EFFECTS OF A HIGH OLEIC ACID BEEF DIET ON CARDIOVASCULAR DISEASE RISK FACTORS OF HUMAN SUBJECTS

A Dissertation

by

THADDEUS HUNTER ADAMS

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

August 2012

Major Subject: Nutrition

Effects of a High Oleic Acid Beef Diet on

Cardiovascular Disease Risk Factors of Human Subjects

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Approved by:

Chair of Committee,	Stephen B. Smith
Committee Members,	Rosemary L. Walzem
	Jason E. Sawyer
	James N. Derr
Intercollegiate Faculty Chair,	Rosemary L. Walzem

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ABSTRACT

Effects of a High Oleic Acid Beef Diet on Cardiovascular Disease Risk Factors of Human Subjects. (August 2012)

Thaddeus Hunter Adams, B.S., Texas A&M University;

M.S., Texas A&M University

Chair of Advisory Committee: Dr. Stephen B. Smith

The consumption of high-fat hamburger enriched with saturated fatty acids (SFA) and *trans*-fatty acids (TFA) may increase risk factors for cardiovascular disease, whereas hamburger enriched with monounsaturated fatty acids (MUFA) may have the opposite effect. Ten mildly hypercholesterolemic men consumed five, 114-g hamburger patties per week for two consecutive phases. Participants consumed low-MUFA (high SFA) hamburger (MUFA:SFA = 0.95; produced from pasture-fed cattle) for 5 wk, consumed their habitual diets for 3 wk, and then consumed high-MUFA hamburger (MUFA:SFA = 1.31; produced from grain-fed cattle) for 5 wk. These MUFA:SFA were typical of ranges observed for retail ground beef. Relative to habitual levels and levels during the high-MUFA phase, the low-MUFA hamburger: increased plasma palmitic acid, palmitoleic acid, and triacylglycerols ($P < 0.01$); decreased HDL cholesterol (HDL-C) and LDL particle diameter percentile distributions ($P < 0.05$); and had no effect on LDL-C or plasma glucose ($P > 0.10$). Plasma palmitoleic acid was positively correlated with triacylglycerols (r = 0.90), VLDL-C (r = 0.73), and the LDL:HDL (r = 0.45), and was

negatively correlated with plasma HDL-C (r = -0.58), whereas plasma palmitic, stearic, and oleic acid were negatively correlated with LDL particle diameter (all $P \leq 0.05$). Because plasma palmitoleic acid was derived from Δ9 desaturation of palmitic acid in the liver, we conclude that alterations in hepatic stearoyl-CoA desaturase activity may have been responsible for the variation in HDL-C and triacylglycerols caused by the low-MUFA and high-MUFA hamburgers.

Cattle with a genetic predisposition to deposit MUFA in their lean and fat tissues, such as Wagyu cattle can be used to produce beef products that are especially enriched with oleic acid and lower in SFA and TFA, and feeding practices can further enhance the composition of beef fat. This indicates that ground beef or hamburger products can be produced that are naturally enriched with oleic acid, and conversely that certain production practices can impair the nutritional quality of beef fat. Finally, we cannot discern from this study design whether the high-MUFA hamburger reversed the effects of the low-MUFA hamburger, or whether the subjects gradually adapted to the elevated intake of total fat. It is clear, however, that the high-MUFA hamburger did not exacerbate any of the effects of the low-MUFA hamburger and can be viewed as at least neutral in its effects on HDL-C and triacylglycerols.

DEDICATION

This work is dedicated first to my grandfather Hershell Garland Adams. He always wanted to find the time to complete his own doctorate and instead chose to complete his family. The balance of work and family challenges me as well, and I believe he chose wisely. As a result of his lifelong achievements, he succeeded in providing a legacy of education in our family that surpassed his lack of a doctorate. He influenced many throughout Texas as a teacher, superintendent and father figure. I would like to follow his example as a Christian, a man, and a leader.

This work is also dedicated to my friend, Byron Javar Brown Taylor. Byron showed me the value of sharing a meal and the magic of breaking bread with others. He showed me kindness, listened to my worries and championed my cause. We spent countless hours together cooking for the community that we share in. We had true fellowship in both joy and sorrow.

These men were a part of me, now gone, and I miss them greatly.

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NOMENCLATURE

apoAI	apolipoprotein A-I
apoB	apolipoprotein B
CLA	Conjugated linoleic acid
d	Day
DRI	Dietary Recommended Intake
FAME	Fatty acid methyl ester
HDL	High density lipoprotein
IDL	Intermediate density lipoprotein
i.m.	Intramuscular
hs-CRP	High-sensitivity C-reactive protein
LDL	Low density lipoprotein
mo	Month(s)
MUFA	Monounsaturated fatty acid
PUFA	Polyunsaturated fatty acids
s.c.	Subcutaneous
SCD	Stearoyl Co-A desaturase
SE	Standard error
SEM	Standard error of the mean
SFA	Saturated fatty acid
TAG	Triacylglycerols

TG	Triglycerides
TFA	*trans*-fatty acid
VLDL	Very low density lipoprotein
vs	Versus
wk	Week
yr	Year

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CHAPTER I

INTRODUCTION

Reports linking certain types of dietary fat to serum lipid levels have often been interpreted to mean that the general public, especially those at risk for cardiovascular disease (**CVD**), should consume diets containing little or no red meat. Early research concluded that dietary saturated fatty acids (**SFA**) such as palmitic acid (16:0) elevate serum cholesterol concentrations, polyunsaturated fatty acids (PUFA), especially linoleic acid (18:2(n-6)) reduce serum cholesterol concentrations, and monounsaturated fatty acids (**MUFA**) have little or no effect[1-3]. The major MUFA in beef, oleic acid (18:1(n-9)), has been studied in more detail and found to lower low-density lipoprotein cholesterol (**LDL-C**) without affecting the beneficial high-density lipoprotein cholesterol (**HDL-C**)[4,5]. This effect is most convincing in studies in which natural foods were used to supplement diets with oleic acid[6-8]. In addition, different SFA have been found to have different effects on serum cholesterol concentrations, as stearic acid (18:0), was shown to have no effect or even to lower serum cholesterol[9,10].

Some beef products have been shown to decrease[11] or have no effect[12,13] on serum cholesterol in free-living individuals. These earlier studies of the effects of beef consumption on serum cholesterol concentration did not consider that beef products can vary in their MUFA:SFA, or take advantage of beef products with widely differing

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This dissertation follows the style of the *British Journal of Nutrition*.

MUFA:SFA ratios within the context of total beef fat intake.

