Spring 2019

Genomic and Proteomic Effects of Red Raspberry (Rubus idaeus) Consumption on the Perivascular Adipose Tissue of the Obese Zucker Rat, a Model of Human Metabolic Syndrome

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GENOMIC AND PROTEOMIC EFFECTS OF RED RASPBERRY (*RUBUS IDAEUS)* CONSUMPTION ON INFLAMMATION IN PERIVASCULAR ADIPOSE TISSUE OF THE OBESE ZUCKER RAT, A MODEL OF HUMAN METABOLIC SYNDROME

by

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A Thesis Submitted in Partial Fulfillment
of the Requirements for a Degree with Honors
(Biochemistry)

The Honors College

University of Maine

May 2020

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ABSTRACT

The Metabolic Syndrome (MetS) affects 35% of U.S. adults and is an indicator of early death. While pharmacological treatments have been developed for the majority of MetS risk factors, obesity-induced inflammation remains to be addressed. Dysfunctional adipose tissue is a source of inflammation, and perivascular adipose tissue (PVAT) is critical in its pathogenesis. This study investigates the effects of red raspberry (*rubus idaeus*) diet-enrichment on inflammation of PVAT. The obese Zucker rat (OZR) model of MetS and the lean Zucker rat (LZR) control (C) model were used. Rats received an eight-week control or whole red raspberry-enriched (WRR) diet (8% w/w red raspberry powder). RT-PCR was performed on LZR and OZR PVAT homogenates to determine gene expression of pro-inflammatory markers (IL-1β, IL-6, MCP- 1, NF- κB, TNF- α) and anti-inflammatory markers (adiponectin and IL-10), and ELISAs were performed to determine concentrations of a subset of these markers (adiponectin, IL-1β, IL-10, MCP-1). RT-PCR analyses of PVAT indicated a significant down-regulation of pro-inflammatory marker NF-κB in OZR-C versus LZR-C models. ELISA analyses indicated a significant increase in anti-inflammatory marker adiponectin concentration in OZR-WRR versus OZR-C models, a significant decrease in anti-inflammatory marker IL-10 concentration in OZR-C versus LZR-C models, and a significant decrease in pro-inflammatory marker IL-1β concentration in OZR-C versus LZR-C models. Findings suggest that WRR enrichment does not have a consistent genomic or proteomic effect on PVAT inflammation status. Further investigations are needed to elucidate the molecular mechanisms dictating the pro-inflammatory and anti-inflammatory effects observed.

ACKNOWLEDGEMENTS

Thank you, first and foremost, to my advisor Dr. Klimis-Zacas: your genuine dedication to teaching, mentoring, guiding and encouraging me throughout the past two years has been greatly valued! Thank you for providing the opportunity to engage in research that lies at the intersection of my passions for nutrition and biochemistry, and for allowing me to take on this project that has greatly expanded my knowledge, skills, and confidence.

Thank you to Natalie VandenAkker, Dr. Panagiotis Tsakiroglou, and Dr. Stefano Vendrame for sharing your knowledge and expertise. Special thanks to Natalie for teaching me all of the relevant techniques, answering all of my questions, sharing the lab with me every day, and for your patience and kindness!

Thank you to each member of my committee for providing guidance, encouragement, commitment, and education in a diversity of ways throughout my undergraduate years.

Thank you to each of my family and friends for the abundance of support, guidance and encouragement that you have provided. And a very special thanks to my Mom and Dad, for modeling the value of hard work, perseverance and kindness since I was young.

I would like to thank the Department of Food Science & Human Nutrition Dr. Alfred Bushway Fellowship, Honors College Dr. Carolyn E. Reed Fellowship, Honors College Charles Slavin Fellowship, Honors College INBRE Thesis Fellowship (Institutional

Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103423), the National Processed Raspberry Council, and the Southport Island Association for funding my research. Thank you to FutureCeutricals for providing red raspberry powder.

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INTRODUCTION

The Metabolic Syndrome (MetS) is a collection of risk factors that affects greater than 30% of U.S. adults and is a significant indicator of early death (1–4). A major contributing factor to the dangers associated with MetS is obesity-induced inflammation (5). While pharmacological treatments have been developed for the majority of MetS risk factors, obesity-induced inflammation remains to be addressed. Considering that obesity rates are persistently increasing in the United States, with over two-thirds of US adults either overweight or obese, effective prevention and treatment are essential to improve and protect the well-being of citizens (4). This is particularly important in the State of Maine, with the highest obesity rate in New England at 29.1% (6).

An established source of inflammation in conditions of obesity is dysfunctional adipose tissue (7). The role of visceral adiposity in MetS has been explored at length, however recent research suggests that perivascular adipose tissue (PVAT) is critical in the pathogenesis of dysfunctional adipose tissue. PVAT surrounds blood vessels and is a highly-active endocrine organ, directly influencing vascular tone and inflammation (7). PVAT releases several anti-inflammatory markers and has been observed to benefit vascular function in healthy individuals (7). However, in imbalanced states such as chronic obesity, PVAT can become dysfunctional and releases pro-inflammatory markers, yielding a loss of the benefits conferred by PVAT (7). Much remains to be understood about the changes that convert PVAT from a functional to a dysfunctional state, as well as modalities for improvement. There is a paucity of studies on whether, or how, diet modulates PVAT biochemistry and physiology for application to this gap in knowledge.

The few studies that have investigated the effect of diet on PVAT biochemistry and physiology used rodent models and high-fat diet (HFD) feeding, with some experiments also employing calorie restriction diets (8, 9). Resultant data have demonstrated effects on adipocyte (adipose cell) size and number, gene expression, and inflammatory marker production upon dietary changes (8, 9). There is a lack of studies on the effect of different diets on PVAT and inflammation status. Studies investigating the effects of functional foods (those which confer physiological benefits) on PVAT are also limited, and only a small number of these have employed berries.

Berries are known for their robust antioxidant content (2). Red raspberries are gaining nutritional interest due to their unique bioactive profiles, with a particularly high level of the polyphenol ellagitannin which has been documented to physiologically benefit cardiovascular health (10). Previous studies in the Klimis-Zacas laboratory have demonstrated the efficacy of a wild blueberry-enriched diet in the attenuation of obesity-induced inflammation, through successfully decreasing PVAT expression and concentration of inflammatory markers in the obese Zucker rat (OZR), a model of MetS (11–14).

This study aims to address the gap in knowledge regarding the effect of a red raspberry-enriched diet on PVAT and inflammation status. The specific goal of this study is to determine gene expression of the pro-inflammatory markers IL-1β, IL-6, MCP- 1, NF- κB, and TNF- α and the anti-inflammatory markers adiponectin and IL-10, and to determine concentrations of a subset of these markers (adiponectin, IL-1β, IL-10, and MCP-1) in the PVAT of two obese Zucker rat (OZR) and two lean Zucker rat (LZR) groups: those receiving a control diet and those receiving a red raspberry-enriched diet.

LITERATURE REVIEW

I. Metabolic Syndrome

The Metabolic Syndrome (MetS) is a collection of risk factors, including abdominal obesity, insulin resistance, dyslipidemia, elevated blood pressure, and decreased HDL cholesterol (1, 2). It is important to note that slightly varying definitions for MetS do exist among different health organizations, including the use of weight circumference versus body mass index, and varying triglyceride and blood pressure definitions (1). The definition used in this study is established by the US National Cholesterol Education Program- Adult Panel III (NCEP ATPIII) (2, 15).

MetS is generally diagnosed when at least three of these five risk factors are present (16). MetS affects greater than 30% of U.S. adults and is a significant indicator of early death (3, 4). There are established connections between MetS and elevated risk for several diseases, including Cardiovascular Disease, Diabetes Mellitus, esophageal and liver cancer, and schizophrenia (17–19). Other conditions which are often observed as comorbidities with MetS are a pro-thrombotic and/or pro-inflammatory state, non-alcoholic fatty liver disease, and reproductive disorders (1). Therefore, MetS is relevant to a variety of fields of study and medical practice.

Current MetS treatment options primarily involve lifestyle interventions, such as physical fitness, dietary changes, smoking cessation, and stress reduction (20). Medications can also be employed to target certain MetS conditions, including ACE inhibitors for high blood pressure, statins to regulate cholesterol, and low-dose aspirin for heart attack and stroke risk reduction (20). A study conducted by The Diabetes

Prevention Program compared outcomes for patients following lifestyle interventions (weight loss using diet and exercise) versus metformin, a medication for high blood sugar, and versus a placebo treatment (21). The initial screening step of the study was the presence of impaired glucose tolerance in subjects, followed by measurements of blood pressure, waist circumference, and fasting HDL and triglyceride levels (21). The study documented that when compared with placebo results, MetS incidence was reduced by 41% for patients employing lifestyle changes, while it was reduced by only 17% for the metformin-receiving patients (21). Following a three-year period, MetS was "resolved" in 38% of the lifestyle-changes group, 23% of the metformin group, and 18% of the placebo group (21). Certain diets have been documented to be particularly effective in the improvement of various MetS risk-factors and comorbidities, including calorie-restricted diets for obesity, diabetes, inflammation, and cardiovascular disease (CVD), increased meal frequency for obesity, and the Mediterranean Diet for diabetes, CVD and obesity (22).

II. Obesity-Induced Inflammation

As mentioned previously, a pro-inflammatory state is recognized as a co-morbidity of MetS (1). Inflammation is a tissue response to stimuli such as injury, pathogens, stress, and the presence of other irritants (23). The acute inflammatory response includes dead or infected cell removal, tissue repair, and wound healing (23). Specifically, the acute inflammatory response is initiated by resident macrophages and mast cells, which produce inflammatory mediators including chemokines and cytokines (24). This triggers leukocyte delivery to the site of injury, among other "plasma

components," which then leads to production of inflammatory mediators such as chemokines and cytokines (24).

This cascade of events enables the "injurious agent" to be removed, followed by the presence of anti-inflammatory and pro-resolution cytokines, and the activity of macrophages (24). These macrophages include both those which are residents to the tissue of interest and those which have been newly-recruited (24). Resolution of inflammation then involves apoptosis and phagocytosis of leukocytes by macrophages, and subsequent departure from the site of inflammation via lymphatic drainage (24). These events all support the transition back to a healthy state of homeostasis in the tissue (24).

Acute inflammation is an essential component in the successful resolution of injuries (24). However, it is when the inflammatory state is either not resolved or is dysregulated, and becomes chronic, that it can become harmful to the body (24). Failure to resolve the inflammatory state can occur when the original stimuli are not neutralized and removed, or if they are removed but inflammatory cells and molecules remain in and near to the site of injury (24). In cases of obesity, excessive adipose tissue expansion leads to some adipocytes becoming apoptotic, which prompts subsequent recruitment of pro-inflammatory macrophages (25, 26). Macrophages are a diverse and adaptable family of large cells with roles in detection and phagocytosis of foreign material, antigen presentation, and the release of cytokines for inflammation initiation (27, 28). Due to their adaptability among phenotypes, macrophages are both very important for healthy repair and maintenance of homeostasis, and capable of contributing to the development of chronic inflammatory diseases (28).

In the case of adipose tissue inflammation, macrophage infiltration leads to an elevated production of pro-inflammatory cytokines (29). Research has demonstrated that inflammatory cytokine expression in adipose tissue is primarily sourced from macrophages, not from adipocytes, which suggests that inflammation is initiated at least in part by macrophage infiltration (29). One theory states that initiation of macrophage infiltration is caused by adipose tissue expansion occurring in concert with weight gain, and the subsequent cascade of events that this causes (29). These events include death of adipocytes, elevated leptin secretion, and recruitment of neutrophils and T cells (29). Upon adipose tissue macrophage infiltration, these macrophages then recruit more macrophages through the release of chemokines such as MCP-1 (29). The subsequent abundance of macrophages can contribute to adipose tissue remodeling, in which the shape and number of adipocytes change (29).

A possible result of this cascade of events is the polarization of macrophages to either an M1 or an M2 macrophage phenotype (29). The M1 macrophage phenotype is referred to as "classically-activated," with inflammation prompting its formation, while the M2 macrophage phenotype is prompted by IL-4 and IL-13 exposure (29). Macrophages with an M1 phenotype secrete pro-inflammatory cytokines, while macrophages with an M2 phenotype secrete anti-inflammatory cytokines and potentially assist in tissue repair (29). Research has indicated that lean mice possess adipose tissue with higher levels of M2 macrophages, and mice receiving a high-fat diet possess higher levels of M1 macrophages (29, 30). Human studies have confirmed the presence of both M1 and M2 populations in adipose tissue, and that these populations secrete pro- and anti-inflammatory cytokines in the same pattern that mice do (29, 31). Further, it has

been reported that M1 adipose tissue macrophages are major causes of both adipose tissue inflammation and insulin resistance in obesity (32).

