ASSESSMENT OF VASCULAR FUNCTION IN RESPONSE TO HIGH-FAT AND LOW-FAT GROUND BEEF CONSUMPTION IN HUMANS

A Dissertation

by

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ABSTRACT

Vascular function is closely related to cardiovascular disease (CVD) risk. In fact, measurements of vascular function are now accepted as independent risk markers for CVD. Beef has long been stigmatized as an unhealthy protein choice, though scientific evidence to support this claim is lacking. The purpose of this study was to assess the vascular impact of adding either low-fat (~5% fat) ground beef (LFB) or high-fat (~25% fat) ground beef (HFB) to a habitual diet. Twenty-three males (40 ± 11 years, 177.5 ± 6.7 cm, 97.3 ± 25.0 kg, 29.9 ± 10.3 % fat, 37.9 ± 7.6 ml/kg/min) participated in this double-blind cross-over design study. Prior to starting the study, participants visited the lab for an initial assessment of blood cholesterol concentrations, vascular function, body composition and aerobic capacity. If inclusion criterion were met, these data were then used as their entry time point measures. After entry, each participant completed two 5-week dietary interventions in a randomized order separated by a 4-week washout period. During the dietary intervention, each participant consumed five beef patties, either LFB or HFB per week. All laboratory testing was completed in the last week of each intervention and in the last week of the washout period. Data were analyzed via 2x2 repeated measures ANOVA ($p<0.05$). The HFB intervention improved flow-mediated dilation (FMD) relative to all other time points. Neither the HFB nor the LFB altered pulse wave velocity (PWV) values. The HFB intervention lowered systolic and diastolic blood pressure (BP) relative to entry values. Relative to entry values, both the HFB and LFB reduced total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C), while

the HFB alone lowered low-density lipoprotein cholesterol (LDL-C). Dietary analysis revealed that relative to all other time points, the HFB intervention increased intake of total fat, monounsaturated fatty acids (MUFAs) and saturated fatty acids (SFAs) with no change in trans-fatty acids (TFA), and also reduced carbohydrate consumption. Consuming high-fat ground beef does not negatively alter PWV values and improves FMD and BP values. Furthermore, consumption of HFB may provide increased cardiovascular benefit by lowering LDL-C levels.

DEDICATION

To my Parents, Tracy and Peggy Lytle. You both have offered unwavering love and support through any endeavor I have set out on. There is no way I would be where I am today without you two in my corner. I am eternally grateful.

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Contributors

This work was supervised by a dissertation committee consisting of Dr. Stephen Crouse (committee chair), Dr. James Fluckey (committee member) and Dr. Christopher Woodman (committee member) of the Department of Health and Kinesiology and Dr. Stephen Smith (committee member) of the Department of Animal Science.

Jason Lytle served as the lead study coordinator and assisted with subject recruitment and scheduling, data collection, data analysis, and manuscript preparation. Karina Wilson, Sean Stanelle, Mathew Wofford, Rebecca Bonta, and Stephanie DesJardin assisted in data collection. Dr. Charlie Shea served as a statistical liaison for the study design and analysis. Dr. Steve Martin serves as the Applied Exercise Science Laboratory coordinator, where the study took place. Dr. Stephen Smith received funding for the project. However, Dr. Smith was not involved in data collection or data entry. Dr. Stephen Crouse served as study PI, and assisted in study design, data analysis, and manuscript preparation.

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NOMENCLATURE

CVD	Cardiovascular disease
BP	Blood pressure
SBP	Systolic blood pressure
DBP	Diastolic blood pressure
IR	Insulin resistance
NO	Nitric oxide
FMD	Flow-mediated dilation
PWV	Carotid-femoral pulse wave velocity
TC	Total cholesterol
LDL-C	Low-density lipoprotein cholesterol
HDL-C	High-density lipoprotein cholesterol
TG	Triglycerides
SFA	Saturated fatty acid
MUFA	Monounsaturated fatty acid
TFA	Trans-fatty acid
SPISE	Single Point Insulin Sensitivity Estimator
HOMA-IR	Homeostasis model assessment-insulin resistance
CHO	Carbohydrate
HFB	High-fat ground beef
LFB	Low-fat ground beef

PUFA	Polyunsaturated fatty acid
MUFA/SFA	Monounsaturated/saturated fatty acid ratio
BMI	Body mass index
DXA	Dual-energy x-ray absorptiometry

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CHAPTER I

INTRODUCTION AND RATIONALE

Background

Cardiovascular disease (CVD) is the leading cause of mortality globally [1]. Physiological factors that include central obesity, elevated blood pressure (BP), dyslipidemia, fasting hyperglycemia, and insulin resistance (IR) are known to increase CVD risk [2]. A commonality between these factors is the atherogenic effect they elicit [3]. Vascular endothelial damage and dysfunction are the first steps of atherosclerosis and the "hardening" of the arteries [4, 5]. This dysfunction has been found to precede the onset of clinically visible atherogenic plaques [3, 6, 7].

Endothelial dysfunction is identified by the impaired vascular response to dilators, most commonly associated with decreased nitric oxide (NO) bioavailability [5, 8]. Endothelial function can be measured through flow-mediated dilation (FMD) or pharmacologically by the infusion of vasodilators [8]. FMD is the measurement of the brachial artery's dilatory response to reactive hyperemia. This measurement is now regarded as an accurate, noninvasive measure of NO bioavailability [9]. Impairment of the brachial artery FMD response has been shown to be significantly related to future cardiovascular events, while improvements are cardioprotective in nature [6].

Furthermore, arterial elasticity is an additional indicator of vascular health and function. Vessel compliance can be assessed through ultrasound imaging of the carotid and femoral pulse wave in order to determine velocity of blood flow. Carotid-femoral

pulse wave velocity (PWV) is a validated method for assessing arterial stiffness and CVD risk [10, 11]. In combination, FMD and PWV provide a comprehensive noninvasive technique to assess vascular health and CVD risk in humans [8, 10, 11].

It has long been understood that there is a clear connection between BP and cardiovascular health. Elevated BP and endothelial dysfunction are integrally related and often occur in conjunction with one another. Due to its regulatory impact on vascular tone, endothelial function has been a target for treating hypertension [12]. Additionally, chronically elevated BP itself can result in damage to the endothelium, which can initiate and progress the atherogenic process [13].The Framingham study demonstrated a well-defined positive relationship between BP and CVD risk [14].

Dietary choices play a pivotal role in a majority of the factors associated with CVD risk. In a review by Hall [15] it was reported that a variety of acute and chronic dietary interventions have been effective at altering vascular function and BP. While the precise mechanistic interactions have yet to be uncovered, there is a clear connection between dietary choices, the aforementioned CVD risk factors, and vascular function [3, 15-17].

Altered serum lipoprotein concentrations are connected to vascular function in human and animal models [18-20]. The deleterious alteration of serum lipids associated with dyslipidemia include high concentrations of total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C), as well as low concentration of high-density lipoprotein cholesterol (HDL-C). It is well understood that dietary alterations can raise and lower human serum lipoprotein concentrations. Dietary fat intake is a common variable used to alter lipoprotein levels.

Chronic beef consumption in humans has been evaluated to find no adverse alterations in serum lipids levels [21-23]. To the authors' knowledge, no research has assessed the impact of chronic ground beef consumption on vascular function. Despite this, beef has become stigmatized as an unhealthy choice due to its large percentage of fat [24], but research to support beef as an unhealthy dietary choice is lacking.

While fatty acid composition of beef varies, beef has been commonly associated with dyslipidemia and increased risk for cardiovascular disease due to having high proportion of saturated fatty acids (SFAs) [25]. Yet, dietary SFAs have differing effects on serum lipids. Stearic acid, one of the most abundant SFAs in beef, has little effect on cholesterol levels in humans [26]. On the other hand, the major monounsaturated fatty acid (MUFA) in beef, oleic acid, has been demonstrated to lower LDL-C and even elevate HDL-C [26-28]. Interestingly, St John et al. [29] demonstrated that grain feeding cattle increases beef's concentration of MUFAs and decreased the proportion of SFAs and trans-fatty acids (TFAs). Thus, the mixture of fatty acids commonly found in grain fed ground beef, could increase HDL-C and lower LDL-C.

IR has been identified as a common link between many of the pathophysiological risk factors of CVD [3]. Aside from its role in hyperglycemia, IR has been also associated with upregulation of cholesterol synthesis and downregulation of cholesterol absorption, independent of obesity [17]. Furthermore, diminished endothelial function is seen in individuals with insulin resistance [3, 30]. IR can be estimated by specific blood lipid ratios (triglyceride [TG]/HDL-C ratio), using the single point insulin sensitivity estimator (SPISE) equation as well as by an assessment of fasting insulin and glucose [homeostasis

model assessment-insulin resistance (HOMA-IR)] [31, 32]. Previous research demonstrates that the percentage of carbohydrates (CHOs) consumed in the diet decreases, when human subjects substitute a protein source for high fat beef [21, 23]. Although the current literature regarding the role of CHOs in IR is inconclusive, it appears that some benefit may be derived from this decrease in CHO consumption [33, 34].

Body composition is an important factor of CVD risk. There is a clear relationship between obesity and many of the previously mentioned CVD risk factors. Abdominal/visceral fat is significantly correlated with IR, elevated BP, and dyslipidemia [35]. Moreover, central adiposity has been linked to endothelial damage, and can even be used as a predictor of vascular function [36, 37]. Similarly, cardiorespiratory fitness is linked to CVD mortality rates and vascular function [38-40]. Low cardiorespiratory fitness is associated with detrimental effects on FDM and PWV values [38]. Training programs that improve cardiorespiratory fitness have been shown to be improve FMD and PWV measures [41, 42].

Purpose

The purpose of this study was to assess the vascular impact, measured via FMD, PWV and BP of 5 weeks of consuming high-fat ground beef (HFB) or low-fat ground beef (LFB) in men. Secondary goals of this study were to 1) determine if consumption of HFB or LFB will affect the human serum lipid profile and assess if this is related to vascular function; 2) evaluate if the HFB or LFB intervention will have an effect on insulin sensitivity and investigate if this is related to vascular function; 3) explore if there is a relationship between body composition and vascular function; 4) explore if there is a

relationship between cardiovascular fitness and vascular function. The results of the proposed research will provide a better understanding of the physiological alterations related to the consumption of ground beef, which in turn will contribute to the literature concerning dietary choices and CVD risk.

Specific Aims and Hypotheses

Primary Aim. *Determine if either HFB or LFB interventions alter the vascular health markers of FMD, PWV or BP.*

Hypotheses

1. We hypothesized that there will be no significant difference in FMD and PWV measures as a result of the HFB or LFB interventions.
2. We hypothesized that the added MUFA of the HFB intervention will lower BP relative to the LFB interventions.

Rationale

Chronically increasing fat consumption, specifically MUFAs, has been shown to reduce BP [43]. Due to this, we postulate that the added fat from the HFB intervention will lower BP. Chronic dietary modification failed to result in significant alterations in PWV [44, 45]. Based on this, we hypothesize that our current intervention will also result in no change to PWV measures. While increasing dietary MUFAs consumption has been shown to alter FMD results [45, 46], the authors' cannot be certain the MUFA content of the beef patties will be high enough to improve FMD, as shown previously [44]. While we do not anticipate a beneficial shift in either PWV or FMD, we also do not expect these measures to be negatively altered by the consumption of either HFB or LFB.

Secondary Aim 1. *Determine the effects of HFB and LFB consumption on the serum TC, LDL-C, HDL-C and the TC/HDL-C ratio.*

Hypotheses

1. We hypothesized that the HFB intervention will increase HDL-C and have no effect on TC and LDL-C, thus favorably altering the TC/HDL-C ratio.
2. We hypothesized that the LFB will lower TC, HDL-C, and LDL-C while having no effect on the TC/HDL-C ratio.

Rationale

The most abundant SFAs and MUFA in ground beef are stearic/palmitic acid, and oleic acid, respectively. In high-fat beef patties (~25% fat), this mixture has previously resulted in increased serum HDL levels with no change in TC or LDL-C in men [22, 23, 47]. Furthermore, diets high in oleic acid have resulted either in no change or reduced TC and LDL-C [28]. Conversely, low-fat beef consumption lowers HDL-C, LDL-C, and TC levels in humans [48]. Together these results suggest that the consumption of HFB will increase HDL-C and potentially have no effect on TC and LDL-C, whereas the LFB will lower HDL-C, LDL-C, and TC concentrations.

Secondary Aim 2. *Determine if either the HFB or LFB interventions will improve the surrogate insulin sensitivity scores of HOMA-IR and SPISE.*

Hypotheses

1. We hypothesized that the decreased CHO consumption as a result of the HFB intervention, will decrease the insulin sensitivity HOMA-IR score.

2. Additionally, we hypothesized that the serum lipid changes associated with the HFB intervention (specific aim 1) will increase the SPISE score.
3. We hypothesized, because the LFB intervention will not alter the macronutrient intake or the TG/HDL ratio, there will be little or no change in any of the insulin sensitivity scores.

Rationale

Previous literature supports beneficial changes in insulin sensitivity when CHO consumption is reduced [33, 34, 49]. Due to the potential of the HFB intervention to inadvertently decrease CHO consumption [21, 23], this may improve HOMA-IR scores. Further, based on the fatty acid composition of the HFB, it can be assumed that serum HDL-C will increase while all other lipoprotein levels remain stable [26, 27]. This may result in an improved SPISE score.

Secondary Aim 3. *To examine the potential relationship between body composition (specifically abdominal obesity) and vascular function.*

Hypotheses

1. We hypothesized there will be no significant change in body composition throughout the course of the study.
2. We hypothesized FMD responses will be inversely related to central obesity (i.e., lower FMD response corresponding to higher levels of central adiposity).
3. We hypothesized PWV values will be positively correlated to central obesity (i.e., higher PWV values corresponding to high levels of central adiposity).

Rationale

Researchers have demonstrated a significant relationship between abdominal obesity, CVD risk, and vascular function [37]. Previous literature supports that abdominal obesity can be used as a predictor of vascular function [36]. Together these results indicate a connection between vascular function, body composition, and CVD risk.

Secondary Aim 4. *To examine the potential relationship between cardiorespiratory fitness and vascular function.*

Hypotheses

1. We hypothesized that there will be no significant change in cardiorespiratory fitness levels throughout the course of the study.
2. We hypothesized that FMD responses will be positively correlated to cardiorespiratory fitness levels (i.e., higher FMD response corresponding to higher cardiorespiratory fitness levels).
3. We hypothesized that PWV values will be inversely correlated to cardiorespiratory fitness (i.e., higher PWV values corresponding to lower cardiorespiratory fitness levels).

Rationale

There is a strong base of support on the inverse relationship of cardiorespiratory fitness and vascular function [38, 41]. The importance of this relationship is further demonstrated by the significant relationship between cardiorespiratory fitness and CVD mortality [39, 40].

CHAPTER II

LITERATURE REVIEW

CVD is a leading cause of death in the U.S., so, interventions aimed at reducing CVD risk have become increasingly popular over the last six decades. Because dietary choices play such a pivotal role in disease prevention and treatment, dietary manipulations to improve health are common. Collectively, physiological targets of diets intended to reduce CVD, include improving BP, dyslipidemia, fasting hyperglycemia and insulin resistance. All of these outcomes are directly related to vascular function. FMD and PWV are measures used for early detection of vascular dysfunction, which precedes the onset of atherogenic plaques [4-6]. Due to its perceived high SFA content, beef has been labeled an unhealthy choice, especially for those who are at risk for CVD [25]. However, the scientific support for this supposition is lacking. The purpose of the current review is to evaluate the literature describing the interactions of vascular function, dietary fats, and markers of CVD risk.

Indices of Vascular Function and Blood Pressure

FMD is a valid, noninvasive measure of vascular function. Specifically, it is the quantification of the vasodilatory response to increased blood flow. This response was first demonstrated by Schretzenmayr [50] and has since been verified by others [51, 52]. The endothelium itself has been identified as major component of the vascular response to this flow stimulus. The endothelium produces a number of dilatory and constrictor substances [53-55]. While the balance of these dilators and constrictors are responsible for

resting vascular tone [56], it is currently accepted that production of dilators, mainly NO, is the primary mechanism for dilation in response to reactive hyperemia [9, 57, 58]. FMD is presented as a percent change in vessel diameter from baseline/resting diameter using the following equation: (max vessel diameter post occlusion – baseline diameter/baseline diameter) *100 [59]. FMD is significantly correlated to relative risk of future cardiovascular events [6]. In a meta-analysis, Inaba et al. [6] identified that a 1% reduction in the FMD response is associated with a 13% increase in relative risk of future cardiovascular events. It is important to note that the majority of these studies measured the FMD response on the brachial artery, which is significantly correlated to carotid artery function [60]. For these reasons, FMD of the brachial artery is now an accepted measure of vascular function, specifically related to NO bioavailability [9]. Additionally, a novel formula for calculating FMD has been established in order to increase generalizability of the FMD value between differing imaging sites and populations [61]. This allometrically scaled FMD value is derived from the following equation: ((max vessel diameter post occlusion/baseline diameter$^{0.87}$)-1/baseline diameter) *100 [61].

Arterial stiffness is widely accepted a risk factor for CVD risk. Previously, pulse pressure was used as a proxy measure for arterial stiffness [62]. However, a more direct method to assess this risk marker is the ultrasonography of the carotid and femoral arteries in order to determine PWV. This hemodynamic measure has previously been associated with higher rates of cardiovascular events [63]. Mitchell et al. [10] supported this finding in the original and offspring Framingham cohort. Additionally, the Framingham group found an improved risk prediction when PWV is added to standard risk factor model [10].

