out and weighed using an electronic balance (Presica 310M, Presica Instruments AG, Switzerland). The small and large intestine lengths were measured using a ruler attached to the dissection board. The liver (left lobe) and kidney samples were collected for histology analysis and preserved in plastic bottles containing 10% buffered formalin solution.

2.6.3 Circulating metabolic substrates

2.6.3.1 Determination of fasting lipids (cholesterol and triacylglycerols)

An Enzyme Linked Immuno-sorbent Assay (ELISA) kit was used to measure the fasting total cholesterol (the kit applies the single reagent, Cholesterol oxidase phenol 4-aminoantipyrine peroxidase (COD-PAP) method) and the total triacylglycerol (the kit applies the single reagent, Glycerine phosphate oxidase peroxidase (GPO-PAP)

method) concentrations. The standards and working solutions were prepared. Thereafter the samples were added to the micro ELISA plates. Only the wells containing cholesterol or triacylglycerols changed colour. The plate was then placed in a plate reader and the samples were directly detected. The colour depths of the generated quinones were directly proportional to the cholesterol or triacylglycerol content respectively. The optical densities of both the cholesterol and triacylglycerols were measured spectrophotometrically (Multiskan Ascent, Lab system, Helsinki, Finland) at a wavelength of 450nm.

2.6.4 Plasma hormones associated with obesity

2.6.4.1 Determination of fasting insulin and adiponectin concentrations

The fasting insulin and adiponectin concentrations were assayed using an Enzyme Linked Immuno-Sorbent Assay (ELISA) kit (DRG®, Rat insulin, USA and DRG® Rat adiponectin, USA, respectively). The micro ELISA plates provided in both kits are pre-coated with an antibody specific to rat insulin and adiponectin (INS and ADP) respectively. The standards were then added to the micro ELISA plate wells and combined with the specific antibody. Thereafter a biotinylated detection antibody specific for rat INS and ADP was added to each micro plate well respectively. The plate wells were then placed into a plate reader (Multiskan Ascent, Lab system, Helsinki, Finland) and only the wells containing rat INS, ADP and biotinylated detection antibody changed colour. The optical densities of both the rat insulin micro plate and the HRP micro plates were measured spectrophotometrically at a wavelength of 450nm. The concentrations of insulin (ng/ml) and adiponectin (pg/ml) were calculated by comparing the optical density of the samples to the standard curve.

The fasting whole body insulin sensitivity was determined using the Homeostasis model assessment of insulin resistance (HOMA-IR) as follows:

HOMA-IR= fasting plasma insulin (μU/ml) x fasting glucose (mmol/L) / 22.5 (Divi et al, 2012)

The insulin concentration (ng/ml) was then expressed as (µU/ml) using the formula:

1 ng/ml= 0.02 µU/ml

2.6.5 Measurement of red blood cell indices

A drop of blood was placed on the haematocrit meter (URIT Digital HB meter, Accurex Biomedical Pty. Ltd) and values of the haemoglobin and haematocrit were measured.

The Mean corpuscular haemoglobin concentration (MCHC) was then calculated using the following equation: $\frac{\textbf{Haemoglobin } (\frac{\mathbf{g}}{\mathbf{dL}})}{\textbf{Haematocrit \%}} \textbf{ x 100}$

2.6.6 Determination of kidney histopathology and hepatic histology scores for NAFLD

2.6.6.1 Processing of Liver and kidney samples for histology

The preserved liver and kidney samples were processed using a Microm STP 120 tissue processor (Thermo Fisher Scientific, Walldorf, Germany). Whilst in the processor, the samples were rotated in various phases starting from formalin followed by the alcohol phase which begins at 70% alcohol and ends at 100% alcohol, and the Xylol phase. In the last phase, the samples were placed in wax. The duration of the tissue processing lasted for 16 hours. Following processing, the samples were embedded in paraffin wax and made into blocks, sectioned using a Microtome (Leica Microsystems, Nussloch, Germany), mounted onto a glass slide and then stained with haematoxylin and eosin for general histopathological examination.

2.6.6.2 Haematoxylin and Eosin (H&E) staining protocol

The protocol described by Bancroft and Gamble (2008) was used. One-half of the liver and kidney slides were stained regressively by dewaxing in xylene, hydrating first in 100% and 95% ethanol and staining with Gill's Haematoxylin. Thereafter the slides were differentiated with acid alcohol, washed with water first then Scotts tap water substitute. The slides were then stained with 1% Eosin, rapidly dehydrated first in 95% ethanol then 100% and cleared in xylene. Cover slips were then carefully mounted onto the slides using a mounting medium with a mixture of distyrene, a plasticizer and xylene (DPX).

2.6.6.3 Hepatic histology scoring for NAFLD

Hepato-cellular changes in the liver tissues of all the experimental groups were assessed and compared using a light microscope (Reichert®, Austria). Both steatosis and hypertrophy were assessed at a 40X magnification. The severity of fibrosis was scored according to accepted current protocols that assess hepatocellular ballooning (0-2), steatosis (0-3) and lobular inflammation (0-3). Macrovesicular steatosis and microvesicular steatosis were both scored separately, and the severity was graded, based on percentage of the total area affected. The following categories were used: 0 (<5%), 1 (5-33%), 2 (34-66%), 3 (>66%).(Santiago-Rolón et al., 2015).

2.7 Statistical analyses

Graphpad Prism 7 (Graphpad Software Inc, San Diego, USA) statistical software was used to analyse the data. The collected data was stratified by sex. Parametric data are expressed as a mean ± SD. The weekly body masses were analysed using a repeated measure analysis of variance (ANOVA) and the means were compared using a Tukey post hoc test. A one way ANOVA was used to analyse multi group parametric data. A Kruskal-Wallis test was used to analyse non-alcoholic fatty liver disease score (NAS) multiple group data followed by a Dunns post hoc test to compare the medians. Statistical significance was set at $p<0.0$.

Chapter 3: Results

3.1 The effects of curcumin on growth performance

3.1.1 Body mass

3.1.1.1 Male rats

Figure 3.1 shows the initial and terminal body masses of forty male Sprague Dawley rats following the interventions. There were no significant differences ($p>0.05$) in the initial body mass across the treatment groups. After six weeks of high fructose feeding, all animals showed a significant increase ($p<0.0001$) in body mass. Rats fed fructose and curcumin (FCUR) had significantly ($p<0.05$) lower terminal body mass compared to the controls. No other significant differences were observed between the groups.

When the body mass gain was expressed as a percentage (figure 3.2) relative to the initial mass, there was no significant difference ($p>0.05$) between the different groups.

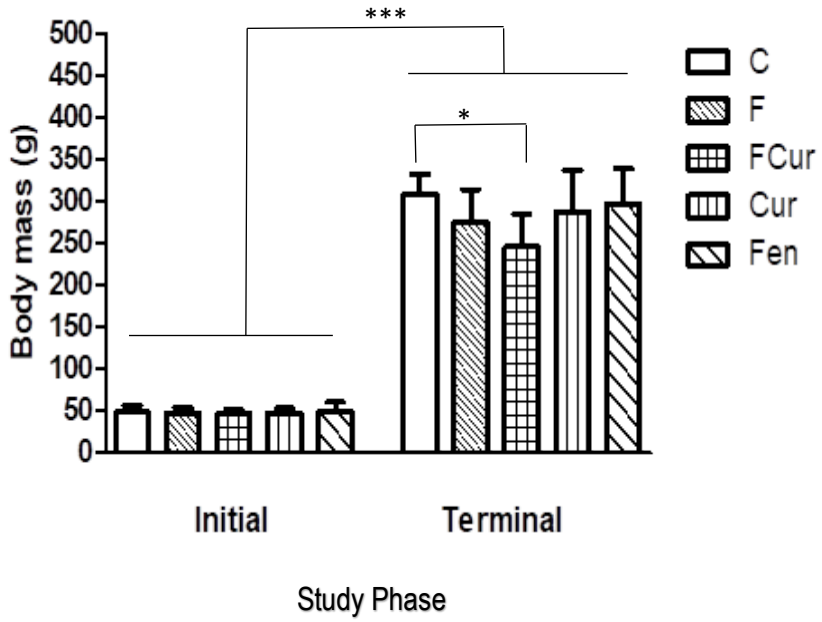

Figure 3.1 Effects of orally administered curcumin on the initial and terminal body mass of forty male Sprague Dawley rats fed high fructose diet over six weeks.

Data expressed as mean ± SD. n = 8 per group; *= $p<0.05$. ***= $p<0.0001$; C = plain gelatine cubes + tap water to drink; F = plain gelatine cubes + 20% fructose solution as drinking fluid; FCur = 500mg/kg curcumin b.w + 20% fructose solution as drinking fluid; Cur = 500 mg/kg b.w curcumin + tap water to drink; Fen = 100 mg/kg b.w fenofibrate + tap water to drink.

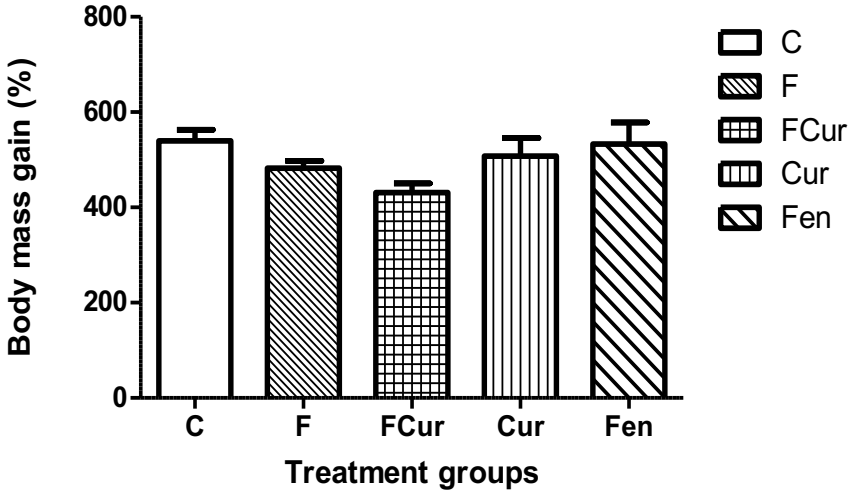

Figure 3.2 Effects of orally administered curcumin on the percentage body mass gain of forty male Sprague Dawley rats fed a high fructose diet over six weeks.

Data expressed as mean ± SD; n = 8; C = plain gelatine cubes + tap water to drink; F = plain gelatine cubes + 20% fructose solution as drinking fluid; FCur = 500mg/kg curcumin b.w + 20% fructose solution as drinking fluid; Cur = 500 mg/kg b.w curcumin + tap water to drink; Fen = 100 mg/kg b.w fenofibrate + tap water to drink.

3.1.1.2 Female rats

Figure 3.3 shows the initial and terminal body mass of forty female Sprague Dawley rats fed a high fructose diet. No significant differences ($p>0.05$) were observed in the initial body mass across the treatment groups. After six weeks of high fructose feeding, all animals showed a significant increase ($p<0.0001$) in body mass.

