THE ATTENUATION OF METABOLIC SYNDROME AND IMPROVEMENT OF GASTROINTESTINAL MICROARCHITECTURE AND PHYSIOLOGY BY CONSUMPTION OF WHOLE FOOD PRODUCTS IN THE DIET OF MALE WISTAR RATS.

A Thesis submitted by

Sharyn M Carnahan, BBioSc (Hons)

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Abstract

Metabolic syndrome is a group of co-morbidities that, when combined, raise the risk of cardiovascular disease and type 2 diabetes. The syndrome is characterised by visceral obesity, hypertension, dyslipidaemia, fatty liver, impaired glucose tolerance and insulin resistance. A healthy diet is paramount to lowering these risks, including the consumption of whole foods, such as oats and coconut, to provide a multitude of nutrients that can achieve this. While understanding how individual nutrients impact health is important, when consumed these nutrients are taken together, so it is also important to understand how they work in synergy with each other, the food matrix that they are found in and other foods that are consumed with them.

Methods: Male Wistar rats fed either a diet containing either cornstarch or a combination of high carbohydrate and high fat was supplemented with either 5% wholegrain oat groats, oat bran or β-glucan powder, 20% wholegrain oat groats, or had the saturated fat component replaced with coconut oil or coconut Nourish for the final 8 weeks of a 16-week protocol. Cardiovascular, metabolic and physiological parameters were then measured to determine whether these products improved cardiometabolic biomarkers of metabolic syndrome. As oats contains prebiotic compounds that are beneficial to gastrointestinal health, the morphology of the small intestine and colon were also measured in the oat studies as was faecal short-chain fatty acid concentrations to quantify changes in the structure and function of the gastrointestinal tract.

Results: Oats and coconut as whole foods attenuated metabolic syndrome markers. Wholegrain oat, oat bran and β-glucan powder improved glucose tolerance, systolic blood pressure and abdominal circumference. β-glucan powder also decreased triglyceride concentrations. All oat products improved duodenal morphology and wholegrain oat groats and β-glucan powder increased faecal short-chain fatty acid concentrations. Virgin coconut oil and coconut Nourish improved blood pressure. Virgin coconut oil decreased abdominal circumference and fasting glucose concentrations and coconut Nourish decreased total cholesterol concentrations.

Conclusions: The studies in this thesis provide evidence that different types of whole foods from the same cereal crop or fruit produce similar results in improving cardiometabolic biomarkers in this model of diet induced metabolic syndrome. They

also provided evidence that modulation of the gastrointestinal tract plays an important role in improving health parameters. The most likely mechanisms of these whole food components are through cardioprotective and hepatoprotective effects produced by anti-inflammatory responses and alterations to short-chain fatty acid production within the colon.

Certification of Thesis

This thesis is entirely the work of ***Sharyn M Carnahan*** except where otherwise acknowledged. The work is original and has not previously been submitted for any other award, except where acknowledged.

Student and supervisors' signatures of endorsement are held at USQ.

Professor Lindsay Brown

Principal Supervisor

Dr. Sunil Panchal

Associate Supervisor

Dr. Kate Kauter

Associate Supervisor

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List of Abbreviations

AACCI	American Association of Cereal Chemists International
ABS	Australian Bureau of Statistics
AHA	American Heart Association
ALT	Alkaline phosphatase
AST	Aspartate aminotransferase
AUC	Area under the curve
BMI	Body Mass Index
CCK	cholecystokinin
CRP	C-reactive protein
DXA	Dual Energy X-ray absorptiometry
EGIR	European Group for the study of Insulin Resistance
EU	European Union
FDA	United States Food and Drug Administration
FFA	Free fatty acids
GI	Glycaemic Index
GLP-1	Glucagon-like peptide 1
GLUT	Glucose transporter type
GPR	G-protein coupled receptors
HDL-C	High density lipoprotein cholesterol
IDF	International Diabetes Federation
IL	Interleukin
ISAPP	International Scientific Association for Probiotics and Prebiotics
LDL-C	Low density lipoprotein cholesterol
MCFA	medium chain fatty acids

NCEP ATPIII	National Cholesterol Education Program Adult Treatment Panel III
NEFA	non-esterified fatty acids
NFκB	Nuclear Factor kappa light chain enhancer of activated B cells
NHANES	National Health and Nutrition Examination Survey
NHMRC	National Health and Medical Research Council
NHLBI	National Heart, Lung and Blood Institute
LVIDd	left ventricular internal dimension end diastole
LVIDs	left ventricular internal dimension end systole
LVPWd	left ventricular posterior wall thickness end diastole
OGTT	Oral glucose tolerance test
PYY	Peptide tyrosine tyrosine
RS	Resistant starch
SBP	systolic blood pressure
SCFA	short chain fatty acid
SEM	standard error of mean
TNF-α	Tumour necrosis factor alpha
TG/TAG	triglycerides
VLDL	very low-density lipoprotein
WHO	World Health Organisation

Chapter 1 – Whole foods as an intervention to prevent metabolic syndrome and gastrointestinal disorders.

1.1 Introduction

A healthy diet is the cornerstone of preventing cardiovascular disease and type 2 diabetes, which together with hypertension, insulin resistance, dyslipidaemia and obesity make up metabolic syndrome (O'Neill and O'Driscoll, 2015). The benefits of consuming whole foods with a wide range of nutrients has been well studied (Tapsell et al., 2016, Sikand et al., 2015), as has the effect that processing has on bioactive components of food (Tosh and Chu, 2015, Decker et al., 2014, Marina et al., 2009b). The regular consumption of fruit, vegetables and wholegrains is advocated in Australian Dietary guidelines (National Health and Medical Research Council, 2013b) and is negatively correlated with the risk of developing chronic diseases (Liu, 2013b). Functional foods modulate biological processes such as regulating lipid metabolism through lowering LDL-C and triglycerides and increasing HDL-C (Sikand et al., 2015). While understanding how individual nutrients can impact health is important, when consumed these compounds are taken together, so it is also necessary to understand how they work in synergy with each other in a whole food. The additive and synergistic effects of phytochemicals and macronutrients within whole foods target numerous signalling pathways that reduce inflammation, decrease energy intake and target satiety hormones (Liu, 2013a).

Isolated bioactive ingredients in human clinical trials have not given consistent results in the prevention of cardiovascular disease, due to loss of bioactivity or not behaving the same as when in a food matrix (Liu, 2013a). There is a complex mixture of phytochemicals in vegetables, fruits and grains and no single phytochemical can replace the combinations that are available. Each phytochemical or macronutrient differs in molecular size and solubility which affects its bioavailability and distribution in different macromolecules at cellular, organ and tissue levels (Liu, 2013a). The balanced combinations of these micro- and macro- nutrients that are found in functional foods cannot be mimicked by pills or tablets. It is generally considered safe to obtain antioxidants and phytochemicals from whole foods and not likely to result in toxic quantities as is possible with consumption of tablets or capsules of pure

phytochemical supplements (Liu, 2013b). For example, studies comparing tomatoes and one of its bioactive compounds, lycopene, show that tomatoes are more effective at decreasing LDL-C oxidation and lipid oxidation that lycopene supplements (Burton-Freeman and Sesso, 2014). Also eggs are high in cholesterol, which is typically bad for heart health, but also have high content of amino acids and other micronutrients which when combined with the cholesterol give a different effect than the cholesterol alone (Miranda et al., 2015).

Diets that focus on one nutrient such as protein or carbohydrates have negative consequences. A trend in low fat, high carbohydrate diets led to the reduction of total fat consumption without considering the type or quality of fat that was consumed and conversely led to an increased intake of refined carbohydrates and added sugar to the diet which in turn led to an increase in cardiometabolic diseases (Malik et al., 2010). Previously, it was thought that replacement of polyunsaturated fatty acids in the diet with high saturated fats led to increased cardiovascular disease; however, this generalisation does not take into account the physiological actions of individual fatty acids (Poudyal and Brown, 2015).

Foods known for their health benefits include wholegrains, fruits and seeds. Wholegrains have protective effects against obesity, type 2 diabetes, cardiovascular disease, hypertension and metabolic syndrome (Sikand et al., 2015). The functional components of wholegrains are the non-digestible complex polysaccharides known as soluble and insoluble fibre such as inulin, β-glucan and resistant starch, as well as phenolics and tocopherols which increase satiety and decrease energy intake through the modulation of GLP-1, PYY and ghrelin (Huang et al., 2011, Beck et al., 2009a, Beck et al., 2010).

Further research on the health benefits of whole foods is warranted as it is more translatable to the human diet, due to the synergy of bioactive and macronutrients and that people consume whole foods rather than nutrient supplements. Research into both the mechanisms of action of the nutrients as well as whole food components is important, because nutrients make up food and there is an interactive relationship between the nutrients in the food.

This study investigated oats as a wholegrain and coconut as a fruit/seed to see if different parts of the whole food gave similar or different health benefits. Oats and coconut have been consumed for thousands of years (Akeret, 2005) with anecdotal evidence for a wide variety of health benefits. Many studies have been undertaken in recent years, particularly on the bioactive components, such as β-glucans in oats (Martínez-Villaluenga and Peñas, 2017, Ho et al., 2016, Varma et al., 2016) and saturated fats in coconut (Babu et al., 2014). Previous studies have utilised different methods and models to examine the benefits of both oat products and coconut products. This study is using the same model, a high-carbohydrate, high-fat diet-induced rat model of metabolic syndrome (Panchal et al., 2011b), to examine different products produced from the two types of plants to determine whether they attenuate metabolic syndrome and the impact on gastrointestinal structure and function.

1.2 Oats and its health benefits

Cereal grains constitute a major part of the daily diet for consumers worldwide, with wheat, rice and maize as the highest consumed crops (Bushuk, 2001). The domestication of grains containing fibre and carbohydrates such as oligosaccharides and their incorporation into the human diet began approximately 12,000 years ago in the Neolithic era (Ladizinsky, 1995). Although the genetic makeup of these grains such as oats may have been modified since then to increase yield and ease of harvest, many of the available "ancient grains" are heritage or heirloom varieties of the more common cereals that are mass produced today (Ladizinsky, 1995, Sweeney and McCouch, 2007, Pingali, 2012).

The human diet has changed dramatically since Neolithic times with increased consumption of processed high carbohydrate, high fat foods leading to chronic diet-induced conditions such as metabolic syndrome (Brown et al., 2009, Sweeney and McCouch, 2007, Pingali, 2012). 'Ancient grains', such as oats, can be classified as prebiotics, as they contain non-digestible carbohydrates not digested in the stomach but fermented by colonic microbes to increase selected bacteria and benefit the host (Roberfroid et al., 2010, Slavin et al., 2013). The definition of prebiotics has been recently updated by International Scientific Association for Probiotics and Prebiotics (ISAPP) to also include non-carbohydrate substances such as polyphenols and

polyunsaturated fatty acids that are converted to conjugated fatty acids assuming that adequate evidence of health benefits in the target host are seen (Gibson et al., 2017).

These grains may also be functional foods, defined as foods that can prevent or reverse health problems as well as providing nutrition. Dietary interventions with these grains may be effective in modulating the gut environment, changing microbial populations and ameliorating obesity and metabolic syndrome (Dixit et al., 2011), possibly through the alteration of gut hormones that work on various segments of the gastrointestinal tract (Willis et al., 2010, Klosterbuer et al., 2012).

1.2.1 Composition of grain

Cereal grains are the fruit or seed of plants belonging to the *Poaceae* (formerly *Gramineae)* family of grasses. This family includes wheat, rice, barley, corn, rye, oats, millet, sorghum, teff and triticale, as well as the 'pseudo-cereals' of amaranth, quinoa and buckwheat which function as cereals (Rebello et al., 2014).

The American Association of Cereal Chemists International (AACCI) classifies wholegrain as "consisting of intact, ground, cracked or flaked caryopsis, whose principal anatomical components – the starchy endosperm, germ and bran, are present in the same relative proportions as they exist in the intact caryopsis" (Rebello et al., 2014). The AACCI classifies a product as wholegrain if it contains 8 g or more wholegrain per 30 g of product, while the FDA states that more than 51% of the total weight must be wholegrain (Rebello et al., 2014). Wholegrains are a major source of calories and offer a variety of nutrients and other food components such as phytochemicals (Liu, 2007). They are associated with the prevention of type 2 diabetes (Venn and Mann, 2004, Murtaugh et al., 2003), cancer (Jacobs et al., 1998) and other chronic diseases (Dixit et al., 2011, Johnston et al., 2010, Opie et al., 2016).

1.2.2 Oats

Oats (*Avena sativa)* belong to the *Poaceae* family of about 12,000 species, commonly known as grasses. Oats have been cultivated since the Bronze Age, approximately 5000 years ago, which followed the Neolithic Age, with the oldest known cultivation found in caves in Switzerland (Ladizinsky, 1995, Akeret, 2005) and south-eastern Italy

(Mariotti Lippi et al., 2015). Wild oats were discovered in Neolithic villages near the Dead Sea in Jordan (Kuijt and Finlayson, 2009, Weiss et al., 2006) indicating the importance of both wild and cultivated oats in the diet of humans.

Oats are an important crop in developing nations as it requires fewer nutrients (sodium, potassium and phosphorus) to cultivate than wheat or maize (Rasane et al., 2013). However, it needs more moisture to produce a unit of dry matter than any other cereal crop except rice (Rasane et al., 2013). Due to this, it is well suited to cool and moist climates. Oats are grown throughout North America and Europe, particularly Russia, Canada and USA (Forsberg and Reeves, 1995).

Historically, oats were used for animal feed (Menon et al., 2016). This use declined with mechanisation of the harvesting process, as there was less need for feedstock. However, the interest in oats as a human health food has increased (Ahmad et al., 2010). The five largest producers of oats are Russia, Canada, Poland, Finland (both included in the European Union figures) and Australia (US Department of Agriculture, 2012) with consumption mirroring the production as seen in Table 1.1.

The composition and therapeutic potential of oats have been studied extensively in particular in relation to hypocholesterolaemic (Braaten et al., 1994), anti-diabetic and cardiovascular effects (Tiwari et al., 2011). Oats and oat products such as bran are good sources of proteins, fats, vitamins and minerals, as well as dietary fibre, particularly β-glucans for its cardioprotective benefits (AbuMweis et al., 2010). Oats decrease blood cholesterol concentrations and prevent heart disease as β-glucans are a water-soluble fibre preventing the absorption of cholesterol in the stomach and intestines (Chen and Raymond, 2008).

The consumption of wholegrain oats is advocated in dietary guidelines and nutritional policies in many countries including USA (2010), Australia (2013), Canada (2011), Mexico (2006) and across South America, Europe and Asia (Clemens and van Klinken, 2014a). The 2015 American Dietary Guidelines suggest consuming at least half of all grains as wholegrains with three serves of 16 g/day to reduce the risk of developing several chronic diseases including cardiovascular disease, diabetes and possibly some cancers (U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2015) .

Table 1.1 World oats production and consumption 2011-2016

World oats production and consumption (thousand metric tons)					
Country	**2011/12**	**2012/13**	**2013/14**	**2014/15**	**2015/16**
Argentina	345	496	445	525	485
Australia	1262	1121	1255	1087	1300
Belarus	448	422	352	470	400
Brazil	354	361	380	300	300
Canada	3158	2830	3906	2979	3430
Chile	451	680	610	421	470
China	600	600	580	600	600
European Union	7927	7909	8380	7845	7640
Kazakhstan	258	200	305	226	250
Mexico	51	84	90	90	90
Norway	231	232	214	236	236
Russia	5332	4027	4932	5267	4550
Turkey	218	210	210	210	210
Ukraine	506	630	467	610	375
Others	372	315	328	315	349
United States	728	892	938	1019	1300
World Total	22308	21119	23502	22310	22095

adapted From USDA, 2016.

1.3 Nutrients in oats

Oats are a nutritious source of protein, carbohydrate, fibre, vitamins and minerals, with minor constituents such as β-glucans that reduce cholesterol and lower the risk of heart disease (Peterson, 2011). The outer layer of a mature, wholegrain oat is unpalatable, dry and brittle; this is the hull (Figure 1.1) composed of cellulose and hemicellulose (Ganßmann and Vorwerck, 1995, Welch, 1995). It acts as protection and a nutrient transportation system to the developing grain (Welch, 1995). Once the grain fully develops, the outer layer becomes hard and unfit for human consumption. Removing

this layer leaves the oat groat, which can be consumed. It consists of three main elements – the bran, starchy endosperm and germ (Figure 1.1) (Welch, 1995).

The outermost layer of the oat groat is composed of the bran. Unlike wheat bran, oat bran is harder to define. The AACCI defines it as "the food which is produced by grinding clean oat groats or rolled oats and separating the resulting oat flour by sieving, bolting, and/or other suitable means into fractions such that the oat bran fraction is not more than 50% of the original starting material and has a total β-glucan content of at least 5.5% (dry weight basis) and a total dietary fibre content of at least 16.0% (dry weight basis) and such that at least one-third of the total dietary fibre is soluble fibre" (Robert et al., 1985). This layer provides protection to the grain and is a rich source of fructans with small amounts of resistant starch (Bernstein et al., 2013), as well as being the largest source of vitamins and minerals (Peterson et al., 1975, Frølich and Nyman, 1988), phytates (Fulcher et al., 1981) and phenolics (Gray et al., 2000) in the oat grain. The outer layers (Figure 1.1) (epidermis, hypodermis, cross and tube cells, seed coat and nucellar tissue) are composed of insoluble polysaccharides, cellulose, hemicellulose, including arabinoxylans and lignins (Bernstein et al., 2013). The aleurone, which forms the interface between bran and endosperm, contains cellulose, hemicellulose, β-glucans, proteins, B vitamins, vitamin E, minerals, phenolics, alkylresorcinols, phytosterols and ferulic acid (Bernstein et al., 2013).

The starchy endosperm (Figure 1.1) is the largest component of the grain, making up approximately 70% of the dry weight (Gulvardy et al., 2014). It contains cellulose, hemicellulose, including arabinoxylans, xyloglucans and glucomannans, as well as resistant starch, proteins, B group vitamins and iron (Bernstein et al., 2013). The highest concentration of protein is in the periphery of the endosperm and decreases towards the centre of the kernel, while the starch is concentrated more towards the centre and decreases towards the aleuronic layer (Miller and Fulcher, 2011). This layer is also rich in lipids, accounting for up to 90% of the total lipid content of the oat groat (Youngs et al., 1977). Most of the lipids are in the subaleurone and endosperm cells in the vicinity of the germ layer (Heneen et al., 2009).

The germ layer (Figure 1.1) from which the grains germinate contains cellulose, fructans, lignans, fatty acids, B vitamins, vitamin E and minerals (Bernstein et al., 2013). The protein in the germ is surrounded by lipids (White et al., 2006), and,

although rich in proteins and lipids, it only accounts for a small proportion of the overall amount (Miller and Fulcher, 2011).

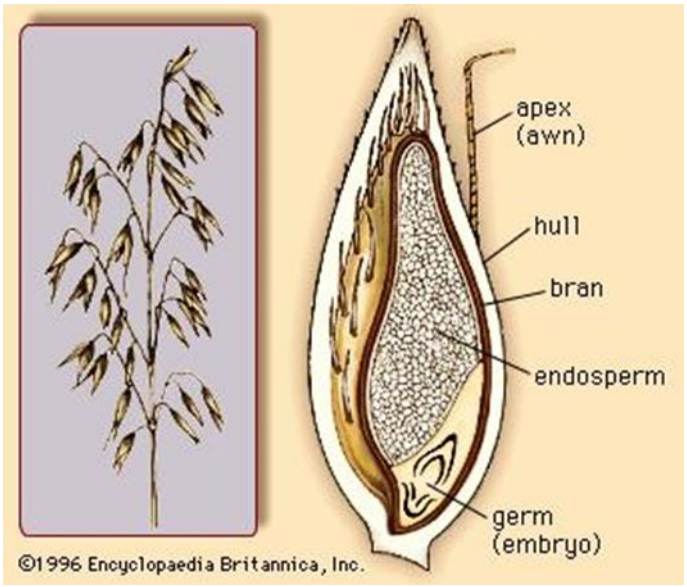


Figure 1.1 Diagram of the structure of an oat grain

Left: oat panicle with many florets. Right: cross-section of an oat grain.

from: Encyclopedia Britannica 2015, retrieved 12 March 2016. http://www.britannica.com/topic/oats/images-videos

While oats contain a wide variety of nutrients, the exact composition depends on many factors including the cultivar, growing conditions, processing and storage time. The range of nutrients found in 28 different cultivars included protein content ranging from 14.5 - 20%, lipids 4 - 8%, starch 53 - 63% and β-glucans 3 - 6% (Guo et al., 2014). In other cultivars, the protein may be as low as 13% with dietary fibre at approximately 10% (Butt et al., 2008).

1.3.1 Proteins

Oat groats contain the highest amount of protein of any commonly consumed cereal grain, ranging from 12.4 - 24.5% (Lásztity, 1998). Oats have a unique amino acid composition of the protein fraction of globulins, albumins, prolamin and glutelin (Wu et al., 1972, Draper, 1973). Proportions of the protein fractions differ between grain

species, with wheat containing higher concentrations of prolamins and globulins than oats.

1.3.1.1 Amino acids

Essential amino acids are not synthesised in the body. For humans, these include histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. These amino acids need to be supplied from dietary sources. Non-essential amino acids can be synthesised by the body and include alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, proline, serine and tyrosine.

The high globulin fraction in oats ensures that the key essential amino acids are in higher concentrations compared to other grains (Gulvardy et al., 2014). Lysine, is a limiting factor in the amino acid balance of grains. However, it is approximately 4.2% in oats, greater than in other grains except rice (Peterson, 2011). When examining the whole protein fraction, while glutamine is lower than other grains, the combined glutamine-glutamic acid content (25% of total protein) is higher (Gulvardy et al., 2014). Overall, the amino acid balance in oats (Table 1.2) makes it a superior wholegrain than wheat and corn for overall protein quality (Pomeranz et al., 1973, Klose and Arendt, 2012).

1.3.1.2 Globulins

Compared to other cereal grains, oats have a high proportion of globulin-type storage proteins found in the starchy endosperm (Shewry and Halford, 2002). They account for up to 50-80% of total oat proteins (Gulvardy et al., 2014), compared to the proportion in wheat, rye and barley of approximately 5% (Shewry and Halford, 2002). The globulins are co-located with prolamins within the endosperm.

Table 1.2 Amino acid composition of oats, wheat, corn and rice

adapted from (Gulvardy et al., 2014, US Department of Agriculture, 2012)

Amino acid	Oats	Wheat flour	Corn meal	Brown rice
Essential amino acids (g / 100 g)				
Histidine	0.405	0.357	0.248	0.202
Isoleucine	0.694	0.443	0.291	0.336
Leucine	1.284	0.898	0.996	0.657
Lysine	0.701	0.359	0.228	0.303
Methionine	0.312	0.228	0.170	0.179
Phenylalanine	0.895	0.682	0.399	0.410
Threonine	0.575	0.367	0.305	0.291
Tryptophan	0.234	0.174	0.057	0.101
Valine	0.937	0.564	0.411	0.466
Non-essential amino acids (g / 100 g)				
Alanine	0.881	0.489	0.608	0.463
Arginine	1.192	0.648	0.405	0.602
Aspartic acid	1.448	0.722	0.565	0.743
Cysteine	0.408	0.275	0.146	0.096
Glutamic acid	3.712	4.328	1.525	1.618
Glycine	0.841	0.569	0.333	0.391
Proline	0.934	2.075	0.709	0.372
Serine	0.750	0.620	0.386	0.411
Tyrosine	0.573	0.275	0.330	0.298

1.3.1.3 Prolamins

Oats contain a lower proportion of prolamins relative to globulins with ~ 15% compared to ~ 80% of total oat protein (Rasane et al., 2013). The prolamins in oats are primarily avenins with low solubility (Shewry et al., 2013) due to their low molecular weight. Prolamins have a high percentage of proline and glutamine with low levels of lysine compared to other cereal grain protein fractions (Capouchova et al., 2004). Prolamins constitute 4 - 15% of the total protein found in oats (Gulvardy et al., 2014,

Janatuinen et al., 1995) compared with wheat (40 - 50%), rye (30 - 50%) and barley (35 - 45%) (Janatuinen et al., 1995).

The low proline percentage in oats makes it less immunogenic than wheat, however it can still be toxic to coeliac disease patients if ingested in large quantities (Wieser, 1996). Prolamins are the fraction of the grain that is harmful to those with coeliac disease (Gulvardy et al., 2014). Oats do not have T-cell stimulating epitopes (Hardy et al., 2015) and do not contain α-gliadin unlike wheat, barley and rye (Moulton, 1959), which makes it suitable for coeliac disease patients (Janatuinen et al., 1995). However, in Australia and New Zealand, it is not recommended for coeliac disease patients to consume oats, as it is a mid-season crop that could be contaminated by gluten containing grains (Coeliac Australia, 2015). Oats also is not recommended for coeliac disease patients as some are sensitive to avenin (Coeliac Australia, 2015).

1.3.1.4 Avenin

Avenins are the prolamin component of oat seeds, existing as both monomers and disulphide-linked aggregates. Avenins, like other cereal prolamins, contain proline and glutamine and have a low solubility (Comino et al., 2015). *In vitro* studies of jejunal mucosa have shown that the avenin portion of oats contains only two units per molecule of the amino acid sequences proline-serine-glutamine-glutamine or glutamine-glutamine-glutamine-proline (Shewry et al., 1992). These two amino acid sequences are the constituents that may be toxic to the mucosa in the small intestine of coeliac disease patients. The molecular weight is lower compared to one molecule of wheat prolamin (gliadin) which contains five units of these amino acid sequences. While oats contain these sequences, there is no antigenic relation to wheat gliadin or barley hordein (de Ritis et al., 1988), which may explain why oats are less toxic to coeliac disease patients compared to wheat and barley (La Vieille et al., 2016).

Evidence of gluten reactive T-cell activation following ingestion of oats is limited. *In vivo* studies show 10% of coeliac disease patients have an activation of avenin specific T-cells with the ingestion of oats (Hardy et al., 2015). These T-cells are cross-reactive with hordein proteins from barley. In an *ex vivo* challenge with barley, these T-cells that are capable of being activated by avenin peptides were activated, yet with

oats there was only a weak antigenic stimulation (Hardy et al., 2015) indicating that the avenin-reactive T-cells were activated by the consumption of barley not oats (Hardy et al., 2015). Further studies have indicated that oat consumption is safe for most coeliac disease patients including children (Högberg et al., 2004, Gatti et al., 2013).

1.3.1.5 Glutelin

Glutelins make up between 5% and 66% of the total protein in oats (Robert et al., 1985). Measurement of this fraction is difficult as they are hard to solubilise completely and are dependent on the extraction solvent and solvent concentration (Robert et al., 1985). Other studies have found that they are less than 10% of the total fraction (Gulvardy et al., 2014).

1.3.1.6 Albumin

The most common protein within the oat grain is water-soluble albumin. It is approximately 1 - 12% of the total oat protein fraction (Lásztity, 1998). Processing of oats leads to degradation of oat proteins into smaller peptides and amino acids in the globulin, prolamin and glutelin fractions, although proteins increased in the albumin fraction (Klose et al., 2009, Klose and Arendt, 2012). Most metabolically active proteins are found in the albumin fraction, which may account for this increase. In general, albumin and globulin have a higher lysine content, and the higher content of these two proteins and a lower content of glutamic acid and prolamin than other cereal grains (Lásztity, 1998) may make oats as a suitable dietary component for coeliac disease patients.

1.3.2 Oats fibre and other carbohydrates

1.3.2.1 Fibre

Fibre is an integral part of the diet, as it is beneficial in the control of most signs of metabolic syndrome (Delzenne and Cani, 2005, Davy and Melby, 2003). In both humans and animal models, dietary fibre regulates body weight, food intake, glucose homoeostasis, insulin sensitivity and cardiovascular disease markers, including serum lipid concentrations, hypertension and inflammatory markers (Davy and Melby, 2003, Delzenne and Cani, 2005). Foods with higher concentrations of dietary fibre have a positive effect on obesity, cardiovascular disease and type 2 diabetes (McKeown et al., 2004, Sahyoun et al., 2006).

Dietary fibre in all foods, including oats, has many physiochemical characteristics and properties (Galisteo et al., 2008), including increasing viscosity in the upper gastrointestinal tract (Drozdowski et al., 2010, Shimoyama et al., 2007), fermentation in the colon (Bednar et al., 2001, Topping and Clifton, 2001) and prebiotic effects (Gibson et al., 2017). Not only does fibre alter gastrointestinal function by improving laxation and increasing stool bulk, fibre also has metabolic significance by enhancing serum lipid concentrations and postprandial glycaemia as well as promoting satiety (El Khoury et al., 2012).

By combining different fibres, the chemistry of food is altered, and many have differing effects based on concentration and type of fibre present (Bernstein et al., 2013). However, due to this complexity, it has been difficult to define which mechanisms exert the beneficial effects on metabolic syndrome. Soluble gel-forming fibres, such as β-glucans, regulate metabolic disturbances through mechanisms linked to this gel-forming capability (Galisteo et al., 2008) as well as their fermentability in the colon (Galisteo et al., 2008), while non-viscous insoluble fibre ameliorates these disturbances via other poorly understood mechanisms (Galisteo et al., 2008).

Dietary fibre is characterised based on the solubility in a buffer and enzyme solution similar to those found in the human gastrointestinal tract (Cho et al., 1997). Insoluble fibre, such as cellulose, resistant starch and some hemicelluloses, increases faecal bulk and excretion of bile acids as well as having a laxative effect and decreasing intestinal

transit time (Cummings and Stephen, 2007). Soluble fibre, such as β-glucans, increases transit time by delaying gastric emptying and slowing glucose absorption (Cummings and Stephen, 2007). However, this is a generalised characterisation of fibre, as only soluble viscous fibre delays gastric emptying time and slow glucose absorption while non-viscous soluble fibre acts as a substrate for microbial fermentation in the colon (Wong et al., 2006).

1.3.2.2 Soluble Fibre – Hemi-celluloses: arabinoxylans, β-glucans

Hemicelluloses are polysaccharide fibres of the cell wall, of which the main ones are arabinoxylans and β-glucans, which are highly fermentable soluble fibres. These convey health benefits such as lowering cholesterol and stabilising post-prandial blood glucose concentrations (Bernstein et al., 2013). Pectins and arabinogalactans make up a small part of the cell wall, but no substantial contribution to health has been found for these components. Minor amounts of xyloglucans, glucomannans and galactomannans are also found (Bernstein et al., 2013).

1.3.2.2.1 Arabinoxylans

Arabinoxylans are found in a range of grains including oats, barley, sorghum, wheat, corn, millet and in the pseudo-cereals of quinoa and amaranth (Roubroeks et al., 2000, Damen et al., 2012, Bernstein et al., 2013). They are the major hemicellulose component of the cell wall of the starchy endosperm and bran (Ebringerova et al., 2005). Arabinoxylans consist of a xylose backbone with arabinose side chains which attach randomly by $1\alpha \rightarrow 2$ or $1\alpha \rightarrow 3$ linkages (Roubroeks et al., 2000). The arabinose units bind water and assist in the production of viscous solutions and fermentation of short chain fatty acids (Damen et al., 2012).

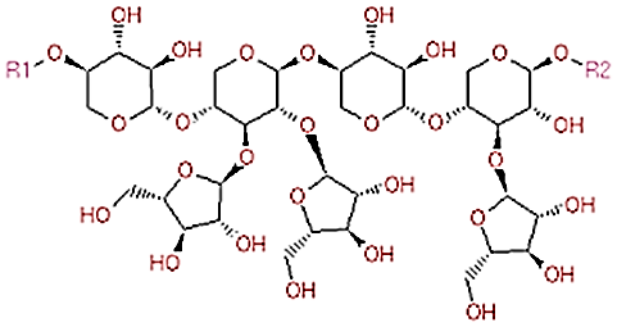


Figure 1.2 Structure of arabinoxylans

(US National Library of Medicine, 2016)

Arabinoxylans (6 g/day or 12 g/day) decreased area under the curve for postprandial glucose concentrations by between 20% and 41%; however, this study only had 12 participants (Lu et al., 2000). A small 6 - week study with 14 participants who included 15 g arabinoxylans in the diet showed decreases in plasma concentrations of glucose, insulin and triglycerides (Garcia et al., 2006). However, another 5 - week study with 15 g/day arabinoxylans in the diet showed no changes in blood lipids, body weight, fat mass or blood pressure (Johnston et al., 2010). A 3 - week study using arabinoxylan-oligosaccharide, a fibre produced through enzymatic manipulation of arabinoxylans, showed no effect on blood lipids (Cloetens et al., 2010, Damen et al., 2012).

Arabinoxylans may have health benefits when added to the diet. However, since most studies were of short duration with small sample sizes, the health claims for cardiovascular benefits have not been established, although it appears that arabinoxylans lower the glycaemic index when ≥ 7 g/day is consumed (Bernstein et al., 2013).

1.3.2.2.2 β-Glucans

β-glucans are a water-soluble, highly viscous fibre with high fermentability that is found mainly in oats and barley in similar concentrations (Brown et al., 1999). Oats contains more β-glucans than whole wheat, with 69% compared to 36%, and corn at 23% (Gulvardy et al., 2014).

A major constituent of dietary fibre in both oats and barley is the β-1,3/1,4-glucan. The β-glucans consist of linear unbranched β – D - glucose with one 1β → 3 linkage for every three or four 1β → 4 linkages (Sundberg et al., 1996). β-glucans form long cylindrical molecules containing up to about 250,000 glucose units (Roubroeks et al., 2000).

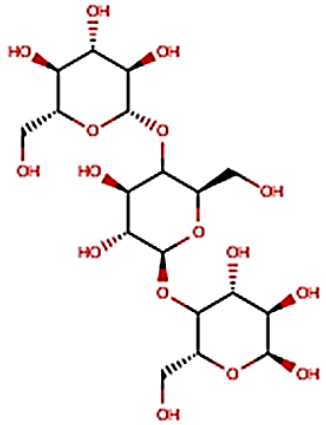


Figure 1.3 Structure of β-1,3/1,4-glucan

(US National Library of Medicine, 2016)

The mechanisms of the physiological effects of β-glucans may include increased viscosity, bile salt binding capacity or fermentability (Davidson and McDonald, 1998, Marlett et al., 1994) as the soluble fibre cannot be digested by humans but can be digested by various species of gut bacteria. β-glucans have a great potential in the treatment of diabetes (Chen and Raymond, 2008), certain cancers (Jacobs et al., 1998), high cholesterol (Braaten et al., 1994, Guo et al., 2014, Zhou et al., 2015) and to boost the immune system in immunocompromised patients (Volman et al., 2010). Many of these benefits appear to be due to β-glucans increasing satiety by modulating appetite regulating hormones, such as cholecystokinin (CCK), GLP-1 and PYY. Increases in postprandial CCK concentrations decreased insulin response and extended subjective satiety in overweight patients in a dose-related response from 2.16 g to 5.68 g/serve (Beck et al., 2009b). Plasma PYY increased after oat β-glucan ingestion, also in a dose-related response between 2.2 g and 5.5 g/serve with a significant difference after 4 hours between controls and the highest dose of β-glucan (Beck et al., 2009a). Another dose-related study showed that 2 g/serve lowered blood glucose concentrations, 4 g/serve was needed for gastrointestinal effects including the release of appetite hormones, while 10 g/serve in a drink that had its viscosity lowered by β-glucanases

increased CCK and GLP-1 compared to a high viscosity version of the same drink (Beck et al., 2010).

Many of the benefits gained from β-glucans depend on their molecular weight. β-glucans induced changes in gut microbiota populations and abundance in a molecular weight-dependent manner (Wilczak et al., 2015, Wang and Ellis, 2014). Low molecular weight β-glucans at 3 g/day and 5 g/day failed to alter gut microbiota, while 3 g/day of high molecular weight β-glucans increased *Bacteroidetes* and decreased *Firmicutes*. This change in microbial abundance correlated with improvements in cardiovascular risk factors, such as BMI, waist circumference, systolic blood pressure and plasma triglycerides (Wang et al., 2016).

The processing of oats has an impact on the cholesterol-lowering ability of the β glucans, as it changed the structure and solubility (AbuMweis et al., 2010). The lipid-lowering effects of β-glucans depended on the food matrix (Kerckhoffs et al., 2003).

β-glucans have a wide range of physiological benefits depending on dose, form, molecular weight and carrier food, therefore continued studies are needed to clarify these interactions.

In 2013, the European Union accepted two health claims for β-glucans: firstly, that they help control blood cholesterol concentrations and secondly, that they attenuate postprandial peaks in blood glucose concentrations following EFSA scientific opinion (EFSA Panel on Dietetic Products, 2011). In addition to this, the EU acknowledges that insoluble fibre improves the wellbeing of the stomach and gut, but has not accepted this as a health claim. The recommended daily intake of β-glucans in both the USA and Europe is 3 g/day which is 30 – 150 g oats/day as oats contain between 2 - 10% β-glucans depending on the component of the oat consumed (El Khoury et al., 2012).

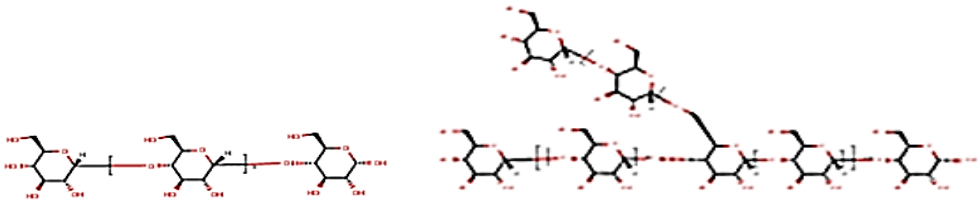
1.3.2.3 Insoluble Fibre - Cellulose and Resistant starch

1.3.2.3.1 Cellulose

Cellulose has little effect on concentrations of either blood lipids or glucose (Bernstein et al., 2013). However, cellulose adds to the faecal bulk and improves digestion within the colon.

1.3.2.3.2 Resistant starch

Resistant starch is soluble and non-viscous fibre that has variable fermentability and as yet there are no established cardiovascular benefits (Johnston et al., 2010). Resistant starches found in the endosperm are composed of amylose, a linear glucose polymer, and amylopectin, a branched-chain glucose polymer.



a) amylose b) amylopectin

Figure 1.4 Structure of a) amylose and b) amylopectin

(US National Library of Medicine, 2016)

Resistant starch behaves as a dietary fibre as it remains undigested until reaching the colon. Resistant starch has four subtypes based on its structure and source, labelled RS1 - 4. RS1 is physically inaccessible to digestive enzymes and found in whole grains or partially milled grains (Haralampu, 2000). RS2 has the nature of a starch granule and comes in raw potatoes, bananas and legumes. Its nature is due to the tightly packed amylose and amylopectin chains within the granule (Haralampu, 2000). RS3 has retrograded amylose and amylopectin in cooked and cooled foods, such as potatoes and bread (Haralampu, 2000). Retrogradation occurs when amylose and amylopectin are heated and then cooled, reforming a more crystalline structure with more hydrogen bonding than before heating, leading to greater gelatinisation of the starches and greater viscosity (Wang et al., 2015). RS4 comes about through chemical modification (Peterson, 2011). Each subtype has its own physical and chemical characteristics, and these influence the rate and site of fermentation in the gut.

Resistant starch contents are lower in cereal flour than in wholegrains, possibly due to the encapsulation of the starch within the grain structure (Haralampu, 2000). Starch granule size plays a role in increasing digestibility as enzymatic reactions in smaller

granules are facilitated by the larger specific surface area (Tester et al., 2004). Oat starch has a granule size of between 3 - 10 µm (Delcour, 2010) compared to other cereals such as quinoa (1 - 2 µm), buckwheat (3-9 µm), teff (2 - 6 µm), sorghum (~ 20 µm) and wheat (2 - 10 µm or 20 - 35 µm depending on variety) (Wolter et al., 2013).

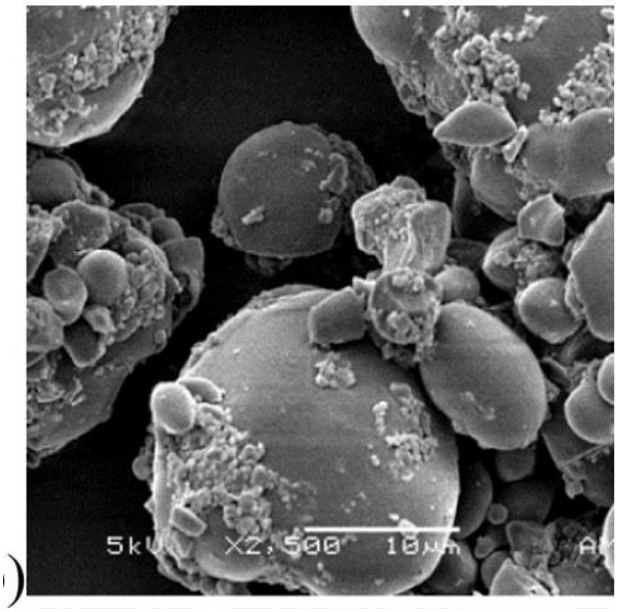


Figure 1.5 Scanning electron microscope image of oat starch

(Wolter et al., 2013)

There is limited long-term data on the effect of resistant starch on glucose metabolism (American Diabetes Association, 2008). However, short-term studies indicate that RS2 can improve insulin sensitivity, but not change body weight, visceral or liver fat deposits, or inflammation (Robertson et al., 2003, Maki et al., 2012). By consuming both resistant starch and β-glucans concurrently, post-prandial plasma glucose and insulin concentrations improve (Behall et al., 2006).

Both the Dietitians Association of Australia and the National Institute of Medicine in the USA have given a guideline of daily fibre intake of 14 g/1000 kcal, which average at 25 g fibre/day for women and 38 g fibre/day for men (Food and Nutrition Board, 2002, Dietitians Association of Australia, 2016).

1.3.2.3.3 Starch digestibility

The rate of starch digestion plays a significant role in the physiological responses achieved through its consumption. The combination of starch granule size, the extent of gelatinisation, composition and structure (Tester et al., 2004), physical encapsulation of the starch within in the grain and the protein and lipid content within the matrix of the grain (Singh et al., 2010) control the rate of digestion. A higher gelatinisation temperature in oats, as well as sorghum and teff compared to wheat, leads to a lower predicted glycaemic index for these grains (Wolter et al., 2013). Digestion rates of oat grains by α-amylase are slower than other grains such as quinoa, buckwheat and teff due to its larger granule size (3-10 μm) which is comparable to wheat (which is bimodal with 2-10 μm – B - type, and 20-35 μm – A - type) (Delcour, 2010).

The composition of the starch found in the grain is also important as starches that are high in amylose have a highly compact structure decreasing the availability of surface area to enzymatic attack. Starches with increased amylopectin, a larger molecule with a larger surface area, are the preferential molecular target for α-amylase (Singh et al., 2010). The higher amylose content in oats (20.5% dry weight) leads to a lower predicted glycaemic index (Wolter et al., 2013). The formation of amylose-lipid complexes impedes susceptibility to enzymes, and the higher lipid content of oats (7.5% dry weight) together with its high amylose may lead to an amylose-lipid complex hindering the enzymatic breakdown of starch (Wolter et al., 2013). Dietary fibre may also impede α-amylase actions by increasing the viscosity of the gastrointestinal contents (Sasaki and Kohyama, 2012). The presence of β-glucans in oats may lead to the reduced digestibility of starch by increasing viscosity resulting in decreased carbohydrate absorption in the gut (Rao and Tattiyakul, 1999).

1.3.3 Lipids

1.3.3.1 Fatty acids

Oats have a relatively high fatty acid content compared to other cereal grains (Morrison, 1978, Youngs, 1986). Thirteen fatty acids are in the lipid fraction of the oat

endosperm (Table 1.3). Palmitic, oleic and linoleic make up 95% of the total, with myristic, stearic and α-linolenic being the other major contributors. Palmitoleic, arachidic, gadoleic, behenic, erucic, lignoceric and nervonic fatty acids are also present in minimal concentrations (Zhou et al., 1998). Australian oat varieties have shown medium chain fatty acid concentrations of 38 - 43% oleic, 36 - 40% linoleic and 17 - 19% palmitic acids (Zhou et al., 1998), indicating that oats have mostly unsaturated fatty acids which may play a major role in realising the health benefits.

Table 1.3 Fatty acid concentration of Australian oat varieties

adapted from (Zhou et al., 1998)

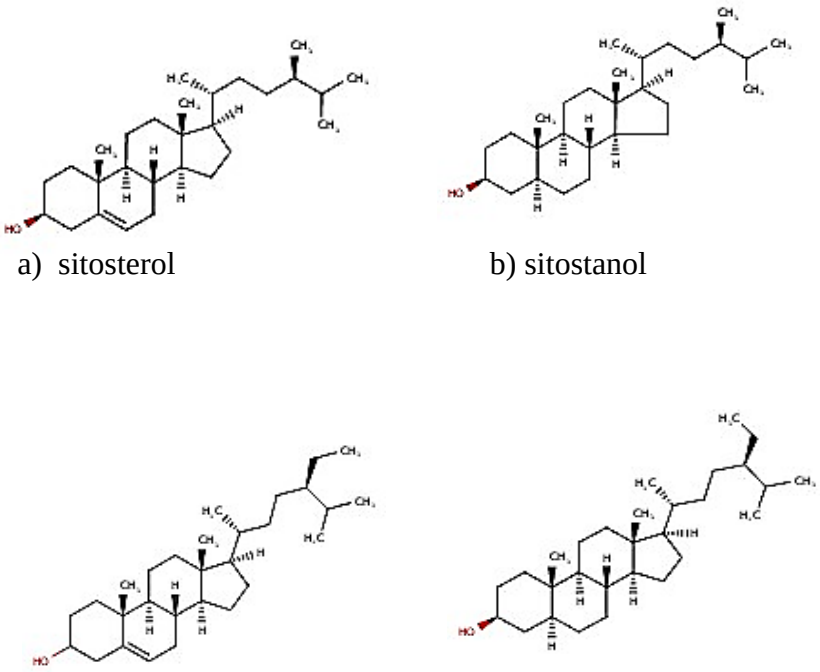
Fatty acid	Common name	% in Australian oat varieties
	Unsaturated	
16:1	Palmitoleic	> 1
18:1	Oleic	38 – 43
18:2	Linoleic	36 - 40
18:3	Linolenic	1 - 2
20:1	Gadoleic	>1
22:1	Erucic	>1
24:1	Nervonic	>1
	Saturated	
14:0	Myristic	> 2
16:0	Palmitic	17 – 19
18:0	Stearic	1 – 2
20:0	Arachidic	>1
22:0	Behenic	>1
24:0	Lignoceric	>1

These medium chain fatty acids are rapidly absorbed, transported to and metabolised by the liver, which makes them similar to carbohydrates rather than other fats as there is less tendency for excess to be stored as body fat (Chwen et al., 2013). Medium chain

fatty acids stimulate intestinal mucosal growth and increased villi height which directly affect nutrient absorption by increasing the absorptive and surface area of the duodenum, jejunum and ileum (Chwen et al., 2013, Czernichow et al., 1996).

1.3.3.2 Phytosterols

Phytosterols (plant sterols) are chemically similar to cholesterol and compete with it for absorption from the gastrointestinal tract. Usual consumption of phytosterols does not affect this absorption; however, when consumed in higher amounts, phytosterols inhibits cholesterol production (Maki et al., 2003). The main sterols found in oats are sitosterol, sitostanol, campesterol and campestanol (Maki et al., 2003, Laakso, 2014).



a) sitosterol b) sitostanol

c) campesterol d) campestanol

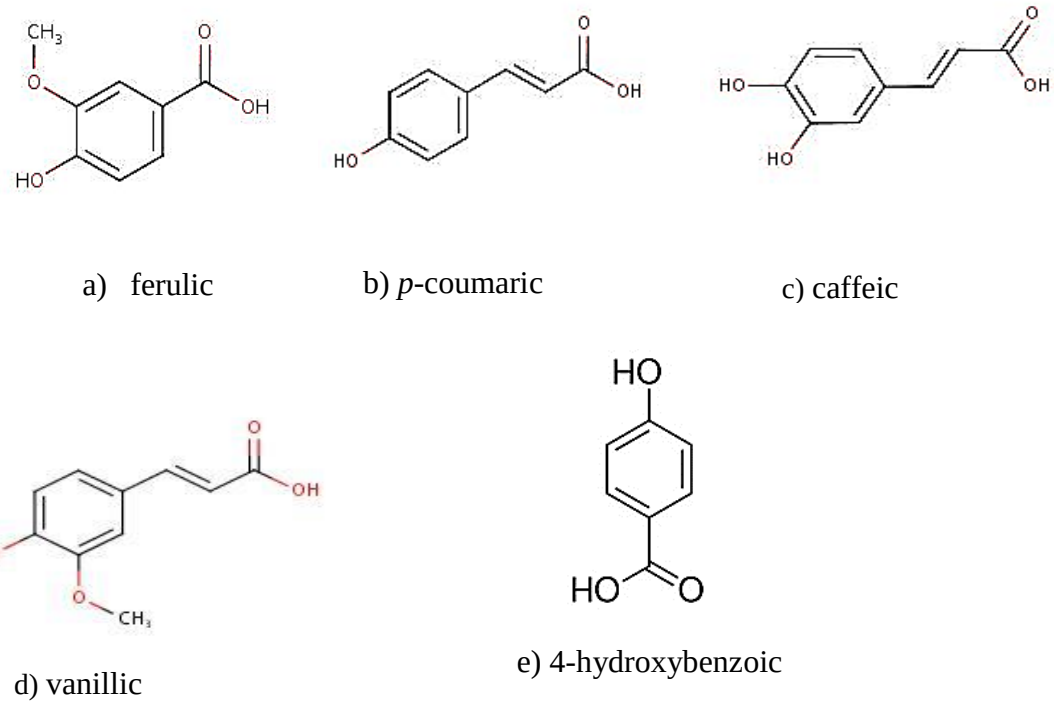
Figure 1.6 Structures of oat phytosterols a) sitosterol b) sitostanol c) campesterol and d) campestanol

(US National Library of Medicine, 2016)

1.3.4 Phytochemicals

1.3.4.1 Phenolics

Oats are a valuable source of phenolic compounds, generally derived from hydroxybenzoic acid and hydroxycinnamic acids, that may contribute to the nutritional and functional benefits (Rasane et al., 2013). The major phenolic acids in oats are ferulic, *p*-coumaric, caffeic, vanillic and hydroxybenzoic acids (Figure 1.7) and their derivatives (Mattila et al., 2005, Kova cova and Malinova, 2007).



a) ferulic b) *p*-coumaric c) caffeic

d) vanillic e) 4-hydroxybenzoic

Figure 1.7 Major phenolic acids found in oats a) ferulic b) p-coumaric c)-caffeic d) vanillic and e) 4-hydroxybenzoic acids

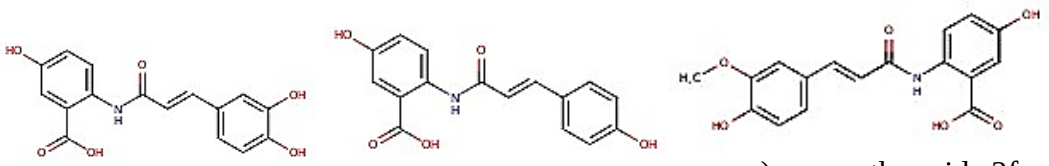
(US National Library of Medicine, 2016, Sigma Aldrich, 2016)

1.3.4.2 Avenanthramides

The avenanthramides are a unique group of antioxidants, found only in oats (Dimberg et al., 2001). There are over 20 different types of avenanthramides; however, the most abundant are 2c, 2p and 2f (Figure 1.8). The number 2 indicates that it is a 5 hydroxyanthranilic acid derivative and the letter is the type of hydroxycinnamic acid attached, either caffeic, p-coumaric or ferulic acids (Dimberg et al., 2001). Avenanthramides are produced in greater concentrations in oat leaves that are suffering from salinity stress and crown rust (Oraby et al., 2017, Antonini et al., 2016).

Avenanthramides have antioxidant potential up to 30 times greater than vanillin and caffeic acid (Dimberg et al., 1993). They may be anti-inflammatory and anti-atherogenic, as they inhibit monocyte adhesion to endothelial cells, inhibit release of pro-inflammatory compounds from macrophages (Liu et al., 2004) and control blood pressure through increased production of nitric oxide and dilatation of blood vessels (Nie et al., 2006). Avenanthramides are more bioavailable in plasma, liver, heart and skeletal tissue in humans and hamsters than in rats (Koenig et al., 2011).

Wild green oat extract (WGOE) contains high concentrations of avenanthramides. WGOE improved cognition a dose of 1600 mg in older adults of below average cognition (Berry et al., 2011). However, a dose of 1500 mg in older adults with normal cognition gave no change in any cognitive measure (Wong et al., 2012). These studies indicate that avenanthramides which suppress inflammatory cytokines by inhibiting NF-κB activation (Guo et al., 2008) and enhance nitric oxide (NO) production in smooth muscles (Nie et al., 2006) causing vasodilation may improve the function of systemic and cerebral arteries and have a potential role in the maintenance of cardiovascular health.



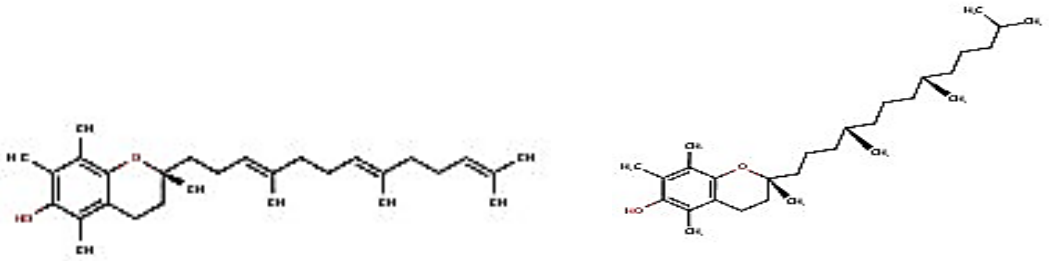
a) avenanthramide 2c b) avenanthramide 2p c) avenanthramide 2f

Figure 1.8 Structure of the most common avenanthramides in oats, a) 2c b) 2p and c) 2f

(US National Library of Medicine, 2016)

1.3.4.3 Vitamin E

Oats are high in tocopherols, particularly α-tocopherol (Figure 1.9) which is found in its highest concentration in the germ, while the tocotrienols, mainly α-tocotrienol (Figure 1.9) with small amounts of β-tocotrienol, are mainly concentrated in the endosperm (Rasane et al., 2013). Of the total tocols in oats, 86 - 91% are α-tocotrienol and α-tocopherol (Rasane et al., 2013). The tocol composition of oat and barley is similar, with α-tocotrienol predominant. However, barley averages twice the total tocol content of oat (58 mg/kg compared to 26 mg/kg) (Peterson, 2001). In wheat, β-tocotrienol is predominant, with γ-tocopherol highest in corn (Barnes, 1983). δ-tocotrienol and γ-tocotrienol have hypocholesterolaemic effects. However, both of these are in low concentrations in barley and absent or with only trace amounts in oats (Peterson, 2001). The processing of oats leads to faster degradation of these compounds, with unprocessed groat tocols stable for over seven months at room temperature, but for only 1 - 2 months after processing (Peterson, 2001). For the tocols to be beneficial to health, relatively large amounts of oats need to be consumed unprocessed or as soon after processing as possible.



a) α-tocotrienol b) α-tocopherol

Figure 1.9 Structure of the most abundant tocols in oats a) α-tocotrienol and b) α-tocopherol

(US National Library of Medicine, 2016)

1.4 Potential for metabolic syndrome and gastrointestinal health

Cereal wholegrains, such as oats, containing a combination of fibre, protein, vitamins and minerals are digested more slowly than refined grains and have protective effects in the body potentially due to the stimulation of growth of appropriate gut bacteria. Wholegrains also generally maintain lower blood glucose concentrations and improve insulin response in the body (Honda et al., 1999). Subjects who consume higher amounts of dietary fibre have lower blood pressure and serum cholesterol concentrations, with reduced glycaemia and improved insulin sensitivity (Zhou et al., 2015). These improved markers of health indicate lower risks of developing stroke, hypertension, type 2 diabetes, obesity and gastrointestinal diseases (Honda et al., 1999).

1.4.1 Metabolic syndrome

Metabolic syndrome is associated with an increased risk of type 2 diabetes and cardiovascular disease. Co-morbidities such as hypertension, insulin resistance, dyslipidaemia, fatty liver and obesity make up metabolic syndrome. According to the International Diabetes Foundation, metabolic syndrome is defined as a combination of central obesity (waist circumference) and two of high blood pressure, raised triglyceride concentrations, low HDL-C, high fasting glucose or diagnosed type 2 diabetes or history of treatment for the above abnormalities (Parikh and Mohan, 2012). Other national health foundations, such as American Heart Association and the National Cholesterol Education program also had similar definitions although differing slightly. In 2009, these groups came together with a consensus definition as shown in Table 1.4.

Additional symptoms may be considered when diagnosing metabolic syndrome, including abnormal uric acid metabolism, pro-thrombotic factors such as plasminogen activator inhibitor-1 and fibrinogen, inflammatory markers such as C-reactive protein, white blood cell count and endothelial dysfunction such as mononuclear cell adhesion and endothelial-dependent vasodilatation (Einhorn et al., 2003, Parikh and Mohan, 2012).