III. Model for Obesity-Induced Inflammation

The obese Zucker rat (OZR) is a model of Human MetS, while the lean Zucker rat (LZR) is a control model (33, 34). OZRs are an effective model for human MetS because they exhibit all of the risk factors associated with MetS (33, 34). Compared with LZRs, OZRs exhibit hyperphagia, elevated circulating insulin levels, hyperlipidemia and elevated inflammation (33). The OZR genotype, fa/fa, causes a leptin receptor defect, and subsequent development of hypertriglyceridemia, hypercholesterolemia, hyperinsulinemia, and insulin resistance, and "moderate" hypertension which develops between eight and twenty weeks of age (13). OZRs become obese within three-to-five weeks of age (33). Only male rats were used in the present study; previous experiments in different studies have indicated that female rats can influence results due to hormonal conditions (35).

The leptin receptor mutation characteristic of the fa/fa genotype causes leptin signaling to be impaired, which is a contributing factor to the higher body weight of OZR (33, 36). This is because leptin plays a significant role in the regulation of energy balance (33, 36). Leptin is a protein produced and released by adipose tissue, with release occurring at a rate that is proportional to the quantity of lipids stored (33). Leptin targets leptin receptors in the brain, with elevated targeting associated with decreased food intake and elevated energy expenditure (33). The levels of circulating leptin are significantly elevated in OZR compared with LZR (33).

IV. Inflammatory Markers

Adiponectin

Adiponectin (also known as Acrp30, adipo-Q, and apM1) is a cytokine produced by adipose tissue, also known as an adipokine (37). Adiponectin was first discovered in 1995, and has since emerged as a molecule with prominent roles in the initiation and alteration of metabolic responses and inflammation, as well as cardio-protection (37, 38). Adiponectin is primarily known as an anti-inflammatory cytokine; circulating plasma levels of adiponectin are typically elevated in healthy individuals compared with in cases of obesity, in which circulating levels decrease (39). Adiponectin can be secreted by PVAT, and has been observed to normalize endothelial function through a subsequent elevation in eNOS phosphorylation, and suppression of ROS generation as a negative modulator of innate immune response (39, 40). The protective effects of adiponectin may also be due to its regulation of energy substrate and calcium ion metabolism (38).

In studies using lean mice, the adiponectin produced by PVAT has been established to exert anti-inflammatory effects that attenuate neointimal formation (41, 42). The neointima is the arterial intima layer that thickens in cases of atherosclerosis, through cell migration and proliferation (43). In addition, adiponectin has been shown to modulate macrophage function towards an anti-inflammatory phenotype, through polarization towards the M2 phenotype, supporting the classification of adiponectin as an anti-inflammatory cytokine (39) (44).

Adiponectin is unique in its proposed role in a compensatory mechanism of action in cases of inflammation (45–47). This compensatory mechanism has been suspected due to evidence of elevated adiponectin expression and concentration in certain cases of

inflammation (45–47). In a study of humans with a BMI less than 25, between 25 and 30, or greater than 30, the left internal thoracic artery PVAT of patients in the highest BMI category had significantly elevated adiponectin compared with those in the other two categories (47). In that study the patients with a BMI greater than 30 also exhibited a slightly improved endothelium-dependent vascular function compared with patients with a BMI between 25 and 30 (47). These findings support the proposed compensatory mechanism, with the potential for the elevated adiponectin expression to have improved the vascular function of those in the highest BMI category. However, it is unclear what exact conditions prompt such a response, as well as the duration of time that this response persists.

IL-10

IL-10 (also known as CSIF or TGIF) is an anti-inflammatory cytokine produced primarily by macrophages with M2 polarization (39, 48). The roles of IL-10 include inhibition of macrophage, Th1, and NK cell activity at times of infection, as well as inhibition of pro-inflammatory cytokine synthesis (48, 49). IL-10 also inhibits NADPH-dependent oxidative stress and elevates NO production, which improves endothelial function (39). While this cytokine is important for a healthy inflammatory response and clearance of pathogens, it can also cause tissue damage when present in excess; therefore, the action of IL-10 must be tightly-regulated to maintain a healthy infection response (48).

With regards to MetS, low circulating IL-10 levels have been associated with MetS, as well as with high body mass index and high percent fat mass (50). IL-10 has

also been shown to exert protection against the formation and stability of atherosclerotic lesions (49). In a study that investigated IL-10 levels in obese and non-obese women with MetS, both populations were determined to have elevated circulating IL-10 levels (49). This finding suggests that in cases of MetS the body responds by elevating IL-10 levels, in an attempt to inhibit the elevated pro-inflammatory cytokine production that has developed (49). In the same study, following a 12-month lifestyle program that included elevated physical activity, obese women without MetS exhibited a decrease in circulating IL-10 levels; however, this decrease was not observed among obese women with MetS (49).

Of note, the STAT3 gene is a downstream target of IL-10 (51). The Janus kinase/signal transducers and activators of transcription (JAK-STAT) pathway is associated with preventing the development of obesity-associated disorders such as leptin resistance and insulin resistance, and the STAT3 gene is a downstream target of IL-10 (51). The JAK-STAT pathway is key in the development of many inflammation-related diseases such as obesity and cancer, and specifically can contribute to pathogenesis in cases of chronic activation (52). JAK-STAT is also involved in the regulation of many "developmental and homeostatic processes" by molecules such as cytokines and hormones (52).

While no studies have been identified that investigate the gene expression or protein concentration of IL-10 in PVAT, some studies have elucidated connections between brown adipose tissue (BAT) thermogenesis and IL-10 (53). An unexpected finding determined that removal of IL-10 signaling in mice actually caused WAT browning, as well as conferred protection against diet-induced obesity, with elevated

mitochondrial respiration observed (53). The study employed an antisense oligonucleotide WAT IL-10 receptor knock-down. Research conducted by the same lab indicates that IL-10 is a repressor of thermogenic gene transcription, through chromatin modulations at "key enhancer and promoter regions" (53). This research used a genome-wide Assay for Transposase-Accessible Chromatin (ATAC)-seq and RNA-seq, and the findings suggest that IL-10 is a "novel regulator of a thermogenic transcriptional program in adipose tissue" (53).

IL-1β

IL-1β is a pleiotropic pro-inflammatory cytokine with roles in sleep, hunger, injury response and inflammation (54). IL-1β is important for protection during infections, however, similar to other inflammatory cytokines, overly elevated expression of IL-1β can cause tissue damage (55). This molecule is produced primarily by macrophages but is also released by dendritic cells (39). Dendritic cells are most often present on the border between PVAT and the adventitia, but they can also exist within PVAT (39). IL-1β is believed to play a role in the development of insulin resistance in cases of obesity (56). Specifically, it has been observed that IL-1β acts through the inhibition of insulin signaling (56, 57). Furthermore, it has been determined that insulin signaling and action in human adipocytes is improved upon blocking IL-1β activity (56, 58, 59). High levels of circulating levels of IL-1B, in concert with high circulating levels of IL-6, have been determined as a prediction for Type 2 Diabetes (60).

White adipose tissue (WAT) has generally been observed to release an elevated amount of IL-1β in cases of human and rodent obesity, with expression higher in visceral

than subcutaneous adipose tissue (57, 60, 61). Previous *in vivo* and *in vitro* adipocyte studies have indicated that IL-1β plays roles in lipolysis activation, insulin resistance, and adipogenesis inhibition in adipose tissue, as well as elevated expression and secretion in cases of inflammation (60, 62–64). It has also been observed that "lipid storage capacity" in adipose tissue, adipose tissue macrophages, and the liver can be regulated by IL-1β (60). Interestingly, due to the adipogenesis-inhibiting action and adipose tissue-liver crosstalk abilities of IL-1β, low levels of this cytokine are associated with lower liver weights in cases of excess calorie consumption (60).

IL-1β is unique among cytokines in that it is not initially inactive upon being produced, and in order to become biologically active must first be activated by inflammasomes via cleaving by caspase-1 (55, 56). Inflammasomes are protein complexes of several subunits that regulate caspase-1 (formerly called IL-1 beta converting enzyme/ICE), which is a proteolytic enzyme (65). Inflammasomes also typically contain nucleotide-binding domain–like receptors (NLRs), and stimuli that can activate NLRs include mitochondrial ROS, bacterial flagellin, extracellular ATP, potassium efflux, and components of the type III secretion system, among other signals (55, 66, 67). It has been determined that the production of IL-1β by macrophages requires the inflammasome components NLRP3, ASC and caspase-1, but not NLRC4, in cases of infection (55). Due to the ability of IL-1β to cause tissue damage in cases of overexpression, it is believed that abnormalities in inflammasome regulation and activation are involved in inflammatory disease pathogenesis (55).

Studies investigating IL-1β expression and content in PVAT are limited, however two studies have been identified (68, 69). One study that investigated human patients

with coronary heart disease documented that IL-1β was expressed at significantly higher levels in coronary artery PVAT (PVAT-CA) vs. internal thoracic artery PVAT (PVAT-ITA) (68). This finding aligns with a previous understanding that PVAT-ITA exhibits atherosclerosis resistance, and therefore it is often used as an artery bypass graft, due to the nature of IL-1β as a pro-inflammatory cytokine (68). These results also provide further evidence for the previously discussed understanding that varying adipose tissue depots exhibit varying phenotypes and functions. In a different study that investigated cultured visceral perivascular adipocytes isolated from human smokers and non-smokers, the perivascular adipocytes of those who smoked regularly exhibited elevated P2X7R-inflammasome complex expression and activity (69). This suggests that regular smoking elevates PVAT inflammatory status, which aligns with previously held findings that smoking is a risk factor for cardiovascular disease (70).

IL-6

IL-6 is a pluripotent pro-inflammatory cytokine and marker of M1 polarization that regulates body weight and lipid metabolism at a systemic level (71). It is suspected that IL-6 secretion is regulated by a diverse array of factors, including hormones, cytokines, diet, physical activity, stress, and hypoxia (72). Higher circulating plasma and adipose tissue concentrations of IL-6 are associated with chronic obesity and insulin resistance (73). Despite this association, both beneficial and harmful effects of IL-6 have been observed with regard to each of these conditions, and the many roles of IL-6 continue to be elucidated (71). It is interesting to note that insulin resistance is more closely linked to adipose tissue concentrations and secretions of IL-6 than with

circulating plasma IL-6 levels (73). It is also interesting to consider that anorexia nervosa has been associated with elevated systemic IL-6 levels (73). It is suspected that IL-6 levels are elevated secondary to extreme calorie deficits, however they may also have a role in the cachexia of anorexia due to a similar role in cancer-associated cachexia (74).

Secretion of IL-6 has been documented from macrophages, adipocytes, skeletal muscle, fibroblasts, and endothelial cells (71). This cytokine is generally present in higher expression levels in human abdominal versus subcutaneous adipose tissue, and in much higher levels surrounding adipose tissue than in circulating plasma (75). It is suspected that IL-6 may play a role in the control of adipose tissue mitochondrial content, through the activation of AMPK and subsequent regulation of PGC-1a and mitochondrial biogenesis (75). IL-6 has also been documented to decrease lipoprotein lipase (LPL) activity in humans, thus potentially affecting adipocyte metabolism (73).

Few studies have been conducted regarding IL-6 presence in PVAT. In a study using cell-cultured aortic PVAT from low-density lipoprotein receptor-deficient mice, it was determined that elevated secretion of PVAT-derived IL-6 mediated aortic stiffening and remodeling (76). It has also been observed that IL-6 inhibition attenuates mechanical stiffness (76). Based upon other studies it is also understood that IL-6 secretions are elevated in PVAT of older mice and from PVAT compared to other adipose tissue depots, and that PVAT IL-6 expression is elevated in cases of higher plasma cholesterol (76, 77). In addition, it has been determined that vascular smooth muscle cells (VSMCs) are also a source of IL-6; considering the close proximity of PVAT to VSMCs, this production is capable of significantly influencing PVAT inflammatory status (78).