Fat from pasture- or hay-fed cattle contains a high proportion of SFA, and this beef fat also is higher in *trans*-fatty acids (TFA)[14]. Conversely, high-MUFA beef fat with very low concentrations of TFA can be obtained from cattle that have been grain-fed for extended periods[14,15]. Certain breed types such as American Wagyu (derived from crossing Japanese Black and Japanese Red bulls on Angus cows) have a genetic propensity to accumulate MUFA in muscle and adipose tissue, and ground beef especially enriched with MUFA can be obtained from Wagyu steers, although feeding practices markedly affect the degree of enrichment with MUFA[14].

In this study, we compared several risks factors for CVD in mildly hypercholesterolemic male subjects after consumption of either low-MUFA (with high SFA, high TFA) hamburger or high-MUFA (with low SFA, low TFA) hamburger for 5 wk with a 3-wk washout period. This experiment tested the hypothesis that risk factors for CVD would be higher in mildly hypercholesterolemic men after consumption of hamburger enriched with SFA and TFA than after consumption of hamburger enriched with MUFA.

CHAPTER II

LITERATURE REVIEW

Cardiovascular disease is the leading cause of death within the United States. It is well known that disease risk can be favorably influenced by diet, but the exact nature of what constitutes favorable dietary change is contentious. In 2000, the Nutrition Committee of the American Heart Association moved away from its former insistence on low fat diets and concluded that diets providing up to 40% of dietary energy as primarily unsaturated fat (20% MUFA, 10% SAT, 10% PUFA) were as heart healthy as low fat diets[16]. An outcome of this official opinion has been the re-evaluation of the nutritional properties of a number of higher fat foods such as dairy, nuts, and dietary oils such as olive oil rich in the monounsaturated fatty acid, oleic acid[17]. Beef has not yet been adequately evaluated with regard to its ability to deliver unsaturated fatty acids in the diet. Such a test was proposed to be conducted in connection with traditional indicators of CVD risk, namely plasma total triacylglycerol and cholesterol and their distribution among low density (**LDL**) and high density (**HDL**) lipoproteins, and apolipoprotein B (**apoB**) and A-I (**apoAI**) amounts in plasma. Importantly, because of newer advances in understanding of how inflammation instigates CVD[18], and that differences in LDL particle diameter represent specific metabolic changes that increase the atherogenicity of LDL[19], we proposed to include additional critically revealing indicators of vascular health and lipoprotein metabolism. High-sensitivity C-reactive protein (**hs-CRP**), is a

serum protein that provides an index of vascular inflammation that has recently been recognized as an index of CVD risk[20]. Similarly, LDL and HDL particle diameter measurement can be used to identify the presence of particularly atherogenic LDL or anti-atherogenic HDL. Small dense LDL are recognized as a risk factor for CVD as this form of LDL is more susceptible to oxidative damage[21] and promotes vascular inflammation[18]. Measurement of HDL particle diameter is important because it can be diagnostic of metabolic changes leading to small dense LDL and the antioxidative capacity of HDL[22]. Because these additional measurements are independent risk factors for CVD, they improve our ability to describe the effects of dietary change on overall vascular health. For example, no change in LDL-cholesterol in combination with a reduction in vascular inflammation as indicated by decreased hs-CRP would be a positive outcome. If that change was associated with an increase in LDL- particle diameter, we would conclude that the dietary change had a net positive effect on lipid metabolism and vascular health. If that change was also accompanied by increased HDL-cholesterol and HDL particle diameter, we would expect that overall lipoprotein turnover had increased, although direct measurement by additional methods would be needed to confirm this.

MUFA constitute 35 to 45% of the total fatty acids in beef produced in the United States[14,15,23]. Perhaps because of the prevalence of oleic acid, lean beef has been shown to decrease[11] or have no effect[12,13] on serum cholesterol in free-living (i.e., free from external control or restraint) individuals.

This research proposed as its primary goal to document that the consumption of beef containing elevated oleic acid will reduce LDL-cholesterol, increase LDL diameter, and decrease hs-CRP in human subjects. To accomplish this, we took advantage of the availability of fat trim from American Wagyu cattle (derived from Japanese Black cattle stock raised in the U.S.). We mixed Wagyu fat trim with regular (domestic) fat trim to achieve MUFA:SFA ratios ranging from 1.0 to nearly 2.0. By use of fat trim from Wagyu cattle, we expected to demonstrate that *the unique benefits of oleic acid in lowering CVD risk factors in human subjects also can be provided by beef*.

Table 1. Fatty acid composition of regular ground beef and ground beef or hamburger containing Wagyu marbling or subcutaneous fat trim

Fatty acid	Regular ground beef	Wagyu/i.m.[x] ground beef	Wagyu/s.c.[y] hamburger
n	6	36	12
14:0	3.5[a]	2.0[b]	1.8[b]
14:1	0.8[a]	0.6[b]	0.9[a]
16:0	24.7[a]	23.0[b]	22.6[b]
16:1	4.0	4.2	4.2
17:0	1.4[a]	0.8[b]	0.5[c]
17:1	0.9[a]	0.7[b]	0.6[c]
18:0	15.3[a]	13.1[b]	7.9[c]
18:1	37.0[c]	44.9[b]	54.8[c]
18:2n-6	2.2[b]	3.2[a]	3.0[a]
18:3n-3	0.3[a]	0.3[a]	0.1[b]
CLAcis-9,trans-11	0.6[a]	0.7[a]	0.3[b]
MUFA:SFA ratio[z]	1.12[c]	1.41[b]	1.92[a]

[x]Ground beef produced with no added fat trim (marbling [i.m.] was the only fat source).

[y]Hamburger produced with Wagyu subcutaneous (s.c.) fat trim.

[z]Monounsaturated:saturated fatty acid ratio = (14:1+16:1+17:1+18:1)/(14:0+16:0+17:0+18:0).

[a,b,c]Means within a row with different superscripts differ ($P < 0.05$).

Preliminary data

We previously demonstrated that the MUFA:SFA ratios in subcutaneous adipose tissue, marbling, and longissimus muscle from Japanese Black or American Wagyu steers were higher than ratios observed in domestic cattle[14,15,24]. In Japanese Black cattle, the MUFA:SFA ratios were greatest in subcutaneous adipose tissue (1.98), less in marbling adipose tissue (1.78), and least in muscle (1.66). We consistently observe the highest concentration of oleic acid in subcutaneous adipose tissue and the lowest concentrations in muscle or lean meat in all cattle breeds that we have tested[15,24], although the MUFA:SFA ratios of those other breeds are substantially less than those observed in Japanese Black or long-fed American Wagyu cattle.

We measured the fatty acid composition of ground beef produced from domestic fat and lean trim, from Wagyu ground beef prepared from highly marbled lean trim, and from Wagyu hamburger prepared from Wagyu lean trim plus Wagyu fat trim (**Table 1**). Because of the high concentration of oleic acid in Wagyu fat trim, the MUFA:SFA ratio in the hamburger containing Wagyu fat trim was 1.92. Because marbling contains less oleic acid, the ratio was 1.42 in Wagyu ground beef containing no outside fat trim. Both MUFA:SFA ratios were significantly higher in than in domestic (regular) ground beef.

