# INDIVIDUAL AND COMBINED EFFECTS OF EXERCISE TRAINING AND DIETARY FAT ON MYOCARDIAL TRIGLYCERIDES IN OBESE MICE

BY

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THESIS

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## ABSTRACT

Visceral adipose tissue has been suggested to have a stronger association with disease than subcutaneous adipose tissue. This is primarily due to its inflammatory nature. Epicardial adipose tissue is a type of visceral fat located on the exterior of the heart. Recent evidence suggests an association between atherosclerosis, epicardial fat, and hepatic triglyceride deposition. PURPOSE -The purpose of this study was to determine the individual and combined effects of endurance exercise training and changes in dietary fat intake on myocardial triglyceride, a marker of epicardial fat, as well as hepatic triglycerides, another visceral fat depot implicated in CVD and diabetes risk, in a mouse model of dietary induced obesity.
METHODS – Male c57BL/6 mice (n=73) fed a 45% high-fat diet for 6-weeks to induce obesity were randomly assigned into four groups: exercise high-fat, exercise low-fat, sedentary high-fat, sedentary low-fat. Exercise intervention consisted of treadmill running for 5 days/week, 40 min/day, at an intensity of 65-70% $VO_2$ max for 12 weeks. After the 12 weeks, animals were sacrificed and both hearts and liver were removed. Using a triglyceride assay, myocardial and liver triglycerides levels were determined.
RESULTS – Exercise training significantly lowered liver triglycerides in both high fat and low fat fed mice ($p < 0.001$ for both groups). However, dietary fat intake did not have a significant effect on liver triglycerides within activity groups. Exercise training also lowered myocardial triglycerides in mice fed a high fat diet ($p = 0.005$), but had no effect on myocardial triglycerides in mice fed a low fat diet. By contrast, mice fed a low fat diet had significantly lower myocardial triglycerides than high fat fed mice, regardless of activity group ($p < 0.01$ for both groups).

CONCLUSION – Exercise training can attenuate epicardial/myocardial and hepatic fat accumulation in obese mice. Low fat diets can decrease myocardial fat accumulation in obese mice.

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# CHAPTER 1: INTRODUCTION

The prevalence of obesity continues to increase year after year. As the number of individuals with this disorder rises, so do the incidences of comorbidities such as cardiovascular disease. The impact of obesity on our society and economy has been devastating, and in light of this, scientists have taken a keen interest in adipose tissue biology[1].

Based on this research, adipose tissue is no longer viewed as a simple storage organ. It is now known to be a highly active tissue secreting adipokines and inflammatory cytokines, which participate in the inflammatory response[2]. In fact, studies have indicated that adipose tissue in the obese reflect an inflammatory profile compared to those of lean individuals[3].

In human adults, the body primarily has two types of adipose tissue: subcutaneous and visceral adipose tissue. Of the two, visceral adipose tissue has been shown to be more pro-inflammatory and metabolically active. Thus, increase visceral adipose tissue has been correlated with many inflammatory diseases[4] such as type-2 diabetes and atherosclerosis[5].

In recent years, epicardial fat, a type visceral adipose tissue, has garnered significant attention. This is primarily due to its proximity to the heart as well as its inflammatory profile. Several studies published have indicated a correlation between the amount of tissue and atherosclerosis[6,7]. Furthermore, disease severity has also been positively correlated with this tissue[8].

Seeing that obesity has a clear and unequivocal role in many disorders, much work has been done to identify methods to decrease adiposity, specifically in visceral adiposity. One approach that has proven to be successful is exercise. Unfortunately, studies looking at the effect exercise on epicardial fat deposition are few in number[9,10].

As mentioned, obesity has reached pandemic proportions and most have attributed this to the changes in diet throughout the decades as well as the general decline in physical activity most people incur. Therefore, exercise has been proposed as a method to reduce adiposity and inflammation[11,12]. Recent publications indicate that physical activity can help reduce epicardial thickness[9,10]. Thus, determining the effect of physical activity on epicardial fat deposition would be of great importance to the public.

The objective of this project is to test the hypothesis that moderate exercise will result in reductions in epicardial fat, as estimated by total triglyceride levels. It is predicted that exercise will cause a decrease in epicardial fat in diet-induced obese mice.

*Purpose*

As mentioned, obesity has reached pandemic proportions and most have attributed this to the changes in diet throughout the decades as well as the general decline in physical activity most incur. Therefore, exercise has been proposed as a method to reduce adiposity and inflammation. Evidence suggests that exercise has anti-inflammatory properties and recent publications indicate that physical activity can help reduce epicardial thickness. Thus, determining the effect of physical on epicardial fat would be of great importance to the public.

The aim of this project is to explore the independent and combined effects of exercise training and dietary fat intake on myocardial and hepatic fat accumulation and to determine if changes in these are correlated with changes in total body weight.

1. **We hypothesized that a 12-week exercise program will reduce epicardial and hepatic fat accumulation, using triglyceride levels in these organs as a proxy for visceral adiposity.**

2. **Furthermore, we hypothesize that the changes in epicardial and hepatic fat will be correlated with overall weight loss**

## CHAPTER 2: LITERATURE REVIEW

### The Obesity Pandemic

It has been well established that obesity has become a major concern, not just here in the United States, but globally. Recent years have seen a rapid escalation in the numbers of overweight and obese around the world[13]. According to the World Health Organization, there is an estimated 1 billion overweight people globally. Of these, around 300 million are said to be obese[14]. In Europe, estimates indicate that average numbers of obesity doubled from 1985 to 2005. It is predicted that this will again double over the next four decades[15]. Though data is limited and fragmented from Latin America, the information reveals that obesity rates are over 20% in adults from Countries such as Brazil, Argentina, Peru, and Paraguay[16]. The Asia-Pacific region has also seen a rise in obesity rates, from 5% in India to 60% in Australia. Even more astonishing are the estimates assessed from China. Though the obesity rates in that country ranks lower than Australia, the increase in numbers from the past 20 years has been 400%[17]. Obesity rates will continue to rise in this region as countries continue to develop economically, especially in China and India.

As previously mentioned, obesity has become a major concern here in the United States. One reason for this is due to the rapid increase in the prevalence of this disease. This swift increase began around the 1980's. Twenty years prior to that, only an increase of less than 2 percentage points of 15% was reported. However, during the late 1980's and into the early 1990's, those numbers increased dramatically to 23%. By 2000 those numbers again increased to 31%, a doubling from 20 years prior[18,19]. As worrying as this trend may be, the numbers seen concerning childhood obesity are just as, or even more staggering. In developed countries around the world, childhood obesity has also become an epidemic. In the United States alone,

twenty five percent of children are overweight. Eleven percent of these are considered obese and seventy percent of these obese children will grow up as obese adults[20,21,22]. In a survey performed from 1988-1994, a doubling to 11% was seen when compared to the last survey performed from 1976-1980. A subsequent survey performed from 1999-2000 reported a rise of 4% occurred in 5 years[23].

Maybe the most pressing concerns are the comorbidities related to obesity. As the pervasiveness of obesity widens, so will the incidence of obesity-related diseases. Obesity has been associated with several diseases such as cardiovascular diseases, diabetes, Alzheimer's disease, and certain types of cancer. More recently, obesity has been connected to depression[24,25,]. As of 2005, obesity reportedly accounted for 400,000 deaths per year and was second only to tobacco related deaths[26]. Linking the current trend in childhood obesity together with its known comorbidities has led many to suggest that this generation will likely have shorter lifespan than their parents. If this prediction were to occur, it would be the first time it would have happened in recorded history[27,28].

As the prevalence of obesity escalates, the burden it will impose on our health care system will also intensify. If obesity was prevented or curtailed, health care expenditure would have been lowered by approximately $46 billion in 1990[29]. In 2001, the Surgeon General reported that the annual medical expenditure indirectly related to obesity was $64 billion[30]. Expense directly related to obesity, however, was claimed to have been $75 billion. When updated to 2003 numbers, the total costs of obesity (indirectly and directly) may have been as high as $139 billion [31].

The scope by which obesity has negatively affected us is incredible. Its impact on us physically and emotionally has been debilitating and dully noted. Less visible but increasingly

important, however, has been its impact on society. Unfortunately, with the ongoing trend, it seems that this disease will cost us more than just our health and well-being.