There were no significant differences ($p>0.05$) in the terminal body mass across the treatment groups as well as in the percentage body mass gain (figure 3.4).

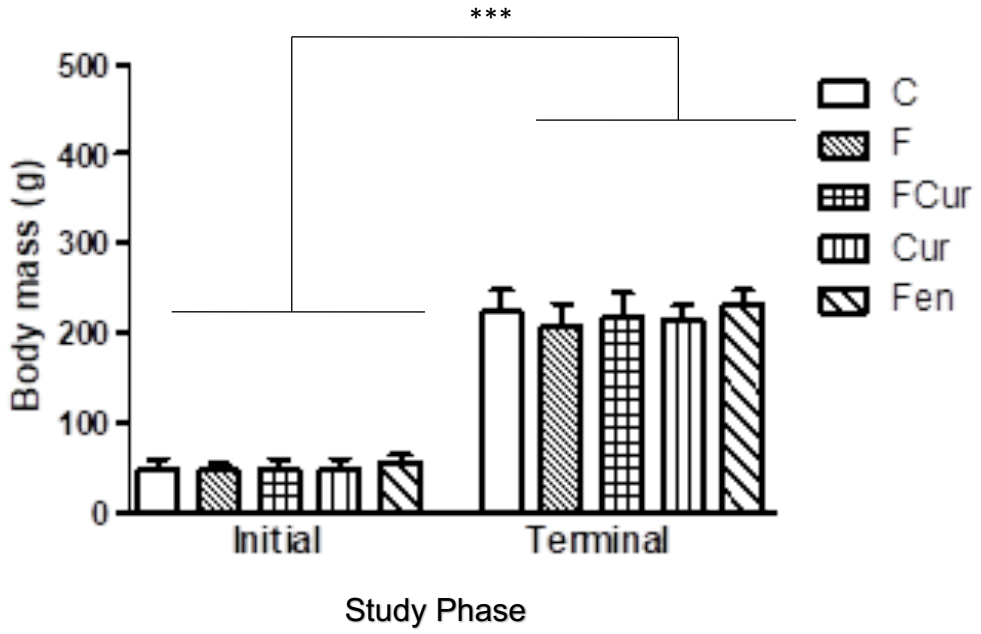

Figure 3.3 Effects of orally administered curcumin on the initial and terminal body mass of forty female Sprague Dawley rats fed a high fructose diet over six weeks.

Data expressed as mean ± SD**.** ***= significantly different at $p<0.0001$. n=8, C = plain gelatine cubes + tap water to drink; F = plain gelatine cubes + 20% fructose solution as drinking fluid; FCur = 500mg/kg curcumin b.w + 20% fructose solution as drinking fluid; Cur = 500 mg/kg b.w curcumin + tap water to drink; Fen = 100 mg/kg b.w fenofibrate + tap water to drink.

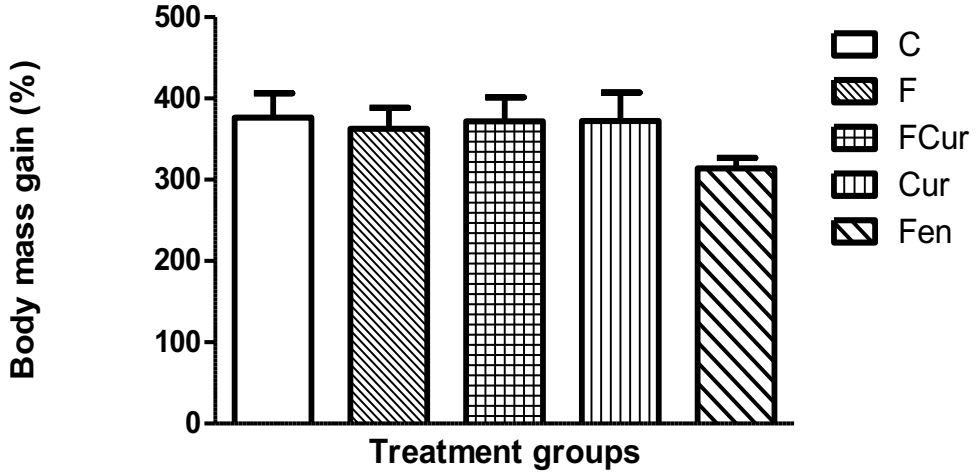

Figure 3.4 Effects of orally administered curcumin on the percentage body mass of forty female Sprague Dawley rats fed a high fructose diet over six weeks.

Data expressed as mean ± SD; n=8; C = plain gelatine cubes + tap water to drink; F = plain gelatine cubes + 20% fructose solution as drinking fluid; FCur = 500mg/kg curcumin b.w + 20% fructose solution as drinking fluid; Cur = 500 mg/kg b.w curcumin + tap water to drink; Fen = 100 mg/kg b.w fenofibrate + tap water to drink.

3.1.2 Linear growth

3.1.2.1 Male rats

Table 3.1 shows the terminal mass, lengths and mass to length ratio of the femurs and tibias of forty male rats following six weeks of high fructose feeding. There were no significant differences ($p>0.05$) in the tibia lengths, the terminal mass and mass to length ratios across the treatment groups.

Rats fed fructose (F) alone had significantly ($p<0.05$) lower femur lengths compared to the control rats. Curcumin (CUR) did not prevent this reduction in femur length as the rats fed fructose with curcumin (FCUR) also had significantly ($p<0.01$) lower femur lengths compared to the control (C). However, there was no significant difference ($p>0.05$) between the rats fed fructose with curcumin and those fed fructose alone. The rats fed fructose and curcumin (FCUR) also had significantly ($p<0.05$) lower femur lengths compared to those either administered curcumin alone (CUR) or, fenofibrate (FEN).

Table 3.1 Effect of orally administered curcumin on tibia and femur masses, lengths and mass to length ratios of male rats fed a high fructose diet for six weeks

Parameter	C	F	FCur	Cur	Fen
Tibia length (mm)	27.00 ± 1.10	26.00 ± 1.00	26.00 ± 1.40	27.00 ± 1.20	27.70 ± 1.10
Tibia mass (mg)	36.00 ± 0.03	33.00 ± 0.05	31.00 ± 0.03	35.00 ± 0.04	35.00 ± 0.03
Tibia ratio (mg/mm)	1.33 ± 0.83	1.26 ± 1.30	1.19 ± 0.75	1.30 ± 1.10	1.30 ± 1.00
Femur length (mm)	32.00 ± 0.97^{a}	30.00 ± 1.10^{bc}	30.00 ± 1.30^{b}	31.00 ± 0.69^{ac}	31.00 ± 0.84^{ac}
Femur mass (mg)	30.00 ± 0.03	27.00. ± 0.03	25.00 ± 0.03	28.00 ± 0.03	27.00 ± 0.03
Femur ratio (mg/mm)	0.95 ± 0.63	0.88 ± 0.72	0.83 ± 0.61	0.90 ± 0.76	0.87 ± 0.90

Data expressed as mean ± SD; ^{abc} = data in the same row with different superscript are significantly different at $p<0.05$; n=8; C = plain gelatine cubes + tap water to drink; F = plain gelatine cubes + 20% fructose solution as drinking fluid; FCur = 500mg/kg curcumin b.w + 20% fructose solution as drinking fluid; Cur = 500 mg/kg b.w curcumin + tap water to drink; Fen = 100 mg/kg b.w fenofibrate + tap water to drink.

3.1.2.2 Female rats

There were no significant differences ($p>0.05$) in the masses, lengths and mass to length ratios of both tibias and femurs of the rats across the treatment groups (Table 3.2).

Table 3.2 Effect of orally administered curcumin on tibia and femur masses, lengths and mass to length ratios of female Sprague Dawley rats fed a high fructose diet for six weeks

Parameter	C	F	FCur	Cur	Fen
Tibia length (mm)	25.00 ± 1.20	25.00 ± 1.20	25.00 ± 1.30	26.00 ± 0.87	25.00 ± 0.78
Tibia mass (mg)	32.00 ± 0.05	29.00 ± 0.04	33.00 ± 0.05	32.00 ± 0.03	33.00 ± 0.03
Tibia ratio (mg/mm)	1.28 ± 1.40	1.16 ± 1.20	1.32 ± 1.50	1.23 ± 0.82	1.32 ± 0.86
Femur length (mm)	30.00 ± 1.40	29.00 ± 1.10	30.00 ± 1.40	30.00 ± 0.88	30.00 ± 0.70
Femur mass (mg)	25.00 ± 0.03	23.00 ± 0.02	25.00 ± 0.03	24.00 ± 0.02	26.00 ± 0.02
Femur ratio (mg/mm)	0.77± 0.92	0.79 ± 0.63	0.83 ± 0.62	0.80 ± 0.62	0.87 ± 0.65

Data expressed as mean ± SD; n = 8; C = plain gelatine cubes + tap water to drink; F = plain gelatine cubes + 20% fructose solution as drinking fluid; FCur = 500mg/kg curcumin b.w + 20% fructose solution as drinking fluid; Cur = 500 mg/kg b.w curcumin + tap water to drink; Fen = 100 mg/kg b.w fenofibrate + tap water to drink.

3.1.3 Radiograph images of tibias and femurs

Subjective visualisation showed no differences in the radio density of the long bones from the males and the females (figures 3.5 to 3.8).

3.1.3.1 Males

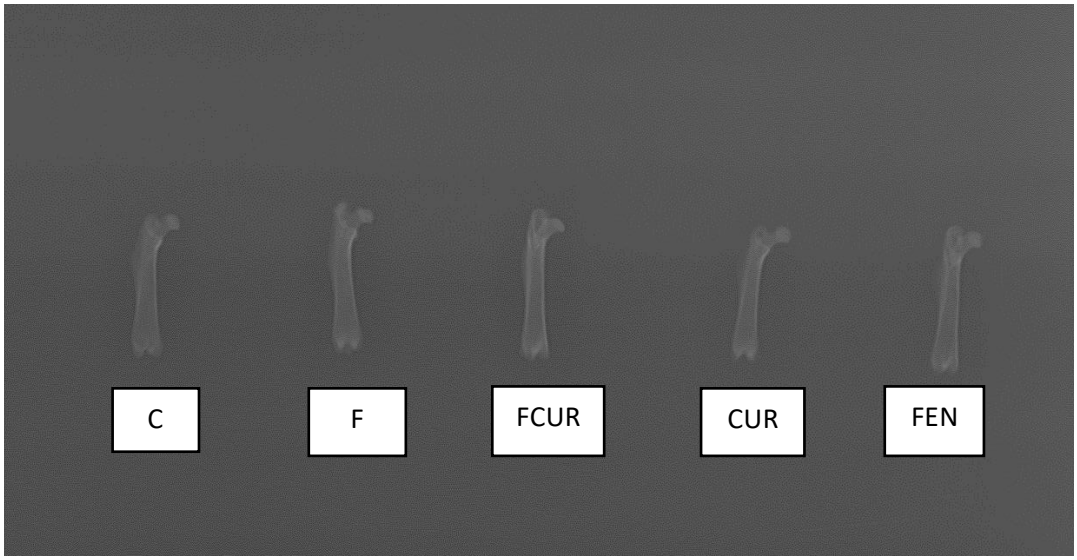

Figure 3.5 Radiograph images representative tibiae of forty male Sprague Dawley rats in the different study groups.

n=8, C = plain gelatine cubes + tap water to drink; F = plain gelatine cubes + 20% fructose solution as drinking fluid; FCur = 500mg/kg curcumin b.w + 20% fructose solution as drinking fluid; Cur = 500 mg/kg b.w curcumin + tap water to drink; Fen = 100 mg/kg b.w fenofibrate + tap water to drink.