MCP-1

MCP-1 is a chemokine with key roles in the regulation of macrophage migration and infiltration (79). MCP-1 production is both constitutive and induced by various cellular stimuli, including oxidative stress and the presence of cytokines or growth factors (79). MCP-1 is primarily sourced from macrophages, however fibroblasts and endothelial cells, epithelial, smooth muscle, mesangial and astrocytic are also capable of MCP-1 production (79). The primary functions of MCP-1 are the regulation of monocyte, natural killer cell, and memory T lymphocyte migration and infiltration (79).

With regards to MetS, MCP-1 has been associated with early atherogenesis, and is a recognized marker of endothelial dysfunction (80). In cases of obesity, MCP-1 expression is generally higher in visceral and subcutaneous adipose tissues of obese patients versus lean controls, as well as plasma concentrations of MCP-1 (81–83). Interestingly, fructose consumption has been documented to increase the already-high levels of plasma MCP-1 in obese patients (81, 84). It has also been determined that MCP-1 plays a key role in the promotion of adipose tissue angiogenesis and development of obesity; in mice with an MCP-1 receptor (CCR2) deficiency, fat deposition and insulin resistance were reduced (81, 85).

A limited number of studies have aimed to target MCP-1 for reduction and subsequent attenuation of obesity-associated syndromes (81, 86–90). In a study in which human MetS patients engaged in an exercise program, a decrease in plasma MCP-1 levels was observed (81, 88). Different studies determined that human bypass surgery resulted in decreased MCP-1 white adipose tissue (WAT) concentration, and administration of Atorvastatin (a type 2 statin) caused decreased serum MCP-1 content (81, 86, 87). Lastly,

using human cell culture, the administration of procyanidins from grape seeds resulted in decreased MCP-1 concentration, and administration of the vasodilator Dilazep caused elevated MCP-1 mRNA expression (81, 89, 90).

A limited number of studies have also investigated the presence of MCP-1 in PVAT (11, 91, 92). In a study conducted in the Klimis-Zacas lab, the PVAT of OZRs that received wild blueberry enrichment had significantly lower MCP-1 concentrations when compared to OZRs that consumed a control diet (11). In that study the MCP-1 concentrations of LZRs that consumed a control diet were also significantly lower than OZRs that consumed a control diet (11). In a study that employed artery injury and subsequent thoracic PVAT implantation, MCP-1 increased rates of neointima formation, but in the same study macrophage infiltration was unexpectedly not influenced by MCP-1 (91). It has also been documented that dysfunctional PVAT adipocytes can cause elevated production of MCP-1 in PVAT (91, 92). Therefore, it follows that the presence of pathological conditions in PVAT, such as pro-atherosclerotic factors, has been associated with elevated MCP-1 concentration in PVAT (92). Inflammation of PVAT has also been associated with significant increases in MCP-1 PVAT concentration, while endovascular injury has been observed to result in significant MCP-1 upregulation in PVAT (92, 93).

NF-κB

Nuclear factor kappa light chain enhancer of activated B cells (NF-κB) is a complex of transcription factors that have roles in the regulation of inflammation, immune responses, oncogenesis, metabolic diseases, and energy homeostasis (94). The

influence of NF-κB on the states of metabolism and inflammation are suspected to be due to the co-evolution and interdependence of nutrient and pathogen monitoring (94). This is in part because it is essential that the energy output required by organisms to defend against pathogens and other foreign materials not disrupt metabolic homeostasis, which could cause starvation (94). NF-κB consists of five subunits: NF-κB 1 (p50), Nf-κB 2 (p52), RelA (p65), RelB, and c-Rel (95). It is the dimerization of these subunits that forms NF-κB (95). Twelve unique combinations of these subunits have been observed *in-vivo*, and NF-κB is capable of target gene activation following assembly of its individual subunits (96).

Regulation of NF-κB takes place through localization, using IκBα (97, 98). IκBα is an inhibitor protein that causes NF-κB dimers to remain in the cytoplasm through non-covalent bonding (97, 98). However, upon the introduction of a diverse range of cellular stimuli, I kappa B kinase (IκK) is activated and subsequently degrades the IκBα that is complexed with NF-κB in the cytoplasm (97, 98). This degradation process begins with the phosphorylation of two N-terminal IκBα serines in the regulatory region, which prompts recognition by the ubiquitin ligase complex (97). The IκB is then polyubiquitinated, which serves as a signal for proteasomal degradation (97). The NF-κB dimers that remain are then able to translocate and accumulate in the nucleus where they then target genes for activation of transcription (97). This allows for activation of genes possessing a κB-binding site, which is a DNA-binding motif that the NF-κB dimers can bind to (97).

The activation of the IκK complex is influenced in part by the presence of several inflammatory cytokine receptors, including IL-1β, IL-10 and TNF-α (94). Further, the

presence of NF-κB in the nucleus regulates expression of these same inflammatory cytokines (94). Therefore, these inflammatory cytokines are part of a positive-feedback loop of expression (94). Interestingly, in the presence of chronic overnutrition such as obesity, activation of NF-κB by IκK takes place in macrophages, adipocytes, and hepatocytes (94). This results in recruitment and activation of macrophages, which is a key step in the "initiation and maintenance of an inflammatory reaction" (94).

The NF-κB pathway appears to also play a significant role in appetite regulation and food intake (94, 99). In studies using mice, a high-fat diet has been observed to cause ER stress and subsequent IKK*B*/NF-κB activation (94, 100). Inflammation in the mediobasal hypothalamus then occurs, which can lead to insulin and leptin resistance, as well as central leptin signaling disruptions (94, 99, 101). Central leptin signaling is responsible for communicating states of overnutrition to the central nervous system; therefore, the disruption in central leptin signaling that occurs following high-fat diet consumption can actually cause an elevated calorie intake, and subsequent weight gain (94). NF-κB also has roles in energy sensing and energy production, which are still in the process of elucidation (94, 102). Due to this connection between the NF-κB pathway and leptin signaling, it is important to note again the use of an experimental (obese) animal model in the current study that possesses a leptin receptor mutation (fa/fa) (34).

The specific subunit investigated in this study is NF-κB-1 (p50). P50 is one component of the primary form of NF-κB, which also includes p65 (103). P50 and p65 are named for their molecular mass, with p50 being 50 kD and p65 being 65 kD in mass (103). In addition to pairing with p65, p50 is also capable of binding DNA as a homodimer, and in both cases has a specificity for κB-binding sites (103). Previous

studies have indicated conflicting roles for p50 in inflammation and energy homeostasis; some studies using mice with a p50 deletion have observed elevated rates of exercise endurance and resistance to obesity, as well as the repression of obesity-associated symptoms such as macrophage adipose tissue infiltration and elevated blood glucose (104). However, other studies have observed an elevated presence of inflammatory cytokines (including TNF-α and IL-6) in adipose tissue and serum, and elevated macrophage adipose tissue infiltration, in cases of p50 knockout mice (105). Therefore, further studies are necessary to elucidate the specific role of p50 in inflammatory control.

TNF- α

TNF- α is a pro-inflammatory cytokine produced primarily by macrophages, but also by adipocytes, endothelial cells, microglia, and astroglia (106). TNF-α has been observed to regulate a diversity of processes including cell proliferation, survival, differentiation, and apoptosis (106). Abnormalities in TNF-α production have been associated with the pathogenesis of several inflammatory diseases, including obesity, diabetes and atherosclerosis (106). This finding has led to the development and approval of several "anti-TNF-α" drugs for disease treatment, such as those for rheumatoid arthritis and inflammatory bowel disease (106). Regulation of TNF-α at the transcriptional level by NF-κB and nuclear factor activated T cells has been documented, as well as at the translational level by the 3′ untranslated region (UTR) of TNF-α mRNA (106, 107).

In cases of obesity, TNF-α has been documented to be secreted at levels that are proportional to degree of adiposity, with levels decreasing as weight is lost (108, 109).

Elevations in TNF-α have been documented to decrease food intake and regulate energy metabolism, and dysregulated TNF-α expression has been associated with obesity (110). In the same study that employed both cell culture and animal models, it was observed that TNF-α affects the release of leptin from adipose tissue, and the expression of leptin through post-translational regulation (110).

V. Types of Adipose Tissue

Previously viewed as simply a "passive storage organ," recent findings have revealed that adipose tissue has a diversity of functions, including energy production, organ protection, inflammation regulation, and communication with other organs through adipokine secretion (111). Adipose tissue is now “recognized as the body's largest endocrine organ,” with significant roles in both metabolism and immunity (26). From a basic biological perspective, adipose tissue is composed of spherical fat-containing cells, with fatty acid transport to and from adipose tissue facilitated by the cardiovascular system (112). Adipose tissue also contains cells beyond adipocytes, including precursors of adipocytes, endothelial cells, blood vessels, fibroblasts, leukocytes, and the stromal vascular fraction of stem cells (25). Specific adipose tissue components vary depending upon depot location, phenotype, and health of the individual (113).

White Adipose Tissue

White adipose tissue (WAT) is composed of unilocular, spherical lipid droplets and a small number of mitochondria (114). WAT plays the primary roles of energy storage and hormone secretion, storing energy as triacylglycerol and releasing it via free fatty acids and glycerol (115). An excessive accumulation of WAT has been associated

with an elevated risk for obesity-related disorders, as well as various metabolic diseases (114) (116). This is particularly true when the WAT accumulation takes place as visceral abdominal adipose tissue, and even more so when this tissue becomes dysfunctional (116). However, it is important to note that WAT does play an important role in the maintenance of healthy human homeostasis; in patients with familial partial lipodystrophy, a disease that causes progressive subcutaneous adipose tissue loss, metabolic complications such as hypertension and severe insulin resistance arise over time, and this correlation has been supported in murine studies (116, 117).

Brown Adipose Tissue

Brown adipose tissue (BAT) is composed of several small, polygonal vacuoles, and has a higher density of mitochondria and capillaries than WAT (114). BAT has the function of non-shivering thermogenesis, in which its ATP-generating mitochondrial activity is harnessed (114). BAT is abundant in newborns, as well as hibernating animals, due to its heat generation capabilities which support maintenance of a healthy body temperature. The presence of BAT is generally elevated with cold exposure, and decreases with age and BMI (118). Although the amount of BAT generally decreases with age, certain BAT depots are maintained and primarily concentrated in the cervical-supraclavicular region (118).

The dissipation of heat by BAT occurs via oxidation of glucose and lipids, with the higher number of capillaries within BAT providing higher oxygen and nutrient levels than WAT receives (115). Another unique aspect of BAT is its expression of uncoupling protein 1 (UCP-1, also known as thermogenin), which is an inner-membrane mitochondrial protein (115). UCP-1 plays a role in the “uncoupling of fuel oxidation

from ATP synthesis" (119). Heat is generated via uncoupled respiration (120). Specifically, UCP-1 causes a "proton leak" to occur across the mitochondrial inner membrane. This is an alternative cascade route that uses the mitochondrial electrochemical gradient for heat production rather than ATP production (121). This enables heat dissipation from the energy that was stored in the proton gradient (122).

Beige Adipose Tissue

A third adipose tissue phenotype has been observed, referred to as beige (also called brown-in-white, or BRITE). Beige adipose tissue exhibits characteristics of both WAT and BAT, with a combination of large spherical and small multilocular lipid droplets, higher mitochondrial concentrations than WAT, and UCP-1 expression (115). The presence of beige adipose tissue has been connected to resistance to obesity and related metabolic diseases (115). WAT can transition into beige adipose tissue following chronic exposure to cold temperatures, exercise, and the administration of browning agents, including certain foods and drugs (123). The transition from BAT to WAT is acknowledged as a normal component of human development, while it is the unique browning of WAT that forms beige adipose tissue (124).

Perivascular Adipose Tissue

Perivascular adipose tissue (PVAT) surrounds blood vessels and is a highly-active endocrine organ, directly influencing vascular tone and inflammation through its release of anti-and pro-inflammatory and vasoactive molecules (7, 125). PVAT is characterized as beige or BAT, depending upon its depot location, which has been associated with several beneficial effects (Figure 1) (125). PVAT has been observed to benefit vascular function in healthy individuals, conferring structural support and enabling the

maintenance of vascular homeostasis through a balanced release of anti- and pro-inflammatory markers (7, 36, 124). This benefit has been observed to persist in conditions of "early obesity," in which a putative compensatory mechanism exerts beneficial effects (124). The specific methods of PVAT resistance to inflammation that have been observed are attributed in part to an elevation in NO production (124).

