CURCUMIN USE IN THE ATTENUATION OF DIET-INDUCED METABOLIC SYNDROME IN RATS

A dissertation submitted by

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Abstract

Metabolic syndrome is the clustering of a multitude of cardiometabolic symptoms including central obesity, hypertension, insulin resistance, chronic low-grade inflammation and dyslipidaemia. Prevalence of metabolic syndrome and obesity is rapidly increasing worldwide, which has prompted extensive research into discovering new treatment approaches to prevent the growth of this epidemic. The objective for this study was to determine the effectiveness of orally administered low (5 mg/kg/day) and high (100 mg/kg/day) doses of curcumin in attenuating signs of diet-induced metabolic syndrome in rats. The rat model of obesity used in this project, designed by the Functional Foods Research Group at the University of Southern Queensland, closely mimics the pathological changes observed in human metabolic syndrome. Male Wistar rats (8-9 weeks old) were divided into six experimental groups. In the treatment group rats, obesity and signs of metabolic syndrome were developed during the first eight weeks of pre-treatment period, followed by curcumin treatment for the remaining eight weeks to reverse these symptoms. The total protocol was of 16 weeks. The efficacy of the low and high doses of curcumin in attenuating metabolic syndrome was assessed by comparing control rats with curcumin treated rats with regard to fat deposition (obesity), cardiovascular health, liver function, lipid profile, glucose tolerance and tissue inflammation. The low dose of curcumin did not attenuate any signs of obesity. In comparison, the high dose of curcumin was able to normalise systolic blood pressure, reduce levels of inflammation in the heart, and lower visceral adiposity compared to high-carbohydrate, high-fat diet-fed obese rats. However, the high dose of curcumin was unable to affect other components of metabolic syndrome including overall fat content, liver health, dyslipidaemia and hyperglycaemia, which conflicted with the results of previous studies. Low bioavailability of curcumin may be an important factor in unresponsiveness of curcumin in this study and improved bioavailability and reduced metabolism through nanoparticles may serve as a future direction for this study.

Declaration

I hereby certify that the experimental work, results, analyses, discussion and conclusions reported in this dissertation are entirely my own effort, except where otherwise acknowledged. I also certify this work is original and has not been previously submitted.

Candidate: Jessica Pahl

Date: 08/11/16

Endorsement

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List of abbreviations

C – Corn starch diet-fed rats

CS – Corn starch diet

H – High-carbohydrate, high-fat diet-fed rats

HCHF – High-carbohydrate, high-fat diet

CC5 – Corn starch diet-fed rats + 5 mg/kg/day curcumin

HC5 – High-carbohydrate, high-fat diet-fed rats + 5 mg/kg/day curcumin

CC100 – Corn starch diet-fed rats + 100 mg/kg/day curcumin

HC100 – High-carbohydrate, high-fat diet-fed rats + 100 mg/kg/day curcumin

CVD – Cardiovascular disease

HDL-c – High-density lipoprotein cholesterol

LDL-c – Low-density lipoprotein cholesterol

IL-6 – Interleukin-6

TNF-α – Tumour necrosis factor-alpha

NAFLD – Non-alcoholic fatty liver disease

WAT – White adipose tissue

BAT – Brown adipose tissue

OGTT – Oral glucose tolerance test

SBP – Systolic blood pressure

NEFA – Non-esterified fatty acids

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Chapter 1. Introduction and literature review

1.1 What is metabolic syndrome?

Metabolic syndrome is the clustering of a multitude of cardiometabolic symptoms including central obesity, hypertension, insulin resistance, chronic low-grade inflammation and atherogenic dyslipidaemia (GrundyKassi et al., 2011). These factors directly increase the risk of cardiovascular disease (CVD) and type 2 diabetes, which are two major causes of mortality that have been increasing in incidence over the last decade (WHO, 2014).

There is not one globally accepted definition of metabolic syndrome. Instead, there are numerous, often country-specific, definitions of metabolic syndrome which have been used to identify those at high risk of developing further complications (Table 1) (Alberti et al., 2009; Alberti et al., 2006; Expert Panel on Detection, 2001; Grundy et al., 2004). Across all definitions of metabolic syndrome, there appears to be general agreement for the parameter thresholds for hyperglycaemia, dyslipidaemia (triglycerides and high-density lipoprotein cholesterol (HDL-c) and hypertension. However, definitions developed by the International Diabetes Foundation (IDF) and the consensus panel (IDF, National Heart, Lung and Blood Institute American Heart Association, World Heart Association, and International Association for the Study of Obesity) have included population and country specific thresholds for waist circumference (Alberti et al., 2009; Alberti et al., 2006).

Waist circumference is indicative of central obesity, a phenotype highly correlated with metabolic syndrome, although the degree of central obesity has different implications for different ethnicities (Alberti et al., 2006). For example, Asian populations have a higher risk for the development of metabolic syndrome with a comparatively low waist circumference (Alberti et al., 2009). The consensus panel removed central obesity as a compulsory criteria for metabolic syndrome in 2009, due to the differences in risk associated with a particular waist

measurement between sexes and ethnicities (Alberti et al., 2009). It is expected that an updated definition for metabolic syndrome will be developed in the near future; as previous definitions are becoming outdated in comparison to the advances in literature regarding obesity and metabolic syndrome in the last seven years.

Table 1. Definitions of metabolic syndrome

	NCEP ATP III (2001)	***AHA/NHLBI (2004)***	***IDF (2005)***	***Consensus panel*[#] *(2009)***
Criteria	Any three of the five criteria below	Any three of the five criteria below	Central obesity, plus two of the four criteria below	Any three of the five criteria below
Central obesity (waist circumference)	Men; >102 cm Female; >88 cm	Men; >102 cm Female; >88 cm	Population and country-specific definitions	Population and country-specific definitions
Hyperglycaemia (fasting glucose)	≥110 mg/dL[$]	≥100 mg/dL	≥100 mg/dL or Rx	≥100 mg/dL or Rx
Dyslipidaemia (triglycerides)	≥150 mg/dL (1.7 mmol/L) or Rx[*]	≥150 mg/dL (1.7 mmol/L) or Rx[*]	≥150 mg/dL (1.7 mmol/L) or Rx[*]	≥150 mg/dL (1.7 mmol/L) or Rx[*]
Dyslipidaemia (HDL-c)	<40 mg/dL (1.0 mmol/L) in males; <50 mg/dL (1.3 mmol/L) in females or Rx[*]	<40 mg/dL (1.0 mmol/L) in males; <50 mg/dL (1.3 mmol/L) in females or Rx[*]	<40 mg/dL (1.0 mmol/L) in males; <50 mg/dL (1.3 mmol/L) in females or Rx[*]	<40 mg/dL (1.0 mmol/L) in males; <50 mg/dL (1.3 mmol/L) in females or Rx[*]
Hypertension	130/85 mmHg or R_x[*]	130/85 mmHg or Rx[*]	≥130/85 mmHg or Rx[*]	≥130/85 mmHg or Rx[*]

[*]R_x stands for pharmacological treatment

[#]Consensus panel includes World Heart Association, International Association for the Study of Obesity, IDF, NHBLI, and AHA

Abbreviations used: NCEP ATP III, National Cholesterol Education Program Adult Treatment Panel III; AHA, American Heart Association; NHLBI, National Heart, Lung, and Blood Institute; IDF, International Diabetes Foundation; HDL-c, high-density lipoprotein cholesterol.

[$]Updated in 2003 to become 100 mg/dL in response to the American Diabetes Association changing the criteria for impaired fasting glucose tolerance from 110 mg/dL to 100 mg/dL.

1.1.1 What is obesity?

Obesity is a disorder characterised by a chronic energy imbalance, whereby energy expenditure is consistently lower than energy intake, necessitating the expansion of adipose tissue to allow the storage of excess energy (Ahn et al., 2010). Obesity is increasing in prevalence at a fast rate due to the rapidly increasing adoption of diets rich in saturated fats and simple carbohydrates, in combination with a sedentary lifestyle (McGill, 2014). However, the variation in individual response to an obesity-inducing environment indicates that there are also clear genetic factors involved in determining susceptibility to obesity (El-Sayed Moustafa and Froguel, 2013). The World Health Organisation (WHO) reported that in 2014, 39% of adults aged 18 years and older were overweight and 13% were obese worldwide (WHO, 2015). The prevalence is significantly higher in countries such as the USA, Australia, Canada and Middle Eastern countries where obesity prevalence is as high as 30% in the population over the age of 18 years (WHO, 2015). Multiple institutions in Australia, such as Medibank Health Solutions, have estimated the cost of obesity to range from $30 to $50 billion in Australia in 2012 (Smeerdijk et al., 2015), with much higher estimates of $147 billion cost in the USA in 2008 (CDC, 2015). With increases in the prevalence of obesity and metabolic syndrome, health costs are continuing to rise globally, increasing the burden on the society.

In a healthy individual, adipose tissue is responsible for maintaining energy homeostasis in response to fluctuations in nutrient supply (Choe et al., 2016). In an obese person, there are large demands placed on adipose tissue for the storage of excess energy as lipids that necessitates adipocyte hypertrophy (increase in size of adipocytes or fat storing cells) and hyperplasia (increase in number of adipocytes) (Sun et al., 2011b). Healthy adipose tissue expansion features enhanced recruitment of adipocyte precursor cells (hyperplasia) and appropriate levels of vascularisation and extracellular matrix growth (Sun et al., 2011a), whereas pathogenic expansion is characterised by rapid expansion of existing fat cells

(hypertrophy), a high degree of macrophage infiltration, adipocyte death, limited vasculature and large amounts of fibrosis (Sun et al., 2011a). In response to further growth beyond the expansion limit, adipose tissue expansion that was previously healthy can transition into this pathogenic phenotype (Sun et al., 2011a). This pathogenic expansion of adipose tissue leads to chronic inflammation, oxidative stress and systemic insulin resistance (Choe et al., 2016; Sun et al., 2011a; Trayhurn, 2013). These pathogenic factors favour the development of adipocyte dysfunction and consequent deregulation of adipokine (chemical messengers secreted from adipocytes) secretion (Trayhurn, 2013).

Adipose tissue secretes a large number of adipokines including leptin, adiponectin, angiotensinogen, interleukin 6 (IL-6) and tumour necrosis factor-*alpha* (TNF-α) which regulate energy balance, insulin sensitivity and carbohydrate and lipid metabolism, blood pressure and inflammation, respectively (Wood et al., 2009). There is increased synthesis of the pro-inflammatory cytokines IL-6 and TNF-α in obesity, which have been found to have important roles in furthering the progression of insulin resistance and chronic low-grade inflammation (Tchernof and Després, 2013). Whereas the level of adiponectin, which is responsible for improving insulin sensitivity, increasing fatty acid oxidation in both liver and muscle tissue and enhancing uptake of glucose by muscles, is decreased in obesity (Hajer et al., 2008).

Whilst it is evident that obesity has the potential to progress to metabolic syndrome, the distribution of body fat has a large role in determining metabolic syndrome risk (Maury and Brichard, 2010). Accumulation of excess adipose tissue in the intra-abdominal region (visceral adipose tissue) is strongly correlated with incidence of metabolic syndrome (Ritchie and Connell, 2007). The mechanism responsible for this occurrence is not completely understood, however, it has been observed across multiple studies that visceral adipose tissue is associated with enhanced monocyte infiltration rate, decreased adiponectin synthesis and increased IL-6 synthesis (Cancello et al., 2006; Fontana et al., 2007; Tankó et al., 2004).

1.1.2 Cardiovascular disease

The presence of metabolic syndrome is highly indicative of an increased risk of CVD regardless of whether the patient is overweight or not, although a higher body mass index correlates with a higher risk of CVD (Ärnlöv et al., 2010). Cardiovascular disease is the outcome of deleterious changes to the heart and blood vessels that affect the transport of blood throughout the body. Restriction of the flow of blood to critical areas such as the heart and brain, as in myocardial infarction and stroke, leads to the death of tissue and possible fatality (Hanson et al., 2013). The different components of metabolic syndrome all have adverse effects on the endothelium of blood vessels, which has a protective role in preventing the development of atherosclerosis (Huang, 2009). Atherosclerosis, one of the major mechanisms in the development of cardiovascular disease, contributes to changes that occur in the blood vessels, and refers to the formation of a plaque in the arterial wall (Lusis, 2000). The role of low-density lipoprotein cholesterol (LDL-c) in the formation of plaques vulnerable to rupture (major cardiovascular event) is well documented (Bentzon et al., 2014; Lusis, 2000; Wentzel et al., 2012). Synthesis of LDL-c is up-regulated in both obesity and metabolic syndrome, whilst the cardio-protective HDL-c is down-regulated (Tchernof and Després, 2013).

1.1.3 Non-alcoholic fatty liver disease and diabetes

Non-alcoholic fatty liver disease (NAFLD) is a multi-stage disease that initially refers to the accumulation of hepatic steatosis (abnormal accumulation of lipids in the liver), that then can progress to steatohepatitis (abnormal accumulation of fat and inflammation in the liver) and eventually fibrosis and cirrhosis (Sattar et al., 2014). Obesity and metabolic syndrome are closely linked to incidence of NAFLD due to the established roles of visceral obesity, dyslipidaemia and insulin resistance in development of NAFLD (Smith and Adams, 2011; Tilg and Moschen, 2010).

Visceral adipose tissue increases the synthesis of fatty acids, which are directly transported to the liver due to the proximity of visceral adipose tissue to the hepatic portal circulation (Ritchie and Connell, 2007). This promotes hepatic insulin resistance, and provides a substrate for lipoprotein synthesis and lipid storage in hepatocytes (Maury and Brichard, 2010). Lipid storage in hepatocytes (liver cells) impairs the function of the liver, in particular hepatic insulin sensitivity, and can lead to the development of fibrosis and inflammation in the liver (Paschos and Paletas, 2009). It appears that TNF-α has an important role in the progression of hepatic steatosis to steatohepatitis, as significantly higher levels of TNF-α were detected in patients with increased fibrosis and inflammation (Crespo et al., 2001). In comparison, adiponectin levels were significantly lower in patients with steatohepatitis compared to hepatic steatosis, emphasizing the protective role of adiponectin in inhibiting the inflammatory actions of TNF-α (Kaser et al., 2005). Also, NAFLD is a major risk factor for the development of type 2 diabetes, and is present in the majority of patients with type 2 diabetes (Tilg and Moschen, 2010).

1.2 Current treatment strategies

While the development of metabolic syndrome involves multiple processes and factors, there is a strong case for the importance of obesity in disease risk and progression. Through targeting reduction of body weight, especially abdominal fat, it has been observed that there are significantly improved markers of health in type 2 diabetes, NAFLD and CVD risk factors (Promrat et al., 2010; Wing et al., 2011). Once obesity is established, it becomes increasingly difficult to achieve and maintain weight loss due to the psychological and environmental barriers that need to be overcome to create lifestyle modifications (Ochner et al., 2015). Considering the sheer number of people that are currently obese globally, there is a large demand for the development of readily available treatment strategies for successful and sustainable weight loss. Currently, bariatric surgery is the most effective method of inducing

weight loss. Bariatric surgery involves restricting the size of the stomach and bypassing part of the intestine to reduce food absorption (Encinosa et al., 2005). A meta-analysis of 11 studies reported that the mean weight loss of patients who underwent bariatric surgery was 26 kg more than patients who used non-surgical treatments (Gloy et al., 2013). Whilst bariatric surgery is an effective solution for obesity; it is expensive and highly invasive and is thus unsuitable to be used as the primary solution to the growing obesity epidemic.

Standard non-surgical treatment involves lifestyle modifications, including changes to the diet and exercise, augmented by the use of prescription weight loss drugs (Brown et al., 2015). Current prescription weight loss drugs, such as Orlistat (gastric lipase inhibitor), on average, are known to reduce body weight by only 3 kg, which is an insignificant amount for an obese person (Li et al., 2005). Also, some other weight loss drugs have side effects such as psychological complications (example – Rimonabant) (Brown et al., 2015). It is hoped that functional foods and nutraceuticals may play a role in reducing obesity, although the evidence for this is quite weak and is a developing area (Brown et al., 2015).

1.3 Functional foods

Functional foods are defined as foods that not only provide nutrition, but also have the capacity to treat or prevent diseases (Rahmatullah et al., 2009). It is well documented that natural products have been used for thousands of years in traditional medicine (Rahmatullah et al., 2009). Traditional medicine encompasses a large number of functional foods, although some examples that are still commonly used in modern society are green pepper, turmeric and green tea (Graziose et al., 2010; Rahmatullah et al., 2009). The possibility for adapting these traditional medicines into novel drugs or functional foods has prompted more in-depth studies to assess their capacity for the treatment of disease such as obesity.

1.4 Spices

In recent years, a wide range of spices, including ginger, turmeric, black pepper, black cardamom, garlic and chilli peppers (capsaicin), have been identified for their potential to treat metabolic syndrome (Bhaswant et al., 2015b; Diwan et al., 2013; Kang et al., 2010; Rahmatullah et al., 2009; Westerterp-Plantenga et al., 2006). Black cardamom and piperine from black pepper have been shown to attenuate effects on diet-induced metabolic syndrome in rats (Bhaswant et al., 2015b; Diwan et al., 2013). In these studies, there were significant improvements in liver function, blood pressure and blood lipids (Bhaswant et al., 2015b; Diwan et al., 2013). Additionally, the therapeutic effect was thought to be mediated through the reduction of inflammation and visceral adiposity. Whilst similar studies have not yet been conducted in humans, these observations demonstrate the therapeutic potential of spices for the treatment of obesity and/or metabolic syndrome. This project will assess the potential of curcumin, a bioactive compound from turmeric, as a treatment for diet-induced metabolic syndrome.

