Time-dependent reversal of diet-induced metabolic syndrome in rats switched to a corn starch diet

A dissertation submitted by:

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Abstract

Obesity is a critical component of metabolic syndrome, a group of interrelated conditions that significantly increase the risk of developing cardiovascular disease, type 2 diabetes and non–alcoholic fatty liver disease. Dietary interventions have been extensively studied in the treatment of metabolic syndrome, however, few studies have investigated the rate at which healthy dietary changes reverse the symptoms of metabolic syndrome. This study aimed to investigate the sequence of metabolic, cardiovascular and hepatic changes that occur over time in obese rats that have been switched to a healthier diet. Male Wistar rats aged 8–9 weeks were fed a high–carbohydrate, high–fat diet for 8 weeks, which was then replaced with a low–fat corn starch diet for an additional 1, 2, 4 or 8 weeks. Diet–induced changes were analysed in comparison to control rats that were fed the corn starch diet for 16 weeks and the high–carbohydrate, high–fat diet for 8 and 16 weeks. Symptoms of metabolic syndrome were evident after 8 weeks of consuming the high–carbohydrate, high–fat diet and worsened with continued consumption of this diet. Daily energy intake rapidly decreased after switching to the corn starch diet and throughout the final 8 weeks of protocol remained consistent with the rats that had never been fed the high–carbohydrate, high–fat diet. Within the first week of the diet switch, plasma triglycerides were halved and there was a significant decrease in omental fat, kidney and liver tissue weights. Diastolic stiffness, heart inflammation and liver fat deposition were markedly decreased 2 weeks after the diet switch, while systolic blood pressure was significantly reduced after 4 weeks. These improvements were maintained 8 weeks after switching to the corn starch diet, despite the rats regaining body weight within 4–6 weeks of the diet switch, and there was also a decrease in plasma non–esterified fatty acids and abdominal fat mass. Based on these results, switching from a high–calorie diet containing

large amounts of saturated fats, fructose and sucrose to a low–fat diet that was rich in complex carbohydrates alleviated the symptoms of metabolic syndrome by decreasing abdominal adiposity, which in turn reduced cardiac inflammation, improved ventricular function and lowered hepatic fat deposition.

Keywords: diet switch, low–fat diet, obesity, metabolic syndrome, cardiovascular disease, inflammation

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: **Date:**

Kaitlyn Wieden

Endorsement

Supervisor: **Date:**

Dr Sunil K Panchal

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List of Abbreviations

ALT	alanine aminotransferase
AMPK	adenosine monophosphate–activated protein kinase
AST	aspartate aminotransferase
ATP	adenosine triphosphate
BMI	body mass index
C	rats fed a corn starch diet for 16 weeks
CS	corn starch diet
DEXA	dual–energy x–ray absorptiometry
GIP	gastric inhibitory polypeptide
GLP–1	glucagon–like peptide–1
H	rats fed a high–carbohydrate, high–fat diet for 16 weeks
H8	rats fed a high–carbohydrate, high–fat diet for 8 weeks
H8C1	rats fed a high–carbohydrate, high–fat diet for 8 weeks, then switched to a corn starch diet for 1 week
H8C2	rats fed a high–carbohydrate, high–fat diet for 8 weeks, then switched to a corn starch diet for 2 weeks
H8C4	rats fed a high–carbohydrate, high–fat diet for 8 weeks, then switched to a corn starch diet for 4 weeks
H8C8	rats fed a high–carbohydrate, high–fat diet for 8 weeks, then switched to a corn starch diet for 8 weeks
HCHF	high–carbohydrate, high–fat diet
HDL–c	high density lipoprotein cholesterol
IL–6	interleukin–6
LDL–c	low density lipoprotein cholesterol

NAFLD non–alcoholic fatty liver disease

NEFA non–esterified fatty acids

OGTT oral glucose tolerance test

PGC–1α peroxisome proliferator–activated receptor–γ coactivator–1α

PPAR–γ peroxisome proliferator–activated receptor–γ

PFK phosphofructokinase

SIRT1 sirtuin 1

TNF–α tumour necrosis factor–α

UCP–1 uncoupling protein–1

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Chapter 1. Introduction and literature review

1.1 The burden of obesity

The global prevalence of obesity has almost tripled within four decades, increasing from 5% in 1975 to 13% in 2014, and is expected to reach 20% by 2025 if current trends continue (NCD Risk Factor Collaboration 2016). Although several gene variants are known to influence individual susceptibility to obesity, this rapid rise in obesity cannot be solely attributed to genetics (Swinburn et al. 2011). The increasing predominance of unhealthy lifestyles, which typically consist of prolonged physical inactivity and frequent consumption of palatable, energy–dense foods, has strongly contributed to the growing obesity epidemic in modern society (Vandevijvere et al. 2015).

Almost 5 million Australian adults are obese and an additional 6 million are classed as overweight, which equates to 63% of the adult population. High body fat is a leading preventable risk factor for disease burden in Australia, second only to tobacco smoking, and the majority of this burden is attributed to related cancers, diabetes, coronary heart disease, stroke, chronic kidney disease and osteoarthritis (Australian Institute of Health and Welfare 2016). If the rise in overweight and obesity within Australia was halted, 6% of the predicted disease burden attributable to obesity–related conditions in 2020 could be avoided. Furthermore, the projected disease burden in 2020 could be reduced by 14%, if the body mass index (BMI) of at risk individuals was decreased by 1 kg/m^2 (approximately 3kg weight loss for a person of average height) (Australian Burden of Disease Study 2017).

1.2 Metabolic syndrome

Obesity is an important component of metabolic syndrome, a combination of interrelated co–morbidities that substantially increase the risk of developing cardiovascular disease, type 2 diabetes and non-alcoholic fatty liver disease (NAFLD) (Grundy 2016; Sperling et al. 2015). Metabolic syndrome is diagnosed based on the presence of any three of the following criteria: elevated waist circumference, increased plasma triglycerides, reduced high density lipoprotein cholesterol (HDL–c), hypertension and fasting hyperglycaemia (Table 1) (Alberti et al. 2009).

Table 1-1. Consensus panel diagnostic criteria for metabolic syndrome

Any three of the following criteria:	
Elevated waist circumference	≥ 94 cm for males ≥ 80 cm for females *Note: these definitions may vary between populations*
Elevated plasma triglycerides	≥ 150 mg/dL (1.7 mmol/L) or drug treatment
Reduced high–density lipoprotein cholesterol (HDL–c)	< 40 mg/dL (1.0 mmol/L) for males < 50 mg/dL (1.3 mmol/L) for females or drug treatment
Elevated blood pressure	≥ 130 mmHg systolic and/or ≥ 85 mmHg diastolic or drug treatment
Elevated fasting glucose	≥ 100 mg/dL or drug treatment

Note: The consensus panel is a collaboration between the International Diabetes Federation; National Heart, Lung and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society and International Association for the Study of Obesity (Alberti et al. 2009).

The key traits that link metabolic syndrome to cardiovascular disease, type 2 diabetes and NAFLD are increased visceral adiposity, dyslipidaemia, hypertension and insulin resistance (Ballestri et al. 2016; Bastien et al. 2014; Lu et al. 2014b). Dyslipidaemia is an abnormal concentration of plasma lipids, including elevated LDL–c, reduced HDL–c and increased triglycerides. Dyslipidaemia and hypertension are both major risk factors for myocardial infarction and stroke, potentially fatal cardiovascular diseases that occur due to the restriction of blood flow to the heart and brain, respectively (Lu et al. 2014b). Elevated fasting blood glucose is indicative of insulin resistance, which results from impaired insulin receptor signalling and has an important role in the development of diabetes and NAFLD (Mitsuhashi et al. 2017; Ortiz-Lopez et al. 2012).

1.3 Obesity and metabolic syndrome pathophysiology

When energy intake exceeds energy expenditure, surplus nutrients are stored for use during times of scarce food availability. Unused glucose is stored within the liver and skeletal muscles as the branched polysaccharide glycogen. Once the glycogen stores are replenished, the remaining glucose is converted to lipids within the liver via the process of *de novo* lipogenesis (Lu et al. 2014a). Dietary and synthesised free fatty acids are transported through the bloodstream within lipoproteins, which vary in density depending on the ratio of lipids within the hydrophobic core and proteins within the amphiphilic outer membrane. LDL–c has a higher proportion of lipids compared to proteins, whereas HDL–c has a greater protein content and transports cholesterol from peripheral tissues to the liver (Griffin 2013; Morgantini et al. 2014).

Circulating lipoproteins are degraded by endothelial lipoprotein lipases, releasing free fatty acids that surrounding cells break down and use for energy production (Griffin 2013). Excess free fatty acids are converted to triglycerides and stored within white adipocytes, the only cell type that is adapted to store lipids without impairing function (Hajer, van Haeften & Visseren 2008). Chronic consumption of a diet rich in fats and simple carbohydrates increases lipid deposition within white adipocytes and promotes adipose tissue expansion (Hoffstedt et al. 2010). Metabolically healthy adipose tissue expands via hyperplasia, which involves stimulating the maturation of adipocyte precursors to increase cell numbers, whereas the metabolic dysfunction associated with obesity also causes adipocytes to expand via hypertrophy, which increases the cell size (Figure 1–1) (Rydén et al. 2014; Shao et al. 2018). Adipocyte hypertrophy is detrimental for cell function, as excessive lipid accumulation induces toxic effects (Hoffstedt et al. 2010; Muir et al. 2018).

Adipose tissue expansion is accompanied by angiogenesis, the formation of new blood vessels, as the larger tissue mass requires an additional supply of blood and nutrients (Gealekman et al. 2011). When adipose tissue expansion occurs more rapidly than the rate of angiogenesis, blood supply to the tissue is diminished and the adipocytes become hypoxic due to insufficient oxygen availability (Pasarica et al. 2009). Adipocytes that incur irreparable damage due to hypoxia undergo necrotic cell death, which stimulates the infiltration of macrophages that surround dying adipocytes and form “crown–like” histological structures (Murano et al. 2008). The macrophages within obese adipose tissue tend to have an inflammation–promoting M1 phenotype, whereas metabolically healthy adipose tissue contains a greater proportion of anti–inflammatory M2 macrophages (Muir et al. 2018).

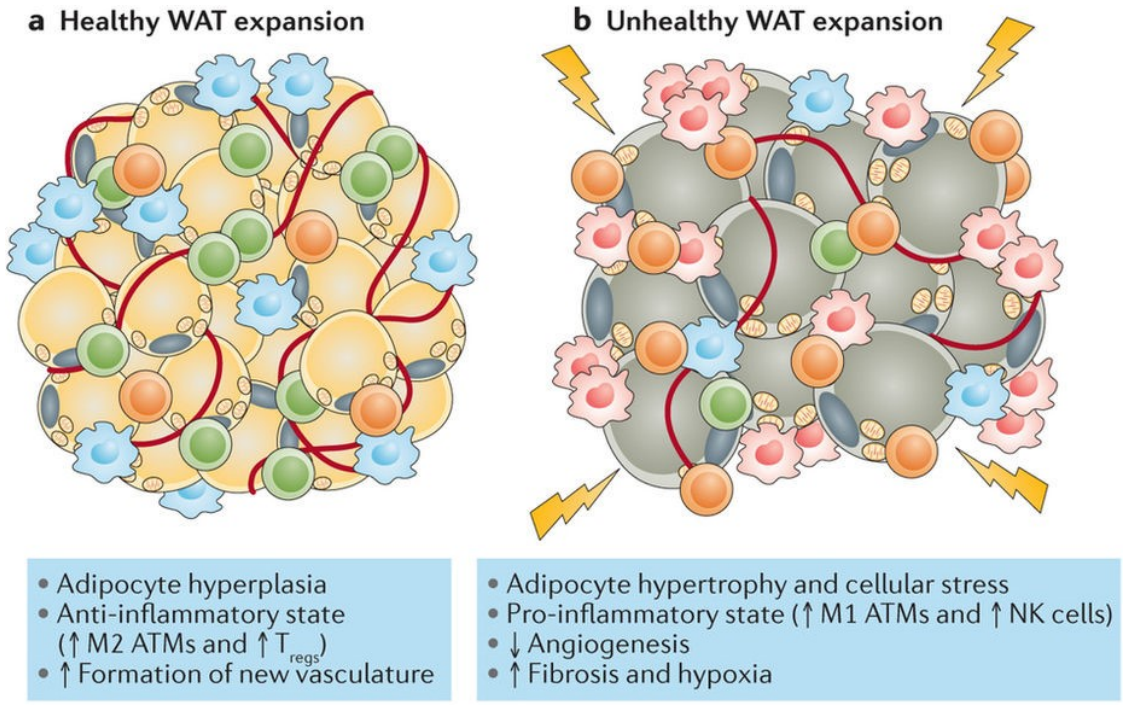

Figure 1-1. Comparison of metabolically healthy adipose tissue expansion and the dysfunctional adipose tissue expansion associated with obesity

a) Metabolically healthy adipose tissue expansion involves the maturation of preadipocytes to increase cell numbers, formation of new blood vessels to provide an adequate nutrient supply and an increase in anti–inflammatory M2 adipose tissue macrophages (ATMs) and regulatory T cells (T_{regs}). **b)** Obese adipose tissue expands via an increase in adipocyte size, which increases cell stress and promotes the infiltration of pro–inflammatory M1 ATMs and natural killer (NK) cells. Adipogenesis is also impaired by unhealthy adipose tissue expansion, which leads to hypoxia and necrotic cell death (Kusminski, Bickel & Scherer 2016, p. 645).