Biomechanical property studies have revealed an anti-contractile effect of PVAT upon surrounding arteries in healthy individuals (14). This anti-contractile effect is important for the regulation of vascular tone, and is mediated by the ROS that are generated from mitochondrial metabolism (126). Mitochondrial dysfunction has been observed in the adipose tissue of obese patients, leading to an elevated pro-oxidative status, and affecting tissue homeostasis (126). Endothelial dysfunction has been linked to an imbalance between vasoconstrictor and vasodilator responses, which can "impair" vascular tone and lead to atherosclerosis development (14). In OZR animals it has been observed that aortic vessels respond with an excessive endothelium-dependent vasodilator response, but a reduced vasoconstrictor response, compared with LZR animals (127–129).

Similarly, although PVAT is associated with benefits in healthy conditions, in cases of chronic obesity PVAT has been observed to become dysfunctional and release pro-inflammatory markers, yielding a loss of the benefits conferred by PVAT (7). In the case of chronic obesity PVAT also exhibits a loss of anticontractile properties, which can contribute to vascular disease (7). This loss of anticontractile effect has been observed in both animal and human cases of obesity (130). However, it is important to again note that

in early cases of obesity-induced inflammation PVAT has been documented to exert a compensatory mechanism of action to maintain homeostasis (124).

PVAT is similar to classical adipose tissue in that it contains not only adipocytes, but also nerves, stem cells, microvasculature, and immune cells (120). However PVAT is unique when compared with other adipose tissue depots is its "secretory profile" (124, 131, 132). In murine studies, aortic PVAT has been observed to express lower levels of adipocyte-related genes such as PPARγ, FABP4, fatty acid synthase (FAS), lipoprotein lipase (LPL), and perilipin, and to produce lower levels of adiponectin and leptin, when compared to subcutaneous (SAT) and visceral adipose tissues VAT (124, 131, 132). Another distinction between PVAT and other adipose depots, such as SAT and VAT, is its modulated "adipocyte differentiation and maturation" marker expression (124). Specifically, expression of these genes is decreased in PVAT, including lipoprotein lipase, glycerol-3-phosphate dehydrogenase (GPDH), and perilipin (124). In a human adipocyte culture study, radial artery PVAT exhibited a unique "secretion pattern" when compared with SAT and VAT. This radial artery PVAT exhibited an elevated production and concentration of cytokines and factors of angiogenesis factors and cytokines, including MCP-1, hepatocyte growth factor (HGF), vascular endothelial growth factor, and thrombospondin (124).

The morphology of PVAT varies depending upon its anatomical location (Figure 1) (125). Thoracic PVAT is most similar to BAT, based upon “full genome DNA microarray,” immunohistochemical and electron microscopy analyses of phenotype (131). PVAT also varies from beige adipose tissue in that it maintains this BAT phenotype even "in the absence of activating stimuli,” and also that it does not exhibit

whitening in the presence of a high-fat diet (125). This is contrasted with abdominal aortic PVAT which aligns more with the morphology of WAT, due to its unilocular adipocyte formation and low expression of UCP-1 (125). Another way in which thoracic PVAT is similar to BAT is in its contribution to "intravascular temperature during cold exposure," through mechanisms described above (125). Further, the location of thoracic PVAT is close to the heart, and surrounds the thoracic artery which all blood flows through prior to passing to the rest of the body (133). This suggests that thoracic PVAT is in a region that is highly important for maintaining healthy homeostasis and function.

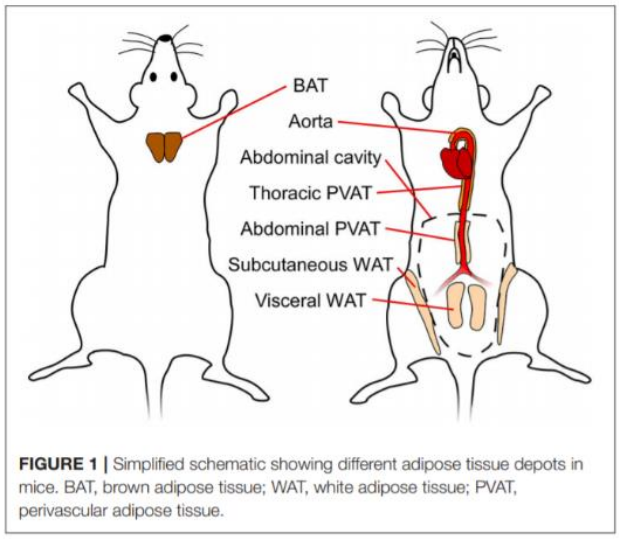

Figure 1: Adipose tissue characterization varies depending upon anatomical location (125)

The Framingham Heart Study recently reported that high levels of thoracic PVAT are significantly associated with a higher prevalence of cardiovascular diseases, even in individuals without high levels of visceral adipose tissue (126, 134). The differential inflammatory status of PVAT has been identified in recent years as playing a role in the prevention, or development, of diseases such as atherosclerosis (126). In the case of

atherosclerosis, a previously-held belief was grounded in the “inside-out” theory, in which immune cells are delivered to the endothelium via the vasculature in cases of intimal injury, producing inflammation that then spreads out to the media and adventitia regions of the artery wall (9, 135, 136). However, recent findings elucidating the role of PVAT have provided evidence for a newer “outside-in” theory (9). This theory states that inflammation can also begin in the PVAT and then spread towards the artery, affecting the surrounding vasculature (137). The outside-in theory has been supported by evidence of “inflammatory cells at the junction of perivascular adipose tissue and the vascular adventitia," "the lack of a fascial plane between the adventitia and surrounding adipose tissue," and "the extension of the vasa vasorum into perivascular adipose tissue" (77, 138).

A limited number of studies have investigated the effects of diet on the activity and state of PVAT. The few studies that have investigated this have primarily employed a high-fat diet (HFD); in a study using female and male wild-type and AMPKα1 knock-out mice, the intake of a HFD led to PVAT adopting an appearance with greater similarity to WAT than BAT, with an increased lipid droplet size (8). The HFD was also associated with a significant decrease (~70%) in adiponectin content of "adipocyte-conditioned media from aortic PVAT” (8). Interestingly, there was no significant change in adiponectin gene expression identified, which suggests that a decrease in translation or actual secretion of adiponectin is the cause of this decreased adiponectin content following HFD (8). A similar study using diet-induced obese mice observed no changes in PVAT adiponectin mRNA expression in thoracic PVAT even after an 8-month HFD

(8). Therefore, further studies of this type are necessary to elucidate the association of adiponectin presence with HFD intake.

While the majority of PVAT studies using diet variations have been focused upon a high-fat or diet-induced obese animal model, a few studies have investigated the effect of an improvement in diet on PVAT (11, 13, 14, 130). In a study that placed obese rats on a calorie-restricted diet which led to weight loss, the PVAT damage that was initially present in rats during obesity was reversed through a reduction in inflammation and increase in nitric oxide synthetase activity (130). This is evidence of the positive impact that dietary and lifestyle changes can have upon PVAT health. A limited number of studies employing functional foods (foods that confer physiological benefits) have also been conducted to investigate their effects on PVAT. Studies in the Klimis-Zacas lab have demonstrated the efficacy of a diet enriched in wild blueberries for the attenuation of local obesity-induced inflammation in the PVAT (11–14). This improvement took place through a decrease in inflammatory marker expression, improved lipid profiles, normalized vascular function, and modulated oxidative stress (11–14).

A limited number of other studies have investigated the conditions and molecules that prompt obesity-related changes in PVAT structure and function. In a study using wild-type and TNF-α receptor deficient mice (TNF-KO), the consumption of a HFD leading to obesity caused increased ROS generation in PVAT (126). This increased ROS generation was attributed to reduced mitochondrial respiration, potentially due to decreased UCP-1 and antioxidant expression, motivated by increased adipocyte TNF-α production (126). These changes are associated with further complications related to obesity-induced vascular dysfunction. Furthermore, it has been identified that the

elevated ROS generation and increased TNF-α production are associated with higher levels of activation of the RhoA/Rho kinase pathway in vascular smooth muscle cells (VSMCs) (126). Atypical activation of the RhoA/Rho kinase pathway has been associated with obesity-associated vascular dysfunction (126).

VI. Red Raspberries

Berries are unique in their robust antioxidant profiles, with quantities reaching four times that of non-berry fruits and ten times that of vegetables (139). Red raspberries (*Rubus idaeus*) are gaining recent interest due to their unique bioactive profiles (140). Red raspberries also possess diverse culinary applications, which make them appealing and adaptable to a variety of diets and lifestyles. There are around 50 different bioactive compounds present in red raspberries, including anthocyanins (ACNs) and ellagitannins (141). The ACN content of red raspberries typically ranges between 23 – 59 mg per 100 g/FW, with cyanidin being most abundant, and lower levels of pelargonidin also present (139, 142).

ACNs are a sub-class of flavonoids and are colored pigments that confer red, purple and blue colors to food (143). The specific color that ACNs confer is dependent upon their conjugation (distribution of double bonds) (143). The health benefits of ACNs include attenuation of inflammation, improvement of vision, prevention of cardiovascular diseases, and reduction of diabetes, cancer and obesity (143). Studies have demonstrated plant ACNs to improve lipid profiles, inhibit platelet aggregation, decrease cancer cell growth, improve visual function in glaucoma patients, activate adiponectin which

improves endothelial dysfunction, and suppress weight gain (143). Many of these health benefits are attributed to the antioxidant activity of ACNs.

ACNs consist of a core flavylium ion structure, which is a flavanol derivative lacking a fourth-position ketone and third-position alcohol (Figure 2) (143). Different side chains are then attached to this core to produce varying anthocyanin identities (Figure 3) (143). There are two pathways used in the synthesis of ACNs: one which yields the two necessary precursors, and one which enables conversion to a stable form (139). The shikimate pathway produces the precursors phenylalanine and malonyl-CoA, which are then converted to unstable anthocyanidins through several folding and chemical steps, and are then coupled to sugars to form anthocyanins which are more stable (139).

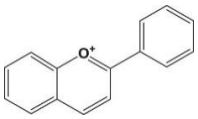

Figure 2: Core anthocyanin structure (143)

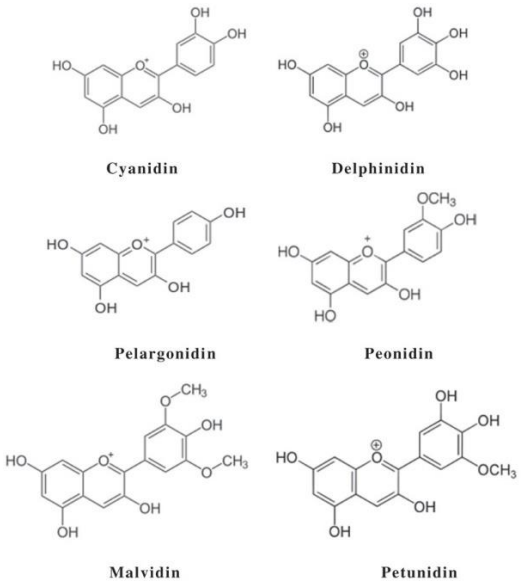

Figure 3: Common ACN compounds in plants (143)

While red raspberries possess an ACN content similar to or less than that of other common berries, their ellagitannin content is uniquely high (144). Ellagitannins are a class of polyphenols that are defined by their presence of one or more "hexahydroxydiphenoyl moieties esterified with a sugar, usually glucose" (145). Sanguiin H-6 and lambertianin C are the two primary ellagitannins present in red raspberries, with quantities of 1871 g/mol and 2805 g/mol respectively (145). Ellagitannins have been documented to physiologically benefit cardiovascular health, with effects such as decreased atherogenesis and decreased thrombosis (146). This is due in part to the release of ellagic acid upon hydrolysis of ellagitannins during digestion, and thus improved bioavailability (146). Red raspberries are also unique in their low sugar and high fiber content. Compared with 1 cup of wild blueberries, 1 cup of red raspberries contains 6 fewer grams of sugar and 1 more gram of fiber (147, 148).