1.5 Curcumin

The commonly used spice turmeric is derived from the rhizomes of the plant *Curcuma longa* (Khalil et al., 2013). Turmeric is a spice that has featured in Indian traditional medicine for thousands of years, and in the last few decades, there has been significant research into identifying whether components from turmeric are effective in treating chronic diseases (Prasad et al., 2014). Curcumin, demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC), the major active constituents of turmeric, belong to a family of diarylheptanoid compounds known as the curcuminoids (Prasad et al., 2014).

Curcumin, as shown in *Figure 1*, is a biphenolic compound with hydroxyl groups at the *ortho*-position on the two aromatic rings that are connected by a β-diketone bridge containing two

double bonds (Begum et al., 2008). The phenolic hydroxyl groups are thought to be critical in the antioxidant activity of curcumin, as they may help in scavenging electrons from reactive oxygen or nitrogen radicals (Amolins et al., 2009; Priyadarsini et al., 2003). Phenolic compounds are constituents of fruits, vegetables and plant-derived beverages including green tea, and have been successfully used in trials for the treatment of obesity in previous studies (Hsu et al., 2009; Sergent et al., 2012). This further cements the importance of the phenolic hydroxyl groups in curcumin. The phenolic hydroxyl groups are conserved across the three curcuminoids - curcumin, demethoxycurcumin and bisdemethoxycurcumin (*Figure 1*). The structural difference between the curcuminoids is the presence or absence of methoxy substitution on the aromatic ring. Of these three compounds, curcumin is therapeutically the most active compound, which suggests that the double methoxy substitution has an important role in the various health benefits of curcumin (Prasad et al., 2014). The methoxy groups are thought to facilitate the scavenging of free radicals, through their ability to release electrons to support the strength of hydrogen bonding from the phenolic hydroxyl groups (Malik and Mukherjee, 2014).

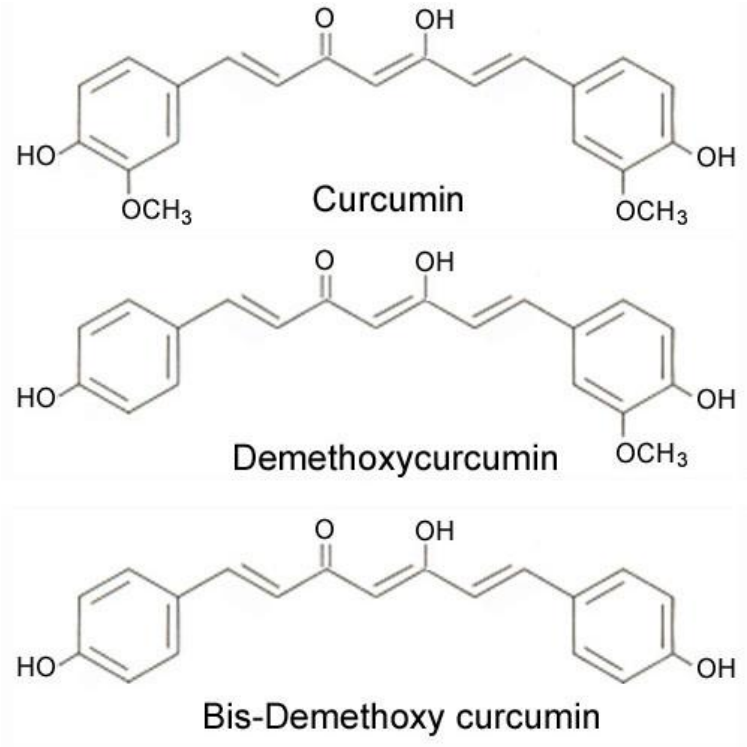


Figure 1. Curcuminoids structures (Wilken et al., 2011).

Although curcumin has been identified as the most active curcuminoid pharmacologically, there has been some argument for increased potency when a combination of all three curcuminoids are used (Rates, 2001; Sandur et al., 2007). A mixture of the three curcuminoids, with curcumin comprising 77%, had increased anti-inflammatory effects over pure curcumin (Sandur et al., 2007). This occurrence could be explained by the removal of possible pharmacodynamic synergism or pharmacokinetic influences when a single compound is isolated (Rates, 2001). In light of this, this study will use all three curcuminoids together in a 'curcuminoid mixture' that will hence forth be referred to as curcumin.

1.6 Effects of curcumin

Using curcumin to attenuate obesity is not a new concept. In fact, the anti-obesity effects of curcumin have been explored in a multitude of studies in the last two decades (Prasad et al., 2014). Studies have shown that curcumin is able to suppress adipogenesis (Ahn et al., 2010), as well as being a potent antioxidant (Kirschweng et al., 2015) and anti-inflammatory agent

(Maithilikarpagaselvi et al., 2016). The broad spectrum activity of curcumin has been attributed to its ability to modulate multiple transcription factors, kinases, cytokines, growth factors and other enzymes (Zhou et al., 2011).

1.6.1 Curcumin targeting adipose tissue

As mentioned earlier, one of the major mechanisms of obesity is adipose tissue hyperplasia (increase in number of adipocytes) which is facilitated by increased adipogenesis. Adipogenesis is the process of conversion of preadipocytes into adipocytes. Interestingly, many studies have shown that curcumin is able to suppress the process of adipogenesis (Ahn et al., 2010; Ejaz et al., 2009; Ferguson et al., 2016; Kim et al., 2011; Lone et al., 2016), thereby suppressing the progression of adipose tissue hyperplasia. It has been reported that weight loss by itself is unable to reduce the number of adipocytes, and instead hypertrophy (cell size) is decreased (Faust et al., 1978; Ferguson et al., 2016; Jo et al., 2009). Adipocyte number is fairly constant in adults, but there is a high turnover rate which is estimated to be 10% per year (Jo et al., 2009). Obesity, in developmental years (child and teenager), drastically increases the number of adipocytes, which sets the precedent for increased risk of persistent obesity with associated complications (Freedman et al., 2001). Since curcumin targets adipogenesis during cell turnover, treatment with curcumin is likely to be more effective if it coincides with either obesity development in childhood or earlier in the onset of obesity.

Although the mechanism of adipogenesis suppression is not clear (Ahn et al., 2010; Ejaz et al., 2009; Ferguson et al., 2016; Kim et al., 2011; Lone et al., 2016), it has been suggested that curcumin suppresses adipogenesis through activation of the Wnt/β-catenin signalling pathway (Ahn et al., 2010), cell cycle arrest via p27 (Ferguson et al., 2016; Kim et al., 2011), and suppression of the transcription factor peroxisome proliferator-activated receptor-γ (PPAR-γ) (Ejaz et al., 2009). However, the primary target of curcumin in suppressing adipogenesis remains unclear. Studies with 3T3-L1 adipocytes from mice reported a dose-dependent result,

whereby treatment with 20μM - 25μM curcumin completely blocked differentiation of adipocytes (Ahn et al., 2010; Ferguson et al., 2016). However, this observation was in cell lines, and therefore has not yet been translated to animal or human studies.

A new strategy for encouraging weight loss in recent years has been to promote energy expenditure by activating brown adipose tissue (BAT) and converting white adipose tissue (WAT) to BAT (Lone et al., 2016). Brown adipose tissue converts energy from food into heat through non-shivering thermogenesis, and therefore increases excess energy expenditure. A critical protein involved in this process is uncoupling protein 1 (UCP1), and curcumin was reported to up-regulate UCP1 at mRNA and protein levels. This occurs in 'beige' adipocytes, which are WAT that has drastically increased expression of UCP1, suggesting the possibility for BAT thermogenic properties (Shabalina et al., 2013). This observation was supported by another study that identified the stimulation of UCP1 gene expression to occur with the release of norepinephrine (Wang et al., 2015), which is mandatory for the activation of beige adipocytes (Cannon and Nedergaard, 2004). This study, conducted with a mouse model (rather than cell culture), observed a dose-dependent response (Wang et al., 2015). The greatest up-regulation of UCP1 mRNA was in response to 100 mg curcumin /kg body weight administered by daily oral gavage for 50 days (Wang et al., 2015). These authors concluded that this effect was due to increased non-shivering thermogenesis, as it was observed that the curcumin-treated mice had increased body temperature compared to controls in response to cold exposure (Wang et al., 2015).

Whilst this study will not examine the changes in adipose tissue at the molecular level (due to time restrictions), adipose tissue will be taken and weighed from the various depots to assess any alterations in fat mass or location. Weight loss in the visceral fat depots is of particular importance due to metabolic abnormalities associated with abdominal adipose tissue.

1.6.2 Curcumin targeting chronic inflammation and oxidative stress

The development of chronic low-grade inflammation in obesity is the outcome of multiple factors, including macrophage infiltration, dysregulation of pro-inflammatory cytokines and the accumulation of free radicals as outlined in section 1.1. Suppression of adipogenesis by curcumin leading to weight loss contributes to a decrease in this chronic inflammation (Chuengsamarn et al., 2014). This loss of body fat in mice has been shown to reduce the number of adipose-associated macrophages that secrete inflammatory cytokines (Chuengsamarn et al., 2014). In addition to this, the ability of curcumin to act as an antioxidant and to target both adipokines and macrophages has been thoroughly documented (Chuengsamarn et al., 2014; Gao et al., 2015; Kirschweng et al., 2015; Maithilikarpagaselvi et al., 2016; Weisberg et al., 2008).

Macrophage activation in adipose tissue is responsible for a major portion of the pro-inflammatory cytokine secretion that is up-regulated in obesity. Therefore, if curcumin is able to inhibit this occurrence, it would potentially reduce the amount of chronic inflammation in obesity. Macrophage infiltration was blocked after 4 g curcumin /kg of diet was administered to high-fat diet-fed mice (Shao et al., 2012). This observation was supported by a similar study in mice, where curcumin was given at 3% of the diet (Weisberg et al., 2008). After staining the perigonadal WAT with macrophage-specific stains, significantly less macrophage infiltration was observed after curcumin supplementation (Shao et al., 2012; Weisberg et al., 2008). This, in turn, would reduce the secretion of TNF-α, and subsequently decrease the development of insulin resistance and incidence of lipolysis which was demonstrated in these studies (Shao et al., 2012; Weisberg et al., 2008). Whilst this has not yet been observed in human studies, the fact that it occurred in two separate mouse studies is encouraging.

The importance of adiponectin in maintaining insulin sensitivity and glucose homeostasis was acknowledged in section 1.1. In recent years, the ability of curcumin to elevate the levels of

adiponectin has been shown in multiple studies (Chuengsamarn et al., 2014; Maithilikarpagaselvi et al., 2016; Panahi et al., 2016). A study in a model of high-fructose diet-fed rats that were treated with curcumin reported an increase in adiponectin levels that almost doubled that of the fructose-fed control group (Maithilikarpagaselvi et al., 2016). This is supported by two human trials. A trial of curcumin was conducted in patients with type 2 diabetes over a six-month period and they reported a steady increase in adiponectin levels in response to curcumin treatment (Chuengsamarn et al., 2014). The other clinical trial was conducted in patients with metabolic syndrome, where increases in adiponectin levels from 12 ng/ml to 21 ng/ml were reported (Panahi et al., 2016). In this trial (Panahi et al., 2016), curcumin intervention only occurred for a period of eight weeks, whereas there was an increase in adiponectin levels that was sustained over another six-month study (Chuengsamarn et al., 2014). This suggests that a further increase in adiponectin levels was feasible if the period of curcumin treatment was extended.

The antioxidant ability of curcumin was briefly discussed in section 1.3, where it was outlined that the phenolic hydroxyl groups allow curcumin to scavenge free radicals. This ability was observed in both microsomes and chemical testing that were used to examine whether curcumin was able to inhibit lipid peroxidation (Ak and Gülçin, 2008; Priyadarsini et al., 2003). The efficiency with which curcumin was able to inhibit lipid peroxidation ranged from 82% to 97% (Ak and Gülçin, 2008; Priyadarsini et al., 2003). This antioxidant ability was observed in a study in carbon tetrachloride-treated rats, where there was a significant reduction in markers of lipid peroxidation compared to the control rats (Naik et al., 2011). The importance of this antioxidant ability is that it limits the role that the systemic oxidative stress has in the progression of obesity to type 2 diabetes or cardiovascular disease.

1.7 Curcumin bioavailability

Although the bioactivity of curcumin is well documented, its therapeutic effectiveness is limited by its low bioavailability due to poor absorption, rapid metabolism and rapid systemic elimination (Khalil et al., 2013). To overcome the problem of low bioavailability, a much higher dose (multiple tablets or very large tablets) is required to produce a therapeutic effect in humans (Antony et al., 2008; Baum et al., 2007). Whilst curcumin in very high doses of up to 12 g/day in humans was found to be non-toxic (Anand et al., 2007), this may result in lower patient compliance and increased expense (Cheng et al., 2001). For these reasons, recent research has focused on improving the bioavailability of curcumin.

1.8 Nanoparticles

There have been numerous attempts to increase the bioavailability of curcumin through evaluating adjuvants such as piperine (Rinwa and Kumar, 2012), curcumin analogues (Amolins et al., 2009) and various nanoformulations (Khalil et al., 2013; Kumari et al., 2010; Liu and Chang, 2011). This honours project is part of a larger study that aims to identify whether nanoparticles are effective in increasing the bioavailability of curcumin, and thus observe a biological response with a lower dose. Nanoparticles have managed to increase the bioavailability of curcumin by 55.4 fold compared to curcumin aqueous suspension (Khalil et al., 2013). This is shown in *Figure 2*, which highlights the longer sustained release of curcumin. The nanoparticles were developed by decreasing curcumin particles to nanometre size using ultrasonification and nanoencapsulating it with poly(lactide-co-glycolide) (PLGA) (Khalil et al., 2013). The PLGA functions to prevent premature degradation and improves the solubility of curcumin (Khalil et al., 2013). However, PLGA is hydrophobic in nature and has short circulation times due to rapid opsonisation and clearance (Khalil et al., 2013). In order to overcome the rapid opsonisation, PLGA nanoparticles were coated with polyethylene glycol (PEG), making the particles hydrophilic and allowing longer circulation times (Khalil et al.,

2013). These nanoparticles will be used in dosing curcumin to the rats used in the extension of this study.

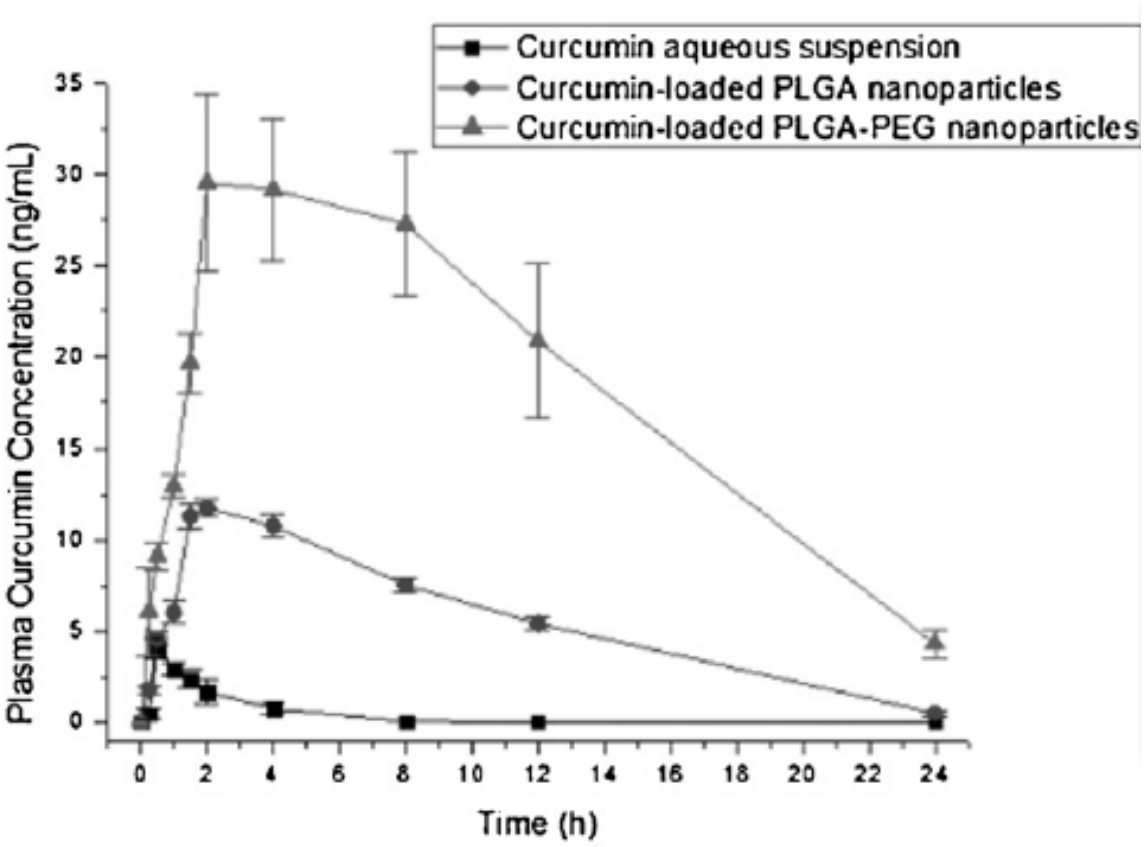


Figure 2. Comparison of vivo plasma concentrations vs. time profiles of the different curcumin formulations (Khalil et al., 2013)

1.9 Project outline

It is evident that curcumin has considerable potential for the treatment of obesity, providing the limitation of poor bioavailability can be overcome. The focus of this study is to determine whether curcumin in aqueous solution that is administered to rats with diet-induced metabolic syndrome, is able to reverse the pathological symptoms that are associated with obesity. The Functional Foods Research Group at the University of Southern Queensland has developed a rat model of obesity that allows the testing of functional foods (Panchal et al., 2011; Panchal and Brown, 2011). The pathological changes that are associated with obesity have been

mimicked in various studies, by feeding rats a high-carbohydrate, high-fat (HCHF) diet (Panchal et al., 2011). Control rats fed a corn starch (CS) diet have a lean body type and consistently showed no symptoms of metabolic syndrome. Using this animal model of diet-induced metabolic syndrome, functional foods such as Queen Garnet plum, purple carrots, coffee extract, spices such as piperine and cardamom, olive leaf extract and seaweeds have been successfully shown to exert positive health benefits in the treatment of metabolic diseases (Bhaswant et al., 2015a; Bhaswant et al., 2015b; Diwan et al., 2013; Kumar et al., 2015a; Kumar et al., 2015b; Panchal et al., 2012; Poudyal et al., 2010).