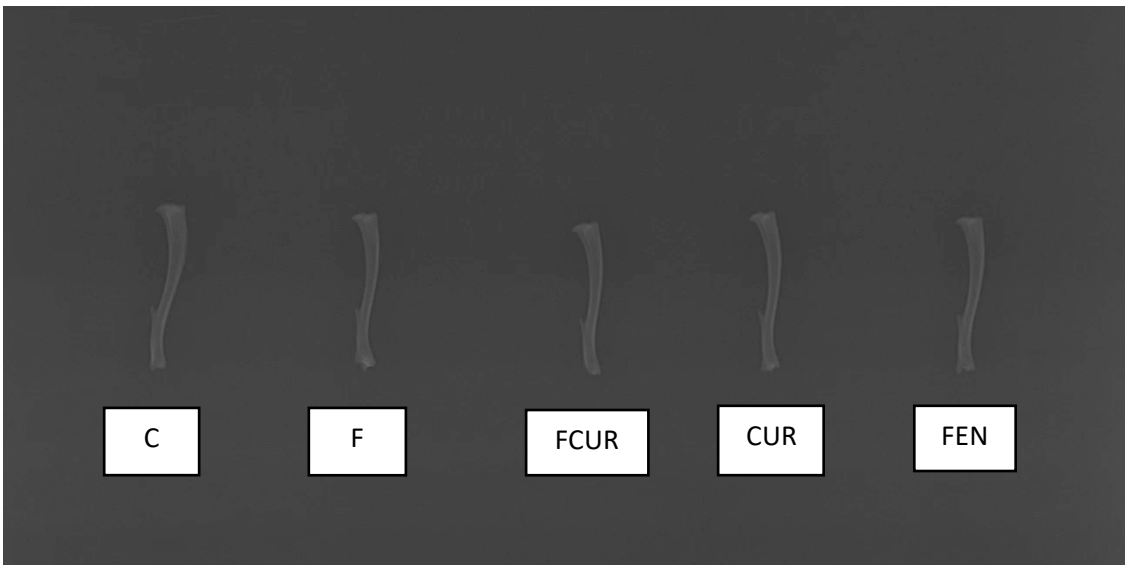

Figure 3.6 Radiograph images of representative femora of forty male Sprague Dawley rats in the different study.

n=8, C = plain gelatine cubes + tap water to drink; F = plain gelatine cubes + 20% fructose solution as drinking fluid; FCur = 500mg/kg curcumin b.w + 20% fructose solution as drinking fluid; Cur = 500 mg/kg b.w curcumin + tap water to drink; Fen = 100 mg/kg b.w fenofibrate + tap water to drink.

3.1.3.2 Females

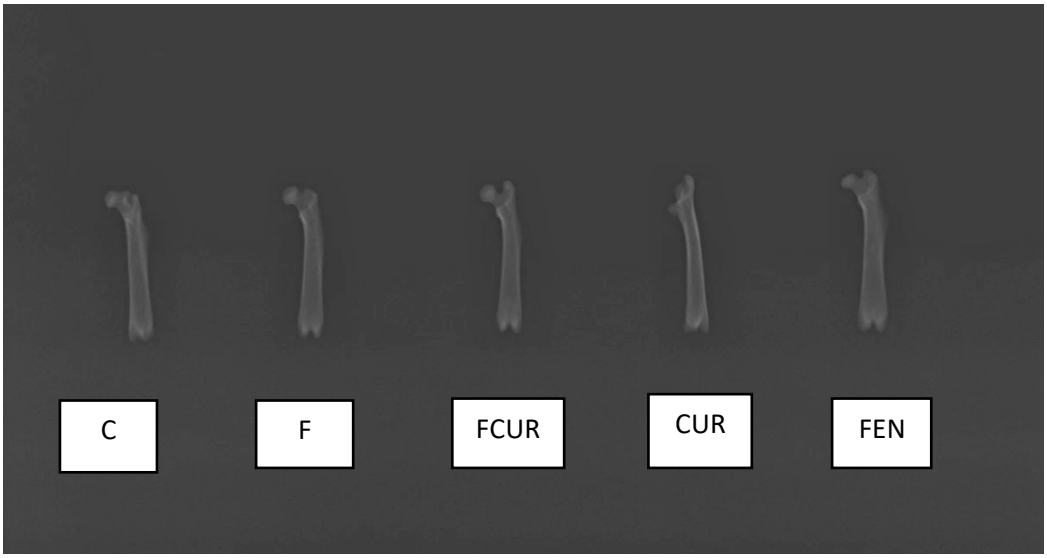

Figure 3.7 Radiograph images representative tibiae of forty female Sprague Dawley rats in the different study groups.

n=8, C = plain gelatine cubes + tap water to drink; F = plain gelatine cubes + 20% fructose solution as drinking fluid; FCur = 500mg/kg curcumin b.w + 20% fructose solution as drinking fluid; Cur = 500 mg/kg b.w curcumin + tap water to drink; Fen = 100 mg/kg b.w fenofibrate + tap water to drink.

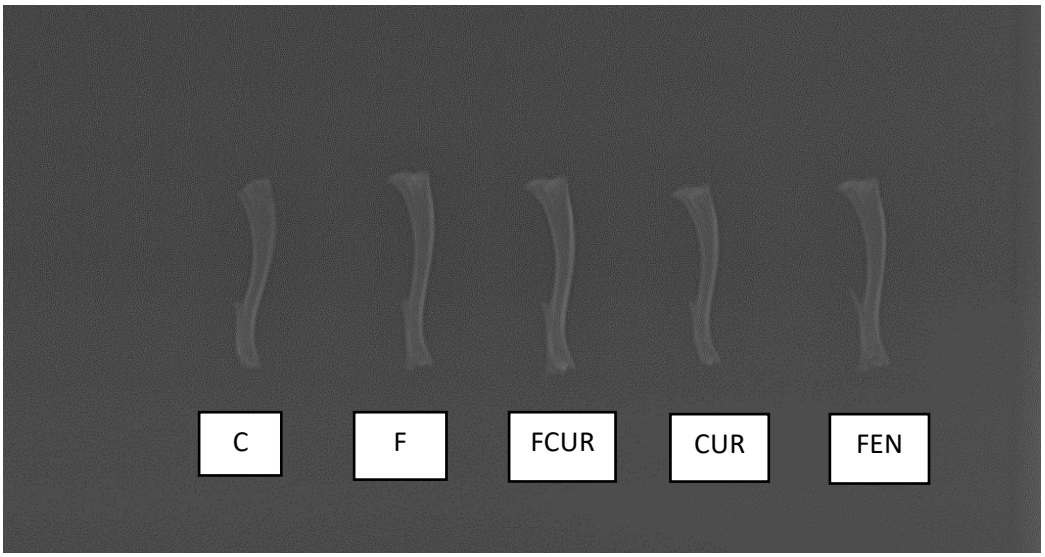

Figure 3.8 Radiograph images of representative femora of forty female Sprague Dawley rats in the different study groups.

n = 8, C = plain gelatine cubes + tap water to drink; F = plain gelatine cubes + 20% fructose solution as drinking fluid; FCur = 500mg/kg curcumin b.w + 20% fructose solution as drinking fluid; Cur = 500 mg/kg b.w curcumin + tap water to drink; Fen = 100 mg/kg b.w fenofibrate + tap water to drink.

3.2 Circulating metabolic substrates

3.2.1 Triacylglycerol and cholesterol concentrations

3.2.1.1 Male rats

Table 3.3 shows the effects of orally administered curcumin on triacylglycerol and cholesterol concentrations of male Sprague Dawley rats on a high fructose diet for six weeks.

No significant ($p>0.05$) differences were observed in the triacylglycerol and cholesterol concentrations of the male rats.

Table 3.3 Effects of orally administered curcumin on triaglycerol and cholesterol concentrations of male Sprague Dawley rats fed a high fructose diet for six weeks

	TREATMENT GROUPS				
Parameter	**C**	**F**	**FCur**	**Cur**	**Fen**
Triacylglycerol (mmol/L)	0.19 ± 0.07	0.28 ± 0.14	0.21 ± 0.06	0.17 ± 0.09	0.19 ± 0.07
Cholesterol (mmol/L)	1.10 ± 0.77	2.81 ± 2.90	4.36 ± 3.30	3.83 ± 3.58	2.69 ± 2.45

Data expressed as mean ± SD; n = 8; C = plain gelatine cubes + tap water to drink; F = plain gelatine cubes + 20% fructose solution as drinking fluid; FCur = 500mg/kg curcumin b.w + 20% fructose solution as drinking fluid; Cur = 500 mg/kg b.w curcumin + tap water to drink; Fen = 100 mg/kg b.w fenofibrate + tap water to drink.

3.2.1.2 Female rats

Table 3.4 shows the effects of orally administered curcumin on triacylglycerol and cholesterol concentrations of female Sprague Dawley rats on a high fructose diet for six weeks.

An overall significant difference ($p<0.05$) was observed in the triacylglycerol concentrations of the female rats. However, no significant differences observed between the treatment groups. No significant ($p>0.05$) differences were observed in the cholesterol concentrations.

Table 3.4 Effects of orally administered curcumin on triaglycerol and cholesterol concentrations of female Sprague Dawley rats fed a high fructose diet for six weeks

	TREATMENT GROUPS				
Parameter	**C**	**F**	**FC**	**Cur**	**Fen**
Triacylglycerol (mmol/L) *	0.19 ± 0.18	0.33 ± 0.16	0.37 ± 0.29	0.14 ± 0.26	0.07 ± 0.07
Cholesterol (mmol/L)	2.65 ± 1.76	3.17 ± 3.00	4.26 ± 3.99	2.63 ± 2.71	2.11 ± 1.16

Data expressed as mean ± SD; *= $p<0.05$ (overall significant difference with ANOVA, but not significantly different between groups in triacylglycerols)n = 8; C = plain gelatine cubes + tap water to drink; F = plain gelatine cubes + 20% fructose solution as drinking fluid; FCur = 500mg/kg curcumin b.w + 20% fructose solution as drinking fluid; Cur = 500 mg/kg b.w curcumin + tap water to drink; Fen = 100 mg/kg b.w fenofibrate + tap water to drink.