**Causes of obesity**

Obesity can be defined as a condition in which excess fat accumulates in adipose tissue, to the extent where health may be impaired[32]. Though the cause of obesity is complex and multi-factorial, it is understood that this condition results when energy intake far exceeds energy expenditure through resting metabolism and physical activity[33]. Contributing to the development of this disease are certainly genetic factors. However, social and cultural environment, lifestyle choices, unhealthy dietary habits, and physical inactivity seem to have major influence in the rising prevalence of obesity[33,34].

As countries develop and populations become more affluent, certain transformations in dietary patterns have been noticed[35]. These changes include an increase in fat consumption (including saturated fat), a rise in sugar intake, and a large increase in animal food consumption. In addition to these, declines in complex carbohydrates, fruit, and vegetable intake have also been noted[35,36,37].

Socioeconomic factors can also increase the incidence of obesity. Nutrient-poor and energy-dense foods are often less expensive and more obtainable for poor groups. This is in stark contrast to more prosperous individuals who are able to afford, and therefore, consume nutrient-rich and overall better quality food items. Augmenting this predicament is the inaccessibility of vendors of healthy food items as many of these are located in more affluent areas[38].

Though genetic factors by themselves seldom bring about obesity, the interaction of environment and genetic predisposition has sparked the interest in recent years. Gene-

environment interaction refers to a situation in which the response or the adaptation to an environmental agent, a behavior, or a change in behavior is conditional on the genotype of the individual[39]. For instance, a study of healthy Japanese men indicated that a missense variant in the interleukin 6 (IL-6) receptor gene interacted significantly with dietary energy intake levels in relation to the risk of abdominal obesity[40].

Aside from food consumption and genetics, the decline of physical activity has also played a role in escalation of obesity. Due to the aid of technology and jobs entailing less physical activity, more and more have adopted an increasingly sedentary lifestyle[41]. Inactivity can lead to increased adiposity by reducing caloric expenditure as well as altering insulin and leptin sensitivity[42].

Several treatments for obesity exist and range in complexity from a change in dietary habits to surgical interventions. Though diet modification may be the simplest and least harmful of the treatments, half of those that lose weight through diet alone will eventually regain all the weight they had lost[43]. Furthermore, similar reductions in fat are seen in those that combined exercise and caloric restriction and those that incurred similar caloric deficits using diet alone. Additionally, those that included exercise improved fitness, insulin resistance and diastolic blood pressure[44]. This indicates the beneficial effects of exercise in both weight loss and in other biological markers that mark obesity related dysfunctions.

**Inflammation**

Inflammation is the physiological response an organism has to negative stimuli, be it physical, chemical, or biological. Response is a coordinated action of many cell types and mediators in an attempt at regaining homeostasis[45]. In a normal acute inflammatory response (physical injury and infection) resident tissue macrophages and mast cells signal for the transport

and migration of leukocytes to the site of the insult leading to the production of chemokines, cytokines and other inflammatory mediators[46]. If the response is successful, the agent is eliminated and inflammation resolution and tissue repair commences. In cases of long-term infections or autoimmune diseases, inflammation can become chronic. Response intensifies in that cytokine levels can increase up to four times the levels of an acute response. In addition, this response also requires the recruitment of different mediators, specifically, T-lymphocytes[47,48].

Signature to the inflammatory response is the release and accumulation of cytokines and macrophages. Cytokines are proteins made by cells and affect the behavior of other cells[49]. These proteins have a dual role in the inflammatory response. Cytokines signal the body to increase defense mechanisms in the form of hematologic and immunologic responses[50]. Furthermore, they induce the expression of genes and synthesize proteins that induce acute and chronic inflammation. In studies utilizing injury and endotoxins as a model for acute inflammation, interleukin-1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α) are shown to increase dramatically, especially immediately after the insult occurred[50,51]. Similar results have been seen in subjects with chronic inflammation. As a matter of fact, elevated cytokine levels have been associated with chronic conditions such as obesity, type-2 diabetes, and atherosclerosis[52,53,54].

As mentioned, macrophages play an integral role in inflammation. During the inflammatory response, macrophages become the first line of defense. Recruited by chemokines, these cells migrate to the site of the insult and secrete inflammatory proteins and phagocytose pathogens or debris. Macrophages can be activated in two ways: “classically” and “alternatively”. When classically activated, macrophages are triggered to destroy bacteria. In

this state, they are considered pro-inflammatory as they produce IL-1, IL-6, and TNF-α, cytokines that are responsible for propagating the response[55]. These macrophages are "classically" activated when exposed to interferon gamma or lipopolysaccharides. On the other hand, when exposed to IL-4, IL-13, and transforming growth factor-beta (TGF-β), macrophages produce proteins IL-10 and IL-1 receptor antagonists. These proteins are anti-inflammatory, thus lending to the term "alternatively" activated macrophages. Because of their "classical" state, macrophages have become a target for researchers investigating the mediators of inflammatory diseases. Consequently, macrophages have been associated with two of the most devastating diseases of modern time –diabetes and cardiovascular disease[56,57,58]. Other than through the overproduction of pro-inflammatory cytokines, macrophages play an integral role in the onset and propagation of atherosclerosis. Macrophages, through the "scavenger-receptor" pathway, internalize oxidized-LDL leading to the formation of cholesterol ester-laden foam cells, the hallmark of atherosclerosis[59,60].

The inflammatory response is the process by which an organism attempts to regain homeostasis. This is clearly meant to be beneficial; however, in cases where the response becomes chronic, the response can become dangerous and assist in the onset and propagation of diseases such as diabetes and heart disease.

**Obesity and Inflammation**

The main physiological role of adipocytes is to act as a storage depot for fat when energy intake exceeds energy expenditure and to release free fatty acids when needed[61]. Another function of adipose tissue is its ability to aid in the inflammatory reponse[62]. This occurs when adipocytes in subcutaneous and visceral adipose tissues hypertrophy with triglyceride due to obesity. At this state, the adipocytes secrete numerous pro inflammatory cytokines such as TNF-

α and macrophage chemoattractant protein-1 (MCP-1)[63,64]. MCP-1 is a powerful chemokine that attracts circulating monocytes and directs them to enter the expanding adipose depot and into the non-adipocyte portion of the tissue termed the stromal-vascular matrix[65]. These macrophages then transform into activated M-1 macrophages[66] and secrete even more MCP-1 to augment monocyte recruitment. These, along with an activated endothelium, generate other proinflammatory cytokines such as interleukin-1 beta (IL-1β), IL-6, and interleuking-8 (IL-8)[62,67]. These cytokines along with TNF-α autocrine feedback, inhibit insulin signaling in adipocytes[68]. This results in insulin resistance and the transformation of stored triglycerides to free-fatty acids[68,69,70]. This causes M-2 activated macrophages to release the anti-inflammatory cytokine interleukin-10 (IL-10) in an attempt to protect the adipocytes from the pro-inflammatory molecules[71]. Pro-inflammatory cytokines, especially IL-6 and TNF-α, inhibit adiponectin, a modulator of fatty-acid catabolism[72]. Both adipocytes and endothelium then produce VEGF[73], which causes angiogenesis to increase adipose tissue vascularity[74]. The free-fatty acid accumulation due to the decrease in adiponectin are then released from the adipose tissue into the hepatic portal vein where they are used as substrates for the synthesis of atherogenic lipoprotein B-containing VLDL-triglyceride particles. These are then released into circulation[75].

As insight into the nature of adipose tissue mounted, researchers sought to look at whether differences can be spotted between the physiology lean and obese adipose tissues. As mentioned above, obesity is a condition where excess fat accumulates in adipose tissue. However, the amount of adipose tissue is not the only characteristic that is differentiates from its lean counterpart. Much work has been done to show that obese adipose tissue is physiologically different than lean adipose tissue. An important distinction between the two is the considerable increase in inflammatory cytokines and acute phase reactants seen in obese adipose tissue. Xu

and colleagues[3] revealed a significant increase in MCP-1, TNFα, and macrophage infiltration in adipose tissue of obese mice when compared to lean animals. Several studies on humans revealed similar results. In a study using Japanese men, inflammatory markers were analyzed and compared between individuals characterized with and without abdominal obesity. Results from this study indicated an increase in CRP and IL-6 in those with abdominal obesity as well as an inverse relationship in adiponectin[76] The decrease in adiponectin is quite important as this protein is anti-inflammatory[76,77] as well as anti-atherogenic[78].