This project will be conducted over a 16-week period using this rat model of obesity. During the first 8 weeks, three groups of male Wistar rats will be fed the HCHF diet and another three groups will be fed the CS diet. The HCHF diet, during these 8 weeks, will induce an obese phenotype. After 8 weeks, the treatment phase will commence where, within each of the diet categories, one group will be given no curcumin (control), a second group will be given 5 mg/kg body weight/day curcumin, and a third group will be given 100 mg/kg body weight/day curcumin through oral gavage of aqueous suspension.

In order to assess the effectiveness of curcumin in attenuating obesity, various physiological measurements will be undertaken at 0 week, 8 weeks and 16 weeks. The differences in fat deposition will be traced between treatment groups through assessment of body composition at 8 and 16 weeks (Dual-energy X-ray Absorptiometry), and then adipose tissues will be extracted and weighed at termination of the project. An oral glucose tolerance test will be performed to identify the sensitivity of rats to glucose. Cardiovascular health will be monitored throughout the 16 weeks through systolic blood pressure measurements and Langendorff heart preparation will be used to assess diastolic stiffness. Heart and liver samples will be prepared for histopathological analysis to assess the level of tissue inflammation and possible fat

deposition. Blood samples will be collected from rats during terminal experiments to identify metabolic parameters such as blood lipids concentrations.

Evaluation of curcumin as a potential agent to reduce the effects of diet-induced obesity in rats has been conducted numerous times before (*Table 2*). However, previous studies have focused primarily on analysing one or two components of metabolic syndrome whereas my project covers a much broader area. In addition, the impact of curcumin on cardiovascular health is an area that has not been explored in a model of diet-induced obesity. The cause and effect relationships in the development of the numerous conditions that characterise metabolic syndrome are poorly understood. Therefore, it is desirable to explore curcumin's effect on a number of symptoms of metabolic syndrome.

Table 2. Similarities and differences between my study and previous studies which have assessed the potential of curcumin to reduce the effects of diet-induced obesity

	Diet-induced obesity?	Curcumin dose	Oral glucose tolerance test	Blood pressure	Cardiovascular health	Body composition	Plasma tests	Tissue inflammation	Observed improvement
My project	Y	5mg/kg/day 100mg/kg/day	Y	Y	Y	Y	Y	Y	-
(Shao et al., 2012)	Y	4g/kg diet	Y	N	N	Y – MRI scan	Y – lipid N – liver	N	Glucose tolerance Body weight Liver health
(Maithilikarpagaselvi et al., 2016)	Y	200mg/kg/day	Y	N	N	N	Y – lipid N – liver	N	Glucose tolerance Body weight Plasma lipid
(Ding et al., 2016)	Y	80mg/kg/day 40mg/kg/day	Y	N	N	Y	Y – lipid N – liver	Y – adipose and liver	Glucose tolerance Body weight Plasma lipid Liver health
(Um et al., 2013)	Y	0.15% diet	Y	N	N	N	Y – lipid Y – liver	N	Glucose tolerance Body weight Plasma lipid Liver health
(Correa et al., 2013)	N – renal failure	120mg/kg/day	N	Y	Y	N	N	N	Blood pressure Cardiovascular

Abbreviations used: Y, tested; N, not tested

1.10 Significance and objectives

Curcumin, through turmeric, has been a component of human diets for a long time. Previous research has identified the effects of curcumin in improving various health aspects as outlined in earlier sections. Due to low bioavailability, most of the health benefits have not been translated to humans.

My project will contribute to the general understanding of the role of curcumin in attenuating diet-induced obesity, and more specifically, it will assess the effects of curcumin on cardiovascular structure and function, liver structure and metabolic functions. Additionally, the results of my project will be compared with a sister-study involving nanoparticles in the delivery of curcumin.

The objectives for this project are to determine the effectiveness of low (5 mg/kg/day) and high (100 mg/kg/day) doses of curcumin in attenuating:

- Symptoms of diet-induced obesity including body weight gain, in particular the distribution and composition of body weight, tissue inflammation and abnormal fat deposition
- Cardiovascular structure and function complications associated with metabolic syndrome
- Liver structure changes associated with metabolic syndrome and NAFLD
- Metabolic complications associated with metabolic syndrome including glucose intolerance, dyslipidaemia and metabolic rate

Additionally, my project will act as a comparison point for the study of nanoparticles containing curcumin.

1.11 Hypothesis

I hypothesise that

- The low dose of curcumin (5 mg/kg/day) orally will have no effects on any of the parameters in obese rats
- The high dose of curcumin (100 mg/kg/day) orally will attenuate diet-induced obesity, changes in heart and liver structure and function, and metabolic changes induced by a HCHF diet

Chapter 2. Materials and Methods

2.1 Materials

Curcumin for this project was obtained from Acros Organics (New Jersey, USA), and was a mixture of curcumin, demothoxycurumin and bisdemethoxycurcumin, at a purity greater than 98%. Beef tallow was purchased from Carey Brothers, Warwick, QLD, Australia. Condensed milk was purchased from Coles Kearney Springs, Toowoomba, QLD, Australia. Meat-free powdered rat food was purchased from Speciality Feeds, Glen Forrest, WA, Australia. Hubble, Mendel and Wakeman salt mixture was purchased from MP Biomedicals, Seven Hills, NSW, Australia. Fructose was purchased from Tate & Lyle, Wacol, QLD, Australia. Corn starch was purchased from Agri Food Ingredients, Kew East, VIC, Australia. All laboratory chemicals were purchased from Sigma-Aldrich Australia, Castle Hill, NSW, Australia, unless otherwise specified at their first appearance in the text.

2.2 Rats, experimental groups and housing

All experimental protocols were approved by the Animal Ethics Committee of the University of Southern Queensland (Project number – 15REA008) under the guidelines of the National Health and Medical Research Council of Australia. In this project, 72 male Wistar rats (8-9 weeks old, 335-340 g) were sourced from the Animal Resource Centre, Perth, WA, Australia. These rats were 7 weeks old when they arrived and were given approximately one week to acclimatise. During the acclimatisation, rats were fed standard laboratory chow diet. Rats were then randomly distributed into six experimental groups with each containing 12 rats.

1. **C –** rats received corn starch (CS) diet for 16 weeks
2. **CC5 –** rats received CS diet for 16 weeks, and were given curcumin 5 mg/kg/day as oral gavage from week 8 to 16 of protocol

3. **CC100 -** rats received CS diet for 16 weeks, and were given curcumin 100 mg/kg/day as oral gavage from week 8 to 16 of protocol
4. **H** – rats received high-carbohydrate, high-fat (HCHF) diet for 16 weeks
5. **HC5** - rats received HCHF diet for 16 weeks, and were given curcumin 5 mg/kg/day as oral gavage from week 8 to 16 of protocol
6. **HC100** - rats received HCHF diet for 16 weeks, and were given curcumin 100 mg/kg/day as oral gavage from week 8 to 16 of protocol

This experimental protocol is outlined in *Figure 3*. All rats were housed in a temperature-controlled (21 ± 2ºC) room with an automated 12-hour light and dark cycle. All rats were housed in individual cages in the animal house at the University of Southern Queensland, Toowoomba campus and had *ad libitum* access to food and water.

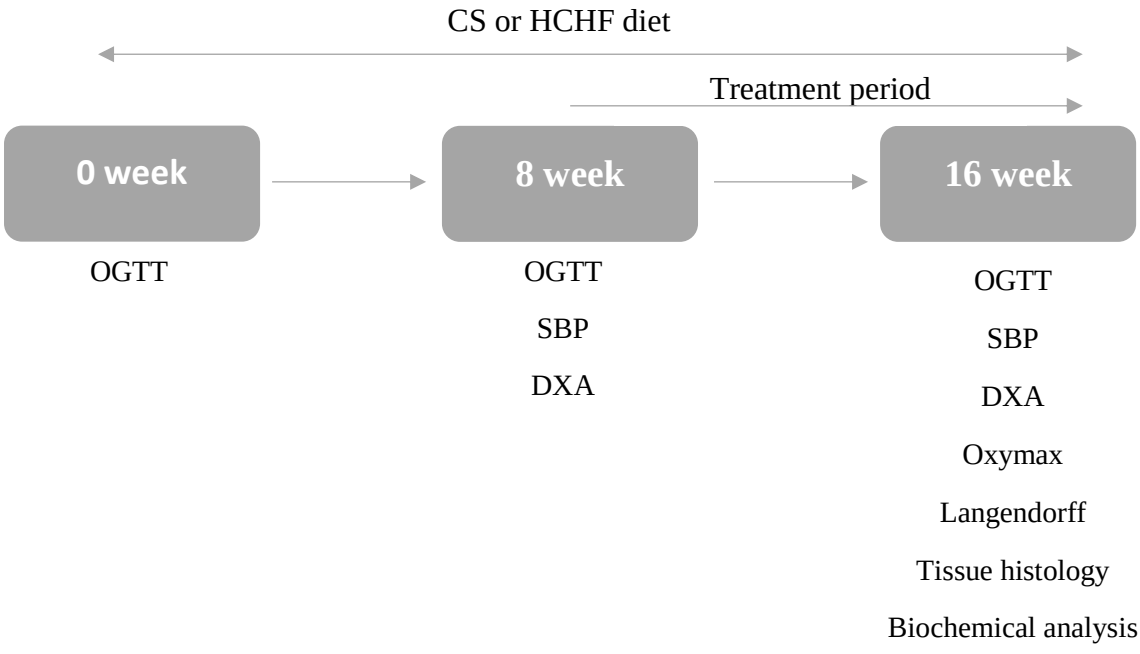


Figure 3. Timeline of the 16 week protocol.

CS, corn starch diet; HCHF, high-carbohydrate, high-fat diet; OGTT, oral glucose tolerance test; SBP, systolic blood pressure; DXA, Dual-energy X-ray Absorptiometry

2.3 Diets

The CS diet contained 570 g of corn starch, 155 g of powdered rat food, 25 g of Hubble, Mendel and Wakeman salt mixture, and 250 mL of water per kilogram of diet. The HCHF diet consisted of 175 g of fructose, 395 g of sweetened condensed milk, 200 g of beef tallow, 155 g of powdered rat food, 25 g of Hubble, Mendel and Wakeman salt mixture, and 50 mL of water per kilogram of diet. In addition, the drinking water for the HCHF group was supplemented with 25% fructose. The energy intake for each diet was calculated from the following values in kilojoules per gram; fructose, 15.40; CS, 15.94; condensed milk, 13.80; beef tallow, 37.70; and powdered rat food, 13.80. The energy densities of the CS and HCHF diets were 11.23 kJ/g and 17.83 kJ/g of food, respectively, and an additional 3.65 kJ/mL in the drinking water for the HCHF diet-fed rats.

2.4 Daily measurements

The body weight and intake of food and water for individual rats were measured every day. The daily energy intake was then calculated using the known energy densities of the CS and HCHF diets.

$$\text{CS daily energy intake (kJ)} = 11.23 \times \text{daily food intake (g)}$$

$$\text{HCHF daily energy intake (kJ)} = \left(17.83 \times \text{daily food intake (g)}\right) + (3.65 \times \text{daily water intake (ml)})$$

The feed conversion efficiency was calculated using the following formula (Panchal et al., 2011).

$$\text{Feed conversion efficiency} = \frac{\text{mean body weight gain (g)}}{\text{daily energy intake (kJ)}}$$

2.5 Curcumin intervention

Rats were treated with either 5 mg/kg/day or 100 mg/kg/day of curcumin. These doses of curcumin were given as an oral gavage of curcumin aqueous suspension. In order to generate a homogenous suspension of curcumin, the aqueous mixture of curcumin was vortexed at maximum speed for approximately 20 seconds, prior to uptake in the syringe for each rat. A 1 mL syringe with an 18 gauge plastic gavage needle was used to gavage curcumin suspension orally to rats.

2.6 Body composition measurement

The body composition of rats were measured at 8 and 16 weeks under light anaesthesia using Zoletil (tiletamine 10 mg/kg, zolazepam 10 mg/kg; Virbac, Peakhurst, NSW, Australia) intraperitoneally. Prior to sedation, the procedure room was heated to 25°C. Body composition was measured using a Norland XR-46 Dual-energy X-ray Absorptiometry (DXA) densitometer (Norland Corp., Fort Atkinson, WI, USA) and data analysed using the manufacturer's recommended software for use in laboratory animals (Small Subject Analysis Software, version 2.5.3/1.3.1; Norland Corp.).

2.7 Systolic blood pressure measurement

The systolic blood pressure (SBP) of rats were measured at 8 and 16 weeks under light anaesthesia using Zoletil (tiletamine 10 mg/kg, zolazepam 10 mg/kg; Virbac, Peakhurst, NSW, Australia) intraperitoneally. Prior to sedation, the procedure room was heated to 25°C. Blood pressure measurement was performed using an NIBP Controller, model number INI25/R (ADInstruments, Bella Vista, NSW, Australia), connected to a PowerLab data acquisition unit (ADInstruments). The machine was calibrated and then the MLT125/R tail cuff (ADInstruments) was slipped over the tail of the sedated rat and the MLT125/R pulse transducer (ADInstruments) placed just posterior to the cuff on the tail. Occlusion was initiated

and blood pressure readings taken once a stable pulse was detected on LabChart Pro (ADInstruments). Five to six SBP readings were taken for each rat, and then the average for each rat was used to calculate the group's average. After the blood pressure measurements, abdominal circumference was measured at both 8 and 16 weeks whilst the rats were under light anaesthesia. A standard measuring tape was used to measure the circumference of the abdomen in the ventral recumbent position (Panchal et al., 2011).

2.8 Oral glucose tolerance test

An oral glucose tolerance test (OGTT) was performed at 0, 8 and 16 weeks. The rats were starved 12 hours prior to testing during which time fructose-supplemented water was replaced with tap water. Overnight fasting blood glucose concentration was determined in tail vein blood using a glucose strip (Freestyle Optium Blood Glucose Test Strips, Abbott Diabetes Care Ltd., Witney, Oxon, UK) attached to a Medisense Precision Q.I.D glucometer (Abbott Laboratories, Bedford, MA). After recording this initial 0 minute blood glucose concentration, all rats were given 40% aqueous glucose solution (2 g glucose/kg body weight) via oral gavage using a 5 mL syringe and an 18 gauge plastic gavage needle. Tail vein blood samples were taken at 30, 60, 90 and 120 minutes following glucose administration (Panchal et al., 2011). After the final reading was taken, group-specific diets and water were given to the rats. The area under the curve (AUC) was calculated from the graph showing blood glucose concentrations against time.

2.9 Calorimetry

Indirect calorimetry was performed using the Oxymax system (Columbus Instruments, Columbus, USA). The initial body weight, weight of the bowl of food and water bottle was recorded. Rats were placed into metabolic cages calibrated using special purpose gas consisting of 20.8% O_2, 0.5% CO_2 and remaining N_2. Metabolic readings were taken for 14 hours,

including a 12 hour dark cycle. The final body weight, and weight of food and water bowls were then recorded. Respiratory exchange ratio (RER), heat and energy expenditure were calculated according to the manufacturer's instructions.

2.10 Terminal euthanasia and Langendorff heart preparation

Intraperitoneal injection of Lethabarb (pentobarbitone sodium, 100 mg/kg, Virbac) was used to induce terminal euthanasia. Immediately following euthanasia, heparin (200 IU) was injected into the right femoral vein to allow collection of ~6 mL of blood from the abdominal aorta after opening the abdomen. The blood was immediately collected into heparinised tubes and centrifuged at 5000 × g for 15 minutes to obtain plasma, which was then stored in five microcentrifuge tubes (for each rat) at –20°C before analysis. The heart was removed for isolated Langendorff heart preparation. Hearts isolated from euthanised rats were perfused with modified Krebs–Henseleit bicarbonate buffer, containing (in mmol/L): NaCl, 119.1; KCl, 4.75; $MgSO_4$, 1.19; KH_2PO_4, 1.19; $NaHCO_3$, 25.0; glucose, 11.0; and $CaCl_2$, 2.16. Buffer was bubbled with 95% O_2–5% CO_2 and maintained at 35°C. Isovolumetric ventricular function was measured by inserting a latex balloon catheter into the left ventricle connected to a Capto SP844 MLT844 physiological pressure transducer and Chart software on a Maclab system (ADInstruments). All left ventricular end-diastolic pressure values were measured during pacing of the heart at 250 beats/minute using an electrical stimulator. End-diastolic pressures were obtained from 0 to 30 mmHg for calculation of diastolic stiffness constant (κ, dimensionless) (Panchal et al., 2011).