3.3 Plasma hormones associated with obesity

3.3.1 Male rats

Table 3.5 shows the effects of orally administered curcumin on the insulin and adiponectin concentrations of male Sprague Dawley rats fed a high fructose diet.

There were no significant ($p>0.05$) differences in the insulin and adiponectin concentrations and the homeostasis model of insulin resistance (HOMA-IR) indexes of the male rats following the six weeks on a high fructose diet.

Table 3.5 Effects of orally administered curcumin on insulin and adiponectin concentrations and HOMA-IR of male Sprague Dawley rats fed a high fructose diet for six weeks

	TREATMENT GROUPS				
Hormone	**C**	**F**	**FCur**	**Cur**	**Fen**
Insulin (ng/ml)	1.08 ± 0.63	1.45 ± 0.69	1.84 ± 1.07	1.10 ± 0.88	1.18 ± 0.43
Adiponectin (pg/ml)	29.18 ± 30.73	44.62 ± 22.41	58.47 ± 33.24	33.13 ± 30.80	44.86 ± 17.58
HOMA-IR index	0.60 ± 0.51	0.83 ± 0.45	1.10 ± 0.78	0.62 ± 0.78	0.62 ± 0.78

Data expressed as mean ± SD; n = 8; C = plain gelatine cubes + tap water to drink; F = plain gelatine cubes + 20% fructose solution as drinking fluid; FCur = 500mg/kg curcumin b.w + 20% fructose solution as drinking fluid; Cur = 500 mg/kg b.w curcumin + tap water to drink; Fen = 100 mg/kg b.w fenofibrate + tap water to drink.

3.3.2 Female rats

Table 3.6 shows the effects of orally administered curcumin on the insulin and adiponectin concentrations of female Sprague Dawley rats fed a high fructose diet.

There were no significant ($p>0.05$) differences in the insulin and adiponectin concentrations and the homeostasis model of insulin resistance (HOMA-IR) indexes of the female rats at the end of the study period.

Table 3.6 Effects of orally administered curcumin on insulin and adiponectin concentrations and HOMA-IR of female Sprague Dawley rats fed a high fructose diet for six weeks

	TREATMENT GROUPS				
Hormone	**C**	**F**	**FCur**	**Cur**	**Fen**
Insulin (ng/ml)	1.41 ± 0.79	2.04 ± 0.63	1.30 ± 0.67	1.54 ± 0.74	1.54 ± 0.67
Adiponectin (pg/ml)	39.29 ± 22.89	53.70 ± 18.81	26.31 ± 18.66	50.28 ± 16.73	28.69 ± 16.05
HOMA-IR index	0.76 ± 0.50	1.10 ± 0.39	0.70 ± 0.46	0.91 ± 0.54	0.84 ± 0.46

Data expressed as mean ± SD; n = 8; C = plain gelatine cubes + tap water to drink; F = plain gelatine cubes + 20% fructose solution as drinking fluid; FCur = 500mg/kg curcumin b.w + 20% fructose solution as drinking fluid; Cur = 500 mg/kg b.w curcumin + tap water to drink; Fen = 100 mg/kg b.w fenofibrate + tap water to drink.

3.4 The effect of orally administered curcumin on visceral organ morphometry

3.4.1 Male rats

Table 3.7 shows the effects of orally administered curcumin on the morphometry of the gastro-intestinal tract, accessory organs, heart, kidneys, epididymal and visceral fat of male rats after six weeks of high fructose feeding.

Rats fed fructose (F) had significantly ($p<0.05$) shorter small intestine lengths compared to the control (C) rats. Curcumin (CUR) did not prevent this reduction as rats fed fructose and curcumin (FCUR) also had significantly ($p<0.01$) shorter small intestine lengths compared to the control (C) rats. However, there were no significant ($p>0.05$) differences between rats fed fructose and curcumin (FCUR) and rats fed fructose (F). The rats fed fructose and curcumin (FCUR) also had significantly ($p<0.05$) shorter smaller intestine lengths compared to rats fed fenofibrate (Fen).

Rats fed fructose and curcumin (FCUR) had significantly ($p<0.01$) lower large intestine masses compared to the control (C) rats. Rats fed fructose and curcumin (FCUR) also had significantly ($p<0.0.5$) lower large intestine masses compared to rats fed curcumin (CUR) and rats fed fenofibrate (Fen). However, there were no significant ($p>0.05$) differences in the large intestine masses of rats fed curcumin (CUR) and rats fed fenofibrate (Fen).

Although there were no significant differences ($p>0.05$) in the absolute masses of the stomachs from rats in the different groups, when expressed relative to body mass, rats fed fructose (F) had significantly ($p<0.01$) smaller relative stomach masses (relative to body mass) compared to control (C) rats. Curcumin did not prevent this reduction as rats fed fructose and curcumin (FCUR) also had a significantly ($p<0.01$) smaller relative stomach mass compared to control (C) rats. However, there were no significant ($p>0.05$) differences between rats fed fructose (F) and rats fed fructose and curcumin (FCUR). Rats fed curcumin (CUR) had significantly ($p<0.05$) smaller relative stomach masses compared to rats fed fructose (F). Rats fed fenofibrate (Fen) had significantly ($p<0.01$) smaller relative stomach masses compared to rats fed fructose (F). Rats fed

fenofibrate also had significantly ($p<0.05$) smaller relative stomach masses compared to rats fed fructose and curcumin (FCUR).

No significant ($p>0.05$) differences were observed in the absolute and relative small intestine masses, large intestine relative mass and lengths. There were also no significant ($p>0.05$) differences in the liver, pancreas, heart, kidneys, caecum and, the epididymal and visceral fat masses (both absolute and relative masses).

Table 3.7 Effects of orally administered curcumin on the gastro-intestinal tract, accessory organs, heart, kidneys, epididymal and visceral fat mass (absolute and relative to body mass) of male Sprague Dawley rats fed a high fructose diet for six weeks

	TREATMENT GROUPS				
Organ	**C**	**F**	**FCur**	**Cur**	**Fen**
S.I. mass (g)	8.39 ± 0.50	8.52 ± 1.02	7.22 ± 1.24	8.29 ± 1.67	8.30 ± 1.22
S.I. mass (%BM)	2.73 ± 0.18	3.12 ± 0.26	3.14 ± 0.2	2.88 ± 0.25	2.80 ± 0.18
S.I. length (mm)	1319.38 ± 28.84^{a}	1310.63 ± 88.24^{bc}	1245.0 ± 66.09^{b}	1345 ± 87.36^{ab}	1320 ± 95.30^{ac}
L.I mass (g)	1.73 ± 0.20^{a}	1.47 ± 0.10^{ab}	1.30 ± 0.14^{b}	1.65 ± 0.14^{a}	1.63 ± 0.33^{a}
L.I. mass (%BM)	0.56 ± 0.05	0.54 ± 0.05	0.53 ± 0.05	0.59 ± 0.11	0.55 ± 0.11
L.I. length (mm)	228.75 ± 9.54	206.25 ± 20.66	200 ± 34.64	223.75 ± 14.82	225 ± 8.02
Stomach (g)	1.43 ± 0.14	1.61 ± 0.25	1.36 ± 0.20	1.46 ± 0.19	1.42 ± 0.19
Stomach (%BM)	0.47 ± 0.05^{a}	0.59 ± 0.05^{b}	0.55 ± 0.04^{bc}	0.51 ± 0.06^{ac}	0.48 ± 0.04^{a}
Caecum (g)	1.14 ± 0.17	1.03 ± 0.13	0.94 ± 0.15	1.20 ± 0.23	1.13 ± 0.24
Caecum (%BM)	0.37 ± 0.04	0.38 ± 0.04	0.38 ± 0.03	0.42 ± 0.05	0.38 ± 0.04
Pancreas (g)	1.05 ± 0.11	1.07 ± 0.23	1.01 ± 0.22	1.11 ± 0.36	1.04 ± 0.31

Pancreas (%BM)	0.34 ± 0.04	0.39 ± 0.04	0.41 ± 0.06	0.38 ± 0.1	0.35 ± 0.09
Liver (g)	9.48 ± 0.49	9.22 ± 1.47	8.20 ± 1.84	9.27 ± 1.99	9.36 ± 1.53
Liver (%BM)	3.09 ± 0.21	3.35 ± 0.24	3.31 ± 0.25	3.21 ± 0.29	3.15 ± 0.23
Kidneys (g)	2.05 ± 0.2	1.99 ± 0.29	1.77 ± 0.30	2.10 ± 0.31	2.07 ± 0.37
Kidneys (%BM)	0.66 ± 0.24	0.73 ± 0.05	0.72 ± 0.04	0.74 ± 0.09	0.69 ± 0.04
Heart (g)	1.30 ± 0.07	1.18 ± 0.14	1.07 ± 0.17	1.25 ± 0.20	1.24 ± 0.14
Heart (%BM)	0.42 ± 0.03	0.43 ± 0.017	0.43 ± 0.017	0.44 ± 0.05	0.42 ± 0.02
Visceral Fat (g)	5.40 ± 1.50	5.86 ± 1.439	5.28 ± 1.89	4.87 ± 1.41	5.55 ± 1.17
Visceral fat (%BM)	1.74 ± 0.38	2.13 ± 0.38	2.18 ± 0.87	1.69 ± 0.48	1.94 ± 0.69
Epididymal fat (g)	1.91 ± 0.53	1.80 ± 0.5	1.49 ± 0.30	1.55 ± 0.47	1.61 ± 0.25
Epididymal fat (%BM)	0.62 ± 0.15	0.65 ± 0.12	0.61 ± 0.09	0.54 ± 0.11	0.55 ± 0.06

Data expressed as mean ± SD; n = 8; ^{abc} = data in the same row with different superscript are significantly different at $p<0.05$; C = plain gelatine cubes + tap water to drink; F = plain gelatine cubes + 20% fructose solution as drinking fluid; FCur = 500mg/kg curcumin b.w + 20% fructose solution as drinking fluid; Cur = 500 mg/kg b.w curcumin + tap water to drink; Fen = 100 mg/kg b.w fenofibrate + tap water to drink. L.I = large intestine; S.I = small intestine; BM = body mass.

3.4.2 Female rats

Table 3.8 shows the effects of orally administered curcumin on the gastro-intestinal tract, accessory organs, heart, kidneys and visceral fat in female rats after six weeks of high fructose feeding.

No significant differences ($p>0.05$) were observed in the macro-morphometry of the gastro-intestinal tract, accessory organs, heart, kidneys or visceral fat across the treatment groups.

