Beyond β-Glucan: Evaluating the Effects of Oat Protein on Reducing Metabolic Syndrome Markers

by

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A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba

in partial fulfilment of the requirements of the degree of

MASTER OF SCIENCE

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ABSTRACT

Oats are recognized to provide many health benefits that are mainly associated with its dietary fibre, β-glucan. However, the protein derived from oats is largely understudied with respect to its ability to maintain health and attenuate risk factors of chronic diseases. The goal of the current study was to investigate the metabolic effects of oat protein consumption in lieu of casein as the protein source in high fat, high sucrose (HF/HS) fed Wistar rats. Four-week-old rats were divided into three groups and were fed three different experimental diets: a control diet with casein as the protein source, a HF/HS diet with casein or a HF/HS diet with oat protein for 16 weeks. Heart structure and function were determined by echocardiography. Blood pressure measurements, an oral glucose tolerance test, and markers of cholesterol metabolism, oxidative stress, inflammation and liver function were also performed. Our results indicate that incorporation of oat protein in the diet was effective in preserving systolic heart function in HF/HS fed rats. Oat protein significantly reduced serum total- and LDL-cholesterol levels. Furthermore, oat protein normalized liver HMG-CoAR activity, which to our knowledge, is the first time this has been reported in the literature. Therefore, our research suggests that oat protein can provide hypocholesterolemic and cardioprotective benefits in a diet-induced model of metabolic syndrome.

ACKNOWLEDGEMENTS

I would like to express my sincerest gratitude to my co-advisors, Dr. Sijo Joseph and Dr. Maneka Malalgoda for their constant encouragement and support throughout this project. Their guidance and reassurance have been paramount in keeping me focused and motivated. Thank you, Dr. Joseph for showing me such kindness and understanding and for giving me the tough pep talks and advice that I really needed to hear at my lowest points. I'm very grateful to have had the pleasure to be a part of your team. I would also like to express gratitude to the other members of my advisory committee, Dr. Thomas Netticadan, Dr. Lovemore Malunga, and Dr. Vanu Ramprasath for their much-appreciated ideas and support.

Thank you to POGA, PepsiCo Quaker, Agriculture and Agri-food Canada and the Canadian Agricultural Partnership's AgriScience Program for funding this study. Thank you to Buffalo Creek Mills for providing the oats used in this study. Thank you to the wonderful professors in the Department of Food and Human Nutritional Sciences at the University of Manitoba who have taught me so much through their courses.

Thank you to Dr. Thomas Netticadan for the opportunity to work in his lab at CCARM and for being a wonderful mentor. I truly value your guidance, the opportunities and the motivational advice you have given me throughout the length of my program. Thank you to Dr. Liping Yu and Dr. Pema Raj for the encouragement and assistance with the study and the statistical analysis of the data. Thank you to Dr. Babak Sobhi, Li Ren and the amazing staff at the R.O. Burrell Animal Facility for their help with the study. Thank you to all of the friends I've made along the way for your love and support!

Finally, I would like to dedicate this thesis to my parents, Tannis and Denis Bouchard, whom have constantly supported, inspired and motivated me throughout my life. To me, they are the pinnacle of strength, dedication, generosity and perseverance. Mom and Dad, you are my rocks. I love you both more than words could ever express. I'm just as proud of you both as you are of me. Thank you for always believing in me and molding me into the woman I am today.

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LIST OF ABBREVIATIONS

ACE - Angiotensin converting enzyme

Ang - Angiotensin

ANOVA - Analysis of variance

BCAA - Branch chain amino acid

CETP - Cholesteryl ester transfer protein

CRP - C-reactive protein

CVD - Cardiovascular disease

CYP7A1 - Cholesterol 7 alpha-hydroxylase

DPP-IV - Dipeptidyl peptidase-4

EFSA - European Food Safety Authority

FFA - Free fatty acids

GLUT-4 - Glucose transporter type 4

HAEC - Human aortic endothelial cells

HF/HS - High fat, high sugar

HMG-CoAR - Hydroxymethylglutaryl coenzyme A reductase

HOMA-IR - Homeostatic model assessment for insulin resistance

IL - Interleukin

IRS - Insulin receptor substrate

LDL - Low density lipoprotein

LDLR - Low density lipoprotein receptor

LPL - Lipoprotein lipase

MetS - Metabolic syndrome

OGTT-AUC - Oral glucose tolerance test - area under curve

OP - Oat protein

PAI-1 - Plasminogen activator inhibitor-1

PI3K - Phosphoinositide 3-kinase

RAS - Renin angiotensin system

RS - Resistant Starch

RDS - Rapidly digestible starch

SCFA - Short chain fatty acids

SMC - Smooth muscle cell

SNS - Sympathetic nervous system

T2D - Type-2 diabetes

TG - Tryglycerides

TNF-α - Tumor necrosis factor-α

VLDL - Very low density lipoprote

1.0.INTRODUCTION

Oat (*Avena sativa L.)* is an important cereal crop that has been historically used as a nutritious food source and for medicinal purposes (Webster, 1986). As of 2022, Canada is the leading oat exporter and the second largest oat producer worldwide, following the European Union (Prairie Oat Growers Association, n.d.; Statista, 2023). Oats were predominantly used as animal feed until the 1980s, where they garnered the attention of both the food industry and consumer market due to beneficial health effects related to oat consumption (Wood et al., 1991).

Metabolic syndrome (MetS) is a condition that involves the clustering of the following risk factors: abdominal obesity, dyslipidemia, hypertension, and hyperglycemia. MetS significantly increases the risk of developing a variety of chronic diseases such as cardiovascular disease (CVD), type 2 diabetes (T2D), cancer, non-alcoholic fatty liver disease, dementia and chronic kidney disease (Beck-Nielsen, 2014) (Figure 1). According to combined data from the Canadian Health Measures Survey cycles 3 and 4, the prevalence of MetS is estimated to be just over 21% in Canadians aged 18 and older (Statistics Canada, 2016)**.** Therefore, prevention and management of MetS and its risk factors through proper nutrition and exercise are incredibly important to promote good health and ward off chronic disease and premature mortality.

Most of the health benefits related to regular consumption of oats have been attributed to their high β-glucan content, which has been evidenced by many clinical trials to be effective in lowering low density lipoprotein (LDL)- and total-cholesterol, regulating blood glucose levels, and providing a positive impact on gut health. This ultimately reduces the risk of developing chronic diseases such as obesity, CVD and T2D (EFSA Panel on Dietetic Products, 2011; Health Canada, 2010; Y. Wang et al., 2016).

In fact, Health Canada approved a health claim in 2010 that links the daily consumption of at least three grams of oat β-glucan and a reduced risk in heart disease by lowering serum LDL- and total-cholesterol levels (Health Canada, 2010). The following year, another health claim substantiated by the EFSA determined that oat β-glucan is able to lower post prandial glycemic response, provided there is four grams of the soluble fibre for every 30 grams of available carbohydrates (EFSA Panel on Dietetic Products, 2011).

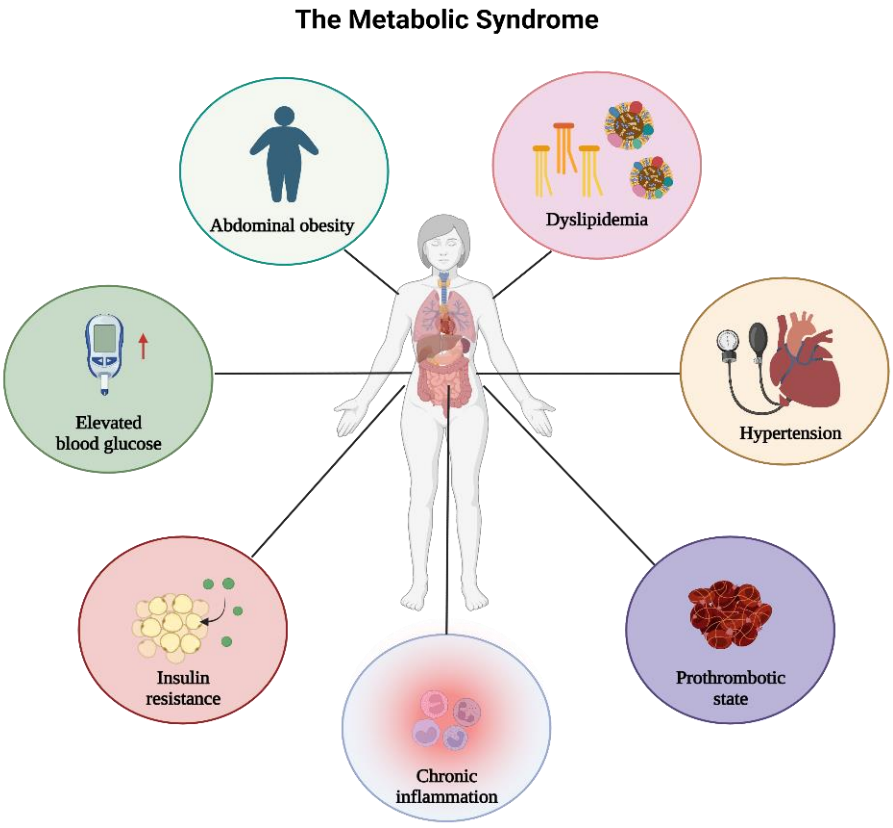

Figure 1: Clinical and Biochemical Features of Metabolic Syndrome. Created with Biorender.com

In addition to their fibre content, oats are a great source of sustainable, high-quality plant protein. Oats contain roughly 12-20% protein, which is exceptionally high compared to most other cereal grains (Mel & Malalgoda, 2022). Oat protein contains relatively higher concentrations of essential amino acids such as lysine, leucine and valine (Shewry, 2007). This is due to the much higher proportions of globulins and lower proportions of prolamins found in oat protein. Prolamin dominated cereals such as wheat, barley and rye tend to have a lower essential amino acid content due to higher concentrations of the non-essential amino acids proline and glutamine (Payne, 2003). Furthermore, oat protein surpasses the World Health Organization essential amino acid requirements in all but lysine and methionine (Sterna et al., 2016). Therefore, oats have excellent potential as a novel plant-based protein ingredient in the food industry.

Higher dietary protein intake has been associated with improvements in cardiometabolic biomarkers, including fat mass, insulin resistance markers, blood glucose, blood cholesterol and blood pressure levels, subsequently leading to a decreased risk of MetS incidence (Gannon et al., 2003; Hajihashemi et al., 2021; Kahleova et al., 2018; Lépine et al., 2023). In particular, emerging scientific evidence suggests that frequent plant protein consumption can significantly reduce the incidence of MetS and its risk factors through different mechanisms than animal based protein products. This is due to the differences in the amino acid profiles of the respective protein sources. Animal protein is richer in indispensable branch chain amino acids (BCAA), while plant protein contains higher amounts of dispensable amino acids such as cysteine, arginine, and glycine, which have been respectively positively and negatively linked to cardiometabolic risk (Lépine et al., 2021, 2023). In addition, the presence of bioactive peptides found within plant-derived proteins may provide protective effects against chronic disease (Bouchard, Malalgoda, et al., 2022).

Moreover, plant protein may allow the body to more efficiently utilize lipids and carbohydrates for protein synthesis, which may offer protective effects against metabolic dysfunction (Lépine et al., 2023). However, there is a gap in the current knowledge detailing the mechanistic basis in which plant protein acts to provide health benefits and protection again MetS and chronic disease risk factors.

Recently, numerous studies have evaluated the functional properties of oat protein for novel food product development (Mel & Malalgoda, 2022). But oat protein is understudied pertaining to comprehensive health effects such as its impact on oxidative stress, inflammation, glucose metabolism, blood pressure as well as heart, liver and kidney function. Though preliminary studies have reported the potential hypocholesterolemic effects of oat protein supplementation in animals (L. Guo et al., 2014; Tong et al., 2016). Therefore, the objective of the current study was to evaluate whether replacing casein (the conventional protein source in rat chow) with oat protein as the dietary protein source has any cardiometabolic effects in high-fat, high sucrose fed rats.

The specific objectives of this research study were to:

1. Extract oat protein from Canadian grown oats through wet extraction processing and perform proximate analysis for nutritional quality.
2. Replace casein with oat protein as the protein source in a high fat, high sucrose fed rat model and evaluate its effects against risk markers associated with metabolic syndrome.
3. Elucidate the biochemical mechanisms by which oat protein may improve these risk markers.

2.0. LITERATURE REVIEW

2.1. OATS OVERVIEW

2.1.1. MARKET VALUE OF OATS AND POTENTIAL OF OAT PROTEIN

Oats were predominantly used as animal feed until the 1980s, where they garnered the attention of both the food industry and consumer market due to beneficial health effects related to oat consumption (Wood et al., 1991). As of 2022, Canada is the leading oat exporter and the second largest oat producer worldwide, following the European Union (Prairie Oat Growers Association, n.d.; Statista, 2023). Canadian oats comprise roughly 40% of global exports. The prairie provinces, Manitoba, Saskatchewan, and Alberta account for around 90% of oat production that takes place in Canada (Yan et al., 2011).

According to the National Research Council Canada, the growing demand for alternative protein sources has been largely attributed to an increasing consumer interest about the health benefits of a protein-rich diet and plant-based alternatives, rising global demand for protein sources due to rapid population growth, consumer awareness about the environmental impacts of animal protein, and willingness to protect animal welfare. Annual global sales of plant-based meat alternatives have grown, on average, 8% annually since 2010. (National Research Council Canada, 2022).

Oats are a sustainable, cost-effective source of high-quality plant protein. Currently, oat protein is an under-utilized ingredient. However, there is increasing interest in using oat as a protein ingredient in the food industry due to excellent functional properties. Oat protein exhibits better fat binding and emulsifying properties than soy protein and has been identified as a cost-effective, heat stable, gelling and foaming agent.

In addition, the structural characteristics of oat protein concentrate are suitable for microbial fermentation, which have been applied to developing oat-based dairy free yogurt products (Mel & Malalgoda, 2022). Therefore, oat protein demonstrates tremendous potential as a novel plant-based protein ingredient which could be used as a replacement to soy protein.

2.1.2. NUTRITIONAL COMPOSITION OF OATS

The following section is taken in part and modified from this review article:

Bouchard, J., Valookaran, A. F., Aloud, B. M., Raj, P., Malunga, L. N., Thandapilly, S. J., & Netticadan, T. (2022). Impact of oats in the prevention/management of hypertension. Food Chemistry, 381, 132198. https://doi.org/10.1016/J.FOODCHEM.2022.132198

2.1.2.1. MORPHOLOGY OF OATS

Oats come from the Gramineae (grasses) family. Among the 70 species of oats that have been identified, the two most common cultivated variants are husked oat (*Avena sativa L.*) and naked oats (*Avena nuda L.*) (Punia et al., 2020). A mature whole oat grain consists of four major fractions namely hull (husk), bran, endosperm and germ. Husked oats are enclosed in a lignified hull and the groat does not thresh free from the hull when harvested. The hull consists of the fibrous lemma and palea which are covered in fine trichomes (Webster, 2002). In naked oats, the hull is thinner, contains much less lignin and is less rigid, permitting the groat to thresh free during harvest. In general, hulls represent 25 – 30 % of the oat grain dry mass and contains lignin (12 – 25 %), cellulose (12 – 25 %), hemicellulose (24 – 33%), ash (5 – 7%), starch (2–17%), proteins (1–8%) and lipids (0.3 – 2%). The hull composition is significantly affected by genotype and growing environment. As the husk is not suitable for human consumption, it is removed mechanically to produce oat groats.