2.11 Organ weights

The liver, abdominal fat (retroperitoneal, omental and epididymal), spleen, brown fat and kidneys were removed and weighed. After the isolated Langendorff heart preparation was completed, the heart was cut so that the right ventricle was separated from the left ventricle and

septum and the two parts of the heart were weighed separately. These organ weights were normalised relative to the tibial length at the time of their removal (in mg of tissue/mm of tibial length) (Panchal et al., 2011). The visceral adiposity index (VAI) was then calculated using this formula;

$$\text{VAI (\%)} = \frac{\text{retroperitoneal fat (g)} + \text{omental fat (g)} + \text{epididymal fat (g)}}{\text{body weight (g)}} \times 100$$

2.12 Histology

The liver, heart, kidney and small and large intestines from four rats in each group were isolated and then fixed in 10% neutral buffered formalin. This was performed approximately 5-7 minutes after euthanasia. Two slides were prepared per tissue specimen and two random, non-overlapping fields per slide were taken to avoid biased analysis. 3 days after fixing, the samples were processed and embedded in paraffin wax. Thin sections (5 μm) of the samples were cut and stained with haematoxylin and eosin stain for determination of inflammatory cell infiltration (20×) and liver fat vacuole presence (20×). EVOS FLC microscope (Tokyo, Japan) was used to capture images of these slides.

2.13 Biochemical analysis

The plasma samples collected during the terminal experiments were used to test for plasma concentrations of biochemical markers. Plasma concentrations of total cholesterol and triglycerides were determined using kits and controls supplied by Olympus (Japan, Tokyo) and an AU 400 Olympus analyser (Panchal et al., 2011). Non-esterified fatty acids (NEFA) were determined using a commercial kit (Wako Diagnostics, Osaka, Japan) (Panchal et al., 2011).

2.14 Statistical analysis

All data are presented as mean ± standard error of the mean (SEM). Any variance within the data was detected using Bartlett's test and variables that are not normally distributed were

transformed using log10 function prior to statistical analysis. Data from C, CC5, CC100, H, HC5 and HC100 groups were tested by two-way analysis of variance for the effects of diet, curcumin and their interaction. When the interactions and/or main effects were significant, means were compared using the Newman-Keuls multiple comparison *post hoc* test. A *P* value of <0.05 was considered as statistically significant. All statistical analyses were performed using Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, USA).

Chapter 3. Results

3.1 Physiological variables

Table 3 displays values for body weight, daily intake of food, water and energy and overall feed efficiency for all groups of rats at week 8 (pre-treatment) and week16 (post-treatment).

Initial body weights of all groups were very similar ($P = 0.23$), but after 16 weeks, the HCHF diet groups had significantly higher body weights than the CS diet groups ($P < 0.001$). Curcumin treatment at 5 and 100 mg/kg/day in both the HCHF (HC5 and HC100) and CS (CC5 and CC100) diet groups did not change body weight compared to controls at 16 weeks. Weekly body weights across the 16-week period for the three HCHF diet groups (H, HC5 and HC100) (*Figure 4A*) did not differ at any time, and neither did the body weights of the three groups on CS diet (C, CC5 and CC100). In comparison, for the majority of this 16-week period, the body weights of the HCHF diet groups were significantly higher than those of the CS diet groups ($P<0.0001$). In other words, body weight gain at week 16, as a percentage of body weight at 8 weeks (*Figure 4B*), was significantly higher for the HCHF diet groups compared with the CS diet groups ($P<0.0001$), while having no difference with the curcumin intervention at both 5 and 100 mg/kg/day doses.

Water intake during the pre-treatment 0-8 week period (*Table 3*) for the three groups on HCHF diet (H, HC5 and HC100) was significantly lower than that for the three groups on CS diet (C, CC5 and CC100) ($P<0.001$). In comparison, water intake during the treatment period (8-16 week) did not differ between the HCHF and CS diet groups. This change in water intake occurred due to the increase in water intake of the HCHF diet groups during the treatment period compared to the pre-treatment period. Food intake during the pre-treatment period (0-8 week) for the three groups on CS diet was higher than that for the HCHF diet groups ($P<0.0001$). This difference in food intake continued during the treatment period (8-16 week), where the food intake of the three CS diet groups was higher than those of the HCHF diet groups ($P<0.0001$).

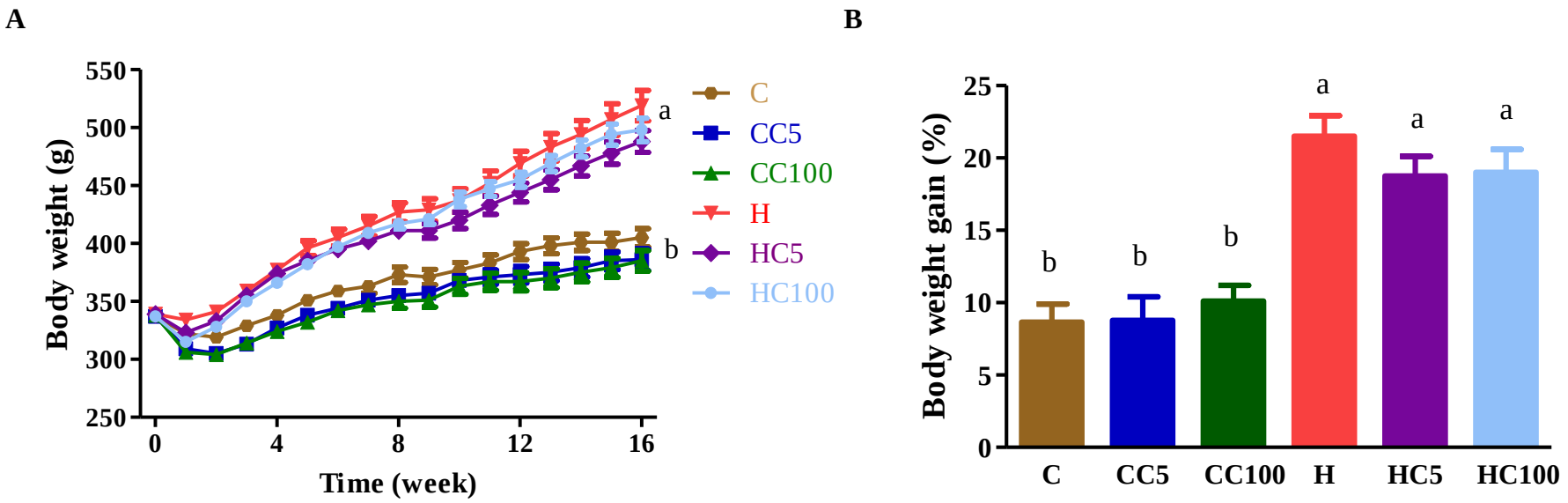
Although the food intake was lower for HCHF diet groups compared to CS diet groups, energy intake during the pre-treatment period (0-8 week) and post-treatment period (8-16 week) for the three HCHF diet groups was higher than those for the three CS diet groups ($P<0.0001$). This increased energy intake was due to the higher energy density of HCHF diet (17.83 kJ/g) compared to CS diet (11.23 kJ/g). During the pre-treatment period, HC5 and HC100 had lower energy intake compared to H rats while no difference was observed in energy intake within the CS diet groups. The difference in energy intake between H and HC100 rats was sustained during the treatment period while H and HC5 rats had no difference in energy intake during this period. Curcumin treatment did not change energy intake in CC5 and CC100 compared to C rats. Feed conversion efficiency during the pre-treatment period (0-8 week) for the three groups on HCHF diet was higher than that for the CS diet groups ($P<0.0001$), and this remained the same during the post-treatment period (8-16 weeks). During the pre-treatment period, feed conversion efficiency for the CC5 and CC100 curcumin groups was significantly lower than that for the C control group ($p<0.05$). Curcumin treatment from 8-16 weeks did not introduce any changes in the feed efficiency in either diet groups (*Table 3*).

Table 3: Body weight and daily intake of food, water and energy and overall feed efficiency

Variable	C	CC5	CC100	H	HC5	HC100	*P* Value		
							Diet	Treatment	Interaction
Initial body weight (g)	337 ± 1	337 ± 1	338 ± 1	339 ± 1	339 ± 1	337 ± 1	0.2250	0.8468	0.2306
Final body weight (g)	394 ± 7^{b}	386 ± 9^{b}	385 ± 9^{b}	510 ± 14^{a}	495 ± 10^{a}	498 ± 10^{a}	< 0.0001	0.3766	0.9275
Water intake (0-8 weeks), mL/day	33.6 ± 4.34^{a}	25.3 ± 1.44^{bc}	28.8 ± 3.20^{ab}	22.0 ± 1.41^{bc}	24.3 ± 1.23^{bc}	20.2 ± 1.11^{c}	0.0004	0.2984	0.0712
Water intake (8-16 weeks), mL/day	32.6 ± 3.92	24.9 ± 1.37	25.6 ± 1.64	29.1 ± 1.42	32.0 ± 2.05	26.6 ± 1.26	0.4777	0.1185	0.0186
Food intake (0-8 weeks), mL/day	42.6 ± 1.99^{a}	40.2 ± 1.34^{a}	40.7 ± 1.39^{a}	26.8 ± 0.760^{b}	22.7 ± 0.609^{b}	23.1 ± 0.378^{b}	< 0.0001	0.0220	0.7649
Food intake (8-16 weeks), mL/day	40.4 ± 1.29^{a}	38.7 ± 1.70^{a}	39.3 ± 1.59^{a}	27.9 ± 1.29^{b}	25.2 ± 0.779^{b}	24.3 ± 0.760^{b}	< 0.0001	0.1493	0.6399
Energy intake (0-8 weeks), kJ/day	477 ± 20.8^{b}	451 ± 15.1^{b}	458 ± 15.1^{b}	550 ± 18.1^{a}	498 ± 9.42^{b}	500 ± 7.91^{b}	< 0.0001	0.0163	0.5140
Energy intake (8-16 weeks), kJ/day	452 ± 12.0^{c}	436 ± 17.2^{c}	431 ± 15.9^{c}	596 ± 23.4^{a}	566 ± 13.3^{ab}	531 ± 15.2^{b}	< 0.0001	0.0452	0.4206
Food conversion efficiency (0-8 weeks), g/kJ	0.075 ± 0.015^{b}	0.040 ± 0.008^{c}	0.023 ± 0.013^{c}	0.160 ± 0.014^{a}	0.143 ± 0.008^{a}	0.158 ± 0.008^{a}	< 0.0001	0.0272	0.0809
Food conversion efficiency (8-16 weeks), g/kJ	0.072 ± 0.011^{b}	0.072 ± 0.013^{b}	0.081 ± 0.008^{b}	0.154 ± 0.012^{a}	0.154 ± 0.010^{a}	0.151 ± 0.011^{a}	< 0.0001	0.9531	0.8256

Values are mean ± SEM, n = 8–12. Means in a row with unlike superscripts differ, $P < 0.05$.

C, corn starch diet-fed rats; **CC5**, corn starch diet-fed rats supplemented with 5 mg/kg/day curcumin; **CC100**, corn starch diet-fed rats supplemented with 100 mg/kg/day curcumin; **H**, high-carbohydrate, high-fat diet fed rats; **HC5,** high-carbohydrate, high-fat diet fed rats supplemented with 5 mg/kg/day curcumin; **HC100**, high-carbohydrate, high-fat diet fed rats supplemented with 100 mg/kg/day curcumin.



	P Value		
Figure	Diet	Treatment	Interaction
A	< 0.0001	0.3766	0.9275
B	< 0.0001	0.6812	0.3859

Figure 4. Effects of curcumin on body weight (A) and body weight gain from 8-16 weeks as a percentage of body weight at 8 weeks (B).

Values are mean ± SEM, n = 8–12. Means without a common letter differ, $P < 0.05$.

C, corn starch diet-fed rats; **CC5**, corn starch diet-fed rats supplemented with 5 mg/kg/day curcumin; **CC100**, corn starch diet-fed rats supplemented with 100 mg/kg/day curcumin; **H**, high-carbohydrate, high-fat diet fed rats; **HC5,** high-carbohydrate, high-fat diet fed rats supplemented with 5 mg/kg/day curcumin; **HC100**, high-carbohydrate, high-fat diet-fed rats supplemented with 100 mg/kg/day curcumin

3.2 Body composition

Table 4 displays data for body composition, at the end of the pre-treatment period at 8 weeks and at the end of the treatment period at 16 weeks, for all groups of rats. At both 8 and 16 weeks, the bone mineral content (BMC) for the three HCHF diet groups was higher than those for the three CS diet groups ($P < 0.001$). Figure 5A displays the change in bone mineral content (BMC) between 8 and 16 weeks. Within the HCHF diet groups, the change in BMC for the HC100 rats was significantly higher than that for the H rats ($P < 0.05$), and while the change in BMC for the HC5 rats was also higher than that for the H rats, the difference was not significant. In comparison, differences in BMC within the three CS diet groups were not significant. The bone mineral density (BMD) did not differ between all six groups of rats at both 8 and 16 weeks.

Total fat mass at 8 weeks (Table 4) for the three HCHF diet groups was significantly higher than those for the three CS diet groups ($P<0.0001$). While there were no significant differences in total fat mass between the HCHF diet groups, the total fat mass at 8 weeks for the CC5 and CC100 groups were significantly lower than that for the C group ($P<0.05$). At 16 weeks, the total fat mass for the three HCHF diet groups was again higher than those for the three CS diet groups ($P<0.0001$), and curcumin intervention at both 5 and 100 mg/kg/day did not change total fat mass in rats (Table 4). The change in total fat mass between 8 and 16 weeks for the three HCHF diet groups (*Figure 5B*) was also greater than those for the three CS diet groups ($P<0.0001$). The change in total fat mass between 8 and 16 weeks was not different within the HCHF and CS diet groups.

In contrast to total fat mass, total lean mass for the HCHF and CS diet groups were not different, and likewise, differences between groups within each diet category were not significant (Table

4). Similarly, changes in lean mass between 8 and 16 weeks (*Figure 5C*) were not significantly different, both between and within dietary categories.

As with total fat mass, the abdominal circumference at both 8 weeks and 16 weeks for the three groups on HCHF diet was significantly higher than those for the three CS diet groups ($P<0.0001$). Differences between groups within each diet category were not significant (*Table 4*).

Visceral adiposity at 16 weeks for the HCHF diet groups was also significantly higher than those for the CS diet groups ($P<0.0001$). While visceral adiposity in both dietary categories for the curcumin groups were lower than the corresponding control, only that for the HC100 group was significantly lower than its H control group ($P<0.05$).

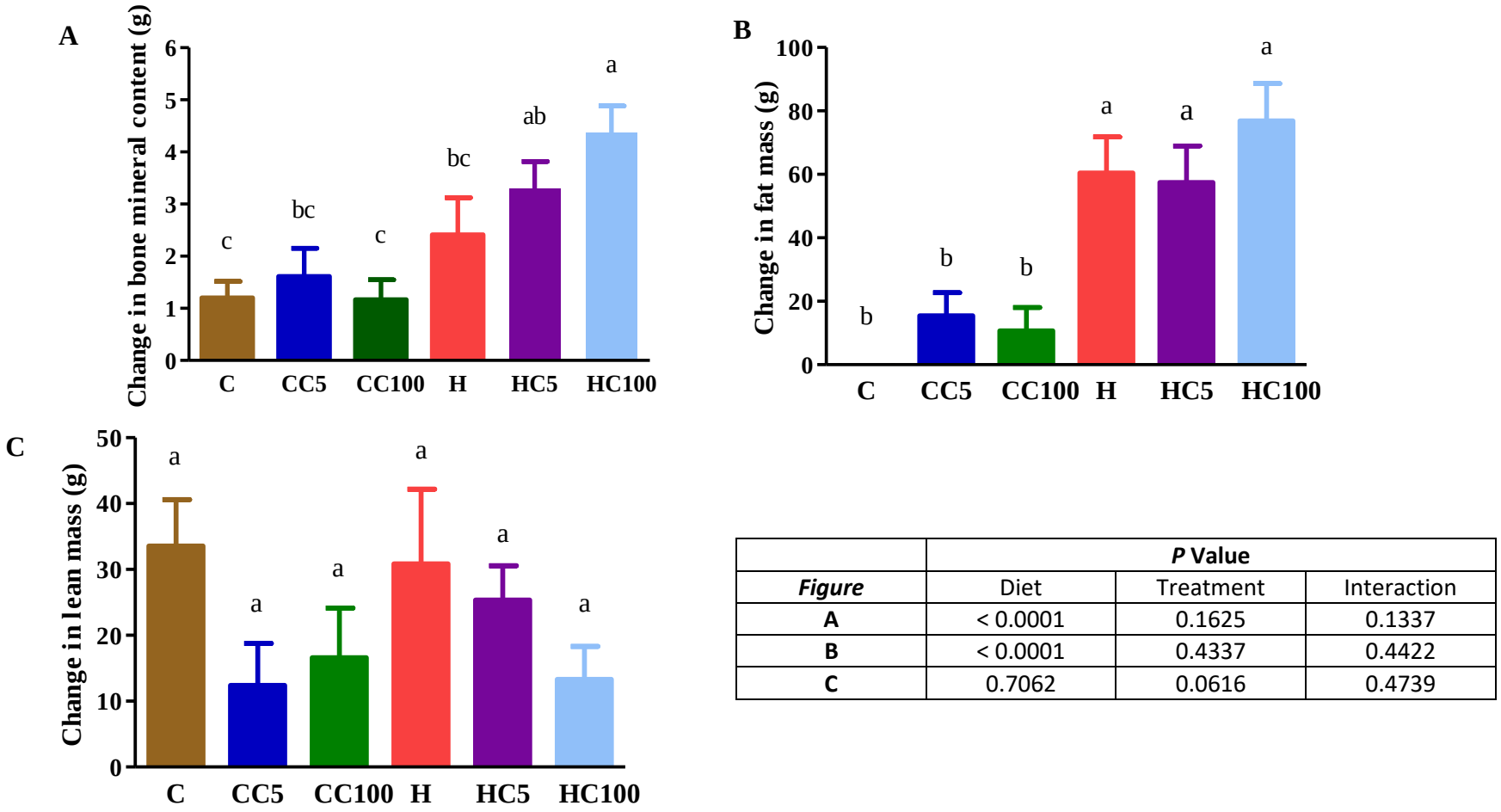
Values for tissue weights at 16 weeks, expressed in mg/mm tibial length, were also compared between and within dietary categories (Table 4). Retroperitoneal fat for the three groups on HCHF diet was significantly higher than those for the three groups on CS diet ($P<0.0001$). Also, retroperitoneal fat for the HC5 and HC100 rats were significantly lower than that for the H rats ($p<0.05$). Epididymal, omental and total abdominal fat for the three HCHF diet groups were all significantly higher than those for the three CS diet groups ($p<0.0001$), and on no occasions were differences between groups within each dietary category significant (*Table 4*).