Table 3.8 Effects of orally administered curcumin on the gastro-intestinal tract, accessory organs, heart, kidneys and visceral fat mass (absolute and relative to body mass) of female Sprague Dawley rats fed a high fructose diet for six weeks

	TREATMENT GROUPS				
Organ	**C**	**F**	**FCur**	**Cur**	**Fen**
S.I. mass (g)	6.44 ± 0.48	6.68 ± 0.60	6.17 ± 0.74	6.41 ± 0.28	6.53 ± 0.77
S.I. mass (%BM)	2.90 ± 0.20	3.23 ± 0.40	2.89 ± 0.56	3.02 ± 0.33	2.84 ± 0.35
S.I. length (mm)	1199.38 ± 42.80	1181.25 ± 51.47	1183.75 ± 50.41	1232.50 ± 159.7	1229.38 ± 46.56
L.I. mass (g)	1.35 ± 0.10	1.22 ± 0.13	1.21 ± 0.12	1.31 ± 0.23	1.31 ± 0.10
L.I. mass (%BM)	0.61 ± 0.07	0.59 ± 0.05	0.56 ± 0.03	0.62 ± 0.11	0.57 ± 0.03
L.I. length (mm)	201.25 ± 14.58	196.25 ± 11.88	189.38 ± 14.00	198.75 ± 19.96	205.63 ± 12.66
Stomach (g)	1.22 ± 0.13	1.19 ± 0.11	1.25 ± 0.18	1.17 ± 0.05	1.17 ± 0.08
Stomach (%BM)	0.55 ± 0.04	0.58 ± 0.07	0.59 ± 0.12	0.55 ± 0.05	0.51 ± 0.05
Caecum (g)	0.88 ± 0.13	0.80 ± 0.10	0.84 ± 0.24	0.95 ± 0.12	0.96 ± 0.21
Caecum (%BM)	0.39 ± 0.06	0.39 ± 0.04	0.38 ± 0.07	0.44 ± 0.05	0.41 ± 0.07

Pancreas (g)	0.93 ± 0.09	0.90 ± 0.16	0.85 ± 0.09	0.77 ± 0.14	0.83 ± 0.13
Pancreas (%BM)	0.42 ± 0.08	0.44 ± 0.08	0.40 ± 0.06	0.36 ± 0.07	0.36 ± 0.06
Liver (g)	7.16 ± 0.52	6.90 ± 0.52	7.08 ± 0.73	6.61 ± 0.31	7.09 ± 0.75
Liver (%BM)	3.22 ± 0.18	3.34 ± 0.37	3.27 ± 0.15	3.12 ± 0.34	3.09 ± 0.33
Kidneys (g)	1.57 ± 0.15	1.54 ± 0.16	1.56 ± 0.19	1.52 ± 0.11	1.63 ± 0.16
Kidneys (%BM)	0.71 ± 0.04	0.74 ± 0.02	0.72 ± 0.02	0.71 ± 0.04	0.71 ± 0.06
Heart (g)	0.99 ± 0.11	0.95 ± 0.09	0.97 ± 0.08	0.97 ± 0.07	1.04 ± 0.05
Heart (%BM)	0.45 ± 0.04	0.45 ± 0.03	0.45 ± 0.03	0.05 ± 0.05	0.46 ± 0.03
Visceral Fat (g)	7.19 ± 2.58	6.01 ± 1.91	6.55 ± 3.11	6.03 ± 1.82	6.39 ± 1.72
Visceral fat (%BM)	3.16 ± 0.88	2.87 ± 0.80	2.9 ± 1.16	2.79 ± 0.69	2.75 ± 0.56

Data expressed as mean ± SD; n = 8; C = plain gelatine cubes + tap water to drink; F = plain gelatine cubes + 20% fructose solution as drinking fluid; FCur = 500mg/kg curcumin b.w + 20% fructose solution as drinking fluid; Cur = 500 mg/kg b.w curcumin + tap water to drink; Fen = 100 mg/kg b.w fenofibrate + tap water to drink. L.I = large intestine; S.I = small intestine; BM = body mass.

3.5 Hepatic steatosis scoring and histology

3.5.1 Nonalcoholic fatty liver disease score and representative liver histology photo sections (H and E staining, 40X magnification)

3.5.1.1 Male rats

Table 3.9 shows the effects of orally administered curcumin on hepatic steatosis scores of male Sprague Dawley rats fed a high fructose diet for six weeks.

The nonalcoholic fatty liver disease scores were similar between the treatment groups. Only the rats fed fructose showed some macrosteatosis.

Table 3.9 Effects of orally administered curcumin on hepatic steatosis scores of male Sprague Dawley rats fed a high fructose diet for six weeks

	TREATMENT GROUPS				
Parameter	**C**	**F**	**FCur**	**Cur**	**Fen**
Steatosis score	0 (0, 0)	0 (1, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)

n=8, C = plain gelatine cubes + tap water to drink; F = plain gelatine cubes + 20% fructose solution as drinking fluid; FCur = 500mg/kg curcumin b.w + 20% fructose solution as drinking fluid; Cur = 500 mg/kg b.w curcumin + tap water to drink; Fen = 100 mg/kg b.w fenofibrate + tap water to drink. . Nonalcoholic fatty liver disease scoring ranges are as follows: 0=<5%. 1= 6-33%, 2=34-66%, 3=65-100%; n=4 per treatment group.

Figure 3.9 shows photo micrographs of representative liver sections from male Sprague Dawley rats fed a high fructose diet for six weeks.

The hepatic histology of the male rats was similar between the treatment groups. Although the rats on fructose only showed some evidence of macrosteatosis.

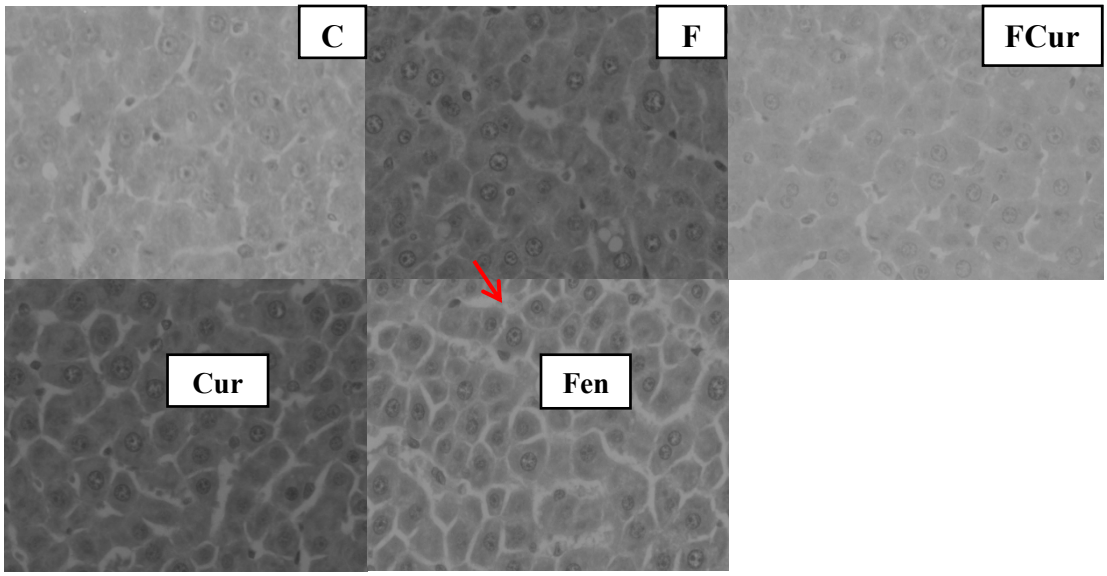

Figure 3.9 Photo sections showing the effects of orally administered curcumin on the liver histology (H and E staining, 40X magnification) of forty male Sprague Dawley rats fed a high fructose diet over six weeks.

The red arrow shows macrosteatosis. C = plain gelatine cubes + tap water to drink; F = plain gelatine cubes + 20% fructose solution as drinking fluid; FCur = 500mg/kg curcumin b.w + 20% fructose solution as drinking fluid; Cur = 500 mg/kg b.w curcumin + tap water to drink; Fen = 100 mg/kg b.w fenofibrate + tap water to drink.

3.5.1.2 Female rats

Table 3.10 shows the effects of orally administered curcumin on hepatic steatosis scores of female Sprague Dawley rats fed a high fructose diet for six weeks.

The rats fed fructose alone showed microsteatosis and macrosteatosis whilst rats fed curcumin alone and fenofibrate showed some evidence of microsteatosis only.

Table 3.10 Effects of orally administered curcumin in the overall hepatic steatosis scores of female Sprague Dawley rats fed a high fructose diet for six weeks

	TREATMENT GROUPS				
Parameter	**C**	**F**	**FCur**	**Cur**	**Fen**
Steatosis score	0 (0, 0)	0 (1, 0)	0 (0, 0)	0 (1, 0)	0 (1, 0)

n-8, C = plain gelatine cubes + tap water to drink; F = plain gelatine cubes + 20% fructose solution as drinking fluid; FCur = 500mg/kg curcumin b.w + 20% fructose solution as drinking fluid; Cur = 500 mg/kg b.w curcumin + tap water to drink; Fen = 100 mg/kg b.w fenofibrate + tap water to drink. Nonalcoholic fatty liver disease scoring ranges are as follows: 0=<5%. 1= 6-33%, 2=34-66%, 3=65-100%; n=4 per treatment group.

Figure 3.10 shows photo micrographs of representative liver sections of female Sprague Dawley rats fed a high fructose diet for six weeks.

Female rats fed fructose (F) alone, curcumin (Cur) alone and fenofibrate (Fen) showed evidence of microsteatosis whilst rats fed fructose and curcumin (FCur) showed no pathology.

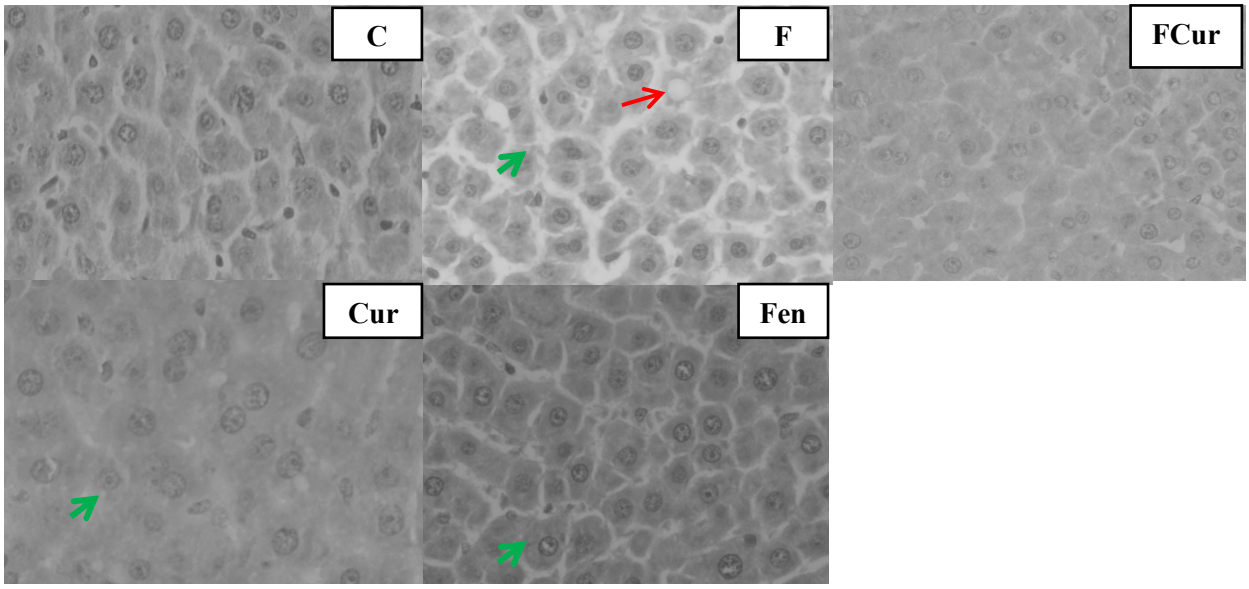

Figure 3.10 Photo sections showing the effects of orally administered curcumin on the liver histology (H and E staining, 40X magnification) of female Sprague Dawley rats fed a high fructose diet over six weeks.