Elevated BP is an independent risk marker for CVD and cardiovascular mortality [14]. Once an individual surpasses a resting BP of 115/75 mmHg, CVD risk doubles for every 20/10 mmHg increase (systolic and diastolic BP, respectively) [64]. This relationship does not remain uniform with age for both systolic and diastolic blood pressure. In fact, after the age of 45, the significance of diastolic blood pressure (DBP) on CVD declines, while the significance of systolic blood pressure (SBP) increases [65]. In the elderly, a 5 mmHg increase in SBP increases the risk for cardiovascular event by 80% [66]. Gokce et al [67] identified a very clear inverse relationship between BP and vascular function assessed by FMD. Because BP and endothelial dysfunction have a mutually causal relationship, the specific cause and effect for BP and endothelial dysfunction is difficult to extrapolate. Nevertheless, it is clear that endothelial function is markedly attenuated in individuals with elevated BP [12].

Acute Dietary Fatty Acids, Vascular Function, and BP

Modulation of the dietary fat content of a single meal produces confounding outcomes on FMD, unknown results on PWV, and has no apparent effect on BP [15]. Among the mixed findings on FMD, a commonality is a decrease in FMD response within 2-6 hours of consuming a meal high in total fat [68-71]. However, the specific fatty acid composition of these meals was not indicated, as most of the researchers used fast food meals for the high-fat intervention. This reduction in FMD was shown to be attenuated by adding 50 g casein or soy protein to a high-fat meal [72]. Limited evidence suggests acute dietary interventions can alter arterial compliance; one group [73] demonstrated impaired arterial compliance after a high-fat meal using aortic flow rate as a compliance measure.

More interestingly, acute intake of specific types of fatty acids (SFAs, MUFAs, and PUFAs) have resulted in mixed outcomes on FMD. The acute ingestion of meals high in SFAs and MUFAs reduced FMD, while a meal high in PUFAs resulted in increased FMD [74-76]. These studies [74-76] demonstrated that the high MUFA and SFA content of olive and coconut oil decreased FMD. This is in opposition to the effect of high PUFA content of walnuts and safflower seed oil, which increased the FMD response after a single meal. To our knowledge, no research has assessed the acute effect of fatty acid types on PWV.

Chronic Dietary Fatty Acid Interventions and Vascular Function

The literature on the chronic dietary influence on FMD and PWV is equivocal due to methodical inconsistencies. However, some conclusions can be drawn from the published literature. De Roos et al. [18, 46] investigated the effects of diets high in CHO, SFAs, MUFAs and TFAs on the FMD response. It was demonstrated that diets high in TFAs, which significantly lower HDL-C concentrations (15.08 mg/dL reduction), reduce the FMD response [46]. Further, high-CHO diets that modestly lower HDL-C (8.12 mg/dL reduction) do not alter the FMD response [18]. These studies used a cross-over design between the dietary interventions. De Roos et al. [46] compared a TFA diet (37% total fat with 9.2 % trans-fat) to SFA diet (41% total fat with < 1% TFA). The TFA diet resulted in a 1.8% reduction in FMD relative to the SFA diet. In a subsequent study, de Roos et al. [18] assessed the FMD response to a low-fat, high-CHO diet (60% energy from CHOs and 25% energy from fat [7.8%energy MUFAs]) compared to an oil rich diet (38% energy as CHOs and 44% energy as fat [19% energy MUFAs]). Although serum lipid levels were

slightly changed between groups, there was no difference in FMD values between the groups [18].

Additional research has supported that high-CHO diets do not negatively alter FMD [45]. Keogh et al. [45] demonstrated that diets high in SFAs reduced FMD compared to diets high in MUFAs, PUFAs, and CHOs. However, it was not reported whether the SFA diet decreased FMD relative to baseline values.

When comparing the effects of diets high in either MUFAs or SFAs, or a diet low in total fat (U.S. National Cholesterol Education Program stage 1 [NCEP-1]), the SFA diet resulted in the lowest FMD, which was not significantly different from baseline measures [44]. Additionally, the MUFA diet elevated FMD response. Only two of the studies assessed PWV, and both demonstrated no significant effect of the dietary interventions on PWV [44, 45, 77].

The principal conclusion of the available literature is that diets high in fat do not negatively alter FMD or PWV measures with the exception of diets high in TFAs, which decrease FMD. Furthermore, in high-fat diets the addition of high levels of MUFAs may improve FMD.

Chronic Dietary Fatty Acid Interventions and BP

Chronic alterations of dietary fat content have resulted in fluctuations in BP [15]. The literature on healthy populations support the conclusion that that the addition of dietary fat (MUFAs or SFAs) in place of CHOs has no negative effect on BP [77, 78]. Ashton et al. [78] employed a 4-week, randomized cross-over design to investigate the effect of a high-fat diet compared to a high-CHO diet. Both the high-CHO and high-fat

diet consisted of 15-18% energy from protein and had equal proportions of PUFAs and SFAs. The high-CHO diet included 55-60% energy from CHOs and 22-25% energy from fat with a similar percentage coming from SFAs, MUFAs, and PUFAs. The high-fat diet consisted of 40-45% energy from CHO, and 40-42% energy from fat with 26-28% of the fat energy coming from MUFAs. These diets resulted in no significant difference in clinical BP measures. However, the fact that baseline BP was not measured confounds the conclusion to the potential effect of MUFAs on BP [78].

Interestingly, in a healthy cohort, an isocaloric intervention consisting of either high SFAs or high MUFAs resulted in lower SBP and DBP as a result of the MUFA diet. Additionally the diet high in SFA did not affect BP [79]. Moreover, in hypertensive and type II diabetic populations, a high-MUFA intervention also lowered BP relative to a high-CHO diet [43, 80]. Rasmussen et al. [43] utilized a 3-week cross-over design study in which participants consumed a diet containing 50% energy from CHOs and 30% energy from fat (10% MUFAs) or 50% energy from fat (30% MUFAs) and 30% energy from CHO. The high-MUFA intervention resulted in a reduction in ambulatory BP.

Evidence suggesting that the enrichment of PUFAs in a high-fat diet will decrease BP is less definitive. One group investigated BP in response to diets high in n-3 PUFAs, n-6 PUFAs, MUFAs, and SFAs. The diet high in SFAs resulted in the highest BP while the diet high MUFAs caused the lowest BP [81]. One limitation of this study was the lack of a washout period, which could have confounded the results.

Taken together, these results indicate that a diet high in total fat, enriched with either SFAs, MUFAs, or PUFAs does not increase BP. Additionally, high fat diets

enriched with MUFAs can result in a reduction of BP. The effect of PUFAs on BP is less clear, thus, a definitive conclusion cannot be made.

Serum Lipids

Serum cholesterol levels are important markers of CVD risk, and are altered by various lifestyle factors, including diet and exercise. A single meal can shift cholesterol concentrations in a variety of ways depending on the composition of the meal. For example, levels are sensitive to meals of equal macronutrient composition that differ only by CHO type (sucrose, glucose, or fructose) [82]. Additionally, longer duration dietary manipulations alter serum cholesterol levels [22, 28, 83]. However, fasted cholesterol concentrations and cholesterol changes in response to dietary interventions are dependent on training status. In general, aerobically trained individuals have lower fasting TG, similar LDL-C and TC and higher HDL-C compared to untrained counterparts [84, 85]. Furthermore, Bounds et al. [86] demonstrated that in trained men cholesterol concentrations remained stable in response to dietary interventions with vastly different macronutrient proportions. Specifically, a high-fat diet (60% fat) and a high-CHO diet (61% CHO) did not alter cholesterol concentrations in trained men [86]. Considering the cholesterol response to acute exercise bouts, previous research demonstrates that TC remains stable, while HDL-C exhibits a delayed increase 24-72 hours post-exercise, and TG and LDL-C present a delayed decrease 24-72 hours post-exercise [86, 87]. Additionally, dietary factors may alter the exercise response to serum cholesterol levels [88]. This research clearly demonstrates that exercise and diet have a pronounced effect on serum cholesterol levels, and a mutually casual effect on one another.

Beef, Serum Lipids and Macronutrient Consumption

Beef has been stigmatized as an unhealthy dietary choice due to its high proportion of SFAs [24, 25]. SFA consumption has resulted in deleterious shifts in cardiovascular risk markers [26, 89]. However, the two most abundant SFAs in beef are palmitic acid, which has been shown to increase LDL-C and HDL-C, and stearic acid, which has little or no effect on cholesterol levels in humans [26, 45]. While increasing LDL-C is not favorable per se, previous research suggests that the cardio protective function of the increased HDL-C would outweigh the rise in LDL-C [90]. Further, oleic acid, which is the most abundant MUFA in ground beef, has been shown to lower LDL-C and even increase HDL-C [26-28]. Taken together, these data suggest that the fatty acid composition in high-fat ground beef may yield a shift in blood lipids that would be beneficial for CVD risk. Despite this, very few randomized trials have investigated the healthfulness of high-fat beef in humans.

Ground beef is the most commonly consumed beef product in the United States [91]. Results of the few studies that examined the health effects of dietary ground beef have failed to uncover any detrimental health effects that would promote increased risk for CVD. For example, Gilmore et al. [22] reported that consumption of high-fat ground beef patties (~24% fat) with a high MUFA content (monounsaturated/saturated fatty acid ratio [MUFA/SFA] = 1.1) increased HDL-C relative to baseline values. During this intervention, a small decrease in LDL-C/HDL-C ratio and in insulin concentrations was noted, with no change in TG or LDL-C concentrations [22]. This result was also supported by Adams et al. [21] who found that consumption of high-fat ground beef patties (35%

fat) with a MUFA/SFA ratio higher than retail ground beef (MUFA/SFA = 1.31), resulted in an increase HDL-C compared to ground beef high in SFA (MUFA/SFA = 0.95; similar to retail ground beef). Furthermore, these results are in agreement with those of Appel et al. [80] who found that consumption of a high-MUFA diet elevated HDL-C and resulted in no change to or a slight reduction in LDL-C [80]. This reduction may be caused by alteration in LDL particles in response to MUFA consumption, which results in an increased clearance rate of LDL-C [83]. It is also important to note that HDL functionality has recently been identified as a more important factor for CVD risk that HDL-C levels alone [92]. On that note, beef consumption has been linked to increased apolipoprotein A1 levels in humans, which is valid marker of HDL functionality [23, 92]. Based on these results, the addition of high-fat ground beef to a typical American diet may be beneficial, or at least not harmful, due to the cardio-protective effect of increased HDL-C and in some cases a reduction in LDL-C [93].

Additional benefits of high-fat beef consumption may stem from the protein content of beef as well as the unintentional reduction of CHO consumption. Appel et al. [80] investigated the effect of three test diets (high-protein, high-CHO, and high-fat) on CVD risk markers, to find that all the diets lowered BP, LDL-C, and CVD risk, with the high-protein diet producing the lowest BP and LDL-C. It is important to note that the acute addition of protein to a high-fat meal neutralizes the reduction in FMD caused by a single high-fat meal [72]. Previous literature revealed high-fat ground beef interventions (5 patties/week) decreased CHO consumption [21, 23]. This was accompanied by a slight decrease in plasma glucose and insulin, which would favorably alter the HOMA-IR score.

Insulin Resistance and Vascular Function

Individuals with IR are at increased risk for morbidity and mortality associated with CVD due to accelerated atherosclerosis [30]. Currently it is understood that a long period of IR precedes the onset of diabetes [94]. Furthermore, IR in otherwise healthy populations is associated with damped endothelium dependent vasodilation [95]. These findings have been validated by a large group study in the Framingham offspring participants, in which IR was associated with reduction in FMD in an age and gender adjusted model [3]. IR was assessed using the HOMA-IR method, which utilizes fasting concentrations of glucose and insulin by the following calculation: HOMA-IR = (fasting plasma insulin [microunits per milliliter]) x (fasting plasma glucose [millimoles per liter])/22.5 [96]. IR was classified as a HOMA-IR score > 4.6. Other methods for assessing IR use TG/HDL-C ratio and BMI (SPISE) to estimate insulin sensitivity [31].

Reducing CHO consumption has produced beneficial reduction on IR in animal models [49]. Alternatively, human research on the effect of CHO consumption on IR is less clear. Some studies suggest that a reduction in CHO intake increases insulin sensitivity [33, 34], while others exhibit no change in insulin sensitivity [97, 98]. However, the value of CHO reduction, specifically sugar, is supported by the positive association between sugar intake, increased energy density of food, increased body mass and increased caloric consumption [34].

Body Composition, Cardiorespiratory Fitness and Vascular Function

Central obesity is a risk factor for CVD that is directly linked to endothelial dysfunction. In fact, Brook et al. [36] determined that central obesity, measured by waist-

to-hip ratio, can be effectively used as a predictor of endothelial dysfunction measured by FMD. Furthermore, waist-to-hip ratio was the only significant independent predictor of FMD in otherwise healthy adults. A waist-to-hip ratio > 0.85 was correlated to a diminished FMD response [36]. Additionally, endothelial dependent dilation has been shown to be diminished in obese subjects who are otherwise healthy [37].

There is a clear connection between cardiorespiratory fitness levels, CVD mortality rates, and vascular function. [38-40]. Cardiorespiratory fitness, measured via maximal oxygen uptake and duration of a graded exercise test, can be used as an all-cause and CVD-related mortality predictor in men [39]. Specifically, individuals with low cardiorespiratory fitness (<27.6 mL/kg/min) have a 3-fold increase in CVD mortality risk when compared to individuals with high cardiorespiratory fitness (>37.1 mL/kg/min) [39]. Likewise, this relationship between fitness level and CVD morality remains significant even when adjusted for lipoprotein concentrations [40]. In a recent review, Montero et al. [38] demonstrated a clear connection between fitness level and vascular function.

Together, the available literature indicates a significant association between body composition, cardiorespiratory fitness, and CVD risk. Increased CVD risk appears to be, at least impart, due to the impairment of vascular function associated with obesity and low cardiorespiratory fitness.

CHAPTER III

METHODS

Participants

Healthy, non-smoking males (ages of 25 and 60 years) were recruited from the Bryan/College Station area to participate in the study. Seventy-five males participated in one of two informational meetings. Subject recruitment numbers are shown in Figure 1. Four individuals did not meet inclusion criteria and 25 men declined to participate. Forty-six men signed Informed Consent forms and 14 men later declined to participate. Thirty-two men were assigned at random to treatment groups (LFB or HFB) and were provided test ground beef patties. Nine men left the study either voluntarily or were excluded due to inability to comply, and 23 men completed all phases of the study. Subject demographics, at entry to the study, are listed in Table 1. All procedures involving human participants were approved by the Texas A&M University Institutional Review Board for use of human participants in research (Protocol number IRB2018-0755). All subjects were provided detailed instructions, including potential risks of participation, and all subjects signed Informed Consent forms prior to participation (Appendix A).

Inclusion Criteria

In order to participate, volunteers must not have been consuming restrictive diets or cholesterol-lowering medications. Additionally, all subjects needed to have normal total cholesterol levels (120 mg/dL - 300 mg/dL) at the beginning of the study. Participants were advised not to change their habitual level of physical activity.

	Entry
Age (years)	39.91 ± 10.76
Height (cm)	177.46 ± 6.73
Body weight (kg)	97.33 ± 25.04
BMI (kg/m^2)	31.15 ± 8.99
Lean mass (kg)	64.51 ± 9.53
Fat mass (kg)	30.63 ± 19.11
Body fat (%)	29.93 ± 10.35
Android fat (%)	35.76 ± 14.12
Gynoid fat (%)	31.03 ± 9.78
VO_{2max} (ml/kg/min)	37.92 ± 7.62

Table 1. Subject Demographics.

Participation required individuals not be consuming restrictive diets or cholesterol-lowering medications. As well, subjects needed to have normal total cholesterol levels (120 mg/dL - 300 mg/dL) at the start of the study. Participants were advised not to change their habitual level of physical activity. Physical activity compliance was assessed by a 7-day activity logs (Appendix B) and body composition assessment during each of the study time points, as well as a submaximal VO_2 treadmill test at entry, and after completion of all diet interventions. Dietary compliance was assessed with 3-day food diaries.

General Procedures

A sample study timeline is displayed in Figure 2. Due to time constraints the initial blood sample was used to determine if the inclusion criteria were met. All 23 subjects who participated in the entry measure met the inclusion criteria and thus, were eligible to complete the study. A two-period, randomized cross-over design was used based on

previous studies [22, 47, 99]. Each participant completed two, 5-week ground beef interventions in a randomly assigned order with a 4-week washout period between the test periods. The men consumed 5 ground beef patties/week, for 5 weeks, for each ground beef type (25 patties for each type). The two treatments were LFB (~5% fat) and HFB (~25% fat) ground beef. Participants were assigned to one of two groups (n ≥ 10 per group), balanced with regard to LDL-C concentrations at the initial screening. Participants received a $50 gift card after completing the first phase of the study, including completion of all diet records, and a second $50 gift card after completing the second phase.