An investigation was recently published on the effects of the consumption of beef on lipoprotein cholesterol metabolism in free-living men[13] (**Table 2**). The study originally was designed to test differences in responses to regular and Wagyu ground beef and

Table 2. Serum lipid and metabolite values of mildly hypercholesterolemic men habitually consuming low (26 g/d) or high (160 g/d) amounts of beef[x,13]

Item	Low-beef group		High-beef group		SE
	Initial value	Test value	Initial value	Test value	
Total cholesterol[a]	241.1[a]	252.9[a]	229.0[b]	232.7[b]	33.9
HDL-cholesterol	41.7	44.1	41.7	40.2	0.5
LDL-cholesterol	172.5[a]	179.5[a]	148.8[b]	144.4[b]	2.6
Apolipoprotein A-I	173.1[b]	199.0[a]	175.3[b]	203.8[a]	2.5
Apoliprotein B	155.8	177.1	154.1	173.1	3.4
ApoA-I:HDL-cholesterol ratio	4.19[b]	4.57[a]	4.38[b]	5.17[a]	0.16
ApoB:LDL-cholesterol ratio	0.90	0.98	1.05	1.26	0.05
Triacylglycerols	134.1	146.5	192.5	240.3	8.0
Glucose	81.2[b]	90.9[a]	83.5[b]	91.1[a]	1.1
Creatinine	1.18	1.26	1.17	1.18	0.01
Blood urea nitrogen	14.9	17.3	14.0	14.7	0.37

[x]All concentrations are mg/dL.
[a,b]Means within a row with different superscripts differ ($P < 0.05$).

steaks. However, in that study, the Wagyu beef was from young bullocks, and thus was low in monounsaturated fatty acids. For this reason, individuals responded similarly to both types of beef, so the data were pooled across beef types and instead focused on differences in habitual intake of beef. Whether the men habitually consumed little beef daily (26 g/d) or relatively high amounts per day (160 g/d), the addition of beef to their diet had no significant effect on LDL or total cholesterol (**Table 2**).

However, addition of beef to the diet increased serum apoAI in both groups of men. Because ApoAI is associated with HDL-cholesterol, this demonstrated that increasing beef consumption in free-living men may actually have had beneficial effects on serum cholesterol. However, the test beef diets tended ($P = 0.08$) to increase the apoB:LDL-cholesterol ratio. This suggests that LDL-cholesterol particles became smaller and denser during the consumption of the test beef. This is not desirable, because increased density of LDL-cholesterol particles now is recognized as an additional risk factor for coronary heart disease[21]. The proposed research will extend our previous results by directly measuring particle diameters for both HDL- and LDL-cholesterol, as well as providing the measure of hs-CRP.

The beef used in our previous investigation was provided as one steak and four 100-g servings of ground beef per week. The MUFA:SFA ratio of the ground beef used in that investigation was 0.87 for the regular ground beef and 0.99 for the Wagyu ground beef. Because our previous investigation demonstrated no differences between regular (domestic) and Wagyu ground beef for measures of lipoprotein metabolism, we can conclude that, if oleic acid in beef truly can lower LDL-cholesterol, then it must be at

higher concentrations than we tested. The ratio of 0.99 often is achieved in domestic ground beef. For us to provide impetus for increasing oleic acid in beef and beef products, we must test beef with higher concentrations of oleic acid.

We propose to demonstrate that increasing the oleic acid concentration in ground beef will lower the three major risk factors for CVD: LDL-cholesterol concentration, LDL diameter, and hs-CRP. Because we will be using an adequate test population of free-living men consuming natural beef products, and because we will be measuring all three risk factors, this information will provide the beef industry with strong and convincing evidence for the healthfulness of beef in the American diet.

CHAPTER III

MATERIALS AND METHODS

Experimental design

Texas A&M University faculty and staff (n = 10) were recruited for this study. Normal, healthy, non-smoking males between the ages of 30 and 60 yr were screened with a battery of blood chemistry tests by a local physician (S. Tseng). Subjects with total serum cholesterol values between 5 and 6.5 mmol/L and not on restrictive diets or medications were selected and given a complete physical examination, including an electrocardiogram and a family history. All participants provided informed consent, and were free-living. Exercise and physical activities were not restricted, but participants were requested not to change their habitual level of physical activity in order to maintain body weight (± 2.2 kg of entry weight). Subject characteristics and baseline lipid and dietary profiles are shown in **Table 3**.

The 10 men were fed low-MUFA hamburger for a 5-wk period and, following a 3-wk habitual diet washout period, were rotated to high-MUFA hamburger. The subjects were contacted weekly to ensure that all 5 beef patties were consumed during each weekly test period. The test subjects were not informed as to which type beef they had been assigned. The beef was supplied to the participants in the form of 114-g hamburger

Table 3. Baseline characteristics for subjects[1]

Item	Mean	SE
Age, *y*	49.3	8.6
Body weight, *kg*	86.1	3.7
BMI	26.8	1.1
Habitual dietary intake		
Energy, *kjoule/d*	9,497	861
Protein, *g/d*	97.5	10.8
Carbohydrate, *g/d*	253.7	21.6
Cholesterol, *mg/d*	376.0	101.4
Fat, *g/d*	91.6	13.8
Saturated	28.0	3.4
Monounsaturated	28.5	4.3
Oleic acid	25.8	3.9
Polyunsaturated	13.9	2.8
Dietary MUFA:SFA	1.04	0.12
Lipoprotein cholesterol, glucose and triacylglycerols, *mmol/L*		
VLDL-C	0.82	0.25
LDL-C	3.57	0.23
HDL-C	1.02	0.06
Glucose	5.09	0.24
Triacylglycerols	2.56	0.7
LDL:HDL ratio	3.54	0.21
LDL diameter, *nm*	19.7	0.6
Plasma fatty acids, *g/100 g total fatty acids*		
16:0	16.6	1.6
16:1(n-7)	1.09	0.17
18:0	7.4	0.3
18:1(n-9)	19.3	1.4
18:2(n-6)	28.5	1.6

[1]Data are means and SE for 10 men.

patties (5 patties/wk). The frozen, vacuum-packaged hamburger patties for an entire diet period were delivered to the participants on or before the first day. No restrictions were placed on how the beef was to be prepared other than that all of the beef be consumed at each sitting.

Preparation of hamburgers

Hamburgers were prepared at the Texas A&M Rosenthal Meat Science & Technology Center, Texas A&M University. By definition, ground beef contains only fat associated with the lean trim from which it is ground[25]. Because fat trim from other parts of the carcass and/or from different cattle was added to the source of lean trim, the term ground beef cannot be used and so the term hamburger is used.