Hotamisligil et al.[54] produced a study yielded similar results. Using tissues biopsies taken from lean and obese pre-menopausal women, they illustrated that TNF-α mRNA expression was markedly higher in the obese women compared to the age-matched lean controls. Even more important than that result was their finding concerning TNF-α protein expression. ELISA protein assays revealed that TNF-α protein expression was significantly higher in the obese subjects compared to their age matched lean counterparts.

As stated earlier, macrophages are a key component of the inflammatory process, especially in conditions characterized by chronic inflammation such as obesity. Therefore, ascertaining whether obese adipose tissue incurs greater macrophage infiltration is vital in determining whether these tissues are more inflammatory. It was mentioned previously that adipose tissue releases MCP-1 - a protein that attracts macrophages. Thus, Harman-Boehm et al.[79] investigated whether increased obesity had an effect on macrophage infiltration. Results from this study showed that MCP-1 mRNA and protein expression was greatly increased in adipose tissue obtain from obese individuals.

A wonderful work by Weisberg et al.[80], added to this ongoing trend. This study utilized both human and mouse models to identify and examine potential changes in inflammatory

markers associated with obesity. Additionally, these researchers looked at inflammation from progression from lean to obese tissues by adding a third group (overweight) to the study. Several types of adipose tissues (epididymal, perirenal, mesenteric, and inguinal subcutaneous) were collected and analyzed from both mice and humans and the results once again yielded findings similar to the others mentioned previously. Immunohistochemical assays performed on both human and mouse adipose tissue revealed a positive correlation between macrophage infiltration, adipocyte size, and increased adiposity.

Many other studies examining the pathophysiology of adipose tissue have been performed. Works investigating changes in inflammation from weight loss due to bariatric surgery[54,81] and other forms of weight loss reveal results like that of the studies detailed above, that obese adipose tissue contain more markers of inflammation than their lean counterparts.

Moreover, further inspection of the data also reveals an important distinction between adipose tissues. However, the variation is not between lean and obese tissues. Instead, the difference is between visceral and subcutaneous tissues. Both works by Weisberg and Harman-Boehm not only revealed a significant difference in inflammatory markers between obese and lean tissues, they also revealed that visceral fat had a marked increase in inflammatory cytokines when compared to subcutaneous adipose tissue. In accordance to these results, findings from a study by Fein et al.[82] found that both IL-6 and VEGF were significantly higher in visceral adipose tissue compared to subcutaneous fat. More support for this came from Zha et al.[83] in which they revealed a greater increase in TNFα gene expression in visceral adipose tissue (compared to subcutaneous fat) in obese humans. These findings indicate that visceral adiposity may be a more important risk factor than total body fat in diseases thought to be inflammatory related.

**Obesity, inflammation and disease**

The fact that obesity greatly increases an individual's risk for disease has been established for quite some time. However, the mechanism by which this occurs still remains indefinable. Within the last two decades, the term "metabolic syndrome", formerly known as "syndrome X", was created to describe a clustering of metabolic disorders that include insulin resistance, central obesity, hypertension, dislipidemia, and hypertriglyceridemia[84]. Thus, it has been proposed that metabolic syndrome is what predisposes obese individuals to co-morbidities such as the development of atherosclerosis, coronary heart disease events[85], and diabetes[86].

A component of metabolic syndrome, inflammation, has garnered much attention in recent years. As stated above, there is a difference in the physiology between obese and non-obese adipose tissue. Over a decade ago, it was discovered that obese adipose tissue in mice expressed higher levels of TNF-α[54] and that this was associated with impairments in insulin function[87]. Uysal et al.[88] yielded similar results in showing that obese mice deficient in TNF-α or its receptors improved insulin sensitivity. Support for this came from results showing improved glucose tolerance in animals with the neutralization of TNFα[89].

Other proinflammatory cytokines have also been associated with insulin resistance and diabetes. Insulin tolerance testing revealed that IL-1α deficient mice have greater insulin sensitivity and lower fasting levels of both glucose and insulin when compared to wild type animals. IL-6 has also been linked with the onset/progression of diabetes. This was shown in patients that have undergone bariatric surgery whose improvement in insulin sensitivity was associated with a decline in this cytokine[90]. As the prevalence of diabetes continues to increase dramatically, much work has been performed to linking insulin resistance and obesity, especially since this condition is critical in the development of the type-II diabetes[91].

Just as inflammation has been implicated in the development of Type II Diabetes, work has been done to show its relationship with and cardiovascular events[92,93]. Utilizing data from the Framingham Heart Study, Vasan et al.[166] associated IL-6 with an increased risk of congestive heart failure. Results from a prospective study performed by Ridker et al.[94] confirm the previous finding. Using healthy men as subjects, IL-6 was again shown to be predictive of cardiac events[95]. TNFα also has been linked to cardiovascular anomalies. After analysis of data obtained from the HEALTH Study, Cesari et al.[96] produced results indicating at a very strong relationship between TNFα and congestive heart failure (CHF). They also claim that this cytokine along with IL-6 may be a better predictor (of CVD) than CRP. However, it must be noted that the association between CRP and CVD decreases with age, and this project included more elderly when compared to many other studies. CRP is an acute phase protein produced in the liver and is a marker of the inflammatory cascade[97]. Thus, many studies have been undertaken investigating the association of this protein with cardiovascular diseases. In accordance with the overwhelming evidence pointing to inflammation as a mediator of CVD, the vast majority of the studies examining cardiovascular events have shown a great association between this protein and CVD[98,99,100]. As mentioned previously, the association between CRP and cardiovascular events decline with age. However, several studies involving young men and women have shown baseline CRP to be predictive of future cardiovascular anomalies and CVD related mortality[101,102]. This positive correlation between CRP and CV events is not surprising as it is primarily released in response to IL-6, which has been stated to have a positive association with CVD.

**Inflammation and atherosclerosis**

Evidence suggests inflammation plays a fundamental role in the onset and progression of atherosclerosis[97]. According to Glass[103], atherosclerosis can be considered a form of chronic inflammation resulting from the lipoproteins, macrophages, T-cells, and the cellular elements of the arterial wall. Signs that an inflammatory response factors in the earliest stages of the disease can be seen in mice shortly after being fed an atherogenic diet[97]. At this stage, lipids begin to accumulate within the arterial wall causing the endothelium to express selectins and intracellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1(VCAM-1). These molecules attract and bind circulating monocytes to the cell wall[104,105]. Whether or not these molecules play a role in atherogenesis were put to rest upon seeing apoE deficient mice missing genes for both E- and P- selectins. Deletion of these genes resulted in a 40-60% decrease in atherosclerosis[106]. Collins et al.[107] yielded similar results with the deletion of the ICAM-1 gene.

After adhering to the endothelial surface, monocytes gain entry into the arterial wall by exposure to oxidized LDL[108]. Oxidized LDL can not only attract these cells directly, they can also induce endothelial cells to release MCP-1[109]. Further evidence of this is seen with the expression of CCR-2 (MCP-1 receptor) on monocytes, which is positively influenced by cholesterol levels. Han et al.[110] substantiates this by showing increased chemotaxis to MCP-1 in hypercholesteremic patients. Alternatively, elimination of the CCR-2 gene reduces the development of atherosclerosis in apoE deficient mice[111].

Upon migrating in the subendothelial space, monocytes differentiate into macrophages. After which, oxidized LDL are taken in to the macrophage via scavenger receptors such as SR-A and CD-36[112]. Removal of these receptors reveals their importance in atherogenesis, as without them, apoE mice exhibit greatly reduced levels of atherosclerosis[113].

Oxidized LDL engulfed by macrophages have several fates, one of them being stored in lipid droplets characteristic of foam cells[97]. Cholesterol in the droplets can be disposed by the macrophages. This is primarily done by "reverse cholesterol transport" via HDL. However, if the level of accumulation is greater than what can be eliminated, foam cells continue leading to fatty streaks.