White adipose tissue is not merely a storage site for excess fatty acids, but is also an essential endocrine organ involved in regulating satiety, metabolism and inflammation. These effects are mediated by adipokines, the bioactive proteins produced by adipocytes and adipose tissue macrophages. The adipokine–dependent regulation of satiety is primarily facilitated by leptin, which targets the hypothalamus to suppress appetite and reduce food intake (Ouchi et al. 2011). Although leptin secretion increases in proportion to adipose tissue mass, obese individuals can become resistant to the anorexigenic effects of leptin. Leptin resistance is attributed to reduced leptin availability within the brain,

resulting from diminished passage across the blood–brain barrier, and impaired leptin receptor signalling (Myers Jr et al. 2010).

Adipokine production is dysregulated in metabolically unhealthy adipose tissue, inducing a state of chronic low–grade inflammation. Increased secretion of pro–inflammatory adipokines, including leptin, tumour necrosis factor–alpha (TNF–α) and interleukin–6 (IL–6), during obesity contributes to the development of metabolic dysfunction, insulin resistance and cardiovascular disease (Ouchi et al. 2011). This systemic inflammation is further enhanced by the decreased production of adiponectin, an anti–inflammatory adipokine that improves insulin sensitivity, reduces metabolic syndrome risk factors and is protective against cardiovascular disease (Ohashi et al. 2014).

Adipokine expression also varies depending on the location of adipose tissue deposition. Although subcutaneous adipose tissue is the largest deposit within the body, the risk of developing metabolic syndrome is substantially higher among individuals with increased abdominal adiposity. This is due to the proximity between intra–abdominal adipose tissue and the visceral organs, which allows adipokines to strongly influence cardiovascular, pancreatic and liver function (Samaras et al. 2010).

1.3.1 Cardiovascular disease

Obesity doubles the risk of developing heart failure, which is attributed to an increase in cardiac output and the expansion of blood volume in proportion to body mass (Kenchaiah et al. 2002; Reddy et al. 2016). Over time, this heightened cardiac workload induces left ventricular hypertrophy, a condition that occurs when the muscle wall of the left ventricle

thickens and loses elasticity (Figure 1–2) (Mahajan, Lau & Sanders 2015). The reduced ventricular wall compliance leads to diastolic dysfunction, which may further progress to systolic dysfunction and high–output heart failure (Alpert et al. 2014; Bastien et al. 2014). Cardiac remodelling is most prominent among severely obese individuals and in the presence of obesity–related co–morbidities that are also independent risk factors for heart failure, including hypertension, obstructive sleep apnoea, coronary artery disease and diabetes (Aurigemma, de Simone & Fitzgibbons 2013; Pujante et al. 2013; Reis et al. 2014).

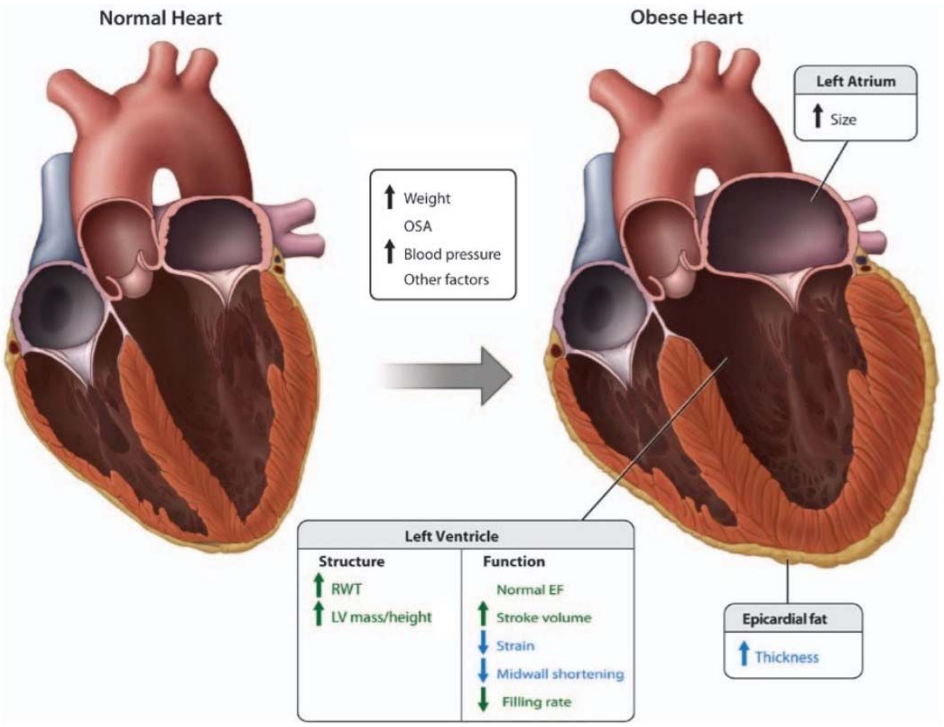

Figure 1-2. Structural remodelling of the heart due to obesity

Obesity is associated with multiple factors, such as hypertension, increased epicardial fat and obstructive sleep apnoea (OSA), that place additional burden on the heart. Left ventricular hypertrophy, which is characterised by an increase in left ventricular mass and relative wall thickness (RWT), occurs as a result of the increased cardiac workload and causes a decrease in ventricular filling rate during diastole. Obesity has also been attributed to left atrial enlargement (Aurigemma, de Simone & Fitzgibbons 2013, p. 148).

1.4 Current obesity treatments and limitations

- Obesity–related co–morbidities currently cost the Australian economy $8.6 billion dollars
- Drug interventions can be used in conjunction with diet changes and exercise, although often have unpleasant side effects. E.g. orlistat, a gastric lipase inhibitor, is associated with abdominal pain, faecal urgency and loose, oily stools. Three anti-obesity drugs have also been withdrawn from the market in the past decade due to serious adverse events: rimonabant failed FDA approval in the US and was withdrawn from European market in 2009 due to concerning psychiatric effects, benfluorex (available in Europe only) was withdrawn in 2009 due to potentially fatal valvular heart disease and sibutramine, which was withdrawn in multiple countries in 2010 due to increased risk of myocardial infarction and stroke in predisposed patients.
- Bariatric surgery is currently the most effective long-term treatment, although may not be a viable option in all cases due to the risk of surgical complications.
- Dietary interventions and exercise are highly effective methods for weight loss and improving overall health, although have limited long-term efficacy due to poor compliance. Weight regain is also common, which is potentially due to a prolonged decrease in metabolic rate after weight loss (Fothergill et al. 2016).
- Thus, there is an urgent need for safer and more effective obesity treatments. Understanding the physiological mechanisms involved in the dietary reversal of obesity could provide potential targets to improve long-term weight loss efficacy.

1.5 Dietary interventions in the reversal of metabolic syndrome

- Discuss previous approaches to changing diet for reversal of metabolic syndrome (include how these benefit patients that have been diagnosed with type 2 diabetes, atherogenic dyslipidaemia, hypertension and NAFLD)
- Calorie restriction, intermittent fasting, functional foods and supplements
- Calorie restriction is the reduction of energy intake without resulting in malnutrition
- Importance of macronutrients – are low-protein, -carbohydrate or -fat diets the most effective?

1.6 Project aims and hypotheses

This study aims to determine:

- If switching from a diet that is rich in simple carbohydrates and saturated fats to a healthier diet can reverse the symptoms of diet–induced metabolic syndrome.
- The sequence of metabolic, cardiovascular and hepatic changes that occur over time after switching to a healthier diet.

It is hypothesised that:

- Consumption of a high–carbohydrate, high–fat diet will induce signs of metabolic syndrome, which will be reversed after switching to a diet that is low in fat, high in complex carbohydrates and less energy dense.
- Switching to a healthier diet will progressively improve metabolic, cardiovascular and hepatic function.

Chapter 2. Methods and materials

2.1 Rats, experimental groups and housing

All experimental protocols were approved by the University of Southern Queensland Animal Ethics Committee (Project number – 17REA003) under the guidelines of the National Health and Medical Research Council of Australia. For this project, 84 male Wistar rats (8–9 weeks old, 335 ± 5 g) were purchased from the Animal Resource Centre, Murdoch, WA, Australia. After arrival, rats were fed a standard laboratory chow diet and given approximately one week to acclimatise. Rats were then randomly allocated into seven experimental groups (*n* = 12 rats/group):

1. **C** – rats received a corn starch (CS) diet for 16 weeks
2. **H** – rats received a high–carbohydrate, high–fat (HCHF) diet for 16 weeks
3. **H8** – rats received HCHF diet for 8 weeks
4. **H8C1** – rats received HCHF diet for 8 weeks, then CS diet for week 9 of protocol
5. **H8C2** – rats received HCHF diet for 8 weeks, then CS diet for weeks 9–10 of protocol
6. **H8C4** – rats received HCHF diet for 8 weeks, then CS diet for weeks 9–12 of protocol
7. **H8C8** – rats received HCHF diet for 8 weeks, then CS diet for weeks 9–16 of protocol

The experimental protocol is outlined in Figure 2–1. All rats were housed within the animal facility at the University of Southern Queensland Toowoomba campus, in a temperature-controlled (21 ± 2°C) room with an automated 12-hour light and dark cycle. The rats were provided individual cages and *ad libitum* access to food and water.