Table 1.4 Consensus definition of metabolic syndrome

CRITERIA	NCEP ATP III (2001)	AHA/NHLBI (2004)	IDF (2005)	Consensus Panel (2009)
Criteria	Any 3 of 5 criteria	Any 3 of 5 criteria	Central obesity plus 2 of the other 4 criteria	Any 3 of the 5 criteria
Central obesity (abdominal circumference)	Men: > 102 cm Women: >88 cm	Men: >102 cm Women: >88 cm	Population and country specific definitions	Population and country specific definitions
Hypertension	≥ 130/85 mmHg	≥ 130/85 mmHg	≥ 130/85 mmHg	≥ 130/85 mmHg
Dyslipidaemia (triglycerides)	≥150 mg/dL (1.7 mmol/L)	≥ 150 mg/dL (1.7mmol/L)	≥150 mg/dL (1.7 mmol/L)	≥ 150 mg/dL (1.7mmol/L)
Dyslipidaemia (HDL-C)	Men: <40 mg/dL (1.0 mmol/L) Women: <50 mg/dL (1.3 mmol/L)	Men: <40 mg/dL (1.0 mmol/L) Women: <50 mg/dL (1.3 mmol/L)	Men: <40 mg/dL (1.0 mmol/L) Women: <50 mg/dL (1.3 mmol/L)	Men: <40 mg/dL (1.0 mmol/L) Women: <50 mg/dL (1.3 mmol/L)
Hyperglycaemia	≥110 mg/dL	≥ 100 mg/dL	≥100 mg/dL	≥ 100 mg/dL

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

1.4.2 Effect of oats on metabolic syndrome

1.4.2.1 Anti-hypercholesterolaemic and anti-lipidaemic effects

Dyslipidaemia is characterised by low plasma concentrations of HDL cholesterol and high concentrations of triglycerides, which indicate an increased risk of developing cardiovascular disease (Galisteo et al., 2008). Many studies indicate the hypocholesterolaemic effects of dietary fibre, particularly soluble fibre on blood lipids and cholesterol concentrations (Braaten et al., 1994, Maki et al., 2003). Oat proteins, lipids and phytosterols content may also play a role in reducing plasma cholesterol concentrations (Guo et al., 2014, Maki et al., 2003, Biel and Jacyno, 2014).

Oats are known for their hypocholesterolaemic effects, with β-glucans as the most effective bioactive component (Braaten et al., 1994). A meta-analysis in 2011 found that β-glucans produced cardiovascular health benefits with between 2 - 10 g/day, the equivalent of 30 - 100 g of wholegrain oats/day decreasing total cholesterol and LDL-cholesterol concentrations (Tiwari and Cummins, 2011). β-glucans in both oats and barley decreased total cholesterol and LDL-C, however, only the β-glucans in oats increased HDL-cholesterol (Tiwari and Cummins, 2011, Ripsin et al., 1992). Barley β-glucans decreased LDL-cholesterol, and as the structure is very similar to that of oats, it could be suggested that the same may occur in a diet consisting of oats (De Angelis et al., 2015). The recommended daily intake (RDI) of 3 g/day of β-glucans revealed a modest decrease in total plasma cholesterol concentrations of 0.30 mmol/L, yet there was no additional change when the dosage was increased to above 3 g/day (Tiwari and Cummins, 2011).

Although the fibre in oats has a hypocholesterolaemic effect, this is likely only to be slight (Brown et al., 1999). However, when wholegrain oats are the source of fibre, the overall effect is greater (Galisteo et al., 2008, Connolly et al., 2012), indicating the benefit is coming from more than just the fibre content. The satiation effects of both soluble and insoluble fibre may be the key to the cholesterol-lowering ability with an increase in bile acid excretion (van Bennekum et al., 2005, Fernandez, 2001). The viscosity of the fibre in the intestinal lumen has an impact on hepatic cholesterol metabolism and the synthesis of lipoproteins (Galisteo et al., 2008). This viscosity may

be the mechanism behind oats cholesterol lowering abilities as β-glucans increase the viscosity of digesta within the gastrointestinal tract. Incretin hormones, such as GLP-1, could have a hypolipidaemic effect (Kok et al., 1998). The incretin hormones control glucose homoeostasis and insulin secretion through the regulation of fatty acid synthase in the liver, which is enhanced by insulin (Kim and Egan, 2008) . However, this mechanism still requires further clarification regarding responses to oats.

1.4.2.2 Anti-glycaemic effects

The glycaemic index is the incremental area under the curve (AUC) of blood glucose concentrations occurring upon ingestion of a carbohydrate-containing food relative to glucose as the reference food (GI glucose = 100) (Wolter et al., 2013). Food are classified as low (> 55, such as legumes, nuts, dairy, pasta), intermediate (55 - 70, such as muesli, different types of bread) or high GI (> 70, such as wholemeal barley bread and white wheat bread) (Wolter et al., 2013, Atkinson et al., 2008). High GI foods cause a rapid release of glucose, while a low GI food is one that has a high concentration of slowly digestible starch that enables a slower and lower increase of glucose concentrations.

The glycaemic response varies based on the food matrix, such as starch susceptibility, protein and lipid content as well as the physical structure of the food (Atkinson et al., 2008). While there is plenty of information regarding the glycaemic response of gluten-containing foods, studies on gluten-free foods are minimal, with those studies conducted on composite recipes rather than specific foods (Di Giacomo et al., 2013). *In vitro* assays can evaluate starch digestibility and calculate a predicted glycaemic index for food. However, *in vivo* results are altered by metabolic factors, such as gastric emptying rate, gut hormone profiles, absorption of glucose through the intestinal mucosa, accessibility of α-amylase to the starch, all of which will modify glycaemia. As the food structure may alter digestion rates through mechanical reasons, such as the slowing of gut transit time, wholegrains tend to have a lower GI than milled products (Granfeldt et al., 2000, Atkinson et al., 2008).

Oats have a lower GI of 71 compared to other grains including wheat - 100, sorghum - 72, quinoa - 95, teff - 74, buckwheat - 80 (Wolter et al., 2013), white rice - 92

and brown rice - 87 (University of Sydney, 2016). Although the GI of oats is classified as high, it can still lower insulin secretion *in vivo* due to its low carbohydrate content (Wolter et al., 2013). An improved glycaemic response through the consumption of oats affects the availability of energy stores by modifying physiological adaptations to energy restriction.

The mechanism in which oats may improve glycaemic control depends on the rate of starch digestion, through the combination of granule size, gelatinisation, composition and structure of the grain, as well as the lipid and protein matrix (Singh et al., 2010). *In vitro* studies indicate that starch digestibility plays a relevant role in the effect that oats have on glycaemia. However, metabolic factors *in vivo*, such as rate of gastric emptying, gut hormone profiles, absorption of glucose through the intestinal mucosa and availability of starch to enzymatic attack also play a significant role (Berti et al., 2004, Fardet et al., 2006).

1.4.2.3 Anti-hypertensive effects

Extensive clinical and animal trials with dietary oat supplementation in hypertension have taken place. However, results are inconclusive with a small number of studies indicating that the consumption of oats reduced systolic blood pressure by 4 - 6%, but with small sample sizes (Saltzman et al., 2001, Keenan et al., 2002). These studies were conducted with hypocaloric diets and on subjects with mild or borderline hypertension, which may have been confounding factors. Many of these studies were evaluating the effect of the wholegrain or bran, which provide several factors that may lead to reductions in blood pressure, such as fibre, magnesium and potassium, and these synergistic interactions can result in a greater reduction in blood pressure.

Other studies found that oat consumption, specifically β-glucans, had no effect on systolic blood pressure (Thies et al., 2014, Swain et al., 1990, Maki et al., 2007, Davy et al., 2002, Kestin et al., 1990). These effects could have been due to limitations in the restrictions on the subjects to those with high BMI, relatively high attrition rates and compliance issues, as palatability of the study food was less than with wholegrain or bran.

1.4.2.4 Adiposity and anti-obesity effects

Several studies suggest an inverse relationship between dietary fibre intake and body weight (Koh-Banerjee et al., 2004, Slavin, 2005), body mass index (van de Vijver et al., 2007) and body fat mass(Nelson and Tucker, 1996) in men (Nelson and Tucker, 1996, Koh-Banerjee et al., 2004). Studies in women (Liu et al., 2003) also demonstrate this relationship. However, many studies on oat bran and β-glucans have failed to show any effect (Anderson et al., 1990, Kirby et al., 1981, Jenkins et al., 2000, Judd and Truswell, 1981, Robitaille et al., 2005, Saltzman et al., 2001, McKeown et al., 2004). These studies were mainly investigating the impact on hypercholesterolaemia and hypertension rather than changes in body weight parameters.

1.4.2.5 Anti-inflammatory and antioxidant effects

Inflammatory markers such as C-reactive protein (CRP), IL-6 and intercellular adhesion molecule 1 (ICAM-1) are associated with cardio-metabolic disease risk, but only CRP is considered an independent marker of cardiovascular disease risk (Pearson et al., 2003). Observational studies have suggested that a high fibre diet may reduce CRP concentrations (Ma et al., 2006, Ajani et al., 2004) while oat bran and wheat bran reduced oxidative stress induced by a high-fat diet in pigs (Rezar et al., 2003).

It is speculated that some of the 20 avenanthramide compounds in oats are anti-inflammatory and anti-atherogenic by inhibiting monocyte adhesion to the endothelial cells of the aorta and down-regulation of inflammatory cytokines and chemokines (Liu et al., 2004). There have been a limited number of studies examining the bioavailability of avenanthramides. However, most examine 2c, 2f and 2p avenanthramides only which can be detected at low concentrations in the circulating blood and taken up by hepatic, cardiac and skeletal tissues of rats (Yang et al., 2014).

Since oat grains are rich in lipids, they contain a number of compounds that protect the lipids from oxidation, with both tocopherols and avenanthramides having antioxidant effects (van den Broeck et al., 2016). It may be that this synergistic relationship is leading to the effect rather than any individual component.

Anti-inflammatory activity was found in a study utilising β-glucan-enriched pasta (75% durum wheat and 25% wholegrain barley flour) which modified gut microbiota composition leading to improved health outcomes (De Angelis et al., 2015). Faecal and blood samples were collected before consumption and after eight weeks on the β-glucan pasta diet. Faecal samples showed an increase in beneficial *Lactobacilli* and a decrease in the harmful *Enterobacteriaceae* species. An increased production of short chain fatty acids including 2-methylpropionic, acetic, butyric and propionic acids as bacterial metabolites may lead to anti-inflammatory activity (De Angelis et al., 2015).

1.4.3 Gastrointestinal Health

The impact of cereal grains, particularly wholegrains, on intestinal microbiota and the contribution this makes to cardio-metabolic disease has recently become a more focused area of study (Carvalho-Wells et al., 2010, Costabile et al., 2008). Many grains contain components classified as prebiotics, including oats that contain β-glucans. Although they have not been definitively proven to be prebiotic, β-glucans are classified as having prebiotic potential (Arena et al., 2014). β-glucans may possess immunomodulatory characteristics, stimulating gut immune responses in several animal species (Bonaldo et al., 2007, Eicher et al., 2006, Li et al., 2006). However, most of these studies have studied the β-glucans found in the cell walls of yeast (*Saccharomyces cerevisiae),* not from grains (oats or barley). Therefore, results may be different due to the change in molecular weight and structure when comparing the two types.

1.4.3.1 Prebiotics

Prebiotics are a substrate that is selectively utilised by host microorganisms to confer a health benefit (Gibson et al., 2017). Until recently, these substrates were carbohydrates (Roberfroid et al., 2010, Gibson et al., 2015), however the updated consensus statement from the International Scientific Association for Probiotics and Prebiotics (ISAPP) now includes non-carbohydrate substances, such as polyphenols and polyunsaturated fatty acids converted to conjugated fatty acids assuming adequate evidence of health benefits in the target host (Gibson et al., 2017). For a prebiotic to

be effective, it must be able to resist gastric acidity, enzymatic hydrolysis and absorption in the upper gastrointestinal tract. It also must be fermentable by one or more intestinal microflora and selectively stimulate the growth of these bacteria (Roberfroid et al., 2010, Slavin, 2013). Humans have consumed prebiotics for many thousands of years as a normal part of the diet (Leach and Sobolik, 2010, Pontzer et al., 2012). However, it is only recently that studies have investigated the mechanisms of their potential health benefits (Costabile et al., 2015, Rastall and Gibson, 2015, Gibson et al., 2017, Richards et al., 2016, Kumar et al., 2016). Prebiotics may attenuate obesity, obesity-related inflammation and obesity-related diseases (Bednar et al., 2001, Neyrinck et al., 2008, Roberfroid et al., 2010) through fermentation in the colon which alters the gut microbiome (Ley, 2010, Rowland et al., 2017). Further information on prebiotics in obesity is contained in the journal article in section 1.4.3.1.1 Prebiotics in Obesity.

As prebiotics improve gut barrier function, pathogenic bacteria are restricted from entering the bloodstream to initiate inflammation (Wells et al., 2017, Catalioto et al., 2011) . Further, the beneficial bacteria can produce compounds that enhance host metabolism and endocrine function (Moreira et al., 2012). The gut microbiome produces by-products such as hydrogen, lactate, methane, carbon dioxide and short chain fatty acids, including acetate, propionate and butyrate. The mechanisms of action of prebiotics and short chain fatty acids on the benefits to gut health, reduced inflammation and obesity have been reviewed (Slavin, 2013).

Propionic and butyric acids may have a positive metabolic effect by modulating pro- and anti-inflammatory markers present in the gut (Galisteo et al., 2008). The consumption of oats increased caecal amounts of short-chain fatty acids, particularly propionic and butyric acid (Berger et al., 2014); however, the amounts differed depending on the type of oat product (Berger et al., 2014).

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Prebiotics in obesity

S. CARNAHAN, A. BALZER, S. K. PANCHAL, L. BROWN

School of Health, Nursing and Midwifery
University of Southern Queensland
Toowoomba, QLD 4350, Australia

Obesity was probably rare in ancient times, with the current increase starting in the Industrial Revolution of the eighteenth century, and becoming much more widespread from about 1950, so concurrent with the increased consumption of carbohydrates from cereals in the Green Revolution. However, dietary components such as oligosaccharides from plants including cereals may improve health following fermentation to short-chain carboxylic acids in the intestine by bacteria which constitute of the microbiome. Such non-digestible and fermentable components of diet, called prebiotics, have been part of the human diet since at least Palaeolithic times, and include components of the cereals domesticated in the Neolithic Revolution. If consumption of these cereals has now increased, why is obesity increasing? One reason could be lowered prebiotic intake combined with increased intake of simple sugars, thus changing the bacteria in the microbiome. Processing of food has played an important role in this change of diet composition. Since obesity is a low-grade inflammation, changing the microbiome by increased consumption of simple carbohydrates and saturated fats may lead to obesity via increased systemic inflammation. Conversely, there is now reasonable evidence that increased dietary prebiotic intake decreases inflammation, improves glucose metabolism and decreases obesity. Would widespread increases in prebiotics in the modern diet, so mimicking Palaeolithic or Neolithic nutrition, decrease the incidence and morbidity of obesity in our communities?

KEY WORDS: Obesity - Prebiotics - Microbiota - Inflammation.

Food is essential to life, with famine as a result of too little food, and obesity as a symptom of excessive food intake. Both famine and obesity are an integral part of human history. They have been connected through the thrifty gene hypothesis, referring to metabolic traits that allowed humans to store energy during times of excess, to be used in times of famine. This attractive hypothesis has generated intense discussion and interesting arguments including that genetic variability in metabolic genes within populations is a means of distributing risk across offspring in the absence of strong selective pressures.[1] However, the thrifty gene hypothesis has been deemed untenable for many reasons, including that hunter-gatherers do not become obese between famines, with body mass index values remaining between 17 and 21.[2] Further, the presence of both slim and obese humans in modern societies suggests that gene mutations are essentially neutral, suggesting genetic drift rather than selection.[3]

The prevalence of obesity has increased markedly in the last two centuries, but there is sufficient evidence for obesity dating back tens of thousands of years.[4] From prehistoric times until the eighteenth century, overweight and obesity were probably rarely seen, and this remains a characteristic of modern hunter-gatherer societies. Prehistoric statuettes such as the so-called Venus figurines found across southern and central Europe show exaggerated female abdomen, breasts, hips and thighs. The oldest of these Palaeolithic statuettes, found in 2008 at Hohle Fels in southern Germany, is between 35,000 and 40,000 years old [5] while the well-known Venus of Willendorf in Austria is about 25,000 years old.[6] Since the first statuettes were found in the 1860's, arguments have focused on whether they are depictions of actual obesity or rather symbols of fertility

Corresponding author: L. Brown, School of Health, Nursing and Midwifery, University of Southern Queensland, Toowoomba, QLD 4350, Australia. E-mail: Lindsay.Brown@usq.edu.au

and abundance. The historical evidence for obesity continues with figurines from the Neolithic period,[7] defined as the beginning of farming between 10,200 and 8,800 BC and ending between 4500 and 2000 BC. In ancient civilisations, only the wealthy could afford to be obese; one documented example is the Ptolemy rulers of Egypt who succeeded Alexander the Great, reigning until 30 BC.[8] The concept that obesity is evidence of conspicuous consumption is an ancient one, but it became clearly developed in the Age of Enlightenment (eighteenth century) as a negative social perception of obesity together with an association with suspect morals and excess, and as an outward representation of the soul.[9]

Prevention of obesity also has a long history, beginning with the writings of Hippocrates (c. 460 BC-c. 370 BC) [5] and continued with the writings of Soranus of Ephesus (2nd century AD) and Caelius Aurelianus (5th century AD).[10-12] Treatments included purging and vomiting, fasting and exercise.[4, 10-12] Many physicians of antiquity recognised that diet was an important factor in both the cause and the attenuation of the disease. Dietary treatments included foods such as cheese, vegetables, mustard seeds and herbs. Alexander Trallianus wrote in the 6th century AD of a diet including indigestible foods, vegetables, barley, rice and legumes as well as fish, seafood and fruit to treat obesity, so an early version of functional foods.[12] The ideas of the ancients appear to be a foundation for modern conservative management of obesity.

This review will discuss the relationships between prebiotics and obesity, without attempting to cover the enormous literature on either prebiotics or obesity. A prebiotic is defined as a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health.[13] The WHO defines overweight and obesity as abnormal or excessive fat accumulation that presents a risk to health for a number of chronic diseases including diabetes, cardiovascular diseases and cancer.[14]

The Neolithic Revolution – successful in providing healthy diets?

The domestication of plants including cereals in the Fertile Crescent during the Neolithic period,[15, 16] termed the Neolithic Revolution, allowed a sedentary life-style with the formation of permanently settled farming towns, later developing into cities. Domestication of cereals is part of a continuum from gathering to cultivation of wild plants, their domestication and then agriculture that probably occurred over thousands of years in widely spread areas.[17] There have been fascinating discoveries and vigorous discussions on the domestication of cereals; some of the more recent reviews discuss the domestication of rice, wheat and maize.[16, 18-22]

Maize was domesticated in modern Mexico, while another ancient crop from the Andean countries of modern Bolivia and Peru has gained recent attention. Quinoa (*Chenopodium quinoa* willd.), technically known as a pseudocereal, has been cultivated for 5000-7000 years with transmission throughout South America via livestock migrations and trade from Venezuela in the north to the southern extremities of Argentina and Chile.[23] Quinoa was considered by the Incas as the mother of grains, with the Emperor using a golden spade to dig the soil to plant the first seed of the new crop each spring. After the Spanish conquest in the 1500's, quinoa cultivation retreated to isolated pockets above 3500m where introduced European crops such as wheat, rye and oats could not be grown. Quinoa has all the properties of an ancient cereal with high protein content (12-17%), dietary fibre content (2-10%), lipids (5-9%) and carbohydrates (60-70%), with 11% as amylose.[23] Quinoa contains essential amino acids, with similar values to casein, the protein found in milk. Quinoa flour is high in xylose and maltose, and low in glucose and fructose, along with ribose and galactose, giving it a low glycaemic index value. The total lipid content of quinoa is 14% with linoleic (39%) and oleic (28%) acids being the most prevalent.[23] The rediscovery of quinoa and other ancient grains by consumers in developed countries may be no more than a consumer fad, but these functional foods may change the way we think about the components of our foods, especially prebiotics.

The human diet has changed dramatically since the introduction of agriculture in the Neolithic Revolution, unlike the human genome. Many factors including geography, agricultural advances and technology, the advent of food processing and food technologies, wars, climatic extremes and modern marketing have changed the food we eat. For most of human history, people only ate what was avail-

able in their locality. There were exceptions, but only for the very wealthy, such as the importation of spices from India to Europe. From the 15th century, when Western Europeans started exploring the world, exposure to new foods occurred with new species being introduced to new cultures. Agricultural advances saw the rise of certain crops and reduced production and consumption of others. In our more recent history, food processing has changed the way many humans hunt and gather their food, and with this has come a dramatic change in the intake of both micro- and macro-nutrients. Further, advances in agricultural productivity have changed the source and quality of the food.

The modern Green Revolution - unsuccessful in providing healthy diets?

Since the Industrial Revolution, there have been several periods of major changes in agricultural production and productivity, including famines such as the potato famine in Ireland from 1845-1852. In the following generation, Northern Europe went through a Green Revolution from 1870 to 1914 with marked increases in productivity.[24] The much more publicised Green Revolution from about 1960 to 2000 was hailed as the world's answer to imminent famine due to a rapidly increasing population. Production increased dramatically, with increases of 208% in wheat production, 109% for rice production and 157% for maize in developing countries between 1960 and 2000.[25] Further, these increased yields came with shorter growing periods. Without this Green Revolution, it has been estimated that food prices would have been 35-65% higher, with the risk of political instability, and caloric availability would have decreased by 11-13%; these changes have benefited all consumers, especially the poor.[25] Gains have been uneven, with China and India showing large improvements with much smaller improvements in sub-Saharan Africa. Even in countries with successful Green Revolution changes in productivity, poverty and food insecurity still exist as does micronutrient malnutrition. Further, environmental impacts have been mixed, with increased production allowing marginal land to return to forest cover, but with increased soil degradation and chemical run-off from productive land. Another critical issue has been the reduced research expenditure as agricultural production increased.[25] Although the Green Revolution is hailed as one of the most positive changes in modern agriculture, did the increased yield and profit come at the expense of nutritional quality and variety in the modern-day diet?

During the Green Revolution, emphasis was placed on increased production, with high-yielding strains with low economic input becoming predominant, specifically of rice, maize and wheat. The use of powerful breeding tools, increased nutrient inputs and shared knowledge resulted in provision of large quantities of food. The doubling in cereal production came largely from increased carbohydrate content, with grain weights typically being inversely proportional to the protein percent.[15, 16, 26] Increases in carbohydrate content require much less nitrogen input than improved protein content. Further, the selective increases in the major cereals may have led to decreased availability and hence consumption of other staple foods such as legumes, grains and cereal-based crops. Production of legumes in the Punjab region of India, a major food production area, has halved during the Green Revolution, with the difference being filled by rice and wheat cultivation.[27] This change decreased the nutritional diversity, purely due to availability.

The microbiome

The processes of absorption of nutrients from food are complex, with important roles of enzymes and hormones released into the gastrointestinal tract. Further, the essential role of the bacteria, especially in the colon, is now being realised. These bacteria, termed the microbiome or microbiota, are diverse with around 500 to 1000 bacterial species present accounting for 2 to 4 million genes (about 100-fold more genes than the human genome) and about 100 trillion bacteria (about 10-fold more cells than the human cells).[28] These bacteria obtain their nutrients from the food we eat, and they also change the components of the food before absorption by the human host. The different bacteria of the microbiome have very specific environmental needs, which are mostly met by the conditions in the distal colon. They are often unable to survive outside this environment, so culture of these species *ex vivo* is relatively unsuccessful with estimates suggesting only 30-40% can

be cultured.[29] The advent of molecular techniques has allowed the potential extent of the relationship between the microbiome and the human host to be investigated.

There is debate about the inheritability of the microbiome with some suggesting it is high,[30, 31] whilst others suggest it is low,[32] leading to ambiguity as to whether there may be directed co-evolution. Among different primate species, there are typical microbiomes, with the host phylogeny being the driving determinant, showing significant evidence for co-evolution.[30] There appears to be geographical differences among human populations,[33] likely to be the result of local traditions and exposure to external elements.[32] The local diet is one of the elements that undoubtedly influence both the long-term changes and the immediate composition of the microbiome. The proportion of fermentable components in the diet influences the species of bacteria present, and the species of bacteria influences the host, allowing complex interactions.[34] The microbiome is very susceptible to short-term changes in the diet,[35, 36] suggesting that the relationship between the diet and the microbiome could be both the cause and the treatment of obesity. The microbiomes of ancient and modern humans are distinct, and geographical variation has probably decreased in the post-Columbian period.[37] These changes continue and so may stunt the evolution of the microbiome, due to reduced diversity and possible gene transfers between species.[34]

Prebiotics

Fermentation of dietary carbohydrates provides the energy for the growth and activity of the microbiome; this is the first part of the definition of these carbohydrates as prebiotics.[13] All known and suspected prebiotics include resistant starches, and non-starch polysaccharides with different sugar monomers and chemical linkages.[38] Prebiotics must be able to resist gastric acidity, resist human enzymatic hydrolysis, resist absorption in the upper gastrointestinal tract, be fermentable by one or more intestinal microflora, and selectively stimulate growth or activity of intestinal bacteria.[39] Prebiotics can be found naturally in many foods including leeks, asparagus, chicory, Jerusalem artichokes, onions, garlic, wheat, rye, barley, oats and soybean.[40] Typically, any food containing dietary fibre is likely to contain some form of prebiotics, although in varying amounts. The definition of a prebiotic concludes by stating that these bacteria must improve the health of the host. There is a long history of prebiotic consumption by humans, but scientific evaluation of the health benefits is relatively recent.

Many ancient foods contain prebiotics as well as nutrients, so the daily prebiotic intake was significant. One of many examples is the yacon, a traditional root used by many Andean tribes in Ecuador, Peru, Bolivia and Argentina. The dietary fibre content of the root, usually eaten raw, is approximately 10.4% of dry matter, with an insoluble fraction of approximately 8.7% and soluble fraction of 1.7% of dry matter.[41] The fibre consists of cellulose, galactose, arabinose and galactose, with lower amounts of xylose, mannose and rhamnose.[41] The fructose oligosaccharide content of yacon varies between 24 and 35% with some studies indicating higher content of 54-62%.[41, 42]

Prebiotics were present in the components of ancient diets and were consumed routinely. In the ancient Indian diet, unlike the modern European diet, prebiotics were a major component through consumption of grains, fruits and vegetables.[43] Also, the Indian diet contained spices and herbs in home-made food which were quite different from the processed food in European diet.[43] Palaeodietary studies, for example in the Chihuahuan Desert of Mexico, have shown that prehistoric people consumed a wide variety of plants, including inulin-rich sotol, agave and onion.[44] Conservative estimates indicate that the average male hunter-forager consumed 135g prebiotics per day, while females consumed 108g/day.[44] When the whole diet was considered as the energy source, it was estimated that a larger proportion of the energy was derived from fermentation of prebiotics in the large intestine on a daily basis. However, the total dietary intake of prebiotics has reduced in the modern era, for example, to less than 20g/day in the USA.[44] Although there are current social trends to target refined carbohydrates as the cause of the obesity epidemic, it is likely more caused by what these originally plant-based, yet highly processed products lack, as opposed to what they contain. The increase in simpler starches with a high digestibility has certainly come at the cost of non-digestible starches and other dietary fibres, which are not useful economically or in a processing context.

The advent of processed foods

Changes in lifestyle due to modernisation created the need for processed and fast foods. The delivery of large volumes of food to a widespread population resulted in industries realising the potential economic gains. Industries aimed to produce low cost, highly-consistent foods with palatable tastes and textures. Raw grains and non-refined materials are mostly unsuitable for these processed foods, hence the industries aimed to include non-fibre carbohydrates, mostly including simple sugars. Highly-refined white bread, wheat, rice and maize products became a norm of the modern westernised diet. Furthermore, the addition of these derivatives to non-wheat-based products increased the amounts of rapidly digestible carbohydrates with no addition of fibrous components. The estimated intake of refined grain was approximately 89% of the cereal intake in the USA in 2005.[45]

The increased consumption of processed foods has been a product of both convenience and cost. Although some credit to this may be derived from the Green Revolution, it would appear that the major driver is the consumer markets pushing toward convenient, cheap and consistent foods. Globalisation may be responsible for the nutritional changes resulting in increased processed food consumption and increased obesity.[46] The increased consumption of processed foods has come together with increased foreign investment in food processing in developing countries.[47] The advances in industrialisation and commercialisation of processed foods since the late 1800s has outpaced increases in dietary knowledge which may explain the current situation.[48] Consumers have then perpetuated the decline in whole food consumption with their increased desire for processed foods.

The marked differences between the whole-food and processed food diets are likely to have contributed heavily to the rising health epidemics of obesity and metabolic syndrome. Epidemiological studies have correlated decreased whole grain intake and increased refined grain intake with weight gain in short-term studies.[49] This evidence suggests that long-term dietary changes contribute to increased body weights and related diseases. The most obvious differences are the increased addition of sweeteners, the decreased amounts of dietary fibres, the addition of preservatives, and the increases in hydrogenated fats.[50]

Processing and prebiotics

The shift toward increased processing has decreased the fibre intake in many countries. It can be clearly seen that the increased processed food consumption mimics the trends in obesity and diet-related diseases. Many link the decreased fibre consumption with a decreased intake of grain and cereal crops. Japan decreased its average fibre consumption per capita by almost 10 grams over the period of 1911-1980.[51, 52] This change can be directly attributed to a decrease in whole grain intake.[52] Alterations in the American diet were marked with fibre intake declining by 28% from 1909 to 1975. The cause is a reduced intake in whole plant-based foods, with the greatest decline coming from the grain intake.[53] In Brazil, the consumption of ultra-processed foods increased over the period of 1987-2003, which led to much lower dietary fibre intake over this period.[54]

Grain crops contain both fermentable and non-fermentable fibres that may confer health benefits. These fermentable fibres are of particular interest as they typically act like prebiotics to change the composition of the gut microbiome. Of particular interest are resistant starches and non-starch polysaccharides. Grains, cereals and legumes have different amounts of non-starch polysaccharides and resistant starches depending on their species, variety and growing conditions. Of particular significance to the prebiotic concept are soluble dietary fibres, many of which are fermentable, and non-starch polysaccharides, all of which are fermentable. The primary grains that increased in production throughout the Green Revolution were rice, maize and wheat with total dietary fibre of 19.6%, 5.7% and 17%, respectively and total soluble dietary fibres of approximately 3.6%, 1.4% and 2.3%, respectively. Other cereals such as millet contain 5.4% total dietary fibre and 3.8% soluble fibres and oats contain 37.7% total dietary fibre and 3.8% soluble fibres.[55] Barley, depending on variety, can contain from 4.5-26.9% soluble non-starch polysaccharides.[56] By replacing 100g of white rice with 100g quinoa, carbohydrate content decreases by 17.5 g and protein content increases by 7.3 g.[57] Gram for gram, ancient grains such as quinoa, amaranth, barley and millet offer fewer carbohydrates and more protein as well as containing beneficial fibres.

Processing reduces the content of soluble dietary fibres.[55] Common food additives such as corn starch and potato starch do not include fibre, processed

white rice contains 0.3% soluble dietary fibre, and processed oats contain less than a third of the total dietary fibres of their unprocessed equivalent.[55] The soluble non-starch polysaccharide content of commonly consumed processed foods while obesity was rapidly increasing were much lower than the raw whole grains. Kellogg's Cornflakes™ contain 0.48% non-starch polysaccharides, puffed rice 0.41%, brown rice 0.89%, white rice 0.92%, Kellogg Krispies™ 0.32%, and white spaghetti 1.82%.[58] With processing comes a reduced intake of the dietary fibre which have potential prebiotic affects and as a result, our overall health is likely to have been impacted.

Obesity-related disease

The changes in nutrient composition and intake due to processing of food are partly to blame for the current prevalence of obesity. Many countries now report more than 50% of their population as overweight or obese with marked increases since the 1980s.[59] The prevalence of obesity today varies from 3-4% in Japan and Korea, to 30% or more in the USA and Mexico.[59] The incidence has appeared to plateau in the USA over the past decade.[60] In Australia, 60% adults and 25% children, or more than 12 million people, are classified as being overweight or obese.[61] Due to these increases, obesity-associated illnesses have also increased, along with an increased financial burden.[62, 63]

Obesity has a major impact on the energy metabolism and regulatory mechanisms that lead to metabolic diseases including type 2 diabetes, cardiovascular disease, dyslipidaemia, hormone-linked cancers and gastrointestinal diseases including inflammatory bowel disease and colon cancer.[64] Type 2 diabetes is closely associated with obesity and insulin resistance, and is affected by the inflammatory status of adipose and other tissues. The associations are clear with increasing incidence of diabetes, hypertension, asthma and arthritis as body mass index increases.[65]

Obesity is a very slowly developing metabolic disorder. While there is evidence showing the relationship between obesity and the above-mentioned diseases, the clear development of obesity is not identified until the person looks obese. During the asymptomatic development of obesity, metabolic dysfunction develops. The process is unclear but it is speculated that the gut microbiome changes with the presence of components in the diet such as saturated fatty acids. Hence, this metabolic dysfunction can be described as a dysfunctional gut microbiome. In contrast, the incidence of obesity and obesity-related diseases in communities that follow a more traditional whole-food diet is lower.[66] Pimo Indians eating traditional diets showed lower body mass index and cholesterol concentrations than people of similar heritage eating a westernised diet. The traditional Pimo diet contains over 50 g/day of potentially prebiotic dietary fibre, primarily from corn and beans.[67] Traditional diets in urban Brazil have a protective effect against obesity, with possible reasons including the high-fibre intake in traditional diets.[68] A similar trend is evident in tribes of the Solomon Islands. In communities where a westernised diet has begun to infiltrate, the plasma cholesterol concentrations are higher and the percentage of males with high blood pressure is increased.[69] Female teachers in Tehran consuming westernised diets high in refined carbohydrates, processed meats and low in dietary fibre have higher body mass index and increased risk of developing metabolic syndrome.[70] These are a few examples, with most studies indicating similar trends following consumption of highly-refined, acellular diets.[66]

The ever-increasing obesity epidemic also increases the financial costs of health care.[50] It has been estimated that, in the year 2000, the burden of obesity from both direct health care and indirect costs was as high as 4.06% of gross national product of China. In the USA and the United Kingdom, estimated costs are likely to increase by $48-66 billion and £1.9-2 billion per year over the next 15 years, respectively.[71] Other countries with rising obesity will have similar increases in health costs.

Chronic inflammation in obesity

Metabolic endotoxaemia, a chronic low-grade inflammatory condition, has been identified as an early predictor of obesity and diabetes.[72] Multiple studies associate obesity with this chronic low-grade inflammatory status. The inflammatory state is characterised by the presence of elevated plasma concentrations of cytokines and by the presence of macrophages and monocytes within the adipose tissue. As the adipose tissue is an endocrine tissue, imbalances and disruptions in adipose tissue status can have marked effects

on metabolic processes. The increased plasma cytokines in obesity are in response to increased plasma microbial components and lipotoxicity. The presence of lipotoxic substances, including the saturated fatty acids, palmitic, stearic and lauric acid,[73-76] and microbial components [77, 78] have the ability to activate toll-like receptors (TLR) which then initiate immune responses.

Obesity has been associated with distinct gut microbiomes and increased gut permeability leading to a low-grade endotoxaemic state.[79] This state leads to the initiation of several inflammatory pathways in adipose tissue. TLR4 responds to increased plasma lipopolysaccharides and lipids, and the induction of the NF-κB gene transcription.[80, 81] The result is an increased expression and secretion of cytokines leading to recruitment and infiltration of macrophages to adipose tissue. The macrophages then further the inflammatory response by increased cytokine expression mediated by NF-κB.[82, 83] TLR2 induces a similar response to the presence of lipopolysaccharide, with the induction of the NF-κB mediated transcription of cytokines;[81] however, it interacts with a wider variety of ligands than TLR4.

Prebiotic interventions

There is increasing evidence that prebiotics have the potential to attenuate obesity, obesity-related inflammation and obesity-related diseases.[84-87] The mechanism of action of prebiotics involves their fermentation in the colon, which changes the gut microbiome.[88] The prebiotics appear to improve gut barrier function, potentially reduce pathogenic bacterial populations and produce compounds which can improve host metabolism and endocrine function.[79] Bacteria produce by-products when fermenting prebiotics including hydrogen, lactate, methane, carbon dioxide and short-chain carboxylic acids including acetate, propionate and butyrate. Much research into the mechanism of action of prebiotics on inflammation and obesity has revolved around the benefits to gut health of these short-chain carboxylic acids.[38]

Prebiotic effects on inflammation

Supplementation with prebiotics increases the plasma and colonic concentrations of short-chain carboxylic acids. Plasma short-chain carboxylic acids initiated activation of specific receptors classified as GPR43 leading to reduced lipolysis and reduced plasma free fatty acids.[89] Reduction of plasma free fatty acids by this mechanism should reduce the inflammatory response by reducing the exposure of membrane TLR to plasma free fatty acids. GPR43-knockout mice showed exacerbated inflammation mediated by immune cells and increased release of reactive oxygen species.[90] Thus, GPR43 plays a role in metabolic inflammation.[90]

Several studies have investigated the effects of short-chain carboxylic acids produced by colonic fermentation on the inflammatory state. The presence of short-chain carboxylic acids reduced the presence of TLR4 mRNA leading to reduced active protein levels and subsequent inflammatory responses.[91] Treatment of neutrophils with butyrate, propionate and acetate at concentrations of 3 mM can counteract the release of TNF-a induced by lipopolysaccharide.[92] Similar effects have been noted in cultured colon epithelial cell lines where short-chain carboxylic acids inhibit NF-κB activity.[92] Similar anti-inflammatory responses have been noted in adipose tissue [93] and cultured macrophages where pro-inflammatory cytokine release can be decreased.[93, 94] These results have not been consistent with evidence suggesting that the presence of short-chain carboxylic acids can enhance the TLR2-induced expression of TNF-a and IL-8.[95]

Whole grains with their combination of fibre, protein, vitamins and minerals appear to have protective effects in the body as they are digested more slowly than refined grains and have the potential to stimulate the growth of appropriate gut bacteria. They also generally maintain lower blood glucose concentrations and improved insulin responses in the body. Subjects with high dietary fibre intake have lower blood pressure and serum cholesterol concentrations, reduced glycaemia, improved insulin sensitivity with lower risk of developing stroke, hypertension, type 2 diabetes, obesity and gastrointestinal diseases.[52]

Prebiotic effects on obesity

Obesity is a complex metabolic disorder with impairments in lipid and carbohydrate metabolism. Different prebiotics have been tested for their effects in obesity as the microbiome fermentation products

of prebiotics can affect host metabolism.[96] Many prebiotics alter blood lipid profiles by binding cholesterol and bile acids in the upper gut, increasing sterol excretion and causing a bulking effect in the intestine improving satiety.[38, 97] Animal studies have shown that dietary interventions by prebiotics, especially fructans, inulin and oligofructose, decrease serum triglycerides.[97] A Japanese study found that when Japanese millet was fed to diabetic mice, plasma concentrations of adiponectin and high density lipoprotein cholesterol increased while glucose and triglyceride concentrations decreased.[98] In human studies, decreases in serum triglycerides by inulin and oligofructose are independent of gender, type of prebiotic, background diet, overweight, dyslipidaemia or diabetes.[99] Despite lowering triglyceride and cholesterol concentrations, prebiotic dietary supplementation in both animal and human trials showed inconsistent modulation of lipid concentrations.[99] Of the studies reviewed, 8 out of 21 showed no change in blood lipid concentrations while the rest indicated lower lipid biomarkers (triglyceride, total cholesterol) for cardiovascular disease risk.[99] More studies of prebiotics targeting selected symptoms or disease such as metabolic syndrome and obesity are required.

Several studies have linked diets supplemented with indigestible oligosaccharides (fructo-oligosaccharides, galacto-oligosaccharides and arabinoxylan) to reduced dietary intake in mice [86, 87] and rats[84, 85]. Most studies in mouse and rat models resulted in decreased body weight gain where high-fat diets were administered with supplementation against a high-fat diet alone; however, the decrease in caloric intake was not sufficient to account for the total weight loss.[84, 86, 87] Human studies of fructo-oligosaccharides supplementation have shown promise in obesity attenuation with long-term supplementation leading to reduction in body weight, body mass index and waist circumference in obese women.[100, 101] A study with fructo-oligosaccharies has indicated gender differences in responses with reduced calorie intake in women and increased intake in men.[102] It may be possible to have responsive and non-responsive hosts to galacto-oligosaccharides despite having similar gut microbiomes before the treatment.[103] This trend is likely to be similar for other prebiotics; however the mechanisms and interactions require further investigation. Supplementation with xylo-oligosaccharides and fructo-oligosaccharides in streptozotocin-induced diabetic rats showed that the improved body weight, reduced mortality and increased population of *Bifidobacteria* and *Lactobacilli* in the caecum may be due to a decreased pH with increased concentrations of short-chain carboxylic acids.[104]

Butyrate is rapidly absorbed from the lumen of the cecum to influence glucose and cholesterol concentrations and lipid metabolism. Supplementation with 5% butyrate to high-fat diet-fed mice inhibited the development of obesity and insulin resistance due to reduced adipose tissue,[104] which suggests it may be an effective compound in the treatment of obesity and insulin resistance. Butyrate also has an important function in maintaining metabolism and the proliferation and differentiation of epithelial cells types.[104] Similarly, propionate reduced cholesterol in rats possibly through inhibition of HMG CoA reductase, redistribution of cholesterol from plasma to liver and enhanced synthesis and secretion of bile acids.[105]

The interrelationships between satiety hormones and the gut microbiota are complex; however, these hormones reduce energy consumption and metabolic processes and may be important in the development and attenuation of obesity. Satiety hormones change with administration of prebiotics. Prebiotics appear to have a stimulatory effect on peptide YY secretion[85, 101, 106] and tend to increase glucagon-like peptide-1.[107, 108] This change is associated with better regulation of post-prandial glucose concentrations, improved insulin tolerance and improved satiety.

Conclusions

There is a widespread concept that our genome and our modern diet are mis-matched, with only the genome remaining unchanged since the Neolithic Revolution. Discussion of the optimal human diet to decrease modern diseases such as obesity leads to passionate discussions, with many of the proposals being incompatible. As one example, should we consume high protein, high saturated fat and almost no carbohydrates as in the Atkins diet? Or is the Ornish diet more appropriate, with 80% carbohydrates and minimal animal protein and fat? Would widespread substitution of the modern diet with Palaeolithic or pre-Palaeolithic nutrition decrease the incidence and morbidity of obesity in our communities? If so, this

would argue that our diet should be optimised to the time when the modern human genome stabilised. As we have had the Green Revolution to increase the production of cereals, do we need a Nutrition Revolution to increase wellness? This could include incorporation of the Palaeolithic diets into current nutritional recommendations but this requires a thorough examination of these diets.[108, 109] Is it possible to become a 21st century hunter-gatherer[110] and still have the same quality of life as currently enjoyed in developed countries? To answer these questions, we need a better understanding of the complex relationships between components of the diet, especially prebiotics, and the bacteria of the gastrointestinal tract, the microbiome. Ultimately, this increased understanding will use past nutritional experience to provide the foundation for a healthy future, including decreasing chronic disorders such as obesity.

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1.4.3.2 Gut morphology

The morphology of the intestinal tract varies from duodenum through to the distal colon as digestive function changes. The small intestine (duodenum, jejunum and ileum) contains villi, which enables a greater surface area to be available for absorption of nutrients. The large intestine or colon has no villi, as the majority of nutrient absorption is complete before entry into this part of the digestive system. A healthy gastrointestinal tract is covered in long healthy villi, shallow crypts and a continuous epithelial lining (Baurhoo et al., 2009). Shallow crypts within the small intestine show the capability of needing fewer nutrients for regeneration and allow the intestinal cells to produce more digestive enzymes, gastrointestinal hormones giving improved nutrient absorption (Baurhoo et al., 2009).

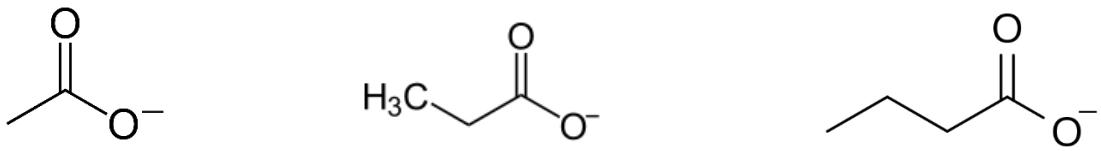
An increase in villi height and villi height to crypt depth ratio due to β-glucan consumption correlated with epithelial cell turnover (Baurhoo et al., 2009, Monro et al., 2012), rather than increased cell size. Intestinal morphology is altered based on dietary intake, with studies on broiler chickens and piglets indicating that the addition of prebiotics, particularly mannan oligosaccharides and fructo-oligosaccharides, improved the structure of the intestine allowing for improved nutrient absorption (Baurhoo et al., 2009, Awad et al., 2008). The colonic mucosal thickness and ileal goblet cell production increased with the addition of oligosaccharides to diet (Breves et al., 2001). The addition of β-glucans improved the intestinal barrier formed by epithelial cells and tight junction proteins (such as claudin-1) (Baurhoo et al., 2009). Along with adheren junctions, gap junctions and desmosomes, they are important in the absorption of nutrients and assist in the maintenance of the intestinal barrier function protecting the gut from pathogens (Shao et al., 2013). Poultry fed brewer's yeast containing β-1,3/1,6-glucan increased production of mucin-producing goblet cells in the small intestine (de los Santos et al., 2007, Cox et al., 2010), indicating that the addition of oats, which contains β-1,3/1,4-glucan, to the diet may also restore villus loss and gastrointestinal damage by improving the mucosal layer.

1.4.3.3 Gut fermentation

The association between diet and the gut microbiome has an important role to play in human health (Costabile et al., 2008). The fermentation of fibre in the large intestine decreases pH and increases bacterial biomass that leads to an increased faecal output

and gas production (CO2, methane and hydrogen) as well as short-chain fatty acids (SCFA) (Blottiere et al., 2003, Connolly et al., 2010, Costabile et al., 2008, Topping and Clifton, 2001, Wong et al., 2006). SCFA are the main product of carbohydrate catabolism. Each SCFA has distinct physiological effects, including shaping the gut environment, influencing colonic physiology, as energy sources for host cells and intestinal microbiota and participation in different host-signalling mechanisms (Ríos-Covián et al., 2016). Dietary supplementation of SCFAs decreased triglycerides and cholesterol (Lu et al., 2016) in high-fat fed mice.

Short-chain fatty acids are two to six carbons long with acetate (C2:0), propionate (C3:0) and butyrate (C4:0) (Figure 1.10) as the main by-products of gut fermentation (Blottiere et al., 2003, Bornet et al., 2002). SCFA are usually present in a 60:20:20 ratio (acetate: propionate: butyrate) in both the colon and faecal matter (Cummings, 1981).



a) acetate b) propionate c) butyrate

Figure 1.10 Structure of the most abundant SCFA produced by oats a) acetate b) propionate c) butyrate

(US National Library of Medicine, 2016)

Acetate (C2:0) is the most abundant colonic SCFA and makes up more than half of total faecal SCFA (Louis et al., 2007). The liver uses acetate as a precursor for cholesterol and long-chain fatty acid synthesis (Wong et al., 2006). It is utilised in the production of acetyl coenzyme A (Figure 1.11) and is metabolised through acetyl-CoA and butyryl-CoA to butyrate (Louis et al., 2010). Acetate has a direct role in appetite regulation as it avoids hepatic clearance and reaches the brain through peripheral circulation (Frost et al., 2014). Acetate can have an anorectic effect by inducing neuropeptide expression and reducing hypothalamic AMPK catalysis (Frost et al., 2014). There is a negative association between serum acetate and visceral adipose tissue and insulin concentrations (Layden et al., 2012).

Propionate (C3:0) plays a role in the liver as a major energy source for gluconeogenesis promoting fatty acid β-oxidation and reducing food intake (Chen et al., 1984). Serum and liver cholesterol concentrations were lower when an increase in propionate was available to upregulate PPARα (Anderson et al., 1984, Higashimura et al., 2015). As a key regulatory factor in lipid metabolism, increases in PPARα indicates improvements in fatty acid β-oxidation (Higashimura et al., 2015). Potential roles have also been found in the activation of GPR41 and GPR43 (Kimura et al., 2011), releasing satiety hormones (Lin et al., 2012) and other metabolic and anti-inflammatory effects (Al-Lahham et al., 2010). Acetate and propionate competitively inhibit each other in the hepatocytes and portal vein into the liver tissue to be utilised for gluconeogenesis. Therefore, any change in this proportion will change the energy available within the liver.

Butyrate (C4:0) (Figure 1.10) is used as an energy source by colonocytes and oxidises to carbon dioxide and ketone bodies (Roediger, 1990). It has a major role in colonocyte proliferation and differentiation and is also associated with decreased colon cancer and other intestinal disorders (Sakata, 2007). It also plays a role in the modulation of epithelial cell cycle and mucosal immune responses (Topping and Clifton, 2001, Bajka et al., 2010, Sakata, 2007). Butyrate is utilised by the colonocytes for intracellular metabolism (Lu et al., 2016), stimulates Na^+ and fluid absorption with reduced contractility which may lead to lower colonic motility increasing uptake of water and minerals (Sakata, 2007, Dass et al., 2007). Colonic absorption of SCFA by surface and crypt epithelial cells is through Na^+-dependent symporters related to Na^+-glucose symporters in the small intestine (sodium monocarboxylate transporter - SMCT). SMCT's lower intracellular concentrations of Na^+ are used as an energy source for the colonocytes (Lu et al., 2016). Butyrate may regulate the expression of specific genes in colonic epithelial cells and may suppress the development of malignancies as the expression of SMCT1 is reduced in colon cancers (Lu et al., 2016).

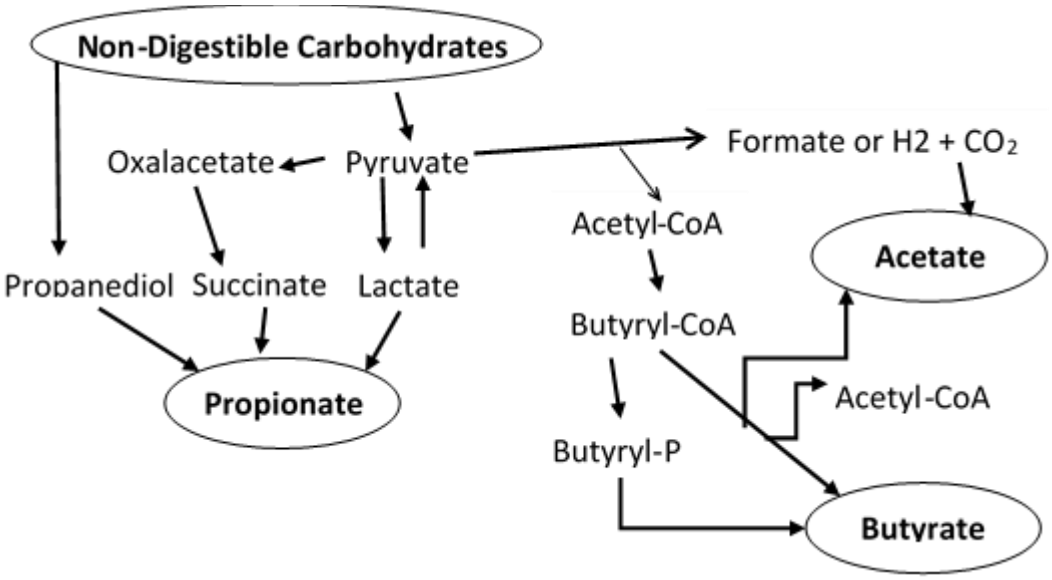


Figure 1.11 Representation of microbial metabolic pathways contributing to SCFA production in the gut

adapted from (Ríos-Covián et al., 2016)

Diet has an impact on SCFA production with changes in gut microbiota altering the SCFA produced (Costabile et al., 2008) and therefore leading to various health outcomes. Reductions in faecal butyrate are associated with colorectal adenocarcinomas in human studies (Chen et al., 2013). Increases in total faecal SCFA concentrations have been correlated with increased obesity (Fernandes et al., 2014, Rahat-Rozenbloom et al., 2014), while SCFA decreased following anti-obesity treatment (Patil et al., 2012, Salazar et al., 2015). Prebiotic substrates also induced changes in SCFA production of healthy individuals (Lecerf et al., 2012) and patients with irritable bowel syndrome (Majid et al., 2011).

Many human feeding studies have shown no observable effects with over 90% of SCFA absorbed in the colon and utilised by the host (Connolly et al., 2012), and due

to the inaccessibility of the human colon for direct investigation, it is hard to quantify SCFA production rates (Verbeke et al., 2015). Studies using *in vitro* methods to produce SCFA from non-digestible components of whole grain cereals determine the quantity and ratio of the various SCFA produced. These studies vary from simple batch fermentation (Salazar et al., 2009, Arboleya et al., 2013) to complex multistage continuous culture systems such as SHIME (Van den Abbeele et al., 2013a, Van den Abbeele et al., 2010, Van den Abbeele et al., 2013b), SIMGI (Barroso et al., 2015) EnteroMix Lacroix (Mäkeläinen et al., 2007) which are computer-generated simulations. However, some of these models showed bias towards butyrate and propionate-producing gut microbiota (Van den Abbeele et al., 2010), issues alleviated by simulation of intestinal mucosal surfaces (Van den Abbeele et al., 2013a).

Proportions of SCFA vary based on the type of carbohydrate fermented (Timm et al., 2010, Fässler et al., 2006, Van den Abbeele et al., 2013b, Rodriguez-Colinas et al., 2013). Oat bran and β-glucan fractions increased propionate (Connolly et al., 2010, Kedia et al., 2009, Van den Abbeele et al., 2013b) proportions while oligofructose increased acetate (Pan et al., 2009). Different fermentation patterns exist due to the speed of fermentation, microbial populations and cross-feeding interactions (Hernot et al., 2009, Zhou et al., 2015, Puertollano et al., 2014).

Dietary supplementation of acetate, propionate or butyrate in *in vivo* studies are common. However, this does not give an accurate measure of what is occurring with the consumption of food products as fermentation rates are not taken into consideration (Hara et al., 1999). Comparisons of *in vivo* and *in vitro* fermentation have indicated that a suppressed rate of synthesis occurs with higher levels of SCFA in the liver absorbed from the intestine (Hara et al., 1999). Faecal propionate and butyrate concentrations increased in mice fed these SCFA compared to the high fat control diet, with no changes in mice fed acetate suggesting that dietary acetate is more efficiently absorbed by the intestine (Lu et al., 2016). The prebiotic effect of these more complex carbohydrates could be enhanced compared to pure culture fermentation (van Zanten et al., 2012) due to the intricacies of the biological system.

Consideration of diurnal changes must also be made when collecting samples for SCFA analysis, as these impact the concentrations of acetate, propionate and butyrate in plasma and faecal samples (Hara et al., 1999). For example, portal concentrations

were increased 5 hours after feeding compared to 15 hours after feeding when they had returned to pre-feeding concentrations (Hara et al., 1999). Considerable individual variability in gut motility and transit time from caecum to rectum may lead to unexpected results when determining SCFA concentrations. In the small intestine, rates are relatively constant; however, in the colon, contents may take from hours to days without there being any dysfunction (Patten et al., 2015). *Ex vivo* studies of rats fed resistant starch have shown that ileal contractility does not change, yet colonic contractility is modified (Patten et al., 2015).

Studies have shown differences in faecal SCFA patterns in rats that were on the different oat-based diets compared to a rice diet (Mårtensson et al., 2002). These indicate that different diets affect the SCFA in the colon and faecal excretion of these SCFA (Mårtensson et al., 2002). SCFA production by wholegrain oats is projected to have a role not only in the colon but also on serum lipids and cholesterol (Mårtensson et al., 2002, Ji-Lin et al., 2014), as well as blood glucose and insulin concentrations (Connolly et al., 2012, Giacco et al., 2016). With processing, the bran of the oat grain is removed. Compounds such as fibre, phytochemicals, vitamins and minerals are also removed, altering the physiological responses (Connolly et al., 2012). Therefore, it is necessary to compare the differences that each component of an oat grain has on risk factors of metabolic syndrome in conjunction with the gastrointestinal changes that take place due to SCFA production (Figure 1.12).

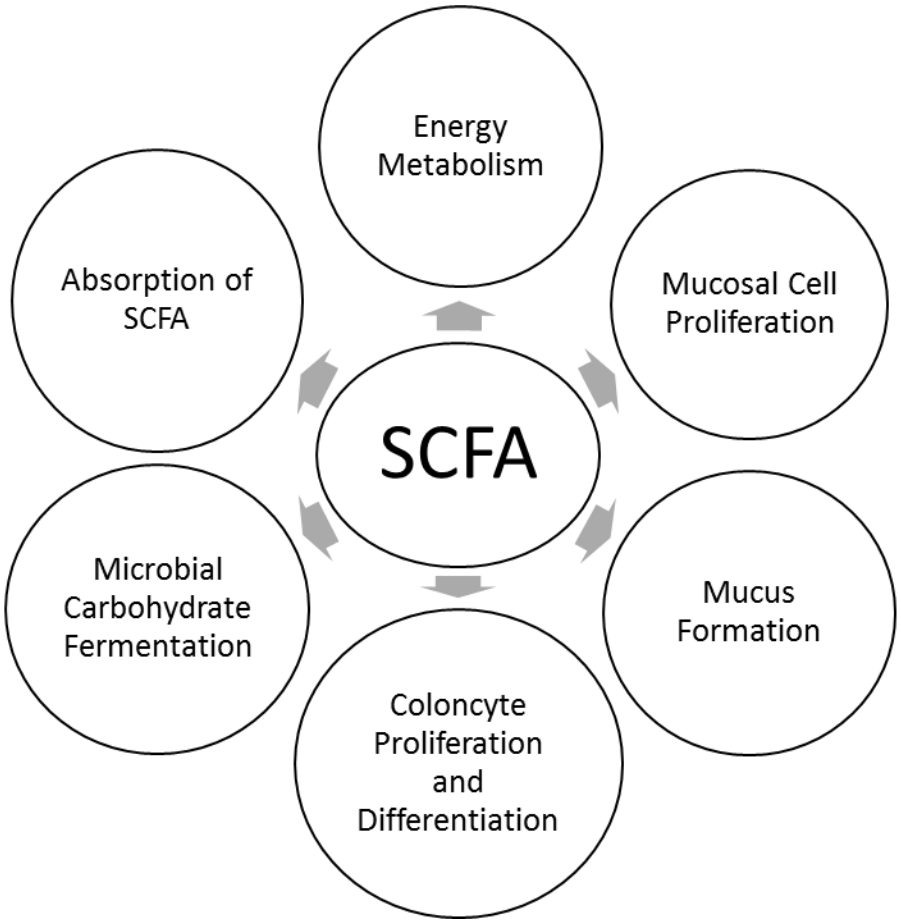


Figure 1.12 Effects of SCFA on colonic morphology and function

1.5 Dietary implications

While a rat model may be useful to examine the interaction of wholegrain oat, oat bran and β-glucans with the intestinal morphology and function, anatomical differences are important to note when translating to human studies. For example, the caecum of the rat, in which most fermentation takes place, needs to be compared with the proximal colon of a human, while the rat colon functions mainly in dehydrating and retaining unfermented residue which correlates to the human distal colon (Monro et al., 2012).