Inflammation again plays a role in the progression of fatty streaks to more complex lesions. Complex lesions are distinguished by the migration of smooth muscle cells from medial layer to the intimal or subendothelial space[103]. Smooth muscle cells may proliferate and add to foam cell load by taking in oxidized LDL. At this point, a fibrous cap may form. This cap consists of smooth muscles in a collagen-proteoglycan mixture[114]. This phase is considered inflammatory as T-cells (glass), macrophages , and smooth muscle[103] have been obtained from lesions[115]. Furthermore, there is evidence indicating these cells as being activated[116].

Atherosclerosis and fibrous cap progression can lead to plaques containing small or absent necrotic lipid cores and a thick fibrocalcific core[115]. Evidence exists indicating this thick calcified cap may actually be protective. Hunt et al.[117] illustrated that coronary artery patients with calcified plaques experienced less symptoms of stroke and ischemic attacks when compared with those without calcification. However, plaques with this type of cap have been associated with severe narrowing of arteries[114].

In stark contrast to the thick calcified cap, lesions may develop into plaques containing a large necrotic core and a thin fibrous cap. Lesions of this sort are more vulnerable to rupture and have been frequently observed in patents succumbing to acute myocardial infarction[114]. Interestingly, these types of caps contain large amounts of macrophages, T-lymphocytes and other inflammatory products[115,118].

There is clear indication that inflammation plays an integral role in the development and progression of atherosclerosis. Therefore, efforts to reduce inflammation -or the products and processes that induce it - should be investigated in greater detail.

**Epicardial Fat, Obesity, and Disease**

As mentioned above, visceral fat has been shown to be more inflammatory than subcutaneous adipose tissue. Visceral fat has also been more closely associated with diseases such as Type II diabetes and cardiovascular disease. Due to these findings, principally the later, scientists began examining a type of visceral fat very specific to the heart –epicardial fat. Epicardial fat is a visceral fat deposit found on the heart[119]. Unfortunately, studies on this tissue are sparse and have only recently begun to compile. With that said, observations between correlating epicardial fat and obesity have been documented since the beginning of the 20th century. In 1933, Smith and Willius[120] noticed that epicardial deposits increased with obesity in obese cadavers. Epicardial fat is commonly deposited along the interventricular and atrioventricular grooves[121]. However, in some cases the entire heart may be completely covered in a layer of epicardial fat[103]. The close proximity of epicardial fat to the coronary arteries, the fact that nothing separates it from the myocardium[119] , in conjunction with its proinflammatory nature[122,123], has compelled researchers to further investigate this tissue.

Though having a heart completely covered in epicardial fat is an extreme case, epicardial adipose thickness has been correlated with the severity of coronary artery disease. Utilizing over two hundred patients with coronary artery disease, Iacobellis et al.[124] were examined to see whether a relationship between epicardial fat and coronary artery disease severity could be established. Subjects underwent a coronary angiography in order to assess the severity of their atherosclerotic lesions. Electrocardiograms performed in order to determine epicardial fat

thickness on the free wall of the right ventricle. The area of the right ventricle is assessed because this is the portion of the heart that increases in adiposity initially. Thickness over the left ventricle and between the apex and base occurs much later[124]. The researchers also compared epicardial fat thickness with other risk factors and coronary atherosclerosis. Results of the study revealed significant correlations between epicardial adipose tissue thickness and age, C-reactive protein (a global marker of inflammation), body mass index, and waist circumference. The study also showed epicardial adipose tissue thickness as an independent factor affecting coronary artery stenosis.

In another study, Iacobellis et al[125] investigated the relationship between epicardial adipose tissue to cardiac, metabolic, and anthropometric markers of metabolic syndrome. Participants, whose morphology ranged from lean to obese, had their right ventricle imaged and analyzed for epicardial fat thickness via echocardiogram. Cardiac, metabolic, and anthropometric measures were also taken. Their results showed a very high correlation between epicardial fat deposition and magnetic resonance imaging of visceral adipose tissue. The results also revealed that waist circumference and diastolic blood pressure (both independent risk factors for coronary artery disease) were strongly correlated with epicardial adipose tissue thickness.

Most methods analyzing epicardial fat and its relation to CVD has been indirect. As seen above, most studies have utilized echocardiograms to assess epicardial fat deposition. However, there are a few projects that have utilized a more direct method of analysis. Employing epicardial tissue biopsies obtained from patients undergoing open-heart procedures Cheng et al.[122] revealed TNF$\alpha$, IL-6, leptin, and visfatin are of greater concentration in epicardial adipose tissue from patients with CAD. Moreover, significant decreases in adiponectin were also seen in this tissue from patients undergoing bypass surgery.

Another method in which epicardial adipose tissue can be associated to atherosclerosis is by seeing how the disease progresses devoid of the adipose depot. In order to do this, some scientists have looked at how a myocardial bridge affects coronary atherosclerosis. Myocardial bridge is a common anatomical condition in which the left anterior descending coronary artery is covered by myocardial tissue instead of epicardial adipose tissue[126].

A study performed by Ishikawa et al.[127] tested whether the myocardial bridge protected an artery from developing atherosclerosis. Their study utilized white rabbits as a model of atherosclerosis. These rabbits were fed a diet high in cholesterol for 20 weeks, after which, they were sacrificed. Two segments of their left coronary arteries were assessed for the presence of atherosclerotic lesions. One segment of the artery ran through epicardial adipose tissue while the other segment entered the myocardium and had no contact with the adipose depot. Their results revealed that segments surrounded by epicardial fat had developed atherosclerotic lesion while the other did not.

The mechanism proposed by which the myocardial bridge may prevent atherosclerotic lesion formation may be due to a change in homodynamic forces. Low shear stress may aid in the formation of atherosclerotic lesions by allowing a greater amount of lipids to enter the vascular wall[126]. Studies have indicated that the shape and pattern of the epithelial cells may influence the amount of shear stress the vessel is exposed to. Studies in rabbits and rats[128] reveal spindle-shaped endothelial cells lining the portion of the descending coronary artery within the myocardial bridge. As expected these portions had a greater amount shear stress compared to the portion exposed to epicardial adipose tissue. Additionally, this portion of the artery consisted of polygonal shaped endothelial cells. In humans, the portion of the descending coronary artery proximal to the myocardial bridge contain endothelial cells are flat and arranged in a pave-like

pattern thus allowing for high shear stress, much higher than the portion of the vasculature exposed to epicardial adipose tissue[129]. In both of these examples, the areas of high-shear stress were free of atherosclerotic lesions, whereas the portion exposed to low shear stress and epicardial adipose tissue exhibited signs of lesions.

**Adventitial Adipose Tissue and Its Role in Atherosclerosis**

It has been shown that atherogenesis results from the transendothelial passage of cholesterol-rich Apo-B lipoproteins from plasma into the intima. Inside the vasculature, these lipids are oxidized, and in conjunction with a chronic inflammatory response within the vessel layers, plaque formation can ensue[126,130]. Due to the proinflammatory nature of epicardial adipose tissue and its close proximity to the coronary arteries, it has been identified as one of the possible contributors to the formation of atherosclerotic lesions. Using this same rationale, scientists have begun to look at another adipose depot as a possible risk factor for atherosclerosis. That depot is adventitial adipose tissue.

Adventitial adipose tissue is classically considered the outermost connective tissue of a vessel[131]. Adventitial fat has been generally ignored in the study of atherosclerosis[132]. This is unfortunate because all the vessels associated with atherosclerotic lesions possess it.

Just as information on epicardial fat is minute, very few studies have been performed on adventitial fat. Of the few, Mazurek et al.[66], compared levels of MCP-1 mRNA and other inflammatory cytokine levels in adventitial adipose tissue from the right coronary arteries and subcutaneous adipose tissue in patients undergoing coronary artery bypass surgery. The results of the study revealed that adventitial fat expressed these inflammatory cytokines at significantly higher levels than subcutaneous fat. Unfortunately, this result was not correlated with lesion formation.