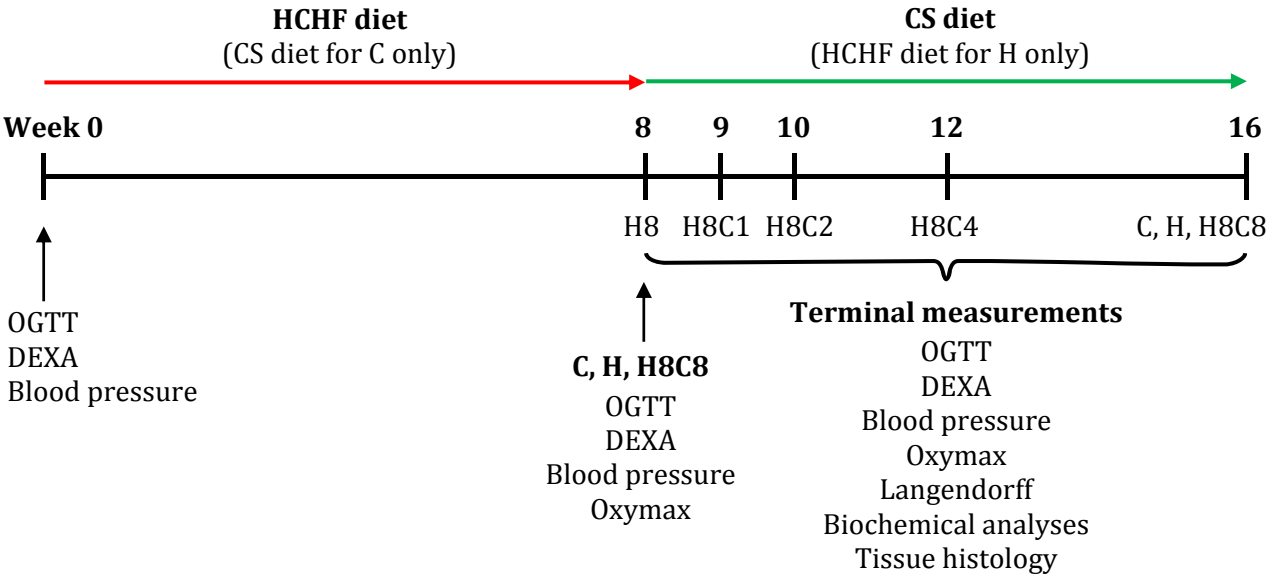

Figure 2-1. Timeline of the experimental protocol

CS, corn starch diet; **HCHF**, high-carbohydrate, high-fat diet; **C**, rats fed CS diet for 16 weeks; **H**, rats fed HCHF diet for 16 weeks; **H8**, rats fed HCHF diet for 8 weeks; **H8C1**, rats fed HCHF diet for 8 weeks then switched to CS diet for 1 week; **H8C2**, rats fed HCHF diet for 8 weeks then switched to CS diet for 2 weeks; **H8C4**, rats fed HCHF diet for 8 weeks then switched to CS diet for 4 weeks; **H8C8**, rats fed HCHF diet for 8 weeks then switched to CS diet for 8 weeks; **OGTT**, oral glucose tolerance test; **DEXA**, dual-energy x-ray absorptiometry; **SBP**, systolic blood pressure.

2.2 Materials

Beef tallow was purchased from Carey Brothers, Warwick, QLD, Australia. Condensed milk was purchased from Coles Kearneys Spring, Toowoomba, QLD, Australia. Meat-free powdered rat food was purchased from Specialty 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.

2.3 Diets

All diets were prepared at the University of Southern Queensland animal house facility. The CS diet comprised 570g corn starch, 155g powdered rat food, 25g Hubble, Mendel and Wakeman salt mixture and 250mL water per kilogram of food. The HCHF diet contained 175g fructose, 395g sweetened condensed milk, 200g beef tallow, 155g powdered rat food, 25g Hubble, Mendel and Wakeman salt mixture and 50mL water per kilogram of food. Rats fed the CS diet were provided with normal drinking water, while the HCHF diet included drinking water supplemented with 25% fructose (w/v). Energy intake was calculated from the following values in kilojoules per gram: corn starch, 15.94; powdered rat food, 13.80; fructose, 15.40; condensed milk, 13.80 and beef tallow, 37.70. The energy density of the CS diet was 11.23 kJ/g and HCHF diet was 17.83 kJ/g with an additional 3.85 kJ/mL of drinking water.

Table 2-1. Composition of the CS and HCHF diets

Components (g/kg)	CS diet	HCHF diet
Corn starch	570 g	–
Fructose	–	175 g
Condensed milk	–	395 g
Beef tallow	–	200 g
Salt mixture	25 g	25 g
Powdered rat food	155 g	155 g
Water	250 mL	50 mL
Drinking water	No additives	25% fructose (w/v)

CS, corn starch diet; **HCHF**, high-carbohydrate, high-fat diet.

2.4 Daily measurements

The body weight, water intake and food consumption of each rat was measured daily. Overall health was monitored each day and the cage bedding was changed on a regular basis. Energy intake was calculated from the energy densities of the CS and HCHF diets. Feed efficiency was calculated using the following formula:

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

2.5 Body composition measurement

Dual–energy x–ray absorptiometry (DEXA) was performed using a Norland XR-46 DEXA densitometer (Norland Corporation, Fort Atkinson, WI, USA) at the beginning and end of protocol. An additional DEXA scan was performed for C, H and H8C8 rats at week 8 of protocol. Rats were anaesthetised via an intraperitoneal injection of Zoletil (tiletamine 10 mg/kg and zolazepam 10 mg/kg; Virbac, Peakhurst, NSW, Australia). Lean mass, fat mass, bone mineral content and bone mineral density were analysed using the manufacturer's recommended software for use in laboratory animals (Small Subject Analysis Software, version 4.5.0/2.3.1, Norland Corp.) (Panchal et al. 2011). Whilst the rats were still sedated, abdominal circumference was measured in the ventral recumbent position using a standard measuring tape (Panchal et al. 2011).

2.6 Oral glucose tolerance test

An oral glucose tolerance test (OGTT) was performed before commencing and at the end of protocol. Rats were food-deprived for 12 hours and fructose–supplemented water was replaced with normal drinking water. After the overnight fast, the basal blood glucose concentration in tail vein blood was measured using a FreeStyle Lite blood glucose test strip (Abbott Diabetes Care Inc., Doncaster, VIC, Australia) attached to a FreeStyle Freedom Lite glucometer (Abbott Diabetes Care Inc.). Rats were administered a 40% aqueous glucose solution (2g glucose/kg body weight) via oral gavage. Tail vein blood samples were taken at 30, 60, 90 and 120 minutes after glucose administration (Panchal et al. 2011). After recording the 120-minute blood glucose concentrations, rats were returned to their group-specific diet and water. The area under the curve was calculated from the graph of blood glucose concentrations over time.

2.7 Calorimetry

Indirect calorimetry was performed using an Oxymax system (Columbus Instruments, Columbus, USA) at 8 weeks for C, H and H8C8 rats and for all groups prior to termination. The system was calibrated using special purpose gas comprising 78.7% nitrogen, 20.8% oxygen and 0.5% carbon dioxide. Rats were weighed and placed into individual metabolic cages and metabolic readings were measured for 12 hours during the dark cycle. Food and water intakes were recorded and rats were returned to their normal cages. Heat production and respiratory exchange ratio (RER) were calculated as outlined in the manufacturer's instructions (Sekar et al. 2017).

2.8 Systolic blood pressure measurement

Systolic blood pressure was measured at the beginning and end of protocol, with an additional measurement performed for C, H and H8C8 rats at week 8, using a MLT844 Physiological Pressure Transducer (ADInstruments, Sydney, NSW, Australia) connected to a PowerLab data acquisition unit (ADInstruments). Rats were anaesthetised using Zoletil (tiletamine 10 mg/kg and zolazepam 10 mg/kg, Virbac) administered via intraperitoneal injection. After calibrating the blood pressure machine, an inflatable tail cuff was positioned at the base of the tail and a MLT1010 Piezo–Electric Pulse Transducer (ADInstruments) was placed immediately posterior to the tail cuff. When a stable pulse was detected, occlusion was initiated and five to six blood pressure readings were recorded for each rat using LabChart Pro (ADInstruments) (Panchal et al. 2011).

2.9 Euthanasia and Langendorff heart preparation

At the end of protocol, rats were euthanised via intraperitoneal injection of Lethabarb (pentobarbitone sodium, 100 mg/kg, Virbac). Immediately after euthanasia, heparin (200 IU) was injected into the right femoral vein and approximately 6mL of abdominal aorta blood was collected into heparinised tubes. Plasma was obtained by centrifuging the blood at 5000× g for 10 minutes and stored at -20°C until biochemical analysis was performed. For Langendorff heart preparation, the isolated hearts were perfused with modified Krebs–Henseleit bicarbonate buffer, which consisted of (in mmol/L): 119.1 NaCl, 4.75 KCl, 1.19 $MgSO_4$, 1.19 KH_2PO_4, 25.0 $NaHCO_3$, 11.0 glucose and 2.16 $CaCl_2$. The buffer was bubbled with 95% O_2–5% CO_2 and maintained at 35°C. A latex balloon catheter was inserted into the left ventricle and the isovolumetric ventricular function was

measured using a Capto SP844 MLT844 physiological pressure transducer and Maclab Chart software (ADInstruments). An electrical stimulator was used to pace the heart at 250 beats/minute and left ventricular end–diastolic pressure values were obtained from 0 to 30 mmHg to calculate the diastolic stiffness constant (κ, dimensionless) (Panchal et al. 2011).

2.10 Organ weights

After the isolated Langendorff heart preparation, the right ventricle was separated from the left ventricle and septum for weighing. The liver, abdominal fat, spleen, kidneys and brown fat were removed and weighed. Organ weights were standardised in relation to tibial length (mg tissue/mm tibial length) (Panchal et al. 2011). Visceral adiposity index was calculated using the formula:

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

2.11 Histology

The heart and liver from four rats in each group were isolated and fixed in 10% neutral buffered formalin. After three days, the samples were processed and embedded in paraffin wax. Thin sections, 5 μm in size, were cut and stained with haematoxylin and eosin for determination of inflammatory cell infiltration (20×) and liver fat vacuole presence (20×) (Panchal et al. 2011). Two slides were prepared per tissue specimen and two random, non–overlapping fields were taken per slide to prevent biased analysis. Images of these slides were captured using an EVOS FLC microscope.

2.12 Biochemical analyses

Plasma samples collected during the terminal experiments were analysed to determine the concentrations of lipids and liver damage biomarkers. Plasma total cholesterol, triglycerides, non–esterified fatty acids and activities of the liver enzymes alanine transaminase and aspartate transaminase were determined using kits and controls as described previously (Panchal et al. 2011).

2.13 Statistical analysis

All data are presented as mean ± standard error of the mean (SEM). Variances within the data were detected using Bartlett's test. The effects of changing diet were evaluated using one–way analysis of variance and the Newman–Keuls multiple comparison *post hoc* test. Student's *t*–test was used to compare body weight, energy intake and feed efficiency in H8C1, H8C2, H8C4 and H8C8 groups before (0–8 weeks) and after switching to the corn starch diet. All statistical analyses were performed using Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, USA) and a *P*–value of <0.05 was considered statistically significant.

Chapter 3. Results

3.1 Physiological variables

Initially, the body weights were similar across all groups of rats (Figure 3–1). Throughout the 16–week protocol, the C rats had significantly lower body weights in comparison to the H and H8C8 rats ($P < 0.0001$) (Figure 3–1A). Despite having similar body weights at 8 weeks, the H8C8 rats had significantly lower body weights at 16 weeks compared to the H rats ($P < 0.0001$). Although the H8C1, H8C2 and H8C4 groups had higher body weights than the H8 and H8C8 groups at 8 weeks, these differences were not statistically significant ($P = 0.08$) (Figure 3–1B). Weight loss after the diet switch followed a similar trend across all groups, however the H8C8 group began to regain weight at week 12 and exceeded the body weight at week 8 within 6 weeks of changing diet.

Daily food intake was significantly greater for the C rats in comparison to the H rats ($P < 0.0001$) (Figure 3–1C). Despite having a similar food intake to the H rats during the first 8 weeks, food intake rapidly increased for the H8C8 rats after the diet switch and was similar to the C rats throughout the final 8 weeks of protocol. Daily food intake during weeks 0–8 was similar for the H8, H8C1 and H8C4 rats, whereas the H8C2 rats had a significantly greater food intake and the H8C8 rats had a significantly lower food intake in comparison to these three groups ($P = 0.04$) (Figure 3–1D). Food intake consistently increased for all rats after switching to the CS diet ($P < 0.05$), although the H8C1 group consumed significantly less food than the H8C2, H8C4 and H8C8 groups after the diet switch ($P < 0.0001$).