Physiological responses to various oat products vary based on structure, composition and production techniques (Zhou et al., 2016, Wang and Ellis, 2014, Shewry et al., 2013). Short-term feeding studies in metabolically controlled confines can produce a meaningful difference in response between experimental and control diets, while they control confounding factors such as nutrient composition, food type and preparatory methods. These studies have little translatability to everyday situations in humans and

for long-term weight change, insulin regulation and blood pressure control where individuals have access to food *ad libitum*. Nevertheless, they still may inform and influence energy metabolism and body weight regulation. Wholegrain oats and associated oat products have the ability to improve stored metabolic fuel, alter gut motility, gastric emptying and affect the production of gut hormones to leading to satiation. The knowledge of these beneficial effects is being disseminated to encourage the consumption of these functional foods which leads to improvements across the range of metabolic syndrome symptoms.

1.5 Coconut literature review

1.5.1 Coconut and its health benefits

The coconut (*Cocos nucifera*) has, for many civilisations, particularly in tropical and subtropical regions, been seen as the "tree of life" (DebMandal and Mandal, 2011). Coconut products have been used as a traditional food source, particularly across Asia and the Pacific Islands for hundreds of years (DebMandal and Mandal, 2011), yet until recently, the health benefits have been anecdotal rather than scientifically proven, with many previous studies focusing only on coconut oil (Arunima and Rajamohan, 2012; Liau et al., 2011; Zakaria et al., 2011) and not on the other components of coconut.

Traditionally, all parts of the coconut, including the shell, fibre, pulp and water have been used to treat diseases such as diabetes, asthma, gastrointestinal and skin disorders, as well as for their antipyretic and diuretic responses (Lima et al., 2015). The coconut husk is a source of fibre that gives antimalarial, antibacterial and antidepressant responses (Akinpelu et al., 2015; Lima et al., 2016).

The health benefits of coconut have been known for centuries (Zakaria et al. 2011). However, for many years, coconut oil was seen as harmful due to the high percentage content of saturated fatty acids. Many tests performed in the 1980s were undertaken using hydrogenated coconut oil which is different in content to virgin coconut oil (VCO) with none of the benefits (Clarke, 1988; Vaca, 1986). Not all saturated fatty acids are bad -(Poudyal & Brown 2015) and some have health benefits, including boosting metabolism, augmenting the immune system (Zakaria et al, 2011) and being a powerful antioxidant (Nevin, 2006).

The benefits of coconut as part of the diet can be seen in South Pacific Islanders who consume coconut in its natural state, and as a major dietary staple. Many have low incidence of coronary heart disease, degenerative diseases and low serum cholesterol concentrations compared to islanders who have adopted a Western-style diet and decreased intake of coconut while increasing concentrations of total cholesterol (Lindeberg et al., 1999; DiBello et al., 2009). Coconut or coconut products as part of the regular diet along with seafood in Samoan islanders protected against risk factors for cardiovascular disease (DiBello et al., 2009). These changes included higher HDL-cholesterol concentrations and lower abdominal circumference (DiBello et al., 2009). Similar observations were seen in the Kitava population from the Tobriand Islands in Papua New Guinea when compared with a Swedish population. The Kitava population is free from overweight, hypertension, cardiovascular disease and malnutrition (Lindeberg et al., 1999). The regular diet of Kitava people includes tubers, coconuts and seafood (Lindeberg et al., 1999).

1.5.2 Composition of coconut

Coconut oil contains mainly medium-chain fatty acids (MCFA) instead of long-chain fatty acids (LCFA) (Marina et al., 2008). These MCFA are easily digested, absorbed and oxidised, and are transported directly to the liver for energy conversion through the tri-carboxylic acid cycle allowing hepatic cells to produce more energy and therefore utilise fat stores (St-Onge et al., 2002). This rapid absorption from the intestine into the liver takes place without pancreatic lipase catabolism (Liau et al., 2011; Arunima and Rajamohan, 2012). VCO has a bland flavour and pleasant odour with a high resistance to rancidity. A narrow melting temperature range makes it easily digestible and gives good absorptive capacity (Marina et al., 2009).

VCO is composed of approximately 90% saturated fatty acids (SFAs), particularly lauric (~50%) and myristic (~20%) acids (Katragadda et al., 2010). As MCFA, both lauric and myristic acids are rapidly oxidised by both the mitochondrial and peroxisomal pathways without being taken up into fat depots. In humans, VCO reduced body fat, especially abdominal fat, since abdominal circumference was decreased (Liau et al., 2011). Previous studies have reported on the various impacts of SFA on human health (Orsavova et al., 2015), and it has been concluded that lauric and myristic acids raise plasma total cholesterol concentrations, with lauric acid

increasing LDL cholesterol and myristic acid increasing both LDL and HDL cholesterol concentrations. However, it is important to note that it is the ratio of total cholesterol to HDL that is a more specific marker of coronary heart disease than the LDL value (Lawrence, 2013; Mensink et al., 2003). Lauric acid lowered the ratio of total cholesterol to HDL, while myristic acid showed no change (Lawrence, 2013).

Lauric acid is converted to monolaurin in the body (Lieberman 2006). Monolaurin is not formed by the body unless there is a source of lauric acid in the diet, therefore indicating that coconut products should be included in the diet. Monolaurin acts as an antiviral, antibacterial and antiprotozoal monoglyceride destroying lipid-coated viruses, bacteria and protozoa (DebMandal and Mandal, 2011). Monolaurin only affected potentially pathogenic microorganisms while having no adverse effect on desirable gut bacteria (DebMandal and Mandal, 2011). Recent studies showed that lauric acid when compared to palmitic and stearic acids prevented induction of obesity and osteoarthritis in high carbohydrate, high fat diet fed rats (Sekar et al., 2017).

Capric acid, a minor component of CO (~6%), converts to monocaprin and acts as an antimicrobial agent (DebMandal and Mandal, 2011). The major triacylglycerides (TAG) found in VCO are LaLaLa (22-25%), CCLa (14-16%), CLaLa (19-21%), LaLaM (13-15%) and LaMM (7-9%) (La – lauric acid; C – capric acid and M – myristic acid) with the proportions of each individual fatty acid varying with the source and product production history (Marina, 2009). The quality of VCO and amount of beneficial MCFA and TAG in the oil varies depending on geographical location and ecological conditions (Nevin and Rajamohan, 2008).

1.5.3 Coconut oil extraction methods

Commercial grade coconut oil can be extracted by several methods. Coconut oil made from copra needs to be purified and refined to be suitable for consumption due to its susceptibility to aflatoxin contamination. (Marina, 2009). However, VCO is produced through a wet process direct from the coconut kernel under controlled temperatures, without any refining, bleaching or deodorising, allowing it to retain the beneficial components such as polyphenols and phenolic acids.

Compared to copra-based oil, VCO decreased total cholesterol, TAG, phospholipids, LDL-cholesterol, VLDL-cholesterol and increases HDL-cholesterol concentrations in serum (Nevin and Rajamohan, 2008). VCO also improved activity of antioxidant enzymes and decreased lipid peroxidation (Nevin and Rajamohan, 2008).

VCO that has been heated can lead to increases in blood pressure and inflammatory markers such as ICAM, VCAM, CRP and TXB, whereas non-heated VCO does not possess these detrimental effects (Hamsi, 2014). Further, non-heated VCO conserved all the functional components of the coconuts, including tocopherols, sterols and squalene) as well as not losing the structural integrity of fatty acids (Zakaria et al., 2011), preserving the natural antioxidant properties of the VCO.

1.5.4 Therapeutic benefits of coconut

1.5.4.1 Hepatoprotective responses

Oxidative stress induced by generated free radicals plays a lead role in development of hepatic toxicity (Otuechere et al, 2014). In 2,4-dichlorophenoxyacetic acid (2,4-D) induced liver damage in rats, increased serum transaminases and alkaline phosphatase enzyme activity, and hepatic lipid peroxidation liver free fatty acids occur along with increased inflammation and necrosis. With the addition of VCO to the diet, serum total protein, albumin and hepatic superoxide dismutase and glutathione peroxidase activities were reduced (Hanaa et al 2013). Another study found that VCO-treated animals improved hepatic antioxidant enzymes, serum transaminase activity and liver free fatty acids, and decreased inflammation and necrosis (Zakaria et al 2011).

VCO decreased total cholesterol, triglyceride and phospholipid concentrations in plasma, while also reducing LDL and VLDL cholesterol concentrations and increasing HDL cholesterol (Nevin et al 2004). It also lowered LDL:HDL ratio as VCO is not deposited in adipose tissue (Dayrit 2003). MCFA such as lauric and myristic acids are directly absorbed from intestine and sent straight to liver to be rapidly metabolised for energy production and therefore, do not participate in biosynthesis and transport of cholesterol (Enig 2004).

One study investigated the effect of coconut flakes compared with oat bran and corn flakes on human serum cholesterol concentrations. Results indicated that reduction in serum total and LDL cholesterol concentrations for both coconut flakes and oat bran

compared to the cornflakes and triglycerides were reduced in all test foods (Trinidad 2004). This study indicates that coconut flour is a good source of both soluble and insoluble dietary fibre and that they both may have a role in reducing lipid biomarkers.

1.5.4.2 Anti-inflammatory, analgesic and anti-pyretic responses

The addition of VCO had moderate anti-inflammatory effects in rats with ethyl phenyl propiolate-induced ear oedema or carrageenan and arachidonic acid-induced paw oedema. (Intahphuak et al. 2010) VCO had an inhibitory effect on chronic inflammation (Intahphuak et al. 2010) with a reduction of transudative weight, granuloma formation and serum alkaline phosphatase activity (Intahphuak et al. 2010). VCO induced moderate analgesic and antipyretic effects in acetic-acid induced writhing (model for analgesic activity) and yeast-induced hyperthermia (anti-pyretic activity) (Intahphuak et al 2010).

1.5.4.3 Anti-hypertensive responses

There has been limited human research on which to assess the responses of coconut oil or coconut products in relation to cardiovascular health as most of the evidence relates to lipid profiles. However, the high amounts of potassium found in tender coconut water (the liquid endosperm) lowered blood pressure (Loki, 2003).

The consumption of coconut products that contain fibre, such as coconut flesh and coconut flour, within a diet that includes sufficient PUFA (omega-3) and an absence of excessive calories from refined carbohydrates does not pose a risk for heart disease (Trinidad 2004).

1.5.4.4 Anti-glycaemic responses

The potency of the insulinotropic effect of MCFA depends on chain length. Both capric and lauric acids have the most potent effects on insulin secretion (Garfinkel 1992). In a study comparing coconut oil and sunflower oil, the coconut oil enhanced insulin action and improved binding affinity of the insulin receptors (Ginsberg 1982).

Coconut kernel protein, mainly arginine, has potent anti-glycaemic activity through reversal of glycogen deposition, activities of carbohydrate metabolising enzymes and

the pancreatic damage due to its effect on pancreatic β-cell regeneration by means of arginine (Salil 2010).

1.5.4.5 Anti-obesity responses

Daily intake of medium and long chain triacyclglycerols (MLCT) reduced body weight and body fat accumulation (Assuncao 2009). Biochemical and anthropometric profiles of women with abdominal obesity (waist circumference >88cm) given VCO decreased abdominal fat compared to soybean oil (Assuncao 2009).

1.5.4.6 Immunomodulatory responses

MCFA in virgin coconut oil have shown antimicrobial properties in killing harmful viruses, bacteria, fungi and parasites (Shilling 2013). MCFA break down into free fatty acids and monoglycerides (Ogbolu 2007). Lauric, capric and caprylic acids in coconut oil are converted to their monoglyceride form as monolaurin, monocaprin and monocaprylin which prevent microbes from inhibiting the immune system. These fatty acids act on microbes in different ways. Some kill organisms that cause fungal infections but may not be useful on other microbes. Together these fatty acids form a high-powered defence system against disease with monolaurin giving the best antiviral, antifungal and antibacterial effects (Shilling 2013).

VCO has been successful and effective against lipid-coated viruses such as Epstein-Barr, influenza, hepatitis C and cytomegalovirus (CMV) as it interferes and disrupts the virus membrane, assembly and maturation (DebMandal and Mandal 2011).

1.5.4.7 Antioxidant responses

VCO is rich in polyphenols which contribute to increased antioxidant enzymes which reduce inflammation and lipid peroxidation (Yeap et al., 2015). One study compared VCO to copra, olive and sunflower oils on endogenous antioxidant status and paraoxonase-1 activity in ameliorating oxidative stress in rats. Virgin coconut oil improved antioxidant capacity compared to other oils by increasing catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase activities in tissues (Arunima and Rajamohan, 2013).

1.5.4.8 Other therapeutic benefits

There are many other benefits of coconut products, particularly coconut oil, that have been found over many years of use including wound healing, Alzheimer's disease, dermatitis and stress. For example, topical applications on skin components and antioxidant status during dermal wound healing of young rats have shown within 24 hours of application complete epithelisation and improvements of the parameters of wound granulation tissue (Nevin 2010). The solubility pattern of collagen, glycohydrolase activity and granulation tissue histopathology have also been studied. Animals treated with VCO had much faster healing with decreased time to complete epithelisation, increased pepsin soluble collagen and glycohydrolase activity leading to higher collagen crosslinking and turnover. This may have been due to the cumulative effect of various minor biologically active components present in coconut oil (Nevin, 2010).

Other benefits have shown following the consumption of medium chain triacylglycerol (MCT) found in coconut may offer enhanced lipid oxidation and greater energy expenditure and a greater elevation of post-prandial oxygen consumption in healthy men compared to long-chain triacyclglycerols (Seaton 1986).

1.5.5 Conclusion

With the renewed emphasis on incorporating heart healthy fats into our diet, VCO and other coconut products show their versatility as a cooking medium and for their pharmacotherapeutic properties. However, there has been limited human research and further research is needed to provide conclusive evidence on clinical applications.

While the health benefits of VCO have been well documented, there has been little done to discover the benefits of other components of coconut, such as the dried flesh which remains after the removal of the oil. Coconut Nourish does more than simply deliver coconut oil to the system, with proteins and carbohydrates as well as SFA, it may be a food source that is useful for combating the signs of metabolic syndrome. The study completed as part of this thesis has examined the impact that commercially available coconut products (Banaban extra virgin coconut oil (VCO) and Banaban Coconut Crunch supplied by Nature Pacific, Burleigh Heads, Qld) have on the signs of metabolic syndrome.

1.6 Objectives

The main objective of this research is to investigate the effects of whole foods as functional foods on a validated rat model of metabolic syndrome.

The aims are:

1. To determine the effects of various oat products (wholegrain oat groats, oat bran and β-glucan powder supplement) on cardiometabolic health and gastrointestinal structure and function.
2. To determine the effects of adding 20% wholegrain oat groats in the diet on cardiometabolic health and gastrointestinal structure and function.
3. To determine the effects of virgin coconut oil and coconut Nourish on cardiometabolic health.

The hypotheses of this research are that:

1. Oats will induce metabolic changes and alter gastrointestinal structure and function in the host and these will be greater with more processing.
2. Wholegrain oat groats will induce metabolic changes and alter gastrointestinal structure and function when added to a high carbohydrate, high fat diet and therefore will be a viable alternative to replace other grains in the diet.
3. Virgin coconut oil and coconut Nourish will induce cardiometabolic changes when substituted into a high carbohydrate high fat diet.

Chapter 2 - Materials and Methods

2.1 Ethics

All animal handling and experimental protocols were approved by the Animal Ethics Committee of the University of Southern Queensland under the guidelines of the NHMRC (National Health and Medical Research Council of Australia) before experiments started. All rats were treated and housed as per the NHMRC 2014 guidelines for ethical treatment of animals (National Health and Medical Research Council, 2013a).

University of Southern Queensland Animal Ethics project numbers are as follows:

AEC Project ID	13REA008
AEC Project Title	Effects of purified saturated fatty acids in normotensive male Wistar rats
Commencement Date	09/07/2013
Expiry Date	31/12/2014
Experiments undertaken	March 2014 – May 2014

AEC Project ID	13REA005
AEC Project Title	Diet-induced obesity in young male Wistar rats – Stage III
Commencement Date	10 /09/2013
Expiry Date	30/09/2016
Experiments undertaken	March 2014 - March 2015

AEC Project ID	15REA003
AEC Project Title	Cereal prebiotics for the treatment of diet-induced obesity in rats
Commencement Date	20/07/2015
Expiry Date	20/07/2018
Experiments undertaken	July 2015 – December 2015

The following handling and diagnostic Standard Work Procedures were utilised:

USQ AEC SWP HP001 Rat blood collection - small volume (tail prick) • USQ AEC SWP HP002 Rat abdominal circumference measurement • USQ AEC SWP HP003 Rat injectable anaesthesia with tiletamine-zolazepam (Zoletil) • USQ AEC SWP HP005 Rodent recognition and management of pain • USQ AEC SWP HP006 Rodent (rat or mouse) administration of a substance (oral gavage) • USQ AEC SWP HP007 Rodent (rat or mouse) non-invasive identification methods • USQ AEC SWP HP009 Rodent handling and restraint • USQ AEC SWP HP013 Rat blood collection • USQ AEC SWP HP017 Rodent (rat or mouse) humane killing or euthanasia by pentobarbital sodium (Lethabarb®) • USQ AEC SWP HP020 Rodent (rat or mouse) transportation • USQ AEC SWP DP001 Rat oral glucose tolerance test • USQ AEC SWP DP002 Rat insulin tolerance test • USQ AEC SWP DP003 Rat systolic blood pressure measurement • USQ AEC SWP DP004 Rat in vivo heart function measurement (echocardiograph) • USQ AEC SWP DP005 Rat body composition measurement using dual-energy Xray absorptiometry (DEXA) • USQ AEC SWP DP006 Rat ex vivo heart function measurement (Langendorff) • USQ AEC SWP EX001 Request for veterinary care to be provided for approved AEC project animals • USQ AEC SWP AF005 Carcass disposal • USQ AEC SWP AF013 Rodent Environmental Enrichment

2.2 Rat diets and experimental protocol

2.2.1 – 5% oats

Male Wistar rats were purchased from Animal Resource Centre (Canning Vale, WA) and housed individually in a temperature-controlled (21 ± 2ºC), 12 - hour light/dark cycle environment with *ad libitum* access to food and water at the University of Southern Queensland Animal House.

Rats aged 8 - 9 weeks and weighing 330 – 340 g were randomly divided into four separate groups and were fed with either of the following diets for 16 weeks: corn starch rich diet (C) (n = 12); high carbohydrate, high fat diet (H) (n = 12). C diet consisted of corn starch (570 g/kg), powdered rat food (155 g/kg), salt mixture (25 g/kg) and water (250 mL/kg). The H diet consisted of fructose (175 g/kg), condensed

milk (395 g/kg), powdered rat food (155 g/kg), beef tallow (200 g/kg), salt mixture (25 g/kg) and water (50 mL/kg). In addition, rats on H diet were given drinking water containing 25% fructose. This common set of C and H rats were used for comparison against all intervention groups.

To test interventions in these diets, the C and H diets were supplemented with either 5% wholegrain oat groats (CO and HO rats) (n = 10/group), 5% oat bran (CB and HB rats) (n = 12/group) or 5% beta-glucan powder (CG and HG rats) (n = 8/group) with this 50 g/kg addition replacing 50 ml/kg of water. Interventions were started after 8 weeks of the diet and continued for the remaining 8 weeks.

Oat groats (Batch #160110598) (Figure 2.1) were sourced from Kialla Purefoods (Greenmount, Qld) and is commercially available. Oat bran (Lot #137162) (Figure 2.1) was sourced from Trumps (Brisbane, Qld) and is commercially available. β-glucan powder (Batch #235177) (Figure 2.1) is a commercially available supplement purchased from Blooms Health (Alexandria, NSW), known as Cholesterol Balance Beta-glucan powder (Figure 2.1) Macronutrient and micronutrient contents of the three interventions were similar apart from protein and β-glucan concentrations (Table 2.1).

Figure 2.1 Oat products used as interventions showing differences due to processing. Wholegrain oat groats (left), oat bran (middle), β-glucan powder (right)

Energy intake was calculated from daily measurements of food and water intake using the following values in kJ/g: fructose 15.40, C 15.94, condensed milk 13.8, beef tallow 37.70 and powdered rat feed 13.80 using food intake, and 3.85 kJ/ml for rats with H diets for the fructose-supplemented water (Panchal et al., 2011b). Energy contents of the diets were 11.2 kJ/g food for the C diet and 21.7 kJ/g food and fructose water for the H diet. Energy content for the CO diet was 12.0 kJ/g food, and 22.5 kJ/g food and

fructose water for the HO diet including fructose water. Energy content for the CB diet was 12.0 kJ/g food, and 22.5 kJ/g food and fructose water for the HB diet. Energy content for the CG diet was 11.9 kJ/g food, and 22.3 kJ/g food and fructose water for the HG diet.

2.2.2 - 20% modified diet

The C and H diets were then modified so that interventions up to 20% w/w of the diet could be included. The modified corn starch-rich diet (mC) consisted of corn starch (570 g/kg), salt mixture (25 g/kg), canola oil (5 ml/kg), skim milk powder (25 g/kg), vitamin mix (5 g/kg) and water (250 mL/kg). The modified high carbohydrate, high fat diet (mH) consisted of fructose (175 g/kg), condensed milk (395 g/kg), canola oil (5 ml/kg), skim milk powder (25 g/kg), vitamin mix (5g/kg), beef tallow (200 g/kg), salt mixture (25 g/kg) and water (50 mL/kg). Both diets were tested using 8 male Wistar rats weighing 330 - 340 g.

To test an intervention in these modified diets, the mC and mH diets were supplemented with 20% wholegrain oat groats (mCO and mHO rats) with this 200 g/kg addition replacing 200 ml/kg of water. Interventions were started after 8 weeks of the diet and continued for the remaining 8 weeks with 8 rats in each intervention. The same batch of oat groats were used as in the CO and HO groups described above.

Energy contents of the diets were 9.9 kJ/g food for the mC diet, 18.5 kJ/g for the mH diet (including fructose water), 13.1 kJ/g food for the mCO diet and 21.7 kJ/g for the mHO diet (including fructose water).

Table 2.1 Macronutrient and micronutrient composition of oat products

Component/100 g	***Wholegrain oat groats***	***Oat bran***	***β-glucan powder***
Energy (kJ)	1597	1600	1377
Protein (g)	13	13	22
Fat (total) (g)	9	8	4
- Saturated fat (g)	3	5	1
Carbohydrates (g)	55	65	62
β-glucan soluble fibre (g)	4	6	12

Full nutrient analysis of β-glucan powder was carried out by Agrifood Technology after results of β-glucan testing returned with results different from the nutritional panel. β-glucan testing of oat groat and oat bran was within the expected range therefore the nutritional panel results were used. Oat groat testing was performed by Symbio Laboratories.

2.3 Physiological measurements

Body weight, food and water intakes were measured daily for all rats. Feed conversion efficiency (%) was calculated as

$$\frac{mean\ body\ weight\ gain\ (g)}{daily\ energy\ intake\ (kJ)}\ x\ 100$$

Abdominal circumference and body length (nose to anus) were measured at 0, 8 and 16 weeks using a standard measuring tape under light anaesthesia with Zoletil (tiletamine 10 mg/kg, zolazepam 10 mg/kg, intraperitoneal; Virbac, Peakhurst, NSW, Australia). Body Mass Index (BMI) was calculated as body weight (g) / body length^2 (cm^2).

Visceral adiposity index (%) was calculated from wet weights of abdominal fat contents at euthanasia and expressed as adiposity percentage using the following formula:

$$\frac{retroperitoneal\ fat\ (g) + omental\ fat\ (g) + epidiymal\ fat\ (g)}{body\ weight\ (g)} \times 100$$

2.4 Oral glucose tolerance tests

Oral glucose tolerance tests were performed at 0, 8 and 16 weeks. Rats were deprived of food for ~ 14 hours (12 hours fasting + 2 hours testing). Fructose-supplemented drinking water in the H, HO, HB, HG, mH and mHO groups was replaced with normal water for the food-deprivation period. Following this, basal blood glucose concentrations were measured in blood samples taken from rat tail vein using Medisense Precision Q.I.D. glucometer (Abbott Laboratories, Bedford, MA, USA). Glucose (2 g/kg body weight glucose as 40% aqueous solution) was given to the rats via oral gavage. Tail vein blood samples were taken at 30, 60, 90 and 120 minutes following glucose administration.

2.5 Body composition by dual X-ray absorptiometry

Dual-energy X-ray absorptiometric (DXA) measurements were performed on rats after 16 weeks of feeding using a Norland XR36 DXA instrument (Norland, Fort Atkinson, WI, USA). The scans were analysed using manufacturer's recommended software for use in laboratory animals (Small Subject Analysis Software, version 2.5.3/1.3.1; Norland Corp) (Iftikhar et al., 1994). The precision error of lean mass for replicate measurements with repositioning was 3.2%.

2.6 Systolic blood pressure

Systolic blood pressure was measured at 0, 8 and 16 weeks. Rats were lightly sedated using an intraperitoneal injection of Zoletil (toletamine 10 mg/kg, zolazepam 10 mg/kg; Virbac, Peakhurst, NSW, Australia) and measurements taken using a MLT1010 Piezo-Electric Pulse Transducer and inflatable tail-cuff connected to a

MLT844 Physiological Pressure Transducer. Data were acquired using PowerLab data acquisition unit (ADInstruments, Sydney, Australia).

2.7 Echocardiography

Echocardiographic examination (Hewlett Packard Sonos 5500, 12MHz transducer) was undertaken at 16 weeks at The Prince Charles Hospital, Chermside, Brisbane in accordance with the guidelines of the American Society of Echocardiography using the leading-edge method (American Society of Echocardiography, 2016). Rats were anaesthetised using an intraperitoneal injection of Zoletil (toletamine 10 mg/kg and zolazepam 10 mg/kg; Virbac, Peakhurst, NSW, Australia) and Xylazil (xylazine 6 mg/kg; Troy Laboratories, Smithfield, NSW, Australia). Echocardiography was only undertaken on mC, mH, mCO and mHO rats (n = 8/group).

2.8 Termination

Rats were euthanised by intraperitoneal injection of Lethabarb (pentobarbitone sodium; 100 mg/kg; Virbac, Peakhurst, NSW, Australia). Heparin (200 IU; Sigma-Aldrich Australia, Sydney, NSW, Australia) was administered through the right femoral vein followed by withdrawal of ~5 mL blood from the abdominal aorta. Blood was collected into heparinised tubes and centrifuged at 5000 × *g* for 15 minutes within 30 minutes of collection. Plasma was collected and stored at − 20°C until further analysis.

Heparinised blood collection is utilised to inhibit thrombin formation and coagulation of blood and to allow the plasma to be centrifuged and collected for analysis. Plasma analysis was undertaken to determine total cholesterol, triglycerides, NEFA, liver enzymes (ALT, AST) and inflammatory marker concentrations.

2.9 Left ventricular diastolic stiffness

After euthanasia, left ventricular function of rats from all groups was assessed using isolated Langendorff heart preparation. Hearts isolated from euthanised rats were perfused with modified Krebs-Henseleit bicarbonate buffer containing NaCl 119.1 mM, KCl 4.75 mM, $MgSO_4$ 1.19 mM, KH_2PO_4 1.19 mM, $NaHCO_3$ 25.0 mM,

glucose 11.0 mM and $CaCl_2$ 2.16 mM. Carbogen gas (95% O_2 – 5% CO_2) (BOC) was used to oxygenate the buffer at 35^{o}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, Sydney, NSW, Australia). The heart was electrically stimulated at 250 beats per minute during the measurement of the diastolic pressure-volume relationship of the left ventricle. Pressures were obtained from 0 up to 30 mmHg for calculation of diastolic stiffness constant (κ, dimensionless) (Panchal et al., 2011b).

2.10 Organ weights

Following perfusion experiments, the right ventricle and left ventricle (with septum) were separated and weighed. Liver, kidney, retroperitoneal fat, epididymal fat and omental fat were removed and weighed. Organ weights were normalised relative to tibial length (mg/mm) at the time of removal.

2.11 Intestinal lengths

Small and large intestines were laid out on the bench for measurement following removal of omental fat and immediately prior to sections being collected for ileum and colon contractility studies. Small intestine lengths were taken from junction of stomach and duodenum (pyloric sphincter) to ileo-caecal junction. Large intestine lengths were taken from caeco-colonic junction to rectum.

2.12 Organ bath studies

2.12.1 Thoracic aortic ring reactivity

A section of thoracic aorta was collected immediately after euthanasia and removal of heart and placed in oxygenated (carbogen 95% O_2- 5% CO_2) Tyrode's buffer containing NaCl 136.9 mM, KCl 5.4 mM, $MgCl_2$ 1.05 mM, NaH_2PO_4 0.42 mM, $NaHCO_3$ 22.6 mM, $CaCl_2$ 1.8 mM, D-glucose 5.5 mM, ascorbic acid 0.28 mM and

Na_2 - EDTA 0.1 mM). Thoracic aortic rings (~ 4 mm length) were suspended in an organ bath chamber with resting tension of ~10 mN, buffer was equilibrated at 37ºC in each chamber. Forces of contraction were measured using a Chart MacLab System (ADInstruments, Castle Hill, Australia). For measurement of sodium nitroprusside and acetylcholine responses, thoracic aortic rings were pre-contracted with noradrenaline and allowed to stabilise. Cumulative concentration-response (contraction) curves were measured for noradrenaline (Sigma-Aldrich Australia, Sydney, NSW, Australia), and cumulative concentration-response (relaxation) curves were measured for acetylcholine and sodium nitroprusside (Sigma-Aldrich Australia, Sydney, NSW, Australia) in the presence of a submaximal (~ 70%) contraction to noradrenaline at half log units from $1x10^{-9}$ mol/L to $3x10^{-5}$ mol/L or until no further increases in contraction were observed.

2.12.2 Ileum and colon reactivity

Approximately 1 cm sections of both ileum and proximal colon were collected and washed gently in Tyrode's buffer. Sections were strung at a tension of ~ 10 mN in organ baths containing 25 ml Tyrode's buffer equilibrated to 37ºC oxygenated with carbogen gas (95% O_2 - 5% CO_2 (BOC)). Each tissue was washed twice in equilibrated Tyrode's buffer prior to and between the addition of each drug concentration and allowed to equilibrate to ~ 10 mN. Force of contraction was measured by a Chart MacLab System (ADInstruments, Castle Hill, Australia). Maximal contractility (mN tension) was measured at half-log increases using acetylcholine from $1x10^{-9}$ mol/L to $3x10^{-5}$ mol/L as a concentration-response curve or until no further increases in contraction were observed.

2.13 Histological analysis

Two rats per group were used exclusively for histological analysis. The following tissues were collected: duodenum, jejunum, ileum, proximal colon, mid colon, distal colon, liver, heart. All intestinal samples collected were ~ 1 cm in length. Sections were immediately washed in cold Tyrode's buffer and placed in 10% buffered formalin.

Duodenal sections were collected 1 cm from junction with stomach. Jejunal sections were taken at the midpoint between duodenum and ileum (based on prior small intestinal measurements). Tissues were processed using a Shandon Pathcentre Automatic tissue processor. Using a pre-set protocol as follows: Dehydration with increasing series of ethanol for 1 hour each 70%, 90%, 95%, 100% x 3, followed by rehydration with xylene 3 times. Samples were then soaked in four sets of wax for 1 hour each.

Following processing, tissues were embedded in paraffin using a Microm International EC350 Tissue Embedding Centre. Tissue specimens were prepared on two slides per specimen and two random, non-overlapping fields per slide were taken to avoid biased analysis. Organs from rats used in perfusion studies were also collected. Thin sections (5 μm) of tissues were cut and stained with haematoxylin and eosin stain (Figure 2.2) for determination of inflammatory cell infiltration (heart and liver), fat vacuole deposition (liver) and gastrointestinal morphology. Picrosirius red staining (Figure 2.3) was used to define collagen distribution in the left ventricle of the heart. Periodic Acid-Schiff's stain (Figure 2.4) was used to determine glycoprotein distribution within the gastrointestinal tract.

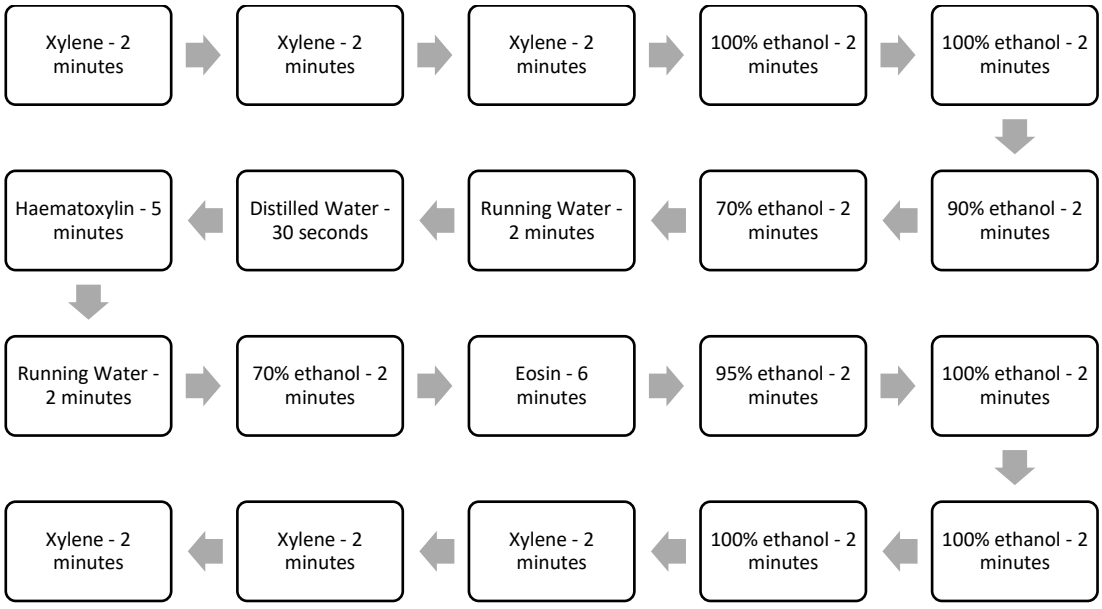


Figure 2.2 - Haematoxylin & Eosin tissue staining protocol

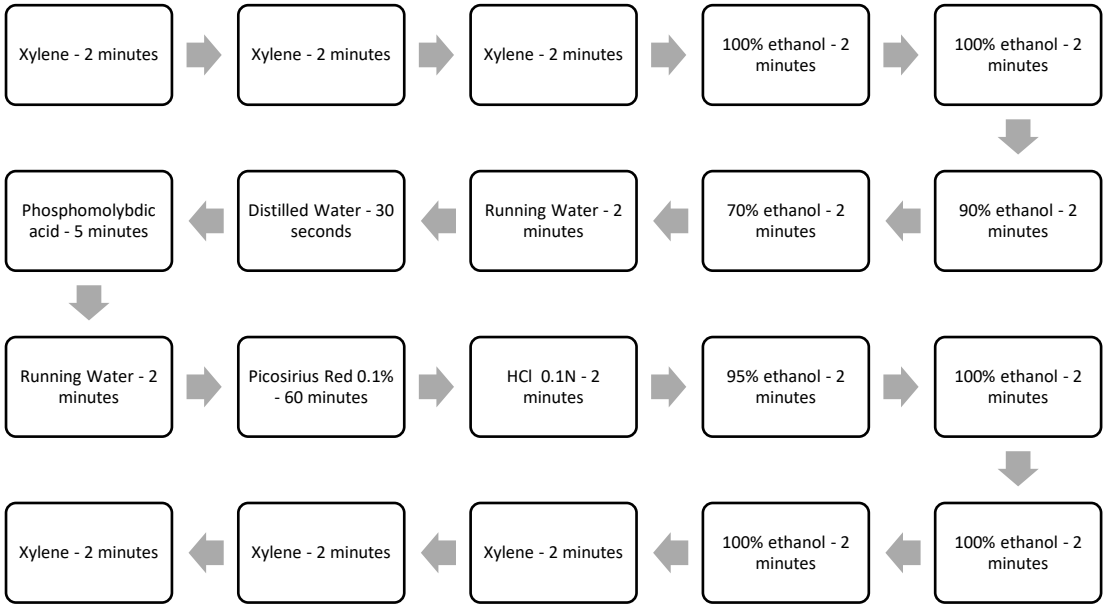


Figure 2.3 - Picrosirius Red tissue staining protocol

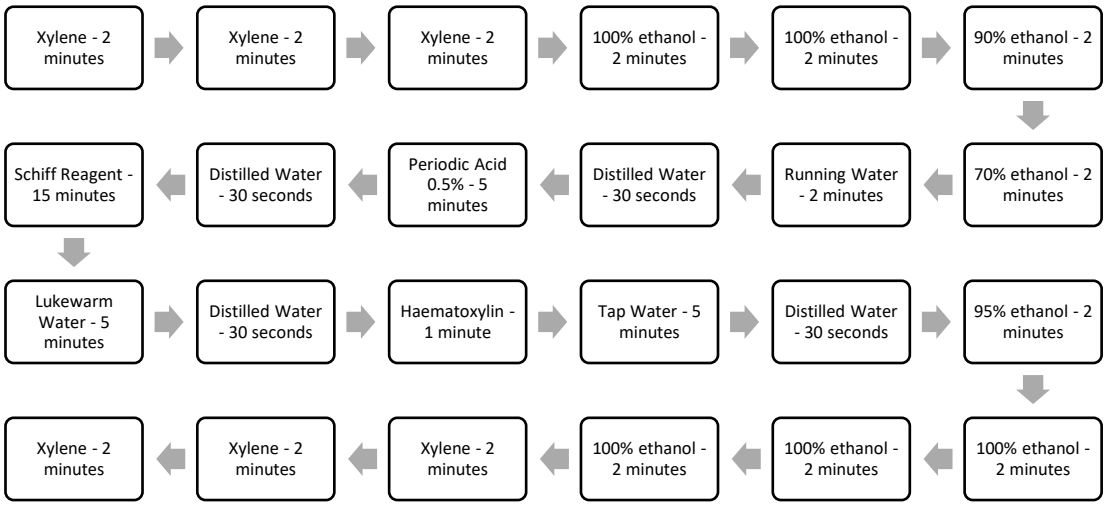


Figure 2.4 - Periodic Acid - Schiff tissue staining protocol

2.13.1 Tissue imaging

EVOS FL Cell imaging system (Life Technologies, Carlsbad, CA) using a Sony ICX285AQ camera at 10 x and 20 x magnification was used to determine the structural integrity of the gastrointestinal tract and the extent of collagen and fat deposition in selected tissue sections.

Nikon Eclipse E600 with a UPLFL 4 x / 0.13 objective using a Olympus DP26 camera was used to measure intestinal structures.

The CellSens Standard 1.9 imaging program system was used for structural analysis. Villi height, crypt depth and mucosal thickness of each section were measured. Each parameter was measured 6 - 8 times using random selection of complete villi / crypt / mucosal structure within the field of view, and an average taken for each section with 4 - 6 sections measured per control and intervention groups. Villi height was determined using villi that were standing vertically, from tip to the top of the crypt. Crypt depth was top of crypt to base. Total mucosal thickness was measured in the small intestine from tip of the villi to start of the muscularis layer while in the large intestine it was measured from lumen to muscularis layer.

2.14 Plasma analysis

Frozen plasma collected in section 2.8 was thawed. Plasma liver enzyme activities – ALT and AST (alanine transaminase and aspartate transaminase), triglycerides, total cholesterol and NEFA (non-esterified fatty acids) were determined using commercially available kits and controls (Olympus) using an Olympus analyser (AU400, Tokyo, Japan). These measurements were undertaken by staff at The University of Queensland Veterinary Laboratory Services, Gatton Campus.

Plasma C-reactive protein (CRP) was determined using a Rat C-reactive Protein ELISA kit (BD Biosciences) as per manufacturer's instructions. Briefly, wash buffer was prepared (PBS in 1 L distilled water). CRP standards were serial diluted from 10 x stock. Plasma samples collected following euthanasia was diluted 1:10000. 100 µL plasma was pipetted into each well of a 96 - well plate in duplicate, then incubated at room temperature for 30 minutes. The plate was washed 4 - 5 times with wash buffer

and 100 µL (1:100 dilution from working concentration) Horseradish Peroxidase (HRP) was added to each well, covered and incubated for 30 minutes at room temperature. The plate was again washed 4 - 5 times and tapped dry. 100 µL 3,3′,5,5′ - Tetramethylbenzidine (TMB) substrate was added and incubated for 5 - 6 minutes at room temperature. 100 µL stop solution was then added and absorbance (450 nm) read using a Fluostar Omega plate reader.

2.15 Fatty acid analysis

2.15.1 Fatty acid extraction

Fatty acids were extracted from 6 - 8 faecal samples per experimental group using diethyl ether (Bacto Laboratories). ~ 0.5 g faeces were placed in a tube with 5 ml MilliQ water, vortexed to break up the samples and acidified with 12 - 15 drops of analytical grade 32% hydrochloric acid (Labserv) and shaken for 30 seconds. 2 ml diethyl ether was added to each sample and centrifuged for 5 minutes at 4000 rpm. Supernatant was removed and stored, and process repeated. Supernatant was combined for each sample and transferred to a 2 ml gas chromatography vial for immediate analysis. This method of extraction recovers >99% of fatty acids that are in samples.

2.15.2 Chromatographic conditions

Fatty acids were identified using gas-chromatography-flame ionisation detector. A Shimadzu GC - 2010 gas chromatograph with an AOC - 20i autoinjector sampled from an AOC - 20s autosampler using a Restek RTX - 5MS capillary column (30 m x 0.25 mm x0.25 µm) (serial number 800436).

An initial oven temperature of 130°C was held for 3 minutes, the temperature was increased to 200°C at a rate of 15°C/min^{-1}. Upon reaching 200°C, the oven was held at this temperature for 3 minutes. The method required a total run time of 10.67 minutes. A volume of 1.0 µL was injected for each sample with an injection temperature of 200°C utilising a 10:1 split ratio using ultra-pure helium carrier gas at a linear velocity of 13.3 cm/s^{-1} at a pressure of 49.9 kPa. The column flow rate was 0.36 mL/min^{-1}. A

purge flow of 3.0 mL/min^{-1} was used, with a total flow rate of 7.0 mL/min^{-1}. The flame ionisation detector used temperature of 200˚C with a sample rate of 40 msec.

Oven conditions for this method were developed using a multi-standard of acetic acid (C2:0) (Rowe Scientific), propionic acid (C3:0) (Sigma-Aldrich) and butyric acid (C4:0) (Sigma-Aldrich) was made up in 4 different concentrations of 0.1 M, 0.01 M, 0.001 M and 0.0001 M to produce a calibration curve. All calibration curves were linear across the ranges tested. Each multi-standard concentration was run to determine retention time of the standard acids (Figure 2.4 and Table 2.2). A standard curve for each acid was done for their quantitation in the samples (Figure 2.6). Accuracy was determined by using a spiked placebo sample and a spiked faecal control sample at a known concentration.

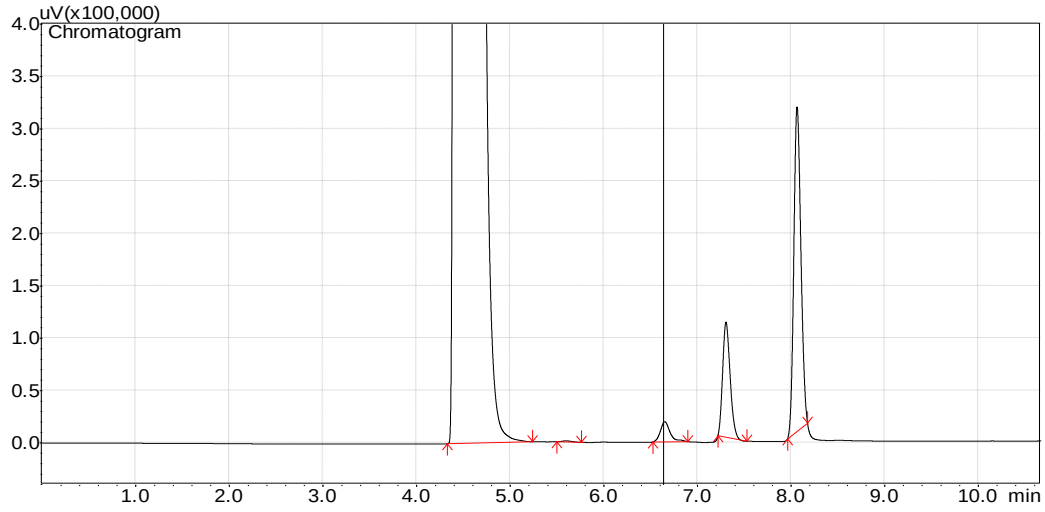


Figure 2.5 Sample chromatogram of standard acids at 0.1M to determine retention time of acetic acid, propionic acid and butyric acid.

Table 2.2 Retention times of acetic, propionic and butyric acids.

Compound	Retention time (minutes)
Acetic acid	6.636
Propionic acid	7.299
Butyric acid	8.067

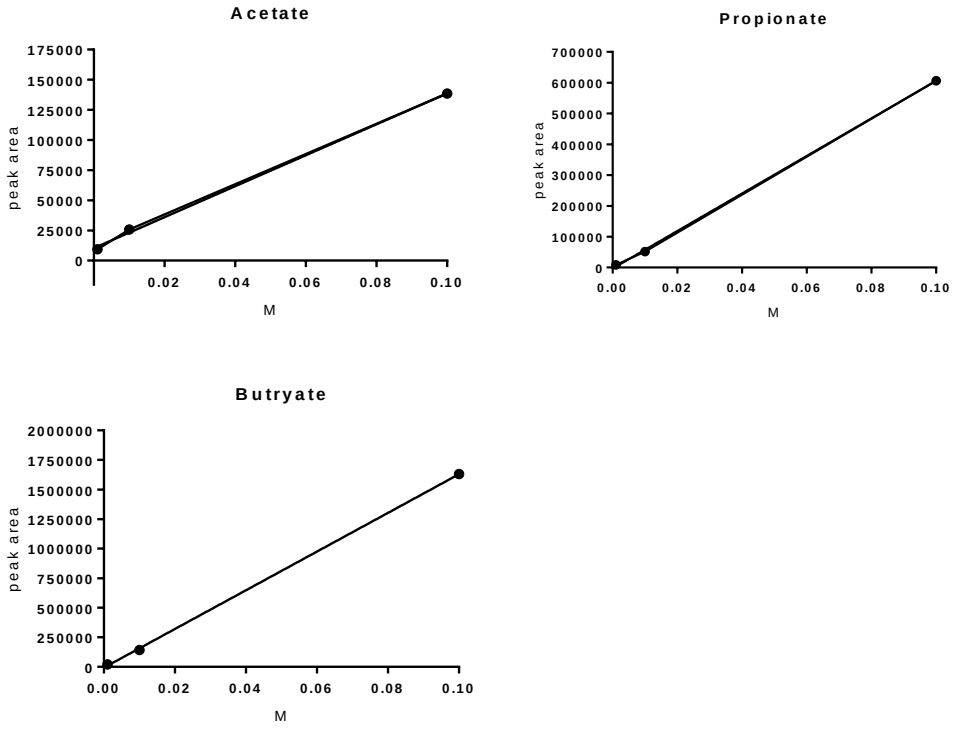


Figure 2.6 Calibration Curves for Acetate, Propionate and Butyrate for faecal SCFA analysis

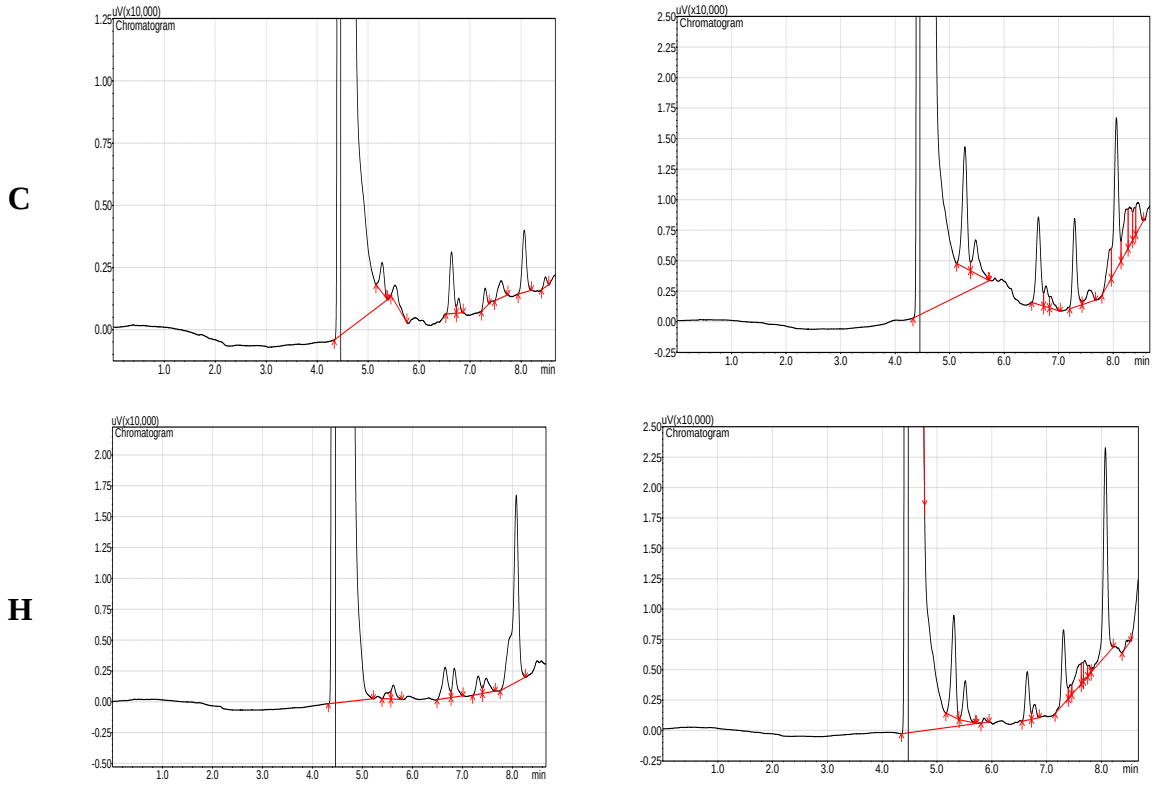


Figure 2.7 Example of validation chromatograms with C and H faecal samples with the addition of 0.01M standard to determine accuracy of retention times and sample selection.

2.16 Statistical analysis

Data are presented as mean ± standard errors of the means (S.E.M). Differences between the groups were determined by 1 - way and 2 - way analysis of variance (ANOVA). Statistically significant variables were treated with Newman-Keuls post hoc test to compare all the groups of animals. $P < 0.05$ was considered statistically significant. GraphPad Prism version 7.00 for Windows (San Diego, CA, USA) was used to perform all statistical analyses.

Chapter 3 - Oat products attenuate metabolic syndrome in high carbohydrate, high fat-fed rats.

3.1 Introduction

Metabolic syndrome and obesity are major worldwide health issues that affect between 20 and 40 percent of the adult population (Moreira et al., 2014, O'Neill and O'Driscoll, 2015). Diets high in fats and carbohydrates, particularly fructose, are one of the major contributing factors towards metabolic syndrome (ter Horst et al., 2016, Moreira et al., 2014). In recent years, diet has become an integral therapeutic component to return the biomarkers of metabolic syndrome to normal. Functional foods that confer a health benefit in addition to nutrition are becoming part of the treatment. Diets that include cereal grains, such as oats, may protect the body against cardiovascular disease and diabetes (Connolly et al., 2012, Zhou et al., 2015, Dixit et al., 2011). As reviewed in Chapter 1, β-glucans decreased blood cholesterol concentrations and improved postprandial glycaemic response by binding cholesterol and bile acids assisting in their elimination from the body (Queenan et al., 2007, Drozdowski et al., 2010). The fibre and micronutrients contained in the cereal grain may have protective effects depending on their solubility (Jonnalagadda et al., 2011). However, the beneficial effects of these grains may vary in response to soluble fibre concentrations.

In recent years, oats have become more fashionable as a food source around the world due to its status as a functional food. Unlike wheat and most other cereal grains, oats do not contain gluten but avenin, which may make oats suitable for coeliac disease patients (Moulton, 1959, Janatuinen et al., 1995). However, in Australia and New Zealand, it is recommended that coeliac disease patients do not consume oats (Gastroenterological Society of Australia, 2012) as some people also have a sensitivity to avenin (Hardy et al., 2015).

Oats are generally consumed as a wholegrain (van den Broeck et al., 2016), however many previous studies have focussed on oat β-glucans. The 2015 American Dietary Guidelines note that 3 g/day β-glucans and 16 g/day of wholegrains reduce the risk of developing chronic diseases, including diabetes, cardiovascular disease and possibly

some cancers (U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2015). Although the gastrointestinal tract is integral to the absorption of nutrients and the environment of the microbiome, there is a paucity of information regarding the impact of oat products on gastrointestinal morphology, especially in relation to high carbohydrate, high fat diets and metabolic syndrome.

One possible mechanism of action is through the production of shortchain fatty acids (SCFA). As β-glucans may have prebiotic effects (Arena et al., 2014), these wholegrain oat groats, oat bran and β-glucans should increase concentrations of SCFA in the colon. The physiological functions of SCFA are discussed in Chapter 1. Previous limited work has concentrated on the gut microbiota composition, showing that wholegrain oat, oat bran and oat β-glucan altered the microbiota (Wolever et al., 2010, Mantzouridou et al., 2013, Kedia et al., 2009). Whether these microbiome changes modify the colonic and plasma concentrations of SCFA and subsequent physiological effects are currently unknown.

The objectives of this study were to determine the effects of wholegrain oat groats, oat bran and partially purified β-glucans on the physiological and metabolic parameters associated with metabolic syndrome. Further, this study assessed whether these changes were associated with changes to the morphology of the gastrointestinal tract and the faecal SCFA in male Wistar rats fed a high carbohydrate, high fat diet. It is hypothesised that, with increased processing of oats in the diet, the parameters associated with metabolic syndrome will be improved to a greater degree. The second hypothesis is that the various oat products will give similar responses in both C and H diets. The third hypothesis is that it is not just the β-glucans in the oat that are beneficial, so that all nutrients work synergistically to improve metabolic syndrome parameters.

3.2 Methods

This study was approved by the USQ Animal Ethics Committee (AEC #13REA005 valid until 30 September 2016) using approved methods, diets, cardiovascular, metabolic, hepatic and gastrointestinal studies as outlined in Chapter 2.

8 groups of rats were used for this study, defined as cornstarch (C) or high carbohydrate, high fat (H) controls (n = 12/group), cornstarch + wholegrain oat groats

(CO) or high carbohydrate, high fat + wholegrain oat groats (HO) (n = 10/group), cornstarch + oat bran (CB) or high carbohydrate, high fat + oat bran (HB) (n = 12/group) and cornstarch + β-glucan powder (CG) or high carbohydrate high fat + β-glucan powder (HG) (n = 8/group). The concentration of β-glucans increased from 4% in the wholegrain oat groat to 6% in oat bran and 12% in the partially purified β-glucan powder.

The following tests on control and intervention groups were performed as in the methods detailed in Chapter 2 of this thesis: body weight, food, water and energy intake, oral glucose tolerance test, body composition (DXA), systolic blood pressure, diastolic stiffness, aortic contractility, ileum and colon contractility, blood lipid analysis, liver enzyme analysis, gastrointestinal structural analysis, short chain fatty acid analysis and plasma and tissue inflammatory marker analysis.

To define the responses to each intervention, this study has been separated into three parts: wholegrain oat groats, oat bran and β-glucan powder. Differences between C and H will be discussed in the wholegrain oat groats section as they are common to all groups.

3.3 Results

3.3.1 Body Composition and dietary intake

Body weights of H, HO (H + wholegrain oat groats), HB (H + oat bran) and HG (H + β-glucan powder) were higher than C, CO (C + wholegrain oat groats), CB (C + oat bran) and CG (C + β-glucan powder) after 16 weeks while CO and CG were heavier than C after 16 weeks. There was no change in final body weight in CB, HB and HG compared to respective controls (Figures 3.1a, 3.1b and 3.1c).

The overall percentage weight gain from week 8 - 16 was not significantly different between any of the diets (Tables 3.1, 3.2 and 3.3). The abdominal circumference of H was increased compared to C; CO was greater than C and HO, HB and HG were decreased compared to H; CB and CG were not significantly different to C (Tables 3.1, 3.2 and 3.3).

The retroperitoneal, omental, epididymal and total abdominal fat pads of H were increased compared to C (Tables 3.1, 3.2, 3.3). No significant differences in any of the fat pads were shown between controls and interventions (Tables 3.1, 3.2 and 3.3).

Lean mass increased in CO, CB and CG compared to C and HG compared with but there was no significant difference between C, H and HO or HB (Tables 3.1, 3.2 and 3.3). Fat mass was increased in H compared to C with no significant difference between HO, HB and H or CO, CB, CG, HG and C; there was a decrease in fat mass in HG compared to H (Tables 3.1, 3,2 and 3.3).

Food intakes (g/day) were decreased in H compared to C and were unchanged in the HO, HB, HG, CO and CG compared to respective controls; CB had a decreased food intake compared to C (Tables 3.1, 3.2 and 3.3). Water intake was the same in H, C all intervention groups except CO had an increased intake compared to C (Tables 3.1, 3.2 and 3.3).

Energy intakes for weeks 8-16 were greater in H, HO, HB and HG compared to C, CO, CB and CG. There was no significant difference between interventions and controls except CO had a decreased intake compared to C (Tables 3.1, 3.2 and 3.3).

Feed efficiency was improved with the addition of wholegrain oat groats, oat bran or β-glucan powder to the diet regardless of control (Tables 3.1, 3.2 and 3.3).