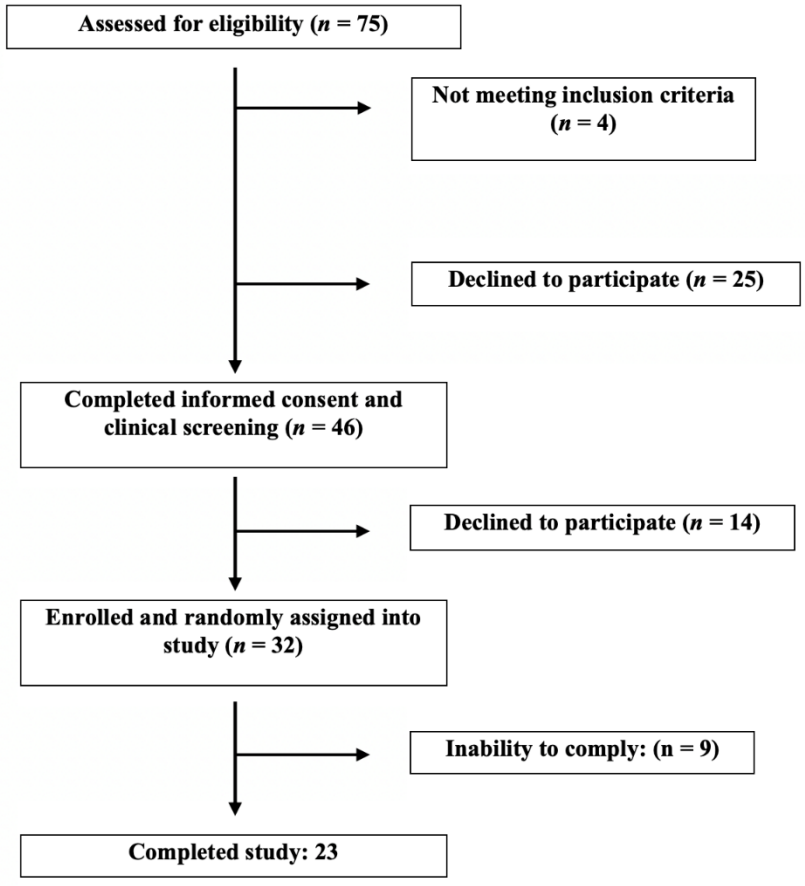

Figure 1. Recruitment Flow Diagram.

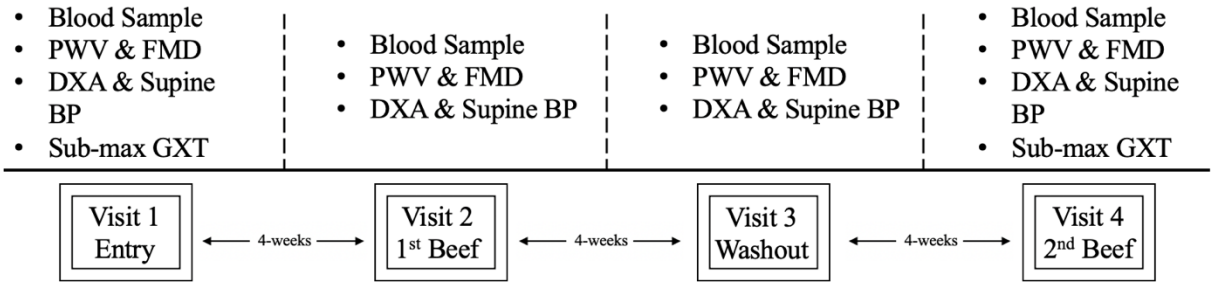

Figure 2. Study Timeline.

Sources of Ground Beef

The source of raw materials for production of the LFB and HFB patties were the pectoralis muscle and 75:25 coarse grind, respectively, purchased from a local supplier (Readfield Meats, Bryan TX). Pectoralis muscle primals were coarse-ground (1.27 cm plate) and then fine-ground (0.32 cm plate) while the 75:25 coarse grind were fine-ground. Then four-ounce (115-g) patties were formed in a patty maker, individually vacuum-packaged, and stored at -20°C. Prior to the initiation of each phase of the ground beef interventions, each participant received an unlabeled box containing 25 frozen, vacuum packaged patties. The initial, targeted fat percentage was 5% and 25% total fat for the LFB and HFB patties, respectively. Chemical analysis of the ground beef after patty formation indicated that LFB patties contained 5.61% fat (6.40 g fat/patty) and the HFB patties contained 23.63% fat (26.93 g fat/patty). The MUFA/SFA was 1.16 and 1.05 for the LFB and HFB ground beef, respectively.

Diet records from previous studies indicated that most study participants pan-broiled the ground beef patties intact, thus samples of the low- and high-fat were pan-broiled [100] and total fat and fatty acid composition of the cooked patties were measured

[21, 22, 99]. Cooking losses for LFB and HFB patties were 5.3 and 79.8%, respectively. Total fat and fatty acid per patty were calculated based on final patty weight and total lipid per patty, which can be found in Table 2. The cooked total lipid and fatty acid composition values were used by the RD for calculation of daily intake of dietary fats.

Food Logs

All participant were required to complete a 2-week run-in period in which they documented their habitual dietary intake using the smartphone application My Fitness Pal (https://www.myfitnesspal.com), or by manually logging if a smart phone was not available. Food diaries were kept 2 weeks before the diet interventions and during the final 2 weeks of each intervention to establish nutrient intakes. Daily intakes of major nutrients and dietary exchanges were analyzed by a registered dietitian (RD) using commercial NutriBase software. Both NutriBase and the smartphone app were used to provide dietary detail. The smartphone app allowed for determining if meat sources were being replaced by the test ground beef patties or if the patties were simply added to the diet.

This was not available in previous studies using only dietary analysis software [22]. All participants received instructions from the RD for dietary logging and for the preparation, including recipes of the ground beef patties (Appendix C and D); the RD contacted the participants at regularly to encourage compliance. Smith and colleagues' previous studies [21, 22, 47] indicate strong compliance to consumption of the ground beef patties themselves. A key focus in this study was maintaining habitual caloric intake. The availability of a phone-based tracker simplified daily compliance monitoring.

Fatty acid	**LFB**		**HFB**	
grams fatty acid/patty	*Raw*	*Pan-broiled*	*Raw*	*Pan-broiled*
Myristic, 14:0	0.16 ± 0.03	0.15 ± 0.02	0.80 ± 0.07	0.46 ± 0.03
Palmitic, 16:0	1.49 ± 0.28	1.45 ± 0.10	6.37 ± 0.60	3.74 ± 0.83
Palmitoleic, 16:1n-7	0.21 ± 0.03	0.20 ± 0.02	0.98 ± 0.09	0.56 ± 0.03
Stearic, 18:0	0.79 ± 0.14	0.77 ± 0.14	3.53 ± 0.33	2.11 ± 0.14
Oleic, 18:1n-9	2.52 ± 0.47	2.17 ± 0.02	9.70 ± 0.92	5.55 ± 0.35
cis-Vaccenic, 18:1n-7	0.11 ± 0.02	0.13 ± 0.02	0.52 ± 0.05	0.33 ± 0.02
Linoleic, 18:2n-6	0.27 ± 0.05	0.27 ± 0.05	0.65 ± 0.07	0.41 ± 0.03
a-Linolenic, 18:3n-3	0.01 ± 0.02	0.01 ± 0.02	0.05 ± 0.02	0.02 ± 0.02
Total trans-18:1	0.17 ± 0.03	0.16 ± 0.03	1.44 ± 0.14	0.84 ± 0.05
Total SFA	2.44 ± 0.45	2.37 ± 0.40	10.70 ± 1.00	6.32 ± 0.38
Total MUFA	2.84 ± 0.53	2.50 ± 0.19	11.29 ± 1.23	6.44 ± 0.40
Total PUFA	0.28 ± 0.05	0.28 ± 0.02	0.71 ± 0.07	0.43 ± 0.03
MUFA/SFA ratio	1.16 ± 0.07	1.06 ± 0.05	1.05 ± 0.24	1.02 ± 0.21
Total lipid per patty	6.40 ± 1.12	6.20 ± 1.05	26.93 ± 4.38	15.93 ± 2.40

Table 2. Fatty Acid and Lipid Content of Beef Patties.
Fatty acid composition and lipid content of raw and pan-broiled ground beef patties initially containing 6.40 g fat/patty (LFB) or 26.93 g fat/patty (HFB). Values are mean ± SD. Total trans-18:1 = sum of 18:1(trans-6), 18:1(trans-9), 18:1(trans-10) and 18:1(trans-11) fatty acids (> 80% 18:1(trans-11)). Total SFA (saturated fatty acids) = sum of myristic, palmitic, and stearic acid. Total MUFA (monounsaturated fatty acids) = sum of palmitoleic and oleic acid. Total PUFA (polyunsaturated fatty acids) = sum of linoleic and alpha-linolenic acid. Total patty lipid was determined gravimetrically before and after cooking. Includes additional, minor fatty acids not included in the table.

Blood Sampling and Analyses

Blood sampling and assay procedures were conducted based on previously published procedures [101]. On the day of blood sampling, subjects were asked to report to the laboratory after an overnight fast (~10 hours), restricted to water only. Blood was collected after 5 minutes of seated rest via venipuncture from the antecubital fossa region of the left arm into serum separator vacutainer tubes using standard, sterile phlebotomy

procedures. After collection, blood was allowed to clot at room temperature for 30-60 min or chilled at 4°C for serum and plasma separation, respectively, prior to centrifugation in a refrigerated centrifuge for 20 minutes (2,000 × g). One serum separator vacutainer was transported prior to freezing to Spectracell Laboratories® for plasma insulin analysis. Aliquots of serum and plasma from additional vacutainers were transferred into separate 2-mL freezer vials. One vial of fresh serum was couriered the same day to a commercial, Clinical Laboratory Improvement Amendments (CLIA) certified laboratory for determination of total cholesterol, HDL-C, LDL-C, and TAG using standard clinical chemistry analyses. The remaining vials were stored frozen at -80°C for additional assays.

Carotid-Femoral Pulse Wave Velocity

The carotid-femoral pulse wave velocity measures were acquired based on previously published guidelines [102]. After a 10-minute supine rest, PWV measures were made via ultrasonography (Logic P6, GE Healthcare, UK) on the right carotid and femoral arteries. The exact imaging site was marked with a felt tip pen. To ensure similar placement on subsequent measures, the distance from specific anatomical landmarks to the image site was recorded. As with other measures taken, subjects were in a fasted state and asked to have avoid alcohol within 24 hours of their visit to the lab. This was confirmed by a compliance checklist (Appendix E) at the beginning of each lab visit. To determine the PWV, time was measured from the top of the R wave on the QRS complex, to the start of the inflection point on pulse wave recording on six separate cardiac cycles for both the carotid and femoral artery. The average of these was used as the time measure for the PWV calculation. The actual distance between the carotid and femoral site was

measured in a straight line from the previously marked locations. This distance was recorded and 80% of this measured distance was used in the PWV calculation, as this has been previously demonstrated to be the most accurate means of assessing the distance between the carotid and femoral arteries in humans [102]. Finally, the difference in the averaged time delay between the carotid and femoral sites was divided by 80% the measured distance between the sites to produce the PWV value in meters per second.

Flow-Mediated Dilation

Assessment of FMD was accomplished using a Logic P6 ultrasound machine (GE Healthcare, UK). All FMD measurements were conducted following previously published guidelines [103]. After an overnight fast, subjects laid supine in a temperature-controlled room for 10 minutes prior to the imaging of the right brachial artery. Subjects then abducted and externally rotated their right arm to increase visualization of the brachial artery. The abducted arm was placed in a padded securing holder atop a table level with the subject's body to increase comfort and minimize movement during imaging. The image of the brachial artery was acquired via a high-frequency linear transducer (10-12 MHz). Once the clearest image of the artery was found, land marks such as veins or arterial branches were noted. The shortest distance from the medial epicondyle to the middle of bicep (where the brachial artery runs) was recorded for reproducibility. Additionally, a probe holding device was used to further ensure consistent vessel imaging. The baseline vessel diameter was recoded for 1-minute and saved to DVD (DVO-1000MD, Sony), and baseline pulse wave was recorded and saved on the ultrasound machine. After the baseline recording, a blood pressure cuff was wrapped around the subject's forearm, distal to the

imaging site, and inflated to 200 mmHg for 5 minutes of occlusion. Following the 5-minute occlusion, the cuff was released and the post occlusion pulse wave was recorded at 15 seconds post occlusion. Next, the post-occlusion vessel diameter was recorded from 30-120 seconds post-occlusion and recorded to DVD. All DVD recordings were converted to MP4 files and analyzed by an individual technician via brachial analyzer tracking software (Brachial Analyzer, Medical Imaging Applications-LLC, IA). All diameter measurements were automatically made at the end of diastole using the gating software upgrade package (Software-Gating module Add-on. Medical Imaging Applications-LLC, IA). Both gated and allometrically scaled FMD values were recorded.

Body Composition

Body composition of all subjects was assessed at the entry visit using a Lunar Prodigy dual-energy x-ray absorptiometry (DXA) machine (General Electric, Madison, WI). All subsequent measures (LFB, washout, and HFB) were made using a Horizon A DXA machine (Hologic, Inc., Bedford, MA, USA). Derived variables of interest from the DXA scans are total body mass, lean body mass, fat mass, percent body fat, and bone mineral content.

Submaximal VO_2

Oxygen uptake ($\dot{V}O_2$) was measured as an index of cardiovascular aerobic capacity before and after the ground beef interventions [88, 104]. An incremental graded exercise test to 80% age predicted max heart rate [105] was conducted on a motor-driven treadmill according to the Bruce et al. [106] protocol. Oxygen consumption during exercise was continuously measured using a calibrated metabolic gas-analysis system (Ultima®,

Medical Graphics, Minneapolis, MN). Measured $\dot{V}O_2$ and HR were recorded as the highest 15-second average oxygen uptake achieved during the exercise test. Estimations of participants maximal oxygen uptake (VO_{2max}) before and after the beef interventions were calculated using an individualized linear regression, using IBM Statistics 23 (IBM, New York), based on heart rate and VO_2 during each stage of the Bruce protocol [106].

Calculated Values

The primary measures of fasting glucose and insulin along with serum lipoprotein concentrations allowed for calculation of HOMA-IR as well as single point insulin sensitivity estimator (SPISE) [31, 32]. Additionally, direct height and weight measures allowed for calculations of body mass index (BMI) for consideration relative to DXA measures of lean and fat mass, as well as aerobic fitness as measured by the graded exercise test via Bruce protocol [106].

Limitations

Subject Compliance

Due to the nature of human subject research, compliance to abide by the specific guidelines of the study may be an issue that could affect the results. This could be failure to consume the specified number of beef patties/week, to fast or to avoid alcohol, caffeine and other stimulants prior to laboratory visits. To limit these potential issues, subjects were contacted at regular intervals by an RD in order to encourage compliance. Additionally, upon arrival to the lab, subjects filled out a compliance checklist (Appendix E) to ensure the fasting specifications had been abided by prior to any testing. The compliance checklist was used prior to testing during each lab visit.

Subject Scheduling

Due to a limited staff, subject scheduling was based on availability. While all testing visits took place during the fifth week of each intervention, the specific day of the week may have been different within and between subjects for each intervention.

Study Duration

While this study utilizes a *chronic* dietary intervention design, outcomes after only five weeks of an intervention cannot be extrapolated to mean similar results will occur from habitually consuming the same diet. Additionally, it is unknown whether these effects will remain stable among differing populations.

DXA Machine Change

Due to machine failure, an alternate DXA machine was used after the first (entry) measurement. The original machine, Lunar Prodigy (General Electric, Madison, WI) was replaced with a Horizon A (Hologic, Inc., Bedford, MA, USA). Due to the technical error differences in body composition estimates between machines, only the three measures after entry (LFB, HFB, and washout) could not be statistically compared, as differences from entry to the three subsequent time points could be due to machine differences.

Laboratory Sampling Error

During the course of study, a fresh blood sample was sent to Spectracell Laboratories® for plasma insulin analysis. Unfortunately, this laboratory lost data for 12 of the 23 subjects on the entry time point measure. Thus, for a 2x2 repeated measures ANOVA a sample size of 11 was used for insulin and HOMA-IR values.

Delimitations

Subject Specificity

Due to the nature of the proposed research, only subjects with serum TC in normal range (total cholesterol above 300 mg/dL or below 120 mg/dL), and not on restrictive diets were eligible to participate. Subjects who had normal TC levels but were taking cholesterol lower medications were also excluded from the study. Also, due to the negative vascular effects of tobacco, individuals who used tobacco products were excluded from participation. All of the aforementioned exclusion criterion was assessed via health history questionnaire (Appendix F) prior to acceptance to the study.

Gender

Due to the fact that the FMD response can be affected by the specific time point of the menstrual cycle in women, this study was limited to men only.

Fasting

In order to control for acute dietary effects on serum lipids and vascular measures, all subjects were asked to avoid all food and drinks, other than water, for 10-12 hours prior to each visit. This was confirmed at the beginning of each lab visit via the subject compliance check list (Appendix E).

Body Composition

Body composition was assessed during each lab visit in order to detect any shifts between study visits. This was used, along with submaximal exercise testing and activity logs, to ensure that subjects did not largely alter their diet or physical activity level throughout the course of the study.

Submaximal Exercise Test

Submaximal exercise was used over maximal testing to reduce physical strain on participants. Submaximal exercise testing, via Bruce treadmill protocol [106], was conducted on the first and last visits. This assessed changes in subject's fitness levels during the study, and the relationships between vascular function and aerobic capacity.