Differences in dorsal brown fat between and within dietary categories were not significant (*Table 4*).

Table 4: Body composition at 8 and 16 weeks.

Variable	C	CC5	CC100	H	HC5	HC100	P Value		
							Diet	Treatment	Interaction
Bone mineral content (8 weeks), g	11.7 ± 0.419^{bc}	10.7 ± 0.210^{c}	10.5 ± 0.226^{c}	13.4 ± 0.584^{a}	12.8 ± 0.337^{ab}	12.8 ± 0.392^{ab}	< 0.0001	0.0430	0.7288
Bone mineral content (16 weeks), g	12.9 ± 0.359^{b}	12.0 ± 0.382^{b}	11.6 ± 0.349^{b}	15.9 ± 0.528^{a}	16.2 ± 0.588^{a}	16.9 ± 0.564^{a}	< 0.0001	0.8150	0.0774
Bone mineral density (8 weeks), g/cm^2	0.174 ± 0.00179	0.174 ± 0.00319	0.170 ± 0.00237	0.178 ± 0.00313	0.174 ± 0.00273	0.172 ± 0.00280	0.3614	0.1764	0.7553
Bone mineral density (16 weeks), g/cm^2	0.181 ± 0.00209	0.181 ± 0.00170	0.178 ± 0.00398	0.190 ± 0.00407	0.188 ± 0.00282	0.185 ± 0.00364	0.0190	0.8248	0.6393
Total fat mass at 8 weeks, g	85 ± 9.51^{b}	57 ± 3.82^{c}	55 ± 4.16^{c}	131 ± 15.8^{a}	123 ± 7.31^{a}	131 ± 9.40^{a}	< 0.0001	0.0926	0.2199
Total fat mass at 16 weeks, g	85 ± 6.66^{b}	70 ± 6.60^{b}	66 ± 5.68^{b}	178 ± 11.1^{a}	185 ± 13.8^{a}	202 ± 12.8^{a}	< 0.0001	0.7494	0.1676
Total lean mass at 8 weeks, g	262 ± 5.02	277 ± 4.52	275 ± 4.86	287 ± 9.43	272 ± 4.68	274 ± 7.76	0.1801	0.9835	0.0279
Total lean mass at 16 weeks, g	295 ± 5.31	292 ± 7.42	292 ± 7.42	315 ± 8.79	289 ± 5.87	291 ± 6.99	0.2735	0.0568	0.2486
Abdominal circumference at 8 weeks, cm	18.9 ± 0.1^{b}	18.9 ± 0.1^{b}	18.4 ± 0.2^{b}	20.3 ± 0.2^{a}	20.1 ± 0.2^{a}	20.4 ± 0.1^{a}	< 0.0001	0.5336	0.0735
Abdominal circumference at 16 weeks, cm	19.3 ± 0.1^{b}	19.4 ± 0.2^{b}	18.4 ± 0.1^{c}	22.1 ± 0.3^{a}	22.0 ± 0.3^{a}	22.1 ± 0.3^{a}	< 0.0001	0.1467	0.0891
Visceral adiposity % at 16 weeks	5.52 ± 0.26^{c}	5.36 ± 0.29^{c}	5.16 ± 0.22^{c}	8.94 ± 0.27^{a}	8.37 ± 0.34^{ab}	7.97 ± 0.29^{b}	< 0.0001	0.0673	0.5440
Tissue wet weight, mg/mm tibial length, at 16 weeks									
Retroperitoneal	242 ± 12.7^{c}	194 ± 7.77^{c}	211 ± 10.4^{c}	501 ± 21.6^{a}	414 ± 27.9^{b}	436 ± 22.0^{b}	< 0.0001	0.0013	0.5070
Epididymal	76 ± 8.05^{b}	72 ± 5.21^{b}	68 ± 7.96^{b}	178 ± 12.3^{a}	161 ± 17.3^{a}	143 ± 12.5^{a}	< 0.0001	0.1576	0.4763
Omental	137 ± 10.1^{b}	126 ± 7.28^{b}	123 ± 6.73^{b}	249 ± 14.6^{a}	226 ± 11.9^{a}	219 ± 10.9^{a}	< 0.0001	0.1088	0.7421
Total abdominal fat	455 ± 25.0^{c}	423 ± 33.7^{c}	402 ± 22.8^{c}	927 ± 35.1^{a}	801 ± 49.1^{b}	797 ± 39.1^{b}	< 0.0001	0.0071	0.4620
Dorsal brown fat	32.5 ± 1.93	27.9 ± 2.15	29.9 ± 2.67	33.1 ± 1.83	29.4 ± 1.34	30.7 ± 1.54	0.5638	0.1320	0.9735

Values are mean ± SEM, n = 12. Means in a row with superscripts without a common letter differ, $P < 0.05$. **C**, corn starch diet-fed rats; **CC5**, corn starch diet-fed rats supplemented with 5 mg/kg/day curcumin; **CC100**, corn starch diet-fed rats supplemented with 100 mg/kg/day curcumin; **H**, high-carbohydrate, high-fat diet fed rats; **HC5,** high-carbohydrate, high-fat diet fed rats supplemented with 5 mg/kg/day curcumin; **HC100**, high-carbohydrate, high-fat diet fed rats supplemented with 100 mg/kg/day.



	P Value		
Figure	Diet	Treatment	Interaction
A	< 0.0001	0.1625	0.1337
B	< 0.0001	0.4337	0.4422
C	0.7062	0.0616	0.4739

Figure 5. Effects of curcumin on changes in bone mineral content from 8 – 16 weeks (A), changes in fat mass from 8 – 16 weeks (B) and changes in lean mass from 8 – 16 weeks (C).

Values are mean ± SEM, n = 8-12. Means without a common letter differ, $P < 0.05$. **C**, corn starch diet-fed rats; **CC5**, corn starch diet-fed rats supplemented with 5 mg/kg/day curcumin; **CC100**, corn starch diet-fed rats supplemented with 100 mg/kg/day curcumin; **H**, high-carbohydrate, high-fat diet fed rats; **HC5,** high-carbohydrate, high-fat diet fed rats supplemented with 5 mg/kg/day curcumin; **HC100**, high-carbohydrate, high-fat diet-fed rats supplemented with 100 mg/kg/day curcumin.

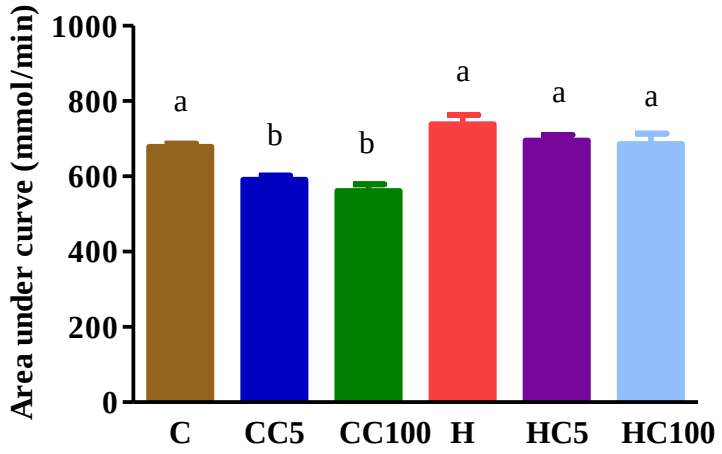
3.3 Metabolic variables

The effects of curcumin on overnight fasting glucose concentration and sensitivity to a glucose load were tested through OGTT and displayed in *Figure 6A* as the area under the curve (AUC). The AUC for the HCHF diet groups was significantly higher than that for CC5 and CC100 groups ($P < 0.0001$), but not the C group. While the AUC for HC5 and HC100 groups was slightly lower than the H group, this was not significantly different. In comparison, the AUC for both CC5 and CC100 groups was lower than the C group ($P < 0.01$).

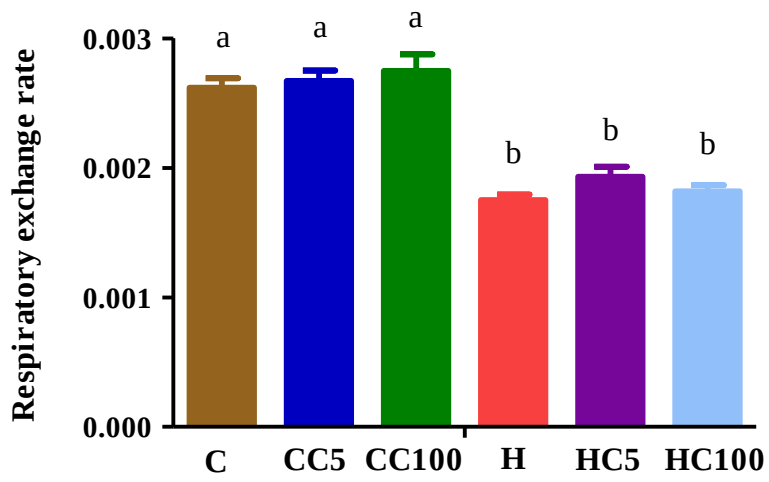
The effects of curcumin on respiratory exchange ratio (RER) was calculated through calorimetry testing, and is displayed in *Figure 6B*. The RER for CS diet groups was significantly higher than for HCHF diet groups ($P < 0.0001$), and there was no difference between treatment and control groups.

The effects of curcumin on plasma total cholesterol, triglyceride and NEFA concentrations are displayed in *Figure 7*. There was no difference in plasma concentrations of total cholesterol between all diet and treatment groups (*Figure 7A)*. However, there was a clear diet impact on plasma concentrations of triglycerides (*Figure 7B)* and NEFA (*Figure 7C)*, where HCHF diet groups were higher than the CS diet groups ($P < 0.0001$). In comparison, curcumin intervention had no effect at both 5 mg/kg/day and 100 mg/kg/day on plasma concentrations of triglycerides and NEFA.

A

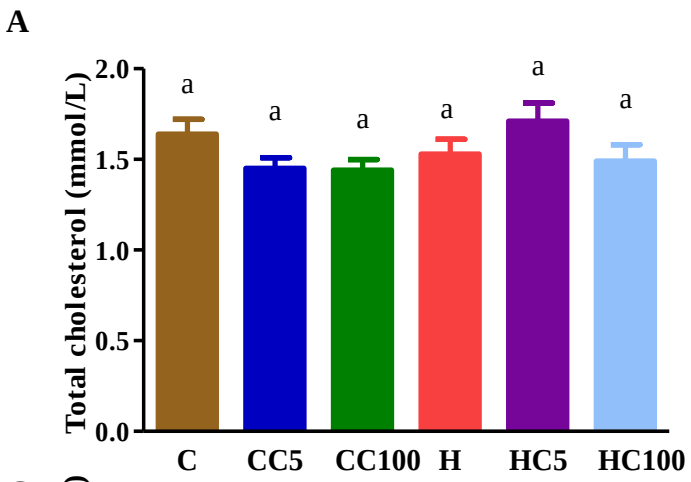


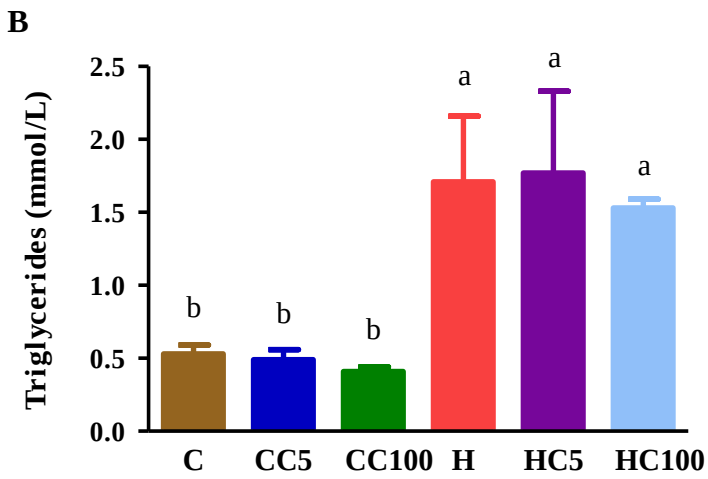
B

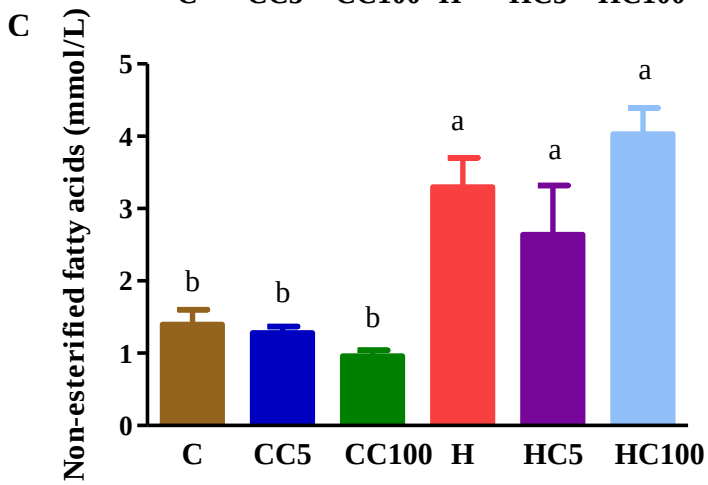


	***P* Value**		
Figure	Diet	Treatment	Interaction
A	< 0.0001	< 0.0001	0.2062
B	< 0.0001	0.2536	0.4319

Figure 6. Effects of curcumin on oral glucose tolerance at 16 weeks (A) and respiratory exchange ratio at 16 weeks (B).
Values are mean ± SEM, n = 8-12. Means without a common letter differ, $P < 0.05$. **C**, corn starch diet-fed rats; **CC5**, corn starch diet-fed rats supplemented with 5 mg/kg/day curcumin; **CC100**, corn starch diet-fed rats supplemented with 100 mg/kg/day curcumin; **H**, high-carbohydrate, high-fat diet fed rats; **HC5,** high-carbohydrate, high-fat diet fed rats supplemented with 5 mg/kg/day curcumin; **HC100**, high-carbohydrate, high-fat diet-fed rats supplemented with 100 mg/kg/day curcumin.







	P Value		
Figure	Diet	Treatment	Interaction
A	0.3151	0.2503	0.0796
B	< 0.0001	0.6891	0.9267
C	< 0.0001	0.1855	0.0181

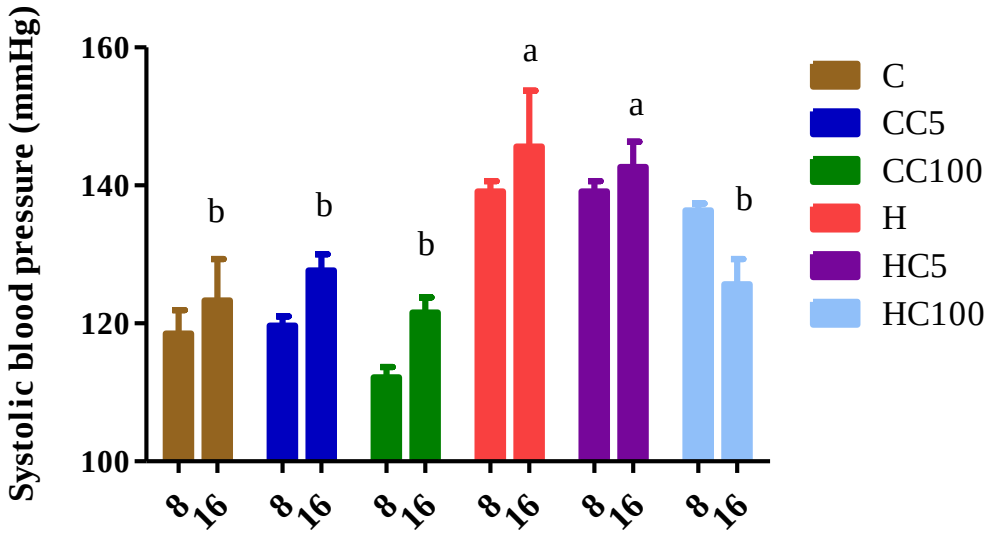
Figure 7. Effects of curcumin on plasma concentrations of total cholesterol (A), triglycerides (B) and NEFA (C).
Values are mean ± SEM, n = 12. Means without a common letter differ, $P < 0.05$. **C**, corn starch diet-fed rats; **CC5**, corn starch diet-fed rats supplemented with 5 mg/kg/day curcumin; **CC100**, corn starch diet-fed rats supplemented with 100 mg/kg/day curcumin; **H**, high-carbohydrate, high-fat diet fed rats; **HC5,** high-carbohydrate, high-fat diet fed rats supplemented with 5 mg/kg/day curcumin; **HC100**, high-carbohydrate, high-fat diet-fed rats supplemented with 100 mg/kg/day curcumin.