Low-MUFA hamburger was formulated from lean and fat trim from domestic cattle and from Wagyu steers fed pasture-based diets. The high-MUFA hamburger was formulated from lean and fat trim of domestic cattle and Wagyu steers fed a corn-based diet for an extended period of time (a minimum 8 mo after weaning). The Wagyu fat trims were obtained from a local producer of genetically similar full-blood Japanese Black (Wagyu) cattle. The domestic fat trim and all lean trim were obtained from the Rosenthal Meat Science and Technology Center. Hamburger patties were formulated to achieve 35% targeted total fat, so that each 114-g patty contained approximately 40 g total fat. Patties were individually vacuum-packed, quick-frozen and boxed by diet type.

The low-MUFA hamburger contained over 2 g more stearic acid per patty than the high-MUFA hamburger (6.14 g versus 4.01 g; **Table 4**), and the high-MUFA hamburger

contained over 2 g more oleic acid per patty (17.2 g vs 15.0 g). Each hamburger type provided a similar amount of palmitic acid (~9.4 g/patty). The low-MUFA hamburger also contained 0.48 g more total TFA and 0.014 g more α-linolenic acid (18:3(n-3)) than the high-MUFA hamburger.

Survey of area ground beef

In order to empirically determine the range of MUFA:SFA in commercially available products we conducted a survey of ground beef from retail outlets within the College Station area and Wagyu ground beef that was purchased from an internet vendor. Determined fatty acid compositions were used to calculate amounts of individual fatty acids in 114-g patties containing 20% fat. This level of fat was selected, as it is the most frequently purchased form of hamburger.

Table 4. Fatty acid composition of hamburger low in monounsaturated fatty acids (Low MUFA) and hamburger enriched in monounsaturated fatty acids (High MUFA)[1,2]

	Hamburger type			
Fatty acid	Low MUFA	SE	High MUFA	SE
	g/114-g hamburger patty, uncooked			
Myristic, 14:0	1.00	0.02	1.07	0.06
Myristoleic, 14:1(n-5)	0.43	0.01	0.29	0.02*
Palmitic, 16:0	9.60	0.15	9.21	0.15
Palmitoleic, 16:1(n-7)	1.18	0.07	1.74	0.04***
Stearic, 18:0	6.14	0.48	4.01	0.02**
trans-Vaccenic, 18:1(*trans*-11)	1.41	0.11	1.21	0.04
18 :1(*trans*-10)	0.31	0.08	0.03	0.01*
Oleic, 18:1(n-9)	15.0	0.5	17.2	0.2**
cis-Vaccenic, 18 :1(n-7)	0.58	0.05	0.81	0.02**
Linoleic, 18:2(n-6)	0.91	0.03	0.92	0.06
α-Linolenic, 18:3(n-3)	0.063	0.003	0.049	0.004*
18:2(*cis*-9,*trans*-11)	0.16	0.01	0.18	0.01*
18:2(*trans*-10,*cis*-12)	0.11	0.01	0.11	0.02
Total SFA[3]	16.7	0.6	14.3	0.2*
Total MUFA[4]	17.1	0.6	20.2	0.2**
Total PUFA[5]	0.97	0.03	0.97	0.06
Total *trans*-fatty acids[6]	1.72	0.03	1.24	0.05***
MUFA:SFA	0.95		1.31***	

[1]Data are means and SE.
[2]Data were analyzed as a Student's *t*-test. *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.
[3]Total SFA = 14:0 + 16:0 + 18:0 + 18:1(*trans*-10) +18:1(*trans*-11).
[4]Total MUFA = 14:1(n-5) + 16:1(n-7) + 18:1(n-9) + 18:1(n-7) + 18:2(*cis*-9,*trans*-11).
[5]Total PUFA = 18:2(n-6) + 18:3(n-3).
[6]Total *trans*-fatty acids = 18:1(*trans*-11) + 18:1(*trans*-10).

Determination of cholesterol fractions, triacylglycerols and glucose

Blood was collected from an arm vein prior to initiation of each dietary treatment and weekly thereafter. A trained phlebotomist at the A.P. Beutel Health Center, Texas A&M University, drew blood samples. Plasma was harvested from blood collected with EDTA and lipoproteins preserved[26] prior to lipoprotein separation using density gradient ultracentrifugation employing human density intervals[27] and determination of lipoprotein diameters[27,28].

Plasma total lipoproteins isolated as the d < 1.2 g/mL fraction of plasma were separated on the basis of diameter with a gel-filtration chromatographic system[29] in order to determine the relative distribution of plasma total cholesterol and triacylglycerol among VLDL, LDL and HDL lipoprotein classes. Separate analyses were made for cholesterol and triacylglycerols, and in each, the eluting lipids were continuously monitored at 505 nm following enzymatic chromophore development within an in-line post-column reactor[29]. Plasma total cholesterol, triacylglycerols, and glucose were determined by separate enzymatic assays (Sigma Chemical Co., St. Louis, MO).

Fatty acid composition of plasma and test hamburger

Fatty acids were measured in the baseline whole plasma samples and from whole plasma samples taken after 5 wk of each test hamburger treatment. Additionally, fatty acid concentrations and concentrations of fat and moisture[30] of the test hamburgers were measured for every batch of product (a minimum of three batches per beef fat combination). Total lipid was extracted and methylated as described[31,32], and fatty acid methyl esters (FAME) were analyzed with a Varian gas chromatograph (model CP-3800 fixed with a CP-8200 autosampler, Varian Inc., Walnut Creek, CA).

Separation of FAME was accomplished on a fused silica capillary column CP-Sil88 [100 m x 0.25 mm (i.d.)] (Chrompack Inc., Middleburg, The Netherlands) with helium as the carrier gas (1.2 mL/min). After 32 min at 180°C, oven temperature was increased at 20°C/min to 225°C and held for 13.75 min. Injector and detector temperatures were at 270 and 300°C, respectively. Individual FAME were identified using genuine standards (Nu-Chek Prep, Inc., Elysian, MN) and expressed as a g/100 g total FAME analyzed or as g/114 g hamburger patty.

Diet records

Prior to each diet phase, and once during each phase, participants completed a 3 day record (to include one weekend day). The diet records were analyzed for nutrient composition by a registered dietitian and used to establish baseline observations, and encourage compliance with the requirement of total patty consumption. The diets

records were analyzed using Nutrient Calc version 1.1 (University of Minnesota, St. Paul, MN). Plasma fatty acid compositions were used to verify recorded patterns of fatty acid intake.