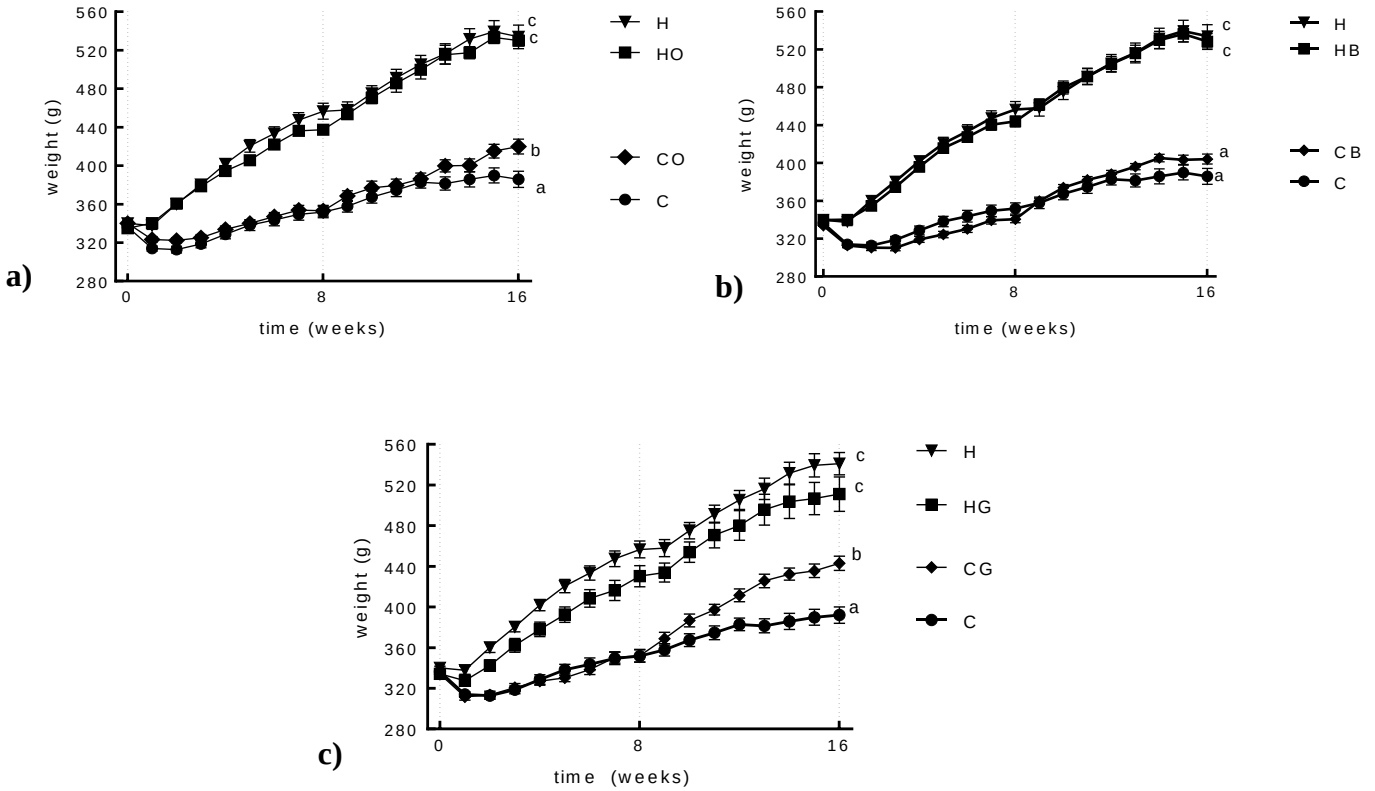


Figure 3.1 Weekly body weight of male Wistar rats fed a diet of cornstarch or high carbohydrate, high fat (common to all groups) supplemented with a) wholegrain oat groats, b) oat bran or c) β-glucan powder after 8 weeks

Values are mean ± S.E.M. n = 8 - 10 per group. Means without a common superscript letter differ

C = cornstarch; CO = C + wholegrain oat groats; CB = C + oat bran; CG = C + β-glucan powder; H = high carbohydrate, high fat; HO = H + wholegrain oat groats; HB = H + oat bran; HG = H + β-glucan powder.

Table 3.1 Body composition and food, water, energy intakes of male Wistar rats following a dietary intervention of wholegrain oat groats

					P-value		
variable	C	CO	H	HO	diet	intervention	interaction
Initial body weight (g)	340±3[a]	340±3[a]	336±1[a]	335±3[a]	0.0984	0.8516	0.8516
8-week body weight (g)	348±6[a]	353±6[a]	452±7[b]	442±7[b]	<0.0001*	0.7068	0.2628
Final body weight (g)	392±8[a]	420±8[b]	541±11[c]	543±11[c]	<0.0001*	0.1274	0.1847
Body weight gain 8-16 week (%)	11.1±1.1[a]	11.8±2.3[a]	11.5±1.2[a]	12.2±1.1[a]	0.07775	0.6566	0.9819
Abdominal circumference (cm)	18±0.2[a]	19±0.1[a]	23±0.4[b]	22±0.4[c]	<0.0001*	>0.9999	0.0020*
Retroperitoneal fat (mg/mm)	199±21[a]	209±17[a]	449±43[b]	445±17[b]	<0.0001*	0.9197	0.8141
Omental fat (mg/mm)	106±10[a]	148±11[a]	246±22[b]	231±8[b]	<0.0001*	0.2508	0.8168
Epididymal fat (mg/mm)	87±9[a]	127±8[a]	252±22[b]	266±9[b]	<0.0001*	0.0705	0.3758
Total fat pads (mg/mm)	392±33[a]	484±27[a]	948±80[b]	942±26[b]	<0.0001*	0.4226	0.3615
Lean mass (g)	255±10[a]	294±10[b]	263±11[ab]	260±8[ab]	0.2033	0.0824	0.0448*
Fat mass (g)	121±12[a]	112±7[a]	254±18[b]	237±7[b]	<0.0001*	0.3078	0.7515
Food intake (g/day)	41±1[a]	38±1[a]	25±1[b]	24±1[b]	<0.0001*	0.0551	0.3288
Water intake (g/day)	27±2[a]	33±2[b]	29±1[ab]	26±1[a]	0.1323	0.3620	0.0086
Energy intake 8-16 weeks (kJ/day)	447±18[a]	409±9[c]	534±8[b]	511±14[b]	<0.0001*	<0.0001*	<0.0001*
Feed efficiency (g/kJ)	0.08±0.01[a]	0.17±0.01[c]	0.13±0.01[b]	0.18±0.01[c]	0.0052	<0.0001*	0.0555

Values are mean ± S.E.M. and n = 8 - 10 per group. Means without a common superscript letter differ, * indicates $P < 0.05$

C = cornstarch; CO = C + wholegrain oat groats; H = high carbohydrate, high fat; HO = H + wholegrain oat groats.

Table 3.2 Body composition and food, water, energy intakes of male Wistar rats following a dietary intervention of oat bran

					P-value		
variable	C	CB	H	HB	diet	intervention	interaction
Initial body weight (g)	340±3^{a}	334±0^{a}	336±1^{a}	340±2^{a}	0.6304	0.6304	0.0203*
8-week body weight (g)	348±6^{a}	339±3^{a}	452±7^{b}	442±6^{b}	<0.0001*	0.1211	0.9339
Final body weight (g)	392±8^{a}	404±5^{a}	541±11^{b}	537±8^{b}	<0.0001*	0.6439	0.3572
Body weight gain 8-16 week (%)	11.1±1.1^{a}	11.9±1.2^{a}	11.5±1.2^{a}	12.0±1.1^{a}	0.8395	0.5898	0.8461
Abdominal circumference (cm)	18±0.2^{a}	18±0.1^{a}	23±0.4^{b}	22±0.3^{c}	<0.0001*	0.0683	0.0683
Retroperitoneal fat (mg/mm)	199±21^{a}	218±13^{a}	449±43^{b}	530±28^{b}	<0.0001*	0.1069	0.3128
Omental fat (mg/mm)	106±10^{a}	100±13^{a}	246±22^{b}	246±18^{b}	<0.0001*	0.8612	0.8612
Epididymal fat (mg/mm)	87±9^{a}	84±7^{a}	252±22^{b}	249±32^{b}	<0.0001*	0.8884	>0.9999
Total fat pads (mg/mm)	392±33^{a}	403±30^{a}	948±80^{b}	1026±75^{b}	<0.0001*	0.4832	0.5971
Lean mass (g)	255±10^{a}	296±11^{b}	263±11^{ab}	266±8^{ab}	0.2842	0.0375*	0.0697
Fat mass (g)	121±12^{a}	94±10^{a}	254±18^{b}	246±5^{b}	<0.0001*	0.0638	0.7759
Food intake (g/day)	41±1^{a}	36±1^{b}	25±1^{c}	23±1^{c}	<0.0001*	0.0011*	0.1415
Water intake (g/day)	27±2^{a}	27±2^{a}	29±1^{ab}	26±1^{a}	<0.0001*	0.3480	0.3480
Energy intake 8-16 weeks (kJ/day)	447±18^{a}	429±10^{a}	531±9^{b}	497±7^{b}	<0.0001*	0.0913	0.6393
Feed efficiency (g/kJ)	0.08±0.01^{a}	0.15±0.01^{b}	0.13±0.01^{b}	0.17±0.01^{c}	0.0015*	<0.0001*	0.1503

Values are mean ± S.E.M. and n = 8 - 10 per group. Means without a common superscript letter differ, * indicates $P < 0.05$

C = cornstarch; CB = C + oat bran; H = high carbohydrate, high fat; HB = H + oat bran;

Table 3.3 Body composition and food, water, energy intakes of male Wistar rats following a dietary intervention of β-glucan powder

					P-value		
variable	C	CG	H	HG	diet	intervention	interaction
Initial body weight (g)	340±3[a]	334±1[a]	336±1[a]	334±1[a]	0.3399	0.0611	0.3399
8-week body weight (g)	348±6[a]	352±7[a]	452±7[b]	428±9[b]	<0.0001*	0.1775	0.0622
Final body weight (g)	392±8[a]	443±7[b]	541±11[c]	511±17[c]	<0.0001*	0.3508	0.0009*
Body weight gain 8-16 week (%)	11.1±1.1[a]	12.8±3.4[a]	11.5±1.2[a]	11.6±3.9[a]	0.8763	0.7030	0.7220
Abdominal circumference (cm)	18±0.2[a]	19±0.1[a]	23±0.4[c]	21±0.5[b]	<0.0001*	0.0735	<0.0001*
Retroperitoneal fat (mg/mm)	199±21[a]	250±21[a]	449±43[b]	384±51[b]	<0.0001*	0.8299	0.1245
Omental fat (mg/mm)	106±10[a]	141±12[a]	246±22[b]	206±25[b]	<0.0001*	0.9027	0.0526
Epididymal fat (mg/mm)	87±9[a]	111±12[a]	252±22[b]	173±32[b]	<0.0001*	0.1753	0.0151*
Total fat pads (mg/mm)	392±33[a]	502±39[a]	948±80[b]	762±103[b]	<0.0001*	0.5877	0.0400*
Lean mass (g)	255±10[a]	324±7[b]	263±11[a]	340±10[b]	0.2293	<0.0001*	0.7041
Fat mass (g)	121±12[a]	88±10[a]	254±18[b]	138±23[a]	<0.0001*	0.0001*	0.0182*
Food intake (g/day)	41±1[a]	40±2[a]	25±1[b]	25±1[b]	<0.0001*	0.7141	0.6412
Water intake (g/day)	27±2[a]	25±1[a]	29±1[a]	31±1[a]	0.0356*	0.8035	0.2625
Energy intake (kJ/day)	447±18[a]	421±12[a]	534±8[b]	507±17[b]	<0.0001*	0.0813	0.9732
Feed efficiency (g/kJ)	0.08±0.01[a]	0.20±0.01[d]	0.13±0.01[b]	0.16±0.01[c]	0.6346	<0.0001*	<0.0001*

Values are mean ± S.E.M. and n = 8 - 10 per group. Means without a common superscript letter differ, * indicates $P < 0.05$

C = cornstarch; CG = C + β-glucan powder; H = high carbohydrate, high fat; HG = H + β-glucan powder.

*** C & H are the same for all 3 intervention groups. ***

3.3.2 Cardiovascular parameters

Systolic blood pressure was higher in H than the C; HO, HB and HG were decreased compared to H; HO was normalised to C while CO, was decreased compared to C; CB and CG were similar to C (Tables 3.4, 3.5 and 3.6).

Left and right ventricular wet weights were not significantly different across any of the intervention or control groups. Left ventricular diastolic stiffness was increased in the H compared to C; All intervention groups were the same as both controls (Tables 3.4, 3.5 and 3.6).

Inflammatory cell infiltration increased in H compared to C, infiltration normalised in HO, HB and HG hearts (Figure 3.4). Collagen deposition was greater in H rats compared to C, with decreased deposition in HO, HB and HG (Figure 3.5)

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Table 3.4 Cardiovascular parameters of male Wistar rats following a dietary intervention of wholegrain oat groats

					P-value		
Variable	C	CO	H	HO	Diet	intervention	interaction
Systolic Blood Pressure (mmHg)	131±2^{a}	128±1^{b}	152±1^{c}	135±2^{a}	<0.0001*	<0.0001*	0.0001*
Left ventricular + septum wet weight (mg/mm)	18±2^{a}	23±1^{a}	20±2^{a}	23±1^{a}	0.5846	0.0337*	0.5846
Right ventricular wet weight (mg/mm)	4±1^{a}	6±0.6^{a}	5±0.7^{a}	6±0.3^{a}	0.5372	0.0696	0.4372
Left ventricular diastolic stiffness constant κ	25±1.6^{a}	28±0.4^{ab}	31±1.5^{b}	30±2.0^{ab}	0.0204*	0.5359	0.2223

Table 3.5 Cardiovascular parameters of male Wistar rats following a dietary intervention of oat bran

					P-value		
variable	C	CB	H	HB	diet	intervention	interaction
Systolic Blood Pressure (mmHg)	131±2^{a}	133±1^{a}	152±1^{b}	140±1^{c}	<0.0001*	0.0006*	<0.0001*
Left ventricular + septum wet weight (mg/mm)	18±2^{a}	23±1^{a}	20±2^{a}	24±1^{a}	0.37331	0.0100*	0.7656
Right ventricular weight (mg/mm)	4±1^{a}	6±0.6^{a}	5±0.7^{a}	6±0.4^{a}	0.4033	0.0152*	0.4033
Left ventricular diastolic stiffness constant κ	25±1.6^{a}	28±1.2^{ab}	31±1.5^{b}	28±0.7^{ab}	0.0338*	>0.9999	0.0338*

Table 3.6 Cardiovascular parameters of male Wistar rats following a dietary intervention of β-glucan powder

					P-value		
Variable	C	CG	H	HG	diet	intervention	interaction
Systolic Blood Pressure (mmHg)	131±2^{a}	130±2^{a}	152±1^{c}	139±3^{b}	<0.0001*	0.0007*	0.0009*
Left ventricular + septum wet weight (mg/mm)	18±2^{a}	24±1^{a}	20±2^{a}	25±1^{a}	0.4199	0.0050*	0.7872
Right ventricular wet weight (mg/mm)	4±1^{a}	5±0.3^{a}	5±0.7^{a}	5±0.6^{a}	0.5365	0.5365	0.5365
Left ventricular diastolic stiffness constant κ	25±1.6^{a}	26±2.9^{ab}	31±1.5^{b}	30±1.8^{b}	0.0286*	>0.9999	0.6461

Values are mean ± S.E.M. and n = 8 - 10 per group. Means without a common superscript letter differ, * indicates $P < 0.05$

C = cornstarch; CO = C + wholegrain oat groats; CB = C + oat bran; CG = C + β-glucan powder; H = high carbohydrate high fat;

HO = H + wholegrain oat groats; HB = H + oat bran; HG = H + β-glucan powder *** C & H are the same for all 3 intervention groups***

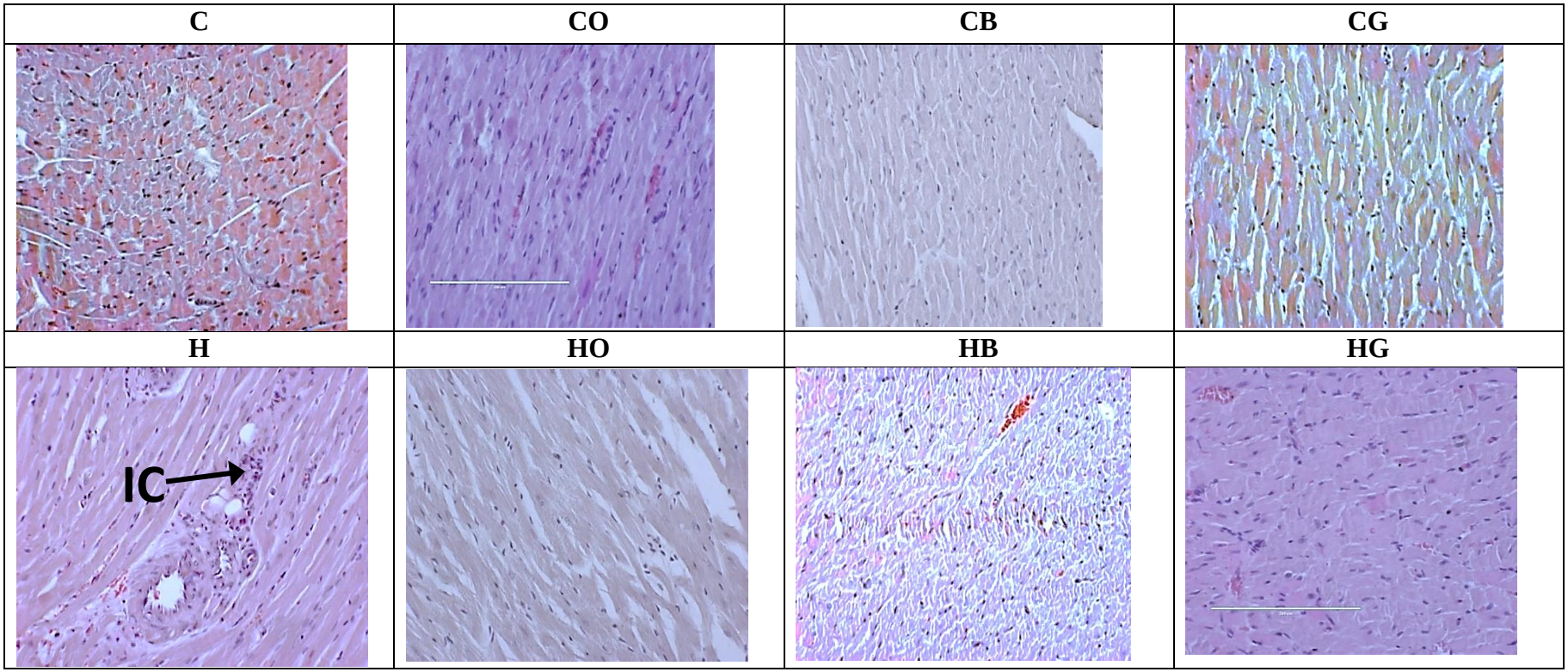


Figure 3.2 Inflammatory cells (IC) in the left ventricle of the heart of male Wistar rats induced by high carbohydrate, high fat diet and following a dietary intervention of wholegrain oat groats, oat bran or β-glucan powder for 8 weeks

Haematoxylin & eosin staining, 20 x magnification.

C = cornstarch; CO = C + wholegrain oat groats; CB = C + oat bran; CG = C + β-glucan powder;

H = high carbohydrate high fat; HO = H + wholegrain oat groats; HB = H + oat bran; HG = H + β-glucan powder

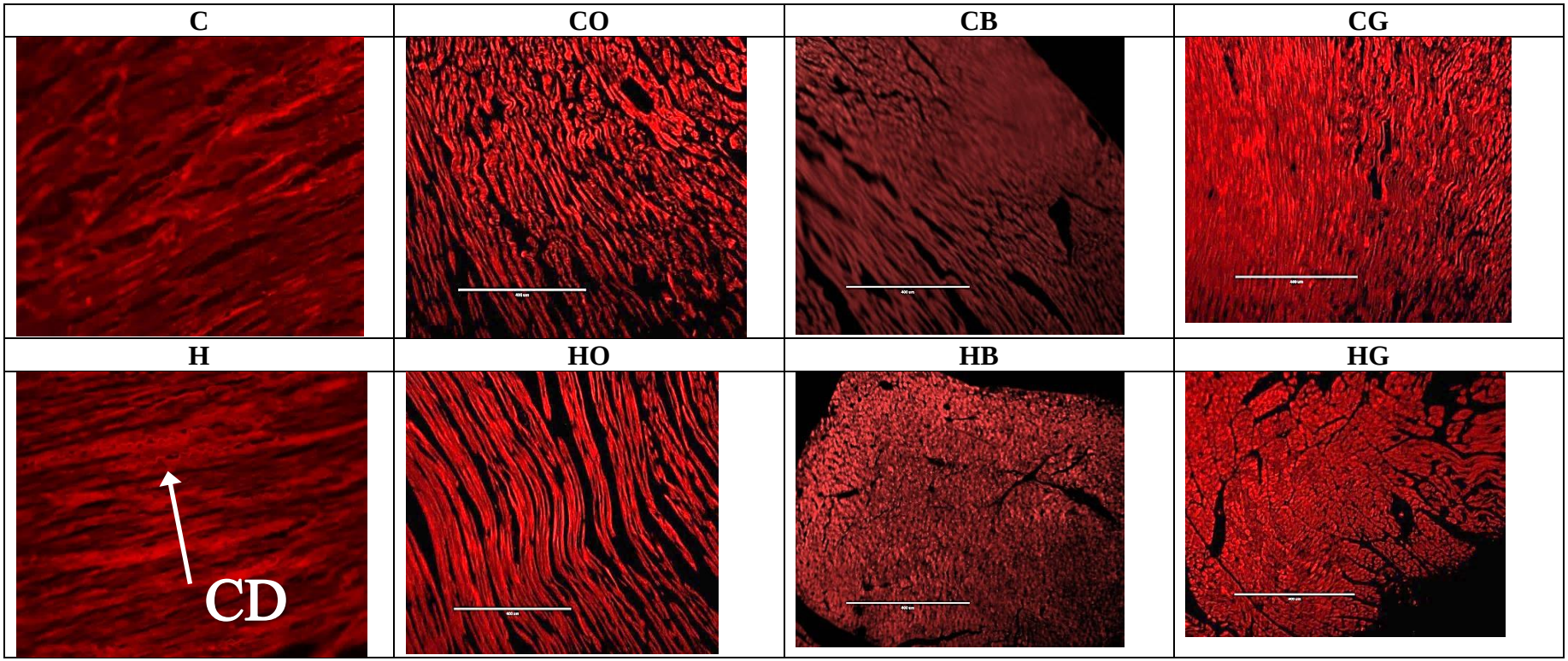


Figure 3.3 Collagen deposition (CD) in left ventricle of the heart of male Wistar rats induced by high carbohydrate, high fat diet and following a dietary intervention of wholegrain oat groats, oat bran or β-glucan powder for 8 weeks

Picrosirius red staining, 20 x magnification.

C = cornstarch; CO = C + wholegrain oat groats; CB = C + oat bran; CG = C + β-glucan powder; H = high carbohydrate high fat; HO = H + wholegrain oat groats; HB = H + oat bran; HG = H + β-glucan powder

.

3.3.3 Liver Parameters

Liver wet weight of H was increased compared to C, HO, HB and HG showed no change compared to H; CO and CG showed no change to C; however, CB was increased compared to C (Tables 3.7, 3.8 and 3.9).

Plasma ALT was higher in H than C; HO and CO, CB remained unchanged compared to their relevant controls; HB decreased compared to H and was not significantly different to C; HG was increased compared to H and CG was not significantly different to either H or C (Tables 3.7, 3.8 and 3.9).

Plasma AST was similar in H and C and remained unchanged in HO, CO and CG; CB and HB were decreased compared to relevant controls; HG increased compared to both H and C. (Tables 3.7, 3.8 and 3.9)

Fat vacuoles in the liver were obvious in H but were not visible in C, CO, CB, CG or HO and HG; HB had fewer and smaller fat vacuoles than H (Figure 3.10).

Table 3.7 Liver weight and plasma ALT, AST of male Wistar rats following a dietary intervention of wholegrain oat groats

					P-value		
variable	C	CO	H	HO	diet	intervention	interaction
Liver wet weight (mg/mm)	229±11[a]	238±8[a]	320±11[b]	323±6[b]	<0.0001[*]	0.5566	0.7684
Plasma ALT (U/L)	25±4[a]	33±4[a]	36±3[a]	30±2[a]	0.2838	0.7865	0.0665
Plasma AST (U/L)	75±9[a]	78±7[a]	67±3[a]	68±8[a]	0.2332	0.7883	0.8932

Table 3.8 Liver weight and plasma ALT, AST of male Wistar rats following a dietary intervention of oat bran

					P-value		
variable	C	CB	H	HB	diet	intervention	interaction
Liver wet weight (mg/mm)	229±11[a]	271±11[c]	320±11[b]	325±9[b]	<0.0001*	0.0309*	0.0861
Plasma ALT (U/L)	25±4[a]	22±2[a]	39±3[b]	27±2[a]	0.0128*	0.0434*	0.2496
Plasma AST (U/L)	75±9[a]	58±2[b]	69±3[a]	56±2[b]	0.5095	0.0182*	0.7343

Table 3.9 Liver weight and plasma ALT, AST of male Wistar rats following the a dietary intervention of β-glucan powder

					P-value		
variable	C	CG	H	HG	diet	intervention	interaction
Liver wet weight (mg/mm)	229±11[a]	246±9[a]	320±11[b]	301±18[b]	<0.0001*	0.9370	0.1608
Plasma ALT (U/L)	25±4[a]	32±4[ab]	36±3[b]	43±5[c]	0.0001*	<0.0001*	<0.0001*
Plasma AST (U/L)	75±9[a]	108±19[ab]	69±3[a]	134±30[b]	0.5006	0.0029*	0.2934

Values are mean ± S.E.M. and n = 8 - 10 per group. Means without a common superscript letter differ, * indicates P < 0.05

C = cornstarch; CO = C + wholegrain oat groats; CB = C + oat bran; CG = C + β-glucan powder; H = high carbohydrate high fat; HO = H + wholegrain oat groats; HB = H + oat bran; HG = H + β-glucan powder. *** C & H are the same for all 3 intervention groups***.

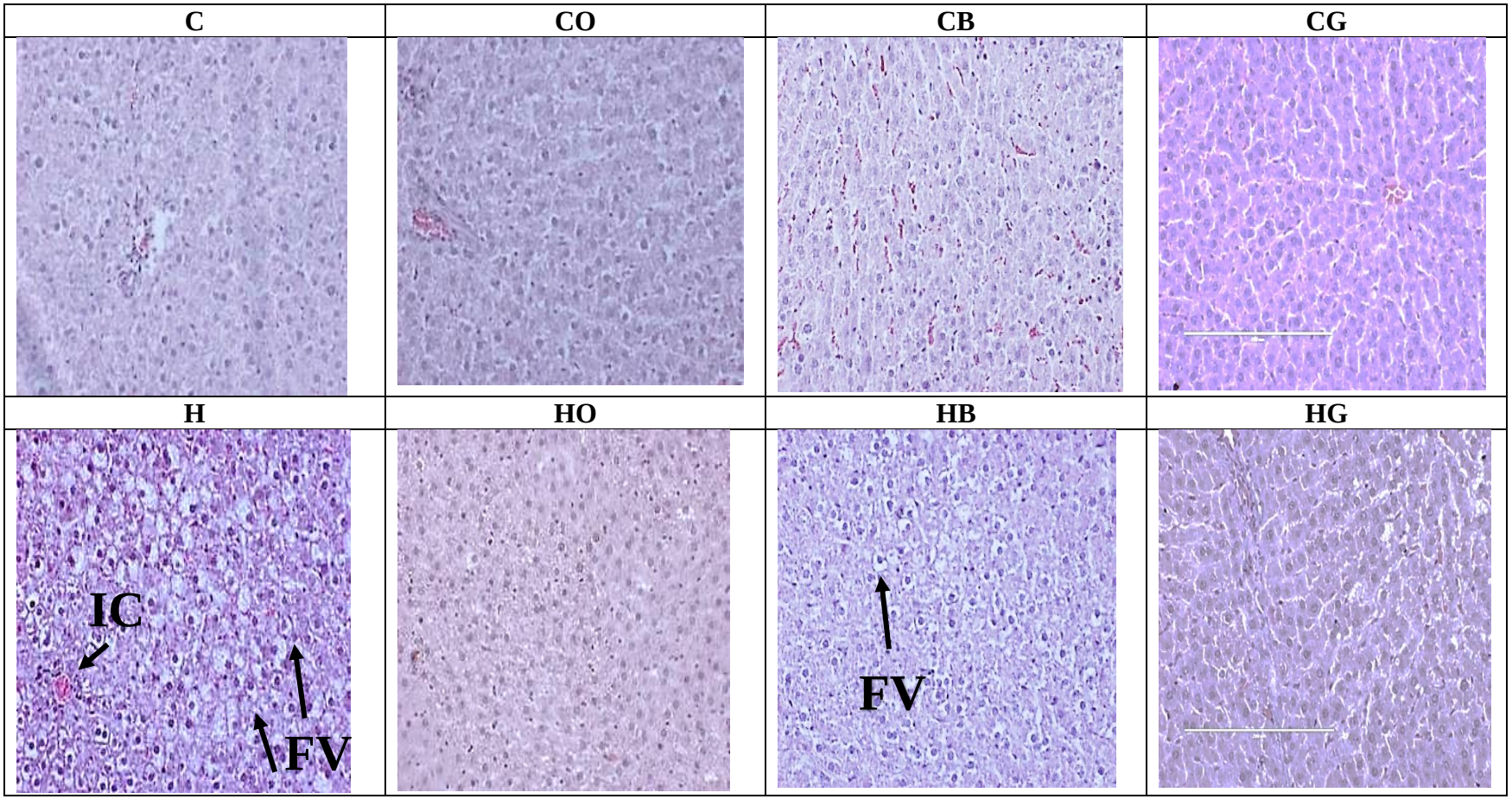


Figure 3.6 Fat deposition (FV) and inflammatiory cells (IC) in male Wistar rat livers induced by high carbohydrate, high fat diet and following a dietary intervention of wholegrain oat groats, oat bran or β-glucan powder

Haematoxylin & eosin staining, 20 x magnification

C = cornstarch; CO = C + wholegrain oat groats; CB = C + oat bran; CG = C + β-glucan powder; H = high carbohydrate high fat; HO = H + wholegrain oat groats; HB = H + oat bran; HG = H + β-glucan powder

3.4 Plasma biochemistry and inflammatory markers

Plasma total cholesterol concentrations were not significantly different between any of the groups (Tables 3.10, 3.11 and 3.12). Plasma triglyceride concentrations were higher in H than C with no change in the HO, HB CO and CB compared to respective controls; HG concentrations decreased compared to H and CG was similar to both C and HG (Tables 3.10, 3.11 and 3.12).

Plasma NEFA concentrations were increased in H compared to C, CO and CB were unchanged compared to C; HO and HB were unchanged compared to H; HG was decreased compared to H and CG increased compared to C and similar to H and HG (Tables 3.10, 3.11 and 3.12).

Plasma C-reactive protein concentrations were not significantly different between any of the groups (Tables 3.10, 3.11 and 3.12).

Fasting blood glucose concentrations were increased in H while concentrations in HO, HB and HG were normalised to C after 8 weeks of intervention CO, CB and CG remained unchanged from C (Table 3.10, 3.11 and 3.12). Similarly, the glucose area under the curve (AUC) was elevated in H; CO, CB and HB were at not significantly different compared to C values while CG, HO and HG had an increased glucose AUC than C but was decreased compared to H (Tables 3.10, 3.11 and 3.12).

Table 3.10 Plasma biochemistry and C-reactive protein of male Wistar rats following a dietary intervention of wholegrain oats groats

					P-value		
variable	C	CO	H	HO	diet	intervention	interaction
Plasma total cholesterol (mmol/L)	1.5±0.1[a]	1.5±0.2[a]	1.6±0.1[a]	1.5±0.1[a]	0.6296	0.6296	0.6296
Plasma triglycerides (mmol/L)	0.3±0.0[a]	0.5±0.0[a]	1.5±0.3[b]	2.2±0.5[b]	<0.0001*	0.0757	0.3136
Plasma NEFA (mmol/L)	1.2±0.2[a]	1.4±0.2[a]	4.1±0.6[b]	5.8±0.6[c]	<0.0001*	0.0264	0.0745
Plasma C-reactive protein (ng/ml)	356±14[a]	359±27[a]	305±21[a]	283±19[a]	0.0049*	0.6511	0.5523
Fasting blood glucose (mmol/L)	4.0±0.1[a]	4.0±0.1[a]	5.1±0.2[b]	4.5±0.2[a]	<0.0001*	0.0607	0.0607
Glucose AUC (mmol/L/min)	651±13[a]	619±19[a]	831±28[b]	730±24[c]	<0.0001*	0.0035*	0.1223

Table 3.11 Plasma biochemistry and C-reactive protein of male Wistar rats following a dietary intervention of oat bran

variable	C	CB	H	HB	*P-value*		
					diet	intervention	interaction
Plasma total cholesterol (mmol/L)	1.5±0.1[a]	1.5±0.1[a]	1.6±0.1[a]	1.5±0.1[a]	0.6359	0.6359	0.6359
Plasma triglycerides (mmol/L)	0.3±0.0[a]	0.6±0.3[a]	2.0±0.3[b]	2.0±0.3[b]	<0.0001*	0.5588	0.5588
Plasma NEFA (mmol/L)	1.2±0.2[a]	2.2±0.5[a]	5.1±0.5[b]	4.4±0.4[b]	<0.0001*	0.7228	0.0536
Plasma C-reactive protein (ng/ml)	356±14[a]	345±47[a]	305±21[a]	293±27[a]	0.0960	0.7034	0.9868
Fasting blood glucose (mmol/L)	4.0±0.1[a]	4.0±0.2[a]	5.1±0.2[b]	4.2±0.1[a]	<0.0001*	0.0037*	0.0037*
Glucose AUC (mmol/L/min)	651±13[a]	712±16[a]	831±28[b]	705±10[a]	<0.0001*	0.0781	<0.0001*

Table 3.12 Plasma biochemistry and C-reactive protein of male Wistar rats following a dietary intervention of β-glucan powder

					P-value		
variable	C	CG	H	HG	diet	intervention	interaction
Plasma total cholesterol (mmol/L)	1.5±0.1[a]	1.6±0.1[a]	1.6±0.1[a]	1.7±0.1[a]	0.3441	0.3441	>0.9999
Plasma triglycerides (mmol/L)	0.3±0.0[a]	0.7±0.1[ab]	2.0±0.3[c]	1.3±0.3[b]	<0.0001*	0.5242	0.0239*
Plasma NEFA (mmol/L)	1.1±0.2[a]	4.1±0.6[bc]	5.1±0.5[c]	3.1±0.8[b]	0.0068*	<0.0001*	0.3448
Plasma C-reactive Protein (ng/ml)	356±14[a]	317±18[a]	305±21[a]	342±15[a]	0.0416*	0.8707	<0.0001*
Fasting blood glucose (mmol/L)	4.0±0.1[a]	4.1±0.2[a]	5.1±0.2[b]	4.4±0.1[a]	0.0001*	0.0772	0.0204*
Glucose AUC (mmol/L/min)	651±13[a]	740±10[c]	831±28[b]	727±25[c]	0.0005*	0.7345	<0.0001*

Values are mean ± S.E.M. and n = 8 - 10 per group. Means without a common superscript letter differ, * indicates P < 0.05

C = cornstarch; CO = C + wholegrain oat groats; CB = C + oat bran; CG = C + β-glucan powder; H = high carbohydrate high fat;

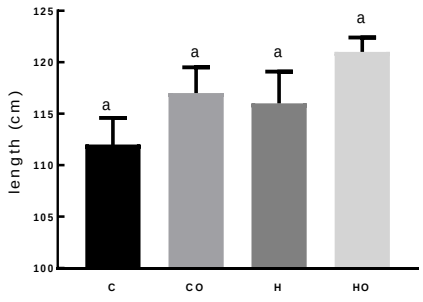
HO = H + wholegrain oat groats; HB = H + oat bran; HG = H + β-glucan powder

*** C & H are the same for all 3 intervention groups***

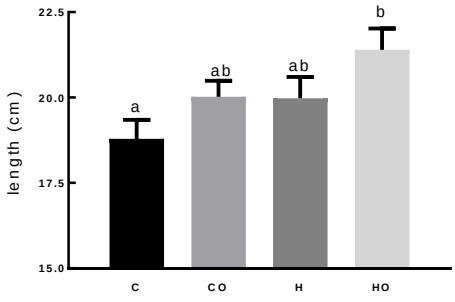
3.3.5 Gastrointestinal parameters

3.3.5.1 Gastrointestinal length

Small intestine length was similar in C and H and interventions were not significantly different to their respective controls (Figure 3.7a, 3.8a and 3.9a). Colon length was similar for both C and H and none of the interventions were different to the respective controls (Figure 3.7b, 3.8b and 3.9b).



a)

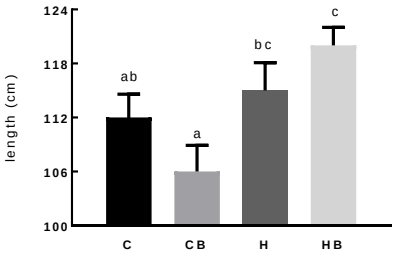


b)

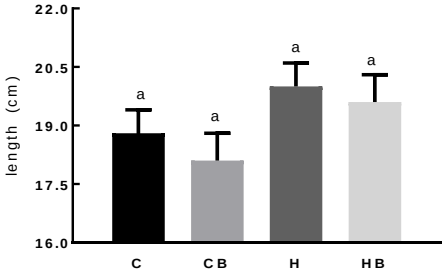
	P- value		
	diet	intervention	interaction
small intestine	0.1550	0.0777	>0.9999
colon	0.0320*	0.0273*	0.8698

Figure 3.7 Gastrointestinal length of male Wistar rats following a dietary intervention of wholegrain oat groats. a) small intestine – pyloric sphincter to caeco-ileal junction and b) colon – caeco-colonic junction to rectum

a)

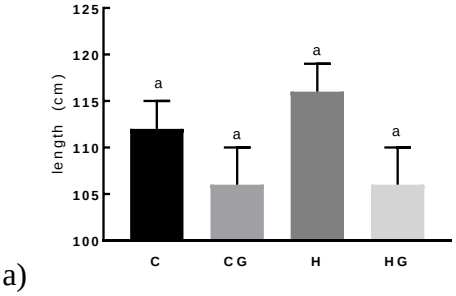


b)

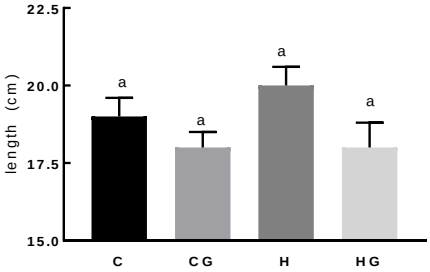


	P- value		
	diet	intervention	interaction
small intestine	0.0035*	0.8550	0.0505
colon	0.0467*	0.4065	0.8201

Figure 3.8 Gastrointestinal length of male Wistar rats following a dietary intervention of oat bran a) small intestine - pyloric sphincter to caeco-ileal junction and b) colon – caeco-colonic junction to rectum



a)



b)

	P- value		
	diet	intervention	interaction
small intestine	0.5683	0.5683	0.0271*
colon	0.4450	0.4450	0.0263*

Figure 3.9 Gastrointestinal length of male Wistar rats following a dietary intervention of β-glucan powder a) small intestine -pyloric sphincter to caeco-ileal junction and b) colon – caeco-colonic junction to rectum

Values are mean ± S.E.M. n = 8 - 10 per group. Means without a common superscript letter differ, * indicates P < 0.05

C = cornstarch; CO = C + wholegrain oat groats; CB = C + oat bran;

CG = C + β-glucan powder; H = high carbohydrate high fat; HO = H + wholegrain oat groats; HB = H + oat bran; HG = H + β-glucan powder

*** C & H are the same for all 3 intervention groups***

3.3.5.2 Gastrointestinal structure

3.3.5.2.1 Villi height

Duodenal villi height was decreased in H than C, while CO, CB CG and HO, HB, HG were similar to C (Tables 3.13, 3.14 and 3.15).

Jejunal villi height was similar for all groups (Tables 3.13, 3.14 and 3.15). Ileal villi height was statistically similar in H and C; CB, CG and HO were similar to both controls; CO was longer than C; HG was greater than C but similar to H and CG (Tables 3.13, 3.14 and 3.15).

Villi height profile along the small intestine was decreased in H diet compared to C and the three intervention diets. There were no changes in height between duodenum, jejunum and ileum in H (Figure 3.10). C duodenum villi height was increased compared to jejunum and ileum, and the jejunum villi height was increased compared to the ileum (Figure 3.10). CO duodenum villi height was increased compared to jejunum and ileum with no difference between jejunum and ileum (Figure 3.10). CB duodenum villi height was increased compared to the jejunum and ileum, and jejunum villi height increased compared to than ileum (Figure 3.10). CG duodenum villi height was greater than the ileum. Jejunum villi height was the same as duodenum and longer than ileum (Figure 3.10).

HO ileum villi height was decreased compared to both duodenum and jejunum. There was no difference between HO duodenum and jejunum (Figure 3.10). HB ileum villi height was less than both duodenum and jejunum. There was no difference between HB duodenum and jejunum (Figure 3.10). HG duodenum villi height was greater than jejunum and ileum while the jejunum villi height was greater than ileum (Figure 3.10).

Table 3.13 Villi height along small intestine of male Wistar rats following consumption of wholegrain oat groats

					P-value		
section	C	CO	H	HO	diet	intervention	interaction
Duodenum (µm)	735±55[a]	616±22[a]	352±81[b]	532±34[a]	0.0001[*]	0.5694	0.0087[*]
Jejunum (µm)	403±31[a]	400±41[a]	380±98[a]	473±15[a]	0.6578	0.4271	0.3973
Ileum (µm)	199±25[a]	304±5[b]	273±53[ab]	254±43[ab]	0.7443	0.2478	0.0998

Table 3.14 Villi height along small intestine of male Wistar rats following a dietary intervention of oat bran

					P-value		
variable	C	CB	H	HB	diet	intervention	interaction
Duodenum (µm)	735±55[a]	709±68[a]	352±81[b]	599±49[a]	0.0007*	0.0974	0.0402*
Jejunum (µm)	403±31[a]	371±45[a]	380±98[a]	515±52[a]	0.3362	0.4120	0.1877
Ileum (µm)	199±25[a]	224±20[a]	273±53[a]	252±34[a]	0.1599	0.9552	0.5202

Table 3.15 Villi height along the small intestine of male Wistar rats following a dietary intervention of β-glucan powder

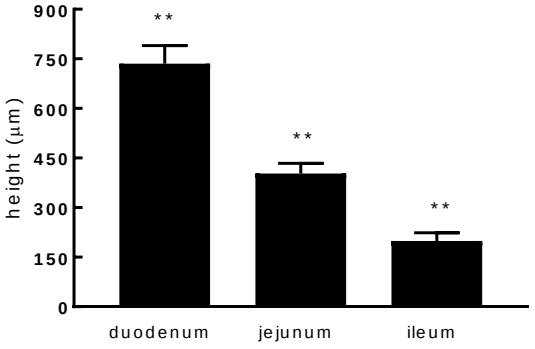
					P-value		
section	C	CG	H	HG	diet	intervention	interaction
Duodenum (µm)	735±55[a]	509±53[ab]	352±81[b]	696±47[a]	0.3789	0.1.518	0.0004*
Jejunum (µm)	403±31[a]	478±28[a]	380±98[a]	499±18[a]	0.9859	0.0995	0.6988
Ileum (µm)	199±25[a]	268±19[ab]	273±53[ab]	340±15[b]	0.0322*	0.0444*	0.9751

Values are mean ± S.E.M. n = 8 - 10 per group. Means without a common superscript letter differ, * indicates P < 0.05

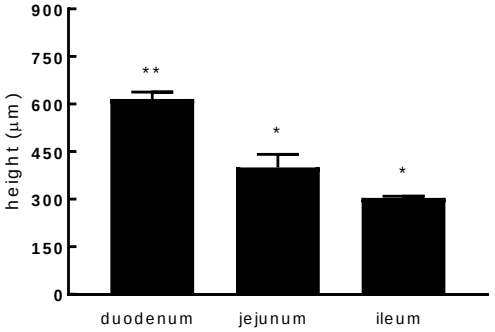
C = cornstarch; CO = C + wholegrain oat groats; CB = C + oat bran; CG = C + β-glucan powder; H = high carbohydrate high fat;

HO = H + wholegrain oat groats; HB = H + oat bran; HG = H + β-glucan powder

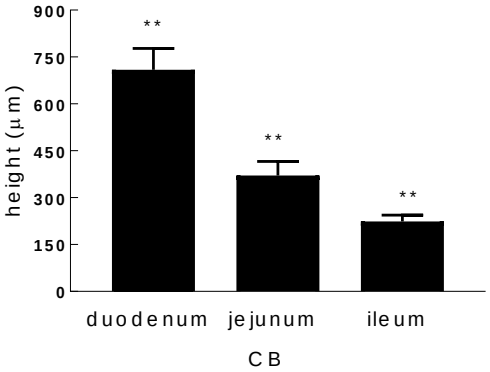
*** C & H are the same for all 3 intervention groups***


900
750
600
450
300
150
0
height (µm)
**
**
**
duodenum
jejunum
ileum

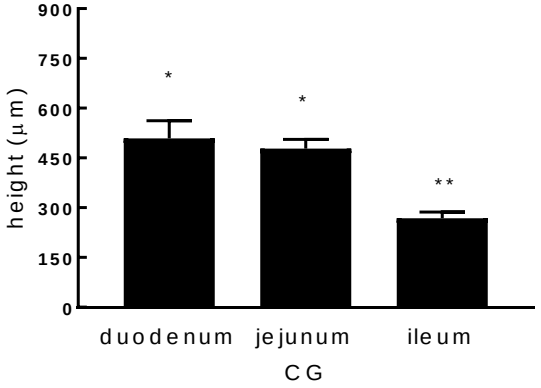
C


900
750
600
450
300
150
0
height (µm)
**
*
*
duodenum
jejunum
ileum

CO


900
750
600
450
300
150
0
height (µm)
**
**
**
duodenum
jejunum
ileum

CB


900
750
600
450
300
150
0
height (µm)
*
*
**
duodenum
jejunum
ileum

CG

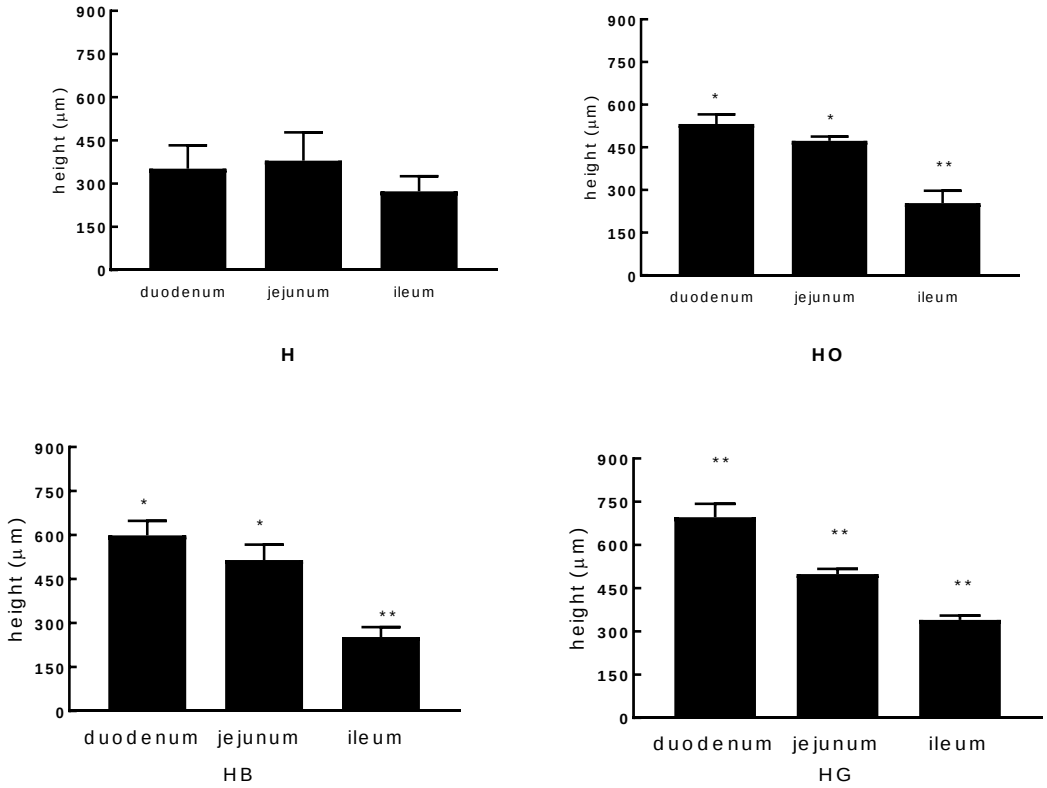


Figure 3.10 Profile of villi height along small intestine of male Wistar rats following a dietary intervention of wholegrain oat groats, oat bran and β-glucan powder

Number of * indicates number of groups that are significantly different. $P < 0.05$ is considered significant.
C = cornstarch; CO = C + wholegrain oat groats; CB = C + oat bran; CG = C + β-glucan powder; H = high carbohydrate high fat;
HO = H + wholegrain oat groats; HB = H + oat bran; HG = H + β-glucan powder *** C & H are the same for all 3 intervention groups***

3.3.5.2.2 Crypt depth

Duodenal crypt depth in H was less than C, with CO, CB and HO, HB the same as C; CG and HG were not significantly different to H and C (Tables 3.16, 3.17 and 3.18). Jejunal and ileal crypt depths were unchanged in all diets (Tables 3.16, 3.17 and 3.18). Colonic crypt depth varied across proximal, mid and distal sections with crypts increasing in depth in the more distal portion of the colon (Tables 3.16, 3.17 and 3.18). However, within each section, there were no significant differences between diets (Tables 3.16, 3.17 and 3.18).

Crypt depth profile along the entire gastrointestinal tract was similar in H with no difference between duodenum, jejunum, ileum, proximal colon, mid colon or distal colon (Figure 3.11). C crypt depth decreased from duodenum, jejunum to ileum. C crypt depth increased from ileum through to the distal colon (Figure 3.11). CO mid-colon and distal colon were deeper than duodenum, jejunum, ileum and proximal colon (Figure 3.11). CB distal colon crypts were deeper than duodenum and jejunum but similar to ileum, proximal and mid colon (Figure 3.11). CG mid colon depth was deeper than all sections of the small intestine as well as the proximal colon. Distal colon crypts were deeper than jejunum and ileum but similar to duodenum, proximal and mid colon (Figure 3.11). HO had deeper crypt depths in mid colon and distal colon compared to proximal colon. HO ileum crypt depth was shorter than duodenum, mid colon and distal colon (Figure 3.11). HB had deeper crypt depths in mid colon and distal colon compared to jejunum and ileum with all other sections similar (Figure 3.11). HG had deeper crypt depths in the distal colon compared to all other sections which were similar depths (Figure 3.11).

Table 3.16 Crypt depth along small intestine and colon of male Wistar rats following a dietary intervention of wholegrain oat groats

					P-value		
section	C	CO	H	HO	diet	intervention	interaction
Duodenum (µm)	151±8[a]	121±10[ab]	103±10[b]	137±23[a]	0.2654	0.8881	0.0309[*]
Jejunum (µm)	112±9[a]	110±2[a]	127±9[a]	136±11[a]	0.0223	0.6826	0.5214
Ileum (µm)	93±9[a]	98±5[a]	108±14[a]	98±8[a]	0.4396	0.7957	0.4396
Proximal colon (µm)	116±17[a]	117±13[a]	85±20[a]	113±5[a]	0.2488	0.3375	0.3713
Mid colon (µm)	158±34[a]	219±2[a]	154±39[a]	188±25[a]	0.5284	0.0941	0.6262
Distal colon (µm)	189±22[a]	211±10[a]	147±26[a]	181±18[a]	0.0812	0.1704	0.7653

Table 3.17 Crypt depth along small intestine and colon of male Wistar rats following a dietary intervention of oat bran

					P-value		
section	C	CB	H	HB	diet	intervention	interaction
Duodenum (µm)	151±8^{a}	134±9^{aB}	103±10^{b}	166±22^{a}	0.5582	0.0995	0.0062*
Jejunum (µm)	112±9^{a}	116±9^{a}	127±9^{a}	125±8^{a}	0.1816	0.9099	0.7346
Ileum (µm)	93±9^{a}	136±22^{a}	108±14^{a}	104±15^{a}	0.5925	0.2245	0.1456
Proximal colon (µm)	116±17^{ab}	149±27^{ab}	85±20^{a}	165±20^{b}	0.1031	0.3058	0.9789
Mid colon (µm)	158±34^{a}	156±29^{a}	154±39^{a}	199±16^{a}	0.5307	0.4897	0.4506
Distal colon (µm)	189±22^{a}	206±19^{a}	147±26^{a}	179±22^{a}	0.2831	0.1346	0.7401

Table 3.18 Crypt depth along small intestine and colon of male Wistar rats following a dietary intervention of β-glucan powder

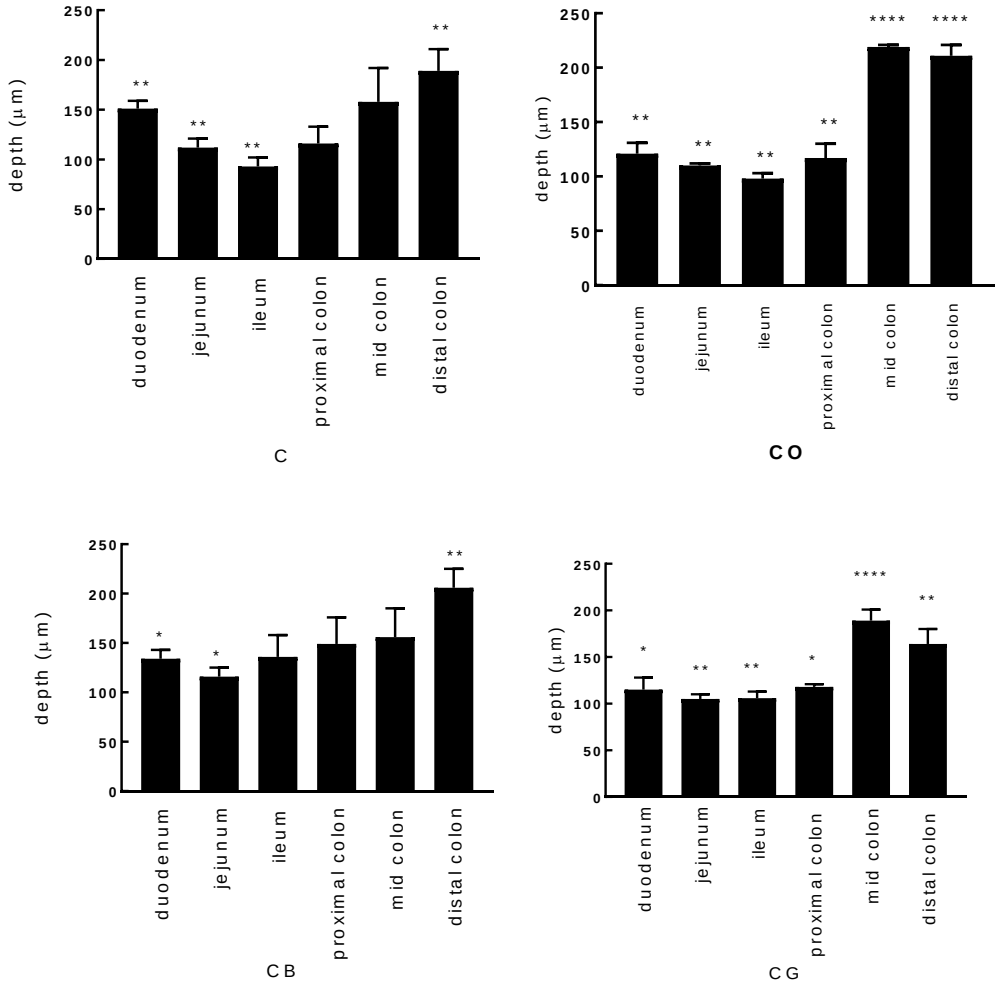
					P-value		
section	C	CG	H	HG	diet	intervention	interaction
Duodenum (µm)	151±8[a]	115±13[a]	103±10[a]	147±12[a]	0.4723	0.7180	0.0015*
Jejunum (µm)	112±9[a]	105±5[a]	127±9[a]	124±9[a]	0.0509	0.5482	0.8095
Ileum (µm)	93±9[a]	106±7[a]	108±14[a]	119±11[a]	0.2003	0.2697	0.9256
Proximal colon (µm)	116±17[a]	118±3[a]	85±20[a]	104±13[a]	0.1421	0.4840	0.5701
Mid. colon (µm)	158±34[a]	189±12[a]	154±39[a]	168±21[a]	0.6663	0.4400	0.7690
Distal colon (µm)	189±22[a]	164±16[a]	147±26[a]	198±10[a]	0.8393	0.5119	0.0651

Number of * indicates number of groups that are significantly different. $P < 0.05$ is considered significant.