The bran is the outer layer of the oat groat and is approximately 30% of the oat groat's dry weight and stores most of the vitamin and mineral content (Frølich & Nyman, 1988). It is made up of pericarp, seed coat, nucellus, sub-aleurone layer and aleurone layer. The aleurone layer is very metabolically active and contains many endogenous hydrolases that cleave storage molecules in the starchy endosperm to supply nutrients for the seedling and to aid in the germination process. In terms of biomass, the bran contains protein (22 %), β-glucan (17 %), arabinoxylan (13.2 %), cellulose (2.9 %), lipids (14%) and ash (6.8 %). The bran is also concentrated in B-vitamins, minerals and various phytochemicals. The endosperm contains approximately 55–70% of the weight of the groat (Klose & Arendt, 2012; Stenhouse, 2011). The endosperm contains 70–78 % starch, 9–12 % protein, 6 – 8 % fat, and 4 – 6% dietary fibre. This component is considered relatively metabolically inactive compared to the bran and the germ (Stenhouse, 2011). The germ is the plant embryo which eventually sprouts into a new plant. It is the smallest fraction and accounts for 3% of the groat (Klose & Arendt, 2012). The germ contains the embryo and scutellum which is comprised of the parenchyma and the epithelium and stores substantial amount of protein and lipids. Similar to bran, the germ tends to be quite metabolically active and is rich in nutrients including proteins, lipids, various minerals, B-vitamins, vitamin E and other antioxidants (Klose & Arendt, 2012; Stenhouse, 2011). Although oats are consumed much less frequently than wheat and rice, they are particularly preferred due to the benefit of usually being eaten as a whole grain rather than in highly processed forms (Stenhouse, 2011).

2.1.2.2. OAT CARBOHYDRATES

Oat carbohydrates consist of 99 % polysaccharides and are low in fermentable oligosaccharides, disaccharides, monosaccharides, and polyols. Oats contain no more than 1% of free sugars, such as sucrose, stachyose and raffinose. The majority of the sugars are found in the bran of the oat, and a small amount is located in the endosperm as well (Menon et al., 2016). The important polysaccharides found in oat groat include starch and β-glucan. The main form of carbohydrate in oats is starch, which makes up about 40–60% of oat groat composition depending on the variety (Arendt & Zannini, 2013; Rasane et al., 2015).

2.1.2.3.. OAT STARCH

Starch accounts for 40–60 % (w/w) of the oat groat and is primarily located in the endosperm region. Starch has two polysaccharide strands namely amylose and amylopectin and which represent 98–99 % of the starch granule content in oats (Punia et al., 2020). The primary structure of amylose consists of a linear chain of (1 → 4) linked α-D- glucopyranosyl residues and an occasional branching (less than 1 %) at (1 → 6) linked α-D- glucopyranosyl residues. Amylopectin is much similar in structure to amylose except that the branching frequency at α-1, 6 linkage is much higher (greater than 3 %). Consequently, oat amylose tends to have a lower degree of polymerization of ~ 3000 compared to that of amylopectin (~5000). These starch polymers exhibit different nutrition and physicochemical properties such as digestibility, stability, solubility, gelatinization temperature, retrogradation rate, and viscosity. The amylose content of oat starch may vary from 2-38 % depending on genotype and environment. Heat treated oats tend to have higher amylose content compared to raw oats. The variations in amylose content in literature has also been attributed to the analytical method used (Webster, 2002).

The oat starch granule has a predominantly A-type polymorphism similar to other cereal grain starches but relatively higher crystallinity values (29 – 36 %) than barley and lower than that of rice, wheat, and corn. Oat starch granules are small and irregular or oval in shape with a well-defined granule surface. They range from 2-12 μm in diameter but tend to exist in clusters which range from 20-150 μm in diameter. Because of their tendency to cluster, it very difficult to classify the shape of oat starch granule as type A or B. Oat starch clusters have a polygonal and ovoid shape. These differences in amylose content and morphology of oat starch, together with the presence of lipids, protein, and ash, have direct influence on the rheological and physico-chemical properties of starch (Webster, 2002). Notably, oat starch contains a higher amount of lipids (1-3%) than other cereal starches such as wheat, corn and rice (0.5-1.2%) (Y. Li et al., 2021; Sayar & White, 2011). The lipids are bound to starch either in the middle of the amylose helix or in the gap between amylose and amylopectin in the starch granules (Angelov et al., 2018). Consequently, oat starch has a relatively higher amount of amylose-lipid complex after cooking, which may reduce solubility, gel formation, retrogradation and digestibility of the starch, although oat starch is considered to be highly digestible. The grand majority of lipids found in oat starch are lysophospholipids and free fatty acids (Y. Li et al., 2021; Sayar & White, 2011).

Nutritionally, starch is categorized by digestion rate namely rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). Oats have been reported to contain approximately 7% RDS, 22 % SDS and 25 % RS (Ovando-Martínez et al., 2013). SDS and RS are considered important fractions for human health, as they moderate glycemic response and may provide other physiological benefits that will be discussed in detail in section 2.2.3..

2.1.2.4. OAT FIBRE

Based on the capacity to dissolve in water and subsequent impact on physiological outcomes, the dietary fibre content in cereals is classified into soluble and non-soluble components. The non-soluble fibre fraction consists of lignins, cellulose and hemicellulose. In contrast, the soluble fibre portion primarily consists of the polysaccharide fraction, mainly β-glucan as the major component in case of oats. Among the major cereal grains, wheat and oats carry the highest content of total dietary fibre and the ratio of soluble to insoluble fibre is comparatively higher in oats (Gulvady et al., 2014). In addition, whole oats contain very high levels of β-glucan (approximately 5 times more) compared to wheat.

2.1.2.5. OAT β-GLUCAN

B-glucan is the predominant soluble fibre component in oats and is primarily located in the cell walls of the starchy endosperm and is concentrated in the aleurone and sub-aleurone layer in the bran (Sangwan et al., 2014). The β-glucan content in oat groats ranges from 3-7 % (Ajithkumar et al., 2005; Andersson & Börjesdotter, 2011; Redaelli et al., 2013; Shewry et al., 2008), of which 50 – 90 % is water soluble. The content of β-glucan in oat is highly affected by genotype, environment and their interaction with genotype variation (Redaelli et al., 2013). Consequently, Canadian oat variety registration requires a minimum of 4.8 % db of β-glucan (Prairie Grain Development Committee, 2023).

Structurally, β-glucan is a mixed linkage polymer of D-glucopyranosyl residues linked together by β-(1–4) linkages (70 %) and interrupted by β-(1–3)- linkages (Kaur et al., 2019).

These D-glucose residues are mostly found in β-(1–4)-cellooligosaccharides (DP = 3 or 4) blocks (>90%) and higher DP oligosaccharides (DP ≥ 4) representing less than 10 %. The ratio of cellotriose to cellotetraose for oat (1.9 – 2.4) is smallest among cereal grain β-glucan (wheat (3.0–4.5), Barely (2.8 – 3.3) and rye (3.0–3.2)). As such, cellotriose residue ranges from 53 to 61 % in oat β-glucan and cellotetraose ranges from 34 to 41 %. β-glucan with higher percentage of cellotetraose or higher DP β-(1–4)-cellooligosaccharides tends to be less soluble probably due to increased intermolecular non-convalent interactions (Woodward et al., 1983). The ratio of cellotriose to cellotetraose is dependent on genotype, environment and their interaction. The variation of this ratio observed in literature may also be attributed to the differences in extraction and analytical methods. Therefore, the difference between β-glucan and cellulose is the presence of β-(1–3)- linkages in β-glucan molecule. Thus, the number of β-(1–3)- linkages in a β-glucan molecule dictates the physiochemical properties of β-glucan part from its molecular weight and the cellotriose and cellotetraose ratio. For oat β-glucan, the ratio of β-(1–4) to β-(1–3)- linkages ranges from 2.3 to 2.7 (Skendi et al., 2003).

In aqueous solution, oat β-glucan exists in different conformations such as rod like shape, random coil, and aggregated depending on molecular weight and concentration. Oat β-glucan aggregate conformation structures are less dense compared those of barley β-glucan (Zielke et al., 2017). The molecular weight and conformation of oat β-glucan heavily influence a number of physical properties, including solubility, solution viscosity and viscoelasticity, and gelation qualities (Wang & Ellis, 2014). For example, higher molecular weight and lower cellotriose to cellotetraose ratio β-glucan tends to have higher viscosities compared those with lower molecular weight and higher cellotriose to cellotetraose ratio (Wolever et al., 2010).

Furthermore, high molecular weight oat β-glucan will not form a gel, but at a reduced molecular weight, gels can be formed under certain conditions (Wang & Ellis, 2014).

2.1.2.6. OAT PROTEIN

Oats have relatively high levels of protein, which gives them a high nutritional value in comparison to other cereal grains. Protein content can range between 11-15% in hulled oats and 12.4-24.5% in dehulled oats, depending on genotype and environmental conditions (Gulvady et al., 2014). The majority of the proteins are located in the bran and germ of the oats, and a smaller proportion can be found in the starchy endosperm enclosed in membrane-bound protein bodies. The highest concentration of protein is found in the periphery of the endosperm and it decreases moving closer to the interior of the kernel (Gulvady et al., 2014). The protein bodies have a diameter of 0.3 – 5 μm which varies considerably with grain fraction (Lásztity, 1998).

According to Osborne classification, there are four types of proteins in oat which are classified by their solubility namely globulins, albumins, prolamins and insoluble glutelin. Globulins are saltwater soluble and make up 70–80% of total protein in oats. This is a unique quality, as the major storage protein fraction in other cereal grains (with the exception of rice) is prolamins. Oats contain 3 S, 7 S and 12 S globulins with the latter being most abundant (Klose & Arendt, 2012). The 12 S globulin have a molecular weight of 324 kDa and are comprised of six 32 kDa acid subunits and six 22 kDa basic subunit polymers. These α- and β- subunits are bonded together via disulfide bonds to form 54 kDa subunits. Finally, these 54 kDa subunits are held together by noncovalent molecular interaction to form a 324 kDa hexamer (Klose & Arendt, 2012).

Globulin isolated from the cell wall is considerably more water soluble compared to the insoluble globulin derived from the protein bodies of the endosperm which could possibly result from carbohydrate-globulin binding and protein-protein interactions between globulin molecules. The minor amount of 3 S and 7 S globulins are believed to be vicilin-like proteins. The major components of 7 S globulins are 55 kDa polypeptides, although minor components with molecular weights of 65 kDa are also present. A 3 S fraction consists of at least two major components weighing 15 and 21 kDa (Klose & Arendt, 2012)

Prolamins are alcohol soluble proteins. The prolamins present in oats are called avenins. The prolamin content ranges from 4 to 15% of total protein in oats which is very low compared to other cereal grains (wheat (75–80%), rye (45–50%), and barley (50–55%)) (Klose & Arendt, 2012). Oat prolamins have a molecular weight of 20–40 kDa and are known to exist as both monomers and disulfide-linked aggregates (Comino et al., 2015). They possess structural similarities with α- and γ-gliadins of wheat, γ-secalins of rye, and β-hordeins of barley protein (Rafique et al., 2022). Avenins contain 35-50% proline and glutamine, which is much lower than prolamins in the wheat, barley, and rye which can have proline and glutamine contents that can exceed 70% of their total amino acids (Comino et al., 2015).

Oats are generally regarded as gluten-free and safe to consume for individuals with celiac disease. However, this statement is subject to controversy. Many of those with celiac are able to consume oats without adverse effects, provided the oats are not contaminated with gluten containing cereals during processing (Holm et al., 2006; Størsrud et al., 2003).

On the contrary, it has been reported that avenins may trigger an immune reaction in some celiac patients by a mechanism similar to the response to gluten found in wheat, rye, or barley. A study by Sjöberg et al. determined that oat consumption can alter the mRNA immune status profile of intestinal mucosa cells, which suggests the activation of T-cells and the presence of leaky tight-junctions. Furthermore, the presence of anti-avenin antibodies has been reported in children with celiac disease (Hollén et al., 2003). Specifically, the epitopes Av-α9A (PYPEQQEPF) and Av-α9B (PYPEQQQPF) found in avenin may be responsible for immune response in celiac disease patients, but very few are reported to have a reaction, making oat intolerance a rather rare event (Gilissen et al., 2016; Londono et al., 2013; Lundin et al., 2003). Therefore, it is important that individuals with celiac disease ensure they are not also sensitive to oats before incorporating them into their diet on a case-by-case basis. Consumption of up to 100 g of oats per day is considered safe for adult celiac patients. For consumer protection, oat can be labelled gluten free or low in gluten only when it contains a maximum of 20 or 100 mg avenins/Kg, respectively, according to the Codex Alimentarius Commission (Codex Stan 118–1979) and the European Regulation No 828–2014. For this purpose, Codex and EU recommends avenin content should be based on immunological assays. Oats contain 13 – 53 mg/Kg avenin content, which varies greatly with genotype (Ahola et al., 2020; Londono et al., 2013) . Certain oat cultivars are known to produce more of an immunological response than others (Pulido et al., 2009). It is important to note that no correlation exists between prolamins/avenin content of oats estimated by Osborne fractionation process and that measured by immunological assays.

Albumins are water soluble, and they make up 1–12 % of the protein. The major components in the albumin fraction have molecular weights of 14-17, 20-27, and 36-47 kDa (Lásztity, 1998). This fraction contains mainly metabolically active proteins such as enzymes and enzyme inhibitors. Enzymes such as proteases, maltase, α-amylase, lichenase, phytase, phosphatase, tyrosinase, and lipase have been found in the albumin fraction (Klose & Arendt, 2012; Lásztity, 1998). Since oats contain a much higher lipid content than other cereal grains, their lipase activity is significantly higher compared to that of wheat, rye and barley, for example (O'Connor et al., 1992). Oat lipase activity is unevenly distributed in the kernel, as it is associated with parts located in the aleurone layer, close to the surface of the caryopsis. Oat lipase activity is highest in flour fractions closest to the outer bran layer and lowest in the starchy endosperm fractions (Lásztity, 1998). Lipase activity in oats is responsible for the degradation of lipids, starting with hydrolysis followed by oxidation. This ultimately results in the rancidification of oat products, which is the main limiting factor for storage and handling (Ekstrand et al., 1993). Hydrothermal processing is used to inhibit enzyme activity in oats to prolong their stability and shelf life.

Glutelins are insoluble proteins in their native form and only becomes alcohol soluble when reduced by a reducing agent. Glutelins make up under 10% of protein present in oats (Lásztity, 1998). Values ranging from 5-66% have been reported. However, the amount of glutelins observed is usually directly related to the efficacy of the preceding extractions of the albumins, globulins and prolamins while using the Osbourne fractionation method and variations (Klose & Arendt, 2012). Oat glutelin fractions are reported to contain polypeptides with a molecular weight of 10-90 kDa. (Jing et al., 2016).

There is very little information available in the literature pertaining to the molecular characterization of oat glutelins such as amino acid and peptide sequences as well as biological and functional properties.

The quality of cereal proteins is determined by their amino acid composition and digestibility. Due to the high globulin content and low prolamin content in oats, there is an increased essential amino acid content (ranging from 1.73-24.21 mg/g) and a superior protein quality compared to other cereals such as wheat, barley and rye (Klose & Arendt, 2012). Oats contain a comparatively high amounts of tryptophan, arginine, asparagine-aspartic acid, threonine and the limiting amino acid in cereals, lysine (Klose & Arendt, 2012; Mel & Malalgoda, 2022). The amino acid content of oats varies with the cultivar. Naked oats are reported to contain higher amounts of essential amino acids compared to hulled oats. Furthermore, oat protein is reported to surpass the FAO essential amino acid requirements in all but lysine and methionine, allowing oat protein to have nearly the same quality as egg, soy, and milk protein (Sterna et al., 2016).

2.1.2.7. OAT LIPIDS

Oats have an exceptionally high lipid content compared to other cereals. Dehulled oats are reported to have an oil content of 2–12%, while wheat, rye and barley contain only 1–3% (Bryngelsson et al., 2002). The oil content in oats is genotype dependent even though it may also be affected by agronomic practices. Reports suggest that increasing nitrogen dosage in fields tends to increase oil accumulation in oat grain (Yan et al., 2017). While lipids are mostly concentrated in the germ fraction of grain for most cereal grains, lipids in oat are most concentrated in the endosperm.