The red arrow indicates macrosteatosis. The green arrow indicates microsteatosis. C = plain gelatine cubes + tap water to drink; F = plain gelatine cubes + 20% fructose solution as drinking fluid; FCur = 500mg/kg curcumin b.w + 20% fructose solution as drinking fluid; Cur = 500 mg/kg b.w curcumin + tap water to drink; Fen = 100 mg/kg b.w fenofibrate + tap water to drink.

3.6 Red blood cell indices

3.6.1 Male rats

Table 3.11 shows the effects of orally administered curcumin on red blood cell indices of male Sprague Dawley rats on a high fructose diet for six weeks.

There were no significant ($p>0.05$) differences in the haematocrit, haemoglobin, and the mean corpuscular haemoglobin concentration (MCHC) of the male rats following the six weeks on a high fructose diet.

Table 3.11 Effect of orally administered curcumin on red blood cell indices of male Sprague Dawley rats fed a high fructose diet for six weeks

	TREATMENT GROUPS				
Parameter	**C**	**F**	**FCUR**	**CUR**	**FEN**
Haematocrit (%)	40.63 ± 7.56	39.38 ± 5.93	39.13 ± 6.11	41.25 ± 7.29	39.38 ± 5.66
Haemoglobin (g/dL)	13.49 ± 2.48	13.13 ± 2.01	13.03 ± 2.10	13.73 ± 2.42	13.15 ± 2.07
MCHC (g/dL)	33.00 ± 0.20	33.00 ± 0.63	33.00 ± 0.30	33.00 ± 0.26	33.00 ± 0.50

Data expressed as mean ± SD; n = 8; C = plain gelatine cubes + tap water to drink; F = plain gelatine cubes + 20% fructose solution as drinking fluid; FCur = 500mg/kg curcumin b.w + 20% fructose solution as drinking fluid; Cur = 500 mg/kg b.w curcumin + tap water to drink; Fen = 100 mg/kg b.w fenofibrate + tap water to drink.

3.6.2 Female rats

Table 3.12 shows the effects of orally administered curcumin on red blood cell indices of female Sprague Dawley rats on a high fructose diet for six weeks.

There were no significant ($p>0.05$) differences in the haematocrit, haemoglobin and the mean corpuscular haemoglobin concentration (MCHC) of the female rats following the six weeks on a high fructose diet.

Table 3.12 Effects of orally administered curcumin on red blood cell indices of female Sprague Dawley rats fed a high fructose diet for six weeks

	TREATMENT GROUPS				
Parameter	**C**	**F**	**FCUR**	**CUR**	**FEN**
Haematocrit (%)	38.41 ± 9.96	45.50 ± 10.03	44.63 ± 7.69	42.50 ± 8.18	41.75 ± 7.13
Haemoglobin (g/dL)	14.46 ± 3.28	15.15 ± 3.39	14.85 ± 2.49	14.15 ± 2.65	13.84 ± 2.45
MCHC (g/dL)	33.00 ± 0.68	33.00 ± 0.28	33.00 ± 0.37	33.00 ± 0.91	33.00 ± 0.42

Data expressed as mean ± SD; n = 8; C = plain gelatine cubes + tap water to drink; F = plain gelatine cubes + 20% fructose solution as drinking fluid; FCur = 500mg/kg curcumin b.w + 20% fructose solution as drinking fluid; Cur = 500 mg/kg b.w curcumin + tap water to drink; Fen = 100 mg/kg b.w fenofibrate + tap water to drink.

Chapter 4: Discussion

The fructose diet did not result in significant differences in body mass, Plasma hormones. In the visceral organs of the males, the fructose fed rats had smaller small intestines compared to controls, as well as lower absolute large intestine masses and relative stomach masses ($p<0.05$). Furthermore, significant differences were observed in the hepatic pathology of males and females. Male rats showed macrosteatosis whilst female rats showed microsteatosis and macrosteatosis.

4.1 Growth performance

High fructose diets have been implicated in the development of metabolic syndrome (Tillman et al., 2014). Metabolic syndrome is a complex, multifactorial derangement associated with disorders such as obesity, insulin resistance and type two diabetes mellitus (Felice et al., 2014). Pharmacological strategies have been identified for the management of the various metabolic disorders, though some are expensive and associated with complications that are difficult to manage (Maithilikarpagaselvi et al., 2016). This has led to natural products being investigated as alternative therapies. Curcumin, a natural phytochemical, has been shown to reduce body weight and improve other metabolic related illnesses (Chen et al., 2017; Panzhinskiy et al., 2019). The present study was conducted to assess whether curcumin when administered during the early post weaning phase, which is a critical period of developmental programming, has long-lasting beneficial effects in rats on a high fructose diet. Specifically, by evaluating growth performance, metabolic dysfunction parameters, markers of health and the development of NAFLD by determining the hepatic histology scores for NAFLD.

High fructose content in the diet can result in increased body mass gain compared to controls on normal diets (Alwahsh & Gebhardt, 2017), but this was not the case in this study. In the current study, no significant differences were observed between rats fed fructose and the controls. The insignificant effect of fructose on body mass was in agreement to what (Felice et al., 2014) and (Bass et al., 2013) found following

administration of high fructose to adult male Sprague dawley rats for 4 and 12 weeks respectively. There are two possible explanations that may account for the lack of body mass gain in fructose fed rats in the current study. The first is age dependant (Tillman et al., 2014). Though multiple studies have observed that a fructose enriched diet results in excess body mass gain, adult rats were used in those studies (Bass et al., 2013; Crescenzo et al., 2014; Felice et al., 2014). Thus, it may be that the rats in the current study were at an age where they were able to fully oxidise the fructose as a source of energy with limited storage of the ingested calories (Tillman et al., 2014). Young rats have a higher metabolic rate compared to adult rats (Tian & Yu, 2017). With increasing age, the basal metabolic rate decreases due to a decrease in muscle mass and daily energy expenditure. In young rats, relatively more energy intake is channelled into metabolism and growth whereas in adult rats, it tends to be stored due to decreased physical activity (Tillman et al., 2014).

The second likely explanation for the lack of impact of fructose on body mass may be due to the concentration of fructose (Lowette et al., 2015). While 20% fructose drinking solution used in the current study has been established to result in excess body mass gain (Hussain et al., 2019; Lembede et al., 2017; Wankhade et al., 2016), a number of studies have administered 23% or more over a longer period of time and have noted that a consumption of 20% or less over a period of six weeks did not significantly affect body mass (Lowette et al., 2015). However it is notable that (Bass et al., 2013) observed no significant increase in the body mass of rats given a 40% fructose solution compared to the controls over a 12 week period.

The two week intervention with curcumin during the early post weaning period did not have an effect on the terminal body masses of the rats even though it has been previously reported that curcumin has anti-obesity properties (Weisberg et al., 2008). This may be due to the duration of exposure to curcumin. Several studies have shown that curcumin administered throughout the feeding period at a dose of three percent of body weight reduced body mass of obese mice over a 16 week period (Weisberg et al., 2008). Therefore, while administering curcumin throughout the growth phases may reduce body mass, when administered only during the developmentally plastic early

post weaning period as was the case in the current study, it had no effect on subsequent body mass gain.

It has been established that measurement of long bones is a more reliable indicator of long-term growth performance than body mass. Growth during childhood is controlled by various factors such as genes and hormones, with insulin-like growth factor (IGF-1) and the sex hormones playing an important role (Abarca-Gómez et al., 2017). A dietary insult such as fructose may alter hormone functioning. The long term effects of a high fructose diet can present as decreases in bone strength and produces weak bone microarchitecture (Laurent et al., 2014).

Distinct differences in the sexual dimorphic effects of diet and supplements on bone growth have also been reported (Horton et al., 2017). In the current study, male rats that received the high fructose diet alone and male rats that received fructose supplemented with curcumin showed significantly shorter femur lengths compared to controls (table 3.1). No significant differences were observed in the female long bone measurements (table 3.2). This may be due to the different functions of the sex hormones. Sex hormone concentrations increase during puberty and promote growth by increasing growth hormone (GH) secretion (Laurent et al., 2014). Testosterone increases bone growth thus increasing bone density. Decreased testosterone levels are associated with lower bone density and increased body fat (Shakibaei & Mobasheri, 2010). In the current study male rats fed a high fructose diet alone and rats fed fructose supplemented with curcumin showed shorter femur lengths but no increased body fat. It may be that a high fructose diet altered hormone functioning by decreasing circulating testosterone concentrations and in turn results in decreased levels of bone growth (El Hafidi et al., 2006). Decreased circulatory testosterone levels are possibly due to impairment of testicular development observed after 4 weeks of high fructose feeding in weaning male Wistar rats (Sakamuri et al., 2016). High fructose consumption may have impaired testis development in the early stages. However, the testosterone levels were

not measured in the current study, and so it cannot be categorically concluded that low testosterone was indeed responsible for the differences in bone parameters.

Furthermore, the addition of curcumin in the diet did not prevent this decrease in femur length even though it is documented that curcumin prevents and improves the high fructose induced physiological changes in bone parameters in mice fed curcumin for three months (Yang et al., 2011). The insignificant differences in bone parameters observed in the female rats may be due to optimal oestrogen levels. Oestrogen plays an important role in the growth and maturation of bone as well as bone turnover (Kalervo & Pirkko, 1996). The female rats in the current study were in puberty and oestrogen is found in elevated concentrations in pubertal females compared to prepubertal rats. It may be that the effects of fructose on metabolism are less severe in females through the function of oestrogen (Galipeau et al., 2002). Therefore, shielding any defects caused by high fructose. However, the current study did not measure oestrogen levels.