C = cornstarch; CO = C + wholegrain oat groats; CB = C + oat bran; CG = C + β-glucan powder; H = high carbohydrate high fat;

HO = H + wholegrain oat groats; HB = H + oat bran; HG = H + β-glucan powder

*** C & H are the same for all 3 intervention groups***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200
150
100
50
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duodenum
jejunum
ileum
proximal colon
mid colon
distal colon
C
CO
CB
CG

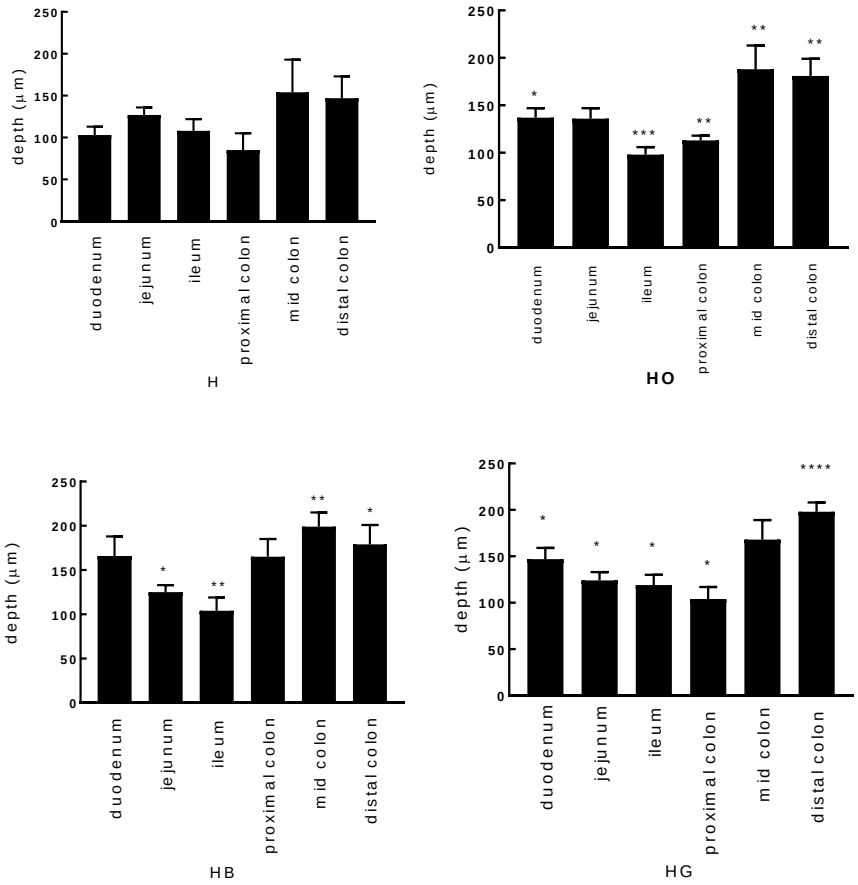


Figure 3.11 Profile of crypt depth along small intestine and colon of male Wistar rats following a dietary intervention of wholegrain oat groats, oat bran or β-glucan powder

Number of * indicates number of groups that are significantly different. P-value <0.05 is considered significant.
C = cornstarch; CO = C + wholegrain oat groats; CB = C + oat bran; CG = C + β-glucan powder; H = high carbohydrate high fat;
HO = H + wholegrain oat groats; HB = H + oat bran; HG = H + β-glucan powder; *** C & H are the same for all 3 intervention groups***

3.3.5.2.3 Villi height to crypt depth ratio

Duodenal villi height to crypt depth ratio was decreased in H compared to C; HO and HG increased compared to H and were not significantly different to C (Table 3.18, 3.19 and 3.20). CO, CB, CG and HB were the same as there relevant controls. Jejunum and ileum villi height to crypt depth ratios were not significantly different between any of the diets (Table 3.18, 3.19 and 3.20).

Table 3.19 Villi height to crypt depth ratio along the small intestine of male Wistar rats following a dietary intervention of wholegrain oat groats

					P-value		
section	C	CO	H	HO	diet	intervention	interaction
Duodenum (µm)	5.0±0.6[a]	5.2±0.3[a]	3.4±0.7[b]	4.4±0.8[a]	0.0498[*]	0.3140	0.4999
Jejunum (µm)	3.6±0.1[a]	3.7±0.4[a]	2.6±1.0[a]	3.6±0.3[a]	0.3355	0.3355	0.4294
Ileum(µm)	2.1±0.2[a]	3.1±0.2[a]	2.7±0.6[a]	2.8±0.6[a]	0.7398	0.2290	0.3229

Table 3.20 Villi height to crypt depth ratio along the small intestine of male Wistar rats following a dietary intervention of oat bran

					P-value		
section	C	CB	H	HB	diet	intervention	interaction
Duodenum (µm)	5.0±0.6[a]	5.3±0.5[a]	3.4±0.7[b]	3.2±0.3[b]	0.0001*	0.9064	0.5577
Jejunum (µm)	3.6±0.1[a]	3.3±0.6[a]	2.6±1.0[a]	4.1±0.1[a]	0.8660	0.3158	0.1367
Ileum (µm)	2.1±0.2[a]	1.9±0.5[a]	2.7±0.6[a]	2.1±0.2[a]	0.3437	0.3437	0.6339

Table 3.21 Villi height to crypt depth ratio along small intestine of male Wistar rats following a dietary intervention of β-glucan powder

					P-value		
section	C	CG	H	HG	diet	intervention	interaction
Duodenum (μm)	5.0±0.6[a]	4.5±0.4[a]	3.4±0.7[b]	5.3±0.4[a]	0.4681	0.2103	0.0382*
Jejunum (μm)	3.6±0.1[a]	4.6±0.5[a]	2.6±1.0[a]	4.1±0.4[a]	0.2226	0.0488*	0.6793
Ileum (μm)	2.1±0.2[a]	2.5±0.1[a]	2.7±0.6[a]	3.0±0.4[a]	0.1606	0.3649	0.8959

Values are mean ± S.E.M. n = 8 - 10 per group. Means without a common superscript letter differ, * indicates $P < 0.05$

C = cornstarch; CO = C + wholegrain oat groats; CB = C + oat bran; CG = C + β-glucan powder; H = high carbohydrate high fat;

HO = H + wholegrain oat groats; HB = H + oat bran; HG = H + β-glucan powder

*** C & H are the same for all 3 intervention groups***

3.3.5.2.3 Mucosal thickness

Duodenal mucosal thickness was decreased in H compared to C while CO, CB, CG, HO, HB and HG were not significantly different to C (Table 3.20, 3.21 and 3.22). Jejunal, ileal, proximal and mid colon mucosal thickness were not significantly different in any of the diets (Table 3.20, 3.21 and 3.22). Distal colon mucosal thickness was decreased in H compared to C, and CO, CB, CG, HO, HB and HG were not significantly different from to their respective controls (Table 3.20, 3.21 and 3.22).

The profile of the mucosa was not uniform along the length of the gastrointestinal tract in all diets and there was large variability within sections (Figure 3.20). The mucosa of the small intestine was greater than the colon in all diets. H mucosal thickness was similar in the duodenum, jejunum and ileum and less in the colon with no difference between the proximal, mid and distal colon. C duodenal mucosa was greater than all other sections. C jejunum was greater than all sections except the duodenum and ileum; proximal colon, mid colon and distal colon were all similar thickness (Figure 3.20). In CO, all gastrointestinal sections were different with duodenum thickest and proximal colon thinnest. Mucosa became thinner from duodenum to proximal colon and became thicker from proximal colon to distal colon (Figure 3.20). In CB, all gastrointestinal sections were different with duodenum thickest and proximal colon thinnest. Mucosa became thinner from duodenum to proximal colon was a similar thickness from proximal colon to distal colon (Figure 3.20). CG mucosal thickness was the most variable with the duodenum the highest and proximal colon the lowest values. Mucosal thickness reduced from duodenum to proximal colon and was similar from proximal colon to distal colon (Figure 3.20). In HO, all gastrointestinal sections were different with duodenum thickest and proximal colon thinnest. Mucosa became thinner from duodenum to proximal colon and thicker in mid colon and distal colon (Figure 3.20). HB duodenal mucosa was thicker than all other sections except jejunum. HB ileum and all colon sections were similar thickness (Figure 3.20). HG duodenal mucosa was thicker than all other sections except jejunum. HG ileum was a different thickness to all other sections and all colon sections were similar thickness (Figure 3.20).

Table 3.22 Mucosal thickness along small intestine and colon of male Wistar rats following a dietary intervention of wholegrain oat groats

section	C	CO	H	HO	*P-value*		
					diet	intervention	interaction
Duodenum (µm)	897±81[a]	861±45[a]	528±99[b]	919±28[a]	0.0328[*]	0.0160[*]	0.0046[*]
Jejunum (µm)	518±57[a]	613±90[a]	670±137[a]	588±26[a]	0.4663	0.9403	0.3121
Ileum (µm)	300±33[a]	433±3[a]	427±63[a]	401±21[a]	0.2110	0.1605	0.0410[*]
Proximal colon (µm)	234±26[a]	213±13[a]	231±57[a]	166±5[a]	0.4425	0.1910	0.4986
Mid colon (µm)	221±23[a]	302±9[a]	268±41[a]	270±10[a]	0.7613	0.1007	0.1174
Distal colon (µm)	314±39[a]	362±5[a]	234±20[b]	246±10[b]	0.0002[*]	0.1954	0.4328

Table 3.23 Mucosal thickness along small intestine and colon of male Wistar rats following a dietary intervention of oat bran

section	C	CB	H	HB	*P-value*		
					diet	intervention	interaction
Duodenum (µm)	897±81[a]	874±60[a]	528±99[b]	848±32[a]	0.0109*	0.0498*	0.0250*
Jejunum (µm)	518±57[a]	543±38[a]	670±137[a]	799±41[a]	0.0135*	0.3279	0.5068
Ileum (µm)	300±33[a]	301±33[a]	427±63[a]	414±43[a]	0.0121*	0.8942	0.8767
Proximal colon (µm)	234±26[a]	226±31[a]	231±57[a]	232±26[a]	0.9682	0.9259	0.9048
Mid colon (µm)	221±23[a]	277±7[a]	268±41[a]	293±15[a]	0.2166	0.1153	0.5390
Distal colon (µm)	314±39[a]	254±12[a]	234±20[b]	249±16[ab]	0.0886	0.3583	0.1308

Table 3.24 Mucosal thickness along small intestine and colon of male Wistar rats following a dietary intervention of β-glucan powder.

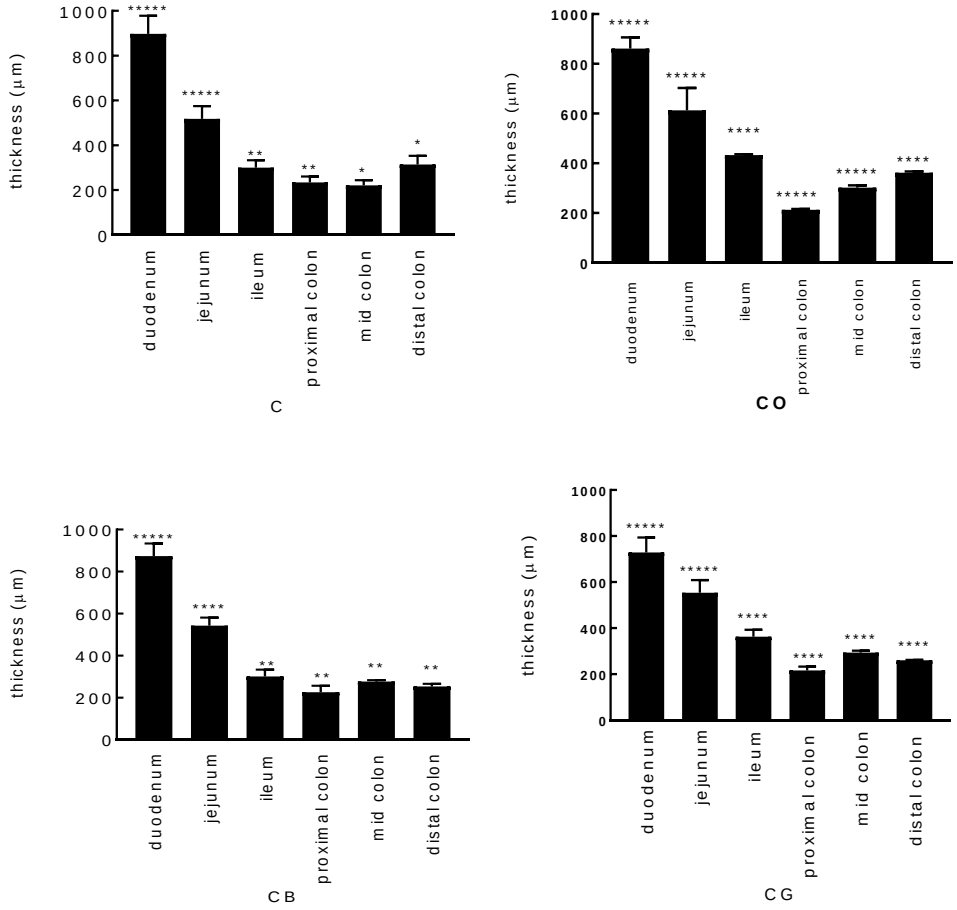
					P-value		
section	C	CG	H	HG	diet	intervention	interaction
Duodenum (µm)	897±81[a]	729±65[a]	528±99[b]	920±58[a]	0.2636	0.1633	0.0017*
Jejunum (µm)	518±57[a]	554±55[a]	670±137[a]	669±24[a]	0.1109	0.8291	0.8195
Ileum (µm)	300±33[a]	363±30[a]	427±63[a]	441±11[a]	0.0161*	0.3352	0.5368
Proximal Colon (µm)	234±26[a]	216±17[a]	231±57[a]	212±21[a]	0.9200	0.5968	0.9886
Mid colon (µm)	221±23[a]	294±8[a]	268±41[a]	276±16[a]	0.5707	0.1230	0.2110
Distal colon (µm)	314±39[ab]	260±20[ab]	234±20[b]	258±13[b]	0.1142	0.5525	0.1319

Values are mean ± S.E.M. n = 8 - 10 per group. Means without a common superscript letter differ, * indicates P < 0.05

C = cornstarch; CO = C + wholegrain oat groats; CB = C + oat bran; CG = C + β-glucan powder; H = high carbohydrate high fat;

HO = H + wholegrain oat groats; HB = H + oat bran; HG = H + β-glucan powder

*** C & H are the same for all 3 intervention groups***


thickness (µm)
1000
800
600
400
200
0
duodenum
jejunum
ileum
proximal colon
mid colon
distal colon
C
CO
CB
CG

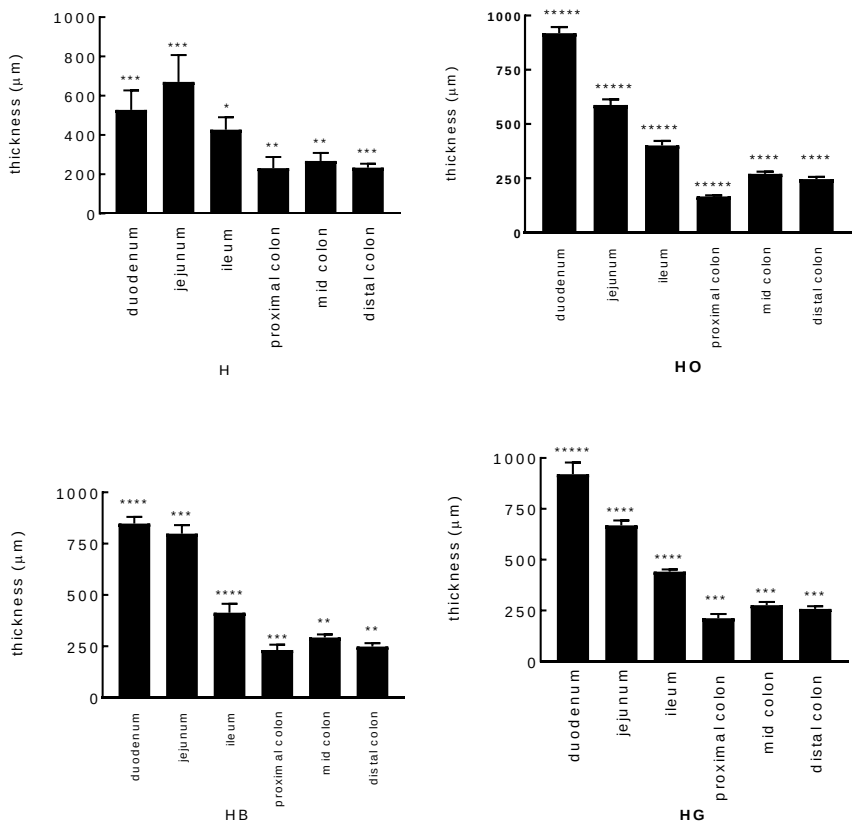


Figure 3.12 Profile of mucosal thickness along small intestine and colon of male Wistar rats following a dietary intervention of β-glucan powder

Number of * indicates number of groups that are significantly different. $P < 0.05$ is considered significant. C = cornstarch; CO = C + wholegrain oat groats; CB = C + oat bran; CG = C + β-glucan powder; H = high carbohydrate high fat; HO = H + wholegrain oat groats; HB = H + oat bran; HG = H + β-glucan powder; *** C & H are the same for all 3 intervention groups***

3.3.5.2.4 Goblet Cells

Duodenal goblet cell numbers were not significantly different in H, C, CO, CB and HG with an increased number in CG, HO and HB compared to H and C (Tables 3.23, 3.24 and 3.25). Jejunal goblet cell numbers were increased in H and HO, HB compared to C, with CO, CB, CG and HG having similar numbers all other diets (Tables 3.23, 3.24 and 3.25). Ileal goblet cell numbers were greater in H than C, with all interventions having similar numbers to both controls (Table 3.23, 3.24 and 3.25).
Colonic goblet cell numbers were unchanged between diets in proximal, mid and distal sections. However, there were more goblet cells in mid and distal colon than the proximal colon in all diets (Table 3.23, 3.24 and 3.25). However, as n = 4/group, further studies will need to be undertaken to determine any significance in goblet cell number changes in this model.

Table 3.25 Goblet cell number along small intestine and colon of male Wistar rats following a dietary intervention of wholegrain oat groats

					P-value		
section	C	CO	H	HO	diet	intervention	interaction
Duodenum (µm)	4 ± 1^{ab}	5 ± 1^{ab}	1 ± 0^{b}	12 ± 5^{a}	0.4479	0.0285^{*}	0.0645
Jejunum (µm)	8 ± 1^{a}	13 ± 3^{ab}	17 ± 2^{b}	16 ± 2^{b}	0.0085^{*}	0.3538	0.1683
Ileum (µm)	9 ± 2^{a}	14 ± 3^{ab}	15 ± 4^{b}	13 ± 6^{ab}	0.5402	0.7126	0.3926
Proximal colon (µm)	5 ± 2^{a}	5 ± 1^{a}	7 ± 2^{a}	6 ± 2^{a}	0.4124	0.7835	0.7835
Mid colon (µm)	9 ± 3^{a}	17 ± 5^{a}	17 ± 4^{a}	10 ± 2^{a}	0.8927	0.8927	0.0508
Distal colon (µm)	10 ± 3^{a}	12 ± 3^{a}	12 ± 1^{a}	11 ± 3^{a}	0.8515	0.8515	0.5753

Table 3.26 Goblet cell numbers along the small intestine and colon tract of male Wistar rats following a dietary intervention of oat bran

					P-value		
section	C	CB	H	HB	diet	intervention	interaction
Duodenum (µm)	4±1[a]	8±1[a]	1±0[a]	16±3[b]	0.1429	<0.0001*	0.0025*
Jejunum (µm)	8±1[b]	13±2[ab]	17±2[a]	18±4[a]	0.0092*	0.2402	0.4304
Ileum (µm)	9±2[a]	11±1[a]	15±4[a]	13±1[a]	0.0553	0.6922	0.6922
Proximal colon (µm)	5±2[a]	8±0[a]	7±2[a]	9±3[a]	0.4729	0.2354	0.8101
Mid colon (µm)	9±3[a]	11±1[a]	17±4[a]	15±2[a]	0.0369*	>0.9999	0.4713
Distal colon (µm)	10±3[a]	14±1[a]	12±1[a]	15±1[a]	0.3938	0.0530	0.7750

Table 3.27 Goblet cell number along small intestine and colon of male Wistar rats following a dietary intervention of β-glucan powder

					P-value		
section	C	CG	H	HG	diet	intervention	interaction
Duodenum (µm)	4 ± 1^{ab}	7 ± 2^{a}	1 ± 0^{b}	5 ± 1^{b}	0.0639	0.0144*	0.6903
Jejunum (µm)	8 ± 1^{b}	10 ± 1^{b}	17 ± 2^{a}	7 ± 1^{b}	0.0426*	0.0106*	0.0007*
Ileum (µm)	9 ± 2^{a}	11 ± 1^{a}	15 ± 4^{a}	7 ± 0^{a}	0.6703	0.2149	0.0497*
Proximal colon (µm)	5 ± 2^{a}	11 ± 1^{a}	7 ± 2^{a}	9 ± 0^{a}	>0.9999	0.0205*	0.2072
Mid colon (µm)	9 ± 3^{a}	10 ± 1^{a}	17 ± 4^{a}	13 ± 1^{a}	0.0558	0.5744	0.3549
Distal colon (µm)	10 ± 3^{a}	12 ± 1^{a}	12 ± 1^{a}	12 ± 1^{a}	0.5744	0.5744	0.5744

Values are mean ± S.E.M. n = 8 - 10 per group. Means without a common superscript letter differ, * indicates $P < 0.05$

C = cornstarch; CO = C + wholegrain oat groats; CB = C + oat bran; CG = C + β-glucan powder; H = high carbohydrate high fat;

HO = H + wholegrain oat groats; HB = H + oat bran; HG = H + β-glucan powder

*** C & H are the same for all 3 intervention groups***

3.3.5.3 Gastrointestinal histology

This section (Figures 3.13 to 3.18) is a visual representation of the data in the previous section (3.3.5.2 Gastrointestinal structure). Duodenum, jejunum and ileum show villi (V), crypts (C), mucosa (MT) and muscularis (M). Proximal, mid and distal colon show crypts (C), mucosa (MT), muscularis (M) and submucosal folds (S).

Gastrointestinal morphology was changed by the addition of wholegrain oat groats, oat bran or β-glucan powder to the high carbohydrate, high fat diet. There were also differences between C and H diets.

Duodenum

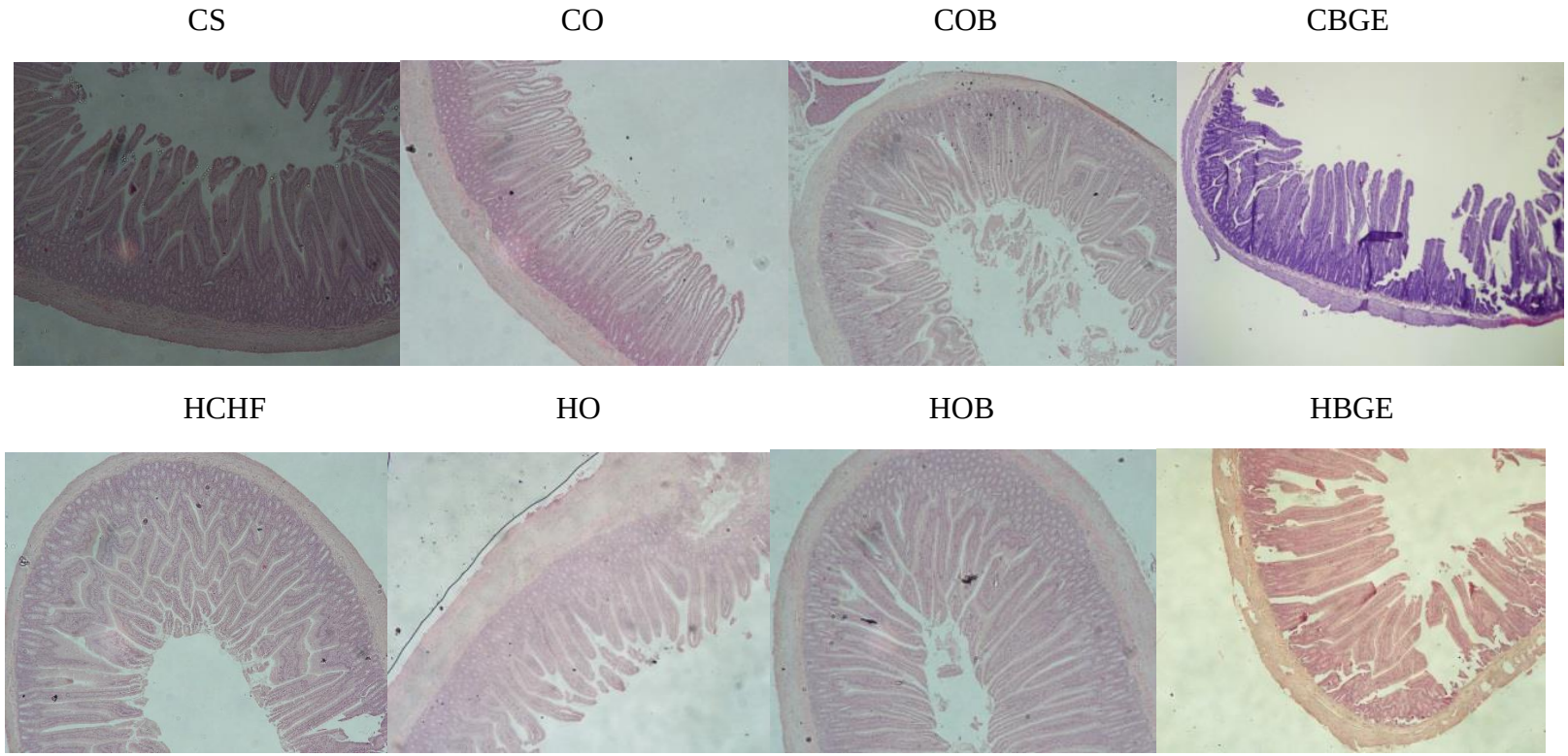
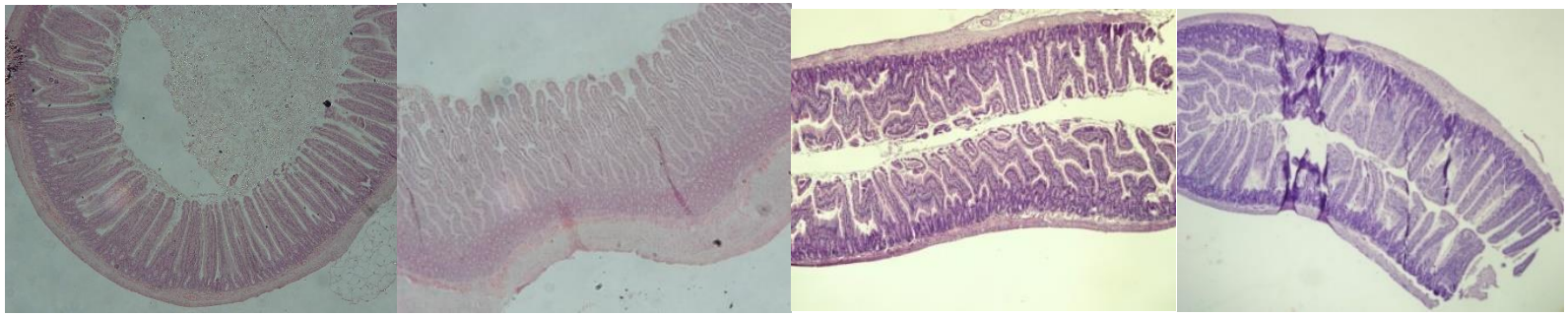


Figure 3.13 Effects of oat supplemented diets on the morphology of the duodenum showing variations between control diets and Haematoxylin & Eosin Staining 4x magnification.

Jejunum

CS CO COB CBGE



HCHF HO HOB HBGE

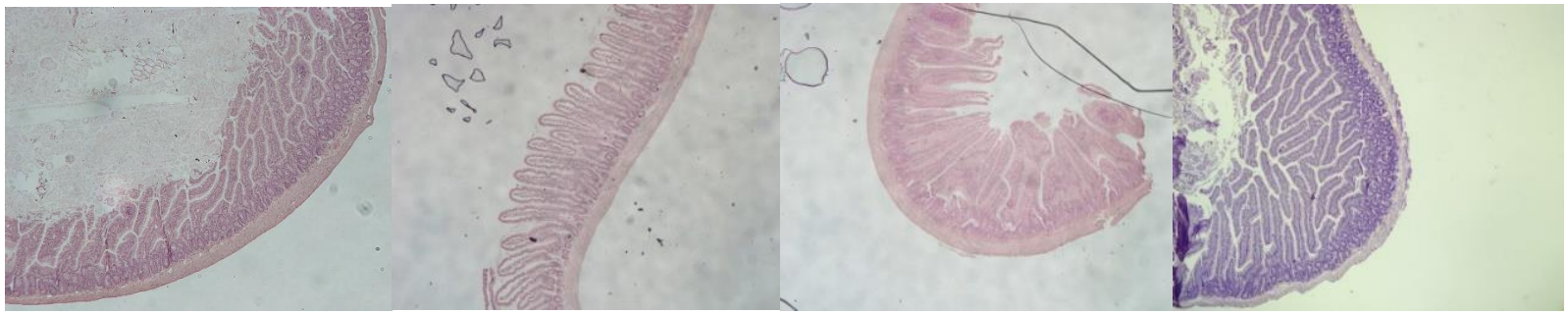


Figure 3.14. Effects of a variety of oat supplemented diets on the morphology of the jejunum, showing variations between control diets and intervention groups. Haematoxylin & Eosin Staining, 4x magnification.

Ileum

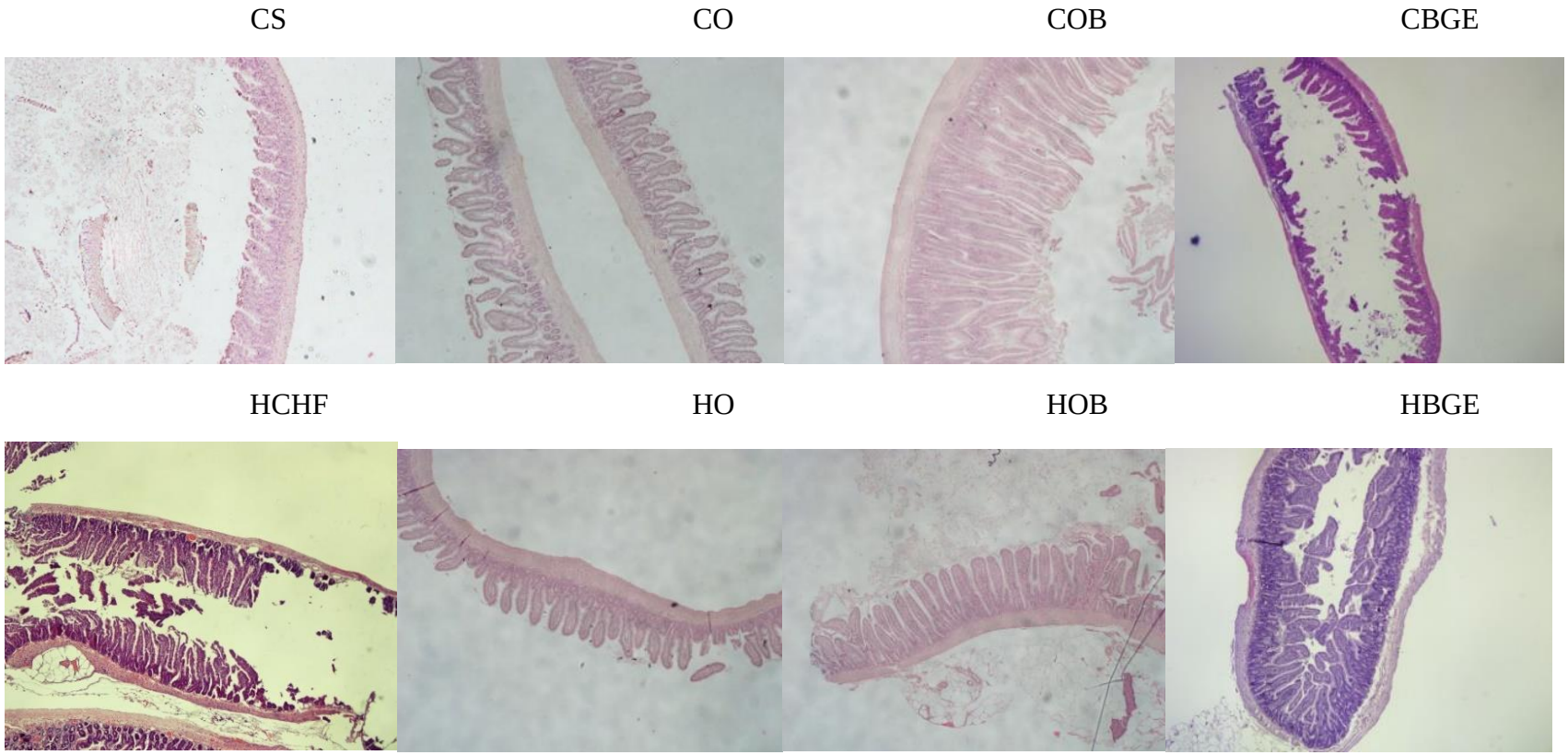


Figure 3.15. Effects of a variety of oat supplemented diets on the morphology of the ileum, showing variations between control diets and intervention groups. Haematoxylin & Eosin Staining, 4x magnification.

Proximal Colon

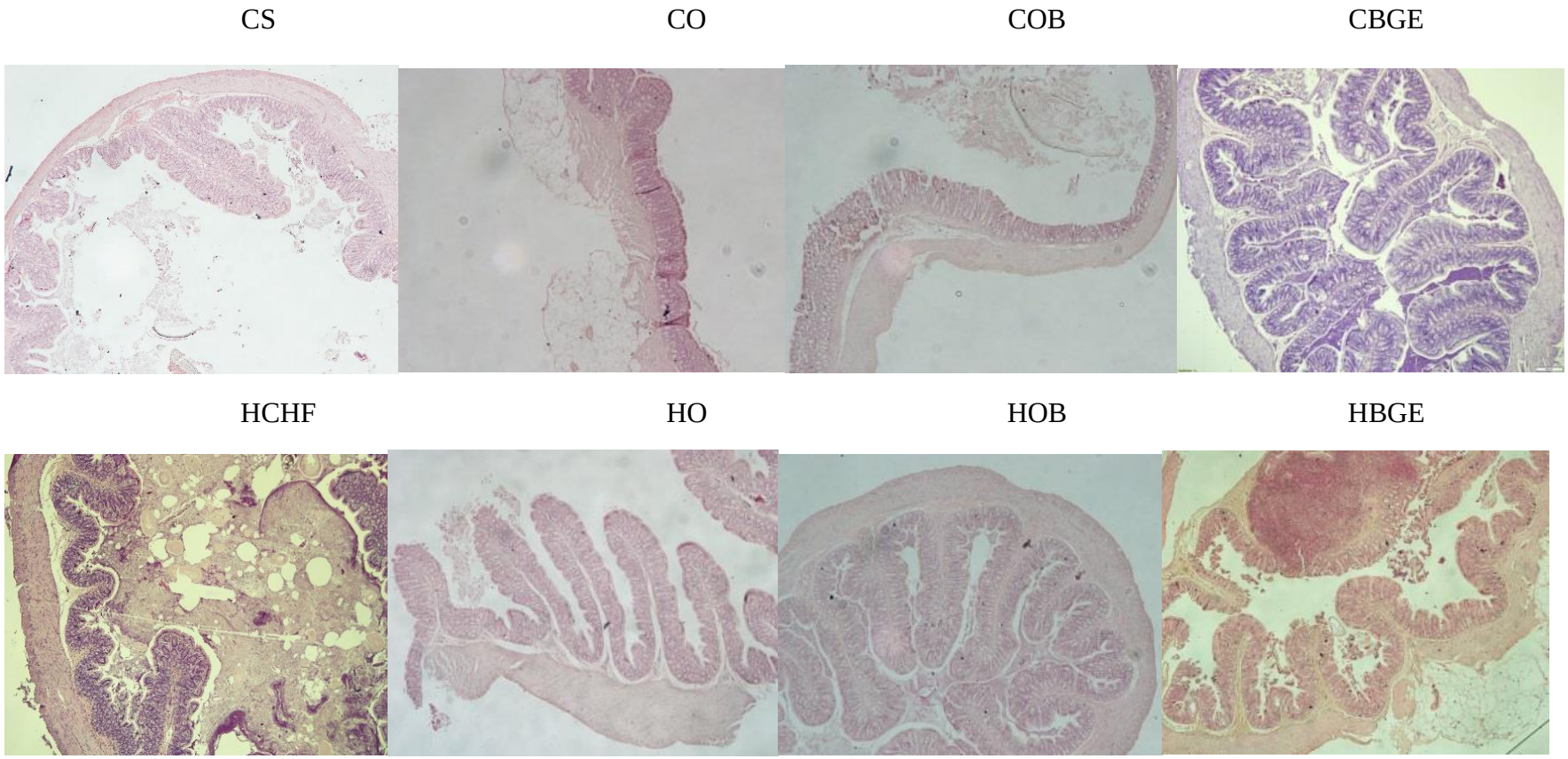
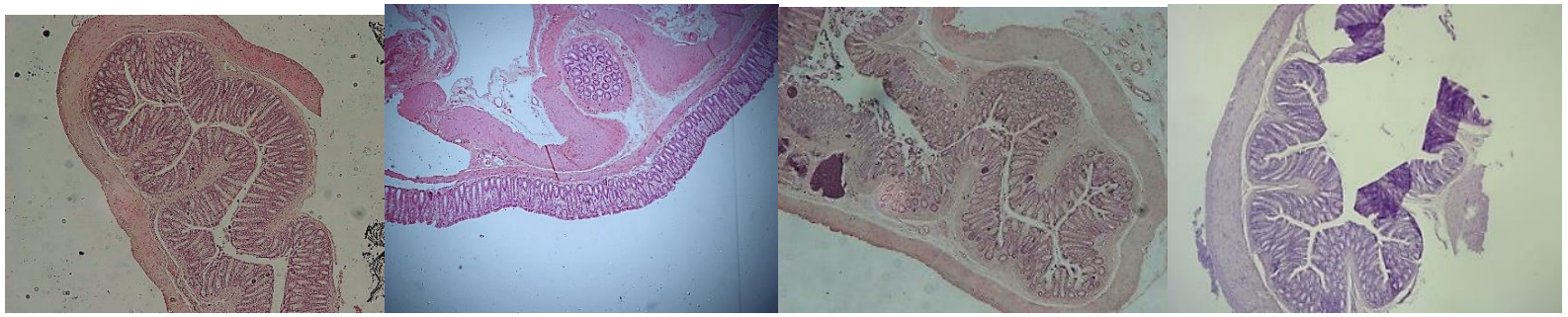


Figure 3.16. Effects of a variety of oat supplemented diets on the morphology of the proximal colon showing variations between control and intervention groups Haematoxylin & Eosin Staining, 4x magnification.

Mid colon

CS CO COB CBGE



HCHF HO HOB HBGE

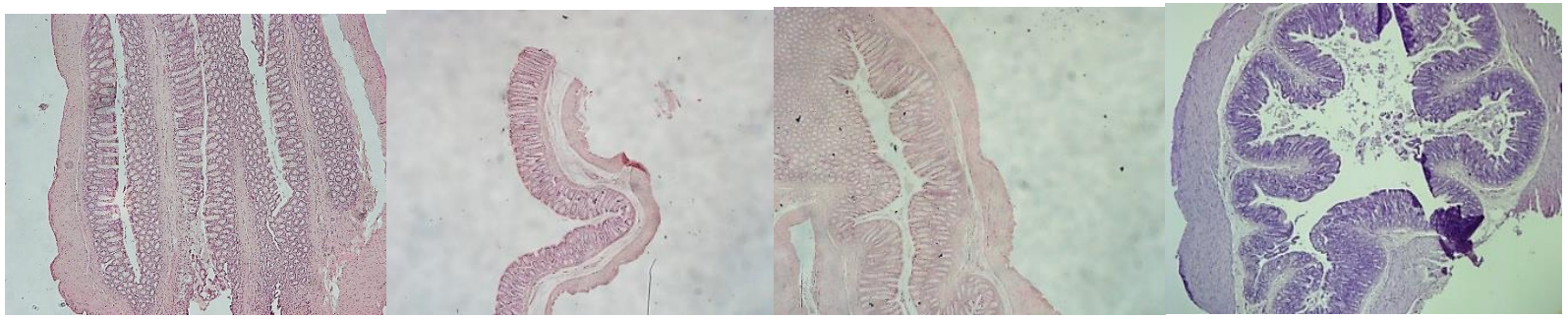


Figure 3.17. Effects of a variety of oat supplemented diets on the morphology of the mid colon, showing variations between control diets and intervention groups Haematoxylin & Eosin Staining, 4x magnification.

Distal colon

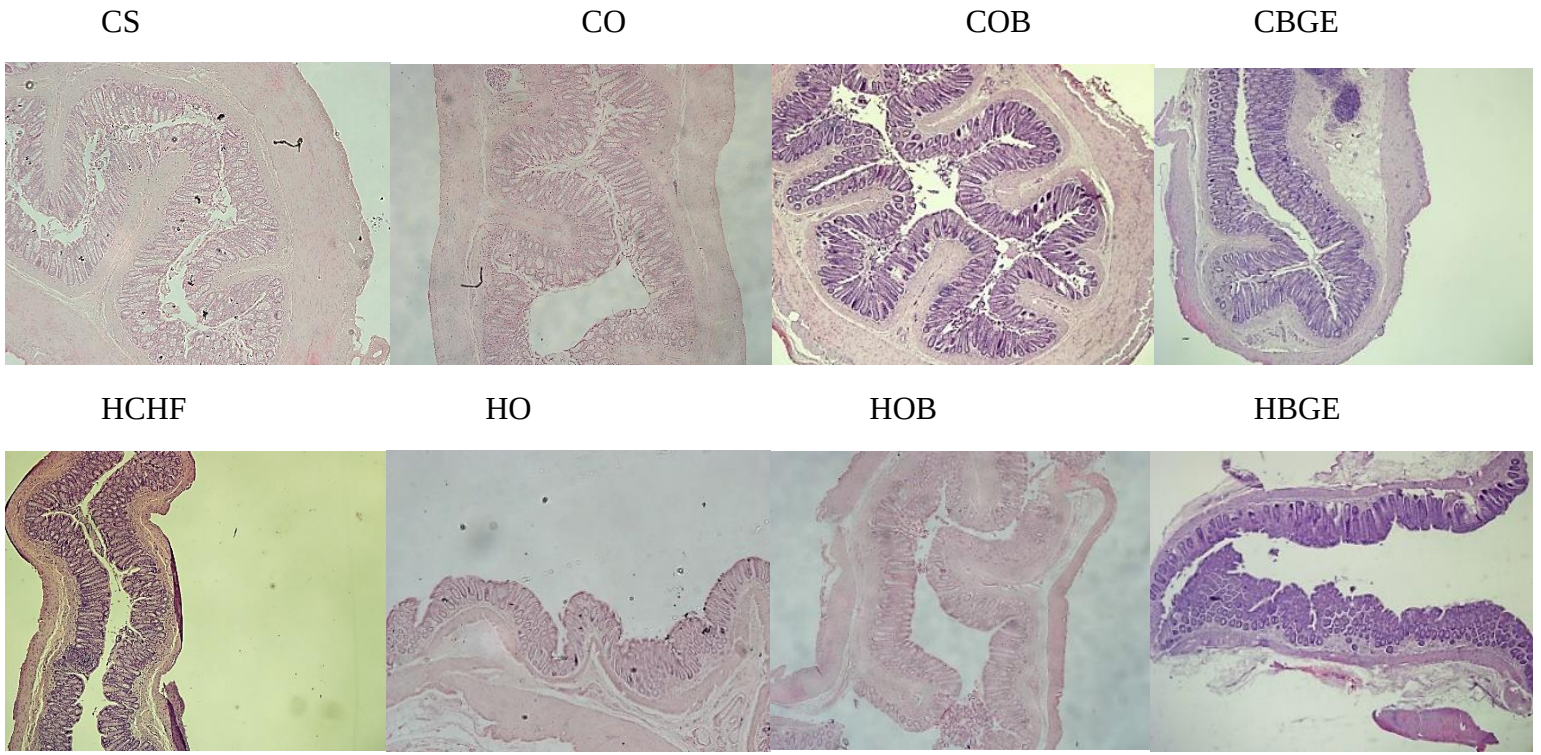
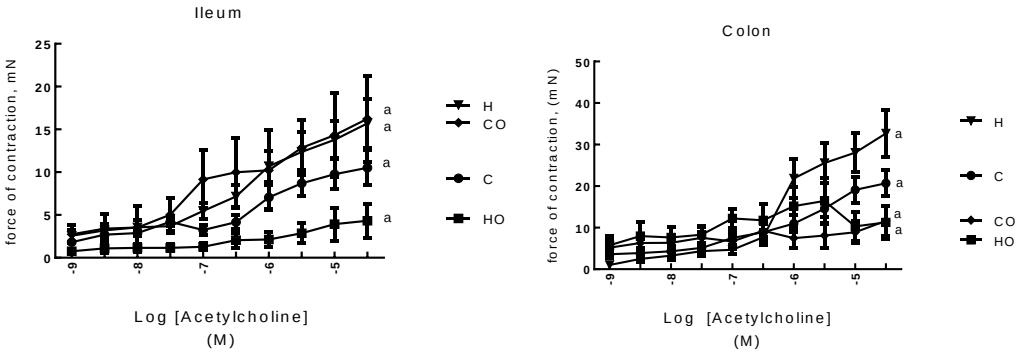


Figure 3.18. Effects of a variety of oat supplemented diets on the morphology of the distal colon, showing variations between control diets and intervention groups. Haematoxylin & Eosin Staining, 4x magnification.

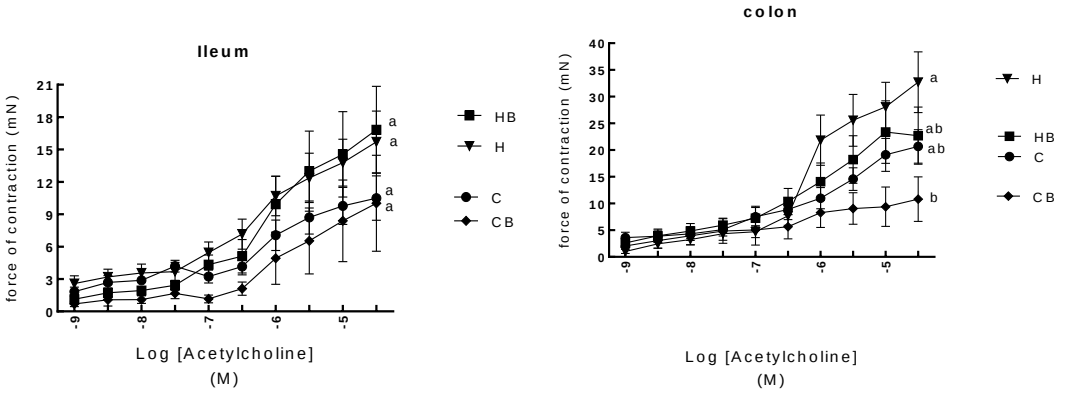
3.3.5.4 Gastrointestinal contractility

Ileal contractility in the H and C diets were the same. There was no difference with the addition of wholegrain oat groats or oat bran (Figure 3.19 and 3.20) β-glucan powder increased ileal contractility compared to both H and C (Figure 3.21). Colon contractility was the same for all diets (Figure 3.19, 3.20 and 3.21) .



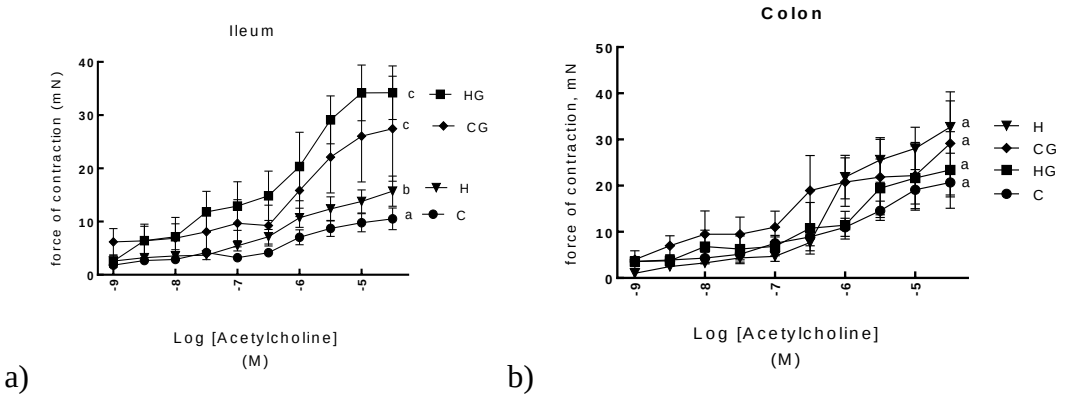
	P-value		
	diet	intervention	interaction
Ileum	0.3963	0.4743	0.0363*
Colon	0.3314	0.0152*	0.3045

Figure 3.19 Effect of acetylcholine on the contractility of ileum and mid colon of male Wistar rats following a dietary intervention of wholegrain oat groats



	P-value		
	diet	intervention	interaction
Ileum	0.0760	0.9220	0.8084
Colon	0.0172*	0.0449*	0.9893

Figure 3.20 Effect of acetylcholine on the contractility of ileum and mid colon of male Wistar rats following a dietary intervention of oat bran



a) b)

	P-Value		
	diet	intervention	interaction
Ileum	0.2216	0.0008*	0.8712
Colon	0.6454	0.9499	0.1940

Figure 3.21 Effect of acetylcholine on the contractility of ileum and mid colon of male Wistar rats following a dietary intervention of β-glucan powder

Values are mean ± S.E.M. n = 8 - 10 per group. Means without a common superscript letter differ, * indicates P < 0.05

C = cornstarch; CO = C + wholegrain oat groats; CB = C + oat bran;

CG = C + β-glucan powder; H = high carbohydrate high fat;

HO = H + wholegrain oat groats; HB = H + oat bran; HG = H + β-glucan powder

*** C & H are the same for all 3 intervention groups***

3.3.5.5 Faecal short-chain fatty acid (SCFA) analysis

Total SCFA, acetate, propionate and butyrate concentrations were lower in H, HO, CG and HG compared to C, CO and CB with no difference between respective controls and interventions (Tables 3.26, 3.27 and 3.28). Total acetate concentrations were lower in HB compared to C and not significantly different to H (Table 3.27). Total SCFA and acetate concentrations of HG were increased compared to H (Table 3.28). Propionate concentrations were lower in H compared to C with HO, CO and CG unchanged compared to respective control (Table 3.26). HG was increased compared to H and not significantly different from C (Table 3.28). Butyrate concentrations were similar in all groups (Tables 3.26, 3.27 and 3.28)

Propionate and butyrate concentrations as a percentage of the total faecal SCFA were greater in H, HO, HB and HG compared to C, CO, CB and CG which in turn decreased the percentage of acetate (Tables 3.26, 3.27 and 3.28)

Acetate to propionate ratio was lower in H and HO than C and CO. HO, HB and HG were not significantly different to H (Tables 3.26, 3.27 and 3.28); CO was higher than C (Table 3.28), however, CB and CG were decreased compared to C (Table 3.29 and 3.30).

Acetate percentage of total faecal SCFA was greatest in C (92%) and lowest H (75%) with similar percentages in CO (77%), CB (86%), CG (83%) HO (85%), HB (75%) and HG (82%) compared to respective controls. Propionate percentage increased in CO (5%), CB (6%), CG (7%), HO (5%), HB (6%) and HG (6%) compared to C (3%) and H (4%). The highest percentage of butyrate was found in H (21%) with least in C (5%) CO (9%), CB (8%), HO (17%) and HB (19%) were not significantly different to C and H. HG (12%) decreased compared to H and CG (10%) increased compared to C.

Table 3.28 Average total SCFA, acetate, propionate and butyrate and acetate to propionate ratio in faecal samples of male Wistar rats following a dietary intervention of wholegrain oat groats

					P-value		
SCFA (µmol/g)	C	CO	H	HO	diet	intervention	interaction
Total SCFA	246±55[a]	212±14[a]	48±4[c]	76±3[b]	<0.0001*	0.9172	0.2894
Acetate	227±50[a]	181±14[a]	36±4[c]	59±3[b]	<0.0001*	0.6640	0.2008
Propionate	7±1[b]	12±1[a]	2±0.4[d]	4±0.5[c]	<0.0001*	0.0002*	0.0676
Butyrate	12±4[a]	19±3[a]	10±1[b]	13±2[b]	0.1597	0.0829	0.4737
Acetate: Propionate	32.1±2.9[a]	15.1±0.1[b]	18.3±3.4[b]	14.8±1.1[b]	0.0061*	0.0002*	0.0082*

Table 3.29 Average total SCFA, acetate, propionate and butyrate and acetate to propionate ratio in faecal samples of male Wistar rats following a dietary intervention of oat bran

					P-value		
SCFA (µmol/g)	C	CB	H	HB	diet	intervention	interaction
Total	246±55[a]	133±23[b]	48±4[b]	36±9[b]	<0.0001*	0.0517	0.1102
Acetate	227±50[a]	114±23[b]	36±4[b]	27±3[b]	<0.0001*	0.0391*	0.0745
Propionate	7±1[a]	8±1[a]	2±0.4[b]	2±0.5[b]	<0.0001*	0.5268	0.5268
Butyrate	12±4[a]	11±1[a]	10±1[a]	7±2[a]	0.2155	0.4039	0.6744
Acetate: Propionate	32.1±2.9[a]	14.5±1.3[b]	18.3±3.4[b]	13.5±1.5[b]	0.0080*	0.0001*	0.0138*

Table 3.30 Average total SCFA, acetate, propionate and butyrate and acetate to propionate ratio in faecal samples of male Wistar rats following a dietary intervention of β-glucan powder

					P-value		
SCFA (μmol/g)	C	CG	H	HG	diet	intervention	interaction
Total	246±55[a]	120±8[b]	48±4[d]	89±2[c]	0.0005*	0.1431	0.0072*
Acetate	227±50[a]	99±6[b]	36±4[d]	73±1[c]	0.0004*	0.0868	0.0039*
Propionate	7±1[ac]	9±1[a]	2±0.4[b]	5±1[c]	<0.0001*	0.0108*	0.5800
Butyrate	12±4[a]	12±3[a]	10±1[a]	11±3[a]	0.6176	0.8675	0.8675
Acetate: Propionate	32.1±2.9[a]	11.1±0.5[b]	18.3±3.4[b]	15.3±3.0[b]	0.0660	0.0001*	0.0039*

Values are mean ± S.E.M. n = 8 - 10 per group. Means without a common superscript letter differ, * indicates P < 0.05

C = cornstarch; CO = C + wholegrain oat groats; CB = C + oat bran; CG = C + β-glucan powder; H = high carbohydrate high fat; HO = H + wholegrain oat groats; HB = H + oat bran; HG = H + β-glucan powder,

*** C & H are the same for all 3 intervention groups***

3.4 Discussion

Metabolic syndrome is a complex combination of abnormalities associated with cardiovascular disease and type 2 diabetes, with the major cause being a diet high in fat and simple sugars (Kaur, 2014). As metabolic syndrome includes diabetes, hypertension, dyslipidaemia, fatty liver, glucose intolerance and obesity, many studies are needed to determine the causes and find ways to attenuate the complexity of symptoms. This study supplemented a high carbohydrate, high fat diet in a rat model of metabolic syndrome that mimics the human condition with 5% wholegrain oat groats, oat bran or partially purified β-glucan powder.

Wholegrains are an important source of calories, nutrients and phytochemicals (Belobrajdic and Bird, 2013). Wholegrains have been associated with prevention of type 2 diabetes (Li et al., 2016), cancer (Jacobs et al., 1998) and other chronic diseases (Johnston et al., 2010, Dixit et al., 2011, Aune et al., 2016). The consumption of wholegrain oats has been advocated in dietary guidelines and nutritional policies worldwide (Clemens and van Klinken, 2014a). The 2015 Dietary Guidelines for Americans suggests consuming at least half of all grains as wholegrains with 3 serves of 16 g/day to reduce the risk of developing chronic disease (U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2015). Wholegrain is classified as 'consisting of intact, ground, cracked or flaked caryopsis, whose principal anatomical components – the starchy endosperm, germ and bran, are present in the same relative proportions as they exist in the intact caryopsis' (Rebello et al., 2014). Most studies use wholegrain rolled oats, oat flakes or oatmeal (Aune et al., 2016, Jonnalagadda et al., 2011); this study used intact oat groats for supplementation.

Oat groats are the hulled kernels of the oat grain that includes the germ and fibre-rich bran portion of the grain as well as the endosperm. Limited processing means that the groat contain many nutrients, including β-glucans, arabinoxylans and dietary fibre as well as high concentrations of proteins and lipids (Connolly et al., 2012). When grain is refined, the bran layer is removed and many bioactive compounds are lost (Connolly et al., 2012). Oat groats still contain these components and can be classified as a functional food as they have a beneficial effect on human health (Rasane et al., 2013).

Oat bran is the outer coating of the oat groat, which is produced by grinding and separating from the groat. Oat bran has at least 5.5% β-glucan and a total dietary fibre content of at least 16% so that at least one-third of the total dietary fibre is soluble (Robert et al., 1985). Bran is a good source of fructans and resistant starch (Bernstein et al., 2013) as well has containing vitamins, minerals (Peterson et al., 1975, Frølich and Nyman, 1988), phytates (Fulcher et al., 1981) and phenolics (Gray et al., 2000). Oat bran has been associated with prevention of hypercholesterolaemia (Kerckhoffs et al., 2003) and hypertension (Keenan et al., 2002). Bran provides many of the health benefits of the wholegrains (Slavin, 2003).

Previous studies have focussed on individual components of the grain, such as β-glucans, dietary fibre, avenanthramides or lipids (Connolly et al., 2012). β-glucans have been classified as a potential prebiotic, meaning that they may selectively promote growth of beneficial microorganisms in the gastrointestinal tract (Connolly et al., 2012). These microbes produce SCFA's through fermentation to be utilised as an energy source for bacteria and host and improvement of gastrointestinal structure and function.

β-glucans are a soluble fibre found in oat grains and when ingested forms a gel inside the stomach and small intestine. This gel traps bile acids leading to reduced re-absorption. As cholesterol is a fundamental component of these bile acids, when concentrations decrease the body uses cholesterol to form bile acids, resulting in a lower LDL cholesterol and therefore an overall lower total cholesterol concentration (Queenan et al., 2007, Drozdowski et al., 2010). Randomised controlled trials with oat products and cardiovascular disease markers found that oat products produced a 3 - 6% decrease in total cholesterol and a 4 - 8% improvement in LDL cholesterol (Thies et al., 2014).