Oat oil constitutes triacylglycerols (32–85%), diacylglycerols (1.8–3%), free fatty acids (2–11%), sterol esters (0.1–4%), phospholipids (6.0–26%), and glycolipids (5.8–17%) (Batalova et al., 2019). The three most abundant fatty acids in oats are palmitic (C16:0), oleic (C18:1), and linoleic (C18:2) acids, which make up 90–95% of total fatty acids content. Other saturated fatty acids reported in oat oil include myristic (C14:0), stearic (C18:0), and eicosenoic (C20:1) acid (Lehtinen & Kaukovirta-Norja, 2011). Alpha-linolenic acid (C18:3) constitutes 0.5 – 4 % of lipids in oats, which provides a good source of omega-3 (Batalova et al., 2019).

2.1.2.8. OAT VITAMIN AND MINERAL CONTENT

Based on the solubility characteristics, oats contain significant amounts of both water and fat-soluble vitamins, including B-vitamins such as thiamine, riboflavin, niacin, pyridoxine and folate which play a significant role in energy and amino acid metabolism. They are also a rich source of tocopherols (vitamin E) which have antioxidant properties (Lasztity, 1998). Like other grains, oats are rich in minerals including magnesium, potassium, phosphorus, and iron and in lower concentrations, calcium, copper, and zinc (Lásztity, 1998). The majority of the mineral content in oats is associated with the soluble fibre fraction and that oat bran fibre binds minerals tightly despite the presence of high levels of phytic acid (Menon et al., 2016).

2.1.2.9. OAT PHENOLIC CONTENT

Phenolics are compounds with one or more aromatic rings with one or more hydroxyl groups and classified as phenolic acids, flavonoids, stilbenes, coumarins and tannins (Liu et al., 2004a). Oats are considered as a great source of phenolic acids. Total phenolic content of oats was reported to be nearly 6.5 µmol gallic acid equiv/g grain.

Most of the phenolic compounds in oats are derived from hydroxybenzoic and hydroxycinnamic acids such as ferulic, caffeic, p-coumaric, vanillic and synergic acids (Kováčová & Malinová, 2008). Phenolic compounds in oats possess antioxidant properties which prevents damage from free radicals and lipid peroxidation. Many of the phenolic acids found in oats are associated with the soluble fibre fraction (Menon et al., 2016). The phenolic content in oats is significantly influenced by genotype, environment and their interaction (X. ping Li et al., 2017; Nkhata Malunga et al., 2022).

Avenanthramides belong to a class of phenolic alkaloids that are mainly found in oats and are not present in other cereal grains. They are low molecular weight phenolic amides which consist of an anthranilic acid linked to a hydroxycinnamic acid by an amide bond. These compounds are phytoalexins, which are produced by the oat plant as a defence mechanism against pathogenic organisms such as fungi (Perrelli et al., 2018). Avenanthramides are simply classified as avenanthramide A-E. A, B and C are the major forms extracted from oats. Although 20 different forms of avenanthramides are reported in the literature (Meydani, 2009). The concentration of avenanthramides is reported to range from 26-150 mg/kg in oat products, which is heavily influenced by environmental stress, growing location and genotype. They appear in almost all milling fractions of the oat but are most concentrated in the bran (Menon et al., 2016).

2.1.3. THE IMPLICATIONS OF OATS IN HEALTH

The following section is taken in part and modified from this review article:

Bouchard, J., Valookaran, A. F., Aloud, B. M., Raj, P., Malunga, L. N., Thandapilly, S. J., & Netticadan, T. (2022). Impact of oats in the prevention/management of hypertension. Food Chemistry, 381, 132198. https://doi.org/10.1016/J.FOODCHEM.2022.132198

Oats contain several bioactive components that provide beneficial health effects (Figure 2). The following section highlights the health promoting and disease-fighting benefits that oats have to offer.

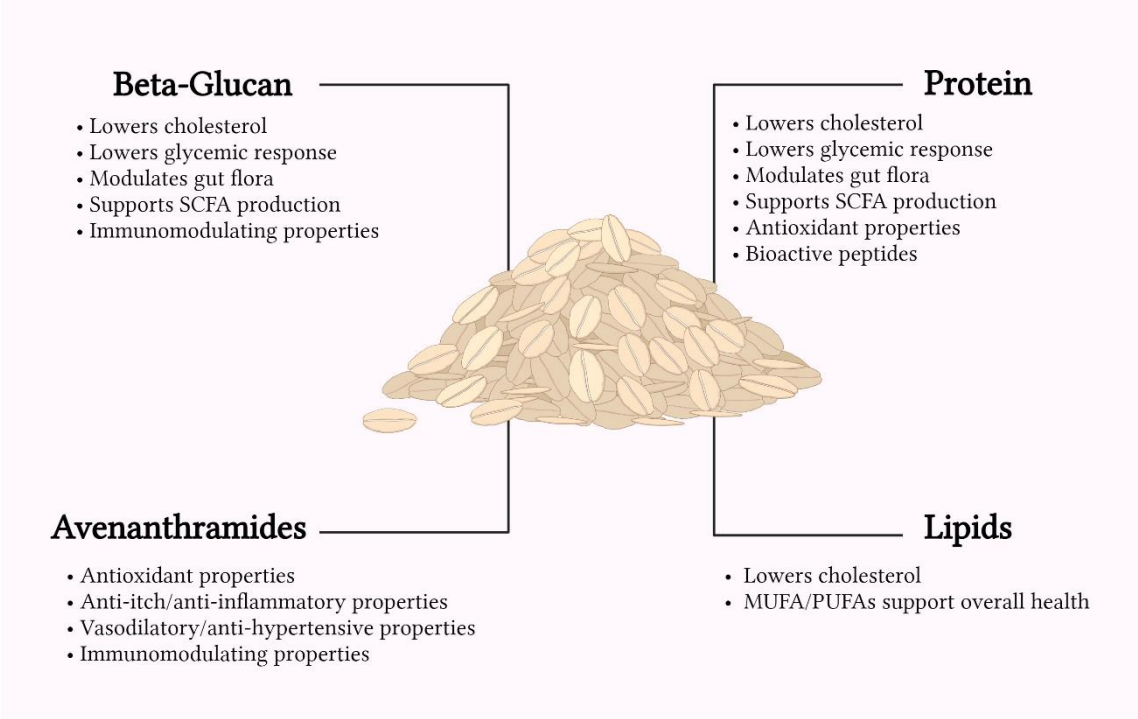

Figure 2: Summary of the health effects exerted by oat components found in pre-clinical and clinical trials. SCFA, short chain fatty acids; MUFA, mono-unsaturated fatty acid; PUFA, poly-unsaturated fatty acid. Created with Biorender.com

2.1.3.1. β-GLUCAN

β-glucan is the main driver of the rise in consumption and marketability of oats because of its associated health benefits such as lowering cholesterol and triglycerides (TG), managing blood pressure, improving glycemic response, favorably modulating gut flora, modifying immune response and providing protective effects against colon cancer (Daou & Zhang, 2012; Murphy et al., 2010; Shoukat & Sorrentino, 2021; Q. Wang & Ellis, 2014; Whitehead et al., 2014). β-glucan health claims to reduce blood cholesterol have been recognized by USFDA, Health Canada, EFSA, Malaysia MoH and Food Standards Australia New Zealand. Furthermore, the EFSA has recognized that oat and barley β-glucans are able to reduce post prandial glycemic response provided that there are 4 g of β-glucans for each 30 g of available carbohydrates (EFSA Panel on Dietetic Products, 2011).

The molecular weight, solubility, and viscosity of β-glucan are important physicochemical properties that heavily influence their cholesterol lowering properties and other health effects. A higher molecular weight of oat β-glucan results in a higher viscosity and subsequently a greater cholesterol and glucose reducing effect. Furthermore, when ingested, soluble fibre will imbibe water, swell, and dissolve in relationship to its size and previous hydrothermal treatments. The solubility of β-glucan influences its capacity to increase its viscosity in aqueous systems. The increase in volume also provides a satiating effect which can aid in weight loss or maintenance. (Daou & Zhang, 2012; Mäkelä et al., 2020).

The health effects of β-glucan are attributed to its capacity to form a viscous mixture in the gastrointestinal tract. This slows gastric emptying and impedes digestive enzymes from encountering their substrates as well as decreases the rate of nutrient transportation and absorption (Mäkelä et al., 2020). Thus, β-glucan is able to significantly attenuate post-prandial blood glucose and insulin response as confirmed by clinical trials (Mohiuddin, 2022; Q. Wang & Ellis, 2014; Wood, 2010)

The cholesterol lowering effect of β-glucan is primarily associated with its ability to entrap bile acid micelles and prevent their reabsorption, promoting microbial conversion to metabolites, thus increasing their fecal excretion. Bile acids synthesized in the liver from cholesterol, are actively reabsorbed by the terminal ileum and undergo enterohepatic circulation (Hyun & White, 2010). The increased excretion of fecal bile acids thereby increases the rate of *de novo* synthesis of bile acids to replenish the hepatic bile acid pool, which results in a reduction in serum total- and LDL-cholesterol (Joyce et al., 2019; Tong et al., 2016). Research has shown increased fecal bile acid excretion following consumption of oat products or β-glucan. Evidence of elevated *de novo* bile acid synthesis and modulation of cholesterol metabolism following consumption of oat β-glucan is reported both in animals through increased activity of hepatic rate limiting enzymes in cholesterol and bile acid synthesis and transport pathways such as LDL receptor (LDLR), HMG-CoAR and CYP7A1 (J. Chen & Huang, 2009; Tong et al., 2015, 2016) or in humans, through the measurement of a bile acid synthesis marker, 7-alpha-hydroxy-4-cholesten-3-one (Joyce et al., 2019).

Animal and human intervention studies show that oat β-glucan significantly impacts the composition of the gut microbiome, which can influence glucose and cholesterol metabolisms as well as overall cardiovascular health (Jayachandran et al., 2018). β-glucan consumption is able to favourably modulate gut flora by selecting for probiotic bacteria such as *Bifidobacterium, Bacteroides* and *Lactobacillus* species (Joyce et al., 2019; Thandapilly et al., 2018). As a soluble fibre, β-glucan is fermented by colonic bacteria to increase the production of short chain fatty acids (SCFA), namely acetate, butyrate and propionate. SCFA are a class of bacterial metabolites known to be energy sources for the heart, brain and muscles. They also increase the acidity in the colon, which provides an environment suited to promote the proliferation of healthy bacteria rather than harmful bacteria (Birkeland et al., 2023). SCFAs act on host physiology through G protein-coupled receptors and post-translational modifications to regulate appetite, blood glucose, lipid metabolism, blood pressure and exert anti-inflammatory and anti-cancer effects (reviewed by Joyce et al., 2019 and Xiong et al., 2022).

2.1.3.2. STARCH

Nutritionally, starch is categorized by digestion rate namely rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). Oats contain approximately 7% RDS, 22 % SDS and 25 % RS. SDS and RS are important fractions for human health, as they moderate glycemic response and may provide other physiological benefits (Ovando-Martínez et al., 2013). Foods containing SDS have a medium–low glycemic index, which is reported to reduce risk of cardiovascular disease and colon cancer as well as prevent and manage diabetes (Ashwar et al., 2016).

RS are recognized as a type of dietary fibre and are able to escape enzymatic digestion in the small intestine to be fermented by gut bacteria to produce SCFAs. Foods containing RS have a lower glycemic index due to the lack of available starch. Similar to β-glucan, animal studies have found that RS favorably modulates glucose and cholesterol metabolism provide protection against colon cancer due to SCFA production (Ashwar et al., 2016).

2.1.3.3. AVENANTHRAMIDES

Oatmeal has been historically used as a therapeutic agent to soothe skin irritation due to the anti-inflammatory and anti-itch properties of avenanthramides. Avenanthramides are shown to have potent anti-oxidant and radical scavenging effects. Ji et al., 2003 observed a reduction of muscular reactive oxygen species in avenanthramide-fed, exercised rats. The avenanthramide-fed group also exhibited increased antioxidant capacity through increased superoxide dismutase and glutathione peroxidase activities compared to the control. Two studies by Chen et al., 2004; 2007 determined that avenanthramides are bioavailable in humans and hamsters and were able to enhance serum glutathione activity by 21% and by acting in synergy with vitamin E and other antioxidants. Furthermore, avenanthramides were shown to protect human LDL against oxidation *in vitro*.

Avenanthramides have been reported to inhibit expression of pro-inflammatory cytokines IL-6, IL-8 and MCP-1, which are known to be involved with vascular smooth muscle cell (VSMC) proliferation, through reducing IL-1β induced NF-κB activation and by decreasing proteasome activity in human aortic endothelial cells (HAEC) (W. Guo et al., 2008; Liu et al., 2004b).

Avenanthramides were also found to able to be effective in reducing mitogen-stimulated VSMC proliferation and improving NO production through increasing endothelial nitric oxide synthase mRNA expression levels in both rat SMC and HAEC (Nie et al., 2006). Additionally, there is preliminary evidence to suggest that oat avenanthramides and phenolic acids have antihypertensive potential by improving systolic blood pressure in prehypertensive or stage 1 hypertensive adults (Soycan et al., 2020). Though further research is needed to evaluate the clinical significance of these compounds.

2.1.3.4. LIPIDS

Oats are considered an excellent source of essential polyunsaturated fatty acids, which appear to have a beneficial effect on cardiac health, which reduces the risk of cardiovascular disease (Lunn & Theobald, 2006). In an animal trial, oat lipids were reported to contribute to the hypocholesterolemic properties of oats, most likely attributed to the co-existence of oleic acid, linoleic, vitamin E, or plant sterols (L. Guo et al., 2014). Another animal study from the same laboratory group determined that oat oil lowered plasma total and LDL-cholesterol and liver free and total cholesterol, cholesterol ester and TG as well as increase faecal lipid and bile acid excretion in hypercholesterolemic rats (Tong et al., 2014). These findings suggest that oat lipids may provide health benefits, though more research is needed on examining the disease fighting effects of oat lipids.

2.1.3.5. PROTEIN

Recently, evidence suggests that higher plant protein consumption is associated with protective effects against metabolic syndrome (Ahnen et al., 2019; Kahleova et al., 2018; Yang et al., 2023). However, the disease fighting benefits of oat protein are not well studied. Results from preliminary studies suggest that oat protein exhibit health promoting benefits that ward off chronic disease. *In vitro* and *in silico* studies have identified antioxidant, immunomodulating, renin inhibiting, ACE inhibiting, α-amylase inhibiting, and DPP-IV inhibiting peptides derived from oat protein, suggesting potential anti-diabetic and cardioprotective effects (Bleakley et al., 2017; Esfandi et al., 2021; Rafique et al., 2022; F. Wang et al., 2015; W. Wang et al., 2022).

Results from animal trials suggests that oat protein confers protective effects against hypercholesterolemia and hyperglycemia as well as improves antioxidant defense. Oat protein has been reported to effectively reduce plasma total cholesterol and LDL-C, liver total cholesterol and cholesterol esters by increasing fecal total lipid and bile acid excretion as well as regulating liver CYP7A1 activity, a rate limiting enzyme in bile acid synthesis, in hypercholesterolemic animal models. In addition, the hypocholesterolemic effects of oats are maximized in varieties containing roughly the same amount of β-glucan but contain higher protein and lipid contents (L. Guo et al., 2014; Tong et al., 2016, 2021). Furthermore, oat protein was shown to alter the gut flora in hypercholesterolemic hamsters by significantly increasing the *Bacteroides:Firmicutes* ratio and the *Muribaculaceae* and decreases in *Eubacteriaceae* and *Erysipelotrichaceae*. *Bacteroides* and *Muribaculaceae* are considered beneficial bacteria which have been hypothesized to be associated with hypocholesterolemic effects, while *Eubacteriaceae* and *Erysipelotrichaceae* are considered harmful for cholesterol regulation.