In another study, Tavora et al.[133], addressed the role of adventitial in atherosclerosis. Their study aimed to correlate the degree of adventitial inflammation in epicardial arteries with the underlying intimal atherosclerotic plaque. The researchers studied sections of coronary arteries from patients that died of coronary artery disease and assessed the adventitial adipose tissue associated with the plaques and segments. The results revealed that adventitial lymphocytic inflammation increased with stenosis. It also indicated that hemorrhage into late core, rupture, erosion, and thin caps had greater adventitial inflammation independent of stenosis severity. Lastly, adventitial adipose macrophage density was increased in plaques with atheromas and positively correlated with adventitial lymphocytes and macrophage content. The authors concluded that features associated with plaque instability are associated with adventitial adipose tissue inflammation.

Though these were the only two studies that specifically addressed the possible function of adventitial adipose tissue in the onset or progression of atherosclerosis, this combined with our knowledge about adipose tissue, especially epicardial adipose tissue, begs us to further investigate the role adventitial adiposity

**Mechanism by Which Epicardial Fat Influences Atherosclerosis**

Unfortunately, the mechanism by which epicardial adipose tissue directly mediates atherosclerosis remains elusive. This fat deposit has been associated with changes in cardiac morphology, such as increased left ventricular hypertrophy. Excess weight placed on the ventricles due to fat deposition increases the effort needed to pump blood inducing, thus inducing hypertrophy. In fact, left ventricular hypertrophy has been positively correlated with left epicardial mass in several studies[134,135].

Regrettably, this alteration in morphology has not been implicated in the development of atherosclerosis. Thereby, the most obvious perpetrators are the inflammatory products produced in and by epicardial fat. As mentioned, no fascia separates epicardial fat and the myocardium. For these reasons, Glass[103] proposed a hypothetical mechanism for how inflammatory products originating from epicardial fat may mediate the development of atherosclerosis. This hypothetical mechanism involves paracrine and vasocrine signaling to shuttle inflammatory mediators into the intima. For the pathway involving paracrine signaling, Glass states that adipokines produced by adipocytes and stromal-vascular cells in epicardial fat diffuse in interstitial fluid and travel across the adventitia, media, and into the intima. There, they interact with all components of plaque formation. In contrast, vasocrine involves inflammatory products entering the vaso vasorum where they travel across the vessel into the lumen. They are shuttled downstream to interact with the lesion as well as all the cells in both the intima. Interestingly, this model allows for macrophages and lymphocytes to migrate to the intima along the vasa vasorum via openings in the media.

**Heart Triglyceride Level and Its Relation to Epicardial Fat.**

Studies investigating epicardial deposition have primarily used echocardiograms to determine and quantify changes in this tissue[9,10,136]. These studies utilize humans as subject and the epicardial fat pad in obese is fairly easy to image. Conversely, our subjects are mice whose fat pads are often times often barely visible to the human eye. Therefore, we have to employ a method in determining epicardial fat content. We accomplished this by determining the concentration of myocardial triglyceride content. This is very similar to the manner by which the level of hepatic steatosis (fatty liver) is ascertained. To our knowledge, this method of determining epicardial fat deposition has not been performed before. However, there is reason to

believe this may be a valid technique. In a study investigating the relationship between myocardial triglycerides, epicardial fat, and heart function Kankaanpaa et al[137], found a strong positive relationship between heart triglycerides and epicardial fat in obese men. The cause for this, they claim, is similar to the development of hepatic steatosis, in that the liver is the first-pass organ that free fatty acids from an adipose depot (intra-abdominal fat) deposit into. Similar to this, the epicardial fat deposits free fatty acids into the myocardium. Furthermore evidence suggests that this mechanism appear to be similar in both obese humans and animals[138,139].

**Exercise Decreases Adipose Tissue Inflammation**

There are several studies that that have been performed to assess the effects of physical activity on inflammation[140,141]. These studies often measure the expression of CRP, an acute phase protein released by the liver[142] and considered one of the best markers of inflammation. These studies all indicate that the average CRP levels in the subjects that performed the most physical activity was still markedly lower than in the subjects that performed the least physical activity[143,144].

Utilizing male patients with chronic heart failure study, Adamopoulos et al. [145], looked at whether exercise could affect markers of inflammation. After participating in a 12-week exercise program, these patients showed a significant reduction in MCP-1 and other proinflammatory markers.

Indicating that these results will apply to women, Okita et al.[146] placed healthy women in an exercise intervention for two months after which CRP levels were assessed. The results showed that the greatest significant declines in CRP were in those that lost a moderate amount of weight. Those that lost the most weight did not have significantly lower levels of CRP, although downward trend was seen. The researchers suggested that strenuous exercise might have

negative effects in those that did not show a significant decrease in CRP. This study also showed the possibility that weight reduction may be an independent factor in the reduction of inflammation.

The vast majority of these studies measured changes in subcutaneous or epididymal adipose tissue with respect to exercise. However, there is one study that examines the effect of exercise in epicardial adipose tissue. Kim et al.[9] assessed how a 12-week exercise program affected markers of insulin resistance, epicardial thickness, and CRP levels among others. The results indicated that exercise aerobic exercise significantly reduced epicardial thickness. It also revealed that an acute bout of aerobic exercise could improve markers of insulin resistance. Unfortunately, the exercise program did not reduce CRP levels. The main problem with this study was the fact that there was no control group. Since the subjects lost an average of 3kg, the decrease in epicardial adipose tissue may have stemmed from weight loss instead of exercise. This would follow in line with the finding in the previous study discussed.

Lastly, our lab produced a pilot study investigating intradialytic exercise and its effect on epicardial fat thickness[10]. Data from this study indicated a significant reduction in epicardial fat in those that participated in the exercise intervention. This and the result from the previous study suggest the ability of exercise to mediate epicardial fat deposition.

**Potential Mechanism Behind the Anti-Inflammatory Effect of Exercise**

*Reduction of Macrophage Infiltration*

The role of macrophages in the inflammatory response has been established. Evidence has shown that macrophages accumulate in obese adipose tissue[80]. Studies have also shown that these the inflammation associated with obesity stems from macrophages occupying the non-adipocyte portion of adipose tissue[147]. This macrophage population has been shown to correlate

with increased adiposity and adipocyte size. As mentioned in some of the studies discussed earlier, macrophages release MCP-1, a cytokine that attracts monocytes to the cause of the inflammatory response. Exercise has been shown to decrease MCP-1 in people suffering from metabolic syndrome[148]. Therefore, by decreasing levels of MCP-1, exercise may be a powerful factor in decreasing inflammation in adipose tissue by reducing macrophage infiltration.

*The Cholinergic Anti-Inflammatory Pathway*

Tracey and Pavlov[149] coined the term "cholinergic anti-inflammatory pathway". These researchers state that the stimulation of the vagus nerve inhibits proinflammatory cytokines from being released. They suggest that the autonomic nervous system induces the inflammatory response through the release of glucocorticcoids. The body then counters this through the vagus nerve or parasympathetic nervous system release of acetocholine, which prevents the release of proinflammatory cytokines. This may be entirely possible as exercise has been shown to increase vagal tone. This stimulation of the vagus nerve may cause the release of acetocholine and thus promote an anti-inflammatory response. This may be extremely important in mediating atherosclerosis progression as the vagus nerve infiltrates the adventitia of coronary arteries and aorta.

## CHAPTER 3: METHODS

### *Animals and Diet*

Four-week-old male C57/Bl mice (n=73) were purchased from Jackson Laboratories (Bar Harbor, Me) at 4 weeks of age. After acclimatization of 1 week, all animals were individually caged and fed a 45% fat diet (high fat diet) to induce obesity. These animals were then assigned to: high-fat sedentary, high-fat exercise, low-fat sedentary (10% fat diet), or low-fat exercise for 12 weeks. Animal feed was obtained from Research Diets Inc. (New Brunswick, NJ); specific information regarding diet can be found in **Table 1**. All animals were allowed to eat *ad libitum* and both food intake and body weight were recorded twice a week for the extent of the study. Animals were housed on a reverse light-dark cycle (lights on at 21:00 h and off at 09:00 h) at 25° C. The diets are well tolerated by the animals and both diet and the duration of the intervention is a well-accepted model for diet-induced obesity. All experiments were approved by the Institutional Animal Care and Use Committee and supervised by the Division of Animal Resources at the University of Illinois at Urbana-Champaign. Guidelines followed for the care and usage of the animals were directed by the National Institute of Health.