Activity Logs

Seven-day activity logs (Appendix B) were used to evaluate maintenance of normal activity levels throughout each phase of the study. With this, large differences in physical activity during a specific phase of the study would have been identified.

Dietary Logs

Dietary logs were kept using the My Fitness Pal smart phone application (https://www.myfitnesspal.com), or manual logging if a smart phone was not available two weeks prior to the start of the study and during the last two weeks of each intervention. This enabled the assessment of the micro and macronutrient intake by a RD, using NutriBase (CyberSoft Inc., AZ) nutritional analysis software during each study phase.

Statistical Design

The primary statistical model was a 2 Condition (HFB, LFB) x 2 Test (Entry, Washout) (2 x 2) repeated measures ANOVA; when values for all four time points (Entry, LFB, Washout, and HFB) were available. Follow-up simple main effects was used for significant interactions and a paired samples t-test was used for significant condition or test effects, to identify the source. If all four time points were not available (ex. VO_{2max}, energy expenditure, and DXA body composition), a paired t-test was used.

CHAPTER IV

RESULTS

Flow-Mediated Dilation

All FMD values including baseline vessel diameter and time to peak vessel diameter were assessed by a 2 (condition) x 2 (test) repeated measures ANOVA. Baseline diameter of the brachial artery and time to peak dilation for each study time point are depicted in Figure 3 and Figure 4, respectively. The ANOVA revealed no difference for baseline vessel diameter or time to peak dilation between any of the study visits.

Average values for gated and allometrically scaled flow-mediated dilation are depicted in Figure 5, as % dilation for each time point. Repeated measures ANOVA revealed a significant main effect of test for allometrically scaled FMD ($p = 0.044$). Specifically, the follow up paired t-test demonstrated the FMD response after the HFB intervention was greater compared to the entry, washout, and LFB time points ($p=0.013$, 0.049 and 0.028, respectively).

Average values for gated FMD are depicted in Figure 6, as % dilation for each study visit. The ANOVA demonstrated a significant main effect of test for FMD ($p = 0.035$). A follow up paired t-test demonstrated the FMD response after the HFB intervention was greater compared to the entry and LFB time point ($p=0.008$ and 0.028, respectively). Additionally, there was trend for increased FMD response after HFB intervention compared to the washout time point ($p=0.057$).

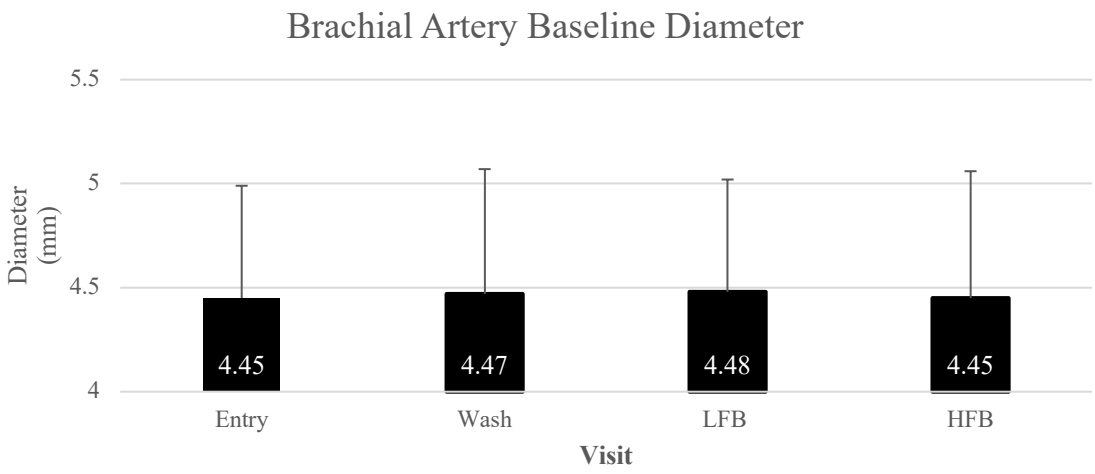

Figure 3. Baseline Brachial Artery Diameter.
Values are for Entry, Washout (Wash), Low-fat beef intervention (LFB), and High-fat beef intervention (HFB). Entry values were taken two weeks prior to starting diet intervention, all other measures were taken in the last week of the given intervention (Wash, LFB, and HFB). Cross-over design (N = 23); values represent mean ± SD. No significant difference (NS) $p > 0.05$.

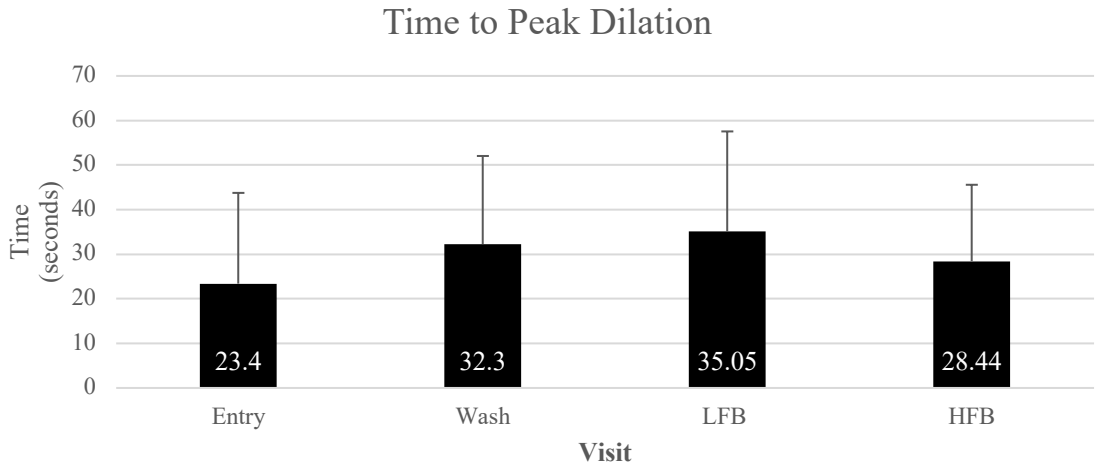

Figure 4. Time to Peak Artery Dilation.
Values are for Entry, Washout (Wash), Low-fat beef intervention (LFB), and High-fat beef intervention (HFB). Entry values were taken two weeks prior to starting diet intervention, all other measures were taken in the last week of the given intervention (Wash, LFB, and HFB). Cross-over design (N = 23); values represent mean ± SD. NS $p > 0.05$.

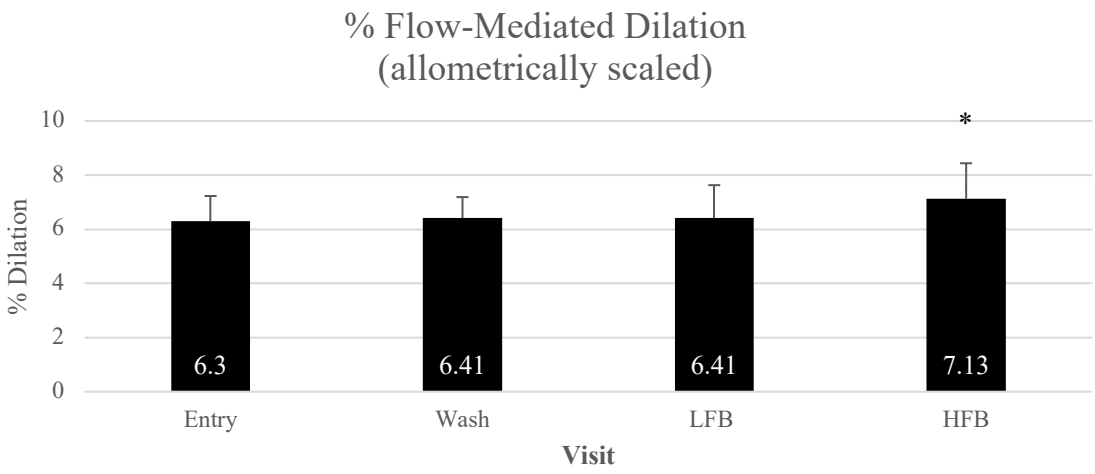

Figure 5. Allometrically Scaled FMD.
Values are % dilation for Entry, Washout (Wash), Low-fat beef intervention (LFB), and High-fat beef intervention (HFB). Entry values were assessed two weeks prior to starting diet intervention. All other measures were taken in the last week of the associated intervention (Wash, LFB, and HFB). Cross-over design (N = 23); values represent mean ± SD. *significantly higher than all other values by paired t-test, $p < 0.05$.

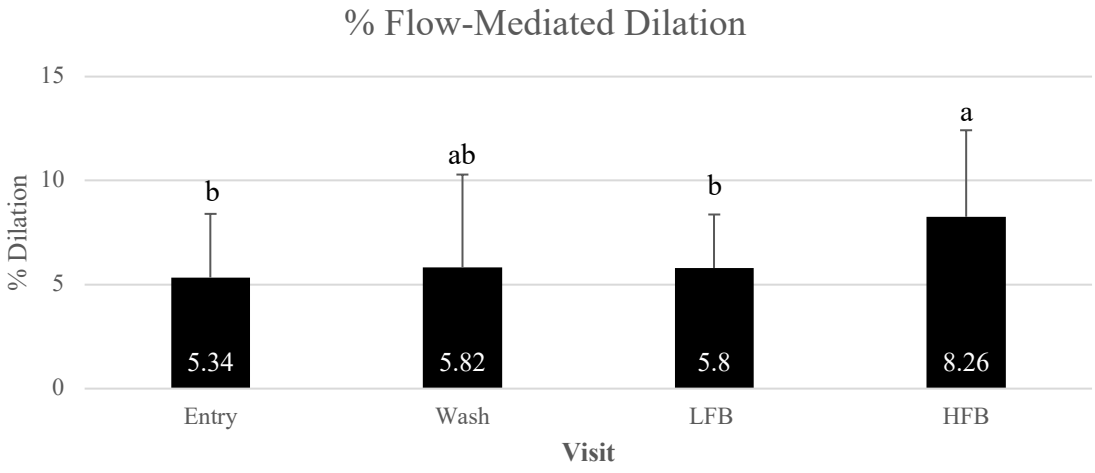

Figure 6. Gated FMD.
Values are % dilation for Entry, Washout (Wash), Low-fat beef intervention (LFB), and High-fat beef intervention (HFB). Entry values were taken two weeks prior to starting diet intervention, all other measures were taken in the last week of the given intervention (Wash, LFB, and HFB). Cross-over design (N = 23); values represent mean ± SD. Means without a common letter differ, $p < 0.05$.

Pulse Wave Velocity

PWV measurements for each study visit are displayed in Figure 7. A 2 (condition) x 2 (test) repeated measures ANOVA revealed no significant condition, test or interaction effect between any study visits.

Resting Blood Pressure

Measurements for resting blood pressure are depicted for SBP (Figure 8), DBP (Figure 9), mean arterial pressure (MAP; Figure 10), and heart rate (HR; Figure 11). All blood pressure values, including SBP, DBP, MAP, and HR values were assessed via 2 (condition) x 2 (test) repeated measures ANOVA. The ANOVA revealed a significant condition effect ($p < 0.01$) for SBP. A follow up paired t-test showed that SBP during the HFB intervention was lower compared to the LFB ($p = 0.04$) and the entry visit ($p < 0.01$). Conversely, SBP during the washout was significantly lower than both LFB and entry time points ($p = 0.02$, 0.01 respectively). No significant difference in SBP existed between the washout and HFB intervention ($p = 0.8$).

Likewise, for resting DBP, ANOVA revealed a significant condition effect ($p < 0.01$) with the paired t-test showing it significantly lowered in the HFB intervention and the washout time point compared to entry ($p = 0.017$ and 0.003, respectively). No other differences were found. Similarly, ANOVA for MAP revealed a significant condition effect ($p < 0.01$) with the follow up paired t-test indicating that MAP was significantly lower after the HFB intervention and the washout time point relative to entry ($p = 0.002$ for both). Statistical analysis for resting heart rate, measured via 3-lead electrocardiogram, found no significant condition, test or interaction effects between time points.

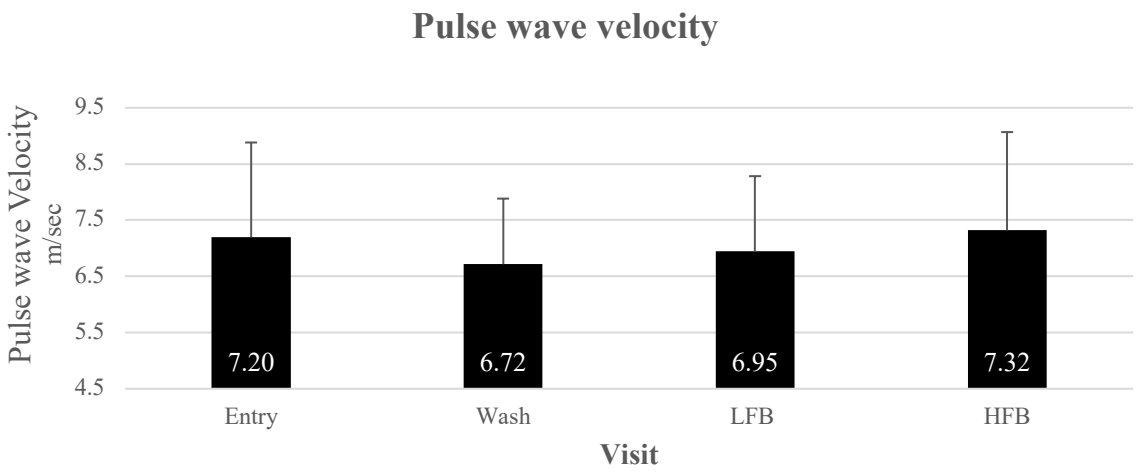

Figure 7. Carotid-Femoral Pulse Wave Velocity.
Values are for Entry, Washout (Wash), Low-fat beef intervention (LFB), and High-fat beef intervention (HFB). Entry values were taken two weeks prior to starting diet intervention, all other measures were taken in the last week of the given intervention (Wash, LFB, and HFB). Cross-over design (N = 23); values represent mean ± SD. NS $p > 0.05$.

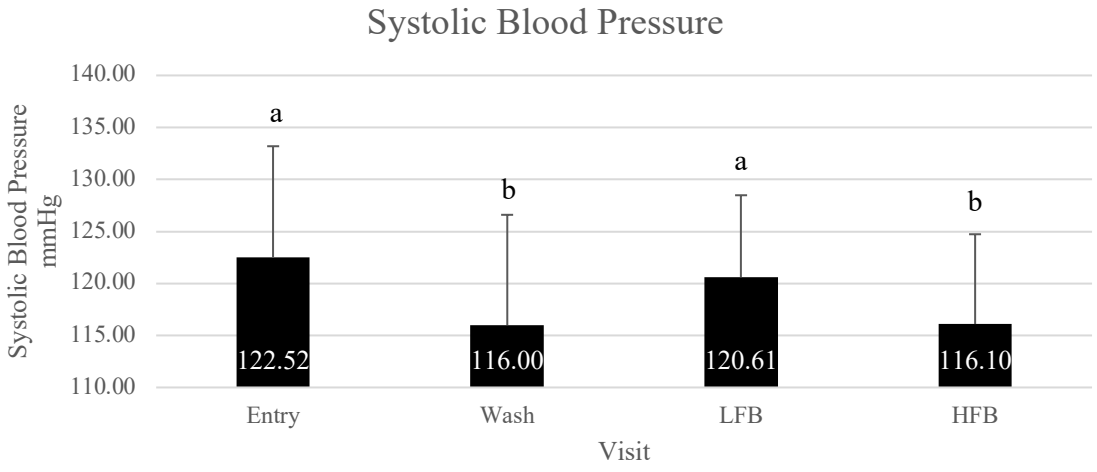

Figure 8. Resting Systolic Blood Pressure.
Values are for Entry, Washout (Wash), Low-fat beef intervention (LFB), and High-fat beef intervention (HFB). Entry values were taken two weeks prior to starting diet intervention, all other measures were taken in the last week of the given intervention (Wash, LFB, and HFB). Cross-over design (N = 21). Values represent mean ± SD. Means without a common letter differ, $p < 0.05$.

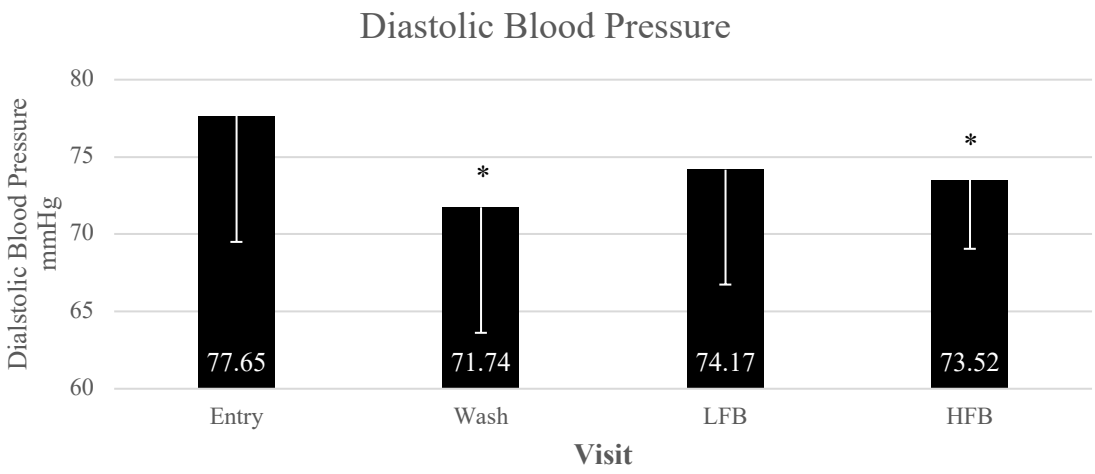

Figure 9. Resting Diastolic Blood Pressure.
Values are for Entry, Washout (Wash), Low-fat beef intervention (LFB), and High-fat beef intervention (HFB). Entry values were taken two weeks prior to starting diet intervention, all other measures were taken in the last week of the given intervention (Wash, LFB, and HFB). Cross-over design (N = 21); values represent mean ± SD.* Significantly different from entry value, $p < 0.05$.