3.4 Cardiovascular structure and function

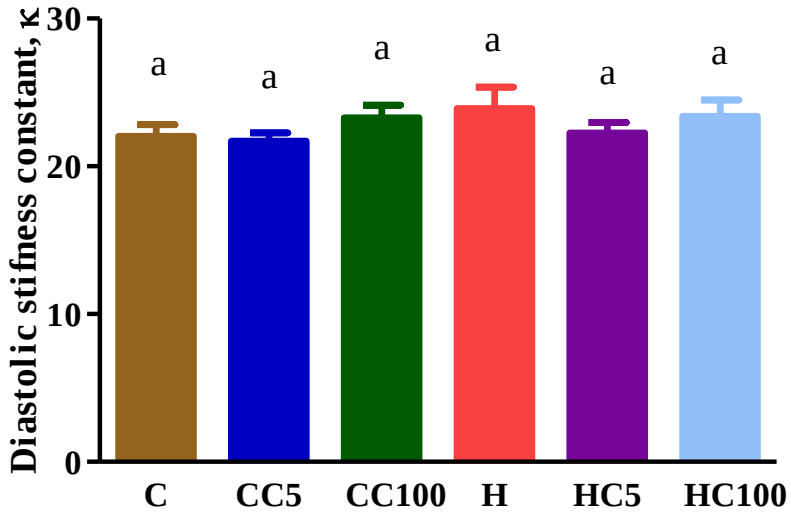
SBP was measured at 8 and 16 weeks, and this is displayed in *Figure 8A.* By the beginning of the 8th week, HCHF diet feeding induced a significantly higher SPB (approximately 140 mm/Hg) than the CS diet groups (approximately 115 mm/Hg) ($P < 0.0001$). At this time point, there was no difference between HCHF diet groups and between C and CC5. However, CC100 was lower than both C and CC5 ($P < 0.05$). At the end of the 16th week, the SBP of both H and HC5 rats had slightly increased from 8 weeks whereas the SPB of the HC100 rats had decreased. Overall, H and HC5 rats had a higher SPB than HC100, C, CC5 and CC100 rats ($P < 0.01$), where SBP in HC100, C, CC5 and CC100 rats were not different to each other.

The diastolic stiffness constant results obtained from the Langendorff heart preparation are displayed in *Figure 8B.* There was no difference in diastolic stiffness constant between all groups ($P > 0.288$). Effects of curcumin on markers of inflammation within the heart for both CS diet groups (*Figure 9*) and HCHF diet groups (*Figure 10*) is displayed using a hematoxylin and eosin stain at a magnification of 20×. There was no observable inflammatory cells within the sections of heart for CS diet groups. Whereas there are large aggregations of inflammatory cells in the hearts of both H and HC5 rats. Whilst there is greater numbers of inflammatory cells within the hearts of HC100 rats, these appears to be less than in H and HC5 rats.

A



B



	P Value		
Figure	Diet	Treatment	Interaction
A – 8 weeks	< 0.0001	0.0090	0.3455
A – 16 weeks	0.0002	0.0137	0.1056
B	0.2882	0.3809	0.6444

Figure 8. Effects of curcumin on change in systolic blood pressure from 8 – 16 weeks (A) and diastolic stiffness constant at 16 weeks (B).

Values are mean ± SEM, n = 8-12. Means without a common letter differ, $P < 0.05$. **C**, corn starch diet-fed rats; **CC5**, corn starch diet-fed rats supplemented with 5 mg/kg/day curcumin; **CC100**, corn starch diet-fed rats supplemented with 100 mg/kg/day curcumin; **H**, high-carbohydrate, high-fat diet-fed rats; **HC5,** high-carbohydrate, high-fat diet-fed rats supplemented with 5 mg/kg/day curcumin; **HC100**, high-carbohydrate, high-fat diet-fed rats supplemented with 100 mg/kg/day curcumin. Note; significant letters on Figure 8A refer to 16 week data.

A **B** **C**

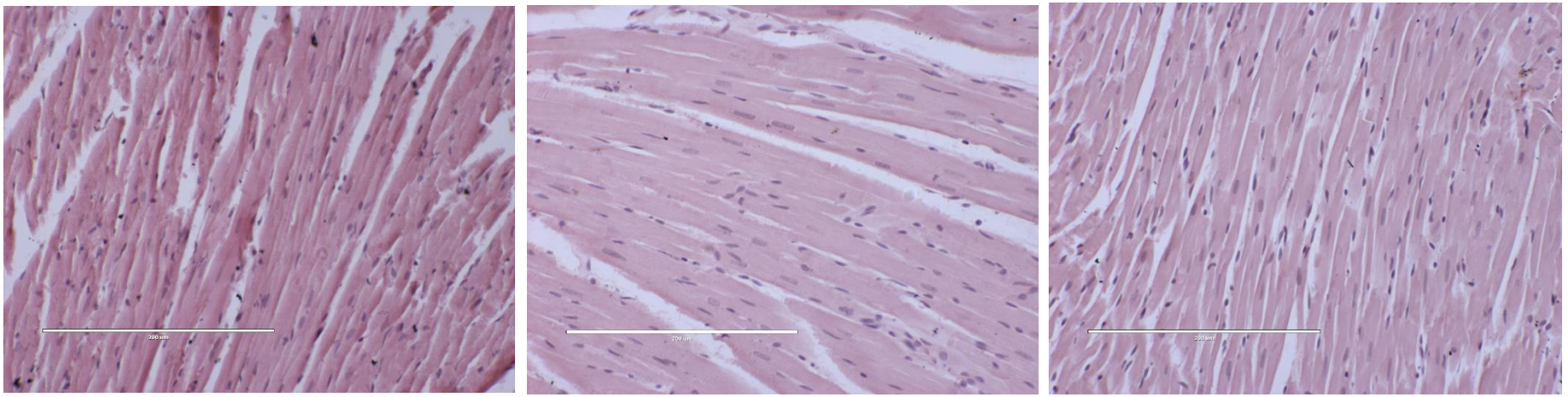


Figure 9. Effects of curcumin on inflammation in the heart of CS diet groups.
A-C represent hematoxylin and eosin staining of the heart (20×). **C**, corn starch diet-fed rats (A); **CC5**, corn starch diet-fed rats supplemented with 5 mg/kg/day curcumin (B); **CC100**, corn starch diet-fed rats supplemented with 100 mg/kg/day curcumin.

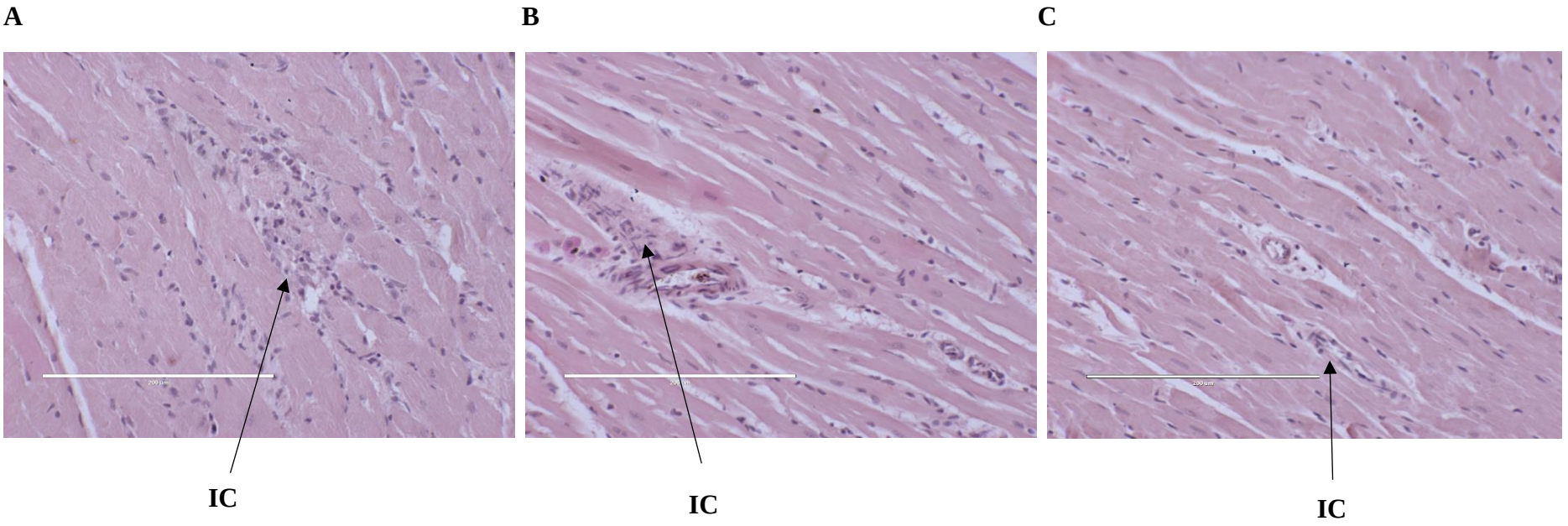


Figure 10. Effects of curcumin on inflammation in the heart of HCHF diet groups.
A-C represent hematoxylin and eosin staining of the heart showing inflammatory cells (marked as 'IC') (20×). **H**, high-carbohydrate, high-fat diet-fed rats (A); **HC5**, high-carbohydrate, high-fat diet-fed rats supplemented with 5 mg/kg/day curcumin (B); **HC100**, high-carbohydrate, high-fat diet-fed rats supplemented with 100 mg/kg/day curcumin (C).

3.5 Hepatic structure

Effects of curcumin on fat deposition and inflammation within the liver for CS diet groups (*Figure 11*) and HCHF diet groups (*Figure 12*) is displayed using a hematoxylin and eosin stain at a magnification of 20×. There appears to be minimal variations between CS diet groups. The livers of the HCHF diet groups had considerably more fat deposition than CS diet group livers. Within the HCHF diet groups, there was no observable difference between rats for both markers of fat deposition and inflammation.

A **B** **C**

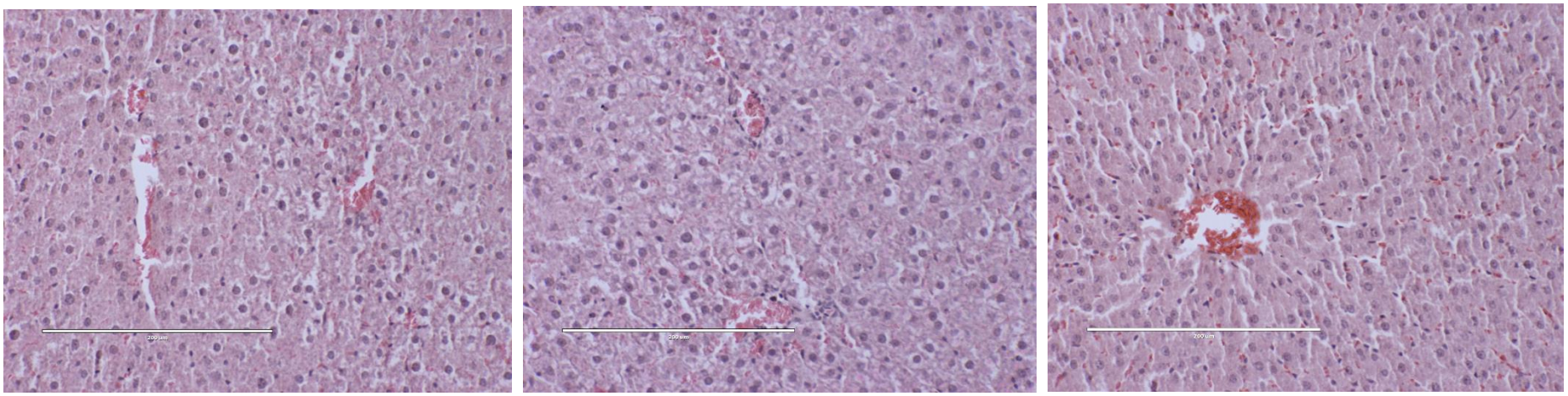


Figure 11. Effects of curcumin on fat deposition and inflammation in the liver of CS diet groups.
A-C represent hematoxylin and eosin staining of the liver showing fat deposition (marked as 'FC') and inflammatory cells (marked as 'IC') (20×). **C**, corn starch diet-fed rats (A); **CC5**, corn starch diet-fed rats supplemented with 5 mg/kg/day curcumin (B); **CC100**, corn starch diet-fed rats supplemented with 100 mg/kg/day curcumin (C).

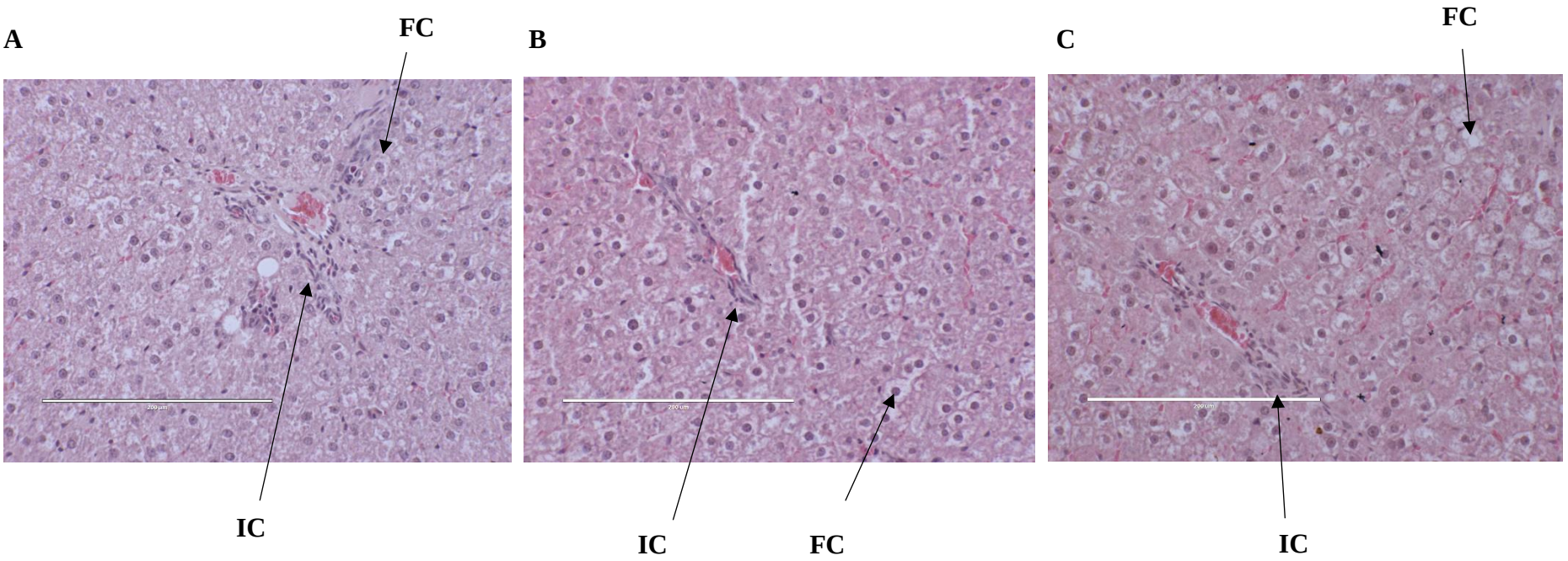


Figure 12. Effects of curcumin on fat deposition and inflammation in the liver of HCHF diet groups.
A-C represent hematoxylin and eosin staining of the liver showing fat deposition (marked as 'FC') and inflammatory cells (marked as 'IC') (20×) in **H**, high-carbohydrate, high-fat diet-fed rats (A); **HC5**, high-carbohydrate, high-fat diet-fed rats supplemented with 5 mg/kg/day curcumin (B); **HC100**, high-carbohydrate, high-fat diet-fed rats supplemented with 100 mg/kg/day curcumin (C).

Chapter 4. Discussion

The objective for this study was to determine the effectiveness of low (5 mg/kg/day) and high (100 mg/kg/day) doses of curcumin in attenuating symptoms of diet-induced metabolic syndrome in rats. The HCHF diet successfully generated a phenotype associated with diet-induced metabolic syndrome; including obesity, metabolic abnormalities, hypertension and ectopic fat deposition in the liver. The findings of this study indicate that the high dose of curcumin administered daily to rats with diet-induced metabolic syndrome was able to normalise SBP, lower visceral adiposity and reduce inflammation in the heart. Interestingly, both the low and high doses of curcumin produced no change in either body weight or total body fat content which conflicted with the majority of similar studies (Ding et al., 2016; Maithilikarpagaselvi et al., 2016; Shao et al., 2012; Um et al., 2013).