No studies have been conducted to date on the effect of red raspberries on PVAT. Thus, considering that wild blueberry dietary enrichment has been documented to confer attenuated inflammation and improved vascular function local to PVAT, it is valuable to elucidate the effect(s) of red raspberry dietary enrichment on PVAT. The specific goal of this study is to determine the effect(s) of a red raspberry-enriched diet on pro-inflammatory and anti-inflammatory marker gene expression and concentration in the PVAT of the OZR model of MetS and the LZR control model.

MATERIALS AND METHODS

Zucker Rats

Sixteen male OZRs and sixteen male LZRs (Charles River laboratories, Raleigh, NC) were purchased in Spring 2018. The animals were housed at the University of Maine Small Animal Facility, individually in an environmentally-controlled room at 22°C with a 12-hours:12-hours light:dark cycle. Mesh-bottomed cages were used to prevent coprophagia. Food and water were replenished daily throughout the eight-week study. Weight was measured weekly using a KD-8000 scale (MyWeigh, Phoenix, AZ).

Diets

For eight weeks, eight OZR and eight LZR were placed on an experimental diet enriched with red raspberry (*Rubus idaeus*), and the remaining eight of each strain received a control diet (Dyets, Bethlehem, PA). The control diet complied with the requirements established in the American Institute of Nutrition's AIN93-G rodent growth diet, with ingredients as indicated in Figure 4 (149). The experimental diet consisted of red raspberry (*Rubus idaeus*) powder substituted at 8% (w/w) for dyetrose in the AIN93-G diet. This percentage is equivalent to human consumption of approximately 1.5 cups of red raspberries per day, based upon the body surface area calculation (150). Diets were vacuum-packed and stored at -20°C in the dark prior to use. Control and experimental diets were distributed daily in powder form into respective glass rodent feeding jars, and both food and tap water were made available *ab libitum.*

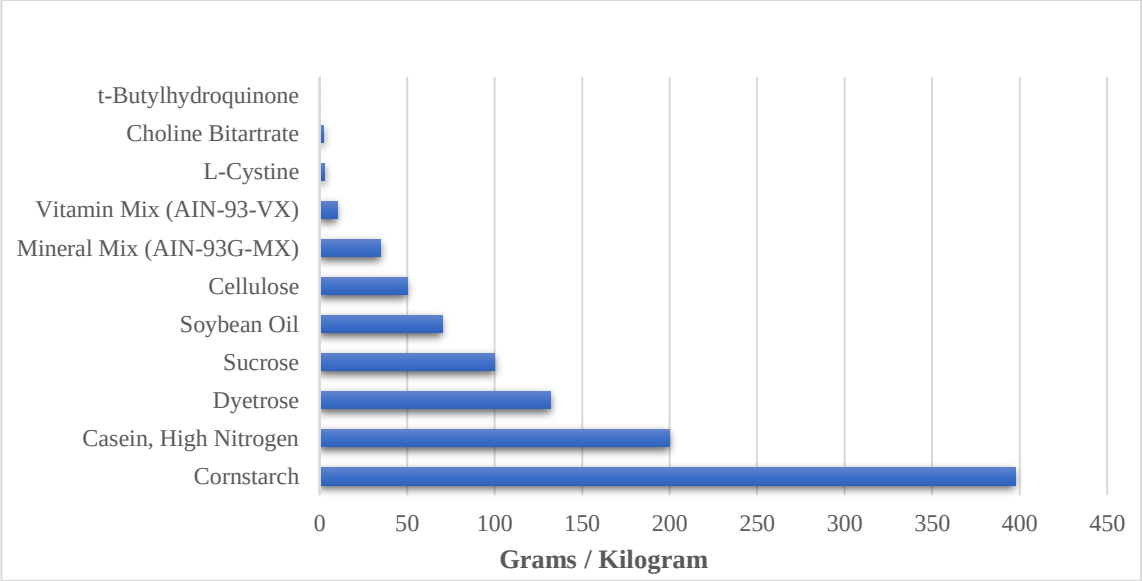

Figure 4: Nutritional composition of rat control diet, based upon the American Institute of Nutrition's AIN93-G rodent growth diet (149)

Red Raspberries

Red raspberries (*Rubus idaeus*) were sourced, freeze-dried and powdered by FutureCeutricals as HiActives® Raspberry N116 Powder (Momence, Il) and processed into diet by Dyets (Bethlehem, PA). The red raspberry powder was analyzed by FutureCeutricals, indicating a 0.5% minimum total anthocyanin, 1.5% minimum total phenolics, 350 µmole TE/g ORAC, and 10 mg/100g Ellagic Acid. Each 100g of red raspberry powder contained 365 calories, 12.12 g fiber, 44.62 g sugar, and 7.92 g protein.

Tissue Collection

Following the eight-week experimental feeding period, two animals per day were anesthetized and tissue samples collected. Animals were fasted overnight but access to water remained. Animals were anesthetized with 95% CO2/5% O2 for two minutes, followed by exsanguination through cardiac puncture. The thoracic aorta was excised and cleaned of blood clots, and then placed in a Petri dish containing physiologic saline

solution (PSS: 118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl, 1.2 mM MgSO4, 1.2 mM KH2PO4, 12.5 mM NaHCO3, and 11.1 mM dextrose). The PVAT were then carefully removed from the aorta, cleaned of blood in PSS, patted dry and weighed. It was then immediately placed in cryogenic vials (Thermo Scientific, Rochester, NY), snap-frozen in liquid nitrogen and stored at -80°C.

PVAT Homogenate

A PVAT homogenate was prepared using 5 mL of Phosphate-Buffered Saline (PBS) (Sigma-Aldrich, St. Louis, MO) and 100 mg of PVAT. PBS was filtered, and then chilled prior to and throughout homogenate preparation. The TissueRuptor II (Qiagen, Germantown, MD) with TissueRuptor Disposable Probes (Qiagen, Germantown, MD) were used for disruption of PVAT, for a duration of 40 seconds or until the tissue homogenate was uniformly homogenous. Each homogenate was then vortexed for 1 minute, followed by centrifugation at 5,000 RPM for 10 minutes at 4 degrees C using a Sorvall RT1 centrifuge (ThermoScientific, Waltham, MA). The subnatant was then carefully collected using a glass pipette, aliquoted into microcentrifuge tubes, and stored at -80 degrees C until further analysis.

Total Protein Content

Total protein of each PVAT homogenate sample was determined using the Pierce BCA Protein Assay (ThermoFisher Scientific, Waltham, MA). BCA Assays were performed using undiluted PVAT homogenate aliquots, and according to manufacturer instructions. Spectrophotometric readings were determined at a wavelength of 562 nm using an xMark Microplate Absorbance Spectrophotometer (BioRad, CA).

Inflammatory Marker Concentrations

Inflammatory marker concentrations were determined using respective ELISA kits. Adiponectin concentrations of PVAT homogenate samples diluted 1:50 with Calibrator Diluent RD5-26 (R&D Systems, Minneapolis, MN) were determined using the Rat Adiponectin ELISA Kit (R&D Systems, Minneapolis, MN) according to respective manufacturer instructions. Spectrophotometric readings were determined at a wavelength of 450 nm and a correction wavelength of 570 nm, using an xMark Microplate Absorbance Spectrophotometer (BioRad, CA).

IL-1β concentrations of PVAT homogenate samples diluted 1:5 with 1x Sample Diluent (Abcam, San Francisco, CA) were determined using the Rat IL-1 beta ELISA Kit (Abcam, San Francisco, CA) following respective manufacturer instructions. Spectrophotometric readings were determined at a wavelength of 450 nm using an xMark Microplate Absorbance Spectrophotometer (BioRad, CA).

IL-10 concentrations of PVAT homogenate samples diluted 1:5 with 1x Sample Diluent (Abcam, San Francisco, CA) were determined using the Rat IL-10 ELISA kit (Abcam, San Francisco, CA). Spectrophotometric readings were determined at a wavelength of 450 nm using an xMark Microplate Absorbance Spectrophotometer (BioRad, CA).

MCP-1 concentrations of PVAT homogenate samples diluted 1:500 with Sample Diluent (MyBioSource, San Diego, CA) were determined using the Rat MCP-1 ELISA Kit (MyBioSource, San Diego, CA). Spectrophotometric readings were determined at a wavelength of 450 nm with a correction wavelength of 630 nm using an xMark Microplate Absorbance Spectrophotometer (BioRad, CA).

Messenger RNA Extraction from PVAT

Messenger RNA (mRNA) was isolated from frozen PVAT fragments using the RNeasy Lipid Tissue Mini-Kit (Qiagen, Germantown, MD). The TissueRuptor II (Qiagen, Germantown, MD) and TissueRuptor Disposable Probes (Qiagen, Germantown, MD) were used for processing tissue into a homogenate according to manufacturer instructions. RNA purity and yield (ng/μL) were determined using the Nanodrop 1000 Spectrophotometer (Thermo Scientific, Rochester, NY). RNA yield values were used in the determination of total RNA volume taken per cDNA reaction, for a final concentration of 0.5 μg/uL. The RT^2 First Strand Kit (Qiagen, Germantown, MD) was used for reverse transcription of RNA to cDNA, with Genomic DNA Elimination Mix and Reverse Transcription Mix (Qiagen, Germantown, MD). Stopped cDNA reactions were then stored at -80 degrees C until further use.

Gene Expression

Custom-designed RT^2 Profiler PCR Arrays (Qiagen, Germantown, MD) were used for determination of NF-κB (NF-κB-1), TNF-α (TNFa), IL-6 (IL6), MCP-1 (Ccl2), adiponectin (adipo1), IL-10 (IL10), and IL-1β (IL1b) expression in PVAT. The housekeeping gene β-actin was used, and genomic DNA controls (GDC), reverse-transcription controls (RTC), and positive PCR controls (PPC) were employed. The RT^2 Profiler PCR Array system includes the RT^2 First Strand Kit (Qiagen, Germantown, MD), Custom RT^2 PCR Array (Qiagen, Germantown, MD), and RT^2 SYBR® Green Mastermix (Qiagen, Germantown, MD). A Bio-Rad CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, CA) was used for thermal cycling and determination of Cq values.

Statistical Analysis

Gene expression relative to the housekeeping gene beta-actin was determined with the ΔΔCt method, with LZR-C animals as the reference expression level (151). Data was analyzed using 2-way ANOVA, with the independent factors diet (red raspberry vs. control) and animal model (OZR vs. LZR) in the Prism application (GraphPad, San Diego, CA). Outliers were removed based upon the GraphPad (GraphPad, San Diego, CA) Outlier Calculator with an alpha value of 0.05. Tukey's HSD post hoc test was also used to further determine significant main effects and interactions.

RESULTS

Animal Weight and Food Consumption

Average animal weight was significantly higher during each week of the experimental feeding period for OZR-C animals compared with LZR-C animals (Figure 5). No significant differences in weight were observed between diet groups compared with respective control models. The average final weight of LZR-C animals following the experimental feeding period was 434 ± 68 g, while that of OZR-C animals was 608 ± 74 g.

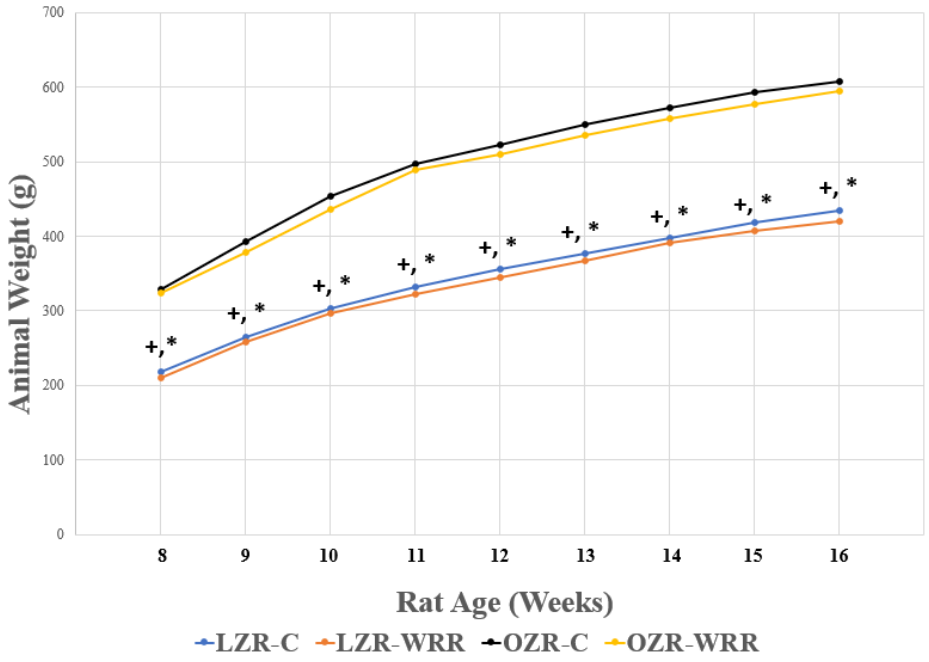

Figure 5: Rat growth throughout experimental duration. No significant differences in growth were observed in diet groups compared with control groups. +Significant difference in growth between LZR-C vs. OZR-C ($p < 0.05$), *Significant difference in growth between LZR-WRR vs OZR-WRR ($p < 0.05$)

Average food intake was significantly higher for OZR-C (36.29 ± 1.05 g/day) vs. LZR-C (26.48 ± 0.73 g/day) and OZR-WRR (35.29 ± 0.625 g/day) vs. LZR-WRR (24.38 ± 0.470 g/day) (Figure 6). No statistical significance was observed in food intake between control and red raspberry-enriched diets.