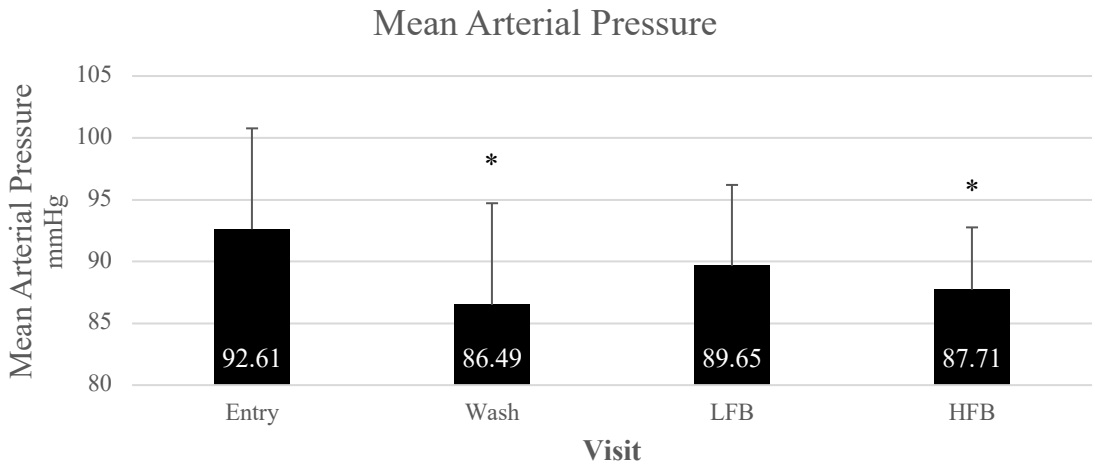

Figure 10. Resting Mean Arterial Pressure.
Values are for Entry, Washout (Wash), Low-fat beef intervention (LFB), and High-fat beef intervention (HFB). Entry values were taken two weeks prior to starting diet intervention, all other measures were taken in the last week of the given intervention (Wash, LFB, and HFB). Cross-over design (N = 21); values represent mean ± SD.* Significantly different from entry value, $p < 0.05$.

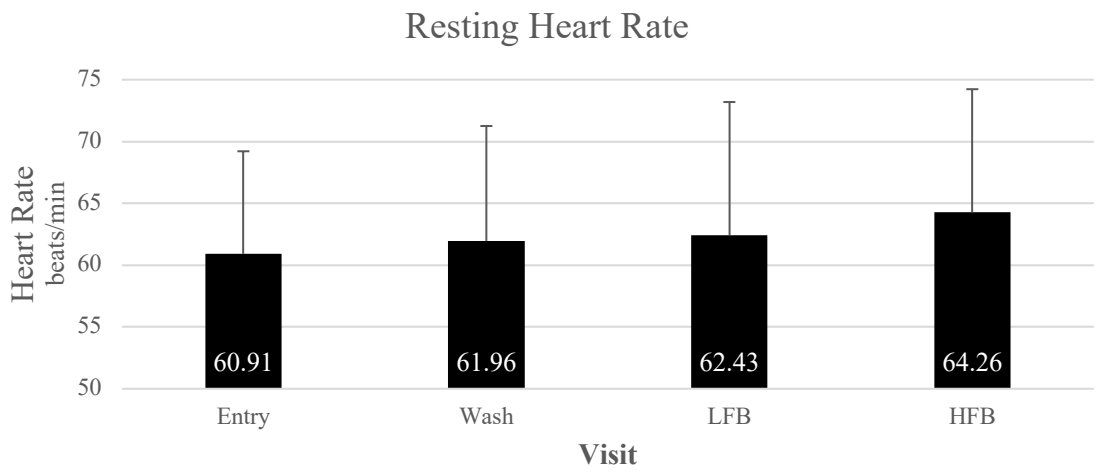

Figure 11. Resting Heart Rate.
Values are for Entry, Washout (Wash), Low-fat beef intervention (LFB), and High-fat beef intervention (HFB). Entry values were taken two weeks prior to starting diet intervention, all other measures were taken in the last week of the given intervention (Wash, LFB, and HFB). Cross-over design (N = 21); values represent mean ± SD. NS $p > 0.05$.

Serum Lipids

Fasting blood samples were collected at each study time point for serum analysis of TC, HDL-C, LDL-C, and TG. Table 3 displays the mean ± SD values for these variables at each time point. A 2 (condition) x 2 (test) ANOVA revealed a significant main effect of test for TC, HDL-C, and LDL-C with no significant condition, test, or interaction effect for TG. With regard to TC, the subsequent paired t-test showed that the after HFB and LFB interventions, values were significantly lower than at the entry time point ($p=0.018$ and 0.024, respectively). HDL-C was also significantly lower after HFB and LFB interventions compared to time entry time point ($p=0.000$ and 0.00, respectively). Additionally, HDL-C was lower after the HFB intervention compared to the washout time point ($p=0.046$). With regard to LDL-C, only the HFB intervention resulted in significantly lower LDL-C compared to entry ($p=0.024$). Trends were seen for lower LDL-

C at the HFB time point compared to the washout (p=0.067) and for the LFB compared to entry (p=0.064). The TC, HDL-C, and LDL-C changes from entry for the HFB, LFB, and washout time point are displayed in Figure 12.

Dietary Analysis

Dietary analyses of macronutrients are displayed in Table 6. A 2 (condition) x 2 (test) ANOVA with a follow-up simple main effect (if an interaction effect was found) or pair T-test (if condition or test effects were found) was implemented to analyze all dietary data ($p<0.05$). A significant test effect (p=0.045) was seen in % CHO consumption, where paired t-test identified consumption during the HFB intervention was significantly lower than entry levels (p=0.030). Additionally, an interaction effect was seen in % protein (p=0.011) and % fat intake (p=0.034). Follow-up simple main effects showed % protein and % fat intake were significantly higher in the LFB and HFB intervention, respectively, compared to all other time points. Significant interaction effects were also found for total fat, SFA, and MUFA (p=0.013, 0.044, and 0.049, respectively) with the simple main effects showing intake values were higher for the HFB intervention compared to other time points. No other significant differences were found for macronutrients.

Analyses of vitamins and minerals are displayed in Table 7. A condition effect (p=0.008) was found for vitamin D, with paired t-test indicating consumption was higher at entry and in the LFB intervention compared to washout and HFB intervention (p=0.014 and 0.041, respectively). Additionally, a test effect was found for folate consumption (p=0.029), indicating it was higher at entry than both HFB and LFB interventions (p=0.045 and 0.006, respectively). No other effects were observed for vitamins and minerals.

	Entry	Washout	LFB	HFB
Total Cholesterol	205.22 ± 41.61	199.91 ± 44.19	193.13 ± 46.71*	191.57 ± 40.72*
Triglyceride	106.48 ± 43.76	113.30 ± 55.99	107.83 ± 43.29	119.83 ± 55.42
HDL-Cholesterol	49.83 ± 10.12	48.17 ± 11.15	46.35 ± 10.77*	45.78 ± 8.89*†
LDL-Cholesterol	133.96 ± 41.04	129.09 ± 40.46	125.30 ± 43.82	121.78 ± 37.08*
TC/HDL	4.27 ± 1.20	4.32 ± 1.29	4.30 ± 1.20	4.33 ± 1.28

Table 3. Fasting Serum Lipids.
Values are mg/dL for Entry, Washout, Low-fat beef intervention (LFB), and High-fat beef intervention (HFB). Entry values were taken two weeks prior to starting diet intervention, all other measures were taken in the last week of the given intervention (Washout, LFB, and HFB). Cross-over design (N = 23); values represent mean ± SD. *significantly lower than entry; †significantly lower than washout, $p < 0.05$.

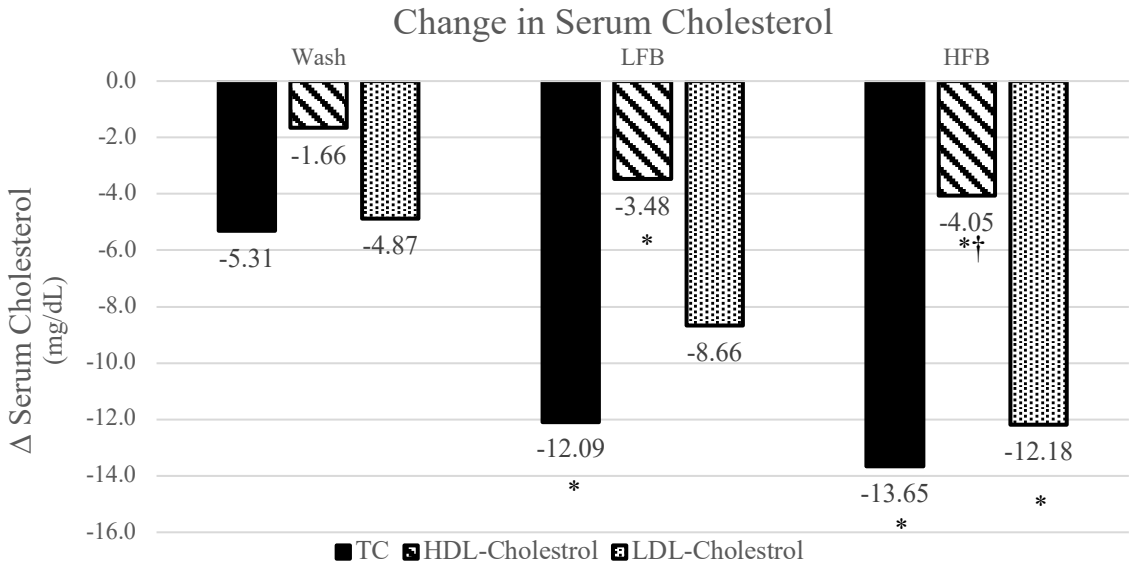

Figure 12. Serum Lipid Change.
Values represent TC, HDL-C, and LDL-C change in mg/dL from Entry for Washout (Wash), Low-fat beef intervention (LFB), and High-fat beef intervention (HFB). All measures were taken in the last week of the given intervention (Wash, LFB, and HFB). Cross-over design (N = 23). Values represent LDL-C at given time point minus entry LDL-C. *significantly lower compared to entry; †significantly lower than washout, $p < 0.05$.

	Entry	Washout	LFB	HFB
BMI (kg/m^2)	31.15 ± 8.99	30.92 ± 8.60	30.76 ± 8.49	30.9 ± 8.52
TG/HDL	2.30 ± 1.26	2.56 ± 1.62	2.52 ±1.34	2.8 ± 1.61
SPISE	5.50 ± 1.87	5.46 ± 1.86	5.44 ±1.75	5.32 ± 1.74

Table 4. Body Mass Index, TG:HDL Ratio and SPISE.
Values are for BMI fasting TG/HDL ratio and calculated SPISE at Entry, Washout, Low-fat beef intervention (LFB), and High-fat beef intervention (HFB). Entry values were taken two weeks prior to starting diet intervention, all other measures were taken in the last week of the given intervention (Washout, LFB, and HFB). Cross-over design (N = 23); values represent mean ± SD. NS $p > 0.05$.

	Entry	Washout	LFB	HFB
Glucose (mmol/L)	5.29 ± 0.40	5.22 ± 0.46	5.21 ± 0.46	5.23 ± 0.48
Insulin (μU/mL)	12.82 ± 13.16	12.20 ± 10.70	10.92 ± 9.59	13.13 ± 13.38
HOMA-IR	3.06 ± 3.32	2.88 ± 2.60	2.62 ± 2.45	3.16 ± 3.30

Table 5. Glucose, Insulin and HOMA-IR.
Values are for fasting glucose, insulin and calculated HOMA-IR at Entry, Washout, Low-fat beef intervention (LFB), and High-fat beef intervention (HFB). Entry values were taken two weeks prior to starting diet intervention, all other measures were taken in the last week of the given intervention (Washout, LFB, and HFB). Cross-over design (N = 11); values represent mean ± SD. NS $p > 0.05$.

	Entry	Washout	LFB	HFB
EN (kcal/day)	2070.8 ± 490.5	1965.9 ± 399.0	1886.8 ± 401.2	2070.7 ± 486.9
% EN CHO	41.3 ± 8.6	41.2 ± 7.4	40.3 ± 8.2	38.3 ± 8.8†
% EN Protein	18.6 ± 3.5	18.9 ± 4.7	22.0 ± 4.8*	18.4 ± 5.8
% EN Fat	38.6 ± 6.0	37.4 ± 6.6	36.8 ± 6.6	42.2 ± 8.7*
Cholesterol (mg/d)	437.2 ± 304.8	330.4 ± 179.9	344.8 ± 196.7	321.7 ± 170.0
Protein (g/d)	96.2 ± 28.5	91.2 ± 22.8	103.9 ± 32.5	95.2 ± 38.2
CHO (g/d)	212.5 ± 63.9	202.4 ± 53.3	188.7 ± 48.8	197.5 ± 64.0
Fat (g/d)	90.0 ± 28.0	82.2 ± 24.6	76.9 ± 19.1	97.1 ± 30.8*
SFA (g/d)	29.8 ± 9.5	27.9 ± 8.8	26.9 ± 8.5	34.1 ± 13.0*
MUFA (g/d)	15.7 ± 8.5	15.3 ± 8.2	15.7 ± 7.3	22.9 ± 11.2*
PUFA (g/d)	8.3 ± 4.3	8.2 ± 4.2	7.8 ± 4.9	7.9 ± 3.6
TFA (g/d)	0.8 ± 0.8	0.5 ± 0.5	0.3 ± 0.4	0.6 ± 0.8
n-6 fatty acids (g/d)	6.2 ± 4.0	5.7 ± 3.4	5.4 ± 4.7	5.1 ± 2.7
n-3 fatty acids (g/d)	0.7 ± 0.7	0.7 ± 0.5	0.7 ± 0.6	0.5 ± 0.3

Table 6. Daily Macronutrient Intake.
Values are for daily intakes of major nutrients of men at Entry, Washout, Low-fat beef intervention (LFB), and High-fat beef intervention (HFB). Data were derived from 3-day diet records that included 1 weekend day. Cross-over design (N = 23); values represent mean ± SD. *significantly different than all other time points via 2x2 repeated measures ANOVA simple main effects; †significantly lower than entry time point, $p < 0.05$

	Entry	Washout	LFB	HFB
Vitamin A (µg/d)	498.9 ± 543.3	453.5 ± 642.3	363.3 ± 313.8	382.2 ± 424.0
ß-Carotene (µg/d)	769.1 ± 1265.5	1995.0 ± 6078.5	1285.1 ± 2088.7	1228.8 ± 2015.1
Thiamin (mg/d)	0.8 ± 0.5	0.8 ± 0.5	0.7 ± 0.4	0.8 ± 0.4
Riboflavin (mg/d)	1.34 ± 0.7	1.2 ± 0.5	1.1 ± 0.4	1.0 ± 0.4
Niacin (mg/d)	13.6 ± 6.5	13.9 ± 5.9	13.3 ± 9.1	11.1 ± 5.2
Pyridoxine (mg/d)	1.2 ± 0.6	1.2 ± 0.6	1.4 ± 1.2	1.1 ± 1.2
Vitamin B_{12} (µg/d)	4.2 ± 3.8	3.7 ± 3.3	4.6 ± 4.5	3.0 ± 3.7
Vitamin C (mg/d)	58.1 ± 41.7	58.9 ± 74.5	73.0 ± 111.8	77.4 ± 118.0
Vitamin D (µg/d)	4.2 ± 3.8*	2.6 ± 2.1	4.9 ± 6.1*	3.4 ± 5.7
Vitamin E (mg/d)	5.8 ± 4.7	4.3 ± 4.0	3.7 ± 2.4	3.9 ± 3.0
Folate (µg/d)	278.3 ± 138.7	239.4 ± 168.9	172.9 ± 85.0†	185.2 ± 121.2†
Calcium (mg/d)	708.9 ± 285.5	611.5 ± 251.3	673.4 ± 345.6	635.4 ± 301.9
Iron (mg/d)	12.7 ± 4.8	11.2 ± 4.0	12.6 ± 3.5	11.6 ± 4.0
Sodium (mg/d)	3864.7 ± 1382.6	3203.1 ± 1340.1	3237.6 ± 1089.0	3182.6 ± 1192.7

Table 7. Daily Micronutrient Intake.
Values are for daily intakes of vitamins and minerals of men at Entry, Washout, Low-fat beef intervention (LFB), and High-fat beef intervention (HFB). Data were derived from 3-day diet records that included 1 weekend day. Cross-over design (N = 23); values represent mean ± SD. *significantly higher than right adjacent time point in table; †significantly lower than entry time point, $p < 0.05$.