Despite the increased food intake, daily energy intake was significantly lower for the C rats in comparison to the H rats ($P < 0.001$) (Figure 3–1E). Daily energy intake before the diet switch was similar for the H8, H8C1 and H8C2 groups, although the H8C2 rats had a significantly greater energy intake compared to the H8C4 and H8C8 rats ($P = 0.003$) (Figure 3–1F). After changing diet, the H8C1 group had a significantly lower energy intake in comparison to the H8C2, H8C4 and H8C8 groups ($P < 0.0001$). Energy intake was also significantly lower for the H8C2 rats after the diet switch in comparison to the H8C4 rats. The daily energy intake for each group significantly decreased after switching to the CS diet, with a 41% reduction for the H8C1 rats, 34% for the H8C2 rats, 14% for the H8C4 and 19% for the H8C8 rats compared to the energy intake during the first 8 weeks ($P < 0.05$).

Daily water intake for the C rats was significantly higher over the first 8 weeks of protocol in comparison to the H and H8C8 groups provided with fructose–supplemented drinking water ($P = 0.01$) (Table 3–1). During weeks 8–16, the daily water intake increased for the H and H8C8 rats and was similar to the C group. Food conversion efficiency was significantly greater for the H and H8C8 groups than the C group during the initial 8 weeks ($P < 0.0001$). Despite a decrease after week 8, the food conversion efficiency of the H group remained higher than the C group at 16 weeks. Although the H8C8 rats had a similar food conversion efficiency to the H rats during the first 8 weeks, this decreased substantially after the diet switch and was lower than the C rats during weeks 8–16 ($P < 0.0001$).

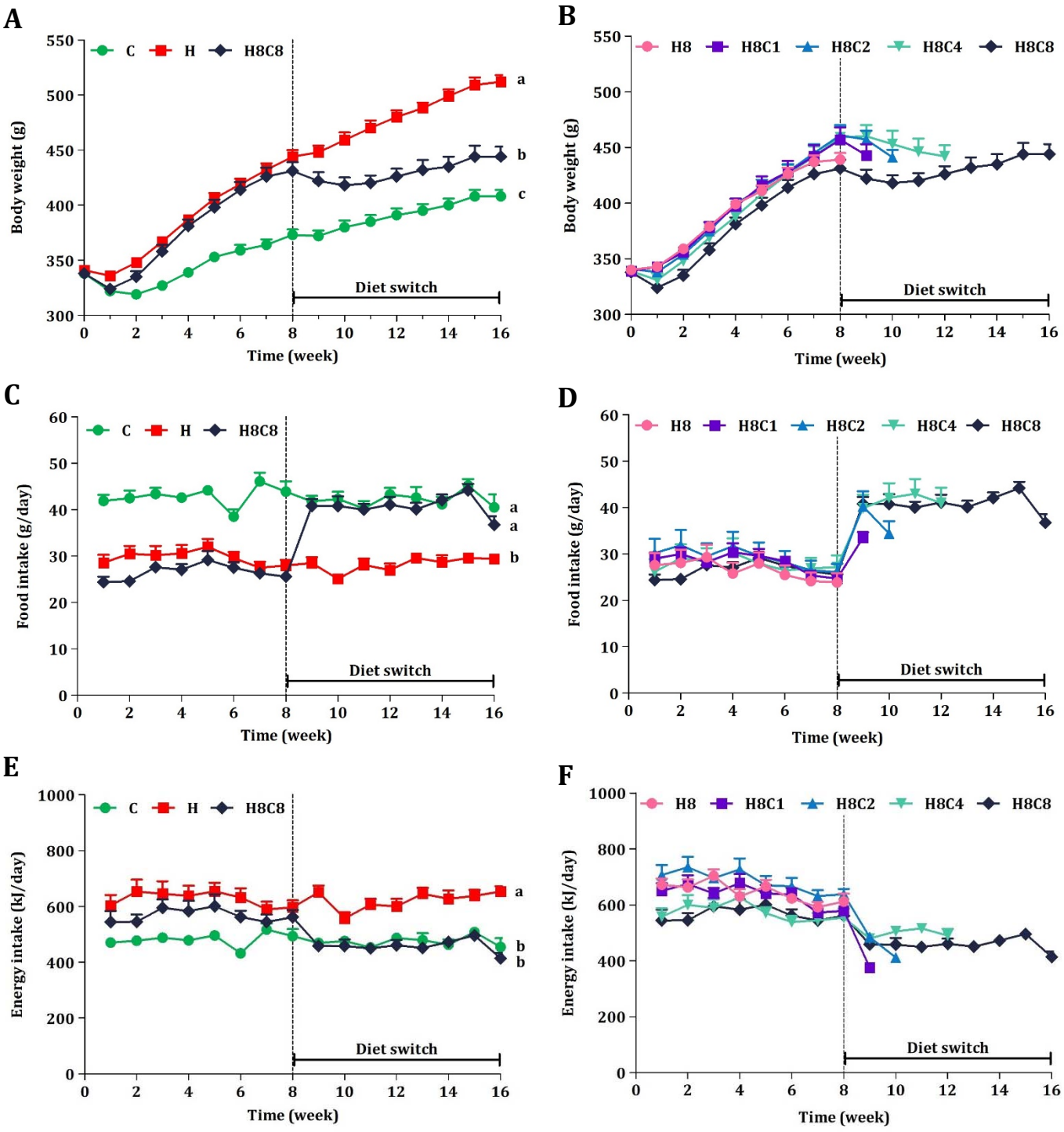

Figure 3-1. Effects of switching diet on body weight, food intake and energy intake at 16 weeks (A, C, E) and at varying time points (B, E, F).

Values are mean ± SEM, n = 10–12. Means with an uncommon letter differ significantly ($P < 0.05$).

C, rats fed a corn starch diet for 16 weeks; **H**, rats fed a high-carbohydrate, high-fat diet for 16 weeks; **H8**, rats fed a high-carbohydrate, high-fat diet for 8 weeks; **H8C1**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 1 week; **H8C2**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 2 weeks; **H8C4**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 4 weeks; **H8C8**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 8 weeks.

Table 3-1. Body weight, energy intake and feed efficiency at 16 weeks

Variable	C	H	H8C8	*P*–value
Initial body weight (g)	338 ± 1	341 ± 1	338 ± 1	0.0620
Body weight at 8 weeks (g)	373 ± 5 [a]	444 ± 6 [b]	431 ± 8 [b]	< 0.0001
Final body weight (g)	408 ± 6 [a]	512 ± 6 [b]	444 ± 9 [c]	< 0.0001
Water intake 0-8 weeks (mL/day)	33.7 ± 2.4 [a]	25.8 ± 1.6 [b]	25.1 ± 2.2 [b]	0.0105
Water intake 8-16 weeks (mL/day)	32.1 ± 2.3	30.4 ± 1.0	30.6 ± 2.4	0.8003
Food intake 0-8 weeks (g/day)	42.7 ± 1.0 [a]	29.6 ± 1.4 [b]	26.6 ± 1.0 [b]	< 0.0001
Food intake 8-16 weeks (g/day)	41.7 ± 1.1 [a]	28.1 ± 0.8 [b]	40.8 ± 1.0 [a]	< 0.0001
Energy intake 0-8 weeks (kJ/day)	479 ± 11 [b]	626 ± 31 [a]	568 ± 27 [a]	0.0006
Energy intake 8-16 weeks (kJ/day)	469 ± 13 [b]	618 ± 16 [a]	460 ± 9 [b]	< 0.0001
Food conversion efficiency 0-8 weeks (g/kJ)	0.073 ± 0.011 [b]	0.166 ± 0.006 [a]	0.164 ± 0.012 [a]	< 0.0001
Food conversion efficiency 8-16 weeks (g/kJ)	0.077 ± 0.006 [b]	0.111 ± 0.004 [a]	0.026 ± 0.012 [c]	< 0.0001

Values are mean ± SEM, n = 11-12. Means in a row with unlike superscripts differ ($P < 0.05$).

C, rats fed a corn starch diet for 16 weeks; **H**, rats fed a high-carbohydrate, high-fat diet for 16 weeks; **H8C8**, rats fed a high-carbohydrate, high-fat diet for 8 weeks and switched to a corn starch diet for an additional 8 weeks.

The H8C8 rats had a significantly lower water intake compared to the H8, H8C1, H8C2 and H8C4 groups during the first 8 weeks of protocol ($P = 0.01$), although all groups had a similar water intake after the diet switch (Table 3–2). Food conversion efficiency during weeks 0–8 was similar for the H8C1, H8C2 and H8C4 rats, although the H8 and H8C1 rats had a significantly lower food conversion efficiency compared the H8C4 rats ($P = 0.01$). After the diet switch, the food conversion efficiency significantly decreased for each group and the H8C8 group had a greater food conversion efficiency in comparison to the H8C1, H8C2 and H8C4 groups ($P < 0.0001$).

Table 3-2. Time-dependent effects of switching diet on body weight, food intake and feed efficiency

Variable	H8	H8C1	H8C2	H8C4	H8C8	*P*–value
Initial body weight (g)	340 ± 1	339 ± 1	341 ± 1	339 ± 1	338 ± 1	0.3063
Body weight 8 weeks (g)	439 ± 6	457 ± 11	461 ± 9	459 ± 10	431 ± 8	0.0824
Final body weight (g)	439 ± 6	443 ± 10	441 ± 7	442 ± 10	444 ± 9	0.9953
Water intake 0-8 weeks (mL/day)	33.3 ± 0.9 ^{a}	32.2 ± 1.1 ^{a}	31.5 ± 1.1 ^{a}	32.2 ± 1.4 ^{a}	25.1 ± 2.2 ^{b}	0.0011
Water intake after diet switch (mL/day)	N/A	34.5 ± 1.8	33.3 ± 1.1	35.0 ± 1.3	30.6 ± 2.4	0.2949
Food intake 0-8 weeks (g/day)	28.7 ± 0.9 ^{ab}	28.2 ± 1.0 ^{ab}	31.4 ± 1.2 ^{a}	30.1 ± 1.4 ^{ab}	26.6 ± 1.0 ^{b}	0.0369
Food intake after diet switch (g/day)	N/A	33.5 ± 1.2 ^{a*}	40.0 ± 1.3 ^{b*}	44.0 ± 1.1 ^{b*}	40.8 ± 1.0 ^{b*}	< 0.0001
Energy intake 0-8 weeks (kJ/day)	646 ± 15 ^{ab}	633 ± 19 ^{ab}	683 ± 24 ^{a}	575 ± 26 ^{b}	568 ± 27 ^{b}	0.0030
Energy intake after diet switch (kJ/day)	N/A	376 ± 13 ^{c*}	450 ± 15 ^{b*}	495 ± 12 ^{a*}	460 ± 9 ^{ab*}	< 0.0001
Food conversion efficiency 0-8 weeks (g/kJ)	0.153 ± 0.010 ^{b}	0.185 ± 0.014 ^{ab}	0.184 ± 0.009 ^{ab}	0.213 ± 0.019 ^{a}	0.164 ± 0.012 ^{b}	0.0122
Food conversion efficiency after diet switch (g/kJ)	N/A	-0.038 ± 0.009 ^{b*}	-0.054 ± 0.012 ^{b*}	-0.044 ± 0.008 ^{b*}	0.026 ± 0.012 ^{a*}	< 0.0001

Values are mean ± SEM, n = 10-12. Means in a row with unlike superscripts differ ($P < 0.05$). * indicates a significant difference within a group after the diet switch.

H8, rats fed a high-carbohydrate, high-fat diet for 8 weeks; **H8C1**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 1 week; **H8C2**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 2 weeks; **H8C4**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 4 weeks; **H8C8**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 8 weeks.