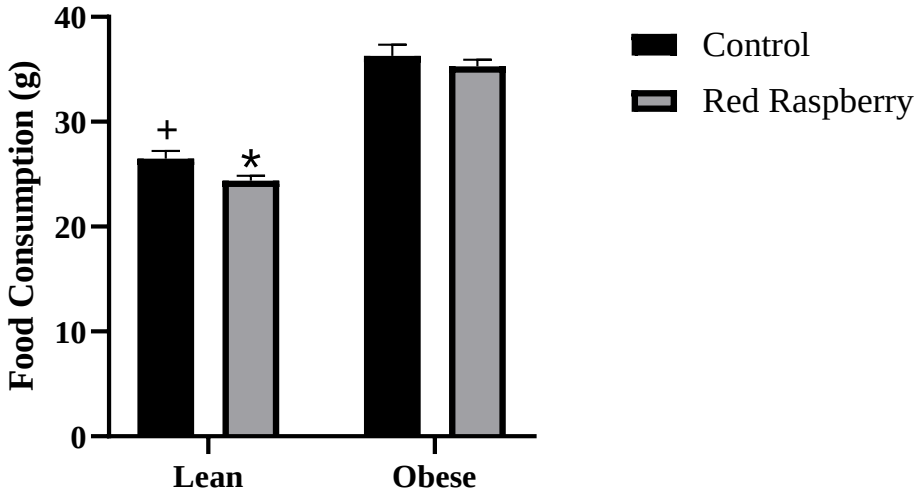

Figure 6: Average food consumption for animal and diet groups. +Significant difference in food consumption between animal type (LZR-C vs. OZR-C, $p < 0.05$), *Significant difference in food consumption between animal types within diet group (LZR-WRR vs. OZR-WRR, $p < 0.05$)

Gene Expression & Protein Concentration

Adiponectin

Gene expression of anti-inflammatory marker adiponectin (gene name Adipoq) in PVAT was analyzed using RT-PCR (Figure 7), and protein concentration was determined using ELISA (Figure 8), as described in Methods. No significant gene expression results were observed either for animal type or diet. ELISA analyses indicated a significant ($p < 0.05$) elevation in adiponectin concentration in OZR-WRR vs. OZR-C animals.

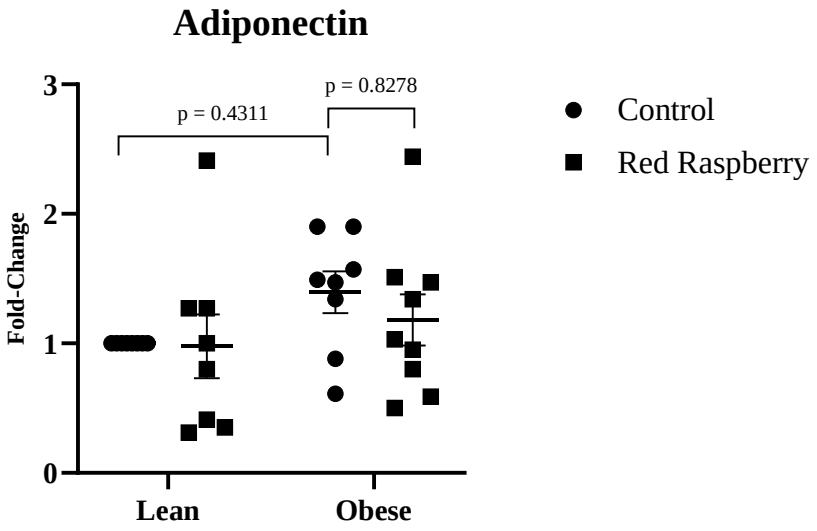

Figure 7: Gene expression of anti-inflammatory marker adiponectin in Zucker Rat PVAT using RT-PCR.

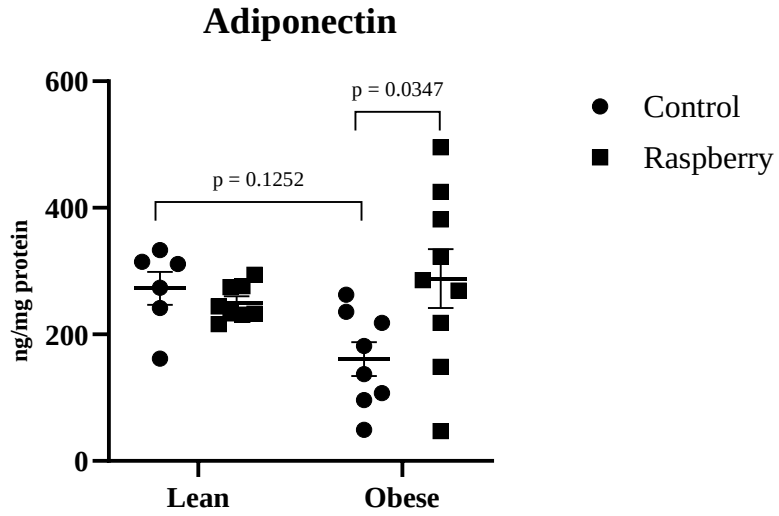

Figure 8: Protein concentration of anti-inflammatory marker adiponectin in Zucker Rat PVAT using ELISA.

Interleukin-10

Gene expression of anti-inflammatory marker IL-10 (gene name IL10) in PVAT was analyzed using RT-PCR (Figure 9), and protein concentration was determined using

ELISA (Figure 10), as described in Methods. No significant gene expression results were observed either for animal type or diet. ELISA analyses indicated a significant ($p < 0.05$) decrease in IL-10 concentration in OZR-C versus LZR-C models.

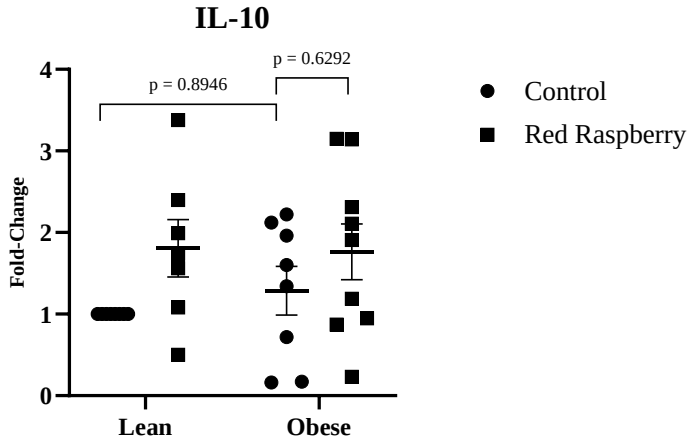

Figure 9: Gene expression of anti-inflammatory marker IL-10 in Zucker Rat PVAT using RT-PCR.

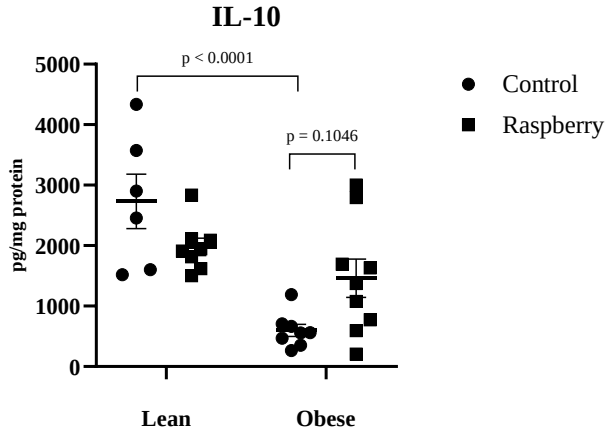

Figure 10: Protein concentration of anti-inflammatory marker IL-10 in Zucker Rat PVAT using ELISA.

Interleukin-1β

Gene expression of pro-inflammatory marker IL-1β (gene name IL1b) in PVAT was analyzed using RT-PCR (Figure 11), and protein concentration was determined using ELISA (Figure 12), as described in Methods. No significant gene expression changes

were observed either for animal type or diet. ELISA analyses indicated a significant ($p < 0.05$) decrease in IL-1β concentration in OZR-C versus LZR-C models.

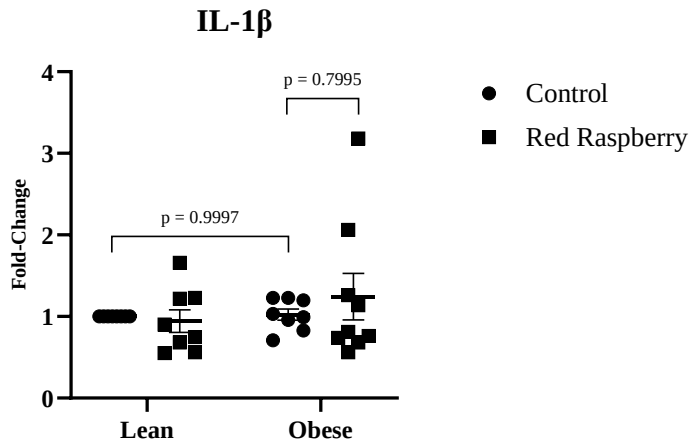

Figure 11: Gene expression of pro-inflammatory marker IL-1β in Zucker Rat PVAT using RT-PCR.

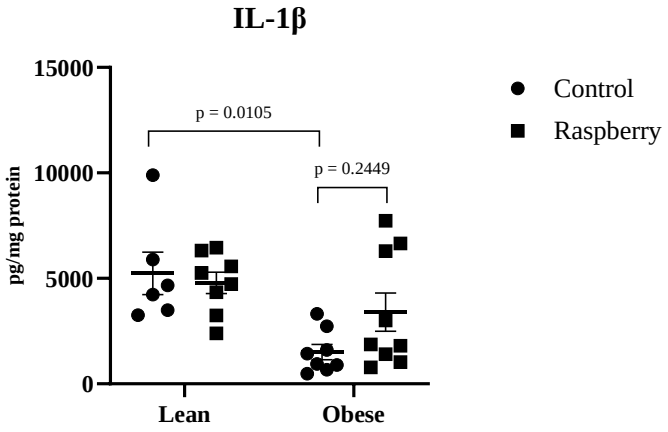

Figure 12: Protein concentration of pro-inflammatory marker IL-1β in Zucker Rat PVAT using ELISA.

Monocyte Chemoattractant Protein-1

Gene expression of pro-inflammatory MCP-1 (gene name Ccl2) in PVAT was analyzed using RT-PCR (Figure 14), and protein concentration was determined using ELISA (Figure 15) as described in Methods. No significant changes in gene expression were observed either for animal type or diet.