### *Exercise Training Intervention*

Animals (in the exercise group) were exercised on a motorized treadmill (Jog-A-Dog, Ottawa Lake, MI) during their dark cycle. Negative reinforcement such as electric shock was not used, as they were not deemed necessary. As a substitute, a foam pad was placed at the back of each lane in order to serve as tactile response. Another purpose of the pad was to prevent the animal's tail from being pinched between the treadmill and the back of the lane. Mice were exercised for a duration of 40 minutes at a speed of 12 m/min at 5% grade for 5 days a week.

For acclimatization, the mice began exercising for 20 minutes per day. The duration was increased by 2 minutes each subsequent day until 40 minutes was attained.

***Heart and Liver Triglycerides***

Animals were sacrificed by cervical dislocation and both hearts and livers were perfused with PBS and removed from the animals. The organs were then sliced in half and the bottom portion is what is utilized for this assay. Each portion was weighed and placed in 4 ml of isopropanol. Tissues were then homogenized for 7-10 minutes in the isopropanol using a motorized hand homogenizer (PowerGen 125, Fisher Scientific). The homogenate was centrifuged at 2,000 g for 10 minutes, after which the liquid was collected. Triglyceride content in the isopropanol solution was measured using a commercially available triglyceride assay kit (Wako Diagnostics, Richmond VA). Triglyceride content was corrected for sample weight.

***Statistics***

Both myocardial and liver triglyceride content were analyzed using a 1-way analysis of variance (ANOVA). For diet and weight measures, repeated measure ANOVAs (diet x time) were conducted. Post-hoc Tukey HSD comparisons were used to determine group differences and $< .05$ was accepted as statistically significant. SPSS version 16.0 (Chicago, Il.) was utilized for all analyses.

## CHAPTER 4: RESULTS

***Body weight.***

Fig. 1 illustrates the changes in body weight brought about by the intervention. At the pre-intervention time point, which was preceded by 6 weeks of high-fat feeding, these diet-induced mice showed significantly higher body weights than animals solely fed a CHOW diet ($p<.001$). Significant differences can also be seen at the 4, 8, and 12 week time points in all groups ($p<.05$) except for the low-fat sedentary and low-fat exercise groups. All groups gained weight throughout the intervention indicating a time effect ($p<.001$). In addition, significant time x exercise ($p<.001$) and time x diet ($p<.001$) interactions were also present.

***Hepatic steatosis.***

Triglyceride levels within the liver are noted in Fig. 2. Using a one-way ANOVA, our results indicate a significant difference between the groups ($p < 0.001$). Both exercise groups (HF-EX and LF-EX) had significantly less triglyceride content to their sedentary counterparts ($p < 0.001$ for both). Surprisingly, diet did not have a significant effect on hepatic triglyceride levels. Comparison of HF-EX and HF-SED as well as LF-EX and LF-SED, produced no significant differences.

***Heart triglycerides.***

After performing ANOVA on data obtained from triglyceride assays performed on heart tissue, we found significant differences between groups ($p < 0.001$). Tukey post hoc comparisons of the groups indicated that exercise has a significant effect on animals on a high-fat diet ($p < 0.001$). However, exercise did not produce a significant change in triglyceride content in animals given a low-fat diet ($p=.089$). Diet produced significant changes in heart triglyceride levels in both exercise and sedentary animals. Myocardial triglyceride content in

these animals was significantly larger in high-fat animals compared to low-fat fed animals in both exercise and sedentary groups ($p < 0.01$ for both).

Table 1. Diet Composition.

| | HFD | | LFD | |
|---|---|---|---|---|
| | gm% | kcal% | gm% | kcal% |
| protein | 24 | 20 | 19.2 | 20 |
| CHO | 41 | 35 | 67.3 | 70 |
| fat | 24 | 45 | 4.3 | 10 |
| kcal/gm | 4.73 | | 3.85 | |
| | gm | kcal | gm | kcal |
| sucrose | 172.8 | 691 | 350 | 1400 |
| soybean oil | 25 | 225 | 25 | 225 |
| lard | 177.5 | 1598 | 20 | 180 |
| total | 858.15 | 4057 | 1055.05 | 4057 |

Rodent diets D12450B and D12451; Research Diets, Inc. (New Brunswick, NJ)

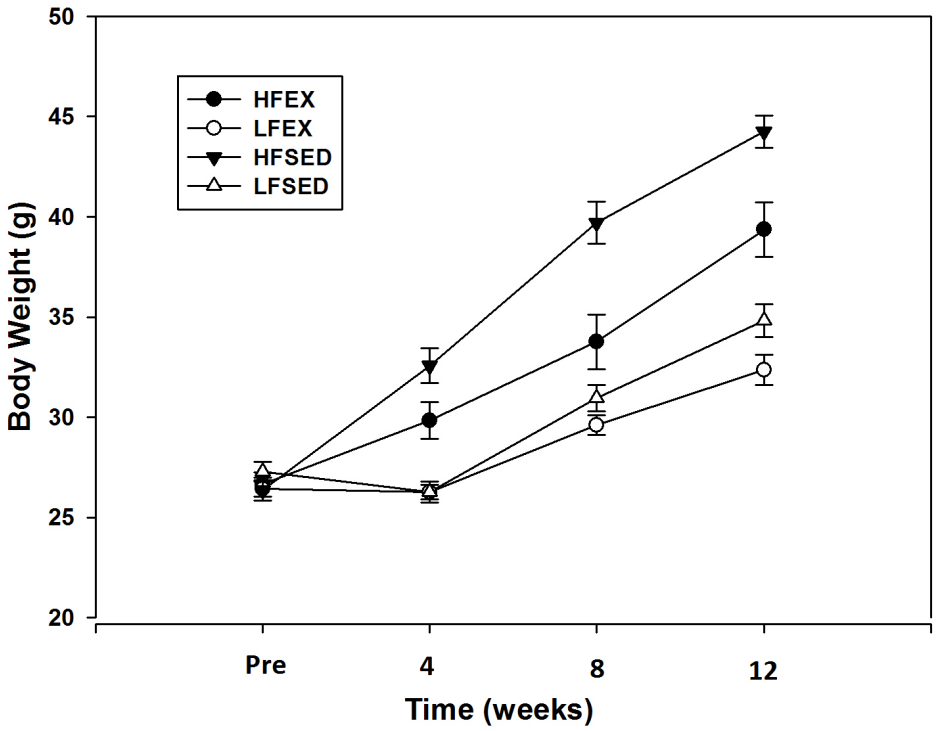


Figure 1 shows the progression of body weight from pre-intervention and 4, 8, 12 week time periods.

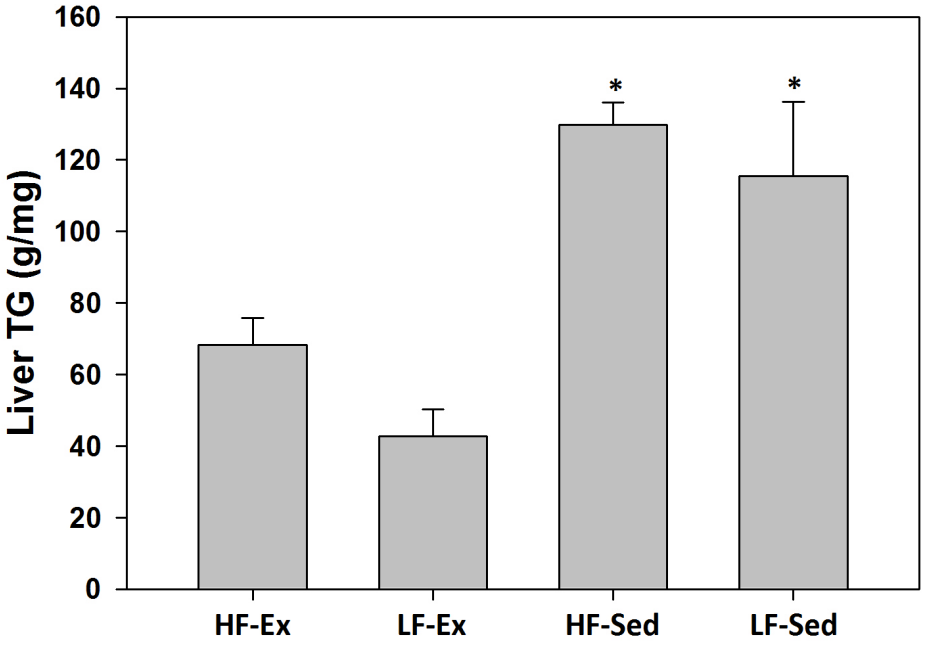


Figure 2 illustrates liver triglyceride content. Exercise produced significant changes in triglyceride content in both high fat and low-fat fed groups (p=.000, .000). Diet produced no significant differences