Furthermore, oat protein consumption increased fecal SCFA content and increased liver LDLR and lipoprotein lipase (Tong et al., 2021). Recently, two studies have assessed the hypoglycemic effects of oat protein. A randomized, crossover acute feeding trial determined that the addition of 24 g of oat protein in a sugar sweetened beverage attenuated significantly increased insulin AUC, improved satiety after consumption and improved the secretion of incretin hormones in healthy male subjects (Tan et al., 2018). The second study concluded that oat oligopeptides significantly reduced fasting blood glucose, HOMA-IR index, OGTT-AUC and urine volume as well as improved oxidative stress markers in diabetic Sprague-Dawley rats (J. bo Wang et al., 2019). Jodayree et al., 2014 reported that the addition of oat bran protein hydrolysate to a high fat diet increased radical scavenging activity in erythrocytes and SOD activity in liver samples of mice, suggesting the antioxidant potential of oat protein.

Based on the current literature, it is evident that β-glucan is not the sole hypocholesterolemic and hypoglycemic component in oats and that the protein fraction may act in synergy with β-glucan to maximize the health effects of oats. More studies are needed to discover the full potential of oat protein and its' capacity to promote good health and attenuate chronic disease risk factors as well as to elucidate the mechanisms in which oat protein exert its' effects.

2.2. METABOLIC SYNDROME OVERVIEW

Metabolic syndrome, otherwise known as syndrome X, is defined as the clustering of 3 or more of the following components: abdominal obesity, increased levels of serum glucose, increased blood pressure, and atherogenic dyslipidemia (notably reduced levels of high-density lipoprotein cholesterol (HDL-C) and hypertriglyceridemia) (Alberti et al., 2009; Grundy, 2016;

McCracken et al., 2018) (Table 1). In addition, MetS is associated with pro-thrombotic and pro-inflammatory states due to abnormalities related to coagulation factors and blood platelets as well as an increase in activity of inflammatory mediators found in various tissues (Grundy, 2016). MetS most often occurs in populations that have excessive calorie intake and a sentient lifestyle with inadequate amounts of physical activity, but underlying metabolic susceptibilities and genetic predisposition can also play a critical role in the development of the syndrome. Having MetS will double the risk of developing atherosclerotic CVD and increases risk for type-2 diabetes five-fold (Grundy, 2016). MetS is also associated with many other chronic diseases such as cancer, gout, polycystic ovary syndrome, sleep apnea, dementia, non-alcoholic fatty liver disease and chronic kidney disease, which increases the risk of overall mortality (Beck-Nielsen, 2014).

Table 1: Criteria for Clinical Diagnosis of the Metabolic Syndrome. Adapted from Alberti et al., 2009

Clinical Features	Consensus Definition (AHA/NHLBI + IDF) 2009
	Any three of the following:
Obesity	Elevated waist circumference (Population- and country-specific definitions)
Plasma glucose concentration*	Fasting plasma glucose ≥ 100 mg/dL
Hypertension*	Blood pressure ≥130/85 mmHg
Triglycerides*	≥ 150 mg/dL
HDL-C*	<40 mg/dL in males; <50 mg/dL in females

* Drug treatments for these conditions are considered alternate indicators.

2.2.1: OBESITY

The onset of obesity, in particular, abdominal obesity, is a driving force in the pathophysiology of the MetS. The modern pandemic of obesity caused by the consumption of the western diet paired with a sentient lifestyle has resulted in an increased prevalence of MetS and its eventual progression to both CVD and type-2 diabetes (Nolan & O'Gorman, 2014). A key element in the pathogenesis of MetS is altered function of normal visceral adipose tissue caused by weight gain. In the case of MetS, visceral adipose tissue metabolism is altered in the following ways: decreased glucose uptake, increased lipid uptake and storage and increased lipolysis (Nolan & O'Gorman, 2014). There is also an increase in the release of non-esterified free fatty acids (FFA) into circulation. These alterations to the adipose tissue metabolism are a result of insulin resistance. Increased lipid uptake and storage results in hypertrophy of intra-abdominal adipocytes, which causes resistance to the anti-lipolytic properties of insulin. Moreover, ectopic fat, defined as excess adipose tissue found in locations that are not classically associated with adipose tissue storage such as the liver, heart, pancreas and muscles, is strongly associated with insulin resistance (Britton & Fox, 2011; Nolan & O'Gorman, 2014). High plasma levels of FFA are seemingly a mediator between adipose-tissue fat and ectopic fat. The accumulation of ectopic fat in the aforementioned organs contributes to systemic metabolic derangements, especially related to insulin, glucose and lipid metabolism (Britton & Fox, 2011).

Adipose tissue produces and secretes bioactive peptides called adipokines that influence metabolic risk factors, namely adiponectin, interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), resistin, leptin, angiotensinogen, and plasminogen activator inhibitor-1 (PAI-1) (Grundy, 2016). Adiponectin is homeostatic factor which regulates insulin sensitivity and glucose and lipid metabolism through its anti-inflammatory, anti-fibrotic, and antioxidant effects.

Serum concentrations of adiponectin are inversely correlated to the amount of adipose tissue present in the body and insulin resistance. As a result, low levels of adiponectin serve as a potent indicator of an increased risk of diabetes and cardiovascular disease (Sharma & Chetty, 2005). IL-6 and TNF-α are pro-inflammatory cytokines that contribute to insulin resistance and systemic inflammation. They are the main factors responsible for the induction of acute phase proteins production (e.g., C-reactive protein (CRP)) (Tangvarasittichai et al., 2016). Resistin is an inflammatory biomarker considered to be an important link between obesity, insulin resistance and diabetes (Jamaluddin et al., 2012). Leptin plays a significant role in modulating food intake, appetite, body mass, metabolic rate and thermogenesis through leptin receptors located in the hypothalamus. Leptin also acts on peripheral tissues to regulate lipid and glucose metabolism and inflammatory responses (Ghadge & Khaire, 2019). Increased production of angiotensinogen in adipose tissue in obese individuals contributes to systemic hypertension as a precursor that can be cleaved by renin–angiotensin system (RAS) enzymes to produce Ang II and other angiotensin peptides which exert vasoconstrictive effects (Frigolet et al., 2013). Lastly, PAI-1 inhibits fibrinolysis through its inhibition of plasminogen activator. Increased secretion of PAI-1 may promote a pro-thrombotic state that is observed in MetS (Grundy, 2008). Therefore, the literature suggests that the degree of visceral obesity can drastically influence the development of metabolic risk factors through the secretion of adipokines.

2.2.2: INSULIN RESISTANCE

Since its first description, MetS has been strongly associated with insulin resistance (Fahed et al., 2022). However, the most recent definition of MetS from the American Heart Association/National Heart, Lung, and Blood Institute does not specifically reference it.

This is most likely due to the difficulty of accurately measuring insulin resistance in clinical practice (Alberti et al., 2009). The best unifying hypothesis is that insulin resistance is a driving force in the underlying pathophysiology of the MetS. The components outlined by the current definition of MetS: abdominal obesity, dyslipidemia, hypertension and elevated fasting glucose are also the most common clinical features associated with insulin resistance. (McCracken et al., 2018; Nolan & O'Gorman, 2014).

Insulin is an endocrine peptide hormone that is secreted by pancreatic β-cells that orchestrates an integrated anabolic response to the availability of glucose and other nutrients (Petersen & Shulman, 2018). The anabolic effects exerted by insulin are increasing glucose uptake in liver, muscles, and adipose tissues while simultaneously inhibiting lipolysis and hepatic gluconeogenesis (Fahed et al., 2022). Insulin plays key roles in many important processes such as protein synthesis, synthesis and storage of fat, and cell growth, proliferation and differentiation (Meshkani & Adeli, 2009).

Type-2 diabetes develops once the body's production of insulin does not meet the increased insulin requirements prompted by insulin resistance. Due to impaired tissue sensitivity to normal insulin levels, hyperinsulinemia, hyperglycemia and lipid abnormalities such as hypertriglyceridemia develop. Pancreatic β-cells will compensate by producing additional insulin to attempt to maintain euglycemia. Eventually, decompensation will occur, leading to the development of pre-diabetes followed by diabetes (McCracken et al., 2018; Meshkani & Adeli, 2009).

Insulin resistance significantly impacts several tissues and organs. Insulin resistance in adipose tissue will result in increased TG hydrolysis from adipocytes that leads to an increased flux in plasma FFAs which produce a lipotoxic effect. In skeletal muscle, it is associated with reduced glucose transport and glycogen synthesis (McCracken et al., 2018; Meshkani & Adeli, 2009).

The flux of FFAs will impact the phosphorylation of insulin receptor substrates (IRS) which will hinder phosphoinositide 3-kinase (PI3K) activity. This leads to a decrease of the translocation of the insulin-mediated glucose transporter 4 (GLUT-4) from intracellular vesicles to the surface of the plasma membrane, leading to reduced glucose uptake (Choi & Kim, 2010; McCracken et al., 2018). In the liver, insulin resistance and FFA flux will diminish the effectiveness of insulin signaling pathways in hepatocytes, resulting in impaired glycogen synthesis, failure to suppress glucose production, enhanced lipogenesis, increased synthesis TG-rich very-low-density lipoproteins (VLDL) and increased production of inflammatory and prothrombotic proteins such as CRP and PAI-1. Therefore, the presence of insulin resistance in adipose tissue, muscle and liver will result in major metabolic abnormalities that are common features of T2D and MetS, which significantly increase the risk of atherosclerotic CVD (McCracken et al., 2018; Meshkani & Adeli, 2009).

2.2.3. HYPERTENSION

Hypertension, defined as repeated elevated blood pressure values of 130/85 mmHg, possesses a significant prevalence in the general population (Alberti et al., 2009). The regulation of blood pressure requires the complex integrated response of many organ systems including the cardiovascular system, sympathetic nervous system (SNS), kidneys, and adrenal glands. Uncontrolled hypertension results in organ damage and is a significant risk factor for stroke, end-stage renal disease, and congestive heart failure (Aloud, 2019). Hypertension is strongly associated with MetS through many links including obesity, insulin resistance, sympathetic overactivity, RAS activation. Common characteristics in hypertensive MetS patients are sodium retention, intravascular volume expansion and increased cardiac output caused by the aforementioned factors.

Insulin resistance is connected to hypertension by several mechanisms. Recent evidence has shown that insulin has an anti-natriuretic effect, which indicates that insulin stimulates renal sodium re-absorption. In those with insulin resistance, this effect may be significantly increased and play a prominent role for the development of hypertension in MetS (Yanai et al., 2008). Insulin has also been reported to stimulate the production of endothelin-1, a potent vasoconstrictor. High serum insulin levels are associated with an increase in circulating endothelin-1 in both healthy and insulin-resistant individuals (Yanai et al., 2008). Furthermore, the insulin receptor is associated with signaling pathways linked to the activation of endothelial nitric oxide synthase activity. Therefore, insulin resistance ultimately impairs nitric oxide bioavailability (Kobayashi, 2015).

The SNS plays a vital role in the regulation of blood pressure, sodium balance and maintaining homeostasis. Sympathetic hyperactivity in MetS impairs renal-pressure natriuresis, increases renal tubular sodium reabsorption and causes hypertension. Moreover, increases in sympathetic activity result in systemic norepinephrine spillover and elevated resting heart rate. Overactivation of the SNS is also strongly associated with other deleterious cardiovascular effects such as cardiac hypertrophy, arterial remodeling, and endothelial dysfunction (Moreira et al., 2015).

The RAS plays a crucial role in blood pressure regulation through enhancing renal salt absorption and modulating vascular tone. RAS hyperactivity is widely known for its' role in the development of hypertension. As previously mentioned, adipose tissue is a major site of production for angiotensinogen which is then locally converted into angiotensin I and II.

Excess angiotensinogen production results in adipocyte hypertrophy and elevation of blood pressure via the action of angiotensin II, which induces systematic vasoconstriction, sodium and water retention and increased production of aldosterone. RAS hyperactivation also leads to chronic elevation of SNS activity, causing renal vasoconstriction and renin-dependent hypertension (Jiang et al., 2016).

2.2.4. DYSLIPIDEMIA

The three major components of atherogenic dyslipidemia associated with MetS are increased TG-rich lipoproteins, decreased HDL, and increased small dense LDL particles, otherwise known as the lipid triad.

As a result of insulin resistance and compensatory hyperinsulinemia, the hepatic overproduction of VLDL, the principal vehicle for the transport of TG in the blood stream, ensues. As previously mentioned, the inability to suppress hepatic glucose production, insufficient muscle glucose uptake and the increased flux of FFA from adipose tissue are the most notable consequences of insulin resistance in these tissues. These events result in an increased flux of FFA and glucose to the liver, which ultimately promotes hepatic VLDL production. Furthermore, VLDL are mostly removed from circulation by the LDLR. Insulin resistance may also impair LDLR activity, suppressing VLDL particle clearance (Ruotolo & Howard, 2002). Furthermore, lipoprotein lipase (LPL) is expressed in many tissues including adipose tissue and cardiac and skeletal muscle. The function of LPL is to hydrolyze the TG core of circulating TG-rich lipoproteins, chylomicrons, and VLDL (H. Wang & Eckel, 2009). Insulin stimulates LPL activity through increasing LPL mRNA expression.

LPL activity in skeletal muscle has been reported to be diminished in insulin resistant patients, suggesting a defective insulin regulation of LPL. Therefore, insulin resistance greatly impacts the lipoprotein metabolic cascade, resulting in decreased clearance of VLDL promotion of a hypertriglyceridemic state (Ruotolo & Howard, 2002).

The decreased levels of HDL observed in an insulin resistant state is a consequence of hypertriglyceridemia. A high production of VLDL can destabilize HDL particles by promoting the exchange of TG from VLDL particles and cholesterol esters from HDL particles mediated by the increased activity of cholesteryl ester transfer protein (CETP) (Chapman et al., 2010; Ruotolo & Howard, 2002). This exchange leads to highly atherogenic cholesteryl ester-enriched VLDL particles. In addition, insulin mediates the clearance of TG from TG-rich lipoproteins by stimulating the secretion of LPL in adipose tissues. Consequently, insulin resistance would blunt LPL activity leading to increased circulation of TG-rich particles and TG loading of HDL particles. Furthermore, insulin resistance augments hepatic lipase-mediated hydrolysis of HDL phospholipids and TG, which leads to the formation of smaller, dense HDL particles and an increased rate of catabolism, ultimately resulting in a substantial decrease in circulating HDL particles (Chapman et al., 2010; Rashid et al., 2003; Vollenweider et al., 2015).

In the case of insulin resistance and hypertriglyceridemia, LDL particles also undergo CETP-mediated alterations of composition and distribution that is similar to HDL particles, resulting in increased production atherogenic small, dense LDL particles(Grundy, 1998; Ruotolo & Howard, 2002). Small, dense LDL particles are seemingly more atherogenic than normal sized LDL particles, as they more prone to modifications, such as oxidation and glycation (especially apparent in hyperglycemic conditions), which could lead to increased production of antibodies against the modified particles resulting in immune responses.

Furthermore, modified LDL particles are more likely to evade clearance mediated by the LDLR pathway, which promotes macrophage scavenger receptors to take up the particles, thus inducing atherosclerosis (Ruotolo & Howard, 2002).

2.2.5. INFLAMMATORY STATE

Obesity causes chronic inflammation that plays a role systemic metabolic dysfunction. Adipose tissue is an important endocrine organ that produces several adipokines that provide both pro- and anti-inflammatory effects. Adipose tissue dysfunction will inevitably dysregulate the production and secretion of these adipokines which would greatly contribute to the pathogenesis MetS.