Statistical analyses

Retail ground beef fatty acid composition was analyzed by analysis of variance (SuperAnova, Abacus Concepts, Inc., Berkeley, CA). When the ground beef type was significant ($P \leq 0.05$), means were separated by the Fisher's Protected LSD method. Plasma lipid fractions were analyzed with a split plot model, with diet in the whole plot, and sample number as the subplot (SuperAnova). Because we included each participant in both diets, participant was included as a block effect. Fatty acid composition of the test hamburgers was tested by the Student's *t*-test, and after-test plasma concentrations of lipoprotein cholesterol, glucose, triacylglycerols, and fatty acids were compared by a paired *t*-test.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Texas A&M University Institutional Review Board for use of human subjects in research (Protocol Number 2004-0026). Written informed consent was obtained from all subjects.

Table 5. Fatty acid composition of ground beef purchased from local retailers[1]

	Fatty acids								Ratio	
Item	16:0	SE	18:0	SE	18:1t[2]	SE	18:1(n-9)	SE	MUFA:SFA	SE
	g/114-g ground beef patty, normalized (20% fat)									
Angus	5.2	0.1	2.9^{c}	0.1	0.9^{c}	0.1	9.3^{b}	0.2	1.13^{b}	0.05
Chub pack	5.3	0.1	3.4^{b}	0.1	1.7^{a}	0.1	8.0^{c}	0.2	0.84^{c}	0.04
Ground chuck	5.3	0.1	3.5^{b}	0.2	1.6^{ab}	0.1	8.2^{c}	0.1	0.86^{c}	0.03
Ground round	5.5	0.1	3.4^{b}	0.2	1.1^{bc}	0.2	8.2^{c}	0.2	0.90^{bc}	0.05
Corn-fed Wagyu	5.5	0.2	2.1^{d}	0.1	0.3^{d}	0.1	10.3^{a}	0.1	1.46^{a}	0.02
Pasture-fed Wagyu	5.8	0.5	2.7^{c}	0.1	0.3^{d}	0.1	7.9^{c}	0.2	1.02^{b}	0.02
P-values[3]	0.22		0.0001		0.0001		0.0001		0.0001	

[1]Data are means and SE for a minimum of three samples per ground beef type. Data are normalized to 20% total fat (22.8 g fat/114-g ground beef patty). Chub pack, ground round, ground chuck, and Angus ground beefs were purchased from three major retailers in the vicinity of Texas A&M University. Wagyu ground beef (grain- and pasture-fed) were purchased from a web-based supplier. Lipids were extracted and fatty acids analyzed as described in the text. A minimum of three samples of each type of product was analyzed. Not all fatty acids present in the ground beef are listed in the table.

[a,b,c]Means within a column with common superscripts or no superscripts are not different ($P > 0.05$).

[2]Sum of 18:1(*trans*-10) plus 18:1(*trans*-11)

[3]Data were analyzed by analysis of variance, with ground beef type as the main effect.

CHAPTER IV

RESULTS

Fatty acid composition of retail ground beef

Chub pack, ground chuck and ground round all had MUFA:SFA less than 1.0 (**Table 5**). The lowest MUFA:SFA in ground beef was observed in chub pack ground beef (0.84) and the highest ratio (1.46) was measured in a branded ground beef from corn-fed Wagyu cattle. There was no difference in the amount of palmitic acid per 114-g serving across retail ground beef types. The chub pack ground beef contained more stearic acid and TFA, and less oleic acid, than the branded Angus and Wagyu ground beefs.

Nutrient intake, body weights, plasma triacylglycerols and glucose

The intakes of total fat, SFA, MUFA, and oleic acid were greater during consumption of the test hamburgers than for the habitual diets (all *P*-values ≤ 0.05; **Tables 3 & 6**). During the high MUFA phase, participants consumed less SFA and more MUFA than during the low MUFA hamburger phase. Participants consumed approximately 40 g/d more fat during the test phases than during their habitual intake, indicating that most participants ate the beef patty in addition to their habitual meals.

Table 6. Daily intake of nutrients for test diets of men rotated from hamburger containing fat trim low in monounsaturated fatty acids (Low MUFA) to hamburger containing fat trim high in monounsaturated fatty acids (High MUFA)[1]

Item	Low MUFA	SE	High MUFA	SE
Energy, *kjoule/d*	10,751	665	10,634	748**
Protein, *g/d*	99.7	11.3	101.1	13.6
Carbohydrate, *g/d*	241.9	14.1	240.2	13.3
Cholesterol, *mg/d*	334.9	42.4	338.2	45.0
Fat, *g/d*	132.3	13.7	129.2	14.6**
Saturated	45.0	4.6	42.7	4.5*
Monounsaturated	48.4	6.6	50.6	7.2*
Oleic acid	43.2	6.4	44.7	7.0*
Polyunsaturated	13.8	2.9	13.9	3.2
Dietary MUFA:SFA	1.06	0.07	1.18	0.08*

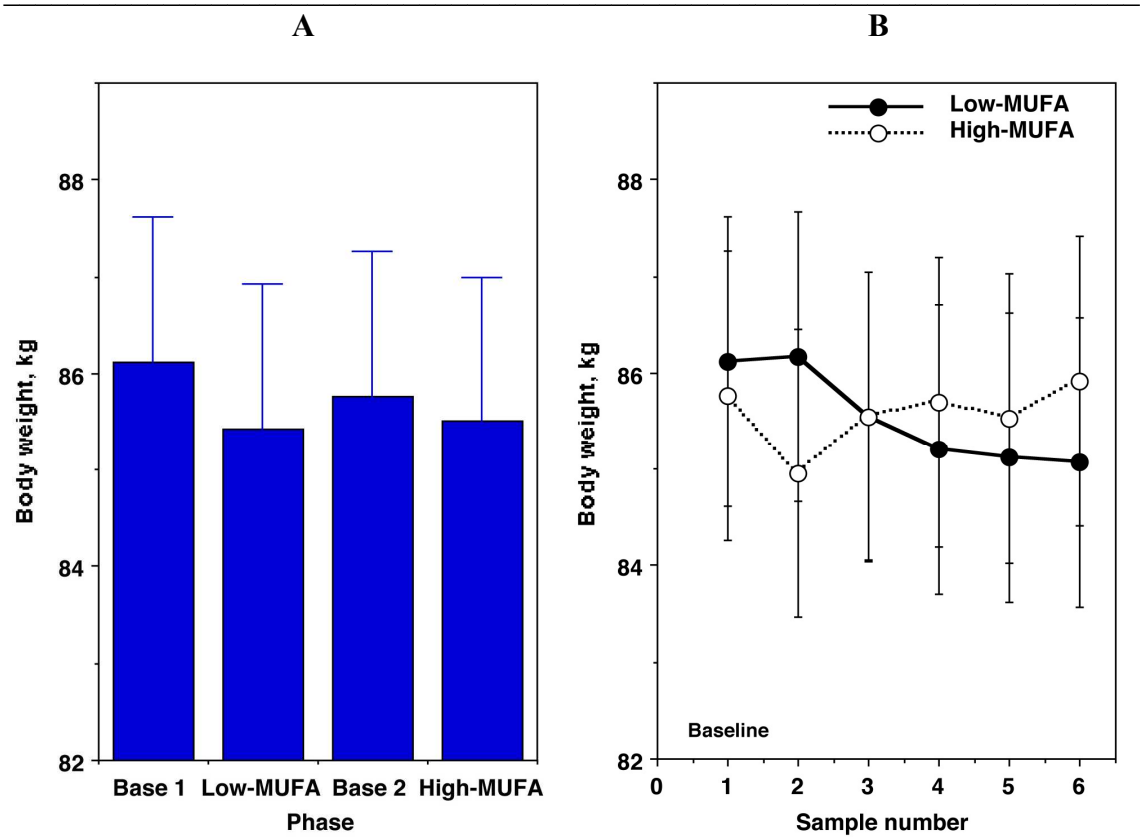
[1]Data are means and SE for three diet records from 10 men per test hamburger.
[2]Data were analyzed as a paired *t*-test. *$P \leq 0.05$; **$P \leq 0.01$.