3.2 Body composition

Bone mineral density did not significantly differ between the C, H and H8C8 groups (Table 3–3). The H rats had a greater bone mineral content than the C and H8C8 rats after 16 weeks ($P = 0.04$), although there was no significant difference between the three groups at 8 weeks. While total lean mass was significantly lower in the C rats at 8 weeks ($P = 0.01$), this increased and was similar to both the H and H8C8 rats at 16 weeks. Total fat mass was similar among the C, H and H8C8 groups at 8 weeks but after 16 weeks, there was a significant difference between all three groups due to an increase in fat mass for the H and H8C8 rats ($P < 0.0001$). The C rats had a significantly lower abdominal circumference compared to the H and H8C8 rats at both 8 and 16 weeks ($P < 0.001$). Despite having a similar abdominal circumference at 8 weeks, the H group had a significantly greater abdominal circumference than the H8C8 group at 16 weeks ($P < 0.0001$).

Visceral adiposity was significantly lower for the C and H8C8 rats than the H rats at 16 weeks ($P < 0.0001$). Total abdominal fat tissue, including retroperitoneal, epididymal and omental fat pads, was significantly greater in the H rats compared to the C and H8C8 rats ($P < 0.0001$). The H rats also had a higher interscapular brown fat weight than the C and H8C8 rats ($P = 0.04$). The heart tissue weight, separated into left ventricle with septum and right ventricle, was similar between the C, H and H8C8 groups, as was the weight of the spleen. In contrast, the liver and kidneys of the H rats weighed significantly more than those of the C and H8C8 rats ($P < 0.001$).

Table 3-3. Effects of switching diet on body composition

Variable	C	H	H8C8	*P*–value
Bone mineral content at 8 weeks (g)	12.0 ± 0.4	13.4 ± 0.6	12.5 ± 0.5	0.1436
Bone mineral content at 16 weeks (g)	13.9 ± 0.7 [b]	15.8 ± 0.4 [a]	14.0 ± 0.6 [b]	0.0382
Bone mineral density at 8 weeks (g/cm^2)	0.172 ± 0.002	0.179 ± 0.003	0.176 ± 0.003	0.1436
Bone mineral density at 16 weeks (g/cm^2)	0.178 ± 0.002	0.178 ± 0.003	0.181 ± 0.003	0.6994
Total lean mass at 8 weeks (g)	269 ± 7 [b]	306 ± 12 [a]	300 ± 9 [a]	0.0119
Total lean mass at 16 weeks (g)	293 ± 4	301 ± 9	305 ± 10	0.5852
Total fat mass at 8 weeks (g)	90 ± 8	128 ± 12	113 ± 13	0.0633
Total fat mass at 16 weeks (g)	89 ± 5 [c]	189 ± 6 [a]	125 ± 12 [b]	< 0.0001
Abdominal circumference at 8 weeks (cm)	18.9 ± 0.5 [b]	21.4 ± 0.4 [a]	20.2 ± 0.3 [a]	0.0006
Abdominal circumference at 16 weeks (cm)	19.4 ± 0.2 [c]	22.3 ± 0.2 [a]	20.3 ± 0.2 [b]	< 0.0001
Visceral adiposity index (%)	5.6 ± 0.3 [b]	8.6 ± 0.3 [a]	5.7 ± 0.4 [b]	< 0.0001
Tissue wet weight at 16 weeks (mg/mm tibial length)				
Retroperitoneal fat	241.4 ± 9.8 [b]	466.0 ± 18.6 [a]	253.9 ± 21.6 [b]	< 0.0001
Epididymal fat	107.7 ± 7.7 [b]	183.2 ± 14.5 [a]	109.6 ± 14.2 [b]	0.0001
Omental fat	141.4 ± 8.2 [b]	227.5 ± 7.7 [a]	149.6 ± 12.8 [b]	< 0.0001
Total abdominal fat	490.5 ± 20.2 [b]	876.7 ± 23.0 [a]	513.1 ± 45.4 [b]	< 0.0001
Interscapular brown fat	29.1 ± 2.3 [b]	37.1 ± 2.0 [a]	29.5 ± 1.7 [b]	0.0432
Left ventricle and septum	23.0 ± 0.9	23.3 ± 1.1	22.3 ± 0.9	0.7484
Right ventricle	5.0 ± 0.3	5.0 ± 0.2	4.9 ± 0.3	0.8679
Spleen	17.7 ± 0.8	18.5 ± 0.7	17.1 ± 0.8	0.4560
Liver	242.1 ± 6.2 [b]	333.2 ± 8.2 [a]	259.5 ± 6.7 [b]	< 0.0001
Kidneys	57.6 ± 2.3 [b]	63.8 ± 1.5 [a]	54.4 ± 1.0 [b]	0.0012

Values are mean ± SEM, n = 10-12. Means in a row with unlike superscripts differ ($P < 0.05$).

C, rats fed a corn starch diet for 16 weeks; **H**, rats fed a high-carbohydrate, high-fat diet for 16 weeks; **H8C8**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 8 weeks.

There were no significant differences in bone mineral density and content, lean mass, total fat mass and abdominal circumference among the H8, H8C1, H8C2, H8C4 and H8C8 groups (Table 3–4). Visceral adiposity and retroperitoneal fat mass were significantly lower for the H8C8 rats than the H8 rats ($P < 0.05$), while the H8C1, H8C2 and H8C4 groups had similar intermediate values. All four diet switch groups had significantly less omental fat tissue than the H8 rats, and there was also a significant reduction in omental fat mass for the H8C8 rats in comparison to the H8C1 rats ($P < 0.0001$). Total abdominal fat consistently decreased at each time point after the change in diet and the H8C8 rats had a significantly lower abdominal fat mass than both the H8 and H8C1 rats ($P < 0.01$). Although there was no significant variation in interscapular brown fat, heart tissue and spleen mass, the liver and kidney weights were significantly lower for the diet switch groups than the H8 group ($P < 0.01$).

3.3 Metabolic variables

The H group had a significantly greater area under curve (AUC) and fasting blood glucose concentration than the C and H8C8 groups at week 16 ($P < 0.02$), which is indicative of reduced insulin sensitivity (Table 3–5). Plasma liver enzymes were higher in the H rats and there was a significant increase in aspartate transaminase (AST) compared to the C and H8C8 rats. While there were no significant differences in total cholesterol, the plasma triglycerides and non-esterified fatty acids (NEFA) were significantly lower in the C and H8C8 groups compared to the H group ($P < 0.01$).

Table 3-4. Time-dependent effects of switching diet on body composition

Variable	H8	H8C1	H8C2	H8C4	H8C8	*P*–value
Bone mineral content (g)	12.9 ± 0.4	13.6 ± 0.6	13.5 ± 0.6	14.2 ± 0.7	14.0 ± 0.6	0.6456
Bone mineral density (g/cm^2)	0.177 ± 0.004	0.183 ± 0.003	0.185 ± 0.003	0.182 ± 0.004	0.181 ± 0.003	0.6630
Total lean mass (g)	310 ± 9	307 ± 7	310 ± 9	300 ± 11	305 ± 10	0.9350
Total fat mass (g)	127 ± 13	123 ± 17	115 ± 13	135 ± 17	125 ± 12	0.9260
Abdominal circumference (cm)	20.2 ± 0.2	20.1 ± 0.4	20.3 ± 0.3	20.5 ± 0.3	20.3 ± 0.2	0.9018
Visceral adiposity (%)	8.0 ± 0.4 ^{a}	6.9 ± 0.5 ^{ab}	6.6 ± 0.3 ^{ab}	6.8 ± 0.4 ^{ab}	5.7 ± 0.4 ^{b}	0.0054
Tissue wet weight (mg/mm tibial length)						
Retroperitoneal fat	394.6 ± 32.8 ^{a}	341.8 ± 31.2 ^{ab}	334.9 ± 25.2 ^{ab}	330.2 ± 27.5 ^{ab}	253.9 ± 21.6 ^{b}	0.0219
Epididymal fat	142.2 ± 9.9	156.4 ± 14.9	142.1 ± 12.6	124.5 ± 13.4	109.6 ± 14.2	0.1215
Omental fat	251.2 ± 11.1 ^{a}	204.4 ± 16.7 ^{b}	176.2 ± 12.8 ^{bc}	184.3 ± 10.7 ^{bc}	149.6 ± 12.8 ^{c}	< 0.0001
Total abdominal fat	787.9 ± 47.1 ^{a}	694.7 ± 62.2 ^{a}	653.2 ± 34.1 ^{ab}	639.0 ± 45.9 ^{ab}	513.1 ± 45.4 ^{b}	0.0055
Interscapular brown fat	32.8 ± 2.1	27.0 ± 2.1	27.1 ± 2.1	27.8 ± 1.9	29.5 ± 2.3	0.2269
Left ventricle and septum	22.6 ± 1.3	24.8 ± 1.0	25.5 ± 0.7	24.8 ± 1.3	22.3 ± 0.9	0.1158
Right ventricle	4.9 ± 0.4	5.7 ± 0.2	5.0 ± 0.4	5.0 ± 0.5	4.9 ± 0.3	0.1749
Spleen	18.8 ± 0.9	16.8 ± 0.8	16.8 ± 0.7	16.8 ± 1.2	17.1 ± 0.8	0.4490
Liver	314 ± 7.8 ^{a}	261 ± 10.3 ^{b}	276 ± 11.8 ^{b}	244 ± 11.5 ^{b}	260 ± 6.7 ^{b}	0.0001
Kidneys	60.5 ± 1.7 ^{a}	54.4 ± 1.3 ^{b}	55.5 ± 1.2 ^{b}	54.1 ± 1.5 ^{b}	54.4 ± 1.0 ^{b}	0.0077

Values are mean ± SEM, n = 10-12. Mean in a row with unlike superscripts differ significantly ($P < 0.05$).

H8, rats fed a high-carbohydrate, high-fat diet for 8 weeks; **H8C1**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 1 week; **H8C2**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 2 weeks; **H8C4**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 4 weeks; **H8C8**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 8 weeks.

AUC was significantly lower in the H8C4 rats in comparison to the H8C2 and H8C8 rats ($P < 0.05$), while the AUC of the H8 and H8C1 rats did not differ from the other groups significantly (Table 3–6). A significant decrease in alanine transaminase (ALT) was observed for the H8C4 rats in comparison to the H8C1 rats ($P < 0.05$) and although aspartate transaminase (AST) was noticeably lower for the H8C8 rats compared to the H8, H8C1, H8C2 and H8C4 rats, these changes were not statistically significant. Total cholesterol was significantly greater for the H8 rats in comparison to the H8C1 and H8C8 rats ($P < 0.0001$). The H8C1 group also had a significantly lower total cholesterol concentration compared to the H8C2 and H8C4 groups. There was a significant reduction in plasma NEFA concentrations for the H8C8 rats in comparison to the H8 rats ($P = 0.05$) and the plasma triglyceride concentration was 51% lower in the H8C1 group than the H8 group ($P < 0.001$). These plasma triglyceride concentrations were maintained in the H8C2 rats and decreased slightly further in the H8C4 and H8C8 rats.

Table 3-5. Effects of switching diet on glucose tolerance, plasma liver enzymes and lipid profile at 16 weeks

Variable	C	H	H8C8	*P*–value
Glucose tolerance				
Area under curve (mmol/min)	625 ± 31 ^{b}	734 ± 33 ^{a}	595 ± 24 ^{b}	0.0062
Plasma liver enzymes				
Alanine transaminase (U/L)	31 ± 4	53 ± 10	35 ± 10	0.1780
Aspartate transaminase (U/L)	84 ± 3 ^{b}	131 ± 19 ^{a}	85 ± 6 ^{b}	0.0438
Plasma lipid profile				
Total cholesterol (mmol/L)	1.60 ± 0.14	1.79 ± 0.09	1.33 ± 0.09	0.0688
Triglycerides (mmol/L)	0.81 ± 0.05 ^{b}	1.78 ± 0.27 ^{a}	0.60 ± 0.19 ^{b}	0.0058
Non-esterified fatty acids (mmol/L)	1.5 ± 0.1 ^{b}	3.9 ± 0.6 ^{a}	1.3 ± 0.2 ^{b}	0.0027

Values are mean ± SEM, n = 3-12. Means in a row with unlike superscripts differ ($P < 0.05$).