As discussed above, when curcumin was supplemented throughout the feeding period, it prevented and improved the high fructose diet induced physiological changes (Banu et al., 2012). In the current study, curcumin was administered for two weeks, which may have been too short a period to observe its beneficial effects on reducing body mass and preventing bone microarchitecture injury. This suggests a possible duration and age dependant mechanism (Lowette et al., 2015).

4.2 Metabolic dysfunction parameters

4.2.1 Plasma cholesterol and triglyceride concentrations

Fructose feeding in rodents is commonly associated with increased hepatic de novo lip genesis, leading to increased plasma triglycerides and cholesterol concentrations (Claudia et al., 2016). The current study observed no significant differences in the plasma triglyceride and cholesterol concentrations across the treatment groups of both male and female rats following six weeks of 20% fructose. The physiological impact of

fructose varies depending on the concentration administered, route of administration and duration of feeding. Studies conducted by Ibrahim, El-Denshary and Abdallah. (2015) and Ge *et al.* (2016) observed increases in body weight in conjunction with elevated plasma triglyceride and cholesterol concentrations, visceral adiposity and insulin following a 10% fructose solution over a five- and twenty-week period respectively. In contrast, studies by Larsen et al. (2013) and Zarfeshani, Mutalib and Khaza'ai. (2012) observed increases in body weight in the absence of elevated plasma triglyceride and cholesterol concentrations, insulin and visceral adiposity over a 10 week intervention with 10 and 21% fructose solutions, respectively. This observation is in agreement with the current study that also observed insignificant differences in the above parameters. This suggests that fructose-fed rats may not develop complete metabolic syndrome (Ackerman et al., 2005).

Interestingly, only a few studies in rats have reported an overall increase in body adipose tissue mass following fructose consumption despite its known lipogenic effects (Sandeva et al., 2015). Other studies have reported no significant increases in adipose tissue, more importantly visceral adipose, and insignificant increases in triglyceride and cholesterol concentrations (Larsen et al., 2013; Zarfeshani et al., 2012). Lack of accumulation of visceral adipose tissue suggests that plasma hormone concentrations and triglyceride and cholesterol concentrations were not disturbed by the interventions and the extra energy derived from the ingested fructose was redirected to growth since the rats were in the pubertal phase. The pubertal phase is characterized by growth spurts due to the growth hormone and an increase in sex hormones which promote pubertal maturation. Therefore, a surplus in energy is used to promote growth in the pubertal stage (Tillman et al., 2014).

Though the two week intervention with curcumin resulted in insignificant differences across the treatment groups in both male and female rats, it is well established that curcumin has lipid lowering effects (Na et al., 2011). Yiu et al. (2011) observed significant decreases in cholesterol and triglyceride concentrations, following curcumin supplementation, in rats fed a high fat diet for 28 days. These observations suggest that curcumin was effective in decreasing hypertriglyceridemia and hypercholesterolemia in

conditions where dyslipidaemia had developed. In the current study the lipid concentrations were unaffected by the fructose feeding and thus curcumin did not have an effect under these conditions.

4.2.2 Plasma hormones

It is well documented that a chronic high fructose enriched diet promotes lipid accumulation through adipose tissue dysfunction. Fructose inhibits the secretion of adiponectin from adipose tissue into systemic circulation (Gordish et al., 2017). Decreased adiponectin levels are associated with the accumulation of visceral adipose fat and dysregulation of insulin-stimulated glucose uptake and utilization (Meeprom et al., 2011). The present study observed no significant differences in the plasma adiponectin and insulin levels. Additionally, no significant differences were observed in the homeostatic model of assessment of whole body insulin resistance (HOMA-IR) of both male and female rats. Since no significant increases were observed in the visceral fat mass, it is unlikely that plasma hormone concentrations would be affected. Presence of excessive visceral adiposity is usually associated with hormone imbalances. Plasma levels of adiponectin are significantly decreased in obese patients, whilst insulin levels are significantly increased (Fiorino et al., 2016). This is commonly due to chronic inflammation associated with adiposity. Accumulation of macrophages in adipose tissue induce impaired adipocyte function and contribute to the development of insulin resistance (Rodrigues et al., 2011).

Though studies have shown that 20% fructose is sufficient to cause hyperinsulinemia and impaired adipose tissue function in pubertal rats over a two week period (Gordish et al., 2017), the current study showed that 20% fructose did not result in insulin-induced metabolic dysfunction. Lembede et al. (2017) and Muhammad. (2017) did not observe significant differences in the plasma insulin and HOMA-IR concentrations in both male and female rats fed a 20% fructose diet. This may be due to the age of the rats and the method in which fructose was administered. Rats used in the current study were of similar age to those used in the above studies and the manner in which fructose was

administered was similar. This may be the reason for the similar trend in results observed.

Though curcumin supplementation had no significant effect across the treatment groups of male and female rats in the current study, studies have shown that curcumin administration attenuated the elevation of plasma glucose levels in fasting and postprandial states in fructose fed rats (Gordish et al., 2017). Furthermore, curcumin supplementation to fructose fed rats was shown to attenuate the insulin-stimulated insulin receptor substrate 1 (IRS-1) serine phosphorylation. IRS-1 serine phosphorylation is known to be defective in the development of insulin resistance and diabetes. Curcumin also activated insulin cascade events by increasing oxidation of glucose and fatty acids (Maithilikarpagaselvi et al., 2016).

4.2.3 Visceral organ morphometry

In nutritional studies, the dietary constituents may have an impact on the gastrointestinal tract as this is the first point of contact of the ingesta with the body. Therefore assessment of visceral organ morphometry is important as part of establishing some of the adverse effects of the diets (Caprio et al., 2017; Shaligram et al., 2018). High fructose consumption hinders the development of the gastrointestinal tract (GIT) organs and impairs the ability of the gastrointestinal tract to deliver nutrients to tissues (Mukonowenzou, 2016) by impairing the digestive and absorptive functions (Ohashi et al., 2018). This occurs when there are structural defects in the gastrointestinal tract. Metabolic complications arising from excessive fructose are increasing in young children and can result in altered organ development and altered linear growth (Ohashi et al., 2018). In the current study, the high fructose diet in the males resulted in shorter small intestine lengths, lower large intestine masses and smaller relative stomach masses.

The increased large intestine masses and relative stomach masses may be attributed to increased gut load (Shaligram et al., 2018). It may be that the rats in the current study

drank more fructose and less rat chow. Although the fluid consumption was not directly measured, when I was replacing the fluids, I did notice that that the rats on fructose tended to drink much more than those on plain water. The decreased length in the small intestine may be attributed to impaired intestine villi which is caused by high fructose consumption (Shaligram et al., 2018). This results in impaired absorption capability due to decreased absorption area (Gupta et al., 2013). This in turn decreased gut function.

The early two week intervention with curcumin did not prevent the reduction observed in the above mentioned organs in the male rats. The combination of fructose and curcumin lowered the lengths and masses observed in the males. This finding suggests that the curcumin was unable to prevent the fructose induced effects on the GIT. This was not the case with the small intestine lengths. It has been observed that 32 days of curcumin administration improves digestibility in the small intestine (Gupta et al., 2013). It may that this was not observed in the current study due to the short exposure to curcumin. Future studies will involve histological assessment of the intestines.

There are two possible explanations for sex differences observed with regards to the effects of the fructose and curcumin on the visceral organ morphometry. Firstly, there may be mechanisms present only in males necessary to drive the effects of fructose on metabolism, secondly, female rats may possess counter mechanisms that protect against the adverse effects of fructose (Galipeau et al., 2002; Roberts et al., 2017). Indeed current literature on the brain gut axis shows sex related differences in gut function and morphology as well as microbiome pubertally due to sex hormones (Morrison et al., 2016). Female rats are protected against metabolic defects typically produced by fructose feeding in males (Galipeau et al., 2002). Six weeks of high fructose feeding did not produce observable metabolic defects in in female Wistar rats (Horton et al., 2017). The presence of oestrogen and/or progesterone, or the absence of male androgens, may be protective in female animals (Hulman et al., 1996). This protection was no longer present after ovariectomy, suggesting that female sex hormones may confer protection against the effects of a high fructose diet (Horton et al., 2017).

Our results are in agreement with a study by Muhammad. (2017) who also observed no significant differences in the gastrointestinal tract morphometry of female rats that were fed 20% fructose solution for 10 weeks from weaning age. Thus the rats as was the case in the current study were exposed to fructose prepubertally before experiencing changes in oestrogen and progesterone concentrations (Horton et al., 2017; Hulman et al., 1996).

4.2.4 Liver histomorphometry and NAFLD scores

Nonalcoholic fatty liver disease (NAFLD) is defined as excessive fat accumulation in the hepatic cells of nonalcoholics (Alwahsh & Gebhardt, 2017; Jegatheesan & De Bandt, 2017; Pappachan et al., 2017). With the liver being the site of fructose metabolism, an increasing body of evidence has shown that unrestrained fructose intake impacts hepatic lipid metabolism and insulin sensitivity (Nomura & Yamanouchi, 2012). Studies in rodents have shown various histological alterations of liver tissue after fructose consumption. Tappy *et al.* (2010) demonstrated increases in hepatic fat content within six weeks of high fructose feeding in rats.

Though studies have shown that signs of metabolic disorder tend to display a lower incidence in females (Chen et al., 2017; Galipeau et al., 2002), the current study observed more evidence of hepatic pathology in females than the males. Whilst male rats fed fructose only showed macrosteatosis, female rats fed fructose only showed macrosteatosis and microsteatosis and rats fed curcumin only and fenofibrate showed microsteatosis. Our results demonstrate that long term fructose intake may affect metabolic function between male and female rats differently. Studies conducted by Vila *et al*. (2011) are in agreement with the current study in that they also observed that female rats showed a more detrimental response in hepatic metabolic alteration compared to males. The differences observed between males and females in response to fructose may be modulated by existing different levels of sex hormones in both genders (Tillman et al., 2014).

In both males and females, rats that received combined fructose and curcumin showed no overt hepatic pathology suggesting that curcumin intake during the early post weaning phase reduced fat caused by the subsequent dietary fructose overload. Curcumin reportedly promotes fatty acid oxidation instead of adipogenesis in its anti-diabetic effect, which contributes to its lipid lowering effect in diabetes (Na et al., 2011). The current study is in agreement with Li et al (2010), who also observed reduced hepatic fat following supplementation with curcumin in rats fed high fructose for four weeks. Though the rats in the present study did not develop overt obesity, six weeks of high fructose feeding was sufficient to cause hepatic steatosis. However, hepatic steatosis is potentially reversible and does not necessarily lead to permanent hepatic injury if interventions are made early (Nomura & Yamanouchi, 2012). The observations in the current study suggest that supplementation with curcumin exerted beneficial effects by preventing the accumulation of fat in the liver as indicated in the fructose and curcumin group, who showed no gross fat accumulation. Curcumin has been shown to be useful as an anti-diabetic, anti-obesity, antioxidant and anti-inflammatory treatment. In previous studies, oral administration of curcumin restricted body weight and improved insulin sensitivity.