In spite of the greater daily fat intake, initial (86.1 ± 3.7 kg) and final (85.9 ± 3.8 kg) mean body weights were not different (**Figure 1A**). However, body weights changed significantly over each sample number ($P = 0.036$; **Figure 1B**). Body weights decreased during the low-MUFA phase and increased during the high-MUFA phase (MUFA group X Sample number, $P < 0.001$; **Figure 1B**). Interestingly, the mean body weights approximately returned to the initial mean body weights at the conclusion of the three phases: Low MUFA, washout, High MUFA.

Participants consumed 117 fewer kjoule/d during the high-MUFA phase than during

the low-MUFA phase (**Table 6**). This difference, though small (1% of total energy intake), was statistically significant, and was caused by lesser intake of total fat during the high-MUFA phase than during the low-MUFA phase. Daily intakes of protein, carbohydrate, total cholesterol, and PUFA were not different between the test phases.

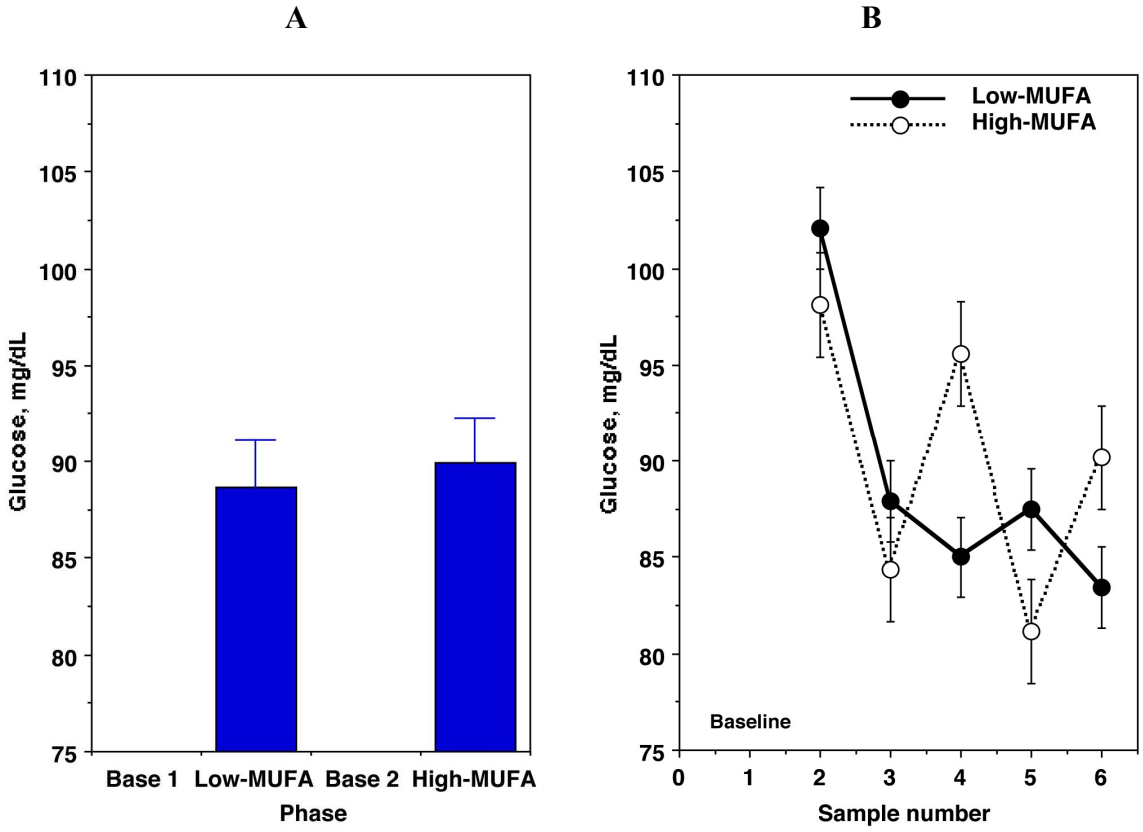
Figure 1. Body weights of mildly hypercholesterolemic men rotated from hamburger containing fat trim high in saturated fatty acids (Low-MUFA) to hamburger containing fat trim high in monounsaturated fatty acids (High-MUFA).



A: Treatment means pooled over sample number (n = 10; MUFA group, P = 0.99)
B: Treatment means at each sampling time:
Sample number, P = 0.036
MUFA group X Sample number, $P < 0.001$
Pooled SEM for each MUFA group is affixed to the means.

Mean plasma glucose concentrations were not different for either phase ($P = 0.86$), but both phases had a significant lowering effect overall throughout the phase **(**Sample number, $P = 0.033$; MUFA group X Sample number, $P = 0.51$; **Figure 2B)**.