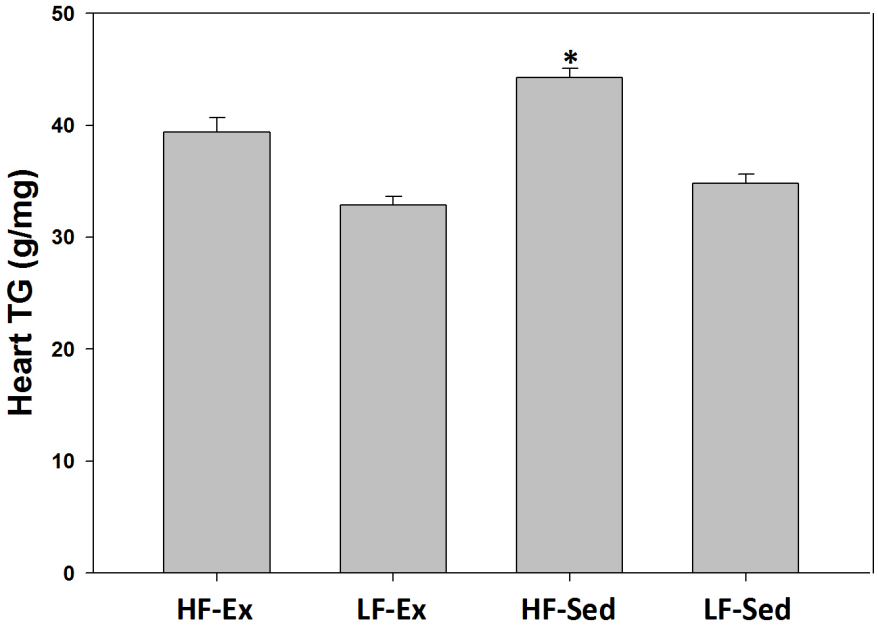


Figure 3 shows myocardial triglyceride levels. Low-fat diet produced significant effects on both sedentary and exercise groups (p=.002, .000). Exercise only produced a significant effect on animals fed a high-fat diet (p=.005).

## CHAPTER 5: DISCUSSION

The primary finding in this study was that both exercise training and dietary fat composition can have significant effects on visceral fat accumulation on both heart and liver. In addition, our data also points to the ability of exercise and a low-fat diet to attenuate total body weight gain. With respect to triglyceride accumulation, diet attenuated increases in the myocardial tissue in both high fat and low fat groups. Exercise, however, only produced a significant change in animals fed a high fat diet. This can be explained by the small increase in triglyceride levels produced by the low fat diet when compared with that incurred by the animals fed a high fat diet. Exercise interventions have been shown to produce greater results in subjects with greater levels of adiposity. With regard to hepatic steatosis, exercise significantly attenuated triglyceride accumulation in both high fat and low fat groups. Unfortunately, a low-fat diet did not produce a significant effect in hepatic triglyceride levels. This result parallels that found by Viera et al.[12] who utilized similar interventions and animals. While this result seems counterintuitive, the amount of sucrose (70%) in the low fat diet is similar to amount used in some high-carbohydrate diets which have often result in increased hepatic steatosis[167]. Though much more knowledge needs to be attained, these findings imply exercise may be the more effective than a low-fat diet in reducing epicardial fat.

Increased adiposity is a well-established risk factor for cardiovascular diseases such as atherosclerosis[150] . Unfortunately, the mechanisms that connect adiposity and cardiovascular anomalies are controversial and not fully understood.

Though escalation in total body adiposity is of great concern, much evidence suggests visceral fat (rather than subcutaneous fat) plays an important role player in the development of cardiovascular abnormalities[11,151,152]. As a result, techniques such as weight reduction therapy,

drugs, and other methods[153] to reduce visceral fat has become a primary research focus. This shift in attention has proved fruitful as weight loss therapies, such as caloric restriction and the manipulation of macronutrients, have been revealed as an effective method of reducing visceral fat[154,155,156,157]. Exercise also has been shown to have an effect on visceral fat. In fact, Viera et al.[12] has recently produced results indicating exercise has the ability to reduce epididymal fat.

With intra-abdominal adipose tissue garnering most of the attention, cardiac adiposity and its effect on cardiac disease has only recently been studied[119]. Epicardial adipose tissue is a layer of visceral fat surrounding the heart. In conjunction with its proximity to the coronary vessels, the inflammatory nature of this tissue has led to speculation that epicardial fat may play a role in the development of cardiovascular disease[66,103]. As a result, studies have revealed this tissue's association with impaired cardiac function, cardiovascular risk, and visceral fat deposition[153]. Unfortunately, little work has been done investigating methods that can reduce epicardial fat deposition.

To the best of our knowledge, our study is only one of three that have investigated the effect exercise can have on epicardial fat deposition, and is the first to perform it on animals. The first study to look at how exercise affects epicardial fat thickness was performed by Kim et al[9]. Their results indicated that a 12-week intervention of running at a moderate intensity decreases epicardial thickness by 8.6%. Unfortunately, a limitation of their study was a lack of a non-exercising control group. Wilund et al.[10] undertook the only other study of looking at this relationship. In a pilot study, patients on maintenance hemodialysis therapy underwent intradialytic exercise for 4 months. These patients, using a cycle ergometer, exercised for 45 minutes at a moderate intensity during their scheduled dialysis session. The findings were similar to the previous study with a reduction in epicardial fat thickness of 9.8% in the exercise

group, with no change in the control group. Our data produced results that seem to parallel those of the studies above. Epicardial fat, in this study was more dependent on exercise and weight than diet. Weight having an effect on epicardial fat is not surprising as there is evidence that indicates epicardial fat can be reduced through weight loss. Iacobellis et al.[158] revealed a 4 mm decrease in epicardial fat thickness in obese participants that lost weight after undergoing a 6-month low calorie diet intervention. Similar results were found in patients that lost weight after bariatric surgery[159].

Unfortunately, there have been no randomized-controlled studies performed relating epicardial fat and diets with different macronutrient content. As mentioned before, epicardial fat is considered a form of visceral fat. Therefore, we can only surmise their relationship based on how visceral fat deposits are affected by these diets. If we relate epicardial fat to visceral fat, then this result is very similar to that found by Viera et al.[12]. Using a very similar diet and exercise intervention, these researchers undertook a study that revealed epididymal fat pad growth was significantly attenuated in animals that consumed a low-fat diet. This present study revealed animals that have undergone a low-fat diet did not have significantly lower levels of heart triglycerides/epicardial fat than those on a high-fat diet. In addition, and similar to our result, these low-fat fed mice weighed significantly less than the mice fed a high-fat diet[12]. These results indicate that diet and weight are more important than exercise in the attenuation of epicardial fat deposition. Unfortunately, these results are in opposition of the other epicardial fat studies mentioned above, whose results point to exercise being the more effective method in managing epicardial fat accumulation.

Our results clearly elucidate the need for further investigations on how exercise affects epicardial fat deposition. As stated, this study is only one of three that has been undertaken

relating epicardial fat and exercise, and our results oppose their findings regarding the matter. However, our result concerning weight and diet-type does parallel several studies indicating their ability to reduce and prevent the accumulation of epicardial fat and other visceral adipose tissue deposits.