Body Composition, Aerobic Fitness and Energy Expenditure

Body composition values, assessed via DXA scan (Hologic, Inc., Bedford, MA, USA), are displayed in Table 8. All body composition values for the washout, LFB intervention, and HFB intervention were analyzed via paired t-test ($p<0.05$). Body fat percentage was found to be higher after the LFB and HFB intervention relative to the washout time point ($p=0.025$ and 0.037, respectively). Additionally, gynoid percent fat was also found to be higher after the LFB intervention compared to the washout time point ($p=0.048$).

Maximal aerobic capacity (VO_{2max}), measured via submaximal graded exercise test at the entry and final dietary intervention time point (LFB or HFB), are displayed in Figure 13. Values were analyzed via a paired t-test ($p<0.05$). No difference was found in VO_{2max} between the first and last visit ($p=0.478$).

Daily energy expenditure estimated by 7-day activity logs are depicted in Figure 14. Energy expenditure, was analyzed via a paired t-test, which showed no significant difference between washout, LFB, and HFB time points (washout vs. LFB $p=0.693$, washout vs. HFB $p=0.795$, LFB vs HFB $p=0.927$).

Dependent Variable Correlations

A simple correlation matrix was implemented to assess relationships between obesity, fitness levels, and vascular health marker. Table 9. displays the results of the correlation matrix.

	Washout	LFB	HFB
Body weight (kg)	98.77 ± 25.81	96.99 ± 24.62	97.35 ± 24.90
BMI (kg/m^2)	30.93 ± 8.60	30.76 ± 8.49	30.90 ± 8.52
Lean mass (kg)	67.31 ± 9.76	66.95 ± 9.39	66.89 ± 9.49
Fat mass (kg)	28.89 ± 17.84	29.31 ± 18.18	29.57 ± 18.85
Body fat (%)	27.43 ± 8.81	27.79 ± 8.93*	27.94 ± 9.03*
Android fat (%)	31.95 ± 10.90	31.48 ± 10.59	31.97 ± 11.27
Gynoid fat (%)	28.75 ± 7.16	29.12 ± 7.32*	29.27 ± 7.29

Table 8. Body Composition.
Values are for body composition assessed via DXA scan at washout, LFB, and HFB time points. Cross-over design (N = 23); values represent mean ± SD. *significantly higher compared to washout time point, $p < 0.05$.

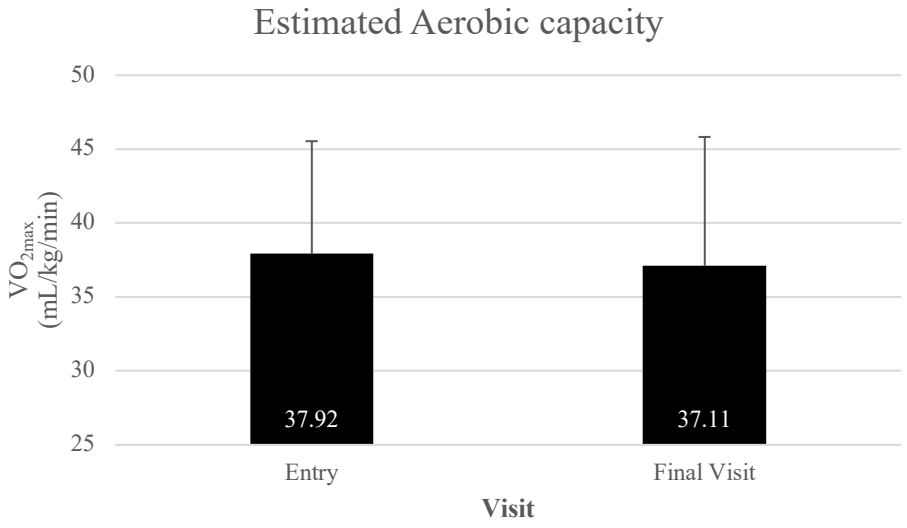

Figure 13. VO_{2max}.
Values are estimated aerobic capacity at Entry and final visit (HFB or LFB) All measures were taken at entry or in the last week of the given intervention (LFB, and HFB). Cross-over design (N = 23); values represent mean ± SD. NS $p > 0.05$.

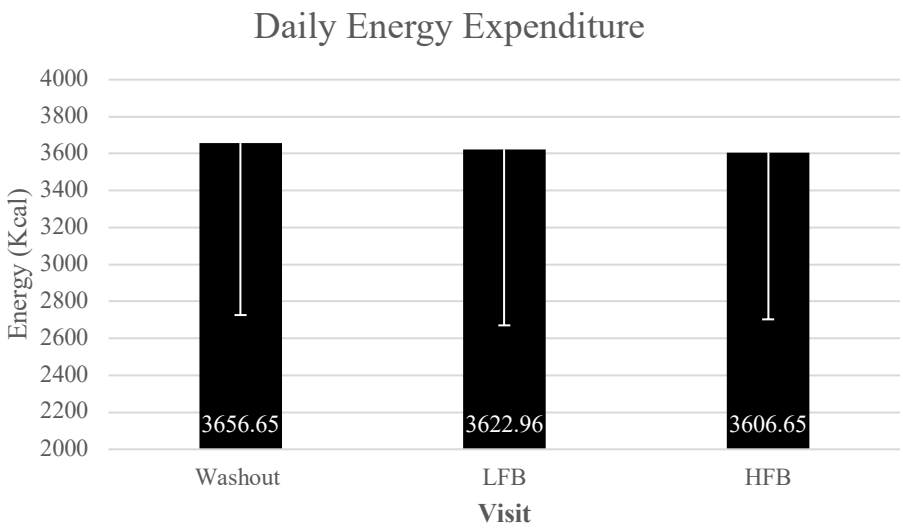

Figure 14. Daily Energy Expenditure.
Values represent estimated energy expenditure measured via 7-day activity logs at washout, LFB, and HFB time points. Cross-over design (N = 23); values represent mean ± SD. NS $p > 0.05$.

	Android	Gynoid	BMI	Weight	VO_2	PWV	FMD
Android	1						
Gynoid	0.896*	1.000					
BMI	0.809**	0.747**	1.000				
Weight	0.763**	0.707**	0.971**	1.000			
VO2	-0.581**	-0.440*	-0.469*	-0.484*	1.000		
PWV	0.435*	0.287	0.548*	0.619**	-0.570**	1.000	
FMD	-0.284	-0.246	-0.171	-0.144	0.190	-0.265	1

Table 9. Correlation Matrix.
Simple correlation matrix for percent android fat (Android), percent gynoid fat (Gynoid), body mass index (BMI), body weight (Weight), pulse wave velocity speed (PWV), and flow-mediated dilation (FMD) (N = 23). **significant $p < 0.01$. *Significant at $p < 0.05$.

CHAPTER V

DISCUSSION AND CONCLUSION

Specific Aims and Hypotheses

The purpose of this study was to investigate the vascular health implications, measured via FMD and PWV, of chronic HFB vs LFB consumption in normocholesterolemic men. An additional goal was to investigate the effects of HFB vs LFB consumption on BP, serum lipids, insulin sensitivity and macronutrient ingestion. Finally, a tertiary goal of this study was the assessment of the relationship between aerobic fitness, body composition and the vascular health markers FMD and PWV.

Primary Aim – In terms of FMD, we reject the hypothesis that the HFB would not alter FMD relative to the LFB. However, we accept the hypothesis that the HFB would not alter PWV relative to LFB. With regard to BP, we accept the hypothesis that the HFB intervention would lower BP relative to the LFB intervention.

Secondary Aim 1 – Regarding serum lipids, contrary to our hypotheses, HFB lowered HDL-C, LDL-C and TC, while LFB did not significantly lower LDL-C. Yet, the LFB intervention lowered HDL-C and TC, as our original hypothesis anticipated. Neither intervention produced a significant change in TC/HDL ratio.

Secondary Aim 2 – As for macronutrient consumption and insulin sensitivity, we accept our original hypothesis, stating CHO consumption would be reduced in HFB and unaltered by LFB interventions. Yet, despite decreased CHO intake, HFB did not affect insulin resistance, assessed by HOMA-IR and SPISE, contradicting our hypothesis.

Secondary Aim 3 – Contrary to our initial hypothesis the HFB and LFB intervention produced a slight increase in percent body fat. In line with our hypothesis, PWV and FMD were positively and negatively correlated with central obesity (android percent fat), respectively, though the FMD correlation failed to reach significance.

Secondary Aim 4 – Consistent with our original hypothesis aerobic capacity did not change throughout the course of the study. Additionally, PWV and FMD were negatively and positively corelated to aerobic capacity, respectively, though the FMD correlation failed to reach significance.

Flow-Mediated Dilation and Pulse Wave Velocity

The significant test effect for FMD indicates that the dilatory response was higher after the HFB intervention compared to the LFB. To the authors' knowledge, this is the first study to assess the vascular outcomes of consuming high-fat vs low-fat ground beef. Because of this, direct comparisons to existing literature are not feasible. However, researchers have examined vascular responses to diets high in altered fat compositions compared to those high in CHOs diets [18, 45, 46], beef compared to bison [107], and addition of lean beef to the DASH diet [108].

Primarily, diets high in TFAs have been demonstrated to have detrimental effects on the FMD response, while diets high in CHO do not alter this response [18, 45, 46]. Mechanistically, this decrease in FMD may be attributed to the reduction in HDL-C (15.08 mg/dL reduction) that is related to high TFA intake [46]. However, high CHO diets — which also reduce HDL-C — have not been shown to alter the FMD response. This may be related to the comparably less severe reduction in HDL-C produced by a high CHO

diet (8.12 mg/dL reduction) compared to the TFA diet [18]. It is important to note that in both these studies the LDL-C remained unchanged [18, 46].

While we are the first to compare the vascular effect of high-fat vs. low-fat ground beef, previous research has been conducted to compare this during bison vs beef consumption [107]. Some similarities exist between our study and that of McDaniel et al. [107] The bison was a lower fat beef alternative containing 8.8-9.5 g of fat per serving, while the beef intervention was substantially higher in fat, containing 19.0-21.8 g of fat per serving. Additionally, their beef intervention was higher in MUFAs compared to the bison. Similarly, our LFB patties contained 6.4 g of fat compared to 26.93 g per HFB patty, with the HFB being considerably higher in MUFAs (11.29 g/patty). However, in our study our HFB patties contained about 2 g more MUFAs per serving than the beef intervention used by McDaniel. Additionally, during our study the daily intake of MUFAs during the HFB intervention was higher (22.9 g/day) than during the LFB intervention, while the total MUFAs intake per day was not listed in the McDaniel et al. [107] paper. Unlike our study, McDaniel et al. [107] found no statistically significant change in serum lipids, though a decrease in both LDL-C and HDL-C were noted. Furthermore, contrary to our results, McDaniel et al. [107] found no significant difference in FMD after 7-weeks of either beef or bison consumption.

Contrary to the findings of the aforementioned studies, the present study revealed an increased FMD response following the HFB intervention. This is likely related to the significant decrease in LDL-C, which in both animal and human models, has been shown to deleteriously affect the FMD response [19]. LDL-C functions as a proinflammatory

vasoconstrictor, which inhibits both the synthesis and release of NO. In a review by Rosendorff [19], LDL-C was shown to severely down regulate endothelial NO synthase, which is the primary vasodilatory mechanism of the FMD response, and completely eliminated the dilatory response to acetylcholine. On the other hand, this deleterious effect can be attenuated by lipid lowering medication, L-arginine, and antioxidants, which adds further evidence to the role of LDL-C in reducing the FMD response.

Additionally, the observed increase in MUFA consumption during the HFB intervention may partially explain the increase in FMD. During the HFB intervention, fat intake was elevated to 42% of total daily energy intake, with 23.5% of the fat being MUFAs. We are not the first group to report an increased FMD response after a dietary shift to high percentage of fat intake, with a larger portion of that fat being MUFAs. Fuentes et al. [44] compared a Mediterranean-like diet, a low-fat diet (national cholesterol education program [NCEP-1]) and a high-SFA diet in hypercholesterolemic men. As a result of the Mediterranean diet, which was 38% total fat with 22% coming from MUFAs, the FMD response was significantly increased. Moreover, this diet decreased LDL-C and TC relative to the low-fat diet (<28% fat) and the high SFA (38% fat, with 20 being SFA) diet. These findings are consonant with our own. Furthermore, Fuentes et al. [44] found a significant negative correlation between LDL-C and FMD. Contrary to Fuentes et al. [44] and the current findings, Keogh et al [45] compared vascular effects of diets high in PUFAs, MUFAs, SFAs, and CHOs and found no differences among the PUFA, MUFA, or CHO diets, with the high SFA diet resulting in a lower FMD response. However, baseline FMD values were not listed. Thus, a determination of whether the high-MUFA

diet improved FMD from baseline cannot be made [45]. Additionally, based on diet records, dietary cholesterol intake was significantly higher during the SFA intervention than all other diets, which could have confounded their results [45]. Based on the current results and previous literature, it can be reasonably determined that high serum LDL-C attenuates FMD, while short-term diets high in MUFA — which appear lower LDL-C — can reverse this reduction in the FMD response. However, this vascular response occurred after dietary interventions lasting only 4 to 5 weeks; thus, it would be imprudent to assume that these beneficial alterations would occur with habitually high levels of MUFA.

The HFB intervention lowered HDL-C, and HDL-C has been shown to improve vascular function. One explanation for these results could be related to alterations HDL functionality. Recently, it has been identified that the functionality of the HDL is far more important than HDL-C concentrations [92]. Various method of assessing functionality have been proposed, with apoprotein A1 levels and cholesterol efflux being the most conclusive [92]. To that end, beef consumption has been linked to increased apoprotein A1 concentrations in humans [23].

Of the existing literature on dietary interventions and vascular function, very few have assessed PWV. Those that have investigated PWV in response to dietary interventions have failed to find significant effects [45, 77]. The current findings support these results. Thus, our study provides additional evidence that PWV is not altered by short-term interventions.

Blood Pressure

Systolic and diastolic BP were lower during the HFB intervention than during the LFB. While the current study is the first to demonstrate a reduction in blood pressure as a result of consuming high-fat ground beef, available literature corroborates this physiological response as a result of increase MUFA intake. Rasmussen et al. [79] reported that increasing overall fat intake, consisting of a high proportion MUFAs, lowered both systolic and diastolic BP. Decreased BP as a result of increased MUFA intake also has been demonstrated in non-insulin dependent diabetes patients [43]. An inverse relationship between BP and MUFA consumption similarly has been noted in cross-sectional studies [109, 110]. Additionally, as reported by Ashton et al.[78], high-fat diets enriched with SFA did not increase BP. This finding in contrary to another relational study [111], but it appears that diets higher in fat with a high SFA content do not increase BP, whereas high-fat diets with a large portion of MUFAs lower BP. This runs counter to the widespread misconception that high-fat foods, specifically beef, are unhealthy food choices, especially for groups at increased risk for CVD. Short-term dietary interventions results are not completely indicative of habitual dietary outcomes, and as BP is affected by many physiological and behavioral factors, it cannot be stated that high-MUFA ground beef would decrease BP all populations.

Dietary Analysis

Total caloric intake did not change between any of the study time points. The HFB intervention resulted in higher total fat, SFA, and MUFA intake, whereas percent energy from CHO decreased during the HFB intervention. This is consistent with other beef

consumption studies [21-23, 99]. However, this is the first study to demonstrate a statistically significant decrease in CHO consumption subsequent to increased fat intake through the addition of ground beef. This finding may provide some benefit for individuals who are insulin resistant [33, 34].

Serum Lipids

In the current study, no change in TG was found. Additionally, both TC and HDL-C were lowered compared to entry by both the LFB and HFB, while only the HFB lowered LDL-C. These results are similar to those of Roussell [48], who added varying amounts of lean beef to the dietary approach to stop hypertension diet (DASH). During the Roussell et al. [48] intervention, serum HDL-C, LDL-C and TC all decreased as a result of increase lean protein consumption.

The depression of HDL-C and LDL-C as a result of consumption of the HFB was unanticipated, as previous research demonstrated that high-fat beef patties with a 1.1 MUFA/SFA ratio increased HDL-C [22]. However, a non-statistically significant reduction in LDL-C was also noted in the Gilmore et al. study [22], with a greater decrease produced by consumption of beef patties with a lower MUFA/SFA ratio (0.71). This may shed some light on our findings, as our HFB patties had a slightly lower MUFA/SFA ratio (1.05) compared to those used by Gilmore et al. [22]. Therefore, this lower MUFA/SFA ratio might have contributed to the significantly lower LDL-C observed in this study during the HFB intervention.

The specific fatty acids composition of our beef patties may have also contributed to these findings. Previous research has demonstrated that palmitic acid is primarily

cholesterolemic [26]. However, when palmitic acid is combined with myristic acid, as it was in the HFB and LFB interventions, the cholesterolemic effect is attenuated [26]. Stearic acid has been reported to lower both LDL-C and HDL-C [112] and oleic acid has also demonstrated to lower LDL-C [26, 112, 113]. These fatty acids were higher in our HFB than in the LFB patties. However, when pan broiled, the amount fatty acids decreased to a greater extent in HFB compared to LFB patties, which may have contributed to the similar results between interventions. While the fatty acid composition of the HFB and LFB may be related to these findings, it cannot be conclusively determined to be only factor influencing the drop in both HDL-C and LDL-C. Dietary components, other than the fat composition, of the beef may be contributing to serum lipids shifts. Unfortunately, the current data limit our ability to discern what this factor may be.