These findings require further mechanistic investigation as a potential strategy for management of NAFLD. Curcumin has been shown to be an effective treatment for metabolic disorders, however the current study aimed to assess whether the early targeted prophylactic administration of curcumin, during the early post weaning period, would have long lasting beneficial health effects in preventing metabolic dysfunction in adulthood. The post weaning is period characterized by a profound change in nutrition. It is at this stage where lipogenic activity changes. A change of diet at this stage may affect health outcomes, thus it presents a window of opportunity to assess whether curcumin may programme for health outcomes later in life, which was the case in the current study. To further assess the well-being of the rats, I investigated the red blood cell indices as a measure of general health.

4.2.5 Red blood cell indices

Spontaneous glycation of haemoglobin can affect the structure and shorten the lifespan of red blood cells (Shanthi et al., 2013). The current study evaluated the haematocrit and haemoglobin concentrations to assess general health. We observed no significant differences in the haematocrit and haemoglobin concentrations of both male and female rats. In addition to the above results, the mean corpuscular haemoglobin concentration was also not significantly different between the treatment groups. Abnormal increases or decreases in cell counts as revealed in a complete blood count may indicate an underlying medical condition (Schumacher et al., 2000). Metabolic disturbances such as type two diabetes can affect red blood cell size which in turn affects function (Agrawal et al., 2016). Haemoglobin and red blood cell mass play an important role in the oxygen transport chain (Ibrahim et al., 2015). Excessive fructose intake may cause longstanding hyperglycaemia which results in the formation of advanced end glycation products (AGEs) (Sakai et al., 2002). Glycation occurs when a sugar molecule, such as fructose, bonds covalently to a protein or lipid molecule without the controlling action of an enzyme (Jain et al., 2006). AGEs can contribute to the biomolecular damage observed in chronic diseases such as diabetes (Jain et al., 2006). Longstanding hyperglycaemia has been shown to promote spontaneous glycation of haemoglobin and cause severe progression of haemoglobin-AGE formation (Hb-AGE) (Sakai et al., 2002). Hb-AGE can result in several alterations which influence the biological function of the haemoglobin molecule. These alterations include: intrinsic secondary structural changes; haem dissociation and substantial remodelling of native conformation (Sakai et al., 2002). Studies have shown that fructose modifies the secondary structure of haemoglobin by exposing the solvent-accessible surface area of sites prone to glycation (Sakai et al., 2002). The rats in the current study showed normal haemoglobin concentrations, confirming our findings reported earlier that they did not develop longstanding hyperglycaemia and thus the red blood cell structure was not significantly affected by the high fructose diet.

Fructose causes oxidative stress by raising uric acid (a pro-oxidant), resulting in the formation of reactive oxygen species (ROS) (Maheshwari et al., 2006). Curcumin has

been shown to be a potent scavenger of a variety of ROS, thus reducing oxidative stress (El-Moselhy et al., 2011). Oral supplementation with curcumin in the current study showed no significant differences in the red blood cell indices across the treatment groups. Though the current study did not measure ROS concentration, it may be that ROS concentration was low or the red blood cells were not affected and thus the antioxidative effect of curcumin could not be observed. Studies have reported the antioxidative effect of curcumin. Jain *et al*. (2006) observed that curcumin reduced glycosylation of proteins and lipid peroxidation in erythrocytes exposed to high glucose mimicking diabetes and proposed that this may have occurred via the antioxidative effect of curcumin. Motterlini et al. (2002) also observed that curcumin prevented oxidant mediated injury by increasing oxygenase production.

4.2.6 Limitations and recommendations

There are some limitations in the present study. Molecular biochemistry measurements were not done and sometimes gene expression and pathways may be affected but not detectable phenotypically in the early stages. Weaning stress has been reported to impair intestinal barrier function and alter mRNA expression of tight junction proteins and enzyme activities in the intestine (Luo et al., 2016). Future studies should measure potential differential expression of phosphorylated c-Jun N-terminal kinases (JNK) and Mitogen-activated protein kinases (MAPK) signalling pathways which can be activated in the intestine by environmental stress and pro-inflammatory cytokines (Luo et al., 2016). Phosphorylation and activation of JNK and MAPK result in downstream substrates which are responsible for altering gene expression (Rodrigues et al., 2011)

The post weaning period is characterized by marked changes in lipogenic enzymes. The present study did not measure lipogenic enzyme activity and future studies should consider measuring glucagon, acetyl coenzyme A (acetyl-CoA), ATP-citrate lyase and fatty acid synthase (FAS). Weaning interrupts the physiological equilibrium of oxidants and antioxidants and may lead to oxidative stress (Lin et al., 2018). Future studies

should consider measuring hepatic free radicals such as hydrogen peroxide, nitrogen oxide and malondialdehyde (MDA) as well as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activity. Oxidative stress causes epigenetic changes resulting in DNA methylation and histone modification (Luo et al., 2016) therefore detection of genes such as SEPTIN-9 and ALX Homeobox 4 (ALX4) may give indication of methylation.

Future studies should consider feeding with fructose for longer to cause overt metabolic dysfunction in order to fully evaluate the impact of the early post weaning curcumin intervention. Though the dose of 500mg/kg used in the current study showed that curcumin was protective even though overt metabolic dysfunction was not achieved from the high fructose diet, future studies should consider using increasing doses of curcumin alongside fructose to observe whether there is incremental (dose response) protective effect.

It is also recommended that in future studies, the food intake and fructose intake should be assessed so that calorie intake and association with the body mass gain and adiposity can be further interrogated.

Assessment of the biochemical markers (alkaline phosphatase, alanine aminotransferase, urea, bilirubin and creatinine) of health may give a better insight of health, however, there needs to be severe damage of the organs before these markers increase in circulation. This was not done as histology (considered the gold standard) was used to assess the liver pathology.

Chapter 5: Conclusions

The hypothesis of the current study was that oral administration of curcumin during the early post weaning period would prevent the development of high fructose induced metabolic dysfunction later in life in male and female rats. The current study targeted the early post weaning period as it is characterized by a change in nutrition from predominantly milk to a diet rich in fat and carbohydrates (Iritani, 1988) which has profound effects on the metabolic, digestive and absorptive functions of animals and ultimately have an effect on subsequent health later in adulthood. Although the high fructose diet did not produce overt metabolic dysfunction, of the measured parameters following the interventions, the fructose diet affected body weight, the long bones, hepatic tissue and GIT morphometry. Of the clinically important metabolic disorders, the present study demonstrated that oral administration of curcumin in the post weaning period exerted some protection in the male and female rats against the development of fructose induced nonalcoholic fatty liver disease. Therefore, there is scope to further explore the early post weaning period as a target for interventions for long term health benefits in human and veterinary medicine.

Though most studies have assessed the metabolic effects of fructose and dietary supplements in males, the current study observed that female rats were less affected by fructose and curcumin in comparison to the male rats. This re-emphasizes the need for studies to investigate both sexes and not extrapolate data from one sex to another. Due to the differences observed, further evaluation is required to assess distinct metabolic effects of fructose and dietary supplements in both sexes. This may be helpful in finding effective methods of treatment. The use of targeted therapy also referred to as precision medicine (Mohammadi et al., 2012) has become increasingly important for disease treatment. Targeted therapies are designed to interfere with molecules or genes that cause disease. The early developmental stages are increasingly being targeted as windows of interventional opportunity for positive health outcomes as these are phenotypically plastic and through epigenetic mechanisms the impact can extend positive benefits through to several generations (Wankhade et al., 2016).

Typically, poor diet is not restricted to high fructose intake, therefore, future studies should consider alternative dietary experimental models by modifying the diet by adding

high fat or high cholesterol, as the typical diet consumed by obese individuals would include a combination of fats and sugars. The role of the gut microbiome in health is a major focus of interest (Morrison et al., 2016), the impact of biologically active phytochemicals on the gut microbiome during the early post weaning phase also needs further exploration.

With childhood obesity on the rise, it has become increasingly important to study metabolic alterations in children using appropriate animal models and alternative innovative interventions as was done in the current study in order to find strategies to prevent metabolic dysfunction from manifesting during childhood and, or later in adulthood. Providing low cost, effective precise targeted prophylactic interventions, with readily available biologically active phytochemicals such as curcumin, would have a long term impact in reducing the burden of health care globally.

Chapter 6: References

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Chapter 7: Appendices

Appendix 1: Ethical clearance certificate

STRICTLY CONFIDENTIAL

ANIMAL ETHICS SCREENING COMMITTEE (AESC)

CLEARANCE CERTIFICATE NO. 2018/05/22/B

APPLICANT: Miss L Gobe

SCHOOL: Physiology
DEPARTMENT:
LOCATION:

PROJECT TITLE: Does curcumin administered in the early post weaning period have long lasting beneficial effects in Sprague Dawley rats on a high fructose diet

Number and Species

40X 21 day old female and 40X 21 day old male Sprague Dawley Rattus Norvegicus

Approval was given for the use of animals for the project described above at an AESC meeting held on 2018/05/29. This approval remains valid until 2020/06/12.
Unreported changes to the application may invalidate the clearance given by the AESC
An annual progress report must be provided

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

Signed: _____ (Chairperson, AESC) Date: 26/6/2018

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

Signed: _____ (Registered Veterinarian) Date: 13 June 2018

cc: Supervisor: Professor K Erlwanger
Director: CAS

Works 2000/lain0015/AESCCert.wps

Appendix 2: Approved modifications and extensions certificate

AESC 2012 M&E

Please note that only typewritten applications will be accepted.

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

a. Name: Lihle Goba

b. School and email address: School of Physiology

c. Experiment to be modified / extended

		AESC NO	
Original AESC number	2018	05	22B
Other M&Es .			None

d. Project Title: Does curcumin administered in the early post weaning period have long lasting beneficial effects in rats on a high fructose diet?

		No.	Species
e.	Number and species of animals originally approved:	80	*Rats*
f.	Number of additional animals previously allocated on M&Es:	0	*Rats*
g.	Total number of animals allocated to the experiment to date:	0	*Rats*
h.	Number of animals used to date:	0	*Rats*

i. Specific modification / extension requested:

I would like to change from using metformin as a positive control to using Fenofibrate.

j. Motivation for modification / extension:

Metformin is available in tablet form which contains excipients which may have an effect on the rats. We have decided to use Fenofibrate, an alternative positive control. Fenofibrate has been previously used in our studies on metabolic dysfunction as it promotes antihyperlipidemic and antidiabetic activity. Fenofibrate will be administered in gelatine cubes (as Metformin would have been). The dose will be 100mg/kg once a day (Lembede, 2018) . All other procedures will not be changed.

Lembede, B. W. (2018) 'Terminalia Sericea aqueous leaf extract protects growing wistar rats against fructose-induced Abstract ':, pp. 1–10. doi: 10.1515/jcim-2018-0035.

Date: 03/07/2018
RECOMMENDATIONS

Signature: 

Date:

Signature:

Chairman, AESC