C, rats fed a corn starch diet for 16 weeks; **H**, rats fed a high-carbohydrate, high-fat diet for 16 weeks; **H8C8**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 8 weeks.

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Table 3-6. Time-dependent effects of switching diet on glucose tolerance, plasma liver enzymes and lipid profile

Variable	H8	H8C1	H8C2	H8C4	H8C8	*P*–value
Glucose tolerance						
Area under curve (mmol/min)	543 ± 16 ^{ab}	544 ± 13 ^{ab}	579 ± 12 ^{a}	498 ± 9 ^{b}	595 ± 24 ^{a}	0.0021
Plasma liver enzymes						
Alanine transaminase (U/L)	40 ± 2 ^{ab}	45 ± 3 ^{a}	37 ± 2 ^{ab}	31 ± 3 ^{b}	35 ± 1 ^{ab}	0.0145
Aspartate transaminase (U/L)	103 ± 4	109 ± 6	105 ± 5	101 ± 5	85 ± 6	0.2732
Plasma lipid profile						
Total cholesterol (mmol/L)	1.72 ± 0.06 ^{a}	1.30 ± 0.07 ^{b}	1.63 ± 0.05 ^{ac}	1.52 ± 0.05 ^{ac}	1.33 ± 0.09 ^{bc}	< 0.0001
Triglycerides (mmol/L)	1.47 ± 0.24 ^{a}	0.72 ± 0.10 ^{b}	0.74 ± 0.09 ^{b}	0.66 ± 0.09 ^{b}	0.60 ± 0.19 ^{b}	0.0008
Non-esterified fatty acids (mmol/L)	2.9 ± 0.4 ^{a}	2.1 ± 0.2 ^{ab}	1.8 ± 0.3 ^{ab}	1.9 ± 0.3 ^{ab}	1.3 ± 0.2 ^{b}	0.0502

Values are mean ± SEM, n = 3-12. Means in a row with unlike superscripts differ significantly ($P < 0.05$).

H8, rats fed a high-carbohydrate, high-fat diet for 8 weeks; **H8C1**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 1 week; **H8C2**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 2 weeks; **H8C4**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 4 weeks; **H8C8**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 8 weeks.

The diet switch had a significant effect on respiratory exchange ratio (RER), the ratio between the volume of oxygen utilised and carbon dioxide produced during metabolic functions (Figure 3–2). RER was significantly lower when rats were fed the HCHF diet in comparison to the CS diet ($P < 0.001$), which was particularly evidenced by the increase in RER for the H8C8 rats after the diet switch (Figure 3–2A). These changes occurred within the first week of switching diet, as RER was significantly greater in the H8C1 rats compared the H8 rats ($P < 0.0001$) (Figure 3–2B). This higher RER was maintained in the H8C2 and H8C4 rats, then significantly increased further in the H8C8 rats.

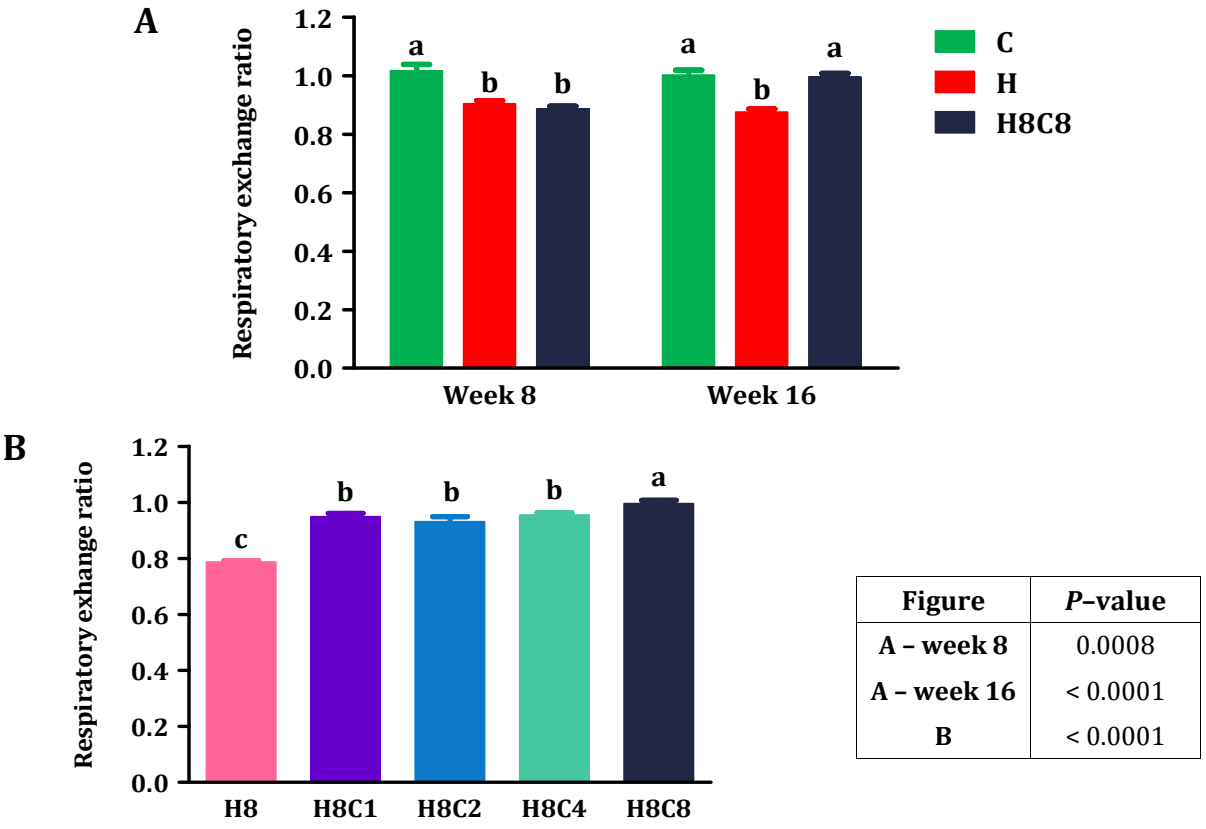

Figure	*P*–value
A – week 8	0.0008
A – week 16	< 0.0001
B	< 0.0001

Figure 3-2. Effects of switching diet on respiratory exchange ratio at 8 and 16 weeks (A) and at varying time points (B)

Values are mean ± SEM, n = 4–6. Means with an uncommon letter differ significantly ($P < 0.05$).

C, rats fed a corn starch diet for 16 weeks; **H**, rats fed a high-carbohydrate, high-fat diet for 16 weeks; **H8**, rats fed a high-carbohydrate, high-fat diet for 8 weeks; **H8C1**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 1 week; **H8C2**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 2 weeks; **H8C4**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 4 weeks; **H8C8**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 8 weeks.

Indirect calorimetry was also used to measure heat production, which is displayed in Figure 3–3. Although the H rats consistently produced more heat than the H8C8 and C rats (approximately 4.5 kcal/hour in comparison to 4.2 and 3.8 kcal/hour, respectively), the heat production was only significantly greater for the H rats in comparison to the C rats at 16 weeks ($P < 0.05$) (Figure 3–3A). Heat production was unchanged in the H8C8 rats after the diet switch and there were also no significant differences in heat production among the H8, H8C1, H8C2, H8C4 and H8C8 groups ($P = 0.08$) (Figure 3–3B).

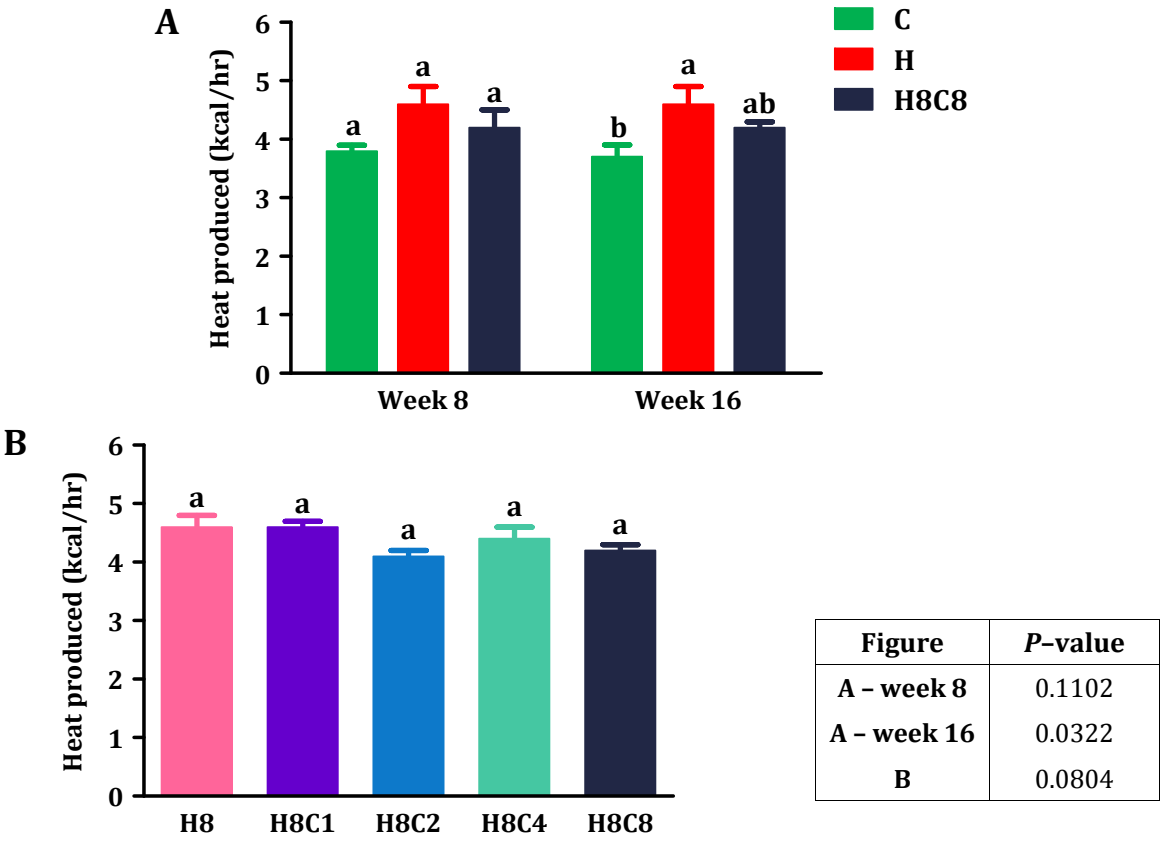

Figure	*P*–value
A – week 8	0.1102
A – week 16	0.0322
B	0.0804

Figure 3-3. Effects of switching diet on heat production at 8 and 16 weeks (A) and at varying time points (B)

Values are mean ± SEM, n = 4–6. Means with an uncommon letter differ significantly ($P < 0.05$).

C, rats fed a corn starch diet for 16 weeks; **H**, rats fed a high-carbohydrate, high-fat diet for 16 weeks; **H8**, rats fed a high-carbohydrate, high-fat diet for 8 weeks; **H8C1**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 1 week; **H8C2**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 2 weeks; **H8C4**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 4 weeks; **H8C8**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 8 weeks.

3.4 Cardiovascular structure and function

Systolic blood pressure was significantly higher in the HCHF diet-fed rats compared to rats fed the CS diet (Figure 3–3). The systolic blood pressure of the H8C8 rats was similar to the H rats (approximately 135–140 mmHg) at 8 weeks and significantly decreased after the diet switch, reaching a similar value to the C rats (approximately 120–125 mmHg) at 16 weeks ($P < 0.0001$) (Figure 3–3A). The decrease in systolic blood pressure after the diet switch was also observed in the H8C4 group, which was significantly lower than the H8 rats and similar to the H8C8 rats ($P < 0.0001$) (Figure 3–3B).