Many studies have investigated cardiovascular disease, diabetes or dyslipidaemia but not metabolic syndrome in its complexity. Limited research exists characterising the gastrointestinal morphology of high carbohydrate, high fat diets when supplemented with wholegrain oat groats, oat bran or β-glucan powder. This study was warranted as it examined the physiological effects, gastrointestinal morphology and faecal SCFA production, after intervention with wholegrain oat groats, oat bran or β-glucan powder using a diet-induced rat model of metabolic syndrome. In part A of this study, C and

H were supplemented with 5% wholegrain oat groats. This translates to approximately 25-30 g/day for the average 70 kg adult human using a relevant scaling equation (Bachmann et al., 1996). In part B of this study, C and H were supplemented with 5% oat bran. This translates to approximately 25 - 30 g/day for the average 70 kg adult human using a relevant scaling equation (Bachmann et al., 1996). In part C of this study, C and H were supplemented with 5% β-glucan powder. This translates to approximately 15-20 g/day for the average 70 kg adult human using a relevant scaling equation (Bachmann et al., 1996).

The high carbohydrate, high fat diet in this study induced many parameters associated with metabolic syndrome, including central obesity, hypertension, dyslipidaemia and impaired glucose tolerance. Cardiovascular changes included increased inflammatory cell infiltration, ventricular fibrosis, cardiac hypertrophy and increased diastolic stiffness. Liver changes included steatosis, inflammatory cell infiltration and portal fibrosis.

Wholegrain oat groats and oat bran both contain β-glucans at lower concentrations than in the β-glucan powder that was investigated. Therefore, it was expected that if β-glucans were the driver of physiological changes in metabolic syndrome, then increased concentrations would produce greater changes.

3.4.1 Body composition and dietary intake

Many studies into the impact of wholegrain oats and oat bran only record data of body composition as part of overall investigations into hypertension and hyperglycaemia. Human and rat intervention studies have given inconsistent results related to the decreased risk of obesity and weight gain (Li et al., 2016, Harland and Garton, 2008).

Energy, food and water intake remained the same with the addition of wholegrain oat groats or oat bran to the diet. Many existing studies are human clinical trials; therefore, it is not possible to directly compare food and water intake with our rat studies.

Rat studies have shown decreased body weight and epididymal fat over a period of 8 weeks on an oatmeal diet (Dong et al., 2016) while humans on a 6 week wheat, corn

or rice diet showed no change in body weight, fat or lean mass (Cooper et al., 2017). Human studies have shown inconsistent results, possibly due to the use of hypocaloric diet programs rather than high carbohydrate, high fat diets (Katcher et al., 2008) or short-term studies which indicated no change in weight, BMI or body fat over 30 days (Li et al., 2016). Comparison of studies is difficult due to variations in the type of administered wholegrain, whether wholegrain was added or substituted into the diet and participant characteristics (age, gender, race) (McKeown and Jacobs, 2010, Rebello et al., 2014). However, overall studies suggest that wholegrains may alter fat distribution independently of body weight change. Human trials using oat bran indicated that body weight and abdominal circumference decreased with no change in lean or fat mass in a 2 - month intervention study (Guevara-Cruz et al., 2012). Abdominal circumference decreased in another trial (Devlin et al., 2016). Rat and mice studies, however, showed no decrease in body weight (Zhou et al., 2016, Jodayree et al., 2014).

Our study is consistent with this as abdominal circumference decreased in HO and HB rats with no change in body weight, total fat pads or fat mass. Lean mass was also unchanged in HO and HB compared to H, however increased when oat groats or bran was added to the CO or CB diet for 8 weeks. This suggests that the combination of resistant starch in the cornstarch diet with added oats causes an increase in fat oxidation and a decrease in carbohydrate and protein oxidation leading to increased lean mass (protein accretion) (Higgins, 2014).

Increasing β-glucans in the diet did not alter body weight when added to a high carbohydrate, high fat diet. However, body weight increased when β-glucan powder was included in the cornstarch diet. Abdominal circumference decreased with a decline in fat mass corresponding to an increase in lean mass. β-glucans are fermented in the colon as a prebiotic that feeds the gut microbiota, increasing the production of acetate, propionate and butyrate (Morrison and Preston, 2016). SCFA mediated production of satiety hormones through FFA2 and FFA3 (GPR41/GPR43) receptors (Adam et al., 2014), decreasing food intake and energy intake, however this was not observed in this study. With improved structure of the gastrointestinal tract, nutrients can be absorbed and utilised by the organs more efficiently. Some studies suggest that

decreases in abdominal circumference (Maki et al., 2007, Zhang et al., 2012) and body weight (Anderson et al., 1990) occur, but most studies have found no effect on weight (Anderson et al., 1990, Jenkins et al., 2000, Judd and Truswell, 1981, Kerckhoffs et al., 2003, Kirby et al., 1981, Robitaille et al., 2005, Beck et al., 2010, Davy et al., 2002, Keenan et al., 2002, Kestin et al., 1990, Swain et al., 1990), BMI (Saltzman et al., 2001, Davy et al., 2002) or abdominal circumference (Beck et al., 2010, Davy et al., 2002).

Many human intervention studies (Maki et al., 2007, Bellisle et al., 2014, Robitaille et al., 2005, Keenan et al., 2002, Brownlee et al., 2010, Aune et al., 2016) have found no changes in these parameters even with increasing concentrations (2.3 g - 10.0 g/day) of β-glucans, while some display an increase in body weight (Davy et al., 2002). However, the anthropometric measurements were not the major component in many of these studies (Lefevre and Jonnalagadda, 2012). Therefore, the addition of this study indicates that the effect of dietary β-glucans on these measures remains unclear. The molecular weight, dose, processing and the food matrix in which the β-glucans are found may influence any changes that occur (Wood et al., 2000, Decker et al., 2014, Lyly et al., 2003).

A possible mechanism of action to explain the β-glucans impact on central obesity could be the increased viscosity of the gastrointestinal chyme (Cloetens et al., 2010). The viscosity causes gel formation by the food bolus mixing with a layer of unstirred water adjacent to the mucosa, slowing the absorption of nutrients due to reduced enzymatic activity (Jenkins et al., 2000, Schneeman and Gallagher, 1985, Eastwood and Morris, 1992). The viscosity of β-glucans can give 79 - 96% changes in glucose and insulin responses to a 50 g glucose drink (Wood et al., 1994). The increased viscosity may send signals to the brain to stop eating sooner and therefore decrease energy intake. Oat β-glucans added to a liquid meal at 10.5 g/400 g portion increased the feeling of satiety in healthy volunteers compared to a meal free of β-glucans (Lyly et al., 2010). However, carrier food of the β-glucans is important in determining satiety as solid or semi-solid meals, which are known to increase satiety (El Khoury et al., 2012), such as our experimental diet, may mask any satiating effect.

As with previous studies, it is difficult to conclude that β-glucans are responsible for all the variations in the physiological changes that may be occurring. However, it is

possible that changing the food matrix and dosage of β-glucans may lead to the observed modifications. It may be that macronutrients such as proteins, carbohydrate or lipid concentrations are the drivers of the changes or the minor nutrients, such as the avenanthramides and other polyphenols. It is more likely that all these components are working synergistically. However, the investigation of these elements was outside the scope of this study but would be worthwhile investigating in the future.

3.4.2 Cardiovascular effects

Hypertension, dyslipidaemia and weight gain are major risk factors leading to cardiovascular disease and metabolic syndrome (Parikh and Mohan, 2012). Although this study did not decrease body weight, wholegrain oat groat, oat bran or β-glucan powder supplementation improved systolic blood pressure after 8 weeks. Diastolic stiffness was unchanged with wholegrain oat groats, oat bran or β-glucan powder consumption. Visual analysis of left ventricles indicated decreased inflammatory cells and collagen deposition after 8 weeks of wholegrain oat groats, oat bran or β-glucan powder consumption.

The mechanisms behind the blood pressuring lowering effects of oats are still unclear, and therefore more investigation is needed, particularly in human trials as this will consider physical activity, energy intake and expenditure as well as the usual stresses of everyday life. Wholegrain oat and oat bran consumption have shown limited effect on blood pressure (Maki et al., 2007, Davy et al., 2002, Tighe et al., 2010, Wolever et al., 2010, Thies et al., 2014). Most studies have investigated oat β-glucan with inconsistent results indicating that systolic blood pressure can decrease by 4 to 8 %. However, these studies have been conducted after consumption of hypocaloric diets (Katcher et al., 2008, Saltzman et al., 2001) or in borderline hypertensive subjects (Keenan et al., 2002). Therefore, it cannot be ruled out that weight loss was the main reason behind the decrease in blood pressure.

Elasticity of arteries and maintenance of vascular tone are important factors in the control of blood pressure. Nitric oxide (NO), prostacyclin and EDHF (endothelium-derived hyperpolarizing factor) which are secreted from the endothelium are responsible for this maintenance (Burger and Touyz, 2012). Oats contain avenanthramides which help in the reduction of coronary heart disease. They possess

anti-inflammatory and anti-proliferative properties and cause vasodilation (Yang et al., 2014). Avenanthramides inhibit monocyte adhesion to endothelial cells, inhibit release of proinflammatory compounds from macrophages (Liu et al., 2004) and control blood pressure through increased production of NO and dilatation of blood vessels (Nie et al., 2006). Further investigation of endothelial function is necessary to confirm or repudiate that the avenanthramides are causing the improvement in blood pressure.

Oats also contain the highest fatty acid content of any cereal, and are a good source of linoleic acid and contain low amounts of saturated fats (Webster and Wood, 2011, Youngs, 1986) which may also be improving cardiovascular health. Further studies investigating the benefits of avenanthramides and oat lipids on cardiovascular disease would be worthwhile.

Our studies contained 4%, 6% and 12% β-glucans and were consistent with those trials that decreased blood pressure. More investigation is needed, particularly in human trials as this will consider physical activity, energy intake and expenditure as well as the usual stresses of daily life.

3.4.3 Liver effects

Dyslipidaemia is a major factor in metabolic syndrome, increased cholesterol and triglycerides lead to cardiovascular dysfunction. The liver plays an important role in regulating plasma lipid concentrations through cholesterol clearance and recruitment, while lipid uptake affects hepatic fat composition. The cholesterol-lowering abilities of oat bran has been well studied in relation to β-glucans (Ho et al., 2016, Zhou et al., 2016, Kestin et al., 1990, Wolever et al., 2010).

The addition of wholegrain oat groats or oat bran to the diet for 8 weeks indicated no change to liver weight. Plasma alanine aminotransferase (ALT) and aspartate transaminase (AST) decreased with the addition of oat bran to the diet but not with the wholegrain oat groats. Total plasma cholesterol decreased, non-esterified fatty acids increased, and no change was found in triglyceride concentrations with the addition of the wholegrain oats. However, there was no alteration to these parameters with the addition of 5% oat bran to the diet. Hepatic lipid deposition decreased with the

addition of wholegrain oats and oat bran. The addition of 7.5%, 15% and 30% wholegrain oat to a high fat diet decreased total cholesterol, triglycerides and free fatty acids as well as plasma ALT and AST activity and decreased hepatic lipid deposition (Peng et al., 2013). Our lower dose of 5% wholegrain oat was consistent with this study except for the free fatty acids; however, this may have been due to differences in wholegrain oat used, animal species or experimental design. These results are consistent with previous studies (El Rabey et al., 2013, Guo et al., 2014, Mosa et al., 2015) that used 10% (El Rabey et al., 2013) and 20% (Mosa et al., 2015) oat bran to reduce the lipid profile and improved liver function indicating that lower amounts of oat bran also have an effect on hypercholesterolaemia. The inhibition of FAS, GPAT and HMG-CoA reductase preventing lipid synthesis and stimulation of PPARα, CPT-1 and AMPK stimulating lipid oxidation has been demonstrated in a rat study with a high fat diet supplemented with either 7.5%, 15% or 30% oat (Peng et al., 2013). As our 5% dose of oat bran did not alter cholesterol, triglyceride or NEFA, it may be determined that it was too low to have an effect as the higher doses decreased each of those parameters.

Many studies report the cholesterol lowering abilities of β-glucans (Ho et al., 2016, Varma et al., 2016, Queenan et al., 2007, Maki et al., 2007, Guo et al., 2014), covered in Part C of this thesis chapter, but other functional components that oats are rich in such as vitamins, minerals, antioxidants and phenolic compounds (Butt et al., 2008) may also contribute to the inhibition of lipid synthesis and stimulation of lipid oxidation (Peng et al., 2013).

The reductions in plasma total cholesterol, triglycerides and NEFA concentrations by β-glucans have been investigated extensively (Kerckhoffs et al., 2003, Guo et al., 2014, Queenan et al., 2007, Ho et al., 2016, van Bennekum et al., 2005, Pick et al., 1996, Braaten et al., 1994, Anderson et al., 1990, Hara et al., 1999). However, this study showed no differences in total cholesterol or triglyceride concentrations. NEFA concentrations decreased in the high carbohydrate, high fat β-glucan powder supplemented diet, but also decreased in the C supplemented diet. Fat vacuole formation in the liver was increased in H which decreased with the addition of β-glucan powder to the diet.

90% of bile acids synthesised from circulating cholesterol are absorbed by the ileum (Dawson, 2011). β-glucans decrease reabsorption of bile acids increasing their transport to the colon and microbial conversion to metabolites leading to increased excretion. This leads to the liver obtaining additional cholesterol by upregulation of low density lipoprotein (LDL) receptors, increasing LDL particle uptake which in turn reduces circulating LDL-cholesterol (Papathanasopoulos and Camilleri, 2010).

Consumption of wholegrain oats have had a greater overall effect on LDL-cholesterol concentration than when specific components, such as β-glucans, are taken individually (Klose and Arendt, 2012, Einhorn et al., 2003). However, as our studies only investigated total cholesterol concentration rather than LDL-cholesterol and HDL-cholesterol, further studies would need to be completed to determine this. Oat bran increased the portion of total bile acid pool that was deoxycholic acid (Marlett et al., 1994), a microbial by-product of bile acid that decreased the absorption of exogenous cholesterol in humans (Hillman et al., 1986). The increased viscosity of β-glucans in the small intestine which slows digestion and absorption is thought to play a role in this decreased reabsorption of bile acids (Wolever et al., 2010, Drozdowski et al., 2010).

β-glucan powder increased fermentation of SCFA with acetate concentration increased with the addition of β-glucan powder to the high carbohydrate, high fat diet. SCFA are readily taken up from the colon and transported to the liver to serve as a substrate for cholesterol synthesis, while propionate has a hypocholesterolaemic action by inhibiting HMGT-CoA reductase and preventing the utilisation of acetate for cholesterol synthesis. Increased propionate is used in hepatic lipid metabolism which may have led to the improvements in the liver structure through decreased steatosis.

3.4.4 Glycaemic effects

Impaired glucose tolerance and fasting glycaemia are major risk factors for cardiovascular disease and Type 2 diabetes (International Diabetes Foundation, 2011). Hypertensive and hypercholesterolaemic subjects are used when examining glucose responses, as these studies did.

Wholegrain oat groats, oat bran or β-glucan powder in the diet improved glycaemic control with postprandial glucose area under the curve (AUC) and fasting glucose concentrations lower after 8 weeks. This may indicate that oat products have a role in normalising blood glucose concentrations.

While there is plenty of information regarding the glycaemic response of gluten-containing foods, studies on gluten-free foods, such as oat bran, are minimal, with those studies conducted on composite recipes rather than specific foods (Di Giacomo et al., 2013). Many studies focus on β-glucans rather than the bran itself, therefore not considering the synergistic effect of all the nutrients found in the wholegrain or bran.

The addition of wholegrain oat to the diet increases viscosity of the food bolus through the gastrointestinal tract, caused by the β-glucans in the grain. The increased viscosity slows the absorption of carbohydrates and lowers postprandial glucose concentrations (Li et al., 2016). Results in previous β-glucans studies have been inconsistent with fasting plasma glucose decreasing in some human and animal trials with no changes in other randomised controlled trials (Cooper et al., 2017, Bao et al., 2014, Shen et al., 2011). Postprandial blood glucose concentrations may be affected by the starch in oats which is slowly degradable due to high amounts of β-glucans (Rose, 2014).

Inconsistent results in previous oat bran studies have shown plasma glucose concentrations decreasing in some human and animal trials while other trials have found no change (Bjorck et al., 1994, Tapola et al., 2005, Holm et al., 1992). As cell structure, particle size and food form are associated with low glucose responses (Björck et al., 1994), one human trial consisted of oat bran flour and oat bran crisp. The oat bran flour lowered glucose area under the curve (AUC) while the oat bran crisp gave no change to the control (Tapola et al., 2005). Another study incorporating oat bran into fettucine did not lower postprandial blood glucose in healthy subjects (Holm et al., 1992). Our study had oat bran that was a flaky consistency and results are similar to the oat bran flour results.

Hypertensive and hypercholesterolaemic subjects are used when examining glucose responses, as this study did. These studies, however, display varying results in the lowering of blood pressure and cholesterol concentrations along with the improvements in glucose response. Indications from 12-week intervention studies on

hypertensive subjects consuming either 5.5 g/day or 7.7 g/day of oat β-glucans did not change fasting glucose concentrations (Davy et al., 2002, Maki et al., 2007). This study indicated an improvement in fasting glucose concentrations with the addition of 14 g/day β-glucan powder.

Consumption of soluble dietary fibre, such as β-glucans, improves postprandial glucose and insulin responses. Postprandial glucose concentrations were decreased in hypercholesterolaemic subjects after consumption of 4 g oat β-glucan as a beverage, while 10 g did not influence postprandial glucose concentrations (Biörklund et al., 2008). In overweight women, improvements in postprandial plasma glucose followed the consumption of 0.26, 0.68, or 2.3 g β-glucan/100 g muffin (Behall et al., 2006). The improved glucose AUC with the addition of β-glucan powder suggests that higher concentrations of β-glucans lower the postprandial glucose concentrations possibly through the alteration of gastric emptying and transit time of glucose through the intestinal tract.

These differing results may be due to the variation of the molecular weight of the β-glucans that are being used (Biörklund et al., 2008, Wolever et al., 2010). The molecular weights of the β-glucans play a major role in the viscosity of the gel that is delaying the gastric emptying and therefore the absorption of the glucose, as higher molecular weight oat β-glucans cause a more viscous gel (Wood et al., 2000). A more viscous gel delays glucose absorption and lowers postprandial glucose concentrations (Wolever et al., 2010), which may also account for limited changes occurring in glucose AUC as the absorption of the glucose is taking place over an extended period. There are limitations on studies utilising the molecular weight of β-glucan as there are currently no available β-glucan control standards with specific molecular weight and branches (Staka et al., 2015). Therefore, most research on β-glucans uses extracts rather than purified samples, and it is therefore not possible to exclude confounding factors (Staka et al., 2015).

Another mechanism for improvements in the glucose AUC and fasting glucose may be the increased production of acetate and propionate, lowering the acetate to propionate ratio. By lowering this ratio, increased gluconeogenesis is possible. Propionate oxidising to propionyl-CoA and pyruvate leads to gluconeogenesis and with this improved hepatic function and maintenance of glucose concentrations

(Giacco et al., 2014). This study showed a lower acetate to propionate ratio with the addition of wholegrain oat groats which is consistent with previous studies.

3.4.5 Inflammation

Many inflammatory markers, such as interleukin-6 (IL-6) and intercellular adhesion molecule 1 (ICAM-1), are linked to cardiovascular disease, but only C-reactive protein (CRP) is currently considered an independent marker of cardiovascular disease risk (Pearson et al., 2003), which is why the current study only measured plasma CRP concentrations. Limited studies have examined the effect that wholegrain oats, oat bran or β-glucans have on the inflammatory markers of cardiovascular disease. No change with increased consumption of oats (Ajani et al., 2004, Ma et al., 2006) has been apparent, which these studies supports. This suggests that the modulation of these markers is not the mechanism for the benefits conferred by oats. However, further studies investigating more specific inflammatory markers such as IL-6 or NF-κB which wholegrains decrease (Herder et al., 2009, Oliveira et al., 2009) may be warranted to confirm or repudiate these findings.

Long term high fat diets can increase intestinal inflammation and oxidative stress (Gulhane et al., 2016). Histological assessment of wholegrain oat groats, oat bran, and β-glucan powder showed limited inflammatory cells throughout the gastrointestinal tract including the high carbohydrate, high fat rats. Previous studies found similar results with limited inflammation (Luck et al., 2015, Gulhane et al., 2016, Johnson et al., 2015). However, other studies have shown increases in pro-inflammatory markers, such as NF-κB and TNF-α (Ding et al., 2010), indicating that these may be more relevant to the development of inflammatory-related diseases such as ulcerative colitis and obesity-associated colorectal cancer. These studies indicate that a high fat diet alone may not be the cause of inflammation in the gastrointestinal tract.

As inflammatory marker results in both cardiovascular and gastrointestinal tissue and plasma samples were not as expected, further studies investigating other inflammatory markers such as IL-6 or NF-κB are needed to confirm or repudiate these findings.

3.4.6 Gastrointestinal parameters

As a prebiotic, oat β-glucans are fermented by the gut microbiome to produce SCFA (Rose, 2014) which contribute to gastrointestinal structure and function. Limited studies exist focussing on the effects that metabolic syndrome has on the gastrointestinal tract with most studies instead examining the gut microbiome. Those studies that have been undertaken tend to focus on specific compounds, such as β-glucans, rather than the complete oat groat or oat bran. Therefore, a study on the changes to gut morphology caused by high carbohydrate, high fat diets and subsequent improvements with the addition of wholegrain oat groats and oat bran to the diet is warranted.

As the healthy control, the C diet may be acting as a prebiotic, improving the gastrointestinal tract structure prior to the addition of the wholegrain oat. Cornstarch is approximately 25% amylose and 75% amylopectin (Takeda et al., 1988). Starch can be used by a wide range of microbiota to produce metabolites for health benefits. Studies have shown that cornstarch alters gut fermentation and bacterial communities (Kalmokoff et al., 2013). Therefore, with a healthy gut environment, any effect that wholegrain oat, oat bran or β-glucan powder may have will be limited as they are also predicted to improve the environment with fermentation by similar microbes. The CO, CB and CG groups may then give less overall improvement than is seen in the H groups. However, the focus of the study was on how wholegrain oat groats, oat bran or β-glucan powder can impact the gastrointestinal tract after a high carbohydrate, high fat diet.

The effects of oat bran on overall health are derived from its effects in the gastrointestinal tract on digestion, absorption and fermentation (Dong et al., 2016). Previous studies have focused on β-glucans prebiotic effect on the gut microbiome (Delcour et al., 2016, Staka et al., 2015) rather than the gastrointestinal tract. Limited studies have investigated oat brans effect on the gastrointestinal morphology of a high carbohydrate, high fat fed rat model of metabolic syndrome.

β-glucans have prebiotic potential, and may act as a substrate for gastrointestinal microbiota to use as an energy source to produce short chain fatty acids which are then utilised for providing health benefit to the host (Gibson et al., 2017).

The influence of high fat and high fructose diets inducing changes in the gut microbiota and its influences on intestinal permeability, inflammation and other metabolic complications have been well studied (Cani et al., 2009, ter Horst et al., 2016, Panchal et al., 2011b, Jurgoński et al., 2014, Moreira et al., 2014, Catalioto et al., 2011). High fat diets contribute to a lack of intestinal barrier integrity with increased low-grade inflammation being caused by metabolic endotoxaemia (Cani et al., 2009) leading to a breakdown of the intestinal barrier and resulting in weight gain, NAFLD and insulin resistance (Cani et al., 2009). Serum endotoxin and TNF-α concentrations decreased with the addition of β-glucans to a high fat diet (Dong et al., 2016). Although this study did not analyse LPS concentrations, by examining the morphology of the intestine, it may be possible to determine the integrity of the intestinal barrier through the overall structure of the various sections of both the small intestine and colon. While there is plenty of research regarding the benefits of β-glucans on cholesterol concentrations and blood pressure, limited research exists on the effect of β-glucans on the gastrointestinal tract and whether it affects the gastrointestinal mucosa. The effects that β-glucans have depend on their molecular form and physicochemical properties. The degree of polymerisation and concentration of oat β-glucan depends on cultivar as well as growing, processing and storage conditions (Clemens and van Klinken, 2014a). Existing studies have used yeast wall β-glucans (Schwartz and Hadar, 2014) rather than grain-based ones for this reason (Handayani et al., 2012).

Limited research has been found on the effects that metabolic syndrome has on the gut with most studies focused on the gut microbiome not the gastrointestinal tract.

This study determined the overall impact of wholegrain oat groats, oat bran or β-glucan powder on the contractility and morphology of the gastrointestinal tract. Further, faecal SCFAs were examined to determine if concentration changes occurred with the addition of these supplements to the diet.

3.4.6 Gastrointestinal morphology

The structure of the gastrointestinal tract is important when considering overall health as nutrient absorption is more efficient in those that are well formed compared to those that are structurally inadequate (Wells et al., 2017). Gastrointestinal health

maintenance is a complex, delicate balance between diet, commensal microbes and the mucosa, including the epithelium and mucus layers. The hypothesis for this section of my study was that morphology can be used to examine intestinal barrier integrity.

The small intestine is the primary absorptive site of nutrients with absorptive cells the major cell type within the epithelial layer. The vast surface area of the small intestine is a result of villi and microvilli projecting from the mucosa (Helander and Fändriks, 2014, Schulze, 2015). The crypt-villus unit is the functional unit of the small intestine (Marshman et al., 2002) with stem cells in the base of the crypt differentiating into various cell types including absorptive, enteroendocrine, Paneth and goblet cells (Marshman et al., 2002). *In vitro* studies have found that structures similar to villi have been produced when cell cultures are exposed to an intestinal chyme indicating that exposure to luminal content is important for development and structural integrity of the small intestine (Kim and Ingber, 2013). As the chyme passes through the small intestine, gut hormones, such as cholecystokinin (CCK), GLP-1 and PYY, are released increasing pancreatic secretions and bile from the bile duct of rodents and gall bladder in humans (West and Mercer, 2004). These hormones lead the digestive processes and possibly have roles in reducing gastric secretions and gut motility (Ellis et al., 2013, West and Mercer, 2004). The addition of β-glucans to the diet increased GLP-1 and PYY (Beck et al., 2010, Greenway et al., 2007). However, whether this is due to structural changes is unknown and was beyond the scope of this study. Goblet cells produce a protective mucus barrier for protection of the epithelial layer from pathogenic bacteria (Hino et al., 2012). SCFA production could mediate intestinal stem cell activity, however only small amounts of SCFA are produced in the small intestine and SCFA may be more significant to differentiation in the colon (Petersen et al., 2014).

The duodenum as the entry point to the small intestine from the stomach receives bile through the rat's bile duct, as they have no gall bladder, which emulsifies fat and digestive enzymes which break down protein from the gastric chyme. Most of the changes in the small intestine were found in the duodenum, particularly in the high carbohydrate, high fat diet with mucosal thickness decreased by approximately 50% primarily due to atrophy of villus height and crypt depth. The addition of wholegrain oat groats, oat bran or β-glucan powder to the high carbohydrate, high fat diet saw an

improved structure with villi, crypts and mucosa returning to similar measurements as the cornstarch diet. Longer villi may indicate increases in brush border hydrolases due to more mature enterocytes being produced (Picariello et al., 2016). Our results differ from previous studies that showed a diet low in fibre increased villi height in the need for absorptive capacity and decreased goblet cell production as well as atrophy in the mucosa (de Oliveira Belém et al., 2015). However, that study used dietary restriction rather than a high carbohydrate, high fat diet as well as 18-month-old rats compared to 6-month-old rats. Other studies have shown trophic effects on villi. Rats fed pectin at 25 g/kg for 14 days significantly increased villus height and crypt depth (Andoh et al., 1999).

This suggests dietary intake and possibly age are key components in the differentiation of intestinal stem cells. Increased villi heights were observed in mice fed a high fat diet, however this study sectioned the small intestine into 3 equal parts and called the first section the duodenum (de Wit et al., 2008). Due to this, the section that was analysed could have been at a further distance from the pyloric sphincter than in our study.

Another mechanism that may be leading to the increased villi height is the high proportion of medium-chain fatty acids, such as oleic and linoleic acids which are found in wholegrain oat groats which stimulate intestinal mucosal growth and increase villi height that can directly affect nutrient absorption by increasing the absorptive and surface area of the duodenum, jejunum and ileum (Chwen et al., 2013).

The jejunum as the longest section of the small intestine is also the main region of absorption of sugars, amino acids and fatty acids. Our studies with wholegrain oat groats, oat bran or β-glucan powder found no morphological changes in the jejunum which was similar to previous studies (Mathers et al., 2007) that indicated longer crypts and shorter villi but no overall change to the structure of the jejunum. Pigs fed both high and low amounts of fermentable dietary fibre have shown no effect to villi height, crypt depth and epithelial layer of the small intestine (Glitsø et al., 1998).

Goblet cell numbers were greater in the high carbohydrate, high fat diet than the cornstarch diet, but there was no difference with the addition of wholegrain oat groats, oat bran or β-glucan powder. As mucus provides physical and chemical protection against pathogenic gut microbiota (Peterson and Artis, 2014), it may also indicate that

a reduced level of protection is needed as the gut microbe population is balanced with the intestinal epithelium (de Oliveira Belém et al., 2015). Other studies have also found that adding dietary fibre, such as cellulose and arabinoxylan both of which are found in wholegrain oat groats, increased goblet cells in the mucosa (Hino et al., 2013, Hino et al., 2012, Ito et al., 2009, Chen et al., 2015). However, due to complexities in measuring the mucus barrier, relatively few studies have assessed this. Immunohistochemistry and other techniques have been used to determine mucin production (Morel et al., 2005, Tanabe et al., 2006, Morita et al., 2008). MUC2 mucin gives physical and chemical protection as well as immunomodulation contributing to intestinal homeostasis (Tadesse et al., 2017). Deficiencies in MUC2 are linked to low-grade chronic inflammation in the small intestine and colon (Velcich A et al., 2002, Sluis et al., 2006). Our study did not determine mucin production as the aim was to determine number rather than functional aspects of goblet cells. However, future research may determine whether MUC2 production and therefore the intestinal mucus barrier is improved with the addition of wholegrain oat groats to the diet.

The ileum is the final section of the small intestine and mainly absorbs vitamin B12 and bile salts and any remaining unabsorbed nutrients from the jejunum. Our studies with wholegrain oat groats, oat bran or β-glucan powder found no changes to villi height, crypt depth, mucosal thickness and goblet cell numbers in the ileum. However, there was a large variability in results achieved, therefore further experiments would be beneficial to confirm or repudiate these findings.

The overall profile along the length of the small intestine has been altered in the high carbohydrate, high fat diet with the villi height atrophied and similar along the entire length rather than longer in the duodenum and decreasing in length towards the ileum as in samples that were fed wholegrain oat groats. This profile decreases the surface area of the small intestine leading to reduced absorptive capacity. The addition of wholegrain oat β-glucans to the diet increases viscosity of the intestinal chyme and therefore slows transit time allowing increased absorption of nutrients and not as much stress is placed on the epithelial layer, and villi. Further rheological studies to determine the viscosity of the intestinal contents would be beneficial to make a correlation between viscosity and intestinal barrier integrity. Previous studies in mice have found that high fat diets lead to a shortening of the small intestine length

compared to a regular chow diet (Beyaz et al., 2016, Soares et al., 2015, de Wit et al., 2008), but no studies have been found that compared intestinal length between high fat and cornstarch diets.

β-glucans act differently in the small intestine compared to the colon. In the small intestine, β-glucans remain intact, as no enzymes are available that hydrolyse it and this increases the viscosity (Daou and Zhang, 2012). A study on pig digesta found that β-glucans were retained within the endosperm of oats until it reached the ileum but the cell wall structures were disrupted by the caecum (Johansen et al., 1997). In human ileostomy patients, 88.5% of ingested β-glucans could be recovered (Lia et al., 2007). In the colon, β-glucans are broken down by gut microbes and fermented into metabolites, such as acetate, propionate and butyrate (Bach Knudsen et al., 2007). Due to this, many of the changes that have been found in the small intestine may not be linked to β-glucans but to other bioactive compounds, such as avenanthramides within the β-glucan powder supplement or in the previous wholegrain oat groat or oat bran studies. As this study was focussed on β-glucans with 12% concentration in the supplement, other bioactive components which may have been present were not examined and future studies could be worthwhile to determine which components lead to the changes that are described below for the small intestine. It is likely that. although the viscosity of β-glucans was involved in the morphological changes, this may not have been the major cause of the observed changes.

Between the small intestine and the colon lies the caecum, where much of the fermentation occurs in a rat. This explains the low concentrations of SCFA that were retrieved from the faecal samples as over 90% of SCFA are utilised either through absorption into the blood stream for use in the liver (acetate and propionate) or colon (butyrate) as an energy source (Ríos-Covián et al., 2016). Further analysis of the caecum should be undertaken by future researchers to determine SCFA concentrations.

The colon was separated into three sections for this study, proximal, mid and distal. Although there were limited changes found in the crypt depth, histological analysis indicated more submucosal folds in the wholegrain oat groat, oat bran or β-glucan power, proximal and distal colon compared to the high carbohydrate, high fat diet fed group. This may be due to increased production of butyrate which is used as an energy

source for colonocytes allowing increased cell proliferation and differentiation (Wong et al., 2006). The increase in submucosal folds gives the colon a greater surface area and increased ability to extract any remaining water and nutrients before waste is then excreted. Histologically, the colon has a more intact epithelial layer and mucus layer in the HO, HB and HG groups compared to the high carbohydrate, high fat group. Limited changes were found in mucosal thickness with increases only in the distal colon of the wholegrain oat groat group. No changes in goblet cell number were noted.

Long term high fat diets can increase intestinal inflammation and oxidative stress (Gulhane et al., 2016). However, my histological assessment showed limited inflammatory cells throughout the gastrointestinal tract including the high carbohydrate, high fat rats. Previous studies found similar results with limited inflammation (Luck et al., 2015, Gulhane et al., 2016). One study found that although there was limited inflammation, increased non-esterified fatty acids indicating oxidative stress was reversed by IL-22 (Gulhane et al., 2016). Another study on mice found T cells in the colon were limited after 3 weeks on a high fat diet, but after 12 – 16 weeks numbers had increased (Luck et al., 2015). Therefore, our results may have been due to the protocol not being long enough for the inflammatory cells to infiltrate. A third study found that, unlike liver and adipose tissue, there was no evidence of intestinal inflammation by monocytes or macrophages from a high fat diet (Johnson et al., 2015). However, other studies have shown increases in pro-inflammatory markers, such as NF-κB and TNF-α (Ding et al., 2010), indicating that these may be more relevant to the development of inflammatory-related diseases such as ulcerative colitis and obesity-associated colorectal cancer. These studies indicate that a high fat diet alone may not be the cause of inflammation in the gastrointestinal tract.

There were limited changes in the colon length of any of the intervention groups. However, as in the small intestine, the diet and intervention both may have contributed to changes in the overall colonic length. No other rat studies have been found that measure intestinal lengths separately in relation to fibre or high fat diets. One study found overall rat intestinal length increased with oat bran compared to wheat bran and pea fibre and a low dietary fibre control (Hansen et al., 2007). Small intestine and colon length was unchanged by β-glucan powder but there was high variability in the

results. These results are the same as the previous wholegrain oat groats and the oat bran studies. This possibly indicates that increasing the concentration of β-glucans in the diet does not change the length of the intestine. However, as all three dietary interventions included compounds other than β-glucans, it is not possible to be definitive that it is the β-glucans.

As our rats were from 16 - 24 weeks of age or equivalent to human young adults, morphological and functional abnormalities that are associated with an ageing population (de Oliveira Belém et al., 2015), such as reduced gastric emptying, slow intestinal transit, impaired digestion and absorption, lower production of digestive enzymes and changing composition of the gut microbiome have not been taken into account. Future research could include an ageing rat population or an extended study protocol. Another issue with this study was ensuring that samples were taken from the same points along the gastrointestinal tract. As can be seen from the data, differences exist between each section and therefore can be assumed to be changing along the entire length not just those sections measured. Although each sample was measured and taken from the same point, there may still have been changes within the sample collected during histological processing and sectioning. Variation between individuals also appears to be an issue as with all groups and parameters exhibiting differing results giving a wide range of data points. Therefore, it may be a useful study to examine numerous sections from a single sample per group to determine the complete profile of the gastrointestinal tract before examining other samples.

Due to these limited gastrointestinal morphological changes, the use of morphology as a sign for intestinal barrier integrity is not viable. Molecular studies such as immunohistochemistry, Western blot and ELISA would be more appropriate to determine LPS, tight junction protein, such as occludin and claudin, and gut hormone concentrations. Overall, there was a large variability in gastrointestinal results achieved, therefore further experiments would be beneficial to confirm or repudiate these findings.

3.4.5 Gastrointestinal contractility

Limited studies have been reported on ileal and colonic muscular contractility using diet-induced metabolic syndrome in rats or with wholegrain oat groats, oat bran or β-glucan supplemented diets. This study found that ileal and colonic contractility were not changed between control and wholegrain oat, oat bran or β-glucan powder consumption, primarily due to large variability.

One study found that elevated concentrations of butyrate reduced colonic contractility suggesting that the butyrate stimulates sodium and fluid absorption reducing contractility and motility (Bajka et al., 2010). Our study indicated that while faecal butyrate concentrations did not increase, total faecal short-chain fatty acid and acetate concentrations were higher in rats fed wholegrain oat groats or β-glucan powder. As acetate can be converted to butyrate in the colon (Ríos-Covián et al., 2016, Chassard and Bernalier-Donadille, 2006), it is possible that this is in part responsible for any decreased colonic contractility. However, unlike the wholegrain oat groats, oat bran consumption did not increase faecal short chain fatty acid production Therefore, it is not possible to say that the stimulation of sodium and fluid absorption by butyrate reduced contractility and motility.

Another study examined wheat in rats fed a western diet found no changes to colonic contractility relative to effects of maize starch (Patten et al., 2015). Rat studies have indicated that SCFA inhibit peristaltic contractility above a physiological threshold of SCFA in the lumen (Cherbut, 2007). However, these effects may differ between species, as human trials using an intracolonic infusion of 100 mM SCFA solution did not modify transit in two healthy subjects (Kamath et al., 1990). As our study examined faecal SCFA and not luminal SCFA concentrations, this may be a worthwhile objective for future research.

3.4.6 Faecal SCFA

SCFAs are metabolites of gut fermentation produced by gut microbiota in the colon from food components that are unabsorbed and undigested in the small intestine. Each SCFA has distinct physiological effects including shaping the gut environment, influencing the physiology of the colon, providing energy sources for the host cells

and gut microbiota and participating in different host-signalling mechanisms (Dong et al., 2016, Kasubuchi et al., 2015, Ríos-Covián et al., 2016). They are the main end-products of carbohydrate catabolism (Ríos-Covián et al., 2016). Acetate and propionate both enter the bloodstream and in the liver they act as energy sources for gluconeogenesis (den Besten et al., 2013). Butyrate is used by the colonocytes for cell proliferation and differentiation (Pryde et al., 2002). Low concentrations of butyrate and propionate lead to inflammatory diseases, such as ulcerative colitis (low butyrate) and asthma (low propionate) (Machiels et al., 2014, Arrieta et al., 2015). As many of their functions and uses have been discussed throughout this chapter in relation to each of metabolic syndrome and gut health parameters, this section will only discuss the results as they were found in relation to each other.

Studies have highlighted the influence of different foods and long-term diets on intestinal microbiota and specifically on the pattern of SCFA. However, although oat β-glucans are a prebiotic and known to produce SCFA, limited studies have specifically investigated the SCFA rather than the impact on the gut microbiome. Firstly, our study was a preliminary one to show that cornstarch and high carbohydrate high fat diets produce different proportions of SCFA and that the addition of wholegrain oat groats will also have an effect on the SCFA production. Secondly, as this is only a preliminary study, the sample size was limited to 6/group. Consequently, further research will need to be continued to confirm and consolidate any results from these experiments.

90 - 95% of SCFA are utilised through rapid absorption from the colonic lumen, meaning faecal samples can only give a representative analysis of production and act as a biomarker as it does not represent epithelial cell exposure (Ríos-Covián et al., 2016). Many studies utilise the addition of 'pure' acetate, propionate or butyrate to the diet to determine serum concentrations. Studies have highlighted the influence of different foods and long-term diets on intestinal microbiota and specifically on the pattern of SCFA (Yang and Rose, Costabile et al., 2015). Limited studies exist where wholegrain oat groats, oat bran or β-glucans as part of an overall diet has been investigated (Cloetens et al., 2012).

Firstly, these studies are preliminary ones to show that cornstarch and high carbohydrate high fat diets produce different proportions of SCFA and that the

addition of wholegrain oat groats, oat bran or β-glucan powder would have an effect on the SCFA production. Secondly, as they were only preliminary studies, the sample size was limited to 6/group. Consequently, further research will need to be continued to confirm and consolidate any results from these experiments.
This study indicated that acetate and propionate concentrations were increased by the addition of wholegrain oats while butyrate remained the same.

Our study indicated that C and CO in the diet produced more faecal SCFA than high carbohydrate high fat diets. The addition of wholegrain oat groats to the high carbohydrate high fat diet also increased the overall faecal SCFA. The larger concentration of SCFA is likely to be due to β-glucans and other soluble fibres, including starch in the diet, that is then able to be fermented. However, it is interesting to note that faecal SCFA in the cornstarch wholegrain oat group contained less total SCFA than the cornstarch control. This may be due to more efficient absorption of SCFA by the epithelial cells of the caecum and colon.

Our oat bran study indicates that high carbohydrate, high fat diets even when supplemented with oat bran produce less faecal SCFA than cornstarch and cornstarch supplemented with oat bran diets. However, unlike the wholegrain oat groat study, there was no change in concentration in total SCFA, acetate, propionate or butyrate concentrations between the control group and its oat bran supplemented group. This may mean that they are being transported across the epithelial layer into the colonocytes and blood stream more efficiently due to the limited inflammation and the gut barrier being intact.
In faecal samples recovered from the H diet and the HO groups, an increased proportion of butyrate was produced compared to the C control. The faecal samples recovered from HG contained a greater proportion of acetate and lower proportion of butyrate than H control. Both of these outcomes may be due to butyrate forming from two molecules of acetyl-CoA, and the liberation of butyrate from butyryl-CoA. The butyryl-CoA to acetyl-CoA transferase pathway is used by the majority of known butyrate-producing gut bacteria (Anastasovska et al., 2012, Louis et al., 2004, Louis et al., 2010). If this is occurring, then the acetate concentrations will be decreased, and butyrate concentrations increased as can be seen in our results.

The fermentation of wholegrain oats and subsequent SCFA production has an integral role at both colonic and systemic levels. As well as β-glucans, wholegrain oat groats and oat bran contain other compounds, such as arabinoxylans and cellulose that have the ability to stimulate the growth of beneficial gut microbiota. Further studies are needed to determine the role these compounds have in SCFA production.

The increased percentage of β-glucans in the diet with the β-glucan powder made little difference to the overall concentration of the SCFA produced, indicating the possible involvement of other components of the wholegrain oat groats or oat bran such as arabinoxylans and cellulose that have the ability to stimulate the growth of beneficial gut microbiota. This study has not definitively answered the question of whether it is the β-glucans alone that has the impact, although they increase the viscosity of the food bolus, leading to slower gastric emptying and absorption of nutrients which all stimulate the growth of gut microbiota and therefore increase SCFA production.

3.4 Conclusion

This study demonstrate that the addition of 5% wholegrain oat groats, oat bran or partially purified β-glucan powder to a high carbohydrate, high fat diet can improve blood pressure, glucose tolerance and cholesterol. Heart, liver and gastrointestinal tract structure was also improved. This may have been due to the overall nutrient content with β-glucans, avenathramides and oat lipids and other compounds working synergistically to make these improvements. β-glucans increase viscosity of the food bolus and slow transit time allowing greater absorption of the other nutrients, with further research on this area needed. Also leading the changes may be the increased production of SCFA which act as energy sources for hepatic and colonic cells and as signalling molecules. Many of the results reported in wholegrain oat groats have been repeated in the study on oat bran. This is unsurprising as the oat groat still contains an intact bran layer. However, it was expected that as the oat bran and β-glucan powder had a higher concentration of β-glucan that results would increase in a dose-related fashion and this was not found. This may have been due to different cultivars of oat grains being used or varying molecular weights of the β-glucans both of which lead to

differing results. As these were unknown in these studies, future work should consider these factors prior to the commencement of work.

This study also indicated that production of SCFA was different between cornstarch and high carbohydrate, high fat diets but limited difference was made with the addition of wholegrain oat groats or oat bran. Further research is needed to determine whether this is due to improved gut integrity and therefore improved absorption rather than excretion of SCFA. Further research is needed to determine whether the consumption of wholegrain oat groats or oat bran also increase the expression of FFAR2, FFAR3, MUC2 and tight junction proteins, leading to improved gut repair, protection and integrity. Also, further study is necessary to determine the gut microbiome changes that have possibly caused the changes in SCFA production.

The main limitation of this study was the limited sample size for the SCFA analysis which needs further exploration to confirm the results. Another limitation was in the gut morphology analysis where samples, although measured to collect tissue from exactly the same section, may have been out of alignment with each other between sample groups. These limitations could be ameliorated by only using the samples for one type of experiments rather than multiple ones that may impact results. It was also noted that in an uncompromised gastrointestinal tract, a high carbohydrate, high fat diet did not lead to increased inflammation. While this may be a good model for studying diet-induced metabolic syndrome, a different model may be necessary for gut studies.

Overall, the addition of wholegrain oat groats, oat bran or β-glucan powder to the diet attenuated diet-induced metabolic syndrome, improved gastrointestinal tract structure Wholegrain oat groats altered SCFA production however, oat bran and β-glucan powder did not alter SCFA output.

Chapter 4 - Wholegrain oat groats improve cardiometabolic and gastrointestinal parameters in a high carbohydrate, high fat diet in male Wistar rats

4.1 Introduction

As the world gains access to diets that are high in fats and simple carbohydrates such as fructose (Moreira et al., 2014, ter Horst et al., 2016), rates of metabolic syndrome are increasing, with 20 to 40% of the adult population having at least one of the co-morbidities encompassed by this syndrome (Moreira et al., 2014, O'Neill and O'Driscoll, 2015). The World Health Organisation (WHO) classifies patients with metabolic syndrome as having decreased glucose tolerance and insulin resistance along with two of the following: hypertension, dyslipidaemia, visceral obesity and microalbuminuria (International Diabetes Foundation, 2011). In recent years, diet has become an integral therapeutic intervention to normalise the biomarkers of metabolic syndrome. Diets that include wholegrains such as oat groats may protect the body against cardiovascular disease and diabetes (Connolly et al., 2012, Zhou et al., 2015, Dixit et al., 2011) through the changes in short-chain fatty acid (SCFA) production and improved gastrointestinal morphology. Oats are a functional food that has been consumed for thousands of years (Ladizinsky, 1995), mostly as a wholegrain (van den Broeck et al., 2016). They do not contain gluten, which makes it suitable for coeliac disease patients if processed separately to gluten-containing grains (Moulton, 1959, Janatuinen et al., 2002). The WHO recommends increasing wholegrain consumption to reduce the risk of obesity, cardiovascular disease and diabetes (WHO/FAO, 2003). According to the 2015 American Dietary Guidelines, 16 g/day of wholegrains will reduce the risk of developing cardiovascular diseases and diabetes (U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2015). Approximately 25% of the Western diet is composed of whole grains (Alexandratos and Bruinsma, 2012). By changing the composition of the diet in the previous study in Chapter 3 to incorporate 20% wholegrain oats, this study results should reflect any health effects that are achieved by wholegrain oats in the human diet. An increase in wholegrain oat consumption and consequently β-glucans and other dietary fibre should increase SCFA production leading to an overall improvement in

gastrointestinal physiology. Knowledge of dietary changes to the concentration of SCFA and subsequent physiological effects is currently limited.

Firstly, this study was a pilot study to determine if modifying the previous model was feasible and secondly to analyse the effects of wholegrain oat groats on the physiological and metabolic parameters associated with metabolic syndrome and whether they were associated with changes in the SCFA produced in male Wistar rats fed a high carbohydrate, high fat diet. I hypothesise that addition of 20% wholegrain oat groats will improve metabolic changes, reverse changes in gastrointestinal structure and increase faecal SCFA concentrations when added to a high carbohydrate, high fat diet, reversing the signs of metabolic syndrome.

4.2 Materials and Methods

Please refer to Chapter 2 for the following methods: rat diets, cardiovascular, metabolic, hepatic and gastrointestinal studies. This study was undertaken under AEC approval #15REA003 valid until 20/07/2018. Four groups of rats were used for this study: modified corn-starch (mC); modified high carbohydrate high fat (mH) controls (n = 8/group), modified corn-starch + wholegrain oat groats (mCO) and modified high carbohydrate high fat + wholegrain oat groats (mHO) (n = 8/group). They were fed the diets described in Chapter 2.

The following parameters were measured in the control and intervention groups using the methods detailed in Chapter 2 of this thesis: body weight, food, water and energy intake, oral glucose tolerance test, body composition (DXA), systolic blood pressure, diastolic stiffness, aortic contractility, ileum and colon contractility, blood lipid analysis, liver enzyme analysis, gastrointestinal structural analysis, SCFA analysis and inflammatory marker analysis.

The C and H diets used in Chapter 3 were modified so that interventions up to 20% w/w of the diet could be included. The modified corn starch-rich diet (mC) and the modified high-carbohydrate, high-fat diet (mH) are described in Chapter 2.

To test an intervention in these modified diets, the mC and mH diets were supplemented with 20% wholegrain oat groats (mCO and mHO rats) with this

200 g/kg addition replacing 200 ml/kg of water as described in Chapter 2. Interventions were started after 8 weeks of the diet and continued for the remaining 8 weeks with 8 rats in each intervention. The same batch of oat groats was used in the CO and HO groups in Chapter 3.

4.3 Results

4.3.1 Body composition and dietary intake

The food intake of the mC rats was not different from the mH or mCO. Food intake was decreased for mHO rats compared to both control groups (Table 4.2). Water intake in the mC rats was increased compared to the mH rats, but this does not include the water (200 ml/kg) that was included in the mC diet. mCO water intake was similar to mC, while mHO was increased compared to mH (Table 4.2). Energy intake for mC was decreased compared to mH. mCO and mHO were increased over mC and decreased from mH (Table 4.2). mC had a negative feed conversion efficiency which correlates with the initial weight loss in weeks 1 - 3 followed by limited body weight gain for the remainder of the study, with mH higher. mCO and mHO had increased feed efficiency compared to their corresponding controls (Table 4.2).

mC rats had the lowest final body weight (Table 4.1) due to weight loss over the 16 weeks. Most of this was in the first 3 weeks followed by maintenance of weight for the following 14 weeks (Figure 4.1). mH rats were heavier than the mC rats after 16 weeks of chronic H feeding (Table 4.1). BMI was lower in mC compared to mH and abdominal circumference was lower in mC compared to mH (Table 4.1). With the addition of 20% wholegrain oat groats at 8 weeks, these four parameters were increased in mCO compared to mC and mHO compared to mH (Table 4.1). Retroperitoneal, omental, epididymal and total fat pads were increased between controls (mC vs mH), between controls and interventions (mC vs mCO and mH vs mHO), and between interventions (mCO vs mHO) (Table 4.1), indicating that the addition of the wholegrain oat groats increased body weights (Table 4.1).

DXA measurements showed limited fat mass in the mC compared to mH (Table 4.1) with no change between the fat mass of the controls and their corresponding

interventions (mC and mCO or mH and mHO) (Table 4.1). Lean mass was lower in the mC compared to mH. The consumption of wholegrain oat increased lean mass in both mCO and mHO groups, with mCO having the highest lean mass of all the groups (Table 4.1). Bone mineral density was similar in both mC and mH groups and was not altered in either mCO or mHO. Bone mineral content in the mC rats was lower than in mH. mCO bone mineral content increased compared to the mC group, but no significant difference was found between the mH and mHO groups (Table 4.1). These parameters, except bone mineral density, indicate that the wholegrain oat has a different effect based on the basal diet (Table 4.1).

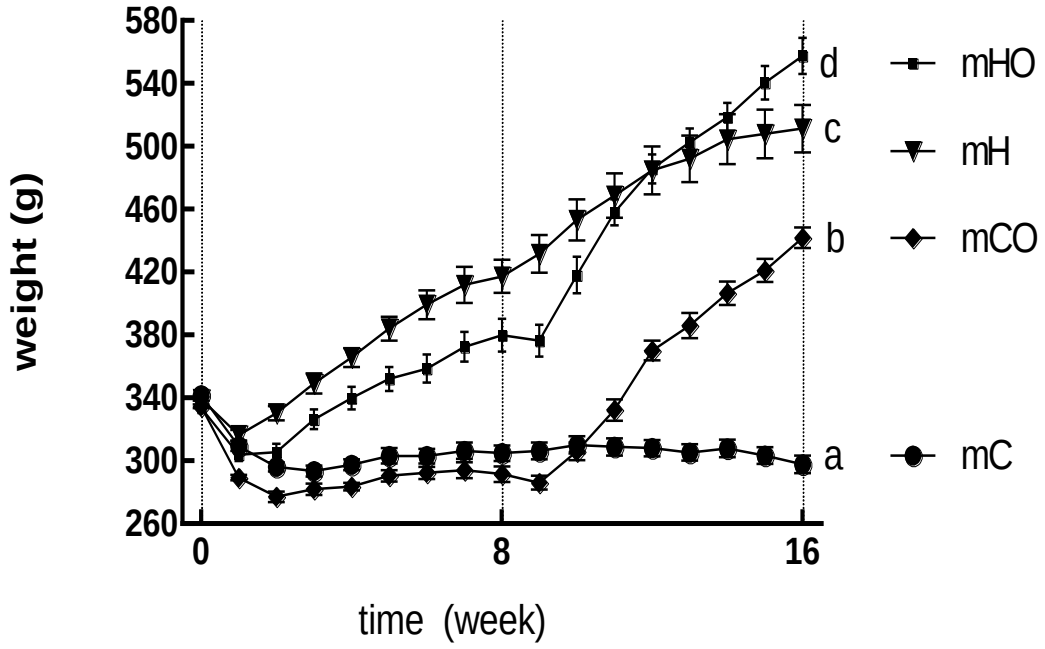


Figure 4.1 Weekly body weight of male Wistar rats fed a diet of cornstarch (mC) or high carbohydrate high fat (mH) supplemented with wholegrain oat groats (mCO/mHO) after 8 weeks

Values are mean ± S.E.M. n = 8 - 10 per group. Means without a common letter differ, $P < 0.05$

mC = modified cornstarch; mCO = mC + wholegrain oat groats;

mH = modified high carbohydrate, high fat; mHO = mH + wholegrain oat groats.

Table 4.1 Body composition of male Wistar rats following a dietary intervention of 20% wholegrain oat groats

variable	mC	mCO	mH	mHO	*P-value* diet	intervention	interaction
Initial body weight (g)	339±1[a]	335±1[a]	337±1[a]	334±1[a]	0.1448	-------	0.6210
8-week body weight (g)	305±5[a]	287±3[a]	417±11[b]	380±12[c]	<0.0001*	-------	0.1676
Final body weight (g)	304±3[a]	443±7[b]	511±15[c]	557±11[d]	<0.0001*	<0.0001*	0.0001*
Body weight gain (%) 8-16 wk	1.0±0.5[a]	15.6±1.8[b]	12.3±1.3[b]	14.8±4.0[b]	0.0112*	0.0001*	0.0040*
Abdominal circumference (cm)	16.7±0.2[a]	20.2±0.2[b]	21.8±0.4[c]	22.8±0.2[d]	<0.0001*	<0.0001*	0.0002*
Body Mass Index (g/cm^2)	0.54±0.01[a]	0.66±0.01[c]	0.73±0.03[b]	0.75±0.01[c]	<0.0001*	0.0015*	0.0190*
Retroperitoneal fat (mg/mm)	169±10[d]	224±22[c]	373±47[a]	504±31[b]	<0.0001*	0.0019*	0.1732
Omental fat (mg/mm)	99±7[d]	134±17[a]	199±14[b]	254±19[c]	<0.0001*	0.0027*	0.4883
Epididymal fat (mg/mm)	63±7[a]	139±21[b]	124±2[b]	271±30[c]	<0.0001*	<0.0001*	0.0734
Total fat pads (mg/mm)	330±21[a]	497±55[c]	696±77[b]	1028±75[d]	<0.0001*	<0.0001*	0.1435
Visceral Adiposity Index	4.6±0.2[a]	4.9±0.5[a]	6.5±0.6[b]	8.8±0.5[c]	<0.0001*	0.0108*	0.0459*
Fat mass (g)	42±6[a]	76±10[a]	160±17[b]	186±17[b]	<0.0001*	0.0343*	0.7689
Lean mass (g)	247±6[a]	349±6[b]	314±9[c]	341±12[b]	0.0027*	<0.0001*	0.0003*
Bone mineral content (g)	9.47±0.23[a]	11.55±0.2[b]	14.26±0.76[c]	15.35±0.64[c]	<0.0001*	0.0050*	0.3490

Values are mean ± S.E.M. n = 8 per group. Means without a common superscript letter differ, * indicate P < 0.05

mC = modified cornstarch; mCO = mC + wholegrain oat groats; mH = modified high carbohydrate, high fat; mHO = mH + wholegrain oat groats.

Table 4.2 Food, water and energy intake and feed conversion efficiency of male Wistar rats following a dietary intervention of 20% wholegrain oat groats

					P-Value		
variable	mC	mCO	mH	mHO	diet	intervention	interaction
Food intake (g/day)	35±1[a]	34±1[a]	32±1[a]	25±1[b]	<0.0001*	0.0005*	0.0067*
Water intake (g/day)	38±2[a]	37±2[ac]	22±1[b]	31±3[c]	<0.0001*	0.0535*	0.0173*
Energy intake (kJ/day) 8-16 wk	365±6[a]	448±9[b]	555±11[c]	511±16[d]	<0.0001*	0.1013	<0.0001*
Feed conversion efficiency (%)	-0.3±0.3[a]	3.9±1[b]	1.8±0.5[c]	4.1±0.9[b]	0.0856	<0.0001*	0.1528

Values are mean ± S.E.M. n = 8 per group. Means without a common superscript letter differ, * indicate $P < 0.05$

mC = modified cornstarch; mCO = mC + wholegrain oat groats;

mH = modified high carbohydrate, high fat; mHO = mH + wholegrain oat groats.

4.3.2 Cardiovascular parameters

Systolic blood pressure was not significantly different in mC or mH rats. The addition of wholegrain oat did not alter blood pressure in mHO. However, blood pressures in mCO were decreased compared to the remaining groups (Table 4.4).