**Limitations**

Our study utilized treadmill running as the mode of exercise. When compared to volunteer wheel running, the drawback to this mode of exercise is the stress inflicted on the animals by forcing them to run. However, our study did not employ electric shocks, nor did we prod the animals to run. In order to encourage our animals to run, we placed a foam pad at the end of their lanes. This provided a physical sensation that persuaded them to run. Volunteer wheel running has its drawbacks as well. Some animals may run extreme distances, distances that most humans would not be able to incur on a daily basis. Alternatively, aged mice perform little voluntary wheel activity. Though volunteer wheel running may provide less stress to the animals, this method may yield inconsistent results, as the amount of running time for each mouse can be highly variable[160]. We feel treadmill running, using our method of encouragement, causes minimal stress to the animals. In addition, treadmill running is a way we can control the amount of activity consistent and applicable to the public health recommendations.

Another limitation to our study is the method by which we measure epicardial fat. Our study used heart triglyceride levels to approximate epicardial fat levels. We decided on using this technique due to the difficulty in measuring epicardial fat pads using echocardiography. However, we are confident in our choice due to a finding by Kankaanpaa [137], that myocardial triglyceride levels are positively correlated with epicardial fat mass. Though their study was

performed on humans, our methods/findings should still be applicable as there is evidence indicating both animals and humans share similar mechanisms in myocardial triglyceride influx.

Though our study shows exercise can reduce epicardial fat we must disclose that the type of mice used for this study may not be the best model for epicardial fat accumulation. The animals used in this project (C57/Bl6) are one of the most widely used inbred mouse strains used for models of disease. Our decision for using this strain came from observations we made during dissections of mice used in other projects. We noticed that aortic adventitial adipose tissue in high-fat fed sedentary obese mice compared with their lean or exercised counterparts. This observation prompted us to undertake this project using this particular mice model. Regrettably, this strain had very little, if any, epicardial fat deposition evident. This coincides with Sack's[161] suggestion that future animal studies (investigating epicardial fat) be performed on pigs, rabbits, and monkeys, animals with discernible epicardial adipose tissue.

**Future studies**

Studies investigating the relationship between epicardial fat and cardiovascular disease are sparse. Adding exercise as a variable reduces the number dramatically. Though exercise has been shown to help reduce epicardial fat mass, physical activity has not been shown to affect inflammation levels in this particular tissue. As mentioned above, exercise can reduce inflammation in visceral fat. As epicardial fat is a form of visceral adipose tissue, exercise may have similar effects on its inflammatory profile. Furthermore, with the vagus nerve having terminations in within the adventitia of coronary arteries, it is conceivable that exercise may have an anti-inflammatory effect via the cholinergic anti-inflammatory complex. Thus, future

investigation of whether exercise can reduce epicardial fat inflammation is warranted. In particular, consideration should be made on the effect of exercise on macrophage infiltration. As mentioned, visceral fat is more inflammatory and has a greater association with inflammatory diseases than subcutaneous fat. Interestingly, macrophage infiltration in obese individuals has been shown to be greater in visceral fat when compared to subcutaneous adipose tissue[79]. Macrophages have been have been implicated in playing a role in diseases such as diabetes. Modification of the MCP-1 gene in mice and its receptor has led to improvements in insulin resistance[162,163]. As for cardiovascular disease, macrophages are present in virtually every stage of atherosclerosis, and are integral in the formation of foam cells. Macrophages have also been found in epicardial fat associated with cardiac events. Recently, exercise has been revealed to reduce MCP-1 in visceral adipose tissue[164]. Therefore, it would of interest to investigate whether exercise would attenuate macrophage infiltration in epicardial adipose tissue.

## CHAPTER 6: CONCLUSION

The prevalence of obesity is staggering. In order to describe the magnitude of how many suffer from this disease, many have used the term "epidemic". However, this doesn't accurately describe the scale of how pervasive this disease has become. For quite some time, excess adiposity and increased weight gain have been an issue in a few select countries--the United States being one of them. This is beginning to change as reports have shown a massive and rapid increase in obesity rates across the globe. Thus, the word "epidemic" can no longer suffice and should be substituted by the more appropriate term "pandemic". This disease has crossed oceans and continents. And, as frightening as this may be, a new "border" has been breached by this disease, that "barrier" is age. For quite some time, weight gain and obesity has been thought to primarily affect adults, as it is true that increases in adiposity usually comes with age. Unfortunately, research has indicated that the incidence of childhood obesity is rising at an incredible rate. Data from these studies tell us that the pervasiveness of childhood obesity has more than doubled in the last twenty years, and that it continues to rise in an accelerated rate.

As the prevalence of obesity continues to rapidly increase, concern with its co morbidities becomes of utmost importance. Diabetes, certain types of cancer, Alzheimer's disease, and depression have all been linked to obesity. In addition, cardiovascular disease, the number one cause of death worldwide, has been closely linked to obesity. Cardiovascular disease is considered an inflammatory disease, and inflammation is the link between this disorder and obesity. Pro-inflammatory markers such as: TNF-$\alpha$, Il-6, MCP-1, VCAM, CRP, t-cells, and macrophages rise with increased adiposity and have all been positively associated with cardiovascular disease. In fact, these and other pro-inflammatory proteins are present in every stage of and may help mediate the progression of atherosclerosis.

Though previously thought of as an inert storage depot, much work has been done to redefine adipose tissue as an extremely active tissue capable of aiding in the inflammatory response by producing inflammatory cytokines and recruiting agents critical to the response. In addition, research has shown that of the two major types of adipose tissue (visceral and subcutaneous), visceral fat is considered much more metabolically active and closely associated with inflammation and cardiovascular disease.

Due to the inflammatory nature of visceral fat and because of its proximity to the coronary arteries, epicardial fat has garnered much attention from scientists in recent years. Epicardial adipose tissue, which is considered a true form visceral fat, has been shown to become increasingly inflammatory as adiposity increases, similar to the more traditional depots of visceral fat. Because there is no visible fascia or barrier that separates this tissue from the coronary arteries, epicardial fat has been thought to supply macrophages and other inflammatory products to the intima via paracrine and vasocrine signaling. There they interact with all the components of plaque formation to mediate the progression of atherosclerosis. Though not much work has been performed on epicardial fat (in comparison to visceral fat depots in organs located in the abdomen), there is indication that epicardial is positively associated with atherosclerosis.

Obesity and its co morbidities have placed an enormous emotional, physical, and monetary burden on our society. Therefore, much effort has been spent trying to find a way to curtail the spread of these disorders. Two of the major causes for this rapid increase in obesity have been due to our increasingly sedentary nature and the rise in caloric intake, especially due to the abundance of high-fat food sources. With this in mind, exercise and a low-fats diet have been prescribed as a method by which adiposity can be attenuated. Both of these techniques have proven effective as indicated by our colleagues' results as both epididymal fat pads were

significantly reduced. Unfortunately, not much work has been performed on the effect of exercise and diet on epicardial fat deposition. As of this time, only two studies have been performed on epicardial fat and exercise, and our lab undertook one of them. Results from these studies reveal that epicardial fat can be reduced by exercise. This present study parallels those findings. In addition, our results also state that consuming a low fat diet can also reduce epicardial fat deposition.

Though there is evidence linking epicardial fat deposition to disease severity, much more work has to be done in solidifying this association. Future studies can focus on macrophage accumulation in epicardial fat. Macrophages are extremely important in the progression of atherosclerosis. Macrophages have been associated with insulin resistance and cardiovascular disease. These phagocytic cells have also been reported in every stage of atherosclerosis, and are a major factor in the creation of foam cells. It has also been shown that macrophage infiltration in epicardial fat is positively correlated with adiposity. Thus, as exercise has been shown to decrease epicardial fat deposition, a project investigating whether exercise reduces macrophage infiltration is justified. Interestingly, exercise may also have anti-inflammatory properties. The "cholinergic anti-inflammatory pathway" is a method by which the body counteracts the inflammatory response. Stimulation of the vagus nerve induces the release of acetocholine, which inhibits the release of pro-inflammatory cytokines. Exercise has been shown to increase vagal tone, thereby leading to the issue of whether it has an effect on macrophage infiltration in epicardial fat.

In conclusion, both exercise and a low fat diet provide for effective treatments in reducing epicardial fat deposition. This may be one of the mechanisms by which it helps reduce the risk of cardiac events. More research still has to be undertaken in order to further understand

these processes. Lastly, both exercise and low-fat serve as valuable treatment options for attenuating weight gain or reducing obesity.

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