By the beginning of the 8th week, HCHF diet feeding had induced hypertension in all three of the HCHF diet groups, whereas the CS diet groups remained normotensive as in previous studies with this model (Bhaswant et al., 2015a; Panchal et al., 2011; Poudyal et al., 2010). The high dose of curcumin administered daily from 8 to 16 weeks, led to a decrease in SBP in the HC100 group. In contrast, both H and HC5 showed an increase in SBP during this period. This appears to be the first diet-induced obesity study in rats that tested if curcumin could induce a reversal of hypertension. Previous studies that tested the efficacy of curcumin in different hypertensive models, including chronic kidney disease (Correa et al., 2013) and chemically induced impairment of vascular function (Nakmareong et al., 2011), did not induce a decrease in SBP but instead led to the stabilisation of pre-existing hypertensive blood pressure in comparison to controls which continued to increase (Correa et al., 2013; Nakmareong et al., 2011).

The enhanced effect of curcumin treatment on SBP in this study compared to these other studies, despite similar dosages of curcumin (all approximately 100 mg/kg/day), could be attributed to the severity of their hypertensive models that may have limited the possibility for curcumin to significantly reduce SBP (Correa et al., 2013; Nakmareong et al., 2011). In addition, the study using the chemically induced impairment of vascular function had a much shorter treatment period (4 weeks compared to the 8 weeks of this study), and a longer time frame could have possibly led to a greater reduction in SBP (Nakmareong et al., 2011).

The mechanisms by which obesity contributes to the development of hypertension are not fully elucidated, although elevated renal sympathetic nerve activity (RSNA) has been recognised as having a major role in this process (Francischetti and Genelhu, 2007; Hall et al., 2010; Kassab et al., 1995). Elevated RSNA impairs the ability of the kidneys to excrete sodium in the urine, culminating in increased sodium reabsorption and consequently higher blood pressure to maintain sodium balance (Francischetti and Genelhu, 2007). There have been several factors implicated in this increase in RSNA including lowered adiponectin levels, visceral adiposity, elevated levels of leptin and angiotensin, endothelial dysfunction (controversial as difficult to determine whether this occurs before or after hypertension) and oxidative stress (Francischetti and Genelhu, 2007; Hall et al., 2010). The ability of curcumin to alleviate SBP in previous studies was attributed to an improved antioxidant response, decreased markers of oxidative stress and improved endothelial function (Correa et al., 2013; Nakmareong et al., 2011). This is supported by the literature, as the antioxidant capacity of curcumin is well documented (Malik and Mukherjee, 2014; Naik et al., 2011; Priyadarsini et al., 2003).

Mitigation of oxidative stress may be a factor in the reduction of SBP observed in this study, although this parameter was not measured in this study (unlike Correa et al. and Nakmareong et al.), and therefore this claim is speculative. Also, it would be incorrect to assume that this is the only mechanism occurring given the multifaceted aspects of both obesity and metabolic

syndrome. Visceral adipose tissue has been implicated as having a pathological role in the development of hypertension (Atzmon et al., 2002; Faria et al., 2002; Francischetti and Genelhu, 2007; Mathieu et al., 2009; Sironi et al., 2004), and this parameter was measured in this study. Both the low and high doses of curcumin produced a significant reduction in total abdominal fat, in particular retroperitoneal fat, compared to the control rats. A mechanism proposed in the link between visceral fat and hypertension is the upregulated secretion of angiotensin from adipose tissue depots in the visceral area and subsequent activation of the sympathetic nervous system (Atzmon et al., 2002; Ibrahim, 2010).

In a recent study, a stronger association was found with retroperitoneal fat (the fat surrounding the kidneys) and hypertension rather than total visceral fat, suggesting that there are paracrine factors involved in this mechanism (Chandra et al., 2014). This may be the reason for the greater reduction in SBP observed in the HC100 group, as they had a significantly reduced retroperitoneal fat mass. However, the lack of a similar reduction in the SBP of the HC5 rats despite their low retroperitoneal fat mass suggests that this hypothesis is not totally correct. Future analysis of adiponectin, angiotensin and leptin plasma concentrations as well as markers of oxidative stress may provide a greater understanding of the mechanism involved in the SBP reduction observed in this study.

Diastolic stiffness constant from Langendorff heart preparation showed no significant difference between groups. In previous studies, HCHF diet feeding consistently produced higher diastolic stiffness constant than CS diet controls (Bhaswant et al., 2015a; Bhaswant et al., 2015b; Diwan et al., 2013; Panchal et al., 2011). Whilst it is possible that HCHF diet feeding did not increase diastolic stiffness, it is also possible that some error contributed to this outcome. Further analysis is necessary to confirm this. Histological analysis of heart sections using a haematoxylin and eosin stain featured higher aggregations of inflammatory cells in HCHF diet groups. Infiltration of inflammatory cells into heart tissue can encourage

myocardial (muscular tissue of the heart), endothelial and fibroblast dysfunction leading to possible cell death or damage (Yndestad et al., 2006). This suggests that there is damage to the heart which may have affected heart function. There was significantly reduced numbers of inflammatory cells in the hearts of HC100 rats which in combination with normalisation of SBP suggests that there was improved cardiovascular health with 100 mg/kg/day curcumin.

Visceral obesity has not only been implicated in the development of hypertension, but also in NAFLD, type 2 diabetes and inflammation (Cancello et al., 2006; Fontana et al., 2007; Smith and Adams, 2011). The significant reduction in visceral adiposity for both curcumin doses in this study was not associated with a change in ectopic fat deposition or infiltration of inflammatory cells in the liver (Figure 10). This suggests that curcumin had no effect on these risk factors for NAFLD or type 2 diabetes (Figure 9 and 10). In previous studies, curcumin was able to inhibit or significantly reduce the deposition of fat in the liver (Ding et al., 2016; Shao et al., 2012; Um et al., 2013). This reduction in liver fat was accompanied by lowered fasting glucose and insulin concentrations and significantly decreased serum cholesterol and triglyceride concentrations (Ding et al., 2016; Shao et al., 2012; Um et al., 2013); these observations are not seen in this study.

The lack of significant change in either body weight or total fat mass after curcumin treatment in this study was also unexpected given that this was recorded in previous studies (Ding et al., 2016; Maithilikarpagaselvi et al., 2016; Shao et al., 2012; Um et al., 2013). Weekly body weights across the 16-week period for the three HCHF diet groups of rats did not differ significantly at any time, and neither did the body weights of the CS diet groups. However, the gain in body weight from 8 – 16 weeks as a percentage of total body weight at 8 weeks for both HC5 and HC100 was lower than for H, although it was not statistically significant. Interestingly, fat mass tended to increase and lean mass tended to decrease from 8 – 16 weeks for the low and high curcumin treatment groups for both diets, although not significantly.

Out of the four studies compared, oral gavage was used in two studies to deliver dosages of 80 mg/kg/day (Ding et al., 2016) and 200 mg/kg/day (Maithilikarpagaselvi et al., 2016) and the other two studies used modified diets with curcumin at 4 g/kg (Shao et al., 2012) and 0.15% diet (Um et al., 2013). The 100 mg/kg/day curcumin treatment group used in this study is comparable to the 80 mg/kg/day dose, although it is half that of the 200 mg/kg/day and is difficult to compare to the curcumin delivered in diet. Treatment length was longer in three of the previous studies (10 – 12 weeks) than the 8 weeks of this study. The division of this study into an 8 week pre-treatment period where obesity and symptoms of metabolic syndrome are developed followed by curcumin treatment in the remaining 8 weeks to attempt to reverse these symptoms is a unique aspect of this study compared to previous studies. In addition, it is likely that the HCHF diet used in this study would produce an unhealthier phenotype than the diet used in previous studies that were either a high-fat diet or a high-fructose diet (not both) (Ding et al., 2016; Maithilikarpagaselvi et al., 2016; Shao et al., 2012; Um et al., 2013).

It is therefore possible that a longer treatment period or higher dosage may have induced a reduction in body weight or total fat, although it is also possible that curcumin (in this delivery method) is unable to exert a significant effect in the animal model of obesity used in this project. This could be a factor in the other unexpected results observed in this study as there was no change in ectopic deposition of fat in the liver, glucose sensitivity and lipid concentrations after curcumin treatment. The low bioavailability of curcumin is the major limiting factor in its therapeutic use, and perhaps is responsible for the poor effectiveness of the 100 mg/kg/day dose of curcumin used in this study (Khalil et al., 2013). In addition, obesity induces damage to the gastrointestinal tract which could have compounded this problem of poor bioavailability (de La Serre et al., 2010). It is hoped that nanoparticles containing curcumin can improve the absorption and delivery of curcumin, and lead to attenuation of metabolic syndrome.

Chapter 5. Conclusions

The objective for this study was to determine the effectiveness of low and high doses of curcumin in attenuating obesity, cardiovascular complications, liver health and metabolic abnormalities. The main findings of this study were that the low dose of curcumin did not attenuate symptoms of diet-induced obesity, whereas the high dose of curcumin did attenuate features of cardiovascular disease, including hypertension and inflammation in the heart. For these reasons, the first element of the hypothesis, being the low dose of curcumin (5 mg/kg/day) orally will have no effects on any of the parameters in obese rats, is accepted. However, the second element of the hypothesis, that the high dose of curcumin (100 mg/kg/day) orally will attenuate diet-induced obesity, changes in heart and liver structure and function, and metabolic changes induced by a HCHF diet, is largely rejected. Overall, both the low and high orally-administered doses of curcumin were unable to attenuate the effects of obesity (excluding reductions in retroperitoneal fat), liver health and metabolic abnormalities, which rejected the majority of the hypothesis. This was unexpected as these symptoms of metabolic syndrome were successfully attenuated with the use of a similar dose of curcumin in previous studies of diet-induced obesity. There were multiple factors that contributed to this unexpected result, although the major factor was the poor availability of curcumin that prevented the reversal of symptoms of diet-induced metabolic syndrome.

Chapter 6. Future Research

Further testing is necessary to strengthen the validity of the health benefits of curcumin observed in this study and to greater understand the unexpected results. It would be beneficial to examine the extent to the improvement observed in cardiovascular health by curcumin intervention. A picrosirius red stain could be used to identity collagen distribution within the heart (marker of fibrosis), which would aid in determining the accuracy of the unexpected results from the Langendorff experiment. Heart tissue from this study has been stored in formalin, and therefore it is possible to conduct this experiment in future. Further, the hearts from these experiments have also been stored at –80°C, and molecular biology analysis for detecting and quantifying protein and mRNA could be used to assess markers of heart health. In addition, given the decreased level of inflammation within the heart tissue observed in HC100 rats, it could be beneficial to test for plasma concentrations of adiponectin and inflammatory biomarkers.

It still remains to be seen whether the nanoparticles containing curcumin will produce improved health benefits over curcumin, as hypothesised. There was no indication that curcumin could attenuate liver structure and function, and metabolic abnormalities associated with metabolic syndrome. If nanoparticles containing curcumin are able to produce significant improvement to symptoms of metabolic syndrome, then further analysis of plasma enzymes of liver health, Western blot analysis and microbial analysis of faecal samples will be conducted to be used as a comparison point for those results. Overall, this future sister study with curcumin nanoparticles to improve its bioavailability would certainly be able to answer if the increased bioavailability of curcumin can attenuate diet-induced metabolic syndrome.

References

Ahn, J., Lee, H., Kim, S., and Ha, T. (2010). Curcumin-induced suppression of adipogenic differentiation is accompanied by activation of Wnt/β-catenin signaling. American Journal of Physiology-Cell Physiology *298*, C1510-C1516.

Ak, T., and Gülçin, I. (2008). Antioxidant and radical scavenging properties of curcumin. Chemico-Biological Interactions *174*, 27-37.

Alberti, K., Eckel, R., Grundy, S., Zimmet, P., Cleeman, J., Donato, K., Fruchart, J., James, W., Loria, C., and Smith, S. (2009). Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation *120*, 1640-1645.

Alberti, K., Zimmet, P., and Shaw, J. (2006). Metabolic syndrome—a new world-wide definition. A consensus statement from the International Diabetes Federation. (Wiley-Blackwell), pp. 469-480.

Amolins, M., Peterson, L., and Blagg, B. (2009). Synthesis and evaluation of electron-rich curcumin analogues. Bioorganic & Medicinal Chemistry *17*, 360-367.

Anand, P., Kunnumakkara, A.B., Newman, R.A., and Aggarwal, B.B. (2007). Bioavailability of curcumin: problems and promises. Molecular Pharmacology *4*, 807-818.

Antony, B., Merina, B., Iyer, V., Judy, N., Lennertz, K., and Joyal, S. (2008). A pilot cross-over study to evaluate human oral bioavailability of BCM-95® CG (BiocurcumaxTM), a novel bioenhanced preparation of curcumin. Indian Journal of Pharmaceutical Sciences *70*, 445.

Ärnlöv, J., Ingelsson, E., Sundström, J., and Lind, L. (2010). Impact of body mass index and the metabolic syndrome on the risk of cardiovascular disease and death in middle-aged men. Circulation *121*, 230-236.

Atzmon, G., Yang, X., Muzumdar, R., Ma, X., Gabriely, I., and Barzilai, N. (2002). Differential gene expression between visceral and subcutaneous fat depots. Hormone and Metabolic Research *34*, 622-628.

Baum, L., Cheung, S., Mok, V., Lam, L., Leung, V., Hui, E., Ng, C., Chow, M., Ho, P., Lam, S., et al. (2007). Curcumin effects on blood lipid profile in a 6-month human study. Pharmacological Research *56*, 509-514.

Begum, A., Jones, M., Lim, G., Morihara, T., Kim, P., Heath, D., Rock, C., Pruitt, M., Yang, F., Hudspeth, B., et al. (2008). Curcumin structure-function, bioavailability, and efficacy in models of neuroinflammation and Alzheimer's disease. Journal of Pharmacology and Experimental Therapeutics *326*, 196-208.

Bentzon, J., Otsuka, F., Virmani, R., and Falk, E. (2014). Mechanisms of plaque formation and rupture. Circulation Research *114*, 1852-1866.

Bhaswant, M., Fanning, K., Netzel, M., Mathai, M., Panchal, S., and Brown, L. (2015a). Cyanidin 3-glucoside improves diet-induced metabolic syndrome in rats. Pharmacological Research *102*, 208-217.

Bhaswant, M., Poudyal, H., Mathai, M., Ward, L., Mouatt, P., and Brown, L. (2015b). Green and black cardamom in a diet-induced rat model of metabolic syndrome. Nutrients *7*, 7691-7707.

Brown, L., Poudyal, H., and Panchal, S. (2015). Functional foods as potential therapeutic options for metabolic syndrome. Obesity Reviews *16*, 914-941.

Cancello, R., Tordjman, J., Poitou, C., Guilhem, G., Bouillot, J., Hugol, D., Coussieu, C., Basdevant, A., Hen, A., Bedossa, P., et al. (2006). Increased infiltration of macrophages in omental adipose tissue Is associated with marked hepatic lesions in morbid human obesity. Diabetes *55*, 1554-1561.

Cannon, B., and Nedergaard, J. (2004). Brown adipose tissue: function and physiological significance. Physiological Reviews *84*, 277-359.

CDC (2015). Adult obesity facts. [Accessed March 2016].

Chandra, A., Neeland, I., Berry, J., Ayers, C., Rohatgi, A., Das, S., Khera, A., McGuire, D., de Lemos, J., and Turer, A. (2014). The relationship of body mass and fat distribution with incident hypertension: observations from the Dallas Heart Study. Journal of the American College of Cardiology *64*, 997-1002.

Cheng, A.-L., Hsu, C.-H., Lin, J.-K., Hsu, M.-M., Ho, Y.-F., Shen, T.-S., Ko, J.-Y., Lin, J.-T., Lin, B.-R., and Ming-Shiang, W. (2001). Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. Anticancer Research *21*, 2895-2900.

Choe, S., Huh, J., Hwang, I., Kim, J., and Kim, J. (2016). Adipose tissue remodeling: its role in energy metabolism and metabolic disorders. Frontiers in Endocrinology *7*, 30.

Chuengsamarn, S., Rattanamongkolgul, S., Phonrat, B., Tungtrongchitr, R., and Jirawatnotai, S. (2014). Reduction of atherogenic risk in patients with type 2 diabetes by curcuminoid extract: a randomized controlled trial. The Journal of Nutritional Biochemistry *25*, 144-150.

Correa, F., Buelna-Chontal, M., Hernández-Reséndiz, S., R. García-Niño, W., J. Roldán, F., Soto, V., Silva-Palacios, A., Amador, A., Pedraza-Chaverrí, J., Tapia, E., et al. (2013). Curcumin maintains cardiac and mitochondrial function in chronic kidney disease. Free Radical Biology and Medicine *61*, 119-129.

Crespo, J., Cayón, A., Fernández-Gil, P., Hernández-Guerra, M., Mayorga, M., Domínguez-Díez, A., Fernández-Escalante, J., and Pons-Romero, F. (2001). Gene expression of tumor necrosis factor α and TNF-receptors, p55 and p75, in nonalcoholic steatohepatitis patients. Hepatology *34*, 1158-1163.

de La Serre, C., Ellis, C., Lee, J., Hartman, A., Rutledge, J., and Raybould, H. (2010). Propensity to high-fat diet-induced obesity in rats is associated with changes in the gut microbiota and gut inflammation. American Journal of Physiology - Gastrointestinal and Liver Physiology *299*, G440-G448.

Ding, L., Li, J., Song, B., Xiao, X., Zhang, B., Qi, M., Huang, W., Yang, L., and Wang, Z. (2016). Curcumin rescues high fat diet-induced obesity and insulin sensitivity in mice through regulating SREBP pathway. Toxicology and Applied Pharmacology, 99-101.

Diwan, V., Poudyal, H., and Brown, L. (2013). Piperine attenuates cardiovascular, liver and metabolic changes in high carbohydrate, high fat-fed rats. Cell Biochemistry and Biophysics *67*, 297-304.