Adipose tissue was once considered an inert tissue primarily related to energy storage but is now emerging as an active element in the regulation of physiological and pathological processes such as inflammation and immune function. As previously mentioned, adipose tissue synthesizes and circulates a variety of pro- and anti-inflammatory factors, such as the adipokines leptin, resistin, and adiponectin, as well as cytokines like TNF-α and IL-6 (Ellulu et al., 2017) The link between obesity and inflammation is the overexpression of these pro-inflammatory factors. Furthermore, immune cells like macrophages are an important component of adipose tissue. Macrophages can be classified as pro-inflammatory (M1) and anti-inflammatory (M2). In the case of obesity, M1 macrophages are most prevalent. Macrophages are recruited by adipokines to then further secrete several inflammatory factors including TNF-α, IL-6, IL-12 and IL-1β to act on adipocytes, and causing a viscous cycle that promotes chronic inflammation and insulin resistance (Liang et al., 2022). With progressive adipocyte hypertrophy accompanied by obesity, the blood supply to adipocytes is reduced, leading to hypoxic conditions. Hypoxia is

proposed to cause necrosis and macrophage infiltration of adipose tissue, leading to overproduction of pro-inflammatory factors that propagates systemic inflammation associated with the development of obesity-related comorbidities (Ellulu et al., 2017).

2.2.6. HIGH FAT, HIGH SUCROSE FED RAT MODEL

The high-fat, high-sucrose (HFHS) fed rat model is used to mimic the pathophysiological changes of MetS induced by the human "Western" diet. The HFHS diet has been shown to induce MetS components such as central obesity, dyslipidemia, inflammation, insulin resistance and overt T2D (Lawson, 2013). The obesogenic effect of the HFHS diet is attributed to the increased content of saturated fatty acids, which are less readily available as an energy source and are thus converted into triacylglycerol to be stored in adipose tissue. Additionally, excessive consumption of rapidly absorbed carbohydrates like sucrose causes hyperglycemic and hyperinsulinemic conditions that result in insulin resistance and diabetes (Pinheiro-Castro et al., 2019). Furthermore, evidence of harmful disruptions in gut microbiome that influence energy metabolism, fat storage, serum lipid levels, and insulin resistance have been reported, suggesting important links between diet, gut microbiome and MetS (Lawson, 2013).

In a diet induced obesity model, Wistar rats are considered good subjects as they have similar behaviors to humans regarding excessive food consumption, which can cause weight gain and metabolic disturbances (de Moura e Dias et al., 2021). Genetic background is an important factor of dietary risk, as some species may become obese and develop various metabolic complications while consuming an obesogenic diet, while other species will not respond in the same way (Lawson, 2013).

Therefore, an effective model of MetS has similarities to humans in regards to the interactions between genes and diet which would ultimately modulate energy, glucose and lipid metabolism.

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3.0 THE CARDIOMETABOLIC EFFECTS OF OAT PROTEIN IN HIGH FAT, HIGH SUGAR FED WISTAR RATS

DISCLAIMER: This manuscript was accepted into Applied Physiology, Nutrition, and Metabolism (December 6th, 2023)

Oat protein modulates cholesterol metabolism and improves cardiac systolic function in high fat, high sucrose fed rats

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Abstract:

Oats are recognized to provide many health benefits that are mainly associated with its dietary fibre, β-glucan. However, the protein derived from oats is largely understudied with respect to its ability to maintain health and attenuate risk factors of chronic diseases. The goal of the current study was to investigate the metabolic effects of oat protein consumption in lieu of casein as the protein source in high fat, high sucrose (HF/HS) fed Wistar rats. Four-week-old rats were divided into three groups and were fed three different experimental diets: a control diet with casein as the protein source, a HF/HS diet with casein or a HF/HS diet with oat protein for 16 weeks. Heart structure and function were determined by echocardiography.

Blood pressure measurements, an oral glucose tolerance test, and markers of cholesterol metabolism, oxidative stress, inflammation and liver and kidney damage were also performed. Our study results show that incorporation of oat protein in the diet was effective in preserving systolic heart function in HF/HS fed rats. Oat protein significantly reduced serum total- and LDL-cholesterol levels. Furthermore, oat protein normalized liver HMG-CoAR activity, which to our knowledge, is the first time this has been reported in the literature. Therefore, our research suggests that oat protein can provide hypocholesterolemic and cardioprotective benefits in a diet-induced model of metabolic syndrome.

Keywords: oat protein; metabolic syndrome; HMG-CoAR; high-fat, high-sucrose diet; systolic heart function; dyslipidemia

3.1. Introduction

Oat (Avena sativa L.) is an important cereal crop that has been historically used as a nutritious food source and for medicinal purposes (Webster, 1986). The market for oats has been gaining momentum in the recent years due to its high content of bioactive ingredients that contribute to many health-related benefits (Boukid, 2021). In particular, frequent consumption of β-glucan, a soluble fibre that is abundant in oats, has been proven to lower serum LDL- and total cholesterol, improve post-prandial glycemic response, improve gut health and reduce risk of chronic disease (EFSA Panel on Dietetic Products, 2011; Health Canada, 2010; Wang et al., 2016). Oats contain 40-60% starch, 3-7% β-glucan, 2-12% lipids, and 15-20% protein (Bryngelsson et al., 2002; Menon et al., 2016; Mirmoghtadaie et al., 2009). In addition to high dietary fibre levels, oats are also recognized as a high-quality sustainable plant protein. Oat protein surpasses the World Health Organization essential amino acid requirements in all but lysine and methionine (Sterna et al., 2016).

Furthermore, oat protein has a significantly lower carbon footprint than animal proteins such as casein and can be collected as a by-product during the commercial production of β-glucan (Mel & Malalgoda, 2022).

Metabolic syndrome (MetS) is defined as a condition that involves the clustering of the following risk factors: abdominal obesity, dyslipidemia, hypertension, and hyperglycemia. MetS is likely to increase the risk of developing chronic diseases such as CVD, type 2 diabetes, cancer, non-alcoholic fatty liver disease, dementia and chronic kidney disease (Beck-Nielsen, 2014). Based on data from the Canadian Health Measures Survey cycle 2, the prevalence of MetS is estimated to be 21% in Canadians aged 18-79 years old (Statistics Canada, n.d.). Efforts to prevent, diagnose and treat MetS and its risk factors are important to ward off chronic disease and premature mortality.

High protein diets have been associated with improvements in cardiometabolic and cardiovascular biomarkers, including blood glucose, blood cholesterol and blood pressure levels (Evangelista et al., 2021; Gannon et al., 2003). Emerging scientific evidence suggests that diets high in protein, specifically plant derived proteins, significantly decrease the cardiometabolic risk factors. These effects may be attributed to the release of bioactive peptides and the amino acid composition of the plant derived proteins (Bouchard et al., 2022). In particular, oat protein, and other plant proteins, have lower Lys:Arg and Met:Gly ratios than animal proteins, which are hypothesized to be partially responsible for the hypocholesterolemic properties associated with plant protein consumption (Tanaka & Sugano, 1989; Tong et al., 2016; Venkatesh et al., 2017).

Earlier preliminary studies have reported the potential hypocholesterolemic effects of oat protein supplementation in animals (Guo et al., 2014; Tong et al., 2016).

However, there is a gap in the current knowledge pertaining to comprehensive health effects related to oat protein such as the impact on oxidative stress, inflammation, glucose metabolism, blood pressure as well as heart, liver and kidney function. Accordingly, the specific objective of the current study was to evaluate whether replacing casein (the conventional protein source in rat chow) with oat protein as the dietary protein source will provide protective effects against cardiometabolic changes in high-fat, high sucrose fed rats.

3.2. Materials and Methods

3.2.1 Animal ethics

The animal experimental protocols for this project were approved by the University of Manitoba Office of Research Ethics and Compliance and Animal Care Committee and were conducted in accordance with guidelines by the Canadian Council for Animal Care (protocol 20-069).

3.2.2. Oat protein extraction

Oat protein was extracted from Manitoban sourced oats provided by Buffalo Creek Mills Altona, MB, CA using a wet extraction method. The protein was extracted from defatted oat flour with alkaline water (pH 9.5) at 35 ºC for 1 hour. The mixture was centrifuged at 5000 x g for 15 minutes. The protein was collected from the supernatant through isoelectric point (pH 4.5 – 5.5) precipitation. The protein residue was mixed with water, and its pH was adjusted to 7.0 – 7.5 with 4 N NaOH. The protein extract was freeze dried and milled prior to its incorporation into the rat chow provided by Research Diets Inc, (New Brunswick, CA).

3.2.3. Experimental model and treatment

Four-week-old Wistar rats were obtained from Charles River Inc. (Quebec, CA). The rats were housed in pairs and submitted to a seven-day acclimation period prior to the commencement of the study. Three experimental groups were fed 20-25g daily of either an AIN93G based control diet with casein as the protein source (10.9% sucrose, 7.17% fat, 17.5% protein) (n=12), an AIN93G based high fat, high sucrose diet with casein (29.6 % sucrose, 26.2% fat, 21.4% protein) (HF/HS, n=11), or an AIN93G based diet with high fat, high sucrose diet with oat protein (29.7% sucrose, 26. 4% fat, 21.5% protein at 88.9% purity) (HF/HS+OP, n=11). Sample size was determined based on a statistical power analysis and outcomes of previous HF/HS diet studies (Louis et al., 2012). Disclaimer: one rat in the HF/HS+OP group did not survive the acclimation period and one rat from the HF/HS group inhaled glucose solution during the OGTT and was euthanized and excluded from the study. The amino acid composition of the oat protein is detailed in Table S1. The complete compositions of the diets are shown in Table 1. Food intake of the rats was monitored daily. Body weights were assessed on a weekly basis. The high fat, high sucrose diet closely reflects the western diet, where highly processed and highly energetic foods are predominately consumed. This diet is known to be effective to induce most of the metabolic disorders associated with MetS (inflammation, obesity, dyslipidemia, insulin resistance, glucose intolerance and hyperglycemia) (Rodríguez-Correa et al., 2020).

Table 1: The composition of the experimental diets

Ingredients (g)	Control diet	HF/HS diet	HF/HS + Oat Protein
Casein	200	200	0
Oat Protein	0	0	202
L-Cysteine	3	3	3
Corn Starch	397.49	397.49	397.49
Maltodextrin 10	132	68.24	68.24
Sucrose	100	235	235
Cellulose	50	50	50
Soybean Oil	70	70	63.694
Lard	0	145	145
t-Butylhydroquinone	0.014	0.014	0.014
Mineral Mix S10026	10	10	10
DiCalcium Phosphate	13	13	13
Calcium Carbonate	5.5	5.5	5.5
Potassium Citrate:1H20	16.5	16.5	16.5
Vitamin Mix V10001	10	10	10
Choline Bitartrate	2.5	2.5	2.5
Total	1010	828.75	824.44
Gram %			
Protein	17.52	21.36	21.5
Carbohydrate	63.34	37.82	38.02
Sucrose	10.89	29.56	29.72
Fat	7.17	26.23	26.36
Fibre	4.95	6.03	6.06
kcal/gram	3.88	4.73	4.75
Kcal % Protein	18	18	18
Kcal % Carbohydrate	65	32	32
Kcal % Sucrose	11	25	25
Kcal % Fat	17	50	50
Total	100	100	100

3.2.4. Transthoracic echocardiography

Heart structure and function were assessed in each experimental group by transthoracic echocardiography after 16 weeks of treatment. The rats were anesthetized during the procedure. Transthoracic two-dimensionally (2D) guided M-mode and Pulse-Wave Doppler measurements were performed using an ultrasound system (VIVID E9, GE, USA) with a 13 MHz transducer as previously described by Aloud et al., 2018; Raj et al., 2020; Thandapilly et al., 2010. The parameters measured included left ventricular ejection fraction (LVEF), fractional shortening (FS), cardiac output (CO), interventricular septal wall thickness at end systole and diastole (IVSs/d), left ventricular posterior wall thickness at end systole and diastole (LVPWs/d), left ventricular internal dimensions at end systole and diastole (LVIDs/d) and isovolumetric relaxation time (IVRT).

3.2.5. Blood pressure measurements

Blood pressure (BP) measurements were performed on conscious animals after 16 weeks of treatment. Systolic and diastolic BP measurements were carried out by using a tail-cuff sphygmomanometer (Kent Scientific, USA) as described by Raj et al.. Throughout each measurement cycle, a volume pressure recording cuff pushed blood away from the tail. The back flow of the blood was stopped using an occlusion cuff. Once the occlusion cuff deflates, the blood flow is let back into the tail which increases the tail volume. Systolic BP was measured as the pressure exerted by occlusion cuff during the increase in tail volume. Diastolic BP was measured as the occlusion cuff pressure during the deflation at which the blood flow in the tail equalizes.

3.2.6. Oral glucose tolerance test

After 12 hours of fasting, an oral glucose tolerance test (OGTT) was performed on all groups the day before sacrifice. The rats were administered a glucose solution (2g/kg body weight) via a polyethylene gastric tube by oral gavage. Blood glucose values were measured with an AlphaTRAK 2 blood glucometer and test strips at 0, 30, 60, and 120 minutes.

3.2.7. Liver homogenate preparation

Approximately 100 mg pieces of rat liver were frozen with liquid nitrogen and crushed into a powder. 1 mL of RIPA lysis buffer with 1 µL of protease inhibitor cocktail (Sigma-Aldrich, CA) was added to the samples. The samples were homogenized at 8800 RPM for three intervals of 10 seconds, placing the samples on ice in between intervals. The samples were then centrifuged at 8000 RPM for 15 minutes and the supernatant was collected. The protein concentration of the samples was analyzed using the DC protein assay (BIO-RAD, CA)

3.2.8. Biochemical assessments

Serum lipid peroxidation levels were estimated by using a thiobarbituric acid reactive substances-based assay kit to determine the amount of malondialdehyde (MDA), a lipid peroxidation product (Abcam, USA). The serum concentration of the inflammatory cytokine, tumor necrosis factor alpha (TNF-α), was determined using ELISA assay kit. The liver activities of cholesterol 7 alpha-hydroxylase (CYP7A1), 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMGCR) and proprotein convertase subtilisin/kexin type 9 (PCSK9) were determined using ELISA assay kits (Biomatik, USA, Novus Biologicals, CA).

Using a Cobas c111 clinical chemistry analyzer (Roche Diagnostics, Quebec, CA), plasma concentrations of alanine animotransferase (ALT), aspartate aminotransferase (AST), urea, glucose, creatinine, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG) were measured.

3.2.9. Statistical Analysis

All values are expressed as means ± SEM. One-way ANOVA was used to compare the differences between the experimental groups using GraphPad Prism 5 software. Tukey's post-hoc was used to further evaluate the statistical significance when a simple effect is detected between groups. $P < 0.05$ was considered to be statistically significant. In the case of OGTT, two-way ANOVA was used to compare the differences between the experimental groups and Bonferroni's multiple comparisons test was used to further evaluate the statistical significance when a simple effect is detected between groups. $P < 0.05$ was also considered to be statistically significant.

3.3. Results

3.3.1. Body weight

The body weights of the animals gradually increased from the baseline to the endpoint of the study (Figure 1a). There was a significant increase in the body weights of both the HF/HS and HF/HS+OP groups compared to the control group, which is expected in a HF/HS rat model (Figure 1b).