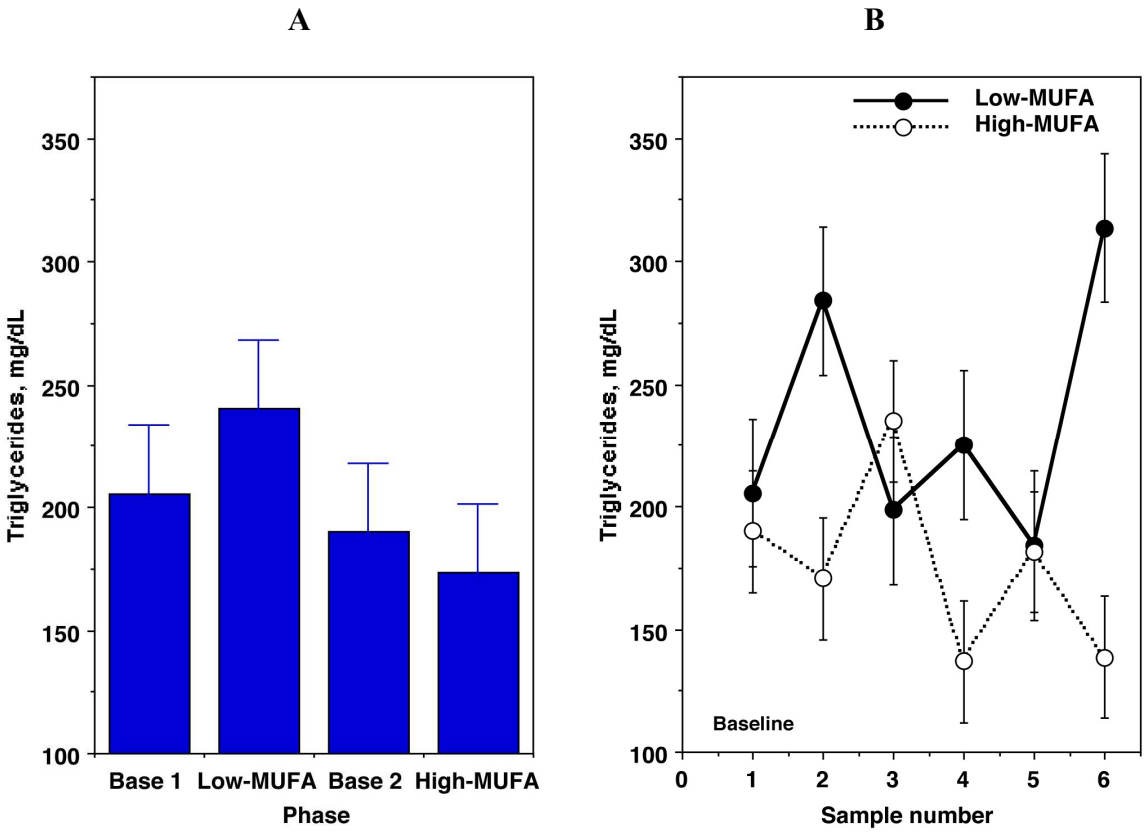
Figure 2. Plasma glucose of mildly hypercholesterolemic men rotated from hamburger containing fat trim high in saturated fatty acids (Low-MUFA) to hamburger containing fat trim high in monounsaturated fatty acids (High-MUFA).



A: Treatment means pooled over sample number ($n = 10$; MUFA group, $P = 0.86$)
B: Treatment means at each sampling time:
Sample number, $P = 0.033$
MUFA group X Sample number, $P = 0.51$
Pooled SEM for each MUFA group is affixed to the means.
Baseline samples were not obtained.

There was no difference in pooled MUFA group means. Plasma triglycerides tended to increase during low-MUFA and decrease during the high-MUFA phase, although no significant effect was observed ($P = 0.10$; **Figure 3**).

Figure 3. Plasma triglycerides of mildly hypercholesterolemic men rotated from hamburger containing fat trim high in saturated fatty acids (Low-MUFA) to hamburger containing fat trim high in monounsaturated fatty acids (High-MUFA).



A: Treatment means pooled over sample number ($n = 10$; MUFA group, $P = 0.33$)
B: Treatment means at each sampling time:
Sample number, $P = 0.80$
MUFA group X Sample number, $P = 0.10$
Pooled SEM for each MUFA group is affixed to the means.

Table 7. Plasma metabolite concentrations in mildly hypercholesterolemic men fed hamburger containing fat trim low in monounsaturated fatty acids (Low MUFA) or fat trim high in monounsaturated fatty acids (High MUFA)[1,2]

Item	Low MUFA	SE	High MUFA	SE
Lipoprotein cholesterol, glucose and triacylglycerols, *mmol/L*				
VLDL-C	0.93	0.34	0.54	0.32
LDL-C	3.31	0.33	3.60	0.28
HDL-C	0.88	0.06	1.06	0.05*
Glucose	4.63	0.12	5.01	0.26
Triacylglycerols	3.90	1.21	1.72	0.43*
LDL:HDL ratio	3.75	0.23	3.35	0.15*
LDL diameter, *nm*	18.1	0.7	18.4	0.3
Fatty acids, *g/100 g total fatty acids*				
16:0	23.6	0.7	15.2	1.2***
16:1(n-7)	1.72	0.22	0.81	0.11***
18:0	7.8	0.2	8.6	0.3*
18:1(n-9)	22.5	1.1	23.9	1.2*
18:2(n-6)	30.3	1.8	34.3	1.8*

[1]Data are means and SE for 10 men per test hamburger.
[2]Data were analyzed as a paired *t*-test. *$P \leq 0.05$; ***$P \leq 0.001$.

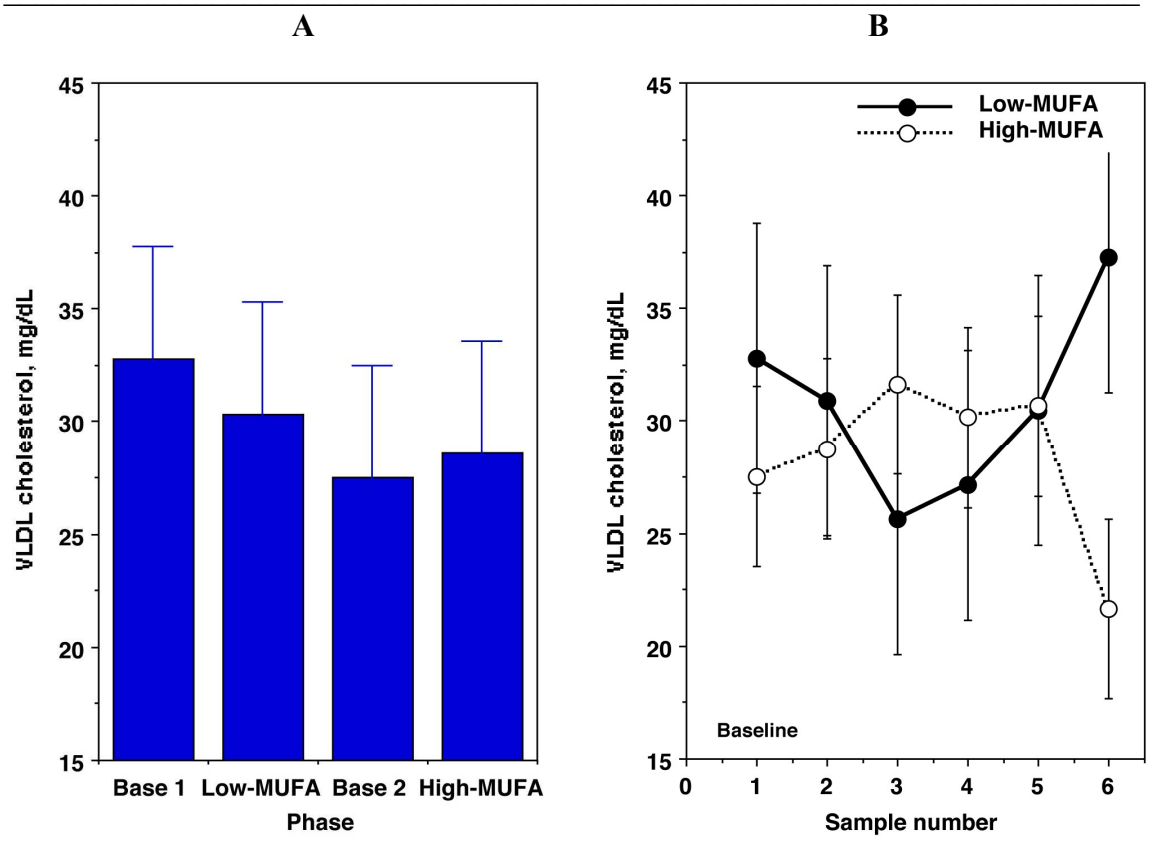
Plasma lipoprotein cholesterol and plasma fatty acid concentrations