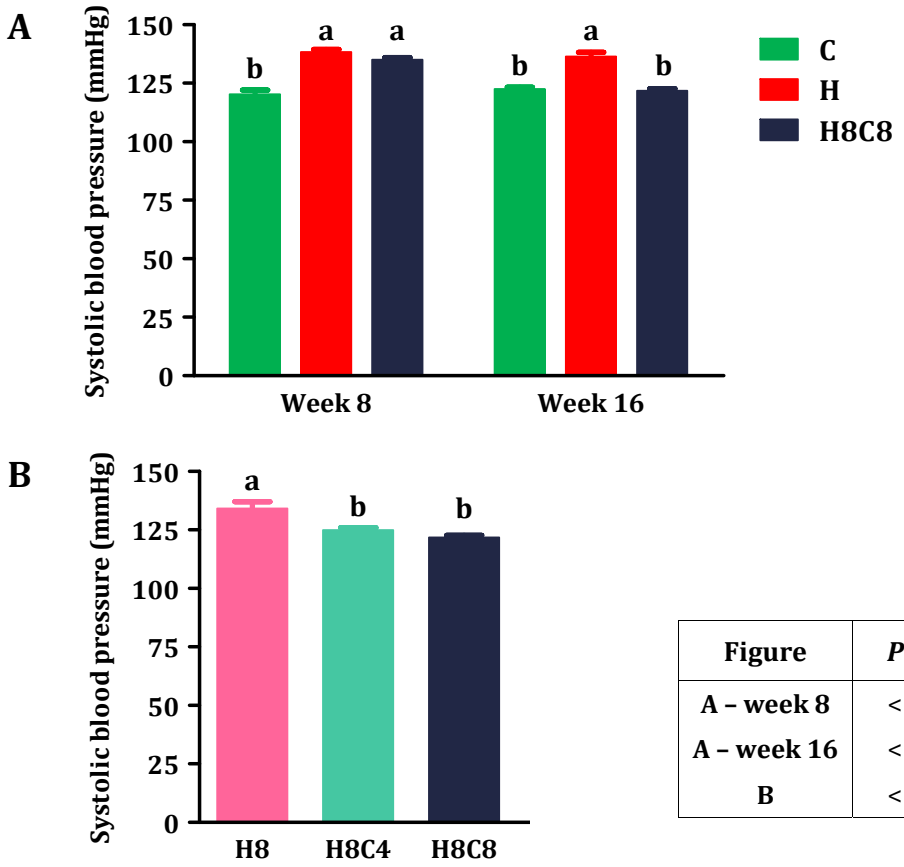

Figure	*P*–value
A – week 8	< 0.0001
A – week 16	< 0.0001
B	< 0.0001

Figure 3-4. Effects of switching diet on systolic blood pressure at 8 and 16 weeks (A) and at varying time points (B)

Values are mean ± SEM, n = 6–11. Means with an uncommon letter differ significantly ($P < 0.05$).

C, rats fed a corn starch diet for 16 weeks; **H**, rats fed a high-carbohydrate, high-fat diet for 16 weeks; **H8**, rats fed a high-carbohydrate, high-fat diet for 8 weeks; **H8C4**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 4 weeks; **H8C8**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 8 weeks.

The diastolic stiffness constant (κ, dimensionless) was significantly greater for the H rats in comparison to the C and H8C8 rats at 16 weeks ($P < 0.0001$) (Figure 3–4A). The effects of the diet switch were apparent after two weeks of consuming the CS diet, with the H8C2, H8C4 and H8C8 groups having a significantly decreased diastolic stiffness in comparison to the H8 and H8C1 groups ($P < 0.0001$) (Figure 3–4B).

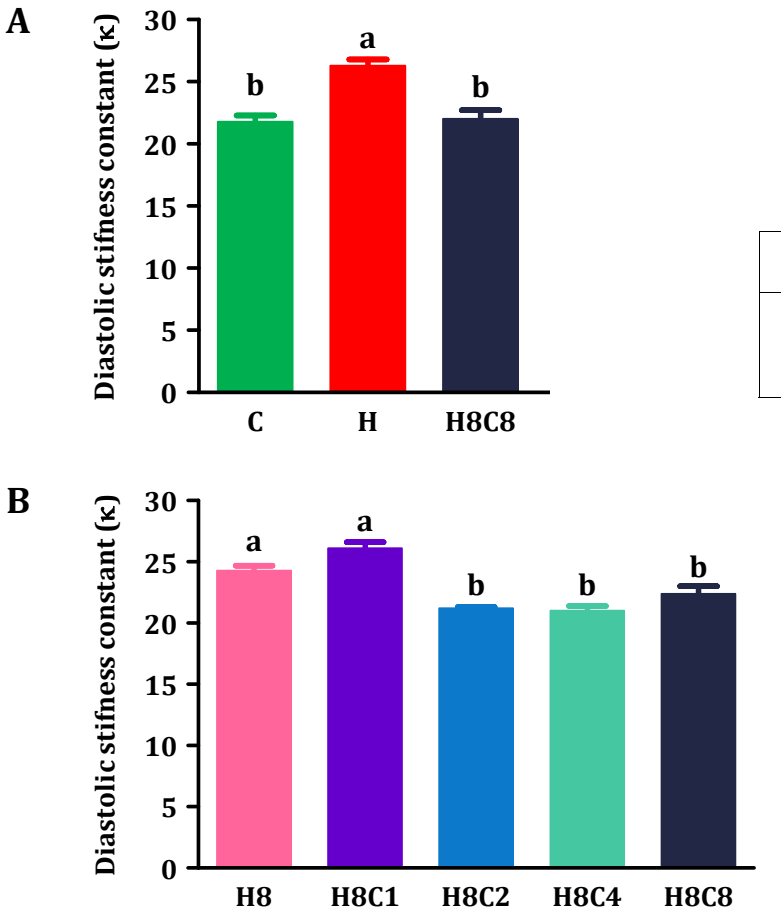

Figure	*P*–value
A	< 0.0001
B	< 0.0001

Figure 3-5. Effects of switching diet on diastolic stiffness at 16 weeks (A) and at varying time points (B)

Values are mean ± SEM, n = 5–10. Means with an uncommon letter differ significantly ($P < 0.05$).

C, rats fed a corn starch diet for 16 weeks; **H**, rats fed a high-carbohydrate, high-fat diet for 16 weeks; **H8**, rats fed a high-carbohydrate, high-fat diet for 8 weeks; **H8C1**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 1 week; **H8C2**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 2 weeks; **H8C4**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 4 weeks; **H8C8**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 8 weeks.

Microscopically, the C rats had no obvious signs of heart inflammation, whereas the H rat hearts contained large clusters of inflammatory cells (Figure 3–5). The diet switch noticeably reduced inflammatory cell infiltration and the heart tissue of the H8C8 rats appeared similar to the C rats at the end of the 16–week protocol. These effects were apparent two weeks after changing diet, as the H8C2 rats had considerably fewer inflammatory cells in comparison to the H8 and H8C1 rats, and the heart inflammation continued to improve in the H8C4 and H8C8 rats (Figure 3–6).

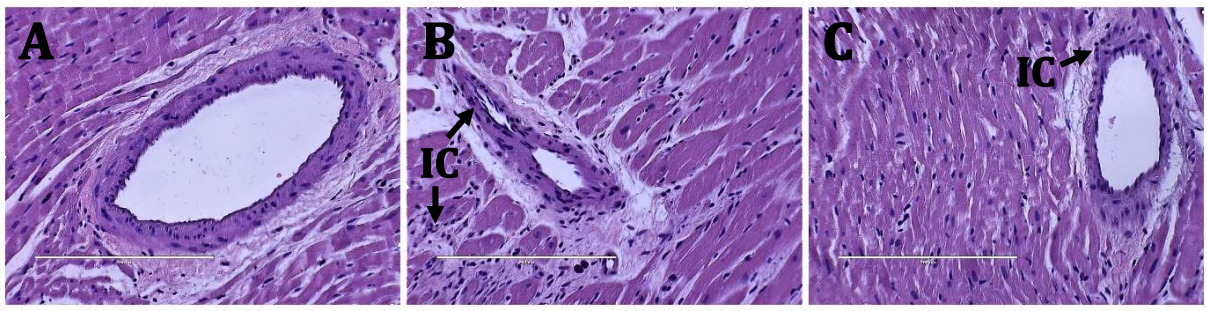

Figure 3-6. Effects of switching diet on heart inflammation

A–**C** represents haematoxylin and eosin staining of the heart showing inflammatory cells (marked as 'IC') (20×). **C**, rats fed a corn starch diet for 16 weeks **(A)**; **H**, rats fed a high–carbohydrate, high–fat diet for 16 weeks **(B)**; **H8C8**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 8 weeks **(C)**.

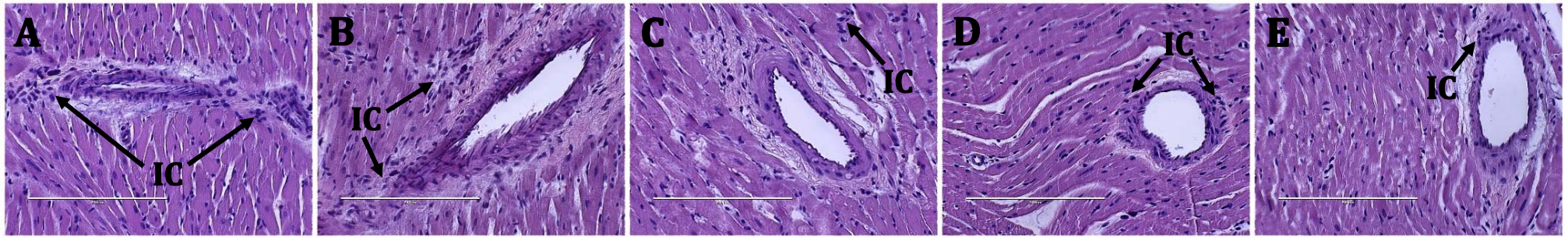

Figure 3-7. Time–dependent effects of switching diet on heart inflammation

A–C represents haematoxylin and eosin staining of the heart showing inflammatory cells (marked as 'IC') (20×). **H8**, rats fed a high–carbohydrate, high–fat diet for 8 weeks **(A)**; **H8C1**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 1 week **(B)**; **H8C2**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 2 weeks **(C)**; **H8C4**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 4 weeks **(D)**; **H8C8**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 8 weeks **(E)**.

3.5 Liver structure

The H rats had extensive lipid deposition and inflammatory cell infiltration within the liver, while the C rats showed no signs of hepatic inflammation or steatosis (Figure 3–7). The liver tissue from the H8C8 rats appeared more similar to the C rats histologically, although there were small fat vacuoles and clusters of inflammatory cells. There was widespread lipid deposition within the livers of the H8 and H8C1 rats, which was visibly reduced within the H8C2 rat livers and continued to improve in the H8C4 and H8C8 rats (Figure 3–8). Liver inflammation persisted after the decrease in fat deposition, as inflammatory cells were still present within the H8C4 and H8C8 rats.

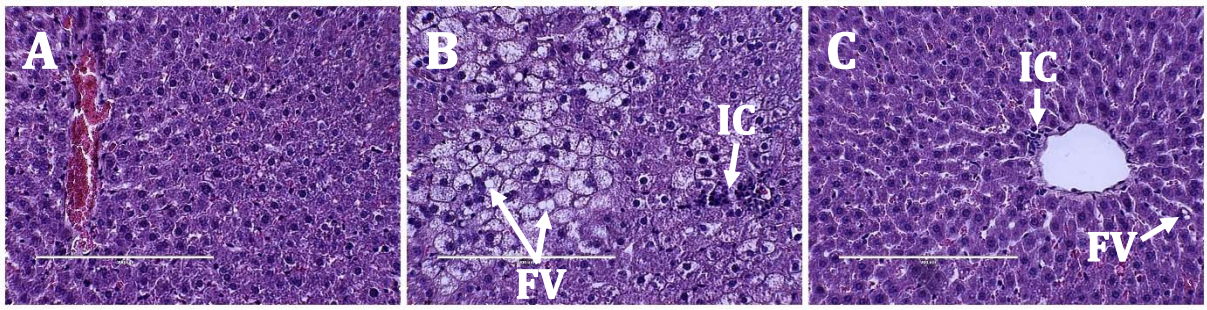

Figure 3-8. Effects of switching diet on liver inflammation and fat deposition

A–C represents haematoxylin and eosin staining of the liver showing inflammatory cells (marked as 'IC') and fat vacuoles (marked as 'FV') (20×). **C**, rats fed a corn starch diet for 16 weeks **(A)**; **H**, rats fed a high–carbohydrate, high–fat diet for 16 weeks **(B)**; **H8C8**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 8 weeks **(C)**.