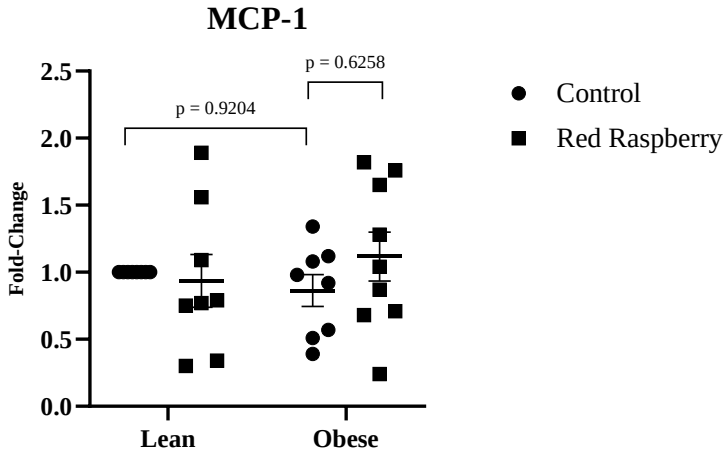

Figure 133: Gene expression of pro-inflammatory marker MCP-1 in Zucker Rat PVAT using RT-PCR.

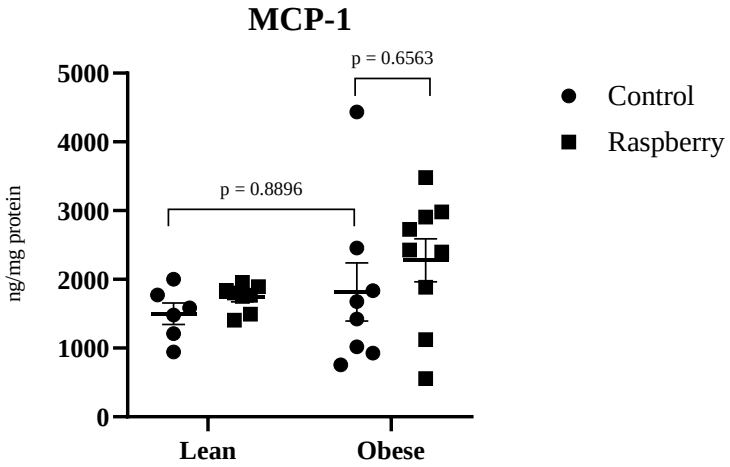

Figure 14: Protein concentration of pro-inflammatory marker MCP-1 in Zucker Rat PVAT using ELISA.

Gene Expression

Interleukin-6

Gene expression of pro-inflammatory marker IL-6 (gene name IL6) in PVAT was analyzed using RT-PCR (Figure 13) as described in Methods. No significant changes in gene expression were observed either for animal type or diet.

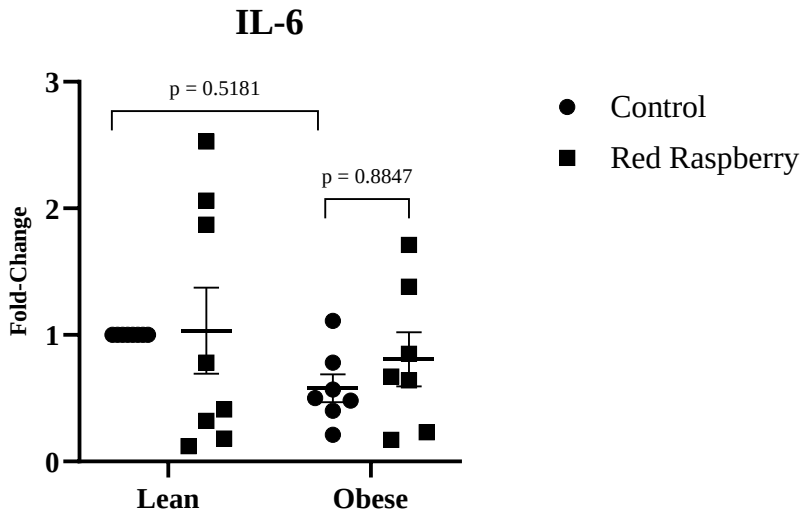

Figure 145: Gene expression of pro-inflammatory marker IL-6 in Zucker Rat PVAT using RT-PCR.

Nuclear factor-κB

Gene expression of pro-inflammatory marker NF-κB (gene name Nfkb1) in PVAT was analyzed using RT-PCR (Figure 16) as described in Methods. A significant ($p < 0.05$) down-regulation of NF-κB in the OZR-C versus LZR-C models was found. No significant differences in gene expression were observed among diet groups compared to animal type.

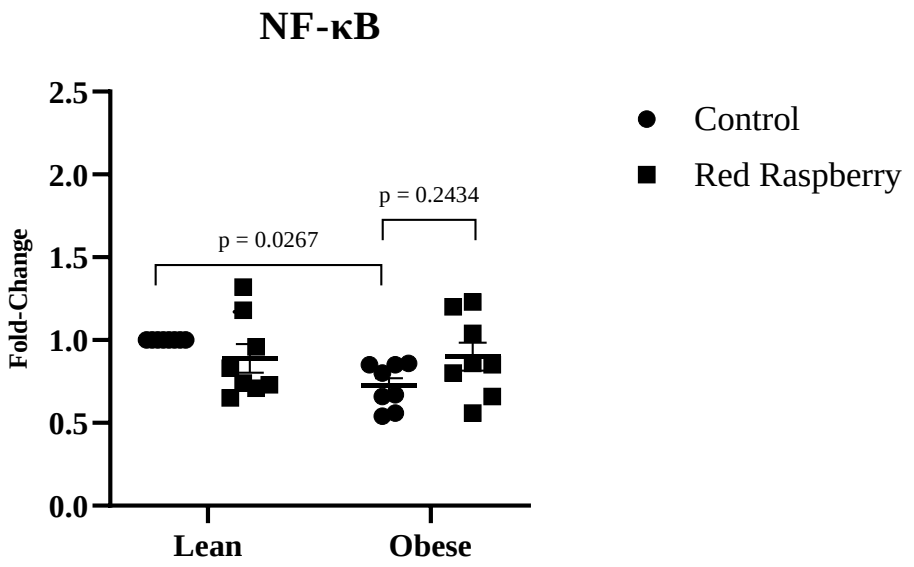

Figure 15: Gene expression of pro-inflammatory marker NF-κB in Zucker Rat PVAT using RT-PCR.

Tumor Necrosis Factor-Alpha

Gene expression of pro-inflammatory marker TNF- α (gene name Tnf) in PVAT was analyzed using RT-PCR as described in Methods. No significant changes in gene expression were observed either for animal type or diet.

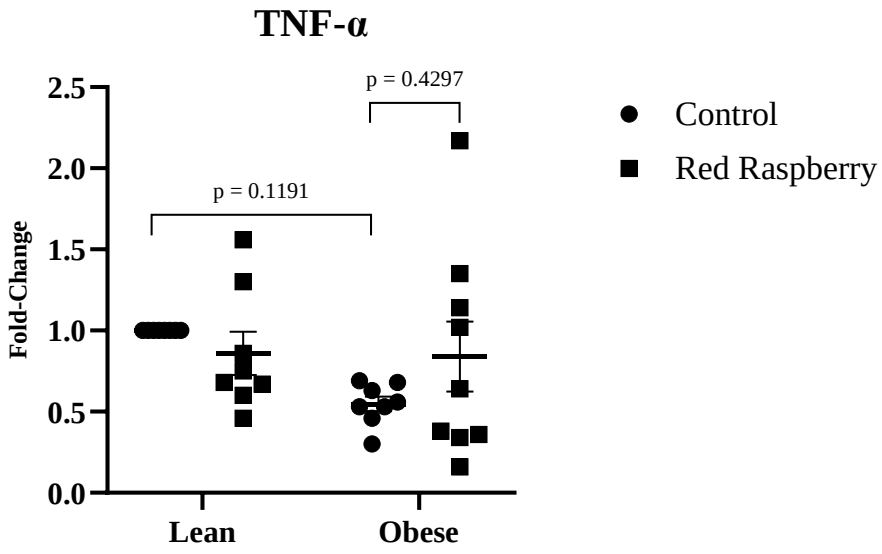

Figure 16: Gene expression of pro-inflammatory marker TNF- α in Zucker Rat PVAT using RT-PCR.

DISCUSSION

This study documents that an eight-week red raspberry dietary enrichment is associated with deviations in inflammatory markers in the PVAT of obese Zucker rats (OZR), including significantly elevated concentration of the anti-inflammatory marker adiponectin. This study also documents that among control (C) animals, IL-10 and IL-1β concentrations are both significantly higher in the PVAT of lean Zucker rats (LZR), and that transcription factor NF-κB expression is significantly decreased in the PVAT of OZR.

Considering that adiponectin is regarded as an anti-inflammatory marker that is most often present in high circulating concentrations in healthy animals and individuals, and lower levels in cases of obesity, this finding suggests that whole-red raspberry (WRR) enrichment promotes a healthier outcome for the PVAT of OZR (39). As discussed in the literature review, adiponectin has been observed to exert protective effects against endothelial dysfunction, neointimal formation, and M1 polarization through a putative compensatory mechanism (39, 41, 44). These findings also support the conclusion that WRR enrichment promotes a healthier outcome for the PVAT of OZR. It is interesting to note that there was no significant difference in PVAT adiponectin expression in different diet or animal groups. This suggests that post-transcriptional modifications are taking place to affect the activity of adiponectin in the OZR-WRR animals in order to cause the observed significant difference in protein concentration.

The documented significant elevation in concentration of anti-inflammatory cytokine IL-10 found in LZR-C versus OZR-C aligns with that which has been

previously established regarding this cytokine; since it is known that IL-10 is primarily produced by macrophages with M2 polarization, it is logical that LZR with lower levels of inflammation would possess higher levels of IL-10 (39, 48). Further, this finding advances the understanding of PVAT and the ways in which this organ responds to obesity, since no studies investigating IL-10 and PVAT were identified during this study's literature review.

Considering that IL-1β is typically viewed as a pro-inflammatory cytokine, it would typically be expected for it to be present at significantly higher concentrations in the OZR-C versus LZR-C (55). In the context of classical visceral and subcutaneous adipose tissue depots, cases of both rodent and human obesity have been associated with elevated release of IL-1β (60). Further, elevated circulating plasma levels of IL-1β are an established prediction of insulin resistance and subsequent development of Type 2 Diabetes (60). However, when considering that which has been established regarding thoracic PVAT, the current study's finding that IL-1β concentration is much lower in OZR-C PVAT than LZR-C PVAT makes more sense. Specifically, thoracic PVAT has been documented to benefit vascular function and exert a compensatory mechanism in cases of early obesity, which resists inflammation in order to maintain vascular homeostasis (7, 124). This is believed to be due in part to its nature as a brown adipose tissue (BAT), as assessed by previous studies' immunohistochemistry, electron microscopy, and genetic analyses (131).

PVAT is also unique when compared with the secretory profiles of other adipose tissue depots (124, 131, 132). In murine studies, aortic PVAT has been observed to express lower levels of adipocyte-related genes such as PPARγ, FABP4, fatty acid

synthase (FAS), lipoprotein lipase (LPL), and perilipin, and to produce lower levels of adiponectin and leptin, when compared to subcutaneous (SAT) and visceral adipose tissues (VAT) (124, 131, 132). This series of documented gene expression and protein variations provides further evidence for PVAT's previously established unique nature.

In this study, it seems that the OZR PVAT may be resisting inflammation by exerting a compensatory effect, resulting in a significantly reduced protein concentration of the pro-inflammatory marker IL-1β. Since thoracic PVAT has a location close to the heart, and surrounds the thoracic artery which all blood flows through prior to passing to the rest of the body, this organ may be highly important for protection and maintenance of a healthy, functioning homeostatic condition in cases of obesity, or at least early transitional stages into obesity (133).

Further, the observed difference in IL-1β concentration is supported by the gene expression of NF-κB in the same animals. Specifically, in this study NF-κB mRNA was significantly down-regulated in the PVAT of OZR-C animals compared to LZR-C animals. NF-κB is a transcription factor with known functions in the activation of genes related to a pro-inflammatory state, with its activation occurring upon translocation to the nucleus from the cytoplasm, as discussed in the literature review (94, 97). The presence of NF-κB in the nucleus is influenced through a positive-feedback loop, with pro-inflammatory markers (including IL-1β) acting as both enhancers and products of this loop, which aligns with the IL-1β concentration results observed (94).

The NF-κB gene expression finding in this study also supports previous knowledge that PVAT exerts protection against inflammation (7, 124). NF-κB has been documented to be activated at higher levels in cases of high-fat diet feeding, and

promotes the recruitment and activation of macrophages (94, 152). However, by reducing the expression of this transcription factor, PVAT may be acting to compensate against the inflammatory state of the OZR-C animals, thus promoting a less-inflamed environment local to the heart and nearby vasculature.