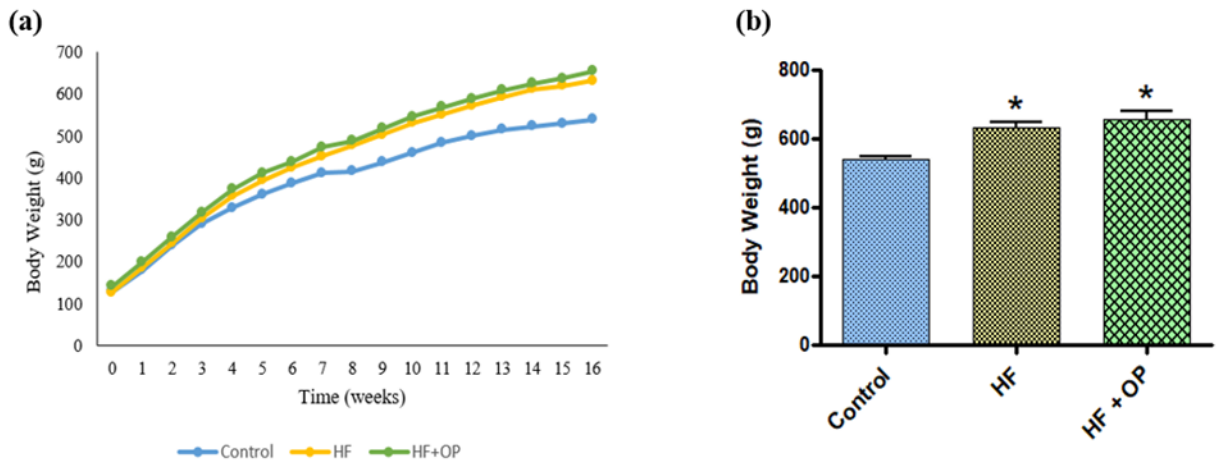

Figure 1: a) Gradual body weight increase of the animals throughout 16 weeks of treatment. b) Comparison of the body weights of the animals at 16 weeks of treatment. Values are means ± SEMs. Significant values are defined as $p < 0.05$. * denotes significance versus the control group, # denotes significance versus the HF/HS group.

3.3.2. Systolic and diastolic blood pressure

BP monitoring was performed after 16 weeks of treatment. No significant differences in systolic or diastolic BP were observed between groups (Figure 2).

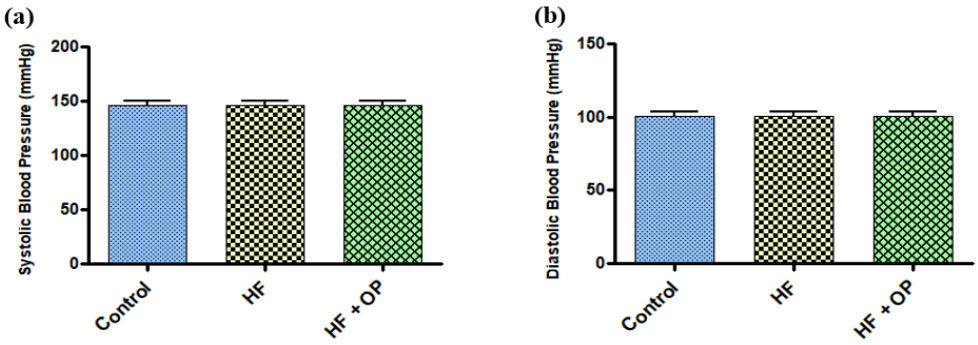

Figure 2: Effects of oat protein treatment on systolic and diastolic blood pressure after 16 weeks of treatment. Values are means ± SEMs. Significant values are defined as $p < 0.05$. * denotes significance versus the control group. # denotes significance versus the HF/HS group.

3.3.3. Heart structure and function

After 16 weeks of treatment, no significant changes were observed between groups for LVIDd (Figure 3a). However, there was a significant increase in LVIDs in the HF/HS group in comparison to the control group, which was normalized in the HF/HS + OP group (Figure 3b). Compared to the control group, the HF/HS group exhibited a significant reduction in both systolic functional parameters, LVEF and FS, which were preserved in the HF/HS + OP group (Figure 3c+d). No significant differences were observed in the cardiac structural parameters IVSs/d and LVPWs/d, the diastolic functional parameter, IVRT, and CO between groups (Figures S1 and S2)

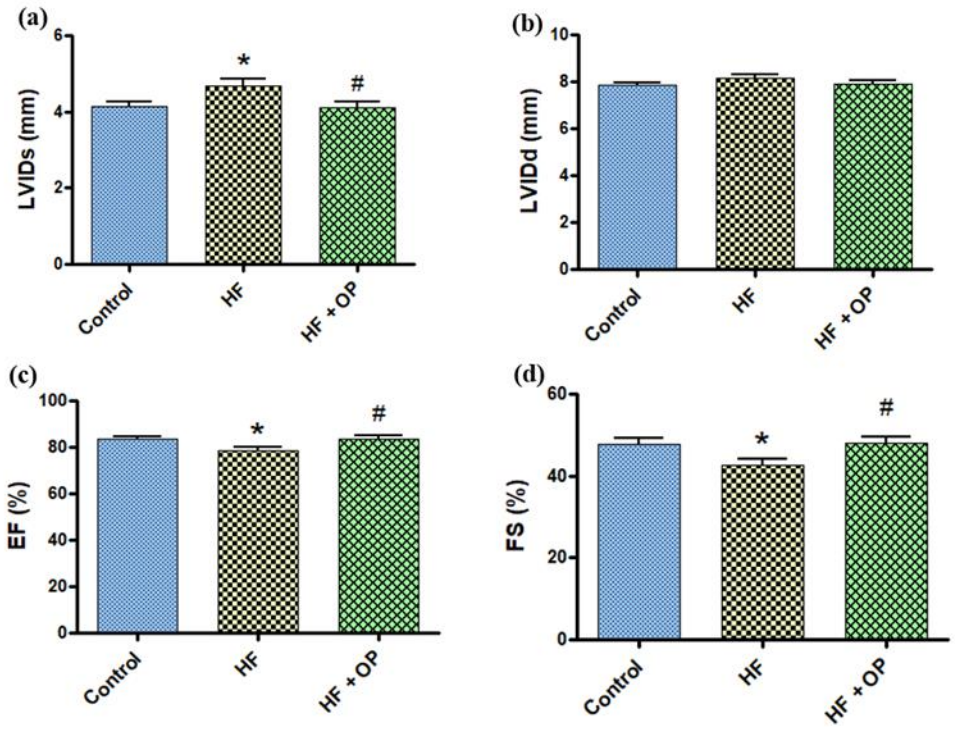

Figure 3: The effects of oat protein on a) left ventricular internal dimension at end systole (LVIDs) , b) left ventricular internal dimension at end diastole (LVIDd), c) left ventricular ejection fraction and d) fractional shortening after 16 weeks of treatment. Values are means ± SEMs. Significant values are defined as $p < 0.05$. * denotes significance versus the control group. # denotes significance versus the HF/HS group.

3.3.4. Serum cholesterol levels and markers of cholesterol metabolism in liver tissue

In the HF/HS group, there was a significant increase in serum TC compared to the control group. The HF/HS + OP group showed a significant decrease in TC compared to the HF/HS group (Figure 4a). For both serum HDL- and LDL-C, the HF/HS group exhibited increased levels compared to the control that did not reach statistical significance.

The HF/HS + OP group showed significantly decreased levels for both HDL- and LDL-C compared to the HF/HS group (Figure 4b + c). Both the HF/HS and HF/HS + OP groups had significantly elevated TG levels compared to the control group (Figure 4d).

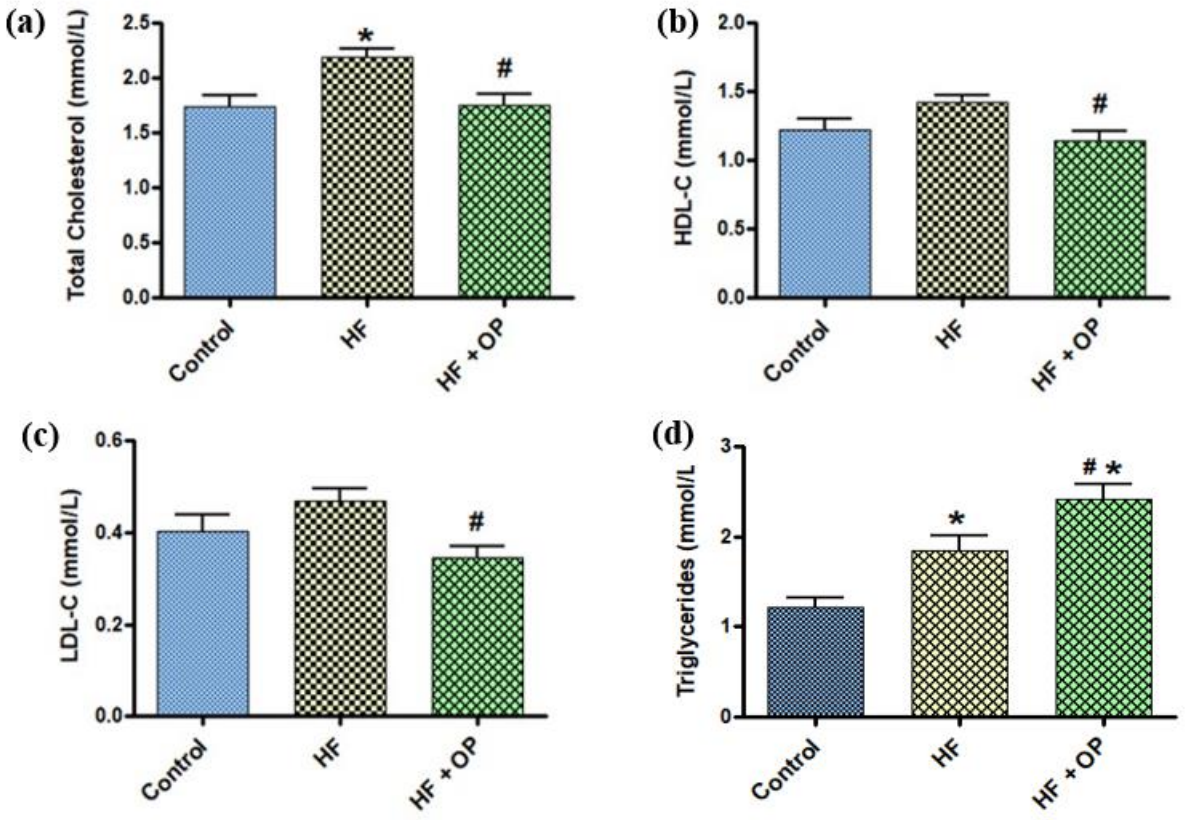

Figure 4: The effects of oat protein on serum a) total cholesterol (TC), b) high-density lipoprotein cholesterol (HDL-C), c) low-density lipoprotein cholesterol (LDL-C) and d) triglycerides (TG) after 16 weeks of treatment. Values are means ± SEMs. Significant values are defined as $p < 0.05$. * denotes significance versus the control group. # denotes significance versus the HF/HS group.

No significant statistical differences were observed among groups for the activities of liver CYP7A1 activity, a rate limiting enzyme in the bile acid synthesis pathway and PCSK9, an enzyme involved in the LDL-C metabolism (Figure 5a+b). However, the HF/HS group exhibited markedly higher activity for liver HMG-CoAR, a rate liming enzyme in the cholesterol synthesis pathway, compared to the control (Figure 5c). In the HF/HS+OP group, the oat protein consumption effectively normalized HMG-CoAR liver activity.

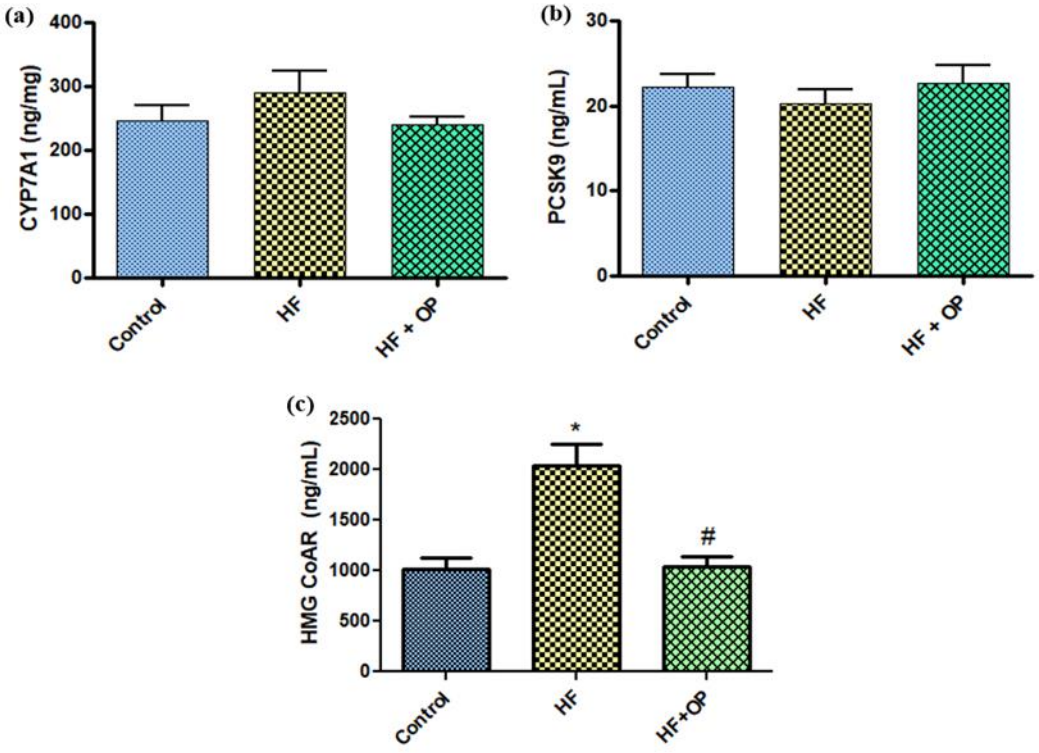

Figure 5: The effects of oat protein on the activities of a) Cholesterol 7 alpha-hydroxylase (CYP7A1) b) Proprotein convertase subtilisin/kexin type 9 (PCSK9), and c) HMG-CoA reductase (HMG CoAR) in liver tissue after 16 weeks of treatment. Values are means ± SEMs. Significant values are defined as $p < 0.05$. * denotes significance versus the control group. # denotes significance versus the HF/HS group.

3.3.5. Random glucose and oral glucose tolerance test (OGTT)

The oat protein treatment was tested for glucose-lowering efficacy. Following a glucose challenge (2 g/kg body weight), the results of the OGTT showed that the HF/HS group had significantly increased serum glucose levels at the 30 minute and 120 minute time points compared to the control. Furthermore, the HF/HS + OP group also exhibited significantly higher glucose levels at the 120 minute time point compared to the control. Interestingly, The HF/HS +OP group had significantly reduced blood glucose levels at the 30-, 60- and 120-minute time points compared to the HF/HS group (Figure 6a). However, there were no significant differences in the total areas under the curve (AUC) analysis among groups (Figure 6b).

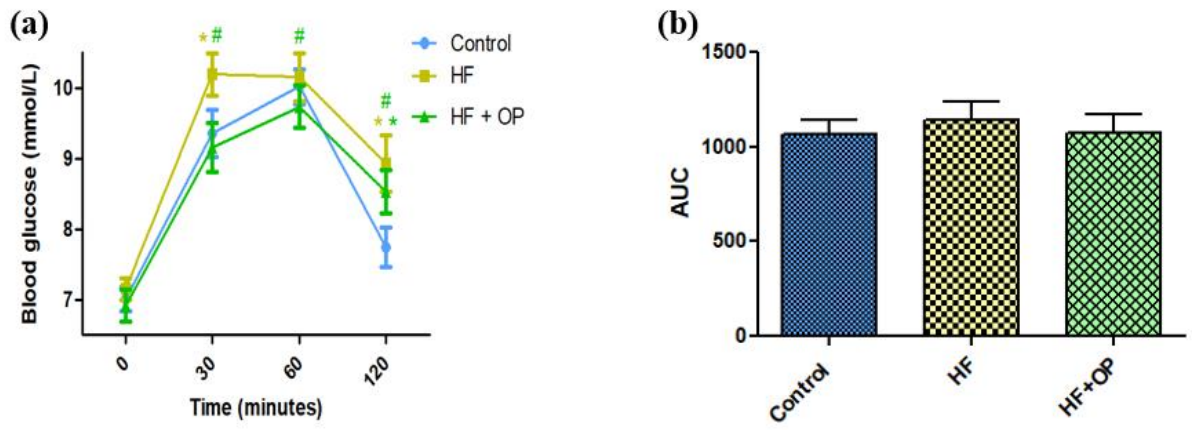

Figure 6: The effects of oat protein on a) an oral glucose tolerance test (OGTT), b) an area under the curve analysis (AUC). Values are means ± SEMs. Significant values are defined as $p < 0.05$. * denotes significance versus the control group. # denotes significance versus the HF/HS group.