1

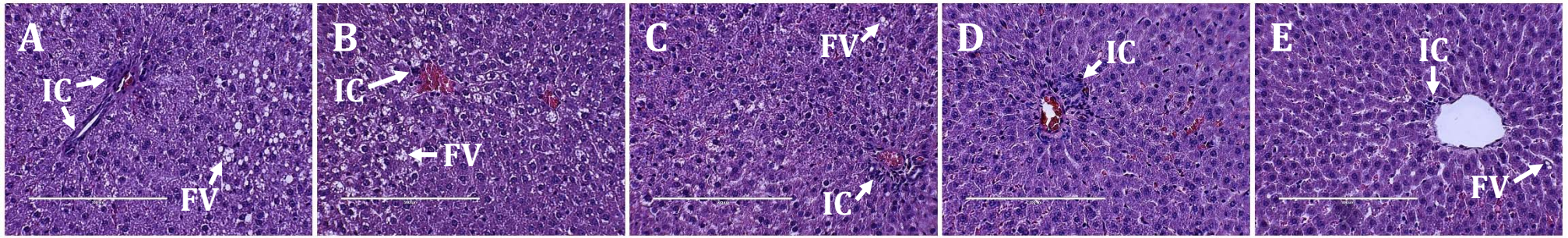

Figure 3-9. Time–dependent effects of switching diet on liver inflammation and fat deposition

A–C represents haematoxylin and eosin staining of the liver showing inflammatory cells (marked as 'IC') and fat vacuoles (marked as 'FV') (20×). **H8**, rats fed a high–carbohydrate, high–fat diet for 8 weeks **(A)**; **H8C1**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 1 week **(B)**; **H8C2**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 2 weeks **(C)**; **H8C4**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 4 weeks **(D)**; **H8C8**, rats fed a high-carbohydrate, high-fat diet for 8 weeks then switched to a corn starch diet for 8 weeks **(E)**.

Chapter 4. Discussion

This study investigated the attenuation of diet–induced metabolic syndrome in rats switched from a diet rich in simple carbohydrates and saturated fats to a low–fat, less energy–dense diet. Consumption of the HCHF diet for 8 weeks induced the characteristic traits of metabolic syndrome, including increased abdominal adiposity, hypertension, glucose intolerance and hypertriglyceridemia. These symptoms worsened with prolonged HCHF diet feeding, as visceral adiposity, plasma lipids, OGTT AUC and diastolic stiffness continued to increase. In contrast, rats fed the CS diet for 16 weeks displayed no signs of metabolic, cardiovascular or liver dysfunction. Switching to the CS diet rapidly reversed several signs of HCHF diet–induced metabolic syndrome, despite the minimal reduction in overall body weight. Within one week of the diet switch, omental fat mass had decreased, the liver and kidneys weighed significantly less, there was an increase in RER and a 50% reduction in plasma triglycerides. Diastolic stiffness, heart inflammation and liver fat deposition significantly decreased after two weeks of consuming the CS diet and systolic blood pressure was reduced within four weeks of the diet switch. Although weight regain occurred 4–6 weeks after changing diet, there was a significant reduction in visceral adiposity, decrease in plasma NEFA concentration and an additional increase in RER by the end of the 16–week protocol.

Symptoms of metabolic syndrome were apparent after feeding rats the HCHF diet for at least 8 weeks, which is consistent with previous studies that have utilised this model (Bhaswant et al. 2015; Panchal et al. 2011; Wanyonyi et al. 2017). The obesogenic nature of the HCHF diet is not only due to the high content of *trans* and saturated fats in the beef tallow, as fructose has also been strongly implicated in the development of metabolic

syndrome (Bahadoran et al. 2017; Ishimoto et al. 2013; Lozano et al. 2016). Despite having a similar molecular structure to glucose and an equivalent caloric value, the metabolism of fructose differs slightly. While glucose metabolism is dependent on the enzyme phosphofructokinase (PFK), which catalyses the rate–limiting step of glycolysis, fructose is primarily metabolised by the liver–specific enzyme fructokinase (Ishimoto et al. 2013; Lyssiotis & Cantley 2013). The fructose metabolic pathway therefore bypasses a crucial PFK–mediated regulatory mechanism that limits the conversion of glucose to substrates for *de novo* lipogenesis, and this lack of inhibitory feedback allows unrestricted conversion of excess fructose to fatty acids for storage within adipose tissues (Lyssiotis & Cantley 2013; Schwarz et al. 2015). Unlike glucose, fructose does not stimulate the release of insulin and other satiety signals, such as glucagon–like peptide–1 (GLP–1) and gastric inhibitory polypeptide (GIP), which diminishes appetite suppression after a meal (Wölnerhanssen et al. 2015). Fructose also strongly activates brain regions associated with food reward, promoting greater energy intake that in turn contributes to weight gain and increased adiposity (Luo et al. 2015; Wölnerhanssen et al. 2015). Excessive fructose consumption has been implicated in the development of leptin resistance and is rapidly reversed after switching to a sugar–free diet, even one that is rich in saturated fats (Shapiro et al. 2011).

Daily energy intake rapidly decreased after switching to the CS diet, which is similar to observations from previous studies investigating the effects of switching mice from an obesogenic diet to a standard chow diet (Guo et al. 2009; Kowalski et al. 2016; Ma, Gao & Liu 2016). The reduced caloric intake in this study was not enforced by restricting food availability, as the rats were provided *ad libitum* access to food throughout the protocol duration, and food intake increased after the diet switch to compensate for the lower

energy density of the CS diet (11.23 kJ/g compared to 17.83 kJ/g for the HCHF diet). Food intake is mediated by gastrointestinal neuroendocrine signals, which are relayed to the brain via the vagus nerve and convey information about the type and quantity of ingested nutrients (Begg & Woods 2013). Vagal nerve fibres express chemoreceptors, which are stimulated by gut hormones secreted from specialised enteroendocrine cells throughout the gastrointestinal tract, and mechanoreceptors that sense distension as food passes through the stomach and intestines during digestion (de Lartigue 2016). Diet–induced obesity alters the vagal response to gut hormone signalling and reduces the sensitivity to gastrointestinal distension (Daly et al. 2011; Kentish et al. 2012).

Previous studies have reported prolonged changes in appetite after switching from an obesogenic diet to a low–fat diet and this has been attributed to the incomplete restoration of vagal nerve sensitivity and increased levels of the orexigenic hormone ghrelin (Briggs et al. 2013; Kentish et al. 2014; Sumithran et al. 2011). In contrast, the diet switch rats in this study did not appear to have a dramatic increase in hunger or lasting satiety impairment. Although weight regain occurred in the H8C8 within four weeks of switching to the CS diet, the daily food intake remained equivalent to the C rats. Rather than the weight gain reflecting an adaptive response to counteract additional weight loss, as has been previously suggested (Fothergill et al. 2016; Guo et al. 2009; Sumithran et al. 2011), this could instead be due to ongoing growth and development of the young rats since the weight gain was parallel to the C rats after adjusting to the CS diet. Similarly, a study by Hoevenaars et al. (2013) found that energy intake and expenditure reflected the most recently consumed diet without any influence from previous consumption of a high–fat diet.

Despite the lack of statistically significant weight loss, there were considerable metabolic improvements following the diet switch.

- The decrease in visceral adiposity suggests that there was an increase in lipolysis and since plasma triglycerides and NEFA decreased, the energy was being utilised. The oxymax results showed an increase in RER, suggesting that the main source of energy was carbohydrates (since the CS diet is high in carbohydrates and low in fats). There was also no decrease in heat production after the diet switch, so the rats continued to release the same amount of energy as heat despite the reduced energy intake.
- The reduction in visceral adiposity observed in this study is consistent with similar studies that investigated mild calorie restriction (Park et al. 2017).
- OGTT AUC results in this study were highly variable, so an alternative, more reliable method of measuring this could be an insulin tolerance test or insulin resistance test (HOMA-IR)

Cardiovascular improvements

- The obesity–related increase in leptin has been associated with hypertension, due to increased sympathetic nervous system activity mediated by the dorsomedial hypothalamus (this also increases BAT thermogenesis). Mice lacking leptin or the leptin receptor are hypotensive despite being severely obese (lack of leptin signalling impairs satiety, so food intake is increased). Systolic blood pressure decreased two weeks after HFD diet–induced obese mice were switched back to a chow diet, which correlated with decreases in body weight and leptin (Simonds et al. 2014).

- Angiotensin is also released from adipose tissue, further contributing to hypertension and ventricular hypertrophy.
- Increased fatty acid oxidation in obesity leads to diastolic dysfunction (Rayner et al. 2018). The diet switch may have reduced diastolic stiffness due to a change in myocardial energetics (Rider et al. 2013).
- Consumption of a high-fructose diet has been shown to increase the activation of apoptosis and suppress survival pathways in cardiac myocytes
- Fat infiltration within the heart can also induce myocyte damage and fibrosis, which contributes to the development of diastolic stiffness and heart failure.
- Discuss the mechanisms involved in reducing hepatic steatosis and the health benefits

Chapter 5. Conclusions

This study investigated the sequence of metabolic, cardiovascular and hepatic changes that occur during the attenuation of diet–induced metabolic syndrome. The main finding was that switching from the HCHF diet to the CS diet led to a reduction in abdominal adiposity, which subsequently decreased fat deposition within the liver, reduced heart inflammation, improved diastolic function and lowered systolic blood pressure. These health benefits occurred despite minimal weight loss after the diet switch, which suggests that diet and body composition have a more important role in determining metabolic health and cardiovascular risk than body weight alone. While the effects of switching diet were rapidly seen in rats, further research will be required to ascertain how these results translate to humans.

Chapter 6. Future directions

To provide further insight into how the diet switch affected the regulation of adiposity, an enzyme–linked immunosorbent assay (ELISA) could be performed on the plasma samples collected in this study to analyse satiety hormone concentrations. The rate at which obesity–induced leptin and ghrelin resistance is reversed after weight loss is unclear, so this study could provide a better understanding of how this occurs over time.

In addition, the samples of white (retroperitoneal), beige (inguinal) and brown adipose tissues collected in this study could be used to determine whether the change in diet induced adipose tissue browning/beiging. Beige and brown adipocytes are distinct from white adipocytes due to the expression of uncoupling protein–1 (UCP–1), which regulates the process of adaptive or "non–shivering" thermogenesis and causes the mitochondria to release energy as heat rather than ATP (Qiang et al. 2012). To observe the changes in UCP–1 expression at the cellular level, immunohistochemistry could be performed on the adipose tissue samples collected for histology. The retroperitoneal fat samples could also be used to determine if the diet switch reduced adipocyte hypertrophy in white adipose tissue, which would involve histology using haematoxylin and eosin staining.

Reverse transcription polymerase chain reaction (RT-PCR) could provide additional insight into the molecular changes that occur as a result of switching to the corn starch diet. Specific gene targets relevant to this study include liver enzymes involved in carbohydrate and lipid metabolism, adenosine monophosphate–activated protein kinase (AMPK), UCP–1, sirtuin 1 (SIRT1), peroxisome proliferator–activated receptor–γ (PPAR–γ) and PPAR–γ coactivator–1α (PGC–1α).

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