With regards to the observed modulations in PVAT inflammatory status, it is interesting to consider previous findings regarding the effect of PVAT on atherogenesis and vascular inflammation initiation (9). Specifically, it was previously believed that this occurred from the "inside out," starting with injury of the intima and subsequent delivery of immune cells through the vasculature, which then produce inflammation that spread to the medial and adventitial regions of the artery wall (10). However, recent findings have provided evidence for a new "outside-in" theory, in which inflammation can also begin in the PVAT and then spread towards the artery, affecting the surrounding vasculature (10). With the present study's results in mind, it seems that the elevated adiponectin concentration in the PVAT of OZR-WRR could be specifically conferring protection against atherosclerosis development. Further, the discussed suspected compensatory mechanism involving NF-κB and IL-1β in OZR-C vs. LZR-C rats could also have a role in protection against atherogenesis through an outside-in approach.

The significant finding in this study involving elevated anti-inflammatory marker adiponectin concentration in PVAT of OZR in cases of red raspberry enrichment suggests that the nutrient and bioactive profiles of this berry may enhance adiponectin's role in the discussed compensatory mechanism of action. Red raspberries possess a diversity of different bioactive compounds, including anthocyanins (ACNs) and ellagitannins (141). Plant ACNs have been documented to confer many benefits, and adiponectin activation is

notably one of these (143). Also relevant is evidence that ACNs improve lipid profiles and endothelial function, and suppress weight gain, and that ellagitannins confer cardiovascular benefits and protection (10, 143).

It is also valuable to compare findings with the limited published research that exists regarding the effect of berry consumption on PVAT. A previous study in the Klimis-Zacas laboratory demonstrated the efficacy of a wild blueberry-enriched (WBB) diet in the attenuation of obesity-induced inflammation, through significantly decreasing expression of inflammatory markers in the PVAT of the same Zucker rat models employed in the present study (11). Significant results from the WBB study included decreased PVAT concentrations of pro-inflammatory markers MCP-1 and TNF-α in OZR that consumed WBB, as well as decreased concentrations of the same pro-inflammatory markers in the PVAT of LZR-C vs. OZR-C animals (11).

In the present study there was not a significant difference in MCP-1 concentration in any case, with p-values of 0.6258 (OZR-C vs. OZR-WRR) and 0.9204 (LZR-C vs. OZR-C). It is also important to note that in the present study TNF-α concentration via ELISA analysis was attempted using several different concentrations of PVAT homogenate, and both with and without protease inhibitors, however in every case TNF-α was not present at spectrophotometrically detectable levels. Variations in inflammatory marker concentration may be due to differences in the bioactive profile of WBB compared to WRR. The total phenolic content and total antioxidant capacity of WRR are both lower than that of WBB, at a quantity of four-fold and three-fold less, respectively (153). Further, WBB possess a bioactive profile with much greater diversity than that of

WRR; these variations in both bioactive type and concentration can translate to varying physiological effects (154).

In a study that investigated the antioxidant capacity of varying berries, it was determined that WRR (*Rubus idaeus* Michx) contained 7.1 μmol of gallic acid/g FW, 0.84 μmol of the anthocyanin Mal-3-glu/g FW, and 21.4 μmol of peroxyl radical absorbance capacity (ORAC-ROO)/g FW (155). This is compared with WBB (*Vaccinium angustifolium* Aiton), with 27.7 μmol of gallic acid/g FW, 4.35 μmol of the anthocyanin Mal-3-glu/g FW, and 64.4 μmol of ORAC-ROO/g FW (155). Further, analysis of a WRR extract bioactive profile determined that the compounds sanguiin H-10 (an ellagitannin), lambertianin C (an ellagitannin component), quercetin-3,4'-diglucoside, sanguiin H-6 (an ellagitannin), and ellagic acid to are present at the highest concentrations (156). Ellagic acid is a product of ellagitannin hydrolysis during digestion, and ellagitannins have been documented to physiologically benefit cardiovascular health, with effects such as decreased atherogenesis and decreased thrombosis (146). No studies that evaluated specific health benefits of quercetin-3,4'-diglucoside have been identified, however considering its antioxidant activity it is suspected that it likely exerts beneficial physiological effects (157).

The WRR bioactive profile is contrasted with that of WBB, which contains high levels of the compounds chlorogenic acid, quercetin and resveratrol, as well as over twenty different anthocyanidins (including delphinidin, malvidin, cyanidin, petunidin and peonidin in greatest abundance) (158). These anthocyanidins then become anthocyanins upon combining with glucose, galactose and arabinose to form anthocyanins such as 3-glucoside, 3-galactoside and 3-arabinoside (154). WBB also possess the flavonoids

flavan and flavonol (159). These compounds can have a unique effect when acting in synergy together, which is another variable that is different when comparing WRR to WBB due to the diverse bioactive profile of WBB (160). With these documented phenolic compound contents in mind, it is important to note that factors such as environmental and harvest conditions, and treatment and storage following harvest, can have an effect on berry phenolic content greatly (158).

In conclusion, the present study provides evidence that consumption of WRR at a human-equivalent rate of 1.5 cups per day can improve the response of anti-inflammatory marker adiponectin to obesity-induced inflammation local to PVAT, through a compensatory mechanism, however this is with an effect lower than that of previous WBB studies. The present study also provides evidence for the role of transcription factor NF-κB subunit p50 in a PVAT-based anti-inflammatory compensatory mechanism in cases of early obesity, in concert with pro-inflammatory cytokine IL-1β. These findings support previous data that have identified thoracic PVAT as a BAT that confers protection against inflammation, as well as prior evidence that berries can be an effective non-pharmacological tool for the attenuation of MetS and obesity-induced inflammation.

FUTURE DIRECTIONS

This study investigated the effects of whole red-raspberry (WRR) consumption in the perivascular adipose tissue (PVAT) of the obese Zucker rat model (OZR) of human Metabolic Syndrome (MetS), and also employed control lean (LZR-C) and obese (OZR-C) models. The WRR consumption was equivalent to human consumption of approximately 1.5 cups of fresh WRR per day. The age of the rats at arrival was eight weeks, and the experimental feeding period took place for a duration of eight weeks. PVAT was collected and subsequent experiments investigated the gene expression and protein concentrations of seven key pro- and anti-inflammatory markers.

Studies were not conducted to directly investigate the adipocyte phenotype of the PVAT. Previous published research has determined that murine thoracic PVAT most resembles brown adipose tissue (BAT) or beige adipose tissue, with BAT generally associated with a healthier phenotype due to its resistance to inflammation and higher expenditure of energy (125, 131, 161). Therefore, investigating the differential histology of OZR-C versus LZR-C and OZR-C versus OZR-WRR PVAT could provide insight to obesity- and WRR-enrichment-related differences in adipocyte structure and function. The phenotypes of these samples could be investigated through direct tissue histology sample analyses, as well as genetic and proteomic investigations of UCP-1 content (162).

Other studies that could provide valuable insight to the PVAT inflammatory state involve the transcription factor NF-κB. As discussed in the literature review, while NF-κB consists of five possible subunits (and most often just the subunits p50 and p65), this study only investigated the mRNA expression of the p50 subunit (95). While this

does provide insight to the presence and functioning of NF-κB, a greater understanding could be created through also determining gene expression of the other four subunits, or at least p65. A related study could investigate levels of IKBα, a key inhibitor of NF-κB's translocation into the nucleus, to elucidate differential regulation of this transcription factor's pathway (and subsequent ability to act upon its target genes, including those which were investigated in this study) (97).

Other upstream molecules of NF-κB that could be valuable to investigate are toll-like receptor ligands and cytokines, which can induce the first signaling for NLRP3 inflammasome activation (163). These cytokines include IL-1β that was investigated in this study, as well as TNF-α that was attempted to be investigated; however other molecules that can induce NLRP3 inflammasome activity are pathogen-associated molecular pattern molecules (PAMPs) and damage-associated molecular pattern molecules (DAMPs) (163). It could also be insightful to investigate the gene expression and activity of a greater range of NF-κB pathway components, such as those involved in signaling [such as thrombin receptor (F2r) and toll-like receptor 1 (tlr1)], sequestration of NF-κB for control of activity [such as NFKB inhibitor alpha (Nfkbia) and component of inhibitor of nuclear factor kappa B kinase complex (chuk)], and downstream targets of NF-κB [such as mitogen activated protein kinase 3 (mapk3) and TNF receptor associated factors (TRAFs)] (164, 165).

Future studies should also examine the cross-talk between PVAT brown versus visceral white adipose tissue inflammation. Considering that PVAT is a highly-active endocrine organ with the ability to communicate with remote adipose depots, questions remain regarding how these other depots are affected by both MetS and WRR

consumption (7). Questions also remain regarding the effect of rodent age on PVAT response to red raspberry dietary enrichment. At the age of 8-16 weeks the consequences of MetS are still developing, so it would be valuable to gain data regarding the effect of WRR consumption on rats who have experienced a more extended duration of inflammation (158). This would help to inform nutritional recommendations for patients of varying age and MetS progression status.

APPENDIX

IACUC Approval

Maine's Public Universities
UNIVERSITY OF MAINE SYSTEM

Paula Portalatin <paula.portalatin@maine.edu>

Protocol A2017-01-06 Approval

1 message

Paula Portalatin <paula.portalatin@maine.edu> Fri, Feb 10, 2017 at 9:28 AM
To: Dorothy Klimis Zacas <Dorothy_Klimis_Zacas@umit.maine.edu>

Protocol #: A2017-01-06
Protocol Title: Role of red raspberries on inflammation and endothelial dysfunction as related to the metabolic syndrome
PI: Dorothy Klimis-Zacas
Species/# Approved: Heterozygous Zucker Rats/40
Homozygous Zucker Rats/40
Approval Period: 2/10/2017-2/9/2020

Dear Dorothy,

The above referenced protocol has been approved by the University of Maine IACUC. As a courtesy the IACUC Office will generally send out reminders for annual and de novo reviews however, it is ultimately the responsibility of the PI to ensure that the protocol is renewed on time.

All of the proposed methods, procedures, and conditions have been approved AS STATED IN THE PROTOCOL APPLICATION. The IACUC must approve any changes or deviations from the approved protocol prior to being initiated.

University of Maine Animal Welfare Assurance #: A3754-01
The University of Maine is registered as a research facility in accordance with the U.S. Department of Agriculture Animal Welfare Act and the Public Health Service Policy on the Humane Care and Use of Laboratory Animals. The University of Maine holds the Office of Laboratory Animal Welfare (OLAW) of the National Institutes of Health assurance for vertebrate animals used in research, teaching and outreach.

The Animal Welfare Assurance (1) confirms the commitment that the University of Maine will comply with the PHS Policy, with the Guide for the Care and Use of Laboratory Animals, and with the Animal Welfare Regulation; (2) describes the institution's program for animal care and use; and (3) designates the institutional official responsible for compliance.

Attached to this email are the cage cards; please post on or near cages housing the animals, thank you.

Sincerely,

Paula

--

Paula Portalatin, M. Ed., CPIA
Research Compliance Officer II
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(207) 581-2657

A2017_01_06 Klimis-Zakas Cage Card.pdf

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Jasmine Waite was raised on Southport Island, ME where she spent many summer days lobstering with her Father and gardening with her Mother. She later lived in Fairfield, IA, where she developed a passion for health and wellness. Jasmine graduated from the Maine School of Science & Mathematics in 2015 and matriculated into the University of Maine's Honors College & Biochemistry B.S. degree program in the same year.

At the University of Maine Jasmine has enjoyed being a member of the research labs of Dr. Sally Molloy and Dr. Dorothy Klimis-Zacas, presenting at several conferences and symposia, serving as an Academic Coach and Peer Mentor to students in the TRIO Student Support Services program, and being an active member of Cru and Chi Alpha Student Ministries. Jasmine has also valued opportunities to develop her knowledge and experience in the field of professional writing.

Following graduation Jasmine plans to enter into a Masters' programs in Human Nutrition and Dietetics, with a special interest in metabolism and adipose tissue biochemistry. Jasmine plans to eventually work directly with patients in the fields of weight loss and eating disorder recovery, providing education, guidance and support for the development of a harmonious relationship with food. Jasmine also hopes to integrate her passion for professional writing, authoring articles that communicate new scientific findings to general audiences, and collaborating with authors from complementary fields.