3.3.6. Markers of oxidative stress and inflammation

No significant differences were shown in the serum levels of the inflammation marker, TNF-α between groups. Similarly, no significant changes in the oxidative stress marker, MDA were observed between groups. However, the levels of MDA were trending upward in the HF/HS group and trending downward in the HF/HS + OP group compared to the HF/HS group (Figure 7).

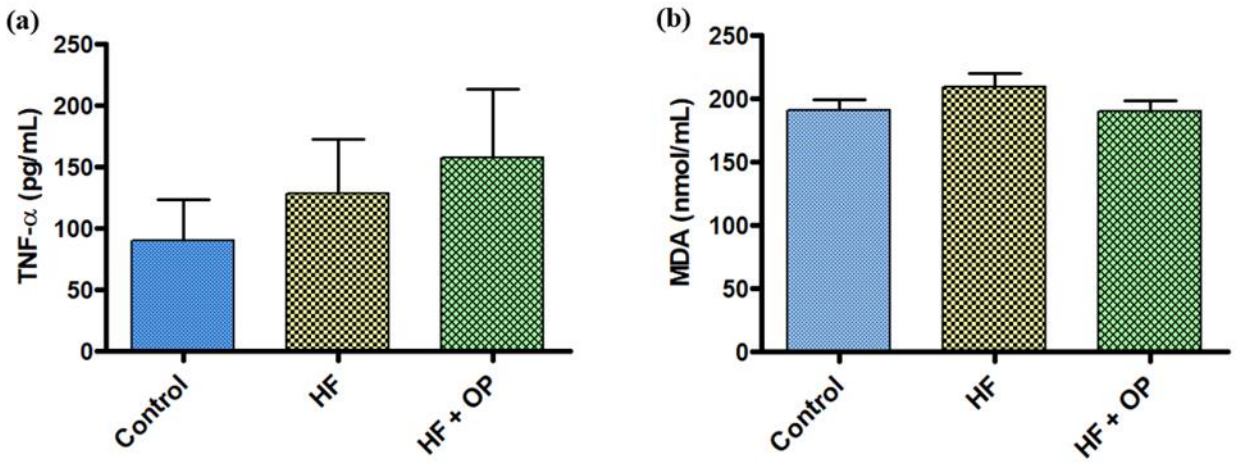

Figure 7: The effects of oat protein on serum tumor necrosis factor-α (TNF-α), and malondialdehyde (MDA) after 16 weeks of treatment. Values are means ± SEMs. Significant values are defined as $p < 0.05$. * denotes significance versus the control group. # denotes significance versus the HF/HS group.

3.3.7. Liver and kidney markers

There was a significant increase in ALT in the HF/HS + OP group compared to both the control and HF/HS groups (Figure 8a). Creatinine levels trended downward in the HF/HS group without reaching statistical significance. The HF/HS +OP group exhibited significantly decreased creatinine levels compared to the control (Figure 8c).

No significant changes were observed for urea and AST levels among groups, though there was a downward trend for AST levels in the HF group (Figure 8b+d).

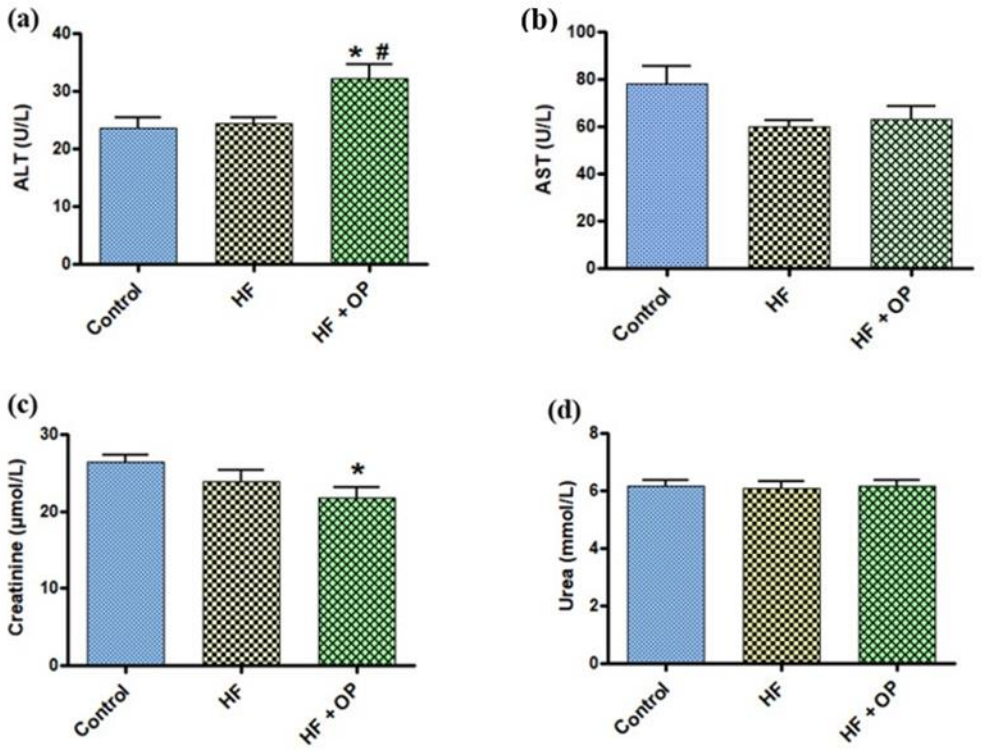

Figure 8: The effects of oat protein on serum a) alanine aminotransferase (ALT), b) aspartate aminotransferase (AST), c) creatinine and d) urea after 16 weeks of treatment. Values are means ± SEMs. Significant values are defined as $p < 0.05$. * denotes significance versus the control group. # denotes significance versus the HF/HS group.

3.4. Discussion

It has been well established that consuming oats on a regular basis can effectively reduce cholesterol and improve blood glucose levels. These effects have been primarily attributed to its β-glucan content, a soluble fibre (Ho et al., 2016; Tiwari & Cummins, 2011).

However, other components in oats, such as oat protein and lipids, have been understudied in regard to their impact on maintaining health and attenuating chronic disease risk factors. As previously mentioned, oats contain a relatively higher amount of protein and essential amino acids than other cereal grains. Compared to other prolamin dominated cereal grains, oat protein consists of a much higher proportion of globulins (roughly 80%) allowing for a more balanced essential amino acid profile. Notably, oats contain high amounts of glutamic acid, leucine, and arginine and possess lower Lys:Arg and Met:Gly ratios than casein which are hypothesized to provide health benefits (Table S1) (Bahramia et al., 2019; Guo et al., 2014; Liu et al., 2009).

In the present study, we observed no significant difference in systolic or diastolic blood pressure between the treatment groups at 16 weeks. Previously some studies have shown that HF/HS diet will induce hypertension (Drosatos & Schulze, 2013; Grundy, 2008; Panchal et al., 2012). Though our results are consistent with one study reported that after 16 weeks HF diet feeding, there was no significant differences in the systolic blood pressure of Wistar rats (Marques et al., 2015). However, we did observe that the HF/HS diet induced significant hypertension-independent cardiac abnormalities by 16 weeks. Metabolic syndrome is considered to triple the risk of cardiac hypertrophy, heart attack, and heart failure (Grundy, 2008). Cardiac lipotoxicity is a known cause of cardiac abnormalities in the setting of metabolic disorders such as dyslipidemia and obesity (Drosatos & Schulze, 2013). Specifically, one previous study reported that there was lipid accumulation in the LV tissue after high fat feeding (Sikder et al., 2018). During high caloric diet mediated metabolic dysregulation, abnormal fatty acid uptake by cardiomyocytes can lead to lipoapoptosis (Sikder et al., 2018).

Furthermore, HF/HS diet promotes pathological cardiac remodeling and an increase in myocardial stiffness in Wistar rats, evidenced by collagen deposition in the LV as well as echocardiographic indexes that indicate increased LV mass, increased systolic stress and depressed systolic function (Leopoldo et al., 2010). In the present study, oat protein treatment was effective in preventing pathological changes in heart structure and function induced by a HF/HS diet as evidenced by the preserved LVIDs, LVEF and FS in the HF/HS + OP fed Wistar rats compared to the HF/HS fed Wistar rats. LV dilation seen as increased LVIDs/d is a known risk factor for congestive heart failure in those who have never had a myocardial infarction (Furuäng et al., 2013). LVEF and FS are parameters that measure LV systolic function. A reduction of FS and LVEF increases the risk of systolic heart failure and signifies that the heart is not contracting effectively resulting in less oxygen-rich blood is circulating throughout the body. LVEF also has a prognostic value in predicting adverse outcomes including mortality and severity of the reduction in LVEF often guides treatment strategies. The current study suggests that the oat protein may have been able to preserve systolic function by counteracting the HF/HS diet induced cardiac lipotoxicity which would in turn reduce the risk of developing heart failure associated with reduced LVEF.

Compared to the control, the HF/HS group exhibited significantly increased total cholesterol and triglycerides as well as HDL- and LDL-cholesterol levels that were trending upward, which did not reach significance. We observed significantly lowered serum LDL-C and TC in the HF/HS+OP group in comparison to the HF/HS group, which is consistent with previous findings (Guo et al., 2014; Tong et al., 2016). However, the oat protein group had significantly lowered HDL-C levels that were comparable to the control group and elevated levels of TG.

The high levels of TG could be attributed to the nature of the HF/HS diet, which is associated with increased visceral fat accumulation and serum TG (Rodríguez-Correa et al., 2020; Zhao & Shen, 2023). In comparison to animal protein, plant protein is reported to limit fat deposition in the liver due to lower hepatic lipid storage in HF/HS fed Wistar rats (Lépine et al., 2023). Therefore, it may be possible that oat protein consumption prevented triglyceride accumulation in the liver, resulting in increased serum TG levels. More studies are needed to delineate the effects of oat protein consumption on triglyceride metabolism. Decreased levels of HDL observed in an insulin resistant state is a consequence of hypertriglyceridemia. High amounts of circulating triglycerides can destabilize HDL particles by promoting the exchange of TG from VLDL particles and cholesterol esters from HDL particles mediated by the increased activity of cholesteryl ester transfer protein (Chapman et al., 2010; Ruotolo & Howard, 2002). In addition, insulin mediates the clearance of TG from TG-rich lipoproteins by stimulating the secretion of lipoprotein lipase (LPL) in adipose tissues. Consequently, obesity induced insulin resistance would blunt LPL activity leading to increased circulation of TG-rich particles and TG loading of HDL particles, leading to the formation of smaller, dense HDL particles and an increased rate of catabolism, ultimately resulting in a substantial decrease in circulating HDL particles (Chapman et al., 2010; Rashid et al., 2003; Vollenweider et al., 2015).

HMG-CoAR is a rate limiting enzyme of the mevalonate pathway which is responsible for the biosynthesis of cholesterol and other isoprenoids. The regulation of HMG-CoAR is a primary target to combat hypercholesterolemia and reduce the risk of cardiovascular disease by modulating de novo cholesterol synthesis (DeBose-Boyd, 2008). High fat diet feeding produces significant increases in HMG-CoAR activity despite more than adequate amounts of cholesterol present in the liver (Wu et al., 2013).

In the current study, this was confirmed by the markedly increased liver HMG-CoAR activity observed in the HF/HS group compared to the control. Interestingly, we observed a significant reduction of liver HMG-CoAR activity in the HF/HS+OP group compared to the HF/HS group and comparable activity to the control group. Our results suggest that oat protein seemingly exerts hypocholesterolemic effects via a statin-like regulatory mechanism to normalize HMG-CoAR activity in rats fed a high-fat high-sugar diet, which explains the decreases of total- and LDL-cholesterol observed. To our knowledge, this is the first time this has been reported in the literature.

Similar to other plant proteins, oat protein has lower Met:Gly and Lys:Arg ratios which are postulated to be responsible for anti-atherogenic and cholesterol lowering properties of plant protein (Tanaka & Sugano, 1989; Tong et al., 2016; Venkatesh et al., 2017). Met is a precursor to homocysteine which inhibits nitric oxide (NO) synthesis which would have a negative impact on endothelial function (Tanaka & Sugano, 1989). In addition, high homocysteine levels may be associated with dyslipidemia (Daly et al., 2009; Tanaka & Sugano, 1989). Gly reduces homocysteine levels which would exert protective effects against Met induced hypercholesterolemia (Venkatesh et al., 2017). Arg is known to be a substrate for the production of NO which would exert protective effects on endothelial function. In addition, supplementation of Arg is shown to lower serum cholesterol levels and TG. Lys is an antagonist to Arg activity, as it increases arginine catabolism by activating kidney arginase (Venkatesh et al., 2017). Therefore, lower Met:Gly and Lys:Arg ratios may be partially responsible for the hypocholesterolemic and cardioprotective effects of oat protein.

A study by Mini & Rajamohan, 2004 reported that two treatment groups, one with coconut protein, which contains high amounts of Arg, and another fed casein with Arg supplementation showed significantly lowered levels of total-, HDL-, LDL, and VLDL-C, as well as significantly reduced HMG-CoAR activity compared to the control in alcohol fed rats. They speculated the hypocholesterolemic effects observed were attributed decreased Lys:Arg ratio. These results align with our research, as oat protein contains high amounts of Arg for a plant protein and a lower ratio of Lys:Arg compared to casein. Therefore, we speculate the arginine content of oat protein may play a role in the reduction of HMG-CoAR activity observed in the HF/HS+OP group.

Liver PCSK9 activities trended downward in the HF/HS compared to the control and trended upward in the HF/HS+OP group, showing similar values to the control group though this did not reach statistical significance. The function of PCSK9 is to bind to the LDL receptor and target it for lysosomal degradation. Decreasing PCSK9 activity would allow for more LDL receptors to be recycled and be present on the cell's surface to remove LDL particles from extracellular fluid, which would result in lower serum LDL-C concentrations (Reiss et al., 2018). Therefore, based on the current data the cholesterol-lowering properties of oat protein seem to be PCSK9-independent. Furthermore, no significant differences in liver CYP7A1 activities were observed among groups, in contrast to other studies in involving oat protein feedings in hypercholesterolemic animal models (Guo et al., 2014; Tong et al., 2016, 2021). CYP7A1 is the rate-limiting enzyme in the synthesis of bile acids derived from cholesterol through the classic pathway.

A proposed mechanism the hypocholesterolemic properties of dietary plant protein is to increase fecal bile acid excretion, which in turn increases the expression of CYP7A1 to replenish the bile acid pool (Guo et al., 2014; Tong et al., 2016). Though this was not observed in the current study.