Insulin Resistance

IR was estimated from fasting glucose and insulin levels (HOMA-IR), BMI, serum HDL-C, TG levels (SPISE), and the TC/HDL-C ratio. Despite the decrease in percent CHO consumption, none of the calculated IR scores were altered by the HFB intervention. Previous research has shown a slight reduction in fasting insulin levels as a result of beef consumption [22]. The discrepancy between these results and our own could be related to the small sample sized used in this study for HOMA-IR values. Due to a lab error, only 11 of our 23 subjects were able to be used for HOMA-IR calculations. Additionally, the reduction in HDL-C did not produce significant SPISE changes. This is likely due to the constancy of the TG levels across all study time points. Similarly, because both HDL-C

and TC levels decreased during both the LFB and HFB intervention, no changes were observed in the TC/HDL-C ratio.

Body Composition and Fitness

Body composition remained stable throughout the course of the study, except for a small 0.36% and 0.51% increase in percent body fat during the LFB and HFB, respectively relative to washout. We are not able to explain these findings, as neither body weight nor lean mass changed during the study. It is plausible that hydration status or machine error of the DXA machine itself explains these alterations [114]. Specifically, the technical error of measurement for fat mass measured from a single DXA machine is shown to 1.9% [115]. This is considerably greater than the minor increases in percent fat observed in this study. Additionally, a compliance check prior to each lab visit indicated all subjects were fasted and normally hydrated, though specific water consumption preceding to each visit were not measured. Moreover, adding to the peculiar nature of the increases in percent body fat, no significant difference was observed in either caloric consumption or daily energy expenditure throughout the study.

Aerobic fitness did not change between the initial and final visit of the study. Participants were asked to avoid any changes in physical activity, which was supported by 7-day activity log and further verified in the maintenance of aerobic capacity. This removes the potential for physical activity to be a confounding factor in changes observed in other measures during this study.

Conclusion

Our results demonstrated that the addition of either low-fat or high-fat ground beef does not result in any deleterious effects on vascular function. In fact, the HFB intervention improved the FMD response, which is known to decrease risk of CVD [6]. An additional benefit of the HFB was a cardio-protective decrease in both SBP and DBP relative to LFB and entry measure, respectively. Furthermore, the HFB intervention resulted in a significant decrease in LDL-C. One caveat to the addition of either LFB or HFB to the diet seen in this study was the reduction in HDL-C. However, this may or may not be detrimental depending on the functionality of the HDL particles themselves. Thus, contrary to common conception, our results suggested that HFB may be a healthier choice than LFB when added to a habitual diet.

Future Research

In the current study we demonstrated a novel serum lipid alteration as a result of both the LFB and HFB intervention. While the specific fatty acid composition of the beef patties was a factor in these shifts, it is the authors' opinion that an additional component of the beef patties may also be contributing to these changes. This may be linked to the increased protein intake or decreased CHO intake in the LFB and HFB intervention respectively. Future research is required to determine whether additional intrinsic factors, other than fatty acid composition in the beef may be altering serum lipids. Additionally, HDL functionality assessments (apolipoprotein A1 and cholesterol efflux) should be implemented to determine if beef alters these markers, which appear to be more important that HLD-C concentration alone.

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APPENDIX A

INFORMED CONSENT DOCUMENT

What happens if I say yes, I want to be in this research?
You will be asked to eat ground beef patties 5 times a week for five weeks. There will be a four week break where you don't eat any patties. Then you will be asked to eat ground beef patties 5 times a week for another five weeks. Some of the patties will be low-fat and some will be high-fat, but you won't be told which ones you are eating. Some of the procedures are optional, as described in the study visits below. You can choose to not participate in the optional procedures and still participate in the research study.

The procedures for each of the study visits are listed below. For the visits where blood is collected, you may be asked to fast (no food or drink except water) for at least 10 hours:

Screening Visit (up to 2 hours):
- Body weight, blood pressure, body temperature, and heart rate will be measured
- A blood sample of approximately 10 mL (about 2 teaspoons) will be collected.
- The researchers will review the results to determine if you are eligible to participate

Visit 1 (about 1 hour):
- A Registered Dietitian will provide diet instructions, sample menus and cooking instructions for the patties
- You will receive the first set of patties (low-fat or high-fat)
- You will be asked to complete a 3-day diet record in NutriBase (software that collects diet and nutrient information) before you begin eating the beef patties. This will be done at home and the research team will provide instructions to you
- You will be asked to complete a 3-day diet record in NutriBase during week 5 of the patty consumption
- Optional procedures (about 1 additional hour):
    - An ultrasound machine will be used to measure flow mediated dilation (FMD) and pulse wave velocity (PWV) of an artery in your forearm and pelvic area. Both of these are measures of the health of your blood vessels. These procedures will require you to lie still on your back for about 10-15 minutes. During the procedures, a blood pressure cuff will be inflated on your arm, three sticky pads will be placed on your chest to measure your heart activity by an electrocardiogram, and an ultrasound probe will be glided across your upper arm, neck, and pelvic area to collect images used to measure blood flow in and size of your arteries.
    - Measurement of your body muscle, bone, and fat tissue using Dual Energy X-ray Absorptiometry (DXA). For this test, you will lie comfortably on a large, padded table while a scanning arm passes slowly over your body. You will be fully clothed, and the scan takes about 7 minutes.
    - Instructions on how to record daily physical activity (DPA). You will be asked to keep track of time during 7 consecutive days when you are engaged in any type of physical activity or physical work.
    - Complete Health and Lifestyle History questionnaire

IRB NUMBER: IRB2018-0755D
IRB APPROVAL DATE: 01/17/2019

What are the risks of being in this study?

- The risks of having blood drawn include slight pain when the needle is inserted. You may develop a harmless black and blue mark, and your arm may be sore. Occasionally, some people feel dizzy or lightheaded when blood is drawn. They may become sweaty, feel cold or tingly, and may faint or throw up. Risks that are possible but unlikely include infection, nerve damage, and puncturing an artery instead of a vein.
- Optional procedures
    - There is an unlikely risk that the submaximal exercise test can cause abnormal blood pressure, abnormal heart rhythm, fainting, shortness of breath, and, in rare instances, heart attack.
    - During the FMD procedure, your hand might become slightly numb during the 5 minutes of blood pressure cuff inflation around the forearm. This slight tingling is normal and resolves quickly following cuff release. The ultrasound gel and sticky pads used may sometimes cause skin irritation.
    - The main risk of the DXA is that you will be exposed to very low levels of x-ray radiation (2.5 mRem), which is about equal to the level of radiation associated an airplane flight from Houston to Dallas, or the atmospheric background radiation during 2 days in College Station. Studies have shown that getting a lot of radiation at one time or getting many small doses over time may cause cancer. There is no known minimum level of radiation exposure that is recognized as being free of all risk. However, the probability of harm associated with the amount of radiation exposure that you will receive in this study is considered low when compared with everyday risks each person receives in a year.

What are the costs of being in the research?

Taking part in this research study will not lead to any costs to you. The beef patties you are asked to consume for the study are provided at no cost to you.

Will being in this study help me in any way?

There are no benefits to you from taking part in this research. We hope the results of this study will help us learn more about how the consumption of high-fat ground beef may reduce risk factors for cardiovascular disease. Should you decide to complete the optional procedures, you will receive additional information about your health at no cost to you.

What happens to the information collected for the research?

Efforts will be made to limit the use and disclosure of your personal information, including research study records, to people who have a need to review this information. We cannot promise complete privacy. Organizations that may inspect and copy your information include the funding organization, the TAMU HRPP/IRB and other representatives of this organization.

IRB NUMBER: IRB2018-0755D
IRB APPROVAL DATE: 01/17/2019

What else do I need to know?
Texas A&M University has no program to pay for medical care for research-related injury. This does not keep you from seeking to be paid back for care required because of a bad outcome.

If you agree to take part in this research, you may receive up to $100.00 for your time and effort. You will receive a $50.00 gift card after you complete Visit 2 and a $50.00 gift card after you complete Visit 4. There will be no additional payments made for completing the optional procedures.

OPTIONAL PROCEDURES

Please indicate whether or not you agree to participate in the optional procedures by initialing one of the choices below. Your decision does not affect your participation in the main study:

_____ **YES,** I agree to participate in the optional procedures for this research study.

_____ **NO,** I do not agree to participate in the optional procedures for this research study.

Your signature documents your permission to take part in this research.

Signature of subject	Date
Printed name of subject	
Signature of person obtaining consent	Date
Printed name of person obtaining consent	

APPENDIX B

APPLIED EXERCISE SCIENCE LABORATORY
SEVEN DAY PHYSICAL ACTIVITY & STEP RECORD*

Name:_____ Age:_____ Ht:_____ Wt:_____

Address: _____ Phone: _____

_____ Email: _____

Occupation:_____ Medications: _____

DIRECTIONS: This Seven Day Physical Activity Record is designed to measure your habitual physical activities over the course of one week. You are asked to record your sleep habits as well as the physical activities you participated in over the course of the past seven days; include both occupational and leisure-time physical activities.

1. **BEFORE READING ANY FURTHER, PLEASE REVIEW ATTACHMENT 1 FOR EXAMPLES OF LIGHT, MODERATE, HARD, AND VERY HARD PHYSICAL ACTIVITIES!**

2. **DO NOT RECORD LIGHT ACTIVITIES.** See Attachment 1 for examples of LIGHT ACTIVITIES. Most of you will spend the majority of your waking hours in light activity. For example, a laboratory worker may be on their feet all day and may feel "fatigued", but the energy cost is in the "light" category. However, we need you to record the number of hours you spend sleeping.

3. For all other physical activities, which may be classified as moderate, hard, or very hard, **DOCUMENT ONLY THE TIME ACTUALLY SPENT PERFORMING THE ACTIVITY**: Include both occupational and leisure-time activities. For example, the laboratory worker in the illustration given above may spend a number of hours stocking shelves with supplies, which would likely be moderate exercise. It is unlikely, however, that they would spend an 8 hour day performing this task, and time should be subtracted for lunch, breaks, etc. Similarly, being at the pool for 2 hours but swimming for 15 minutes should be recorded as 15 minutes, not 2 hours.

4. For this record to be representative of your normal physical activity habits, it is critical that the week's activities be "normal" for you. For example, a week in which you take a holiday or a few days vacation would clearly NOT be a "normal" week for you. **IF THE UPCOMING WEEK'S ACTIVITIES WILL NOT REPRESENT YOUR NORMAL ACTIVITY PATTERNS, THEN PLEASE DO NOT COMPLETE THIS FORM - WAIT FOR A WEEK THAT WILL REFLECT YOUR NORMAL PHYSICAL ACTIVITY PATTERNS.** Note that a week is not necessarily Sunday through Saturday, but may be any consecutive 7 day period.

5. Use the record forms beginning on the next page to record; (1) the physical activity, (2) the total hours/minutes spent performing the activity, (3) and rate how hard you worked at the particular physical activity, and (4) your total steps for the day, if known. Use the following scale to rate how hard you worked.

6. Return this completed record to the laboratory staff at your next laboratory visit.

SCALE TO RATE HOW HARD YOU WORK

1 - Barely breaking a sweat; breathing just slightly elevated.
2 - Moderate sweating; breathing significantly above normal, but could talk normally.
3 - Heavy sweating; breathing very heavy to nearly winded, could NOT talk normally.

PLEASE GO TO THE NEXT PAGE TO BEGIN YOUR SEVEN DAY ACTIVITY RECORD

*From: Blair et al., Assessment of habitual physical activity by a seven day recall in a community survey and controlled experiments. Am. J. Epidem. 122:794-804, 1985.

1

DAY ONE

Date:_____ **Day of Week:**_____

Activity	**TOTAL TIME (Hours:Minutes)**	**HOW HARD (1,2,3)**
Sleeping, including naps		
Total Steps for the Day If Known (e.g., FitBit, Apple Watch)	**Total Steps =**	

DAY TWO

Date:_____ **Day of Week:**_____

Activity	**TOTAL TIME (Hours:Minutes)**	**HOW HARD (1,2,3)**
Sleeping, including naps		
Total Steps for the Day If Known (e.g., FitBit, Apple Watch)	**Total Steps =**	

DAY SEVEN

Date:_____ **Day of Week:**_____

Activity	**TOTAL TIME (Hours:Minutes)**	**HOW HARD (1,2,3)**
Sleeping, including naps		
Total Steps for the Day If Known (e.g., FitBit, Apple Watch)	**Total Steps =**	

CONCLUDING QUESTIONS

1. Would you say that during the past week you were (check one):
   - _____less active than usual
   - _____about as active as usual
   - _____more active than usual

2. Which statement most nearly describes your attitude toward leisure-time physical activity?
   - _____I absolutely detest physical activity and exertion of any type.
   - _____I do not enjoy physical activity or exertion of any type.
   - _____I do not like activities which make me sweat, but I do like some types of light activities.
   - _____I enjoy light physical activity of many types, and occasionally like hard physical activity.
   - _____I thoroughly enjoy all types of physical activities, even those which are hard and very hard.

3. When you have time off from work (weekends/vacations) or during work breaks (lunch, etc), how often do you participate in physical activities, including recreational sports, which would be considered moderate to very hard?
   - _____Never
   - _____Seldom
   - _____Sometimes/Irregularly
   - _____Frequently/Regularly
   - _____Almost Always

THANK YOU VERY MUCH FOR YOUR TIME AND ACCURACY IN COMPLETING THIS QUESTIONNAIRE. THIS INFORMATION IS INDISPENSABLE FOR OUR STUDY, AND WE THANK YOU FOR YOUR WILLINGNESS TO COOPERATE IN COMPLETING THIS FORM.

ATTACHMENT 1

CLASSIFICATION OF PHYSICAL ACTIVITY

LIGHT ACTIVITIES

Household/Occupational

- *Bakery, general*
- *Bookbinding*
- *Carpet sweeping*
- *Cooking*
- *Eating (sitting)*
- *Farming*
  - *driving harvester*
  - *driving tractor*
  - *milking by machine*
- *Ironing*
- *Knitting, sewing*
- *Lying at ease*
- *Machine-tooling*
  - *machining*
  - *working sheet metal*
- *Painting, inside*
- *Printing*
- *Shoe repair, general*
- *Sitting quietly*
- *Standing quietly*
- *Tailoring*
  - *cutting*
  - *hand-sewing*
  - *machine-sewing*
- *Typing (electric and manual)*
- *Wallpapering*
- *Watch repairing*
- *Writing (sitting)*

Sports/Recreational

- *Billiards*
- *Canoeing (leisure)*
- *Card playing*
- *Drawing (standing)*
- *Horse racing (walking)*
- *Music Playing*
  - *accordion (sitting)*
  - *cello (sitting)*
  - *conducting*
  - *flute (sitting)*
  - *horn (sitting)*
  - *piano (sitting)*
  - *trumpet (standing)*
  - *violin (sitting)*
  - *woodwind (sitting)*

MODERATE ACTIVITIES

Household/Occupational

- *Carpentry (general)*
- *Cleaning*
- *Electrical work*
- *Farming*
  - *feeding animals*
  - *milking by hand*
- *Food shopping*
- *Gardening*
  - *weeding*
  - *hedging*
  - *raking*
- *Sawing*
- *Woodworking*
- *Shopping/Walking*
- *Locksmith*
- *Machine-tooling*
  - *operating lathe*
  - *tapping and drilling*
  - *welding*
- *Mopping floor*
- *Painting (outside)*
- *Planting seedlings*
- *Plastering*
- *Scraping paint*
- *Stock clerking*
- *Pressing (tailoring)*
- *Window cleaning*

Sports/Recreational

- *Archery*
- *Croquet*
- *Cycling, leisure 5.5 mph*
- *Dancing (ballroom)*
- *Gymnastics*
- *Music playing*
  - *drums (sitting)*
  - *organ (sitting)*
- *Table tennis*
- *Treading water, normal*
- *Volleyball*
- *Walking, normal pace*

HARD ACTIVITIES

Household/Occupational

- *Coal Mining*
  - *drilling coal, rock*
  - *erecting supports*
  - *shoveling coal*
- *Farming*
  - *feeding cattle*
  - *shoveling grain*
- *Forestry*
  - *ax chopping, slow*
  - *hoeing*
  - *planting by hand*
  - *stacking firewood*
- *Furriery*
- *Scrubbing floors*
- *Steel mill, working in*
  - *fettling*
  - *forging*
  - *tipping molds*
- *Pushmowing yard*

Sports/Recreational

- *Badminton*
- *Canoeing (racing)*
- *Resistance/Circuit training*
  - *Universal*
  - *Nautilus*
  - *Free weights*
- *Cricket*
- *Cycling, leisure 9.4 mph*
- *Dancing (medium aerobic)*
- *Golf (without cart)*
- *Horse racing (trotting)*
- *Skiing, soft snow (leisure)*
- *Tennis*

VERY HARD ACTIVITIES

Household/Occupational

- *Farming*
  - *barn cleaning*
  - *forking straw bales*
- *Forestry*
  - *ax chopping, fast*
  - *barking trees*
  - *carrying logs*
  - *felling trees*
  - *sawing by hand*
  - *trimming trees*
- *Digging*
- *Horse grooming*
- *Marching, rapid*
- *Steel mill, working in*
  - *hand rolling*
  - *merchant mill rolling*
  - *removing slag*
  - *tending furnace*

Sports/Recreational

- *Basketball*
- *Boxing*
- *Circuit training*
- *Hydra-Fitness*
- *Climbing hills*
  - *no load*
  - *5 kg load*
  - *10 kg load*
  - *20 kg load*
- *Cycling (racing)*
- *Dancing*
  - *aerobic (intense)*
  - *twist" and "wiggle"*
- *Horse racing (galloping)*
- *Judo*
- *Jump rope (70-145 per min)*
- *Racquetball*
- *Running (5-11 min. mile)*
- *Skiing, hard snow*
- *Skindiving*
- *Snowshoeing, soft snow*
- *Squash*
- *Swimming (all strokes)*
- *Field hockey*
- *Football*

APPENDIX C

Dear Study Participant,
For you to be a part of this study, we need you to complete a three-day diet record. We need you to record everything that you consume for three consecutive days. These should include two weekdays and one weekend day. (This Thursday would be an ideal time to start!) Instructions for keeping your diet record are attached

For recording, there are two options. The first option is to use MyFitnessPal. Once you have a MyFitnessPal account you can either use the app on your smartphone or access MyFitnessPal online on your computer. Using this program you will search and enter food items that correspond with what you eat and/or drink. If you use MyFitnessPal you can set the options so that I will be able to see the days that you are wanting to be used for this study. I will provide instructions for this on an attached handout.