Left ventricular mass in mC was decreased compared to mH. Both mCO and mHO were increased compared to mC and similar to mH (Table 4.4). Right ventricular mass was lowest in mC compared to mH. mCO was not significantly different to both mC and mH groups, while mHO was significantly different to mH (Table 4.4). Left ventricular diastolic stiffness constant was similar in mC and mH, and was increased in mHO and mCO compared to both controls (Table 4.4).

Echocardiographic results (Table 4.3) indicated a lower heart rate in mC compared to mH. Heart rate in mCO was similar to mC and mHO was not significantly different to mH. LVIDd was smaller in mC than mH, mCO and mHO were both larger than mC and the same as mH. However, LVIDs indicated no significant difference between mC and mH and was unchanged with the interventions. LVPWd was smaller in the mC compared to mH, with mCO the same as mH. mHO was larger than both controls and mCO. Systolic volume, relative wall thickness, ejection time, deceleration time and MCMO showed no significant differences between mC and mH or either of the interventions. The ascending and descending aorta diameters were smaller in the mC group compared to mH. Both mCO and mHO were the same as mH. Fractional shortening in mC was the same as mH and mCO. It was increased in mHO compared to the other groups. The ejection fraction was similar in mC and mH as well as mCO. mHO had an increased ejection fraction compared to mC but it was similar to mH and mCO.

Histological evaluation supported the preceding results. Inflammatory cell infiltration marked as “IC” (Figure 4.2) was high in both mC rats and mH rats, and was limited in mHO and mCO. Collagen deposition is clearly seen in the left ventricles of mC and mH hearts with limited deposition in mCO and mHO hearts (Figure 4.3).

The above results lead to the conclusion that the mC diet needs further refining to be a considered a suitable healthy control for examining cardiovascular function and structure. Limited protein in this diet could be a cause of the inconsistencies of this control diet. This concept will be expanded in the Discussion section of this chapter.

Table 4.3 Echocardiographic cardiovascular parameters of male Wistar rats following a dietary intervention of 20% wholegrain oat groats

					P-value		
variable	mC	mCO	mH	mHO	diet	intervention	interaction
Heart rate (bpm)	230±5[a]	231±5[a]	262±14[b]	271±15[b]	0.0027[*]	0.6573	0.7225
LVIDd (mm)	6.94±0.15[a]	8.05±0.23[b]	8.09±0.21[b]	7.89±0.09[b]	0.0124[*]	0.0206[*]	0.0013[*]
LVIDs (mm)	4.14±0.18[a]	4.54±0.19[a]	4.21±0.35[a]	3.89±0.24[a]	0.2946	0.8842	0.1951
LVPWd (mm)	1.68±0.02[a]	1.84±0.02[b]	1.84±0.03[b]	1.99±0.04[c]	<0.0001[*]	<0.0001[*]	0.8624
Systolic volume (ml)	0.08±0.01[a]	0.09±0.01[a]	0.10±0.01[a]	0.07±0.01[a]	0.9394	0.3624	0.0595
Relative wall thickness	0.49±0.01[a]	0.46±0.02[a]	0.47±0.02[a]	0.51±0.01[a]	0.3691	0.7635	0.0408[*]
Ascending aorta diameter (cm)	0.25±0.01[a]	0.31±0.01[b]	0.29±0.01[b]	0.31±0.02[b]	0.1197	0.0030[*]	0.1197
Descending aorta diameter (cm)	0.24±0.00[a]	0.29±0.01[b]	0.27±0.01[b]	0.28±0.01[b]	0.2418	0.0010[*]	0.0227[*]
Fractional shortening (%)	40.4±1.5[a]	43.8±1.5[a]	43.5±1.6[a]	50.8±2.6[b]	0.0086[*]	0.0056[*]	0.2900
Ejection fraction (%)	78.5±1.6[a]	83.8±2.1[ab]	81.6±1.5[ab]	87.5±2.1[b]	0.0749	0.0038[*]	0.8919
Ejection time (ms)	86.7±2.1[a]	79.7±3.4[a]	82.1±2.3[a]	90.9±3.5[a]	0.0285[*]	0.1662	0.0843
Deceleration time (ms)	54.6±1.3[a]	58.8±1.9[a]	50.4±1.9[a]	55.4±2.0[a]	0.0405[*]	0.0144[*]	0.8243
E_M (m/s)	0.63±0.02[a]	0.79±0.03[b]	0.70±0.02[c]	0.72±0.16[abc]	>0.9999	0.1928	0.3089
A_M (m/s)	0.34±0.02[a]	0.45±0.0[bc]	0.39±0.01[ac]	0.51±0.03[b]	0.0305[*]	<0.0001[*]	0.8389
MCMO (ms)	113±2[a]	114±2[a]	118±5[a]	113±5[a]	0.6176	0.6176	0.4550
Estimated LV mass (g)	0.71±0.00[a]	1.07±0.04[b]	1.05±0.03[b]	1.14±0.03[b]	<0.0001[*]	<0.0001[*]	0.0008[*]

Values are mean ± S.E.M. n = 8 per group. Means without a common superscript letter differ, * indicate $P < 0.05$ mC = modified cornstarch; mCO = mC + wholegrain oat groats; mH = modified high carbohydrate, high fat; mHO = mH + wholegrain oat groats; LV = left ventricle, RV = right ventricle, LVIDd = Left ventricular internal dimension end-diastole, LVIDs = Left ventricular internal dimension end-systole, LVPWd = left ventricular posterior wall thickness end-diastole, E_M = peak velocity early diastolic transmitral flow, A_M = peak velocity late diastolic transmitral flow, MCMO = mitral closing-mitral opening time

Table 4.4 Cardiovascular parameters of male Wistar rats following a dietary intervention of 20% wholegrain oat groats

variable	mC	mCO	mH	mHO	*P-value*		
					diet	intervention	interaction
Left ventricular + septum wet weight (mg/mm)	17.9±0.8[a]	23.4±0.8[b]	25.3±0.8[b]	24.9±1.6[b]	0.0001*	0.0145*	0.0067*
Right ventricular weight (mg/mm)	4.8±0.4[a]	5.7±0.3[abc]	6.8±0.4[b]	5.3±0.5[ac]	0.0600	0.5029	0.0040*
Left ventricular diastolic stiffness constant κ	25.5±0.3[a]	28.6±1.4[ab]	26.8±1.2[a]	31.5±1.9[b]	0.1036	0.0046*	0.5244
Systolic Blood Pressure (mmHg)	143±3[a]	130±1[b]	142±3[a]	142±4[a]	0.0848	0.0433*	0.0433*

Values are mean ± S.E.M. n = 8 per group. Means without a common letter differ, * indicate $P < 0.05$

mC = modified cornstarch; mCO = mC + wholegrain oat groats;

mH = modified high carbohydrate, high fat; mHO = mH + wholegrain oat groats.

LV = left ventricle, RV = right ventricle

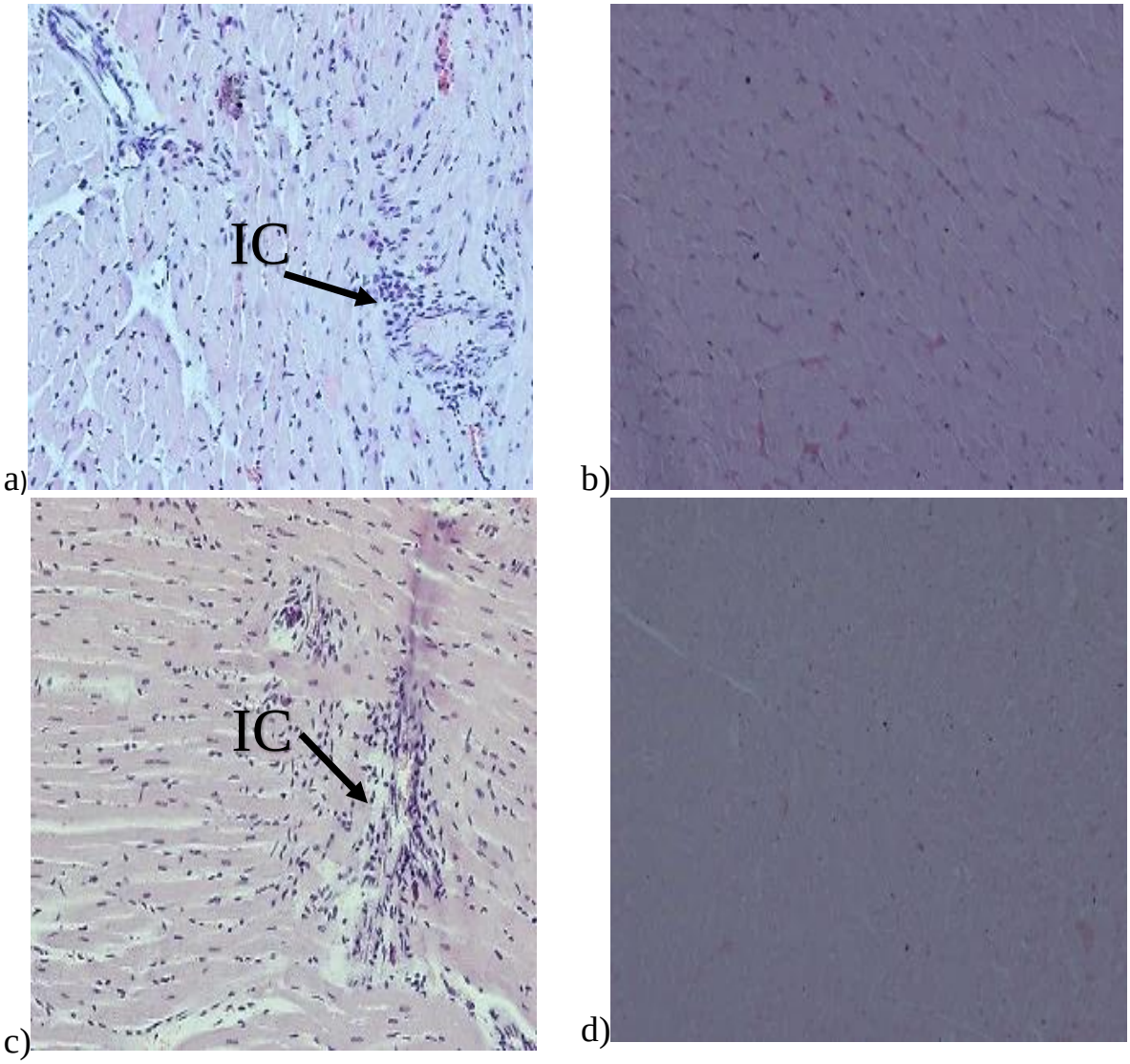


Figure 4.2 Inflammatory cells (IC) in the left ventricle of the heart of male Wistar rats induced by high carbohydrate, high fat diet and following a dietary intervention of 20% wholegrain oat groats

Haematoxylin & eosin staining, 20 x magnification

a) mC = modified cornstarch b) mCO = mC + wholegrain oat groats

c) mH = high carbohydrate high fat d) mHO = mH + wholegrain oat groats

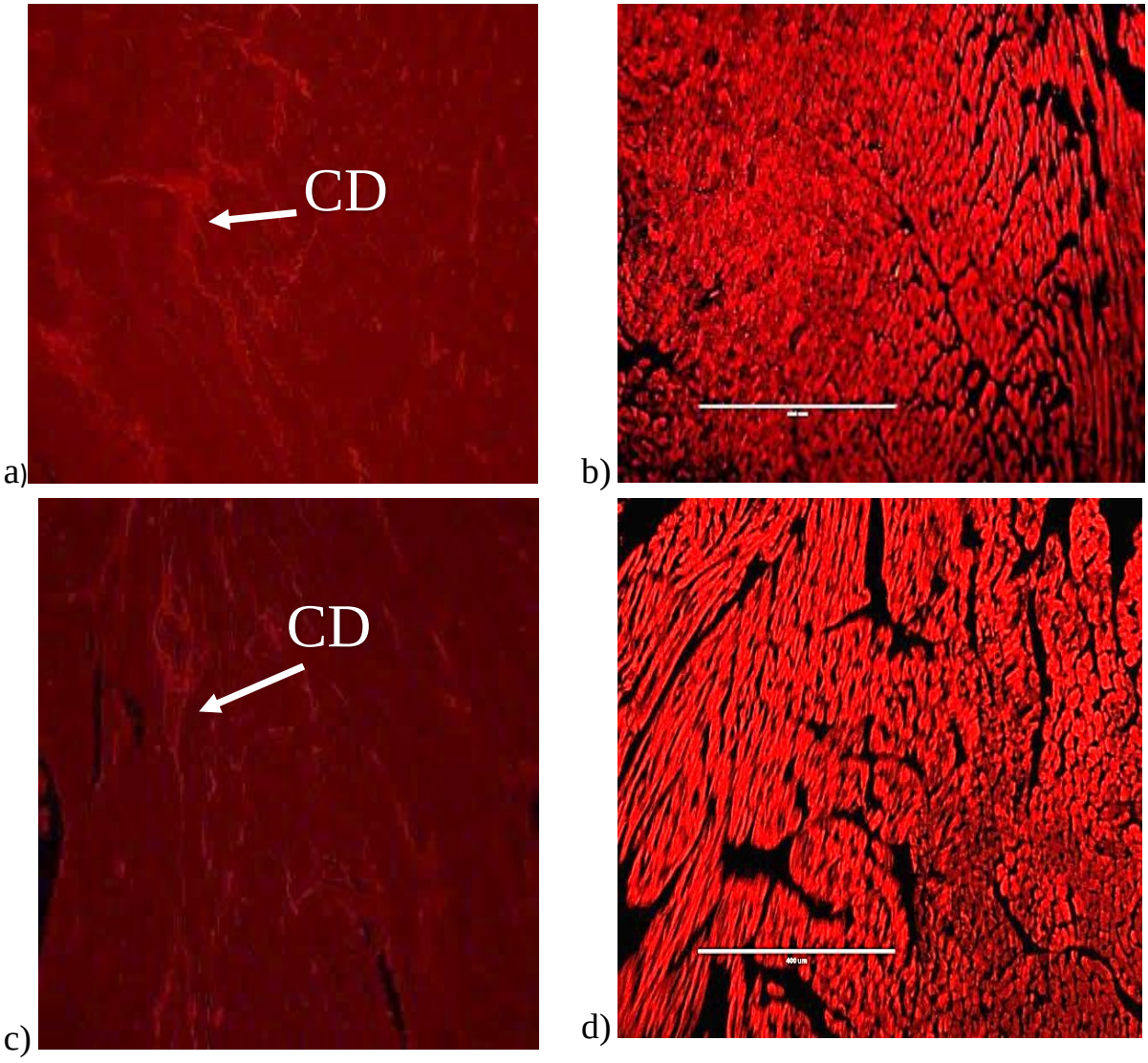


Figure 4.3 Collagen deposition (CD) in the left ventricle of the heart of male Wistar rats induced by high carbohydrate, high fat diet and following a dietary intervention of 20% wholegrain oat groats

Picrosirius red staining, 20 x magnification

a) mC = modified cornstarch b) mCO = mC + wholegrain oat groats

c) mH = modified high carbohydrate high fat d) mHO = mH + wholegrain oat groats

4.3.3 Liver parameters

Liver wet weight in mC was lower compared to mH. mHO was greater than mCO but there was no significant difference between the interventions and mH; however, mCO and mHO were both heavier than mC (Table 4.5). Plasma alanine transaminase (ALT) was not significantly different in mC and mH, mHO was unchanged compared to the controls but increased in the mCO group while plasma aspartate transaminase (AST) was similar in mC and mH and unchanged in mCO and mHO. (Table 4.5).

Histological evaluation indicated fat vacuoles in the liver in both mC rats and in mH ones, with limited macrovascular steatosis in mCO and mHO. Limited inflammation is seen in both control and intervention groups (Figure 4.4).

As with the cardiovascular results, the liver results lead to the conclusion that the mC diet needs further refining to be a considered a suitable healthy control for examining liver structure and function. Limited protein in this diet may be a reason for the inconsistencies of this control diet. This will be discussed later in this chapter.

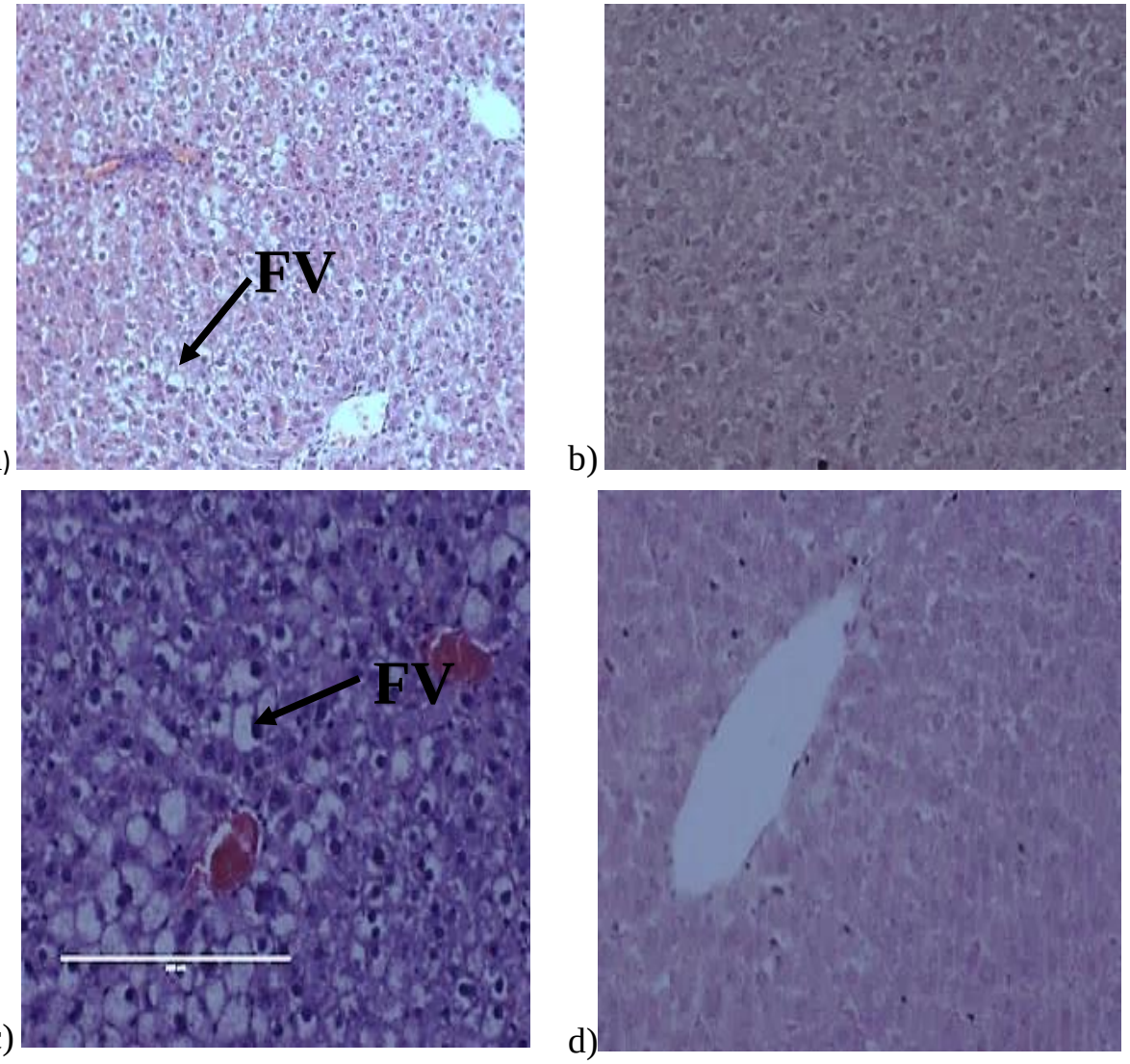


Figure 4.4 Fat deposition (FV) and inflammatory cells (IC) in male Wistar rat livers induced by high carbohydrate, high fat diet and following a dietary intervention of 20% wholegrain oat groats

Haematoxylin & eosin staining, 20 x magnification

a) mC = modified cornstarch b) mCO = mC + wholegrain oat groats

c) mH = modified high carbohydrate high fat d) mHO = mH + wholegrain oat groats

Table 4.5 Liver weight and plasma ALT, AST of male Wistar rats following the a dietary intervention 20% wholegrain oat groats

variable	mC	mCO	mH	mHO	*P-value*		
					diet	intervention	interaction
Liver wet weight (mg/mm)	194±7[a]	311±9[b]	334±12[bc]	352±13[c]	<0.0001*	<0.0001*	<0.0001*
Plasma ALT (U/L)	27±1[a]	35±3[b]	24±1[a]	27±2[a]	0.0130*	00102*	0.1754
Plasma AST (U/L)	92±6[a]	80±8[a]	68±8[a]	81±12[a]	0.1917	0.9775	0.1577

Values are mean ± S.E.M. n = 8 per group. Means without a common superscript letter differ, * indicate P < 0.05

mC = modified cornstarch mCO = mC + wholegrain oat groats

mH = modified high carbohydrate, high fat mHO = mH + wholegrain oat groats.

4.3.4 Plasma biochemistry and inflammatory markers

Plasma total cholesterol concentrations were statistically similar in mC and mH with both mCO and mHO having elevated concentrations. Triglyceride concentrations were lowest in mC, while mH and mHO had similar concentrations. mCO concentrations were not significantly different from both mC and mH. Non-esterified fatty acid (NEFA) plasma concentrations were lower in mC compared to mH, with mCO and mHO indicating no significant differences to their relevant controls (Table 4.6).

Fasting blood glucose concentrations in the mC group were lower than in the mH rats Similarly, the blood glucose response to oral glucose loading showed the glucose area under the curve (AUC) decreased in the mC group but elevated in the mH rats. Fasting blood glucose concentrations for mCO and mHO were similar to mC. The glucose AUC was decreased in mHO compared to mH and not significantly different in mCO compared to mC (Table 4.6).

Plasma C-reactive protein (CRP) concentrations were similar in mC and mH. Both mCO and mHO had increased CRP concentration compared to the controls (Table 4.6). This may be due to the increased total fat pads (Table 4.1) present in both mCO and mHO as CRP is produced by adipocytes.

Table 4.6 Plasma biochemistry and CRP of male Wistar rats following a dietary intervention of 20% wholegrain oat groats

variable	mC	mCO	mH	mHO	*P-value* diet	intervention	interaction
Fasting blood glucose (mmol/L)	4.0±0.2[a]	4.3±0.1[a]	5.0±0.1[b]	4.4±0.1[a]	0.0003*	0.2665	0.0020*
Blood glucose AUC (mmol/L/min)	738±19[a]	743±21[a]	925±44[b]	767±24[a]	0.0038*	0.0307*	0.0219*
Plasma total cholesterol (mmol/L)	1.6±0.1[a]	2.0±0[b]	1.7±0.1[a]	2.0±0[b]	0.4853	<0.0001*	0.4853
Plasma triglycerides (mmol/L)	0.7±0.1[a]	1.2±0.1[ab]	1.8±0.2[b]	2.1±0.5[b]	0.0012*	0.1618	0.7221
Plasma NEFA (mmol/L)	2.2±0.2[a]	2.6±0.6[a]	4.9±0.3[b]	4.8±0.7[b]	<0.0001*	0.7641	0.6175
Plasma C-reactive protein (ng/ml)	319±14[a]	469±44[b]	340±54[a]	475±29[b]	0.7103	0.0005*	0.8364

Values are mean ± S.E.M. n = 8 per group. Means without a common superscript letter differ, * indicate $P < 0.05$

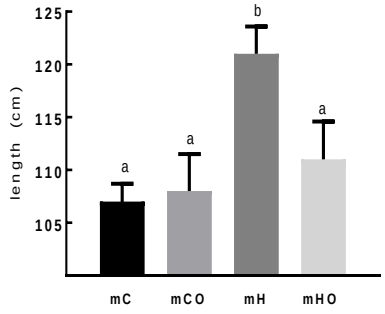
mC = modified cornstarch; mCO = mC + wholegrain oat groats;

mH = modified high carbohydrate, high fat; mHO = mH + wholegrain oat groats

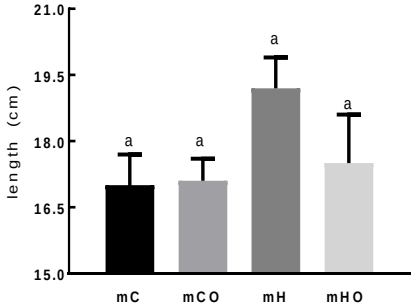
4.3.5 Gastrointestinal parameters

4.3.5.1 Intestinal length

Small intestine length from pyloric sphincter to ileo-caecal junction (duodenum, jejunum and ileum) was longer in mH than mC. mCO and mHO were similar length to mC. There was no significant difference in colon length (proximal, mid, distal colon) between mC, mH, mCO or mHO (Figure 4.5).



a)



b)

	P-value		
	diet	intervention	interaction
small intestine	0.0043*	0.1152	0.0562
colon	0.1049	0.3129	0.2571

Figure 4.5 Gastrointestinal length of male Wistar rats following the a dietary intervention of 20% wholegrain oat groats a) small intestine pyloric sphincter to caeco-ileal junction and b) colon – caeco-colonic junction to rectum

Values are mean ± S.E.M. n = 8 per group. Means without a common superscript letter differ, * indicate $P < 0.05$

mC = modified cornstarch, mCO = mC + wholegrain oat groats, mH = modified high carbohydrate, high fat, mHO = mH + wholegrain oat groats.

4.3.5.2 Gastrointestinal morphology

Morphological analysis indicated that crypt depth was not significantly different in mC and mH mid-colon. The addition of wholegrain oat groats led to deeper crypts in the mCO rats, but the mHO crypts were similar length to both mC and mH controls (Figure 4.6a). Morphological analysis indicated mC had increased mucosal thickness than mH. mCO and mHO had mucosal thickness similar to mC (Figure 4.6b). Goblet cell numbers were low in both mC and mH mid-colons. The addition of wholegrain oat increased goblet cell numbers in mCO, with mHO similar to mC, mH and mCO (Figure 4.6c).

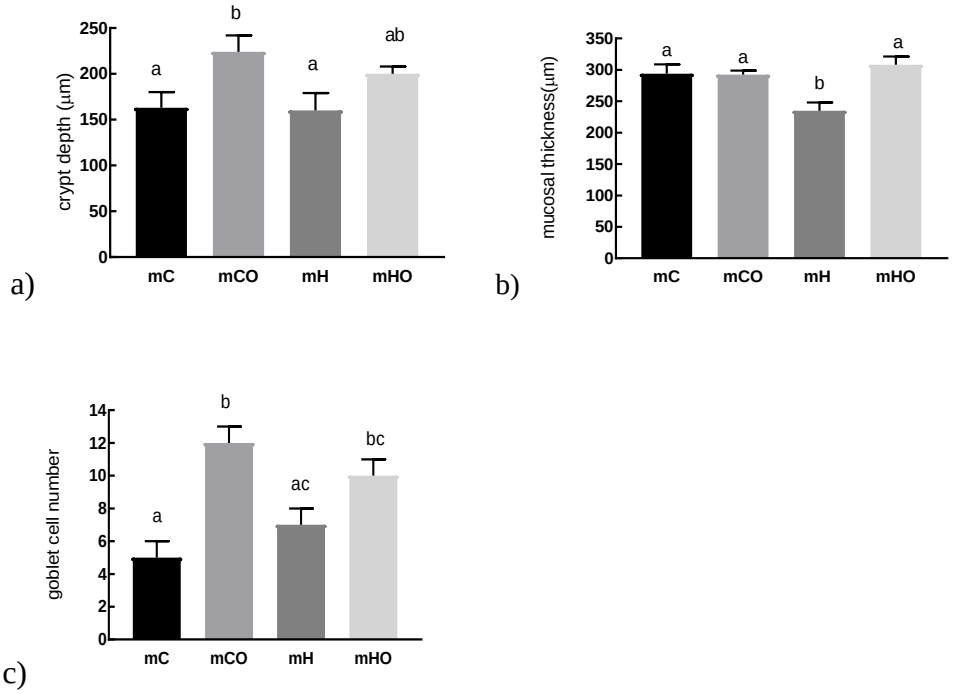


Figure 4.6 Effect of a dietary intervention of 20% wholegrain oat groats on a) crypt depth b) mucosal thickness c) goblet cell number in the mid colon of male Wistar rats

Values are mean ± S.E.M. n = 8 per group. Means without a common superscript letter differ, $P < 0.05$

mC = modified cornstarch; mCO = mC + wholegrain oat groats;

mH = modified high carbohydrate, high fat; mHO = mH + wholegrain oat groats

4.3.5.3 Gastrointestinal histology

Visual examination of the mid-colon indicated an increased number of submucosal folds in mC compared to mH that had limited folds. mCO and mHO had more submucosal folds than mH and a similar visual aspect as the mC (Figure 4.7). Increased submucosal folds indicates a greater surface area of the mucosa in the mid-colon. Crypts are upright and have a more complete structure in mC, mCO and mHO compared to mH (Figure 4.7). The epithelial layer is more complete in mC, mCO and mHO compared to mH (Figure 4.7).

Gastrointestinal morphology was changed by the addition of wholegrain oat to the high-carbohydrate, high-fat diet. There were differences between mC and mH diets. This section (Figure 4.7) is a visual representation of the data in the previous section (4.5.2 Gastrointestinal morphology). Duodenum, jejunum and ileum show villi (V), crypts (C), mucosa (MT) and muscularis (M). Proximal, mid and distal colon show crypts (C), mucosa (MT), muscularis (M) and submucosal folds (S).

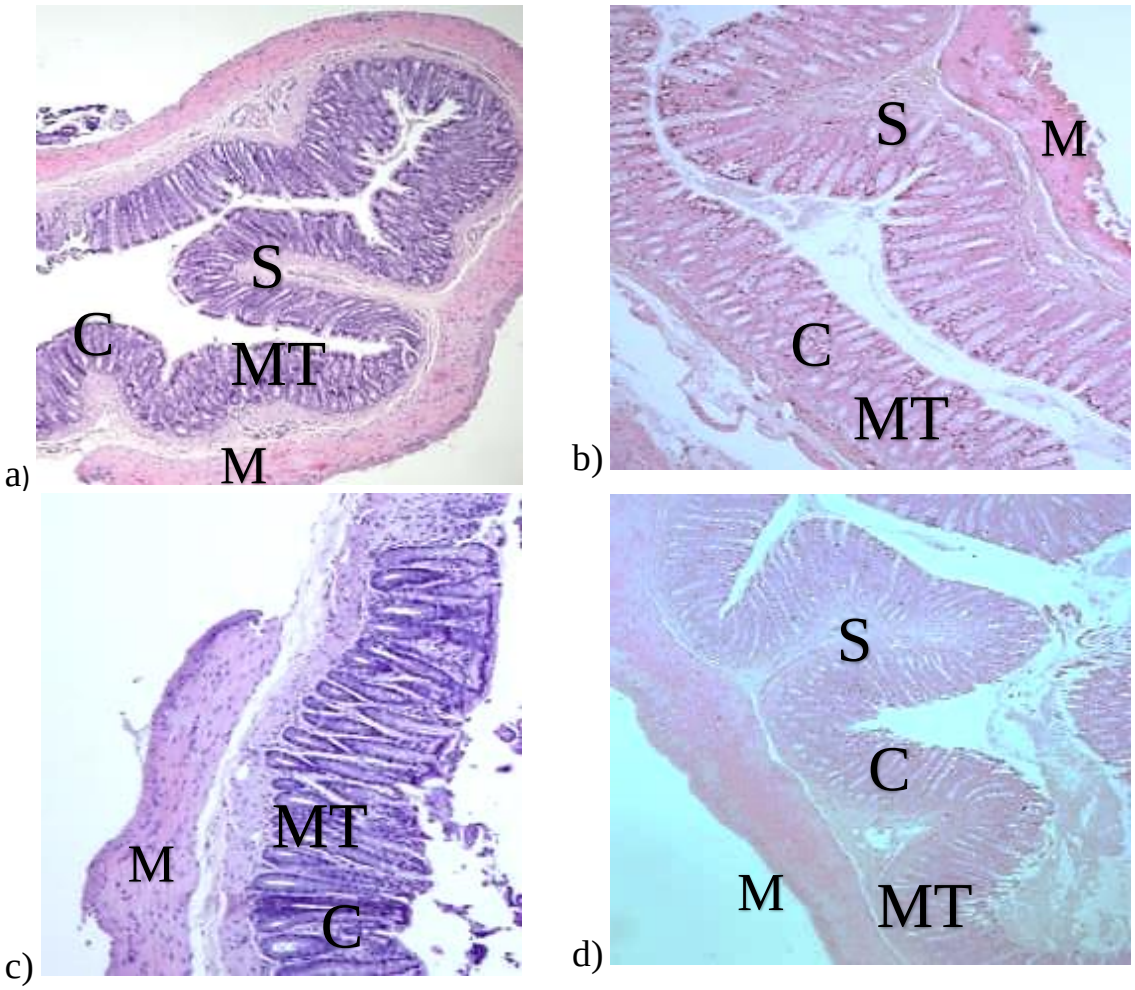


Figure 4.7 Morphology of the mid colon of male Wistar rats following a dietary intervention of 20% wholegrain oat groats

C = crypt, MT = mucosa, S = submucosal folds, M = muscularis

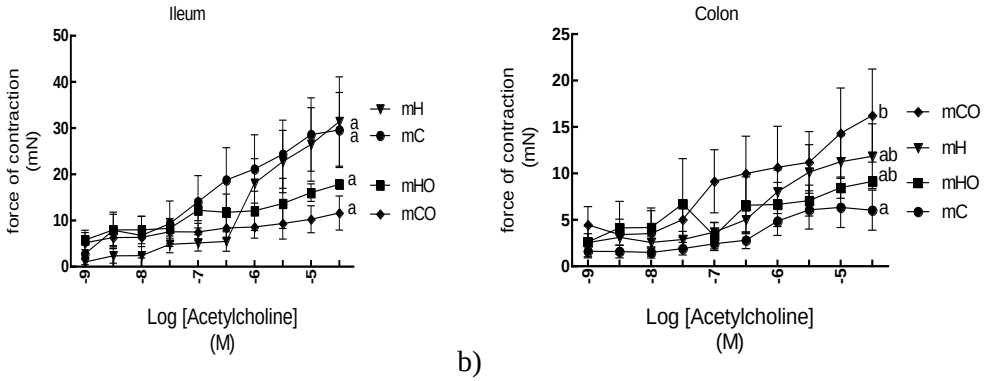
Haematoxylin & eosin. 10 x magnification

a) mC = modified cornstarch b) mCO = mC + wholegrain oat groats

c) mH = modified high carbohydrate, high fat d) mHO = mH + wholegrain oat groats

4.3.5.4 Gastrointestinal contractility

Ileal reactivity was not significantly different in mC and mH. mCO and mHO gave similar responses as the controls (Figure 4.8a). There were no significant differences between mC and mH or mHO for acetylcholine-induced colonic contractility. mCO had an increased colonic contractility at maximum contraction compared mC (Figure 4.8b).



a) b)

Figure 4.8 Effect of acetylcholine on the contractility of ileum and mid colon of male Wistar rats following a dietary intervention of 20% wholegrain oat groats

Values are mean ± S.E.M. n = 8 per group. Means without a common superscript letter differ, P < 0.05

mC = modified cornstarch; mCO = mC + wholegrain oat groats;

mH = modified high carbohydrate, high fat; mHO = mH + wholegrain oat groats

4.3.6 Faecal short chain fatty acid (SCFA) analysis

4.3.6.1 Combined

Total SCFA, acetate, propionate and butyrate concentrations when averaged across all rats (n = 6) in each group were higher (Table 4.7) than when each group of rats were separated into rats that could feed until termination (n = 3) (Table 4.8) compared to rats that were fasted for 2-3 hours prior to termination (n = 3) (Table 4.9). The percentage of acetate relative to mC increased in mH and decreased in mCO and mHO samples. Propionate increased in all groups compared to mC. Butyrate decreased compared to mC in mH, mHO and mCO (Table 4.7).

When fed and fasted samples were combined, there was an overall effect on total SCFA, acetate, propionate or butyrate caused by the diet (Table 4.7). When separated out to fed and fasted samples (Tables 4.8 & 4.9), both wholegrain oat and diet influenced total SCFA. Acetate concentrations were decreased by the addition of wholegrain oat in the fed samples (Table 4.8), while the diet had an effect on acetate and propionate in the fasted samples (Table 4.7). Therefore, for further analysis, fed and fasted groups were analysed separately.

Table 4.7 Average total SCFA, acetate, propionate and butyrate and acetate to propionate ratio in faecal samples of male Wistar rats following a dietary intervention of wholegrain oat groats

					P-value		
SCFA (µmol/g)	mC	mCO	mH	mHO	diet	intervention	interaction
Total	118±13^{ab}	107±9^{b}	155±12^{a}	126±6^{ab}	0.0138*	0.0680	0.3957
Acetate	96±11^{a}	48±4^{a}	127±13^{b}	91±6^{a}	0.0384*	0.0121*	0.2696
Propionate	9±1^{a}	13±1^{b}	16±2^{b}	16±1^{b}	0.0012*	0.1462	0.1462
Butyrate	13±1^{a}	13±1^{a}	12±1^{a}	19±1^{b}	0.0006*	0.0091*	0.0006*
Acetate:Propionate	10.6±0.1^{a}	3.7±0.1^{d}	7.9±0.2^{b}	5.7±0.1^{c}	0.0155*	<0.0001*	<0.0001*

Values are mean ± S.E.M. n = 6 per group. Means without a common superscript letter differ, P < 0.05

Table 4.8 Average total SCFA, acetate, propionate and butyrate and acetate to propionate ratio in faecal samples of male Wistar rats following a dietary intervention of 20% wholegrain oat groats. Rats fed until termination.

					P-value		
SCFA (µmol/g)	mC	mCO	mH	mHO	diet	intervention	interaction
Total	80±3^{a}	62±1^{b}	105±3^{c}	51±1^{b}	0.0021*	<0.0001*	0.0002*
Acetate	67±15^{ab}	48±1^{a}	93±8^{b}	39±7^{b}	0.3828	0.0041*	0.0938
Propionate	6±0.3^{a}	7±1^{a}	6±1^{a}	8±1^{a}	0.5850	0.1263	0.5850
Butyrate	7±0.4^{a}	7±0.4^{a}	6±1^{a}	10±2^{a}	0.4111	0.1211	0.1211
Acetate: Propionate	11.1±1.9^{b}	6.9±0.8^{c}	15.7±1.3^{a}	4.8±0.3^{c}	0.3384	0.0003*	0.0259*

Values are mean ± S.E.M. n = 3 per group. Means without a common superscript letter differ, P < 0.05

Table 4.9 Average total SCFA, acetate, propionate and butyrate and acetate to propionate ratio in faecal samples of male Wistar rats following a dietary intervention of 20% wholegrain oat groats. Rats fasted for 2-3 hours prior to termination.

					P-value		
SCFA (µmol/g)	mC	mCO	mH	mHO	diet	intervention	interaction
Total	38±3[a]	45±3[a]	50±3[a]	69±3[b]	0.0003*	0.0025*	0.0805
Acetate	29±7[a]	33±4[a]	34±7[a]	52±9[a]	0.1240*	0.1538	0.3454
Propionate	3±1[a]	6±0.3[a]	10±3[a]	8±0.4[a]	0.0228*	0.7628	0.1570
Butyrate	6±0.1[a]	6±1[a]	6±2[a]	9±2[a]	0.3468	0.3468	0.3468
Acetate: Propionate	10±1[a]	5.5±0.3[bc]	3.4±0.3[c]	6.5±0.8[b]	0.0032*	0.3297	0.0005*

Values are mean ± S.E.M. n = 3 per group. Means without a common superscript letter differ, $P < 0.05$

mC = modified cornstarch; mCO = mC + wholegrain oat groats;

mH = modified high carbohydrate, high fat; mHO = mH + wholegrain oat groats.

4.3.6.2 Fed vs fasted

4.3.6.2.1 Combined SCFA

Total combined (acetate, propionate and butyrate) SCFA concentrations were lower in mC compared to mH. The addition of wholegrain oats lowered the total SCFA concentration in mHO and mCO in the fed samples compared to both controls (Table 4.7). Concentrations decreased in the mCO compared to mC and mHO concentrations which were decreased compared to mH (Table 4.7). In the fasted faecal samples, there was no difference between mC and mH. mCO was similar to the controls and mHO had an increased concentration of SCFA compared to the controls (Table 4.7). In both fed and fasted samples, acetate was the largest proportion of SCFA with limited propionate and butyrate; however, there was an increased proportion of propionate and butyrate present in mHO samples (Table 4.7).

4.3.6.2.2 Acetate, propionate and butyrate concentrations

Concentrations of acetate in faecal samples of fed rats was lower in mC compared to mH (Table 4.8). Acetate did not change between mC and mCO, and decreased from mH to mHO (Table 4.8). In the fasted samples, mC, mH and mCO had lower concentrations than in the fed groups while mHO had similar concentrations to fed mHO, but there was no difference between any of the fasted groups (Table 4.9). Propionate concentrations were similar for mC and mH. mCO and mHO were similar to the controls in the fed samples (Table 4.8). There was no difference between any of the groups in the fasted samples (Table 4.9).

Butyrate concentrations were similar in all groups in the fed samples (Table 4.8). In the fasted samples, butyrate concentrations in all groups were similar (Table 4.9).

4.4 Discussion

Metabolic syndrome has a complex aetiology, with the major factor that leads to the disease being a diet high in fats and simple sugars (Kaur, 2014). Since metabolic syndrome involves cardiovascular disease, diabetes, glucose intolerance and obesity, many studies need to be undertaken to define the causes and discover ways to attenuate the complexity of symptoms in this syndrome.

The present study has supplemented a high-carbohydrate, high-fat diet as a rat model of metabolic syndrome that mimics the human condition with 20% wholegrain oat groats.

Limited research exists characterising the gut morphology of high-carbohydrate, high-fat diets when supplemented with wholegrains. This study was warranted as it examined the physiological effects, gut morphology and SCFA production, after intervention with a similar amount of wholegrain oat as the average human cereal consumption using a diet-induced rat model of metabolic syndrome. In this study, mC and mH were supplemented with 20% wholegrain oat. This translates to approximately 100 – 120 g/day in the average 70 kg adult human using a relevant scaling equation (Bachmann et al., 1996).

The American Association of Cereal Chemists International (AACCI) classifies a wholegrain as 'consisting of intact, ground, cracked or flaked caryopsis, whose principal anatomical components – the starchy endosperm, germ and bran, are present in the same relative proportions as they exist in the intact caryopsis' (Rebello et al., 2014). This study used intact oat groats for supplementation.

Wholegrains are an important source of calories and offer nutrients and other food components such as phytochemicals (Liu, 2007). Wholegrains have been associated with the prevention of type 2 diabetes (Venn and Mann, 2004, Murtaugh et al., 2003), cancer (Jacobs et al., 1998) and other chronic diseases (Dixit et al., 2011, Johnston et al., 2010, Opie et al., 2016). The consumption of wholegrain oats has been advocated in dietary guidelines and nutritional policies in many countries including the USA, Australia, Canada, Mexico and across South America, Europe and Asia (Clemens and van Klinken, 2014a). The 2015 American Dietary Guidelines suggest consuming at least half of all grains as wholegrains with 3 serves of 16 g/day to reduce the risk of

developing chronic diseases including CVD, diabetes, and possibly some cancers (U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2015).

The modified high-carbohydrate high-fat diet in this study induced many parameters associated with metabolic syndrome, including central obesity, hypertension, dyslipidaemia and impaired glucose tolerance. Cardiovascular changes included fibrosis, cardiac hypertrophy and increased diastolic stiffness and liver changes included steatosis, inflammatory cell infiltration and portal fibrosis as well as changes in plasma ALT and AST activities. These are consistent with the earlier findings using the established H diet (Panchal et al., 2011b). The modified cornstarch diet also induced several unexpected results such as hypertension, cardiac fibrosis, increased diastolic stiffness and steatosis and increased plasma AST activity.

4.4.1 Body composition and dietary intake

Few studies focus specifically on the impact of wholegrain oats on body composition as many only record this data as part of studies into hypertension and hyperglycaemia. Many of the studies investigating wholegrains such as The Framingham Heart Study classify a serving as any 1 of breakfast cereal, oatmeal, brown rice or other grains, slice of dark bread, popcorn or added bran or added germ (McKeown et al., 2010). This makes it difficult to compare studies of individual wholegrains and gives inconsistent results.

Cross-sectional and prospective epidemiological studies have shown a reduced risk of obesity and weight gain with wholegrain consumption (Harland and Garton, 2008, Jonnalagadda et al., 2011, Liu et al., 2003, Koh-Banerjee et al., 2004, Sahyoun et al., 2006). Observational studies also lend credibility to this relationship between wholegrains and weight loss (Liu et al., 2003, Koh-Banerjee et al., 2004). A systematic review and analysis of 15 human trials indicated that, with higher wholegrain consumption, adiposity measured by waist circumference was reduced by 2.7 cm and BMI was 0.630 kg/m^2 lower (Harland and Garton, 2008). The cross-sectional and epidemiological studies, The Framingham Offspring Study and the Baltimore

Longitudinal Study of Aging (BLSA), indicated an inverse association between wholegrain consumption and BMI, waist to hip ratio and waist circumference (McKeown et al., 2004, Newby et al., 2007). The BLSA has been ongoing since 1958 with participants returning every 12-24 months for repeated measures. Low wholegrain consumption compared to high wholegrain consumption indicated lower BMI (25.5 vs 24.8 kg/m^2), lower body weight (75 vs 72.4 kg) and smaller waist circumference (87.4 vs 85 cm) (Newby et al., 2007). As with other studies, multiple wholegrains were consumed therefore it is difficult to indicate if any one wholegrain provides greater benefits.

Prospective cohort studies of healthy males and females who consumed wholegrains such as wheat, oats, corn, rice, barley, rye bulgur, buckwheat, popcorn, amaranth and psyllium over 8 years showed an inverse association between increased wholegrain consumption and long-term weight gain (Koh-Banerjee et al., 2004, Liu et al., 2003). These studies also indicated a dose-dependent response with an increase of 40 g/day wholegrain decreasing weight 0.49 kg in men and 1.52 kg in women and an increase of 20 g/day decreasing weight 0.36 kg, with no associated response in refined grain consumption (Dong et al., 2016, Koh-Banerjee et al., 2004, Liu et al., 2003).

The Adelaide Male Aging Study while not investigating wholegrains noted that increased cereal fibre had an inverse association with BMI (27.3 vs 25.4 kg/m^2), percentage body fat (34 vs 31.5%) and trunk fat mass (42.8 vs 37.8%). There was no association between vegetable or fruit fibre and these measures (Atlantis et al., 2008).

Intervention studies have shown inconsistent results in humans, possibly due to the use of hypocaloric diet programs rather than high carbohydrate, high fat diets (Juvonen et al., 2009, Katcher et al., 2008). Also the type of wholegrain administered, whether wholegrains were added or substituted into the diet and various participant characteristics (age, gender, nationality, health status) (McKeown and Jacobs, 2010) could be confounding factors in these studies. When these intervention and observational studies are combined, there is an overall suggestion that wholegrains may alter body fat distribution independent of changes in overall body weight. Our study is consistent with these studies as total abdominal fat pads increased in the mHO intervention group. However, there was no difference in body weight or lean mass, so this may be due to redistribution of fat from subcutaneous to abdominal stores.

Energy intake increased with the addition of wholegrain oats to mC which is similar to these intervention studies (Juvonen et al., 2009, Katcher et al., 2008). However, with the addition of wholegrain oats to the mH hypercaloric diet, the energy intake decreased which was inconsistent with previous studies. As many of these existing studies have been human clinical trials, it is not possible to directly compare food and water intake with our rat study. With the addition of wholegrain oat to the diet, food intake decreased, and water intake increased. This may have been due to the changed consistency of the diet. The mH diet was a thick liquid prior to the addition of the oat possibly meaning that the animals needed less water but with the addition of oat they needed to consume more due to the diet becoming thicker and stickier as water was removed and replaced by the oat grains.

4.4.2 Cardiovascular effects

The current study highlighted issues with the control healthy diet in relation to cardiovascular results. I used a modification to previous studies using the high carbohydrate, high fat rat model (Panchal et.al., 2011b), so that up to 20% of wholegrains or other interventions could be administered in the diet. To enable this, the powdered food was removed and replaced with a vitamin mix and increased water content. The removal of the powdered food decreased protein content by 20%, which appears to have led to decreased muscle and cardiac mass. As such, this may not be a good healthy control. The results for the modified unhealthy H control are similar to previous studies using the H diet (Panchal et.al., 2011a, Bhaswant et al., 2017) and also to the 5% experiments in Chapter 3.

Therefore, due to issues with the mC diet, results may be inconsistent with earlier studies relating to high-carbohydrate, high-fat diets and metabolic syndrome; however, they may be relevant to cardiovascular disease caused by low protein diets. In pigs, a low protein diet upregulated lipogenic gene expression while down-regulating lipolytic gene expression (Wang et al., 2012). Pigs that were fed a low protein diet had lower lean muscle mass and higher intramuscular fat than those on a high protein diet (Wang et al., 2012). The current study demonstrated similar lower lean muscle mass in the modified controls compared to the controls of Chapter 3 but intramuscular fat was not determined. The increase in fat vacuoles in the mC liver is a possible indication that intramuscular fat may be increased in this study.

Alternatively, isocaloric high animal or plant protein diets reduced liver fat and inflammation in Type 2 diabetes (Markova et al., 2017). The current study indicated that, with the addition of wholegrain oats, and subsequent increase in protein content, liver fat and inflammation decreased. This will need further investigation.

Previous studies specific to oats or other cereal fibres have provided mixed results in the benefits to cardiovascular health (Maki et al., 2007, Behall et al., 2006, Pins et al., 2002, Keenan et al., 2002). Studies have found systolic blood pressure increased (Maki et al., 2007) or decreased (Behall et al., 2006, Pins et al., 2002, Keenan et al., 2002). It is likely that both fibre and micronutrient intakes explain results in reducing antihypertensive medication and leading to positive blood pressure changes (Pins et al., 2002). As with this rat study, a human study was designed to identify the hypotensive effects of whole food intervention rather than specific bioactive compounds (Pins et al., 2002). It suggested that wholegrain oats have a synergistic anti-inflammatory effect with the soluble fibre (β-glucans) fraction, minerals, antioxidants, polyphenols and other phytonutrients responsible for the lowering of blood pressure (Pins et al., 2002). These changes may be mediated by improvements in other risk factors, such as body weight, dyslipidaemia and insulin resistance (Harris and Kris-Etherton, 2010). The current study results are consistent with previous studies indicating lower blood pressure, less cardiac inflammation and collagen deposition with the addition of wholegrain oats to both the mH and mC diets.

Epidemiological studies in North America and Europe have indicated that wholegrains, such as rye, oats, brown rice and barley reduced the risk of cardiovascular disease (Seal and Brownlee, 2015), had an inverse association with the risk of death from ischaemic heart disease (Jacobs et al., 1998) and lowered the risk of coronary artery disease (Lutsey et al., 2007, Steffen et al., 2003). Intervention studies, in humans and animals, raise important issues relating to compliance and the interaction of complex food-based systems where the benefits and interactions of all the components are not necessarily understood.

Several mechanisms may be responsible for the improved cardiovascular function. These include β-glucans forming a gel-like substance in the intestine increasing faecal excretion of cholesterol and bile acids and slowing glucose absorption (Keenan et al., 2002, Alminger and Eklund-Jonsson, 2008). Anti-oxidant and anti-inflammatory

properties of polyphenols and phytonutrients may also play a part; however, these were not examined as part of this study.

The fermentation of polysaccharides in the rat cecum resulting in SCFA production may also modulate blood glucose and insulin responses improving vascular function, blood pressure and weight control which in turn improve cardiovascular health (Pereira et al., 2002). Increasing propionate concentrations by fermentation of wholegrain oats inhibited cholesterol synthesis leading to the improvements in cardiovascular health (Richards et al., 2016, Queenan et al., 2007 However, it is still unclear if the lowering of LDL-cholesterol is the sole mechanism of action of wholegrain oat with regards to reducing the risk factors leading to coronary heart disease (Kelly et al., 2007). As this study only investigated total cholesterol changes, we are unable to confirm that LDL-cholesterol is lower in the intervention groups. However, the efficacy of oat and barley β-glucans in lowering blood lipids is well substantiated (Jonnalagadda et al., 2011).

The higher fibre content of wholegrain oats may be fermented by colonic bacteria producing acetate and propionate which enter the portal circulation and influence hepatic glucose oxidation, decrease fatty acid release and increase insulin clearance and improve insulin sensitivity (Pereira et al., 2002). This may be due to improved insulin receptor sensitivity and a lower overall dietary glycaemic index and related insulin secretion.

While current scientific evidence suggests that wholegrain oats has a beneficial effect on cardiovascular risk reduction, further research is needed to better understand the mechanisms of action and the impact that various components of the oats have on cardiovascular risk factors.

4.4.3 Liver effects

As with the cardiovascular results with the healthy mC in this experiment, there were inconsistencies with the liver results. Both mC and mH controls showed indications of steatosis, inflammation and fat vacuole formation in the liver. This indicates that this model needs further refinements before it is used in future studies.

The addition of wholegrain oats to the diet showed no change in liver wet weight, or ALT/AST activities. However, wholegrain oat decreased fat vacuole formation and inflammation in the liver. Previous studies have attributed the beneficial effects on blood lipid concentrations and hypocholesterolaemic effects to β-glucans (Forsberg and Reeves, 1995, Morrison, 1978), yet others have shown only slight improvements (Ebringerova et al., 2005). Up to 90% of bile acids that enter the small intestine for fat absorption are reabsorbed by the ileum (Dawson, 2011). As a soluble fibre, β-glucans reduced bile acid reabsorption thereby increasing bile acid excretion, lowering bile acid concentrations in the liver and increasing the conversion rate of cholesterol to bile acids (Papathanasopoulos and Camilleri, 2010). The liver then obtains the additional cholesterol by upregulating LDL receptors and increasing LDL particle uptake reducing circulating LDL-cholesterol (Papathanasopoulos and Camilleri, 2010). Further studies have shown that when wholegrain oats are consumed, there is a greater overall effect than when specific components, such as β-glucans, are taken individually (Klose and Arendt, 2012, Einhorn et al., 2003). Increases in the proportion of propionate produced by fermentation of wholegrain oats, and used in the hepatic lipid metabolism, may also lead to the improvements seen in the structure of the liver.

4.4.4 Glycaemic effects

Wholegrain oats in the diet improved glycaemic control with the postprandial glucose AUC returning to normal while fasting glucose concentrations were lower than the mH. This indicates that the wholegrain oats were playing a role in normalising glucose concentrations in the blood. Previous studies have found that the addition of wholegrains to the diet increased viscosity in the intestines caused by β-glucans, thus slowing the absorption of carbohydrates and lowering postprandial glucose concentrations (Li et al., 2016). However, results have been inconsistent with fasting plasma glucose with some human and animal trials finding a decrease in fasting glucose (Shen et al., 2011, Shen et al., 2016, Pick et al., 1996) but other randomised controlled trials found no differences (Bao et al., 2014).

Lower blood glucose concentrations and improved insulin response of wholegrains has been demonstrated previously (Liu, 2007). Human studies comparing wholegrain and refined cereals in subjects with metabolic syndrome have found that wholegrains

lower plasma insulin and triglyceride concentrations which may reduce pancreatic β-cell stress preserving the β-cell function and delaying the onset of diabetes (Giacco et al., 2014, Tosh, 2013). A 12 - week intervention study found that the mechanism of these changes was not mediated by changes to free fatty acid (FFA) or GLP-1 plasma concentrations (Giacco et al., 2014). The reduction of postprandial triglycerides acts on the synthesis and/or catabolism of triglyceride lipoproteins. This reduction leads to an increase in lipoprotein lipase activity possibly due to the modification of lipid composition in lipoprotein particles rather than a reduction in number due to fibre present in wholegrain which interferes with the fat absorption in small intestine leading to synthesis of lipoproteins that are less rich in triglycerides (Giacco et al., 2014).

The viscosifying properties of β-glucans in the wholegrain oat groat may be one mechanism of action by delaying glucose absorption in the upper gastrointestinal tract (Wood et al., 1994). Viscosity has been attributed to 79 - 96% of changes in plasma glucose and insulin response (Wood et al., 2000). β-glucan concentration and molecular weight control the overall viscosity of the chyme that is produced for digestion (Wood et al., 2000). A linear relationship is evident between AUC and viscosity, with each $\log_{10}$ increment in viscosity contributing to approximately 30 mmol/min/L decrease in postprandial glucose AUC (Tosh, 2013). However, very few studies report viscosity or molecular weight in the products that have been tested, including this current study.

Another mechanism linking wholegrain consumption, colonic events and insulin sensitivity may also be involved in glycaemic control. The fermentation of wholegrains may improve the composition of gut microbiota and decrease gut barrier permeability by reducing LPS endotoxin leakage into circulation. Lower LPS alleviated peripheral inflammation and insulin resistance (Boulangé et al., 2016). This may be assisted by the continuous supply and absorption of metabolites, including acetate, propionate and butyrate and ferulic acid derivatives through fermentation (Giacco et al., 2014).

4.4.5 Inflammation

Obesity has been characterised as a low-grade inflammation with increased inflammatory markers (Pereira and Alvarez-Leite, 2014) such as C-reactive protein (CRP) in obese subjects compared to lean ones. CRP is an acute phase protein indicating inflammation and is not only associated with obesity but also with cardiovascular disease and Type 2 diabetes. CRP is synthesised mainly in hepatocytes but CRP is also synthesised and secreted by adipose tissue (Fantuzzi, 2005). Increases in the mass of adipose tissue associated with obesity lead to increases in blood serum CRP concentrations (Ouchi et al., 2003, Selvin et al., 2007). A 14 week study of male Wistar rats on a high fat diet similar to our protocol showed higher blood CRP in obese animals compared to the control, with no significant difference in CRP concentration in the adipose tissue and no correlation between CRP in blood serum, liver or adipose tissue (Dimitrov et al., 2014). Our study is consistent with these findings as blood plasma CRP concentrations increased in the mH control and in both intervention groups where adipose tissue was greater than in the controls. Further studies investigating more specific inflammatory markers such as IL-6 or NF-κB which wholegrains decrease (Herder et al., 2009, Al-Lahham et al., 2010, Oliveira et al., 2009) may be warranted to determine inflammatory status.