Ejaz, A., Wu, D., Kwan, P., and Meydani, M. (2009). Curcumin inhibits adipogenesis in 3T3-L1 adipocytes and angiogenesis and obesity in C57/BL mice. The Journal of Nutrition *139*, 919-925.

El-Sayed Moustafa, J., and Froguel, P. (2013). From obesity genetics to the future of personalized obesity therapy. Nature Reviews Endocrinology *9*, 402-413.

Encinosa, W., Bernard, D., Steiner, C., and Chen, C. (2005). Use and costs of bariatric surgery and prescription weight-loss medications. Health Affairs *24*, 1039-1046.

Expert Panel on Detection, E., and Treatment of High Blood Cholesterol in Adults (2001). Executive summary of the Third Report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). Journal of the American Medical Association *285*, 2486.

Faria, A., Ferreira, S., G, R., and Zanella, M. (2002). Impact of visceral fat on blood pressure and insulin sensitivity in hypertensive obese women. Obesity Research *10*, 1203-1206.

Faust, I., Johnson, P., Stern, J., and Hirsch, J. (1978). Diet-induced adipocyte number increase in adult rats: a new model of obesity. American Journal of Physiology - Endocrinology and Metabolism *235*, E279.

Ferguson, B., Nam, H., and Morrison, R. (2016). Curcumin inhibits 3T3-L1 preadipocyte proliferation by mechanisms involving post-transcriptional p27 regulation. Biochemistry and Biophysics Reports *5*, 16-21.

Fontana, L., Eagon, J., Trujillo, M., Scherer, P., and Klein, S. (2007). Visceral fat adipokine secretion is associated with systemic inflammation in obese humans. Diabetes *56*, 1010-1013.

Francischetti, E., and Genelhu, V. (2007). Obesity–hypertension: an ongoing pandemic. International Journal of Clinical Practice *61*, 269-280.

Freedman, D., Khan, L., Dietz, W., Srinivasan, S., and Berenson, G. (2001). Relationship of childhood obesity to coronary heart disease risk factors in adulthood: the Bogalusa Heart Study. Pediatrics *108*, 712-718.

Gao, S., Zhou, J., Liu, N., Wang, L., Gao, Q., Wu, Y., Zhao, Q., Liu, P., Wang, S., Liu, Y., et al. (2015). Curcumin induces M2 macrophage polarization by secretion IL-4 and/or IL-13. Journal of Molecular and Cellular Cardiology *85*, 131-139.

Gloy, V., Briel, M., Bhatt, D., Kashyap, S., Schauer, P., Mingrone, G., Bucher, H., and Nordmann, A. (2013). Bariatric surgery versus non-surgical treatment for obesity: a systematic review and meta-analysis of randomised controlled trials. British Medical Journal *347*, f5934.

Graziose, R., Lila, M., and Raskin, I. (2010). Merging traditional chinese medicine with modern drug discovery technologies to find novel drugs and functional foods. Current Drug Discovery Technologies *7*, 2-12.

Grundy, S., Brewer, H., Cleeman, J., Smith, S., and Lenfant, C. (2004). Definition of metabolic syndrome report of the National Heart, Lung, and Blood Institute/American Heart Association Conference on scientific issues related to definition. Circulation *109*, 433-438.

Hajer, G., van Haeften, T., and Visseren, F. (2008). Adipose tissue dysfunction in obesity, diabetes, and vascular diseases. European Heart Journal *29*, 2959-2971.

Hall, J., da Silva, A., do Carmo, J., Dubinion, J., Hamza, S., Munusamy, S., Smith, G., and Stec, D. (2010). Obesity-induced hypertension: role of sympathetic nervous system, leptin, and melanocortins. Journal of Biological Chemistry *285*, 17271-17276.

Hanson, M., Fareed, M., Argenio, S., Agunwamba, A., and Hanson, T. (2013). Coronary artery disease. Primary Care: Clinics in Office Practice *40*, 1-16.

Hsu, C., Wu, C., Huang, S., and Yen, G. (2009). Phenolic compounds rutin and o-coumaric acid ameliorate obesity induced by high-fat diet in rats. Journal of Agricultural and Food Chemistry *57*, 425-431.

Huang, P. (2009). eNOS, metabolic syndrome and cardiovascular disease. Trends in Endocrinology and Metabolism *20*, 295-302.

Ibrahim, M. (2010). Subcutaneous and visceral adipose tissue: structural and functional differences. Obesity Reviews *11*, 11-18.

Jo, J., Gavrilova, O., Pack, S., Jou, W., Mullen, S., Sumner, A., Cushman, S., and Periwal, V. (2009). Hypertrophy and/or hyperplasia: dynamics of adipose tissue growth. PLOS Computational Biology *5*, e1000324.

Kang, J., Tsuyoshi, G., Han, I., Kawada, T., Kim, Y., and Yu, R. (2010). Dietary capsaicin reduces obesity-induced insulin resistance and hepatic steatosis in obese mice fed a high-fat diet. Obesity *18*, 780-787.

Kaser, S., Moschen, A., Cayon, A., Kaser, A., Crespo, J., Pons-Romero, F., Ebenbichler, C., Patsch, J., and Tilg, H. (2005). Adiponectin and its receptors in non-alcoholic steatohepatitis. Gut *54*, 117-121.

Kassab, S., Kato, T., Wilkins, F., Chen, R., Hall, J., and Granger, J. (1995). Renal denervation attenuates the sodium retention and hypertension associated With obesity. Hypertension *25*, 893-897.

Kassi, E., Pervanidou, P., Kaltsas, G., and Chrousos, G. (2011). Metabolic syndrome: definitions and controversies. BioMed Central Medicine *9*, 1.

Khalil, N., do Nascimento, T., Casa, D., Dalmolin, L., de Mattos, A., Hoss, I., Romano, M., and Mainardes, R. (2013). Pharmacokinetics of curcumin-loaded PLGA and PLGA–PEG blend nanoparticles after oral administration in rats. Colloids and Surfaces B: Biointerfaces *101*, 353-360.

Kim, C., Le, T., Chen, C., Cheng, J., and Kim, K. (2011). Curcumin inhibits adipocyte differentiation through modulation of mitotic clonal expansion. The Journal of Nutritional Biochemistry *22*, 910-920.

Kirschweng, B., Tátraaljai, D., Földes, E., and Pukánszky, B. (2015). Efficiency of curcumin, a natural antioxidant, in the processing stabilization of PE: Concentration effects. Polymer Degradation and Stability *118*, 17-23.

Kumari, A., Yadav, S., and Yadav, S. (2010). Biodegradable polymeric nanoparticles based drug delivery systems. Colloids and Surfaces B: Biointerfaces *75*, 1-18.

Li, Z., Maglione, M., Tu, W., Mojica, W., Arterburn, D., Shugarman, L., Hilton, L., Suttorp, M., Solomon, V., and Shekelle, P. (2005). Meta-analysis: pharmacologic treatment of obesity. Annals of Internal Medicine *142,* 532-546.

Liu, C., and Chang, F. (2011). Development and characterization of eucalyptol microemulsions for topic delivery of curcumin. Chemical and Pharmaceutical Bulletin *59*, 172-178.

Lone, J., Choi, J., Kim, S., and Yun, J. (2016). Curcumin induces brown fat-like phenotype in 3T3-L1 and primary white adipocytes. The Journal of Nutritional Biochemistry *27*, 193-202.

Lusis, A. (2000). Atherosclerosis. Nature *407,* 233-241.

Maithilikarpagaselvi, N., Sridhar, M., Swaminathan, R., and Zachariah, B. (2016). Curcumin prevents inflammatory response, oxidative stress and insulin resistance in high fructose fed

male Wistar rats: Potential role of serine kinases. Chemico-Biological Interactions *244*, 187-194.

Malik, P., and Mukherjee, T. (2014). Structure-function elucidation of antioxidative and prooxidative activities of the polyphenolic compound curcumin. Chinese Journal of Biology *2014*.

Mathieu, P., Poirier, P., Pibarot, P., Lemieux, I., and Després, J. (2009). Visceral obesity. The Link Among Inflammation, Hypertension, and Cardiovascular Disease *53*, 577-584.

Maury, E., and Brichard, S. (2010). Adipokine dysregulation, adipose tissue inflammation and metabolic syndrome. Molecular and Cellular Endocrinology *314*, 1-16.

McGill, A. (2014). Causes of metabolic syndrome and obesity-related co-morbidities part 1. A composite unifying theory review of human-specific co-adaptations to brain energy consumption. Archives of Public Health *72*, 30.

Naik, S., Thakare, V., and Patil, S. (2011). Protective effect of curcumin on experimentally induced inflammation, hepatotoxicity and cardiotoxicity in rats: Evidence of its antioxidant property. Experimental and Toxicologic Pathology *63*, 419-431.

Nakmareong, S., Kukongviriyapan, U., Pakdeechote, P., Donpunha, W., Kukongviriyapan, V., Kongyingyoes, B., Sompamit, K., and Phisalaphong, C. (2011). Antioxidant and vascular protective effects of curcumin and tetrahydrocurcumin in rats with l-NAME-induced hypertension. Naunyn-Schmiedeberg's Archives of Pharmacology *383*, 519.

Ochner, C., Tsai, A., Kushner, R., and Wadden, T. (2015). Treating obesity seriously: when recommendations for lifestyle change confront biological adaptations. The Lancet Diabetes and Endocrinology *3*, 232-234.

Panahi, Y., Hosseini, M., Khalili, N., Naimi, E., Soflaei, S., Majeed, M., and Sahebkar, A. (2016). Effects of supplementation with curcumin on serum adipokine concentrations: a randomized controlled trial. Nutrition.

Panchal, S., Poudyal, H., Iyer, A., Nazer, R., Alam, A., Diwan, V., Kauter, K., Sernia, C., Campbell, F., and Ward, L. (2011). High-carbohydrate, high-fat diet–induced metabolic syndrome and cardiovascular remodeling in rats. Journal of Cardiovascular Pharmacology *57*, 611-624.

Panchal, S.K., and Brown, L. (2011). Rodent models for metabolic syndrome research. BioMed Research International *2011*.

Paschos, P., and Paletas, K. (2009). Non alcoholic fatty liver disease and metabolic syndrome. Hippokratia *13*, 9-19.

Poudyal, H., Panchal, S., and Brown, L. (2010). Comparison of purple carrot juice and β-carotene in a high-carbohydrate, high-fat diet-fed rat model of the metabolic syndrome. British Journal of Nutrition *104*, 1322-1332.

Prasad, S., Gupta, S., Tyagi, A., and Aggarwal, B. (2014). Curcumin, a component of golden spice: From bedside to bench and back. Biotechnology Advances *32*, 1053-1064.

Priyadarsini, K., Maity, D., Naik, G., Kumar, M., Unnikrishnan, M., Satav, J., and Mohan, H. (2003). Role of phenolic O-H and methylene hydrogen on the free radical reactions and antioxidant activity of curcumin. Free Radical Biology and Medicine *35*, 475-484.

Promrat, K., Kleiner, D., Niemeier, H., Jackvony, E., Kearns, M., Wands, J., Fava, J., and Wing, R. (2010). Randomized controlled trial testing the effects of weight loss on nonalcoholic steatohepatitis. Hepatology *51*, 121-129.

Rahmatullah, M., Noman, A., Hossan, S., Rashid, M., Rahman, T., Chowdhury, M., and Jahan, R. (2009). A survey of medicinal plants in two areas of Dinajpur district, Bangladesh including plants which can be used as functional foods. American Eurasian Journal of Sustainable Agriculture *3*, 862-876.

Rates, S. (2001). Plants as source of drugs. Toxicon *39*, 603-613.

Rinwa, P., and Kumar, A. (2012). Piperine potentiates the protective effects of curcumin against chronic unpredictable stress-induced cognitive impairment and oxidative damage in mice. Brain Research *1488*, 38-50.

Ritchie, S., and Connell, J. (2007). The link between abdominal obesity, metabolic syndrome and cardiovascular disease. Nutrition, Metabolism and Cardiovascular Diseases *17*, 319-326.

Sandur, S., Pandey, M., Sung, B., Ahn, K., Murakami, A., Sethi, G., Limtrakul, P., Badmaev, V., and Aggarwal, B. (2007). Curcumin, demethoxycurcumin, bisdemethoxycurcumin, tetrahydrocurcumin and turmerones differentially regulate anti-inflammatory and anti-

proliferative responses through a ROS-independent mechanism. Carcinogenesis *28*, 1765-1773.

Sattar, N., Forrest, E., and Preiss, D. (2014). Non-alcoholic fatty liver disease. British Medical Journal *349*.

Sergent, T., Vanderstraeten, J., Winand, J., Beguin, P., and Schneider, Y. (2012). Phenolic compounds and plant extracts as potential natural anti-obesity substances. Food Chemistry *135*, 68-73.

Shabalina, I., Petrovic, N., de Jong, J., Kalinovich, A., Cannon, B., and Nedergaard, J. (2013). UCP1 in brite/beige adipose tissue mitochondria Is functionally thermogenic. Cell Reports *5*, 1196-1203.

Shao, W., Yu, Z., Chiang, Y., Yang, Y., Chai, T., Foltz, W., Lu, H., Fantus, I., and Jin, T. (2012). Curcumin prevents high fat diet induced insulin resistance and obesity via attenuating lipogenesis in liver and inflammatory pathway in adipocytes. Public Library of Science One *7*, e28784.

Sironi, A., Gastaldelli, A., Mari, A., Ciociaro, D., Postano, V., Buzzigoli, E., Ghione, S., Turchi, S., Lombardi, M., and Ferrannini, E. (2004). Visceral fat in hypertension influence on insulin resistance and β-cell function. Hypertension *44*, 127-133.

Smeerdijk, J., Jovic, M., Hutchins, D., Petre, T., and Lee, J. (2015). Weighing the cost of obesity: a case for action. (PricewaterhouseCoopers International Limited).

Smith, B., and Adams, L. (2011). Non-alcoholic fatty liver disease. Critical Reviews in Clinical Laboratory Sciences *48*, 97-113.

Sun, K., Kusminski, C., and Scherer, P. (2011a). Adipose tissue remodeling and obesity. The Journal of Clinical Investigation *121*, 2094-2101.

Sun, K., Kusminski, C.M., and Scherer, P.E. (2011b). Adipose tissue remodeling and obesity. The Journal of Clinical Investigation *121*, 2094-2101.

Tankó, L., Bruun, J., Alexandersen, P., Bagger, Y., Richelsen, B., Christiansen, C., and Larsen, P. (2004). Novel associations between bioavailable estradiol and adipokines in elderly women with different phenotypes of obesity. Implications for Atherogenesis *110*, 2246-2252.

Tchernof, A., and Després, J. (2013). Pathophysiology of human visceral obesity: an update. Physiological Reviews *93*, 359-404.

Tilg, H., and Moschen, A. (2010). Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. Hepatology *52*, 1836-1846.

Trayhurn, P. (2013). Hypoxia and adipose tissue function and dysfunction in obesity. Physiological Reviews *93*, 1-21.

Um, M., Hwang, K., Ahn, J., and Ha, T. (2013). Curcumin attenuates diet-induced hepatic steatosis by activating AMP-activated protein kinase. Basic and Clinical Pharmacology and Toxicology *113*, 152-157.

Wang, S., Wang, X., Ye, Z., Xu, C., Zhang, M., Ruan, B., Wei, M., Jiang, Y., Zhang, Y., Wang, L., et al. (2015). Curcumin promotes browning of white adipose tissue in a norepinephrine-dependent way. Biochemical and Biophysical Research Communications *466*, 247-253.

Weisberg, S., Leibel, R., and Tortoriello, D. (2008). Dietary curcumin significantly improves obesity-associated inflammation and diabetes in mouse models of diabesity. Endocrinology *149*, 3549-3558.

Wentzel, J., Chatzizisis, Y., Gijsen, F., Giannoglou, G., Feldman, C., and Stone, P. (2012). Endothelial shear stress in the evolution of coronary atherosclerotic plaque and vascular remodelling: current understanding and remaining questions. Cardiovascular Research *96*, 234-243.

Westerterp-Plantenga, M., Diepvens, K., Joosen, A., Bérubé-Parent, S., and Tremblay, A. (2006). Metabolic effects of spices, teas, and caffeine. Physiology and Behavior *89*, 85-91.

WHO (2014). The top 10 causes of death. [Accessed November 2016].

WHO (2015). Obesity and overweight. [Accessed February 2016].

Wilken, R., Veena, M., Wang, M., and Srivatsan, E. (2011). Curcumin: A review of anti-cancer properties and therapeutic activity in head and neck squamous cell carcinoma. Molecular Cancer *10*, 1.

Wing, R., Lang, W., Wadden, T., Safford, M., Knowler, W., Bertoni, A., Hill, J., Brancati, F., Peters, A., and Wagenknecht, L. (2011). Benefits of modest weight loss in improving cardiovascular risk factors in overweight and obese individuals with type 2 Diabetes. Diabetes Care *34*, 1481-1486.

Wood, I., de Heredia, F., Wang, B., and Trayhurn, P. (2009). Cellular hypoxia and adipose tissue dysfunction in obesity. Proceedings of the Nutrition Society *68*, 370-377.

Yndestad, A., Damås, J., Øie, E., Ueland, T., Gullestad, L., and Aukrust, P. (2006). Systemic inflammation in heart failure–the whys and wherefores. Heart Failure Reviews *11*, 83-92.

Zhou, H., Beevers, C., and Huang, S. (2011). Targets of curcumin. Current Drug Targets *12*, 332-347.