We speculate that other mechanisms that were not evaluated in the current study were involved in the cholesterol lowering properties of the oat protein treatment. The gut microbiome plays a large role in cholesterol, glucose and fatty acid metabolism. The diet of the host is known to heavily influence intestinal microbiota. As evidenced by Tong et al., consumption of plant proteins favorably modulates gut bacteria to select for phyla such as *Muribaculaceae* and *Bacteroidetes* which are shown to have beneficial effect on cholesterol levels, fecal bile acid excretion, short chain fatty acid (SCFA) production and glucose tolerance, and decrease the relative abundance of *Firmicutes* and *Erysipelotrichaceae* which are considered to have negative effects of glucose and cholesterol metabolism (Liang et al., 2019; Martínez et al., 2013; Tong et al., 2021). In particular, oat protein was reported to significantly increase the *Bacteroidetes*:*Firmicutes* ratio (B/F ratio) as well as increase the abundance of *Muribaculaceae* and decrease that of *Erysipelotrichaceae* in hypercholesterolemic hamsters (Tong et al., 2021). The development of MetS and obesity are associated with a loss of intestinal microbial diversity and lower B/F ratios in both humans and animals (Moossavi & Bishehsari, 2019; Qiao et al., 2014; Woting & Blaut, 2016). The gut microbiota produces metabolites such as SCFAs and secondary bile acids which are shown to have important effects on cholesterol metabolism through many mechanisms reviewed by Jia et al., 2023.

Oat protein and other plant-derived protein sources were reported to promote SCFA production, namely acetate, butyrate and propionate, which could provide hypocholesterolemic effects (Kostovcikova et al., 2019; Tong et al., 2021; Tsukahara & Ushida, 2000). Approximately 90% of cholesterol output is caused by the conversion of cholesterol to bile acids in the liver (Jia et al., 2023). The consumption of oat protein and other plant protein sources is shown to stimulate fecal bile acid excretion resulting in a decrease in plasma total cholesterol (Guo et al., 2014; Spielmann et al., 2008; Tong et al., 2016, 2021). Several animal studies have shown that butyrate and propionate supplementation caused a reduction in LDL- and total-cholesterol (Flaig et al., 2023). Furthermore, butyrate and propionate has been reported to downregulate the gene expression of HMG-CoAR and several other genes involved in the cholesterol biosynthesis pathway in human enterocytes (Alvaro et al., 2008). Therefore, we speculate that the oat protein consumption may beneficially alter the gut microbiome that improved SCFA production and fecal bile acid excretion to produce hypocholesterolemic effects, though more studies are warranted to explore this topic.

High-fat feeding has been associated with changes in glucose uptake and metabolism due to the presence of excessive lipids and their oxidation at the tissue level (Kahn & Pedersen, 1993). High caloric intake is also known to decrease the levels pathological changes in pancreas, GLUT-4 expression that can lead to an increase circulating glucose (Auberval et al., 2014; Kahn & Pedersen, 1993). In the present study, the OGTT showed that glucose levels were significantly higher in HF/HS fed rats at 30 and 120 min. Based on these results, we can assume that at 16 weeks of treatment, the HF/HS groups were starting to develop insulin resistance. HF/HS+OP fed rats had significantly lower glucose levels compared to HF/HS fed rats at 30, 60 and 120 min.

These results suggested that oat protein supplementation may be able to prevent adverse changes in glucose tolerance in the setting of HF/HS feeding. Had we prolonged the study, we may have been able to see a more exaggerated effect and significant results in the AUC analysis.

Metabolic syndrome is often accompanied by systemic inflammation known as "metabolic inflammation". Metabolic inflammation is characterized by altered profiles of circulating cytokines such as TNF-α, infiltration of immune cells into tissues, activation of systemic inflammatory pathways and increased lipid peroxidation (Chan et al., 2019; Moreto et al., 2014). In this study, we did not observe significant differences for TNF-α among groups at 16 weeks of treatment, though there was an upward trend in both the HF/HS and HF/HS + OP groups compared to the control, which is most likely attributed to the consumption of the HF/HS diet. There were also no significant changes in the levels of serum lipid peroxidation product, MDA between groups. This points to the possibility that there was no systemic oxidative stress due to HF/HS feeding at 16 weeks. That being said, HF/HS may have caused oxidative stress at tissue level in specific organs. Further studies are needed to delineate this aspect.

Serum urea levels were not significantly different among groups. However, we observed significantly reduced creatinine levels in the HF/HS + OP group. All groups fall within the clinical range of 17.68 – 44.21 µmol/L for rats (Giknis & Clifford, 2008). These results suggest that oat protein consumption may exert an effect on renal function and creatinine clearance. A possible explanation could be the reduced amount of methionine in oat protein compared to casein. Muscle creatine synthesis utilizes roughly 30% of the available methionine pool, therefore the reduced amounts of methionine available in oat protein would explain a decrease serum creatinine (Da Silva et al., 2009). We also examined the effects of oat protein consumption on ALT and AST.

ALT and AST are enzymes that have important roles in the glutamate cycling pathway. Serum ALT and AST activities are commonly used biomarkers to assess liver health and function (Sookoian & Pirola, 2012). In a clinical setting, abnormal levels of liver enzymes, such as ALT and AST, are associated with increased risk of MetS , T2D and CVD. HF/HS diet feeding is shown to significantly increase ALT and AST levels which may be associated with liver dysfunction and the development of hepatic steatosis (Ishimoto et al., 2013; Simoes et al., 2020). In the current study, the HF/HS + OP group exhibited significantly increased levels of serum ALT. It's important to note that this value (mean of 32.3) is not clinically significant, as it falls within the normal clinical range of 18-45 U/L for this parameter in Wistar rats (Giknis & Clifford, 2008). We believe this is attributed to a shift in amino acid metabolism related to plant protein consumption. Using isotopic methods, Lépine et al., 2023 demonstrated that plant protein consumption, compared to animal protein, induced higher amino acid transamination and increased routings of dietary carbohydrates and lipids toward dispensable amino acid synthesis through glycolysis and ß-oxidation in HF/HS fed Wistar rats. Furthermore, several fish models have reported increased ALT following the consumption of a plant-based diet (Abdel-Latif et al., 2022). For this reason, we speculate that the change in ALT levels in the HF/HS+OP group is not pathological and is instead related to the re-routing of the metabolism to rely less on the dietary protein source and more on the utilization of carbohydrates and lipids for amino acid synthesis, which may partially explain the beneficial effects of plant protein on the glucose and cholesterol metabolism (Lépine et al., 2023). Therefore, the metabolomic analysis of oat protein consumption would be very beneficial to explore.

3.5. Conclusion

The present study showed that replacing casein with protein derived from oats provided protective effects against cardiac lipotoxicity and beneficially modulated cholesterol and glucose metabolism in Wistar rats fed a HF/HS diet. Furthermore, oat protein consumption increased ALT levels in HF/HS fed rats which may suggest rerouting of the amino acid metabolism, and triglycerides, which implies there may be an effect on organ lipid deposition. More studies are needed to fully elucidate the effects of oat-protein on cardiometabolic health and the mechanisms by which they take place. Well-designed randomized human feeding studies are warranted to further validate the efficacy of oat proteins and level of incorporation needed to observe beneficial effects in metabolic health and disease. Furthermore, efforts should be concentrated on the development of functional food products containing oat protein as well as nutraceutical and therapeutic applications.

Supplementary Materials:

Table S1: The amino acid composition of oat protein and casein

Amino acid	Oat Protein (%)	Casein (%)
Alanine	3.6	2.5
Arginine	6.8	3
Aspartic Acid	5.4	2.5
Cysteine	1.6	0.6
Glutamic Acid	17.0	10.4
Glycine	4.0	1.5
Histidine	2.3	2.3
Isoleucine	3.8	3.7
Leucine	6.9	7.8
Lysine	2.7	6.5
Methionine	1.2	2.5
Phenylanine	5.8	4.2
Proline	4.5	8.8
Serine	2.8	5
Threonine	2.3	3.6
Trypthophan	1.2	1
Tyrosine	3.5	4.5
Valine	5.0	4.6
Cystine + Methionine	2.8	3.1
Total Amino Acids	80.5	78.1

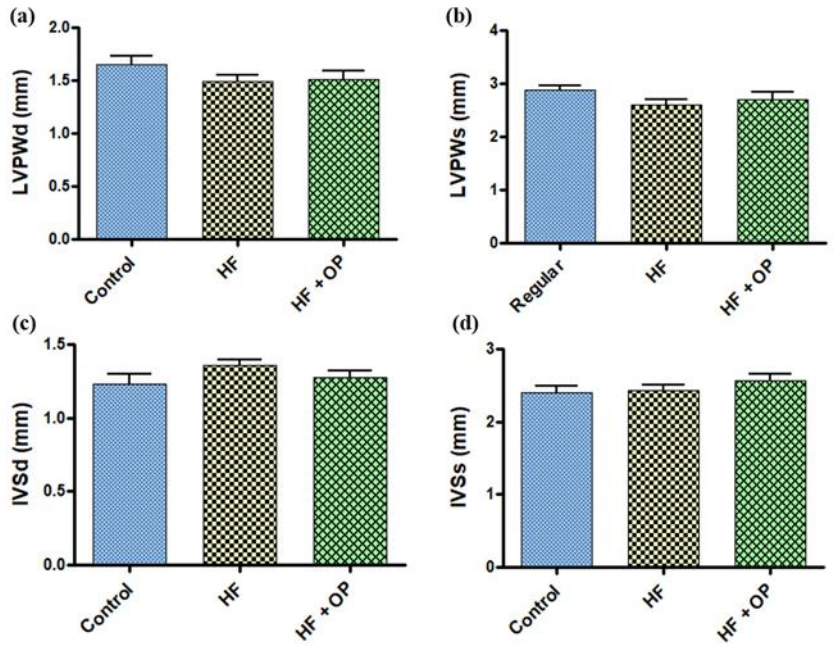

Figure S1: The effects of oat protein on a) left ventricular posterior wall thickness at end diastole (LVPWd), b) left ventricular posterior wall thickness at end systole (LVPWs), c) interventricular septum thickness at end diastole (IVSd) and d) interventricular septum thickness at end systole (IVSs) after 16 weeks of treatment. Values are means ± SEMs. Significant values are defined as $p < 0.05$. * denotes significance versus the control group. # denotes signficance versus the HF/HS group.

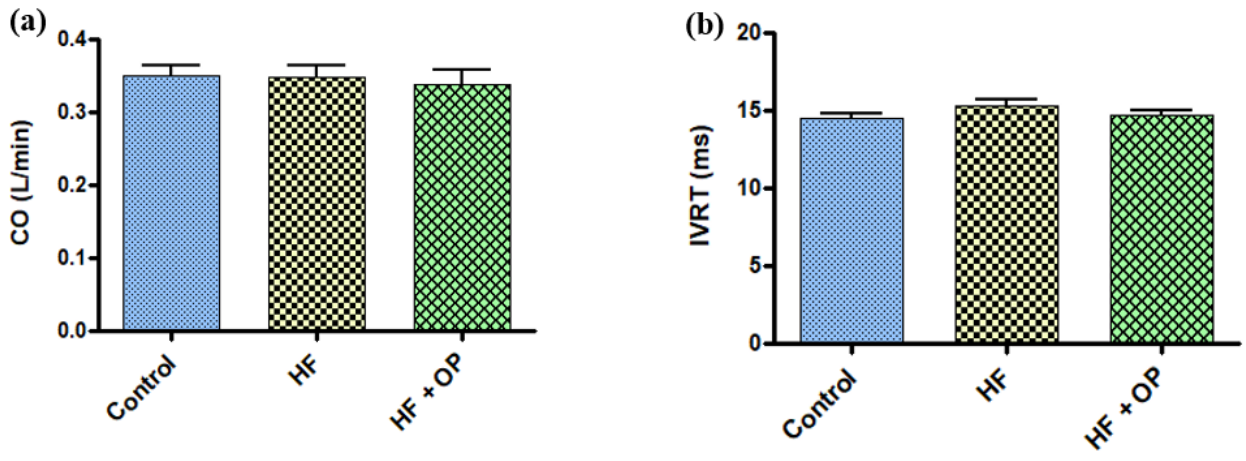

Figure S2: The effects of oat protein on a) cardiac output (CO) and b) isovolumic relaxation time (IVRT) after 16 weeks of treatment. Values are means ± SEMs. Significant values are defined as $p < 0.05$. * denotes significance versus the control group. # denotes signficance versus the HF/HS group.

Author Contributions:

Conceptualization was done by S.J.T. and T.N.. Original draft was prepared by J.B. and P.R.. Methodology was done by J.B, B.S, and L.Y.. Data curation and formal analysis was done by J.B., L.Y. and P.R.. Visualization was done by J.B.. Review and editing were done by J.B., P.R., L.M., M.M., S.J.T., and T.N.; Funding acquisition was done by S.J.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Agriculture and Agri-Food Canada's Agri-Science Program in collaboration with Prairie Oat Growers Association (grant # 3672)

Data Availability Statement: The data used to support the findings of this study are included within the article and the supplementary information files.

Acknowledgments: The authors would like to thank Li Ren and the staff from the R. O. Burrell Animal Facility for their help with this study.

This study was presented at the ACS Fall Meeting, August 13-17 2023.

https://acs.digitellinc.com/sessions/570470/view

Conflicts of Interest: The authors have no conflict of interest to declare.

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4.0 CONCLUSIONS AND FUTURE DIRECTIONS

To conclude, our study has provided some valuable insight into the health effects of oat protein. We were able to observe that oat protein provided protective effects against the HF/HS-induced lipotoxicity to improve systolic function, lowered total- and LDL-cholesterol, and reduced HMG-CoAR activity in Wistar rats, showing that β-glucan is not the only cholesterol-lowering component in oats.

We were presented with some limitations throughout this study. We were unable to collect any insulin related data due to evidence of hemolysis in the serum samples collected during the oral glucose tolerance test. Insulin resistance is a primary driver in metabolic syndrome. This data would be important to determine if there were any indications of insulin resistance resulting from high fat, high sugar feeding, as well as gain insight on the glucose metabolism of the Wistar rats in this study. Had the samples not have been hemolyzed, we would have also looked into serum HbA1c to determine if there were any long-term elevations of blood glucose among groups.

Though we observed no significant changes in blood pressure among groups, the data revealed all rat groups, including the control, had elevated blood pressure. We found that the rats were exhibiting moderate signs of stress during blood pressure readings, which would undoubtably raise the blood pressure values obtained. It's possible that the rats weren't adequately trained to tolerate the tail cuff sphygmomanometer used for blood pressure readings.

A lipid panel in the liver tissue (such as TG, total-, free-, LDL-, VLDL-, HDL-cholesterol) could have provided more insight into the effects of oat protein on the lipid metabolism of HF/HS fed rats. Furthermore, several other important markers of cholesterol

metabolism such as SREBP-1c and -2, hepatic lipase, lipoprotein lipase, ACAT1, ABCG-5 and -8, and LDL-R could have been addressed in the liver tissue to provide a comprehensive mechanistic background into why we observed lowered serum total- and LDL- cholesterol in the HF/HS+OP group. We also could have looked into fecal bile acid excretion as a possible mechanism for cholesterol lowering.

Lastly, the data from this experimental animal trial cannot be directly translated into human clinical significance, as oat protein was the sole protein source in the intervention group. This is unrealistic, as humans consume a large variety of protein sources and cannot rely solely on oat protein for sustenance. As previously mentioned, oats contain roughly 12-20% protein, and though the protein quality is comparable to soy protein, it is an incomplete protein source due to lysine and methionine being limiting amino acids.

Future efforts should be directed to conducting pre-clinical and clinical trials that would further assess the cardiometabolic effects of oat protein consumption with emphasis on the cholesterol and glucose metabolism and the mechanisms by which these effects take place. The adequate level of oat protein incorporation to observe beneficial health effects in humans is yet to be determined. Furthermore, efforts should be concentrated on the development of functional food products containing oat protein as well as nutraceutical and therapeutic applications. Oat protein is a sustainable, high-quality plant-based protein source that can be supplemented into various food products to enrich their nutritional and textural properties. Therefore, oat protein could be a cost-effective, health promoting ingredient that could provide a lot of value to the food supply chain.