The second option for recording your intake is to do it the old fashioned way and write down everything that you eat and email your diet record to me.

Please begin you diet record soon and submit them to me before coming for your blood sampling.

Thank you.

Dana Smith, PhD, RD, LD

Click “My Home” then “Settings” then “Diary settings”.
Set your preference for “Public” or “Friends Only”, and save your changes.
At the end of the day, when you have finished logging your diary, select “Complete This Entry.” This will create a convenient “View Diary” link for your friends (me).

APPENDIX D

Food Diary - How to Keep Track of What You Eat

1 Record everything you eat and drink. The more accurate your food record is the more useful it will be. Keep a record everything that goes into your mouth. Include all meals, drinks, snacks and even nibbles of food you eat while you cook

- Be very specific, and break complicated foods down by ingredient. For example, instead of writing down "turkey sandwich," write out the quantity of bread, turkey and condiments as separate entries. Handle other mixed foods, like casseroles and smoothies, in a similar way. This will help you remember what is in foods or the total amount of calories.
- Don't forget to record snacks or random odds and ends you eat, like a cookie offered at work.
- Record all beverages. Don't forget to track your total water intake as well.

2 Write down accurate quantities. A food scale would be most helpful. Measuring cups are practically a must.

- List how much for all food/drink items. This might be in volume (1/2 cup), weight (2 ounces net weight), volume (8 fluid ounces), or quantity (12 pretzels).
- Measure foods using cups, bowls, or other containers that are a specific measurement. This will help with the accuracy of your journal. Guesstimating or "eye-balling" is not accurate and typically leads to underestimating.
- Sometimes estimating will be necessary such as when you eat out. If you are at a chain restaurant, check online for information on the quantities of ingredients per serving.
- When needed compare servings to common items. For example: a deck of cards is 3 ounces or 1/2 cup, one egg is 2 oz or 1/4 cup, a golf or ping pong ball is 2 Tbsp, a tennis ball is ¾ cup, and a baseball is 1 cup.

3 Tell what kind: Include the type of food/drink. Be as specific as you can. For example: chicken is not very descriptive, but

- Be as specific as you can. For example: chicken is not very descriptive, but rather include preparation method (fried chicken tenders, stewed skinless boneless chicken thighs, or buffalo chicken wings).
- Include any extras. For example: lettuce, tomato, and ketchup on a burger, sugar in coffee, and sauces or gravy.

APPENDIX E

COMPLIANCE CHECK LIST

NAME: _____ DATE: _____ TIME:_____

Check YES or NO for the following

	YES	NO	
1. 12 hours fasted?	☐	☐	If no, time of last meal?_____
2. Consumed caffeine in past 12 hours?	☐	☐	If yes, explain_____ _____
3. Consumed alcohol in past 12 hours?	☐	☐	If yes, explain_____ _____
4. Fluid other than water past 12 hours?	☐	☐	If yes, explain_____ _____
5. Exercise last 48 hours?	☐	☐	If yes, explain_____ _____
6. Have you changed your Exercise habits?	☐	☐	If yes, explain_____ _____
7. Have you changed your eating habits?	☐	☐	If yes, explain_____ _____
8. Do you take any supplement?	☐	☐	If yes, explain_____ _____
9. Do you take any medications?	☐	☐	If yes, explain_____ _____

APPLIED EXERCISE SCIENCE LABORATORY 12/18
DEPARTMENT OF HEALTH AND KINESIOLOGY
TEXAS A&M UNIVERSITY, COLLEGE STATION, TEXAS 77843

HEALTH AND LIFE STYLE HISTORY

INSTRUCTIONS: *Please complete this form as accurately and completely as possible, and bring it to your appointment. The information you provide will be used to evaluate your health by the physician or exercise physiologist who will see you in our laboratory. All information will be treated as privileged and confidential.*

IDENTIFICATION AND GENERAL INFORMATION

1. Name:_____ 2: UIN:_____ 3. Today's Date:_____
Last First M.I. mo/da/yr

4. Age:_____yrs 5. Date of Birth:_____ 6. Gender: Male Female

7. Home Address:_____
Street City State Zip

8. Office Address or Department:_____ Email: _____

9. Home Phone #:_____ 10. Office Phone #:_____ 11. Occupation:_____

12. Personal Physician: _____
Name Street City State Zip

ILLNESSES AND MEDICAL PROBLEMS Check all the conditions or diseases for which you have been diagnosed and/or treated. Also give the date of occurrence or diagnosis. If you suspect that you may suffer from one of the conditions, please indicate this in the right margin after the date.

Condition Diagnosed	Yes	Date (mo/yr)
13. AIDS	___	_____
14. Alcoholism	___	_____
15. Anemia	___	_____
16. Arthritis	___	_____
17. Asthma	___	_____
18. Bronchitis (chronic)	___	_____
19. Cancer:		
20. Breast	___	_____
21. Cervix	___	_____
22. Colon	___	_____
23. Lung	___	_____
24. Uterus	___	_____
25. Other_____	___	_____
26. Cirrhosis (liver)	___	_____
27. Colitis (ulcerative)	___	_____
28. Depression	___	_____
29. Diabetes	___	_____
30. Emphysema	___	_____
31. Epilepsy	___	_____
32. Frequent Bleeding	___	_____
33. Hepatitis B	___	_____
34. Pneumonia	___	_____
35. Tuberculosis	___	_____
36. Renal/Kidney Problems	___	_____
37. Other_____	___	_____

Cardiovascular Problems Diagnosed

	Yes	Date (mo/yr)
38. Stroke	___	_____
39. Heart Attack	___	_____
40. Coronary Disease		
41. Rheumatic Fever	___	_____
	___	_____
42. Rheumatic Heart Disease	___	_____
43. Heart Valve Problem	___	_____
44. Heart Murmur	___	_____
45. Enlarged Heart	___	_____
46. Heart Rhythm Problem	___	_____
47. Other Heart Problems	___	_____
48. High Blood Pressure (controlled)	___	_____
49. High Blood Pressure (uncontrolled)	___	_____
50. High Blood Cholesterol	___	_____
51. Diseases of the Arteries	___	_____
52. Phlebitis	___	_____
53. Systemic or Pulmonary Embolus	___	_____
54. Other _____	___	_____
55. Other _____	___	_____

Do You Now Have or Have You Recently Had:

	Yes	Most Recent Occurrence (mo/yr)
56. Seizures	___	_____
57. Chest pain on exertion relieved by rest	___	_____
58. Chest pain not always associated with exertion?	___	_____
59. Shortness of breath lying down, relieved by sitting up?	___	_____
60. Unexpected weight loss (more than 10 lbs)?	___	_____
61. Unexpected rectal bleeding	___	_____
62. Leg Pain after walking short distances?	___	_____

Women Only (Men May Skip to Number 68)
Please Answer the Following:

	Yes	Date (mo/yr)
63. Was your last pelvic exam or Pap smear abnormal?	___	_____
64. Do you have menstrual period problems?	___	_____
65. List number of menstrual periods in last year	____	
66. When was your last menstrual period?(1st day) month_____day_____yr____		
67. Please give number of: pregnancies_____living children _____		

Men And Women Answer the Following:

Have you ever had:	Yes	Date (mo/yr)
68. A chest x-ray?	___	_____
69. An abnormal chest x-ray?	___	_____
70. An ECG (electrocardiogram)?	___	_____
71. An abnormal ECG?	___	_____
72. An exercise stress test?	___	_____
73. An abnormal exercise stress test?		

MEDICATIONS Check those medications which you are currently taking on a regular basis. If your medication is not listed, please list it in blanks marked "other".

74. ___None
75. ___Aldomet
76. ___Allergy Medication
77. ___Aminophylline
78. ___Antacids
79. ___Aspirin
80. ___Asthma Inhaler
81. ___Birth control pills
82. ___Blocardren (Timolol)
83. ___Bumex
84. ___Butazolidin
85. ___Catapres
86. ___Cardizem (Diltiazem)
87. ___Corgard (Nadolol)
88. ___Coumadin
89. ___Crystodigin
90. ___Diabinese
91. ___Digitalis
92. ___Digitoxin
93. ___Digoxin (Lanoxin)
94. ___Dilantin
95. ___Dyazide
96. ___Dymelor
97. ___Feldane
98. ___Hydrodiuril
99. ___Hydropres
100. ___Hygroton
101. ___Inderal (Propranolol)
102. ___Insulin
103. ___Iron
104. ___Isoptin (Verapamil)
105. ___Isordil
106. ___Lanoxin
107. ___Lasix
108. ___Librium
109. ___Lopressor
110. ___Maxizide
111. ___Minipress
112. ___Motrin
113. ___Muscle Relaxant
114. ___Naprosyn
115. ___Nitro-bid
116. ___Nitroglycerin
117. ___Norpace
118. ___Norvasc
119. ___Oral hypoglycemic agents
120. ___Orinase
121. ___Penicillin
122. ___Persantine
123. ___Potassium
124. ___Pravachol
125. ___Prednisone
126. ___Pro-banthine
127. ___Procardia (Nifedipine)
128. ___Procan SR
129. ___Pronestyl
130. ___Quinaglut
131. ___Quinidine
132. ___Reglan
133. ___Reserpine
134. ___Ser-Ap-Es
135. ___Sleeping pills
136. ___Tagamet
137. ___Tenormin (Atenolol)
138. ___Thiazides
139. ___Thyroid
140. ___Trandate (Labetalol)
141. ___Valium
142. ___Visken (Pindolol)
143. ___Vitamins
144. ___Zantac
145. ___Zyloprim
146. ___Other:_____
147. ___Other:_____
148. ___Other:_____
149. ___Other:_____
150. ___Other:_____
151. ___Other:_____

SURGICAL HISTORY Check the surgical procedures you have had and give the date of the surgery.

	Yes	Date (mo/yr)
152. Appendectomy	___	_____
153. Knee Surgery or ankle surgery	___	_____
154. Arm or shoulder surgery	___	_____
155. Back surgery	___	_____
156. Hysterectomy (women only)	___	_____
157. Vasectomy (men only)	___	_____
Cancer related surgery		
158. Breast	___	_____
159. Cervix	___	_____
160. Colon	___	_____
161. Lung	___	_____
162. Uterus	___	_____
163. Liver	___	_____
164. Kidney	___	_____
165. Other (Specify)_____	___	_____

	Yes	Date (mo/yr)
Heart surgery		
166. Heart catheterization		
167. Angioplasty (PTCA)	___	_____
	___	_____
168. Coronary bypass (CABG)	___	_____
169. Valve repair/replacement	___	_____
170. Other	___	_____

ORTHOPEDIC PROBLEMS Place a check in the blank to indicate any of the following orthopedic problems you may have.

		Yes	Most Recent Occurrence (mo/yr)
171.	Low back pain		
172.	Shoulder pain	___	_____
173.	Elbow pain	___	_____
174.	Wrist or hand pain	___	_____
175.	Hip problems	___	_____
176.	Knee problems	___	_____
		___	_____
177.	Ankle or foot problems	___	_____
178.	Work or exercise limited by orthopedic problem?	___	_____
179.	Other _____	___	_____

FAMILY HISTORY Please identify blood relatives who have been diagnosed as having the following diseases and give their age at time of diagnosis.

	Yes	Age at Diagnosis
Heart Disease		
180. Father		
181. Mother	___	_____
182. Sibling	___	_____
183. Paternal grandparent	___	_____
184. Maternal grandparent	___	_____
	___	_____
	___	_____
High Blood Pressure		
185. Father	___	_____
186. Mother	___	_____
187. Sibling	___	_____
188. Paternal grandparent	___	_____
189. Maternal grandparent	___	_____
Stroke		
190. Father	___	_____
191. Mother	___	_____
192. Sibling	___	_____
193. Paternal grandparent	___	_____
194. Maternal grandparent	___	_____

Have any of your blood relatives noted above had any of the following?

	Yes	Age Diagnosed
195. Heart attack under age 50	___	_____
196. Heart operations	___	_____
197. Stroke under age 50	___	_____
198. Elevated cholesterol	___	_____
199. High blood pressure under age 40	___	_____

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200. Diabetes	___	_____
201. Obesity	___	_____
202. Cancer under age 60	___	_____

HISTORY OF TOBACCO USE

	Yes	No
203. Have you ever used tobacco products including smokeless?	___	____
204. Do you presently use tobacco products?	___	____

If you did or do use tobacco, please indicate the average amount used per day and the age you started.

	Amount	Age Started
205. Cigarettes (number cig. per day)	___	_____
206. Cigars (number per day)	___	_____
207. Pipe (number pipefuls per day)	___	_____
208. Smokeless (fraction of packs/tins/day)	___	_____

209. If you have quit using tobacco, when was it? (mo/yr) _____

210. If yes to above, how old were you when you quit using tobacco? _____

SMOKING/STRESS/TENSION

Smoking - My smoking history is:
Never____[0] Not for last 10 years____[2] Not for last 5 years____[3]
Recently quit____[4] Still smoke____[5]

Stress / Tension
Rate how closely you agree with each of the following statements by filling in the blank preceding each statement with a number from 1 to 10.

Strongly Disagree				Agree Somewhat				Strongly Agree	
1	2	3	4	5	6	7	8	9	10

_____1. I can't honestly say what I really think or get things off my chest at work, school, or home.
_____2. I seem to have lots of responsibilities but little authority.
_____3. I seldom receive adequate acknowledgment or appreciation when I do a good job.
_____4. I have the impression that I am repeatedly picked on or discriminated against.
_____5. I feel I am unable to use my talents effectively or to their full potential.
_____6. I tend to argue frequently with co-workers, customers, teachers, or other people.
_____7. I don't have enough time for family and social obligation or personal needs.
_____8. Most of the time I have little control over my life at work, school or home.
_____9. I rarely have enough time to do a good job or accomplish what I want to.
_____10. In general, I'm not particularly proud of or satisfied with what I do.

ALCOHOL CONSUMPTION

211. Do you drink alcoholic beverages? Yes No

If **YES,** please indicate the type and amount you consume per week.

	Amount
212. Glasses of beer per week (12 oz.)	_____
213. Glasses of wine per week (8 oz.)	_____
214. Ounces of liquor (cordials=1 oz)	_____
215. Ounces of hard liquor (shot=1 oz)	_____

SPORT ACTIVITIES Check those activities in which you regularly participate or in which you have participated over the past year. Also indicate the approximate number of months in the last year you engaged in these activities, the number of times per month, the number of minutes per session, and the intensity of your participation. **Note:** Rate your intensity on a scale of **1** to **10** with **1** being very low and **10** being very high intensity.

	# of months per year	# times per month	Min/session	Intensity (1=low;10=high)
216. Basketball	___	___	___	___
217. Volleyball	___	___	___	___
218. Softball	___	___	___	___
219. Baseball	___	___	___	___
220. Jogging	___	___	___	___
221. Running	___	___	___	___
222. Swimming	___	___	___	___
223. Bicycling	___	___	___	___
224. Golf	___	___	___	___
225. Tennis	___	___	___	___
226. Badminton	___	___	___	___
227. Racquetball	___	___	___	___
228. Handball	___	___	___	___
229. Table Tennis	___	___	___	___
230. Sailing	___	___	___	___
231. Water Skiing	___	___	___	___
232. Horseback Riding	___	___	___	___
233. Bowling	___	___	___	___
234. Calisthenics	___	___	___	___
235. Walking	___	___	___	___
236. Canoeing/Rowing	___	___	___	___
237. Fishing	___	___	___	___
238. Hunting	___	___	___	___
239. Dancing	___	___	___	___
240. Skating	___	___	___	___
241. Soccer	___	___	___	___
242. Lawnwork/Yard Care	___	___	___	___
243. Gardening	___	___	___	___
244. Housework	___	___	___	___
Other_____	___	___	___	___
Other_____	___	___	___	___
Other_____	___	___	___	___

Have you ever participated in a triathlon or cycling competition? _____

If yes, what was the distance? (List all that apply) _____

If yes, was it as part of a team, for competition, or for recreation? _____

If yes, how did you compare to others in your age category? _____

In addition to the above information that you have listed, if you are aware of any other conditions, symptoms, or special circumstances that might be related to your overall health and well-being, please give a detailed explanation here.

_____
_____
_____

Subject's signature _ **Date:** _____

Witness signature_____**Date:** _____