4.4.6 Gastrointestinal Effects

Gut function and efficiency is the key to successful nutrient absorption with the mucosa balancing electrolytes, immune response and endocrine function. Consumption of wholegrains may benefit those with metabolic syndrome by altering the environment of the gut. Previously in this chapter, it has been noted that β-glucans change the viscosity of the chyme being passed through the gut. This alters the transit time and in turn the absorption that takes place.

One further mechanism of action that may be involved in gut function is the production of SCFA, which act as metabolic substrates for the gut microbiota. Gut microbiota and SCFA changes are correlated with energy intake, glucose, insulin, satiety hormones and hepatic cholesterol and triglyceride accumulation (Parnell and Reimer, 2012, Boulangé et al., 2016, Cani et al., 2009, Gibson et al., 2017, Delcour et al., 2016,

Verbeke et al., 2015) Prebiotic fibre has been substantially studied in relation the changing microbial dysbiosis (Boulangé et al., 2016, Bindels et al., 2015). However, as this study shows, further research is needed to explore how these changes occur.

Healthy colons have a more complicated microarchitectural structure (Cox et al., 2010) therefore giving a greater surface area for nutrient absorption with longer and straighter crypts of Lieberkühn as seen with this study. Feed efficiency conversion rates in this study correlate with improved gut morphology. Poor gut morphology leads to inefficient feed conversion rates, as seen in the mC and mH colons, as repairing damaged enterocytes is an energy-consuming activity and valuable resources are diverted from growth to tissue repair and maintenance. Mucins and glycoproteins are produced by goblet cells which account for most of the epithelial cells found in the crypts. The mucus produced offers a barrier protecting the absorptive surfaces from microbial challenges, while endogenous acids, digestive enzymes and bile decrease bacterial growth which leads to improvements in the gut microarchitecture (Cox et al., 2010, Forder et al., 2007).

Limited studies have been performed to determine physiological and histological changes in the colon of rats fed wholegrain oats. However, studies on poultry fed wholegrain wheat (15 - 22.5% of diet) and soybean (2.4% of diet) indicate that dietary fibre induced physiological and histological changes including increased feed efficiency, caecal and jejunal crypt depths, caecal butyrate and total SCFA concentrations, and increased thickness of muscularis externa (Zdunczyk et al., 2013, Jankowski et al., 2009). This study showed increased crypt depth that could possibly be due to changes in the fermentation of the wholegrains leading to increases in total SCFA concentration in the mHO compared to the control group.

The proliferation of colonocytes is more effective with increased butyrate production compared to acetate or propionate (Topping and Clifton, 2001). In a chronically starved colon, SCFA cannot be easily replaced and diminished mucosal nutrition may lead to mucosal atrophy and diminished absorption (Roediger, 1990). However, whether this process begins in the short-term still needs further research. This study has given some clarity to this area with the improvement in the mCO mucosa following the addition of the wholegrain to the diet.

As one of the bioactive compounds in wholegrain oat, β-glucans are seen to be prebiotic compounds selectively fermented by groups of beneficial bacteria present in the gut (Roberfroid et al., 2010). The main by-products of this fermentation are the short-chain fatty acids: acetate, propionate and butyrate. Acetate is the most abundant while propionate and butyrate are usually found in roughly equal proportions (Roediger, 1990). This study agrees with previous research with high concentrations of acetate and similar concentrations of propionate and butyrate produced in these experiments (Jankowski et al., 2009).

In vitro experiments have found that different SCFA profiles are produced by different non-digestible components. β-glucans produced a propionate-rich profile with 51:32:17 acetate to propionate to butyrate ratio, while arabinoxylans have a butyrate-rich profile at 63:12:25 ratio. β-glucans also produce a higher total SCFA concentration than inulin (Hughes et al., 2007, Hughes et al., 2008). Differences in SCFA production will also occur with β-glucans of different molecular weights (Hughes et al., 2008). An *in vitro* study using human faecal microbiota for fermentation found propionate total and proportional concentrations increased from 30% with 150 kDa oat β-glucans to 37% with barley 172 kDa β-glucans (Hughes et al., 2008). Therefore, it is necessary for further research to be completed to determine the differences that may have occurred due to this factor. Studies have highlighted the influence of different foods and long-term diets on intestinal microbiota and specifically on the pattern of SCFA (Yang and Rose, Costabile et al., 2015). Limited studies exist where β-glucans as part of an overall diet have been investigated (Cloetens et al., 2012).

In humans, up to 99% of SCFA produced by bacterial fermentation are rapidly absorbed from the colonic lumen (Scheppach, 1994). However, as the rat gastrointestinal tract differs substantially from a human in terms of length and composition (Nyman et al., 2007), any results gained from studies using rat faecal samples need to take account of this difference in anatomy. Pigs and dogs which have similar bowels to humans appear to be better models (Topping and Clifton, 2001). Rats are coprophagic, re-ingesting faeces produced by caecal fermentation (Nyman et al., 2007), ensuring retention of liquid digesta while solids are voided. This has implications for digestion of resistant starch but not fibre. Insoluble fibre fermentation

differs little between rats that were allowed to perform coprophagy or not (Cree et al., 1986, Jackson and Topping, 1993). Coprophagy is a very important variable to consider and limits the reliability of SCFA data from rats, therefore it would be best to use humans, pigs or dogs for SCFA experimentation.

This study indicated that while the difference in healthy and unhealthy control diets change the total faecal excretion of SCFA as well as individual SCFA, limited changes exist when adding wholegrain oat to the diet when using all samples tested which were a combination of rats that were fed until termination and collection of faecal samples and those that were fasted for 2 - 3 hours prior to collection. Propionate and butyrate concentrations fell after starvation or restricted feeding in rats, with butyrate decreasing the most and being restored more slowly after feeding recommenced (Topping and Clifton, 2001). The current study saw a difference between SCFA concentrations from fed samples compared to fasted samples indicating that a larger proportion of the SCFA is being excreted possibly due to excess being produced. However, due to the small number of faecal samples (total n = 6/group, fed/fasted n = 3/group) that were tested, any results need to be observed with that restriction in place. Further research will be needed to consolidate these results taking into account the differences between human and rat gastrointestinal tracts.

Many studies to determine SCFA concentrations using cell cultures produced conflicting results (Connolly et al., 2010, Connolly et al., 2012, Dass et al., 2007, Hernot et al., 2009). These studies establish the quantity and ratio of SCFA produced on fermentation of non-digestible carbohydrates *in vitro.* There are very few studies that examine the effect of wholegrain oat consumption on SCFA production, but these few studies do not support a positive effect on SCFA production. Two studies investigated impact of feeding wholegrain cereals (wheat and maize) on microbiota and SCFA production and did not detect any changes in faecal SCFA concentrations (Costabile et al., 2008, Carvalho-Wells et al., 2010) as they are readily absorbed in the colon and either transported to the liver to be utilized in cholesterol synthesis and gluconeogenesis (acetate and propionate) or used in the colon to produce and differentiate colonocytes (butyrate). This study is consistent with the very low concentrations in our faecal samples. Faecal SCFA concentrations are used as a surrogate marker for SCFA production, however, this does not represent epithelial cell

exposure (Thies et al., 2014). The production of SCFA is also highly variable not only between individuals but also within individuals with many confounding factors, such as recent dietary intake, colonic transit time and hydration status. Therefore, the interpretation of this data is greatly limited (Thies et al., 2014).

In human studies, there is a lack of evidence to support the effect of oats on faecal SCFA excretion as often no effect is observed as 90% of SCFA are absorbed and utilized by the host (Connolly et al., 2012). No changes in faecal SCFA or butyric acid were shown in a 2 - week oat bran randomised control trial on humans with colorectal adenomas (Kashtan et al., 1992). A 4 - week human randomised controlled trial with an oat bran intervention compared to high amylose wheat diet indicated no change to the faecal excretion of acetate or propionate but butyrate was lower (Noakes et al., 1996). Finally, a 12 - week trial on humans with ulcerative colitis indicated no change in faecal excretion of the variety of SCFA or sum of SCFA, however butyric acid was increased by 26% by week 4 (Hallert et al., 2003). While these studies used oat bran which was lower in β-glucans than wholegrain oat, it could be extrapolated that as the prebiotic effect of β-glucans is expected, then by lowering the content, SCFA production will be decreased and therefore less likely to be found in faecal samples.

As this study indicated total increases of acetate, propionate and butyrate, as well as the proportion of propionate and butyrate in the fed mHO rather than the fed mCO compared to the controls, a possible mechanism may be that the increased total SCFA production is causing a lower pH resulting in a decreased solubility of free bile acids which in turn decreases secondary bile acids (van Bennekum et al., 2005, Wong et al., 2006). SCFA-mediated modulation of gut hormones by free fatty acid receptor 2 (FFAR2) and free fatty acid receptor 3 (FFAR3) may also regulate food intake and energy balance leading to a decrease in obesity (Lin et al., 2012). Cross-feeding may also occur between *Bifidobacteria* and butyrate-producing bacteria (De Vuyst and Leroy, 2011) which may explain the significant increase in acetate and butyrate production. As exploring the microbiota was outside of the scope of this study, further research is needed to confirm any changes and mechanisms of action that this may lead to.

Increases of faecal SCFA have been correlated with increased adiposity (Fernandes et al., 2014, Rahat-Rozenbloom et al., 2014). While this study gives similar results, this

may be due to the minimal fat mass of both control diets prior to the addition of the wholegrain oats. As previously mentioned, further refinement of these diets is necessary prior to any further intervention studies.

4.5 Conclusions

Results from this study demonstrate that the addition of 20% wholegrain oats to a high carbohydrate, high fat diet improved blood pressure, glucose tolerance and plasma cholesterol concentrations. It also improved the structure of the heart, liver and gastrointestinal tract. These responses may be due to changes in the gut microbiome caused by fermentation of dietary fibre, resistant starch, β-glucans and other minor prebiotic components within the grain. The microbial metabolites (SCFA) act as energy sources for the colonic and hepatic cells leading to improved function. Further research determining whether wholegrain oat consumption leads to greater expression of FFAR2 and FFAR3, MUC2 and claudin-1 improving gut repair, protection and integrity is needed.

The main limitation of this study was the low protein intake of the control diets which led to decreased lean mass and body weight, particularly in the mC or healthy control. Further refinements of these diets are necessary before future intervention studies are carried out. Another limitation to this study was the difference between human and rat gastrointestinal tracts. The rat model is valuable for studying diet-induced metabolic syndrome. However, when studying how the gut is involved, a different mode of study, such as an artificial gut, may improve results.

Overall, the addition of wholegrain oats is beneficial in attenuating diet-induced metabolic syndrome and improving the structure of the gastrointestinal tract, and could be a healthy replacement for other grains in the diet.

Chapter 5 - Coconut products improve signs of diet-induced metabolic syndrome in rats

5.1 Introduction

Coconut (*Cocos nucifera* L.) has for many civilisations, particularly in tropical and subtropical regions, been seen as the 'tree of life' (DebMandal and Mandal, 2011) as all parts of the tree can be used for numerous purposes yet until recently the health benefits, have been anecdotal rather than scientifically proven, with many previous studies focusing on only on the coconut oil (Arunima and Rajamohan, 2012, Liau et al., 2011, Zakaria et al., 2011) and not the other components of coconut.

The fruit components are used industrially and in-home cooking in many ways (Lima et al., 2015). Traditionally, parts of the coconut fruit (such as shell fibre, pulp and water) and tree root have been used in many countries for treatment of various diseases (Lima et al., 2015). While coconut fibre is rich in polyphenols, the oil is rich in medium-chain saturated fatty acids (~90% of the oil) (Lima et al., 2015). Saturated fatty acids have been debated as a class of fatty acids for their association with cardiovascular disease risk (Lecerf, 2009, de Souza et al., 2015, Hammad et al., 2015), although the roles of the individual fatty acids were not considered for such conclusions. A recently published article highlights the need to look for a biological classification in order to confirm the disease risk and prevention potential of fatty acids (Poudyal and Brown, 2015).

Cold-pressed coconut oil (also called virgin coconut oil or VCO) is composed of ~ 50% lauric acid, ~ 20% myristic acid and ~ 6% capric acid, all medium-chain saturated fatty acids (Katragadda et al., 2010). The content of medium chain fatty acids in VCO can vary depending on geographical location and ecological conditions (Nevin and Rajamohan, 2008). Lauric acid as the major component of coconut oil has an advantage in its cellular metabolism. Lauric acid is highly oxidized in the cell while the longer chain saturated fatty acids are oxidized at a slower rate (DeLany et al., 2000, Göransson, 1965, St-Onge and Jones, 2002). Myristic acid is 2 carbons longer than lauric acid and can be expected to have intermediate oxidizability, so slower than palmitic acid and stearic acid (DeLany et al., 2000, Rioux et al., 2000, St-Onge and Jones, 2002). In a previous study, lauric acid showed HDL-cholesterol increases in

healthy subjects in a crossover design study after 6 weeks of high-lauric acid diet consumption (Temme et al., 1996). In coronary artery disease patients, a diet rich in extra virgin coconut oil helped in reducing waist circumference with increases in HDL-cholesterol (Cardoso et al., 2015).

Metabolic syndrome is the cluster of risk factors for cardiovascular disease and type 2 diabetes (O'Neill and O'Driscoll, 2015). These risk factors are correlated with higher intake of dietary fat (Golay and Bobbioni, 1997, Lee, 2013, Hariri and Thibault, 2010). Dietary intake of saturated fatty acids are proposed to be involved in the development of metabolic diseases including obesity and dyslipidaemia (Golay and Bobbioni, 1997, Hariri and Thibault, 2010). This study is investigating the role of coconut-derived commercial products, Banaban virgin coconut oil and Banaban coconut nourish, as sources of fats and fibre. We have developed a high carbohydrate, high fat diet-fed rat model for human metabolic syndrome with metabolic, cardiovascular and hepatic complications developed in these animals (Panchal et al., 2011b).

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ORIGINAL PAPER

Coconut Products Improve Signs of Diet-Induced Metabolic Syndrome in Rats

Sunil K. Panchal[1] · **Sharyn Carnahan**[1,2] · **Lindsay Brown**[1,2]

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Abstract Increasing prevalence of obesity and metabolic syndrome warrants identification of potential therapeutic options for intervention. This study tested commercially available Virgin Coconut Oil and Coconut Nourish, as coconuts are rich sources of lauric and myristic acids. Male Wistar rats were fed either corn starch diet (C); high-carbohydrate, high-fat diet (H); high-carbohydrate, high-virgin coconut oil diet (HV); or high-carbohydrate, high-coconut Nourish diet (HN) for 16 weeks. Metabolic, liver, and cardiovascular health parameters were measured during and at the end of the study. Virgin coconut oil lowered body weight (C 386±8g, H 516±13g, HV 459±10g), blood glucose concentrations (C 4.2±0.1 mmol/L, H 5.4±0.2 mmol/L, HV 4.6±0.2 mmol/L), systolic blood pressure (C 127±5mmHg, H 149±4mmHg, HV 133±3mmHg,) and diastolic stiffness (C 25.0±1.7, H 31.4±1.2, HV 25.2 ±2.3,) with improved structure and function of the heart and liver. Coconut Nourish increased total body lean mass (C 255 ±10g, H 270±16g, HN 303±15g) and lowered plasma total cholesterol concentrations (C 1.6±0.2 mmol/L, H 1.7 ±0.1 mmol/L, HN 1.0±0.0 mmol/L), systolic blood pressure (C 127±5mmHg, H 149±4mmHg, HN 130±3mmHg) and diastolic stiffness (C 25.0±1.7, H 31.4±1.2, HN 26.5±1.0), improved structure and function of the heart and liver but increased plasma concentrations of triglycerides (C 0.3 ±0.1 mmol/L, H 1.1±0.4 mmol/L, HN 1.8±0.2 mmol/L) and non-esterified fatty acids (C 1.2±0.3 mmol/L, H 3.3 ±0.8 mmol/L, HN 5.6±0.4 mmol/L). Thus, the fiber and protein in coconut Nourish and the medium-chain saturated fatty acids in virgin coconut oil may improve cardiovascular and liver complications in obesity.

Keywords Metabolic syndrome · Obesity · Coconut · Saturated fatty acids · Lauric acid

Introduction

Metabolic syndrome is a cluster of risk factors for cardiovascular disease and type 2 diabetes including obesity, insulin resistance, hypertension, dyslipidemia, and impaired glucose tolerance [1]. The role of dietary saturated fatty acids as a risk factor for cardiovascular and metabolic disease has been widely debated [2, 3]. Current results suggest that the biological effects of saturated fatty acids depend on the source of the fat and the chain length of individual fatty acids, supporting the classification of these fatty acids based on biological effects rather than chemical structure [3]. Lauric and myristic acids are medium-chain saturated fatty acids found in large amounts in coconuts [4]. The 12-carbon lauric acid is rapidly oxidized in the cell, the 14-carbon myristic acid has an intermediate rate of oxidation while the longer-chain saturated fatty acids such as the 18-carbon stearic acid are oxidized at a slower rate [5]. Cold-pressed coconut oil, also referred to as virgin coconut oil (VCO), is mainly composed of saturated fatty acids (~91%), predominantly lauric and myristic acids [6].

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✉ Sunil K. Panchal
Sunil.Panchal@usq.edu.au

[1] Functional Foods Research Group, Institute for Agriculture and the Environment, University of Southern Queensland, QLD, Toowoomba 4350, Australia

[2] School of Health and Wellbeing, Faculty of Health, Engineering and Sciences, University of Southern Queensland, Toowoomba 4350, Australia

Traditionally, components of coconuts such as shell fiber, pulp, and water have been used to treat diseases including diabetes, asthma, stomach pain, dermatitis, and diarrhea, as well as for their antipyretic and diuretic responses [7]. The husk from coconut fruit as a source of fiber has been associated with health effects including antimalarial, antibacterial, and antidepressant responses [8, 9]. Further, our recent studies showed that lauric acid prevented induction of obesity and osteoarthritis in high-carbohydrate, high-fat diet-fed rats [10]. A review of studies with coconut oil suggested that the consumption of coconut oil did not improve lipid profile and hence cardiovascular risk when compared to *cis* unsaturated fatty acids [11].

Intakes of simple sugars (fructose and sucrose), long-chain saturated fatty acids and *trans* fatty acids in the modern diet are major predictors of the increase in metabolic syndrome [2, 3, 12]. Our previous studies have reported that the feeding of high-carbohydrate, high-fat diet using fructose and sucrose as simple sugars and beef tallow as the source of long-chain saturated fatty acids and *trans* fatty acids induced metabolic, cardiovascular, and hepatic complications in rats that are similar to the metabolic syndrome in humans [10, 13, 14]. Further, foods can also prevent or reverse disease states, and current research has focused on these functional foods for metabolic diseases [15].

This study investigated two commercial coconut-derived products, Banaban virgin coconut oil and Banaban coconut Nourish, as sources of medium-chain saturated fatty acids and fiber, respectively, replacing beef tallow in diet-induced metabolic syndrome in rats. Structural changes in the rat heart were characterized by histology and echocardiography, while heart function was assessed *in vivo* using echocardiography and *ex vivo* in isolated perfused hearts. Systolic blood pressure and responses of isolated thoracic aortic rings were measured to identify vascular reactivity. Metabolic parameters (oral glucose tolerance, body composition, and plasma lipid concentrations) were assessed. Changes in liver structure and function were defined by histology and plasma activities of liver enzymes.

Materials and Methods

Rats and Diets 40 male Wistar rats aged 9–10 weeks old (337 ± 1 g) were purchased from Animal Resource Centre (Canning Vale, WA, Australia) and housed individually in a temperature-controlled (21 ± 2 °C), 12-h light/dark cycle environment with free access to group-specific food and water at the University of Southern Queensland Animal House. Rats were randomly divided into four separate groups ($n = 10$ each) and were fed with one of the following diets for 16 weeks: corn starch diet (C); high-carbohydrate, high-fat diet (H); high-carbohydrate, high-VCO diet (HV) and high-carbohydrate, high-coconut Nourish diet (HN). The composition of C and H diets have been reported previously [13, 14]. In HV and HN diets, beef tallow in the H diet was replaced by Banaban virgin coconut oil (200 g/kg) and Banaban coconut Nourish powder (200 g/kg), respectively. The composition of VCO and coconut Nourish powder are described in Supplementary Table 1.

Physiological Measurements Body weight and intakes of food and water were recorded daily for all rats. Energy intakes and feed conversion efficiency were calculated [13]. Abdominal circumference was measured every eighth week using a standard measuring tape under light anesthesia with Zoletil (tiletamine 10 mg/kg, zolazepam 10 mg/kg, intraperitoneal; Virbac, Peakhurst, NSW, Australia) [13]. Oral glucose tolerance tests were performed on rats at 0, 8, and 16 weeks [13]. Dual-energy X-ray absorptiometric measurements were performed on rats after 16 weeks of feeding using a Norland XR36 DXA instrument (Norland, Fort Atkinson, WI, USA) [13]. Systolic blood pressure and echocardiographic examination were measured [13]. After terminal euthanasia, left ventricular function was assessed using isolated Langendorff heart preparation [13]. Responses of thoracic aortic rings to noradrenaline, acetylcholine, and sodium nitroprusside were measured [13].

Euthanasia Euthanasia was induced by intraperitoneal injection of Lethabarb (pentobarbitone sodium; 100 mg/kg; Virbac, Peakhurst, NSW, Australia). Heparin (200 IU; Sigma-Aldrich Australia, Sydney, NSW, Australia) was administered through the right femoral vein followed by withdrawal of ~5 mL blood from the abdominal aorta. Blood was collected into heparinized tubes and centrifuged at 5000 × *g* for 15 min within 30 min of collection. Plasma was collected and stored at −20 °C.

Organ Weights and Histology Following perfusion experiments, right ventricle and left ventricle (with septum) were separated and weighed. Liver, kidney, and retroperitoneal, epididymal, and omental fat contents were removed and weighed. Organ weights were normalized relative to tibial length (mg/mm). Two rats *per* group were used exclusively for histopathological analysis. Thin sections (5 μm) of tissue were cut and stained with haematoxylin and eosin stain for determination of inflammatory cell infiltration (heart and liver, ×20) and fat vacuole deposition (liver, ×20). Picrosirius red staining was used to define collagen distribution in the left ventricle (×20) [13].

Plasma Biochemistry Plasma activities of alanine transaminase and aspartate transaminase and plasma concentrations of triglycerides, total cholesterol, and non-esterified fatty acids were determined [13].

Statistical Analysis Data are presented as mean ± SEM. GraphPad Prism version 6.00 for Windows (San Diego, CA, USA) was used for statistical analyses of differences between the groups by one-way analysis of variance. Statistically significant variables were treated with Neumann–Keuls *post hoc* test to compare all groups of animals. $P < 0.05$ was considered statistically significant.

Results and Discussion

Physiological and Metabolic Parameters with Virgin Coconut Oil (VCO) VCO-fed HV rats had higher body weight, body weight gain, and abdominal circumference compared to C rats while these parameters were lower in HV rats compared to H rats (Table 1). Energy intake and feed conversion efficiency were higher in HV rats compared to C rats while these parameters were lower than H rats (Table 1). During oral glucose tolerance test, basal blood glucose concentration in HV rats was not different from C rats while it was lower than H rats (Table 1). Although VCO lowered basal blood glucose concentrations, it failed to improve the overall glucose tolerance as evident from the area under the curve (Table 1). Retroperitoneal, epididymal, and omental fats were higher in HV rats compared to C rats while they were not different between H and HV rats (Table 1). Total body fat and lean mass were similar in C and HV rats whereas both these parameters were also similar in H and HV rats (Table 1). Plasma total cholesterol concentrations were not different in C and HV rats while HV rats had higher total cholesterol concentrations compared to H rats (Table 1). Plasma triglyceride concentrations were higher in HV rats compared to C rats while HV and H rats had similar triglyceride concentrations (Table 1). Plasma non-esterified fatty acid concentrations were higher in HV rats compared to C rats while H and HV rats had no differences in plasma concentrations of non-esterified fatty acids (Table 1).

The responses of diet supplementation with virgin coconut oil may be due to replacement of longer-chain saturated fatty acids with medium-chain fatty acids [16]. Virgin coconut oil components may induce increased energy expenditure leading to a lower weight gain than beef tallow. The major medium-chain fatty acids in virgin coconut oil are lauric acid and myristic acid [16], which are rapidly absorbed in the intestine even without pancreatic lipase [17]. Lauric acid is more likely to be oxidized by the mitochondria than longer-chain saturated fatty acids such as stearic and palmitic acids [18]. We have also compared lauric, myristic, palmitic, and stearic acids for their effects on obesity and osteoarthritis with lauric acid markedly reducing both obesity and osteoarthritis compared

Table 1 Dietary intakes, body composition, and plasma biochemistry

Variables	*C*	*H*	*HV*	*HN*
Initial body weight (g)	340 ± 1	337 ± 1*	337 ± 1*	337 ± 2
Final body weight (g)	386 ± 8	516 ± 13a*	459 ± 10b*	512 ± 11a*
Body weight gain (%)	13.3 ± 2.3	52.2 ± 3.7a*	36.5 ± 4.1b*	51.7 ± 2.8a*
Food intake (g/day)	38.8 ± 1.4	24.4 ± 0.5a*	21.4 ± 0.8b*	24.5 ± 0.9a*
Water intake (g/day)	30.0 ± 1.5	25.9 ± 1.9b	21.7 ± 1.2b*	36.7 ± 1.5a*
Energy intake (kJ/day)	378 ± 16	524 ± 10a*	441 ± 14b*	458 ± 14b*
Feed conversion efficiency (g/kJ)	0.12 ± 0.02	0.34 ± 0.02a*	0.27 ± 0.03b*	0.38 ± 0.02a*
Abdominal circumference (cm)	18.5 ± 0.2	22.7 ± 0.5a*	20.7 ± 0.3b*	21.8 ± 0.2a*
Basal blood glucose (mmol/L)	4.2 ± 0.1	5.4 ± 0.2a*	4.6 ± 0.2b	5.2 ± 0.2a*
Area under the curve (mmol/L·minutes)	765 ± 26	866 ± 22*	862 ± 32*	893 ± 29*
Retroperitoneal fat (mg/mm)	177 ± 21	420 ± 43*	415 ± 50*	380 ± 47*
Epididymal fat (mg/mm)	120 ± 11	229 ± 15*	198 ± 20*	252 ± 19*
Omental fat (mg/mm)	134 ± 13	242 ± 24*	237 ± 19*	250 ± 22*
Total abdominal fat (mg/mm)	430 ± 41	892 ± 73*	850 ± 87*	881 ± 84*
Total body fat mass (g)	121 ± 12	235 ± 19*	176 ± 26	169 ± 15*
Total body lean mass (g)	255 ± 10	270 ± 16	256 ± 11	303 ± 15*
Fat:lean mass ratio	0.49 ± 0.07	0.81 ± 0.10*	0.71 ± 0.12	0.58 ± 0.08
Plasma total cholesterol (mmol/L)	1.6 ± 0.2	1.7 ± 0.1b	2.0 ± 0.0a	1.0 ± 0.0c*
Plasma triglyceride (mmol/L)	0.3 ± 0.1	1.1 ± 0.4	1.4 ± 0.3*	1.8 ± 0.2*
Plasma NEFA (mmol/L)	1.2 ± 0.3	3.3 ± 0.8*	4.8 ± 0.8*	5.6 ± 0.4*

All values are mean ± SEM. * indicates significantly different values compared to C. Means between H, HV and HN without a common letter are significantly different, $P < 0.05$. C, corn starch diet-fed rats; H, high-carbohydrate, high-fat diet-fed rats; HV, high-carbohydrate, high-VCO diet-fed rats; HN, high-carbohydrate, high-coconut Nourish diet-fed rats; NEFA, non-esterified fatty acids

to H rats, in contrast to palmitic and stearic acids [10]. Other studies with coconut oil have shown similar health benefits [19–21]. Previous studies with palmitic and stearic acids confirmed their role in inducing insulin resistance [22, 23]. Although lauric and myristic acids are also saturated fatty acids with similar chemical properties to palmitic and stearic acids, all saturated fatty acids cannot be considered to produce the same physiological responses [3]. Fat to lean mass ratio can predict metabolic dysfunction [24]. In our study, fat to lean mass ratio was higher in obese rats compared to healthy lean rats reflecting the metabolic disturbances in obese rats. Further, the improved fat to lean mass ratio in VCO and coconut Nourish supplemented rats suggests that these interventions improve the metabolic status of rats.

Physiological and Metabolic Parameters with Coconut Nourish HN rats had higher body weight, body weight gain, and abdominal circumference compared to C rats while these parameters were similar in HN and H rats (Table 1). Energy intake was higher in HN rats compared to C rats while it was lower than H rats (Table 1). Feed conversion efficiency was higher in HN rats compared to C rats while it was similar in H and HN rats (Table 1). During oral glucose tolerance test, basal blood glucose concentrations in HN rats were higher than C rats while it was similar to H rats (Table 1). Area under the curve was unchanged between the groups (Table 1). Retroperitoneal, epididymal, and omental fats were higher in HN rats compared to C rats while they were not different between H and HN rats (Table 1). Total body fat mass in HN rats was higher than C rats while it was similar to H rats; total body lean mass in HN rats was higher than C rats while it was similar to H rats (Table 1). Plasma total cholesterol concentrations were lower in HN rats compared to C and H rats (Table 1). Plasma triglyceride concentrations were higher in HN rats compared to C rats while HN and H rats had similar triglyceride concentrations (Table 1). Plasma non-esterified fatty acid concentrations were higher in HN rats compared to C rats while H and HN rats had no differences in plasma concentrations of non-esterified fatty acids (Table 1). In summary, replacement of beef tallow with coconut Nourish caused no change in body weight while increasing total body lean mass. Coconut Nourish contains nutrients including protein and fiber in addition to saturated fatty acids. This high content of protein may be responsible for the decreases in fat mass to lean mass ratio [25]. Moreover, the increase in lean mass occurred in the presence of lower energy intake compared to the obese rats, thus increasing the feed efficiency. Coconut Nourish is also the source of fiber that may also help in the improvements seen in these rats.

Cardiovascular and Hepatic Structure and Function with VCO Virgin coconut oil, a rich source of medium-chain fatty acids, reduced blood pressure in Spontaneously Hypertensive Rats and heated palm oil-induced hypertensive rats [26, 27]. Increased nitric oxide production may be a possible mechanism for this decrease in blood pressure [26, 27]. Fructose-induced hypertension in rodents is well-studied as this model has been used for intervention trials throughout the world [28, 29].

HV rats showed similar systolic blood pressure to C rats while it was lower in HV rats compared to H rats (Table 2). LVIDs was higher while fractional shortening and ejection fraction were lower in HV rats compared to C rats (Table 2). HV rats showed similar left ventricular diastolic stiffness to C rats while stiffness was lower in HV rats compared to H rats (Table 2). Left ventricular wet weight was similar in C and HV rats while it was lower in HV rats than H rats (Table 2). HV rats showed inhibition of infiltration of inflammatory cells (Fig. 1c) and collagen deposition (Fig. 1g) compared to H rats (Fig. 1b and f).

Livers from HV rats showed inhibition of infiltration of inflammatory cells and deposition of fat vacuoles (Fig. 1k) compared to H rats (Fig. 1j). HV rats had higher liver wet weight compared to C rats while it was not different compared to H rats (Table 2). Plasma ALP activity was higher in HV rats compared to C rats while it was unchanged compared to H rats (Table 2).

In summary, replacement of beef tallow with VCO as a source of saturated fatty acids lowered body weight, blood glucose concentrations, systolic blood pressure, and diastolic stiffness while improving structure and function of the heart and liver but without decreasing central obesity. These changes may be correlated to the lower inflammatory cell infiltration and subsequent collagen deposition in the heart and liver of virgin coconut oil-supplemented rats.

Previous studies reporting the impact of saturated fatty acids on human health concluded that lauric and myristic acids raised plasma total cholesterol concentrations, with lauric acid increasing LDL cholesterol and myristic acid increasing both LDL and HDL cholesterol concentrations [30, 31]. Lauric acid lowered the ratio of total cholesterol to HDL, while myristic acid did not change this ratio [32]. The current study found that total plasma cholesterol concentrations increased with virgin coconut oil supplementation in rats, although it cannot be confirmed if the changes were due to increases in HDL-cholesterol or LDL-cholesterol.

Cardiovascular and Hepatic Structure and Function with Coconut Nourish HN rats showed similar systolic blood pressure to C rats while it was lower in HN rats compared to H rats (Table 2). LVPWd, IVSd, and relative wall thickness were higher while fractional shortening and ejection fraction were lower in HN rats compared to C rats (Table 2). LVPWd, IVSd, and relative wall thickness were higher in HN rats while ejection time was lower compared to H rats (Table 2). HN rats showed similar diastolic stiffness to C rats while it was lower in HN rats compared to H rats (Table 2). Left ventricular wet

Table 2 Cardiovascular and hepatic structure and function

Variables	*C*	*H*	*HV*	*HN*
Cardiovascular variables				
Systolic blood pressure (mmHg)	127 ± 5	149 ± 4^{a*}	133 ± 3^{b}	130 ± 3^{b}
LVIDd (mm)	7.63 ± 0.13	8.31 ± 0.30	7.94 ± 0.21	7.76 ± 0.26
LVPWd (mm)	1.73 ± 0.08	1.79 ± 0.04^{b}	1.89 ± 0.05^{ab}	1.97 ± 0.03^{a*}
LVIDs (mm)	3.41 ± 0.22	4.34 ± 0.34*	4.40 ± 0.22*	4.00 ± 0.23
IVSd (mm)	1.71 ± 0.09	1.77 ± 0.03^{b}	1.86 ± 0.06^{ab}	1.94 ± 0.03^{a*}
Relative wall thickness	0.45 ± 0.02	0.43 ± 0.02^{b}	0.47 ± 0.02^{ab}	0.51 ± 0.02^{a*}
Fractional shortening (%)	55.6 ± 2.4	45.5 ± 3.4*	44.5 ± 1.7*	48.7 ± 1.6*
Ejection fraction (%)	90.5 ± 1.4	85.7 ± 3.0	82.8 ± 1.5*	86.3 ± 1.2*
Ejection time (ms)	83.8 ± 3.6	88.0 ± 2.3^{a}	89.0 ± 1.5^{a}	81.7 ± 2.3^{b}
Left ventricle wet weight (mg/mm)	19.7 ± 0.4	22.3 ± 0.9^{a*}	20.6 ± 0.3^{b}	23.8 ± 1.0^{a*}
Right ventricle wet weight (mg/mm)	4.28 ± 0.18	4.68 ± 0.35^{ab}	3.84 ± 0.23^{b}	5.66 ± 0.73^{a}
Diastolic stiffness constant, κ	25.0 ± 1.7	31.4 ± 1.2^{a*}	25.2 ± 2.3^{b}	26.5 ± 1.0^{b}
Hepatic variables				
Liver wet weight (mg/mm tibial length)	205 ± 18	293 ± 13^{b*}	301 ± 8^{b*}	372 ± 21^{a*}
Plasma ALP activity (U/L)	124 ± 15	196 ± 19^{a*}	222 ± 9^{a*}	152 ± 11^{b}

All values are mean ± SEM. * indicates significantly different values compared to C. Means between H, HV and HN without a common letter are significantly different, $P < 0.05$. C, corn starch diet-fed rats; H, high-carbohydrate, high-fat diet-fed rats; HV, high-carbohydrate, high-VCO diet-fed rats; HN, high-carbohydrate, high-coconut Nourish diet-fed rats; LVIDd, left ventricular internal diameter during diastole; LVPWd, left ventricular posterior wall thickness during diastole; LVIDs, left ventricular internal diameter during diastole; IVSd, interventricular septal thickness during diastole; ALP, alkaline phosphatase

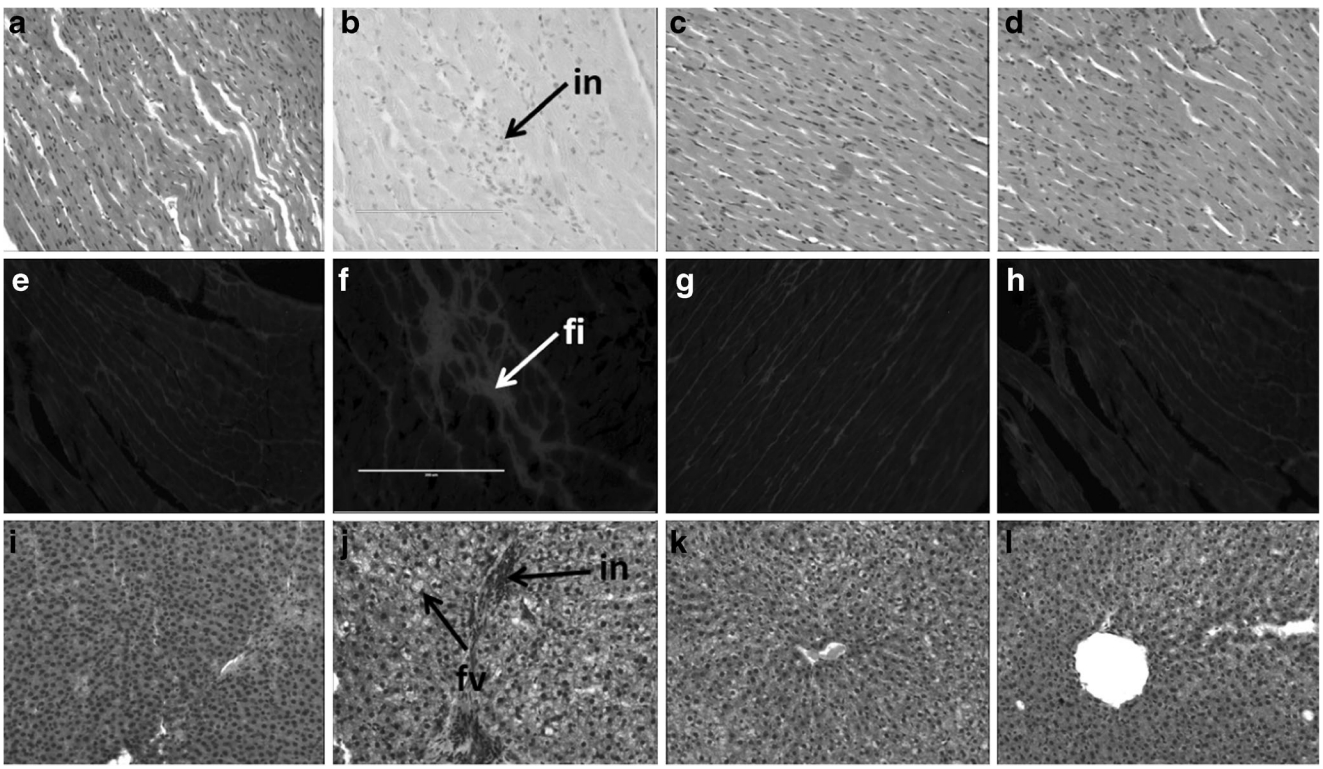

Fig. 1 Effects of coconut products on inflammation and fibrosis in the heart and inflammation and fat deposition in the liver. Haematoxylin and eosin staining of left ventricle showing inflammatory cells ('in'; ×20) from C (**a**), H (**b**), HV (**c**) and HN (**d**) rats. Picrosirius red staining of left ventricle showing fibrosis ('fi'; ×40) from C (**e**), H (**f**), HV (**g**) and HN (**h**) rats. Haematoxylin and eosin staining of liver showing inflammatory cells ('in') and fat deposition ('fv', ×20) from C (**i**), H (**j**), HV (**k**) and HN (**l**) rats. C, corn starch diet-fed rats; H, high-carbohydrate, high-fat diet-fed rats; HV, high-carbohydrate, high-VCO diet-fed rats; HN, high-carbohydrate, high-coconut Nourish diet-fed rats

weights were similar in H and HN rats but higher in HN rats compared to C rats (Table 2). HN rats showed inhibition of infiltration of inflammatory cells (Fig. 1d) with inhibition of collagen deposition (Fig. 1h). HN rats showed improved aortic responses to noradrenaline (Supplementary Figure 1A, $-\log EC_{50}$ values – C = 6.89 ± 0.18; H = 6.42 ± 0.33; HV = 6.31 ± 0.08; HN = 6.98 ± 0.17) with no change in thoracic aortic responses to sodium nitroprusside and acetylcholine (Supplementary Figure 1B and 1C).

Livers from HN rats showed inhibition of infiltration of inflammatory cells and decreased deposition of fat vacuoles (Fig. 1l) compared to H rats (Fig. 1j). HN rats had higher liver wet weight compared to C and H rats (Table 2). Plasma ALP activity was similar in C and HN rats while it was lower in HN rats compared to H rats (Table 2).

Thus, the physiological and metabolic changes with coconut Nourish were accompanied by the prevention of increases in systolic blood pressure and diastolic stiffness while improving structure and function of the heart and liver, but also by increased plasma triglyceride and non-esterified fatty acids concentrations with lowered plasma total cholesterol concentrations. Similar to virgin coconut oil, coconut Nourish was able to normalize the systolic blood pressure and left ventricular diastolic stiffness. While these changes were similar, coconut Nourish was also able to lower total cholesterol concentrations. This reduction may be attributed to the fiber content of the coconut Nourish as shown in previous study with dietary fiber reducing blood pressure and total cholesterol [33].

Coconuts are known for their great versatility for communities in the tropical and subtropical areas of the world. Coconut or coconut products as part of the regular diet along with seafood decreased cardiovascular disease risk factors in Samoan Islanders, including higher HDL-cholesterol concentrations and lower abdominal circumferences [34]. Similar observations were seen in the Kitava population when compared with the Swedish population. The regular diet of Kitava people includes tubers, coconuts, and seafood and this Kitava population is free from overweight, hypertension, cardiovascular disease, and malnutrition [35]. It is important that the extent of health benefits with coconut and coconut products is explored further to establish the role of coconut as a functional food. These results can then form the basis for controlled human trials to identify translatable responses of coconuts.

Conclusions

Both virgin coconut oil and coconut Nourish contain high amounts of lauric and myristic acids while coconut Nourish also contains increased fiber and protein. When beef tallow was replaced with virgin coconut oil or coconut Nourish in a high-carbohydrate, high-fat diet, the increases in systolic blood pressure and diastolic stiffness in the heart were inhibited. Moreover, the coconut Nourish increased the lean mass in rats even with decreased energy intake. Virgin coconut oil reduced the fasting blood glucose concentrations while Nourish was able to reduce the total cholesterol concentrations in plasma. Both the virgin coconut oil- and Nourish-supplemented diets were rich in fructose and sucrose content, so these results are quite relevant to overweight and obese individuals who consume diets rich in simple sugars.

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Author's Contributions S.K.P. and L.B. designed the research protocol and interpreted the results; S.K.P. and S.C. conducted the animal experiments and analyzed the data; S.K.P., S.C. and L.B. wrote the manuscript; and S.K.P. had primary responsibility for final content. All authors read and approved the final manuscript.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Statement on the Welfare of Animals All experimental protocols were approved by the Animal Ethics Committee of the University of Southern Queensland under the guidelines of the National Health and Medical Research Council of Australia. The approval number for this study was 13REA008. This article does not contain any studies with human participants performed by any of the authors.

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Chapter 6 – Conclusions and future directions

6.1 Conclusions

Obesity, hypertension, dyslipidaemia, hyperglycaemia and insulin resistance together are termed metabolic syndrome (as discussed in Chapter 1). This combination of metabolic abnormalities increases the risk of cardiovascular disease and type 2 diabetes and contributes to increased mortality and morbidity (O'Neill and O'Driscoll, 2015). An unhealthy lifestyle including an unhealthy diet and decreased physical activity are strong determinants of the increasing obesity epidemic in Australia. Promoting healthy lifestyles through diet, nutrition and exercise will decrease the burden of these metabolic diseases. A healthy diet with the addition of functional foods gives health benefits and improves disease-related biomarkers. Therefore, research is warranted in evaluating functional foods using appropriate animal models prior to translation to human clinical trials.

Research into dietary interventions that can improve metabolic syndrome markers requires a well-established and well-designed animal model that mimics the human condition to provide a better understanding of physiology, biochemistry and pathogenesis of disease. This study used a model of diet-induced metabolic syndrome and cardiovascular complications. Young male Wistar rats were fed a high carbohydrate (simple sugars), high saturated/*trans*-fat diet for sixteen weeks developing abdominal obesity, hypertension, dyslipidaemia, impaired glucose tolerance, cardiovascular complications and liver steatosis (Panchal et al., 2011b). This diet-induced rat model of metabolic syndrome was used to determine the physiological effects of wholegrain oats (Chapters 3 and 4), oat bran (Chapter 3), oat β-glucan powder (Chapter 3), virgin coconut oil and coconut Nourish (Chapter 5).

Traditional food products that have been consumed for thousands of years were examined in relation to their impact on metabolic syndrome. Through this focus on foods that have been in the human diet for a long time, it may be possible for the health benefits gained from them to be incorporated easily into daily consumption without the need for drastic changes to dietary intake. Wholegrain oats, oat bran, oat β-glucan,

virgin coconut oil and coconut nourish were investigated and indicated that they were beneficial in the attenuation of metabolic syndrome.

Oats are widely grown in Northern Europe and Canada and have been consumed for over 5000 years (Ladizinsky, 1995, Akeret, 2005, Mariotti Lippi et al., 2015). The benefits of adding oats to the diet have been known for many years. Oat β-glucans improve cholesterol and glucose tolerance. The study in Chapter 3 compared wholegrain oat groats, oat bran and oat β-glucan powder added to a high carbohydrate, high fat diet to determine whether they gave similar physiological responses or if the increased processing changed the response given. The study indicated that each of the oat products improved glucose tolerance as well as blood pressure, liver structure and duodenal morphology. As responses were similar for the three interventions given it is concluded that nutritional components in the oats may be working synergistically and therefore consumption of wholegrain oats in the diet is preferred. The relatively low dose of oat product in the diet could have contributed to the limited changes in the remainder of the gastrointestinal tract.

The overall nutrient content of wholegrains with β-glucans, avenanthramides, oat lipids as well as a wide range of amino acids makes oats a viable replacement for other cereals in the diet. As oats do not contain gluten or α-gliadin that makes wheat, barley and rye toxic to coeliac disease patients, the addition of oats to the diet is possible for these patients. Further testing needs to be undertaken with the avenin content as some coeliac disease patients may also be sensitive to this compound. Chapter 4 of this thesis determined that it is possible that oats could make a viable replacement as the biomarkers for metabolic syndrome were improved. It also gave indications that oats would be a viable nutritional source when protein is lacking in the diet to increase body weight, improve heart function and structure and liver function and structure. Increasing oat intake in the diet would be simple for the average person as it would require either substituting oats for other cereal grains or adding oats to the diet. There are many products available, such as breads, porridge, food bars, oat milk and breakfast cereals which come as wholegrains or bran, as well as β-glucan supplements that come as powders to add to drinks. Therefore, the wide variety of oat products available for consumption would enable people to make a healthy choice in their diet.

This study also looked at coconuts as another traditional food source from South East Asia and the Pacific Islands. Coconuts have a variety of health benefits, such as treatment of diabetes, asthma, dermatitis as well as having antibacterial and antidepressant responses (Babu et al., 2014, Marina et al., 2009a). Coconut oil contains saturated fatty acids, mainly myristic and lauric acids, with cardiovascular and metabolic responses unclear. In Chapter 5 of this thesis, virgin coconut oil and a high fibre coconut Nourish were examined for their health benefits. Both virgin coconut oil and coconut Nourish improved cardiovascular and liver structure and function. Coconut Nourish increased body lean mass, and virgin coconut oil decreased body weight. These changes lead to improved heart health and metabolic syndrome parameters.

The addition of both oat and coconut products to the diet gave beneficial cardio-metabolic effects in this rat model of metabolic syndrome. They are easily available sources of fibre, protein, fats, carbohydrates and micronutrients that can be included in the human diet as functional foods conferring health benefits. This thesis has achieved the aims set in Chapter 1, where the effects of oats and coconut on cardio-metabolic health and on gut structure and function were hypothesised.

The hypotheses of this study were that various oat products (wholegrain oat groats, oat bran and β-glucan powder) would induce metabolic changes and alter gastrointestinal structure and function when added to a high carbohydrate, high fat diet and that oats could be a viable alternative to other grains in the diet. Another hypothesis was that virgin coconut oil and coconut Nourish would also induce cardiometabolic changes when substituted into a high carbohydrate high fat diet.

The first hypothesis is proven that oats in the form of wholegrain oat groats, oat bran and β-glucan powder induces metabolic changes and improves gastrointestinal structure and function. However, the second part of the hypothesis that changes would be greater with more processing is disproven as no greater changes were observed with the increased processing. The second hypothesis is proven as wholegrain oat groats induced metabolic changes and altered gastrointestinal structure and function when added to a high carbohydrate, high fat diet. The third hypothesis is proven with virgin coconut oil and coconut Nourish improving cardio-metabolic function when added to a high carbohydrate, high fat diet in rats.

6.2 Limitations and future directions

There are clear indications that oat products in the form of wholegrain oats, oat bran and β-glucan powder and coconut products in the form of virgin coconut oil and coconut Nourish improved most of the markers of metabolic syndrome in a rat model. However, the mechanisms of action of these foods remains unclear and the bioactive compounds remain to be clarified. While β-glucans are an important bioactive component, further studies need to determine how they work synergistically with other compounds to produce the improvements.

Clinical trials using a variety of oat products and bioactive compounds would be beneficial to determine how they attenuate metabolic syndrome and to establish the relative benefits of whole foods vs extracts. It is currently suggested that 3 g/day of β-glucan is beneficial for lowering cholesterol (Shen et al., 2016) or 10 – 25 g/day fibre intake (National Health and Medical Research Council, 2013b) so these reports could be a good starting point for determining dosages. Individual bioactive compound dosages would need to be determined and this could possibly be done by starting with the wholegrain oat groat containing all compounds and gradually increase the processing until individual bioactive components are studied. This would need to be a long-term study over 12 - 24 months with washout periods between each intervention. However, compliance may be an issue if taste and texture of the food matrix is unpleasant for each participant.

Limited morphological changes were found due to the structure of the rat gastrointestinal tract. While a rat model may be useful to examine the interaction of wholegrain oat, oat bran and β-glucans with the intestinal morphology and function, anatomical differences are important to note when translating to human studies. While clinical trials can inform metabolic syndrome attenuation for gastrointestinal morphological examination, invasive techniques, such as endoscopy would need to be used to resect tissue samples from the gastrointestinal tract for morphological examination more than once throughout the study. Other options within a clinical trial setting could indirectly measure morphological changes by examining other markers of gastrointestinal health through blood and plasma samples for inflammatory markers, stool samples or intestinal permeability with urine samples. It may be possible to

develop *in vitro* and *ex vivo* techniques using cell culture to grow "mini-guts" or enteroids / colonoids (In et al., 2016), human organs on chips (Ingber, 2016, Kim and Ingber, 2013) or artificial guts (Van den Abbeele et al., 2013b, Barroso et al., 2015) to determine the changes to human gastrointestinal morphology.

The investigations in this thesis raised further topics as valid targets for research.

These topics include:

- Further refinement of the modified high carbohydrate, high fat diet rat model of metabolic syndrome.

As the modified diet was run as a pilot study, it is necessary to further refine it so that in future studies the healthy control is improved and not causing cardiovascular and liver issues. It is expected that by increasing the protein content in this diet that those issues could be alleviated.
This diet could also be promoted as a protein-deficient, carbohydrate-loaded diet, as found in developing nations, this imbalance could be reversed with dietary interventions.

- Investigation of other bioactive compounds found in oats, such as avenanthramides, avenins and arabinoxylans.

The study of other bioactive compounds found it oats would enable a clearer picture of how the addition of oats to the diet attenuates metabolic syndrome biomarkers and whether the compounds work individually or synergistically. It would be expected that each of the compounds improves biomarkers, such as avenanthramides improving vasodilation for cardiovascular function. Arabinoxylans may have prebiotic effects in the gastrointestinal tract and they may improve glucose metabolism. A study on avenin is important due to gastrointestinal sensitivities in some coeliac disease patients, so that morphological studies would confirm or repudiate previous findings on the impact of avenin in the gastrointestinal tract.

- Investigation of various molecular weight oat β-glucan products.

Molecular weight plays an important role in the health benefits of β-glucans, therefore this study is important in examining low molecular weight β-glucans which would be expected to give limited changes compared to high molecular weight β-glucans which

would improve cardiovascular and metabolic markers, such as BMI, abdominal circumference, blood pressure and triglycerides. It would also be expected that the higher molecular weight β-glucans would lead to beneficial microbial changes in the gut.

- Determining the molecular mechanisms for oat products to improve glucose and lipid metabolism and reduce inflammation.

By understanding the molecular mechanisms that lead to oats improving glucose and lipid metabolism as well as those that reduce inflammation, the diet can then be targeted to improve those specific molecules. It would be expected that tight junction proteins, such as occludin and claudin, increase to improve intestinal permeability. Increases in MUC2 to improve the mucus layer of the gastrointestinal tract and increased gut hormone production of CCK, PYY and GLP-1 to improve satiety, decrease food intake, improve glucose and lipid metabolism would also be expected.

- Quantifying the effects of oat products on the diversity of gut microbiota.

As oats contain prebiotic compounds, it would be expected that increases in *Bacteroides* and decreases in *Firmicutes* would be found. By investigating various oat products, this quantification of microbial diversity could lead to more personalised dietary understanding.

- Extending the fatty acid analysis to include caecal and plasma concentrations in the obese rat model used in the studies described in this thesis.

Short chain fatty acids are recognised as a key process in gut and systemic health, Investigating the fatty acids found in the caecum and plasma it gives a more complete picture of what is occurring. Fermentation occurs in the caecum therefore it would be expected that higher concentrations of acetate, propionate and butyrate would be found compared to the faecal samples. Examination of the plasma concentrations would give an indication of how long the SCFA remain in the blood stream to be used. It would be expected that, with a single dose of oat, limited concentrations would remain. However, if consumed regularly, sustained concentrations should remain.

- Elucidating the effects of oat products on mucin production as the basis for the functional improvement of the gastrointestinal tract.

MUC2 produces the mucus layer in the gastrointestinal tract, which prevents host-pathogen interactions. It is expected that increased MUC2 would be produced leading to an increased mucus layer and improvements to the epithelial cell layer and tight junctions preventing 'leaky gut'.

- Investigate other cereals such as barley, spelt and rye, that could have similar bioactive compounds to oats.

  By investigating other cereals that have similar bioactive compounds, it is expected that similar results to the current study would be achieved. However, due to individual variety between cereals such as viscosity, molecular weight, fermentability and botanical structure, different metabolic responses could be measured. Another study could be undertaken that adds a number of different cereals to the diet to investigate the interactions between them.

- The development of varieties of oats high in specific bioactive compounds to improve the availability of the active components.

This would be an interesting study to undertake. However, it is outside the scope of this current research, as these varieties may not yet exist, but could be performed in collaboration with research groups studying crop health.

These studies will provide novel insights in the knowledge gap and give a better understanding of the molecular mechanisms that will strengthen the results from the studies compiled in this thesis. As improving nutrition becomes a tool in individualised health plans, a better understanding of the functionality of foods such as oats and coconut will allow cost-effective ways to manage metabolic syndrome.

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Appendix

Conference Abstract

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THE IMPACT OF OAT B-GLUCAN IN A RAT MODEL OF METABOLIC SYNDROME

Carnahan S.
University of Southern Queensland, Australia

Introduction:
Oats have been a staple of the human diet in Europe for millennia. The potential health benefits of oats may improve the signs of the metabolic syndrome, especially obesity and inflammation. As a prebiotic, oat-derived β-glucan may modulate the gut environment leading to these changes. This study determined whether different dosages of oat β-glucan in the diet produced different physiological, metabolic and morphological responses in a rat model of metabolic syndrome

Methods:
We tested oats as a potential intervention in metabolic syndrome by supplementing the diet of high-carbohydrate, high-fat (HCHF) fed rats with 5% wholegrain oats, 5% oat bran or 5% β-glucan powder for the final 8 weeks of a 16-week protocol. Rats (n=10/group) were assessed for metabolic parameters of heart and liver function including systolic blood pressure, aortic contractility, diastolic stiffness and collagen deposition in the heart, plasma ALT and AST activities and tissue histology. Physiological and metabolic parameters including body composition and oral glucose tolerance as well as morphological changes to the gastrointestinal tract were also measured

Results:
All interventions lowered systolic blood pressure, blood glucose concentrations and abdominal circumference in HCHF-fed rats. Rats supplemented with 5% wholegrain oats, 5% oat bran and 5% β-glucan powder showed systolic blood pressure of 135±2mmHg, 140±1mmHg and 139±3mmHg, respectively compared to 148±4mmHg in HCHF-fed rats without interventions. Blood glucose concentrations, measured by total area under curve for 120 minutes after a glucose bolus, improved from 989±73mmol/L.min in the un-supplemented HCHF rats to 773±34mmol/L.min for the wholegrain, 729±19mmol/L.min for oat bran and 727±25mmol/L.min for β-glucan powder supplemented rats. Abdominal circumference decreased from 23±0.5cm in the HCHF rats to 21±0.4 cm, 21±0.3 cm and 21±0.5 cm in the wholegrain, oat bran and β-glucan powder supplemented rats, respectively. Liver tissue histology indicated decreased fat vacuoles and inflammatory cells in the supplemented groups. Plasma AST concentrations were increased in β-glucan powder 109±17U/L compared to HCHF 67±3U/L with no changes in ALT. Improved gut morphology along the length of the gastrointestinal tract was observed in all three oat-supplemented groups compared to HCHF. The profile of the gastrointestinal tract altered depending on the diet. Villi height of duodenum, jejunum and ileum of HCHF were stunted compared to the oat-supplemented groups. The mucosal thickness of the duodenum was 528±99µm in the HCHF rats compared to 919±28µm, 848±32µm and 920±58µm respectively in the wholegrain oat, oat bran and β-glucan powder supplemented rats. Crypt depth increased in the proximal colon from 85±20µm in the HCHF to 113±5µm, 165±20µm and 104±13µm respectively in wholegrain oat, oat bran and β-glucan powder groups.

Discussion:
Therefore, wholegrain oats, oat bran and β-glucan powder as dietary supplements may promote gastrointestinal health leading to a reduction in cardiovascular and metabolic disorders following a HCHF diet.

Keywords: Prebiotics, Cardiovascular, Gastrointestinal, Metabolic Syndrome, beta-glucan