For participants compared by paired *t*-test, VLDL-C concentration was highest after consumption of the high-SFA hamburger and lowest after consumption of the high-MUFA hamburger ($P = 0.03$; **Table 7**). The concentration of plasma triacylglycerols and the LDL:HDL ratio were greater ($P < 0.05$) after the high SFA (Low-MUFA) hamburger phase than after the high-MUFA hamburger phase (**Table 7**). Conversely, HDL-C was greater after consumption of the high MUFA hamburger than after consumption of the high-SFA hamburger.

Plasma concentrations of palmitic and palmitoleic acid were significantly higher ($P \leq 0.001$), after the low-MUFA (high-SFA) phase than after high-MUFA phase (**Table 7**). In opposition to this finding, plasma stearic, oleic, and linoleic acid concentrations were significantly higher ($P \leq 0.001$) after consumption of the high-MUFA hamburger than after consumption of the low-MUFA (high-SFA) hamburger. Unlike other plasma fatty acids, palmitic acid did not return to pre-treatment values after the 3-wk washout period, but remained elevated (20.5 ± 0.7 g/100 g plasma fatty acids; data not shown).

In contrast to VLDL-C among individuals, plasma VLDL concentrations when pooled among men in this study were not significant. There was also nothing notable about sample, nor were there significant interactions of MUFA group X Sample number (**Figure 4)**.

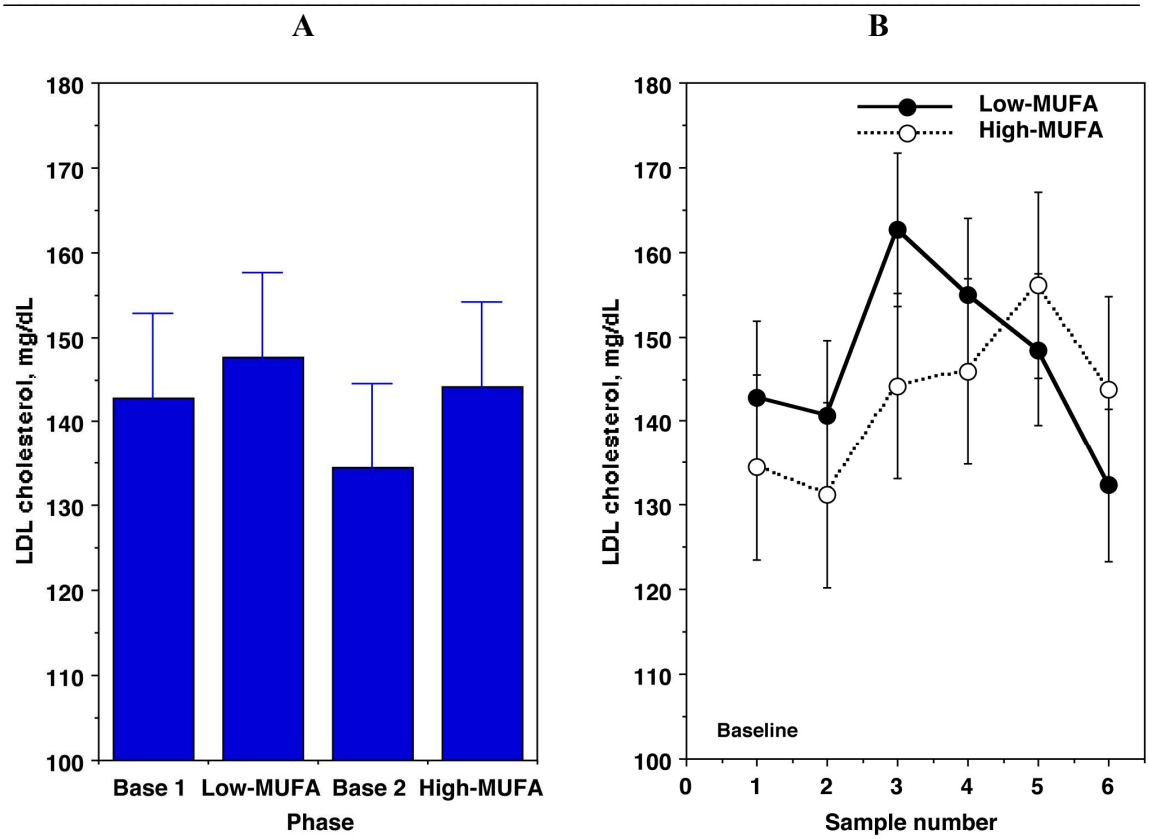
Figure 4. Plasma VLDL cholesterol of mildly hypercholesterolemic men rotated from hamburger containing fat trim high in saturated fatty acids (Low-MUFA) to hamburger containing fat trim high in monounsaturated fatty acids (High-MUFA).



A: Treatment means pooled over sample number (n = 10; MUFA group, P = 0.87)
B: Treatment means at each sampling time:
Sample number, P = 0.99
MUFA group X Sample number, P = 0.28
Pooled SEM for each MUFA group is affixed to the means.

There was no difference observed for plasma LDL cholesterol in each MUFA group (**Figure 5A**). However, LDL-C sinusoidally changed significantly over time for both MUFA groups ($P = 0.006$) in the split-plot analysis, and there was a tendency (MUFA group X Sample number interaction, $P = 0.12$) for the high-MUFA ground beef

Figure 5. Plasma LDL cholesterol of mildly hypercholesterolemic men rotated from hamburger containing fat trim high in saturated fatty acids (Low-MUFA) to hamburger containing fat trim high in monounsaturated fatty acids (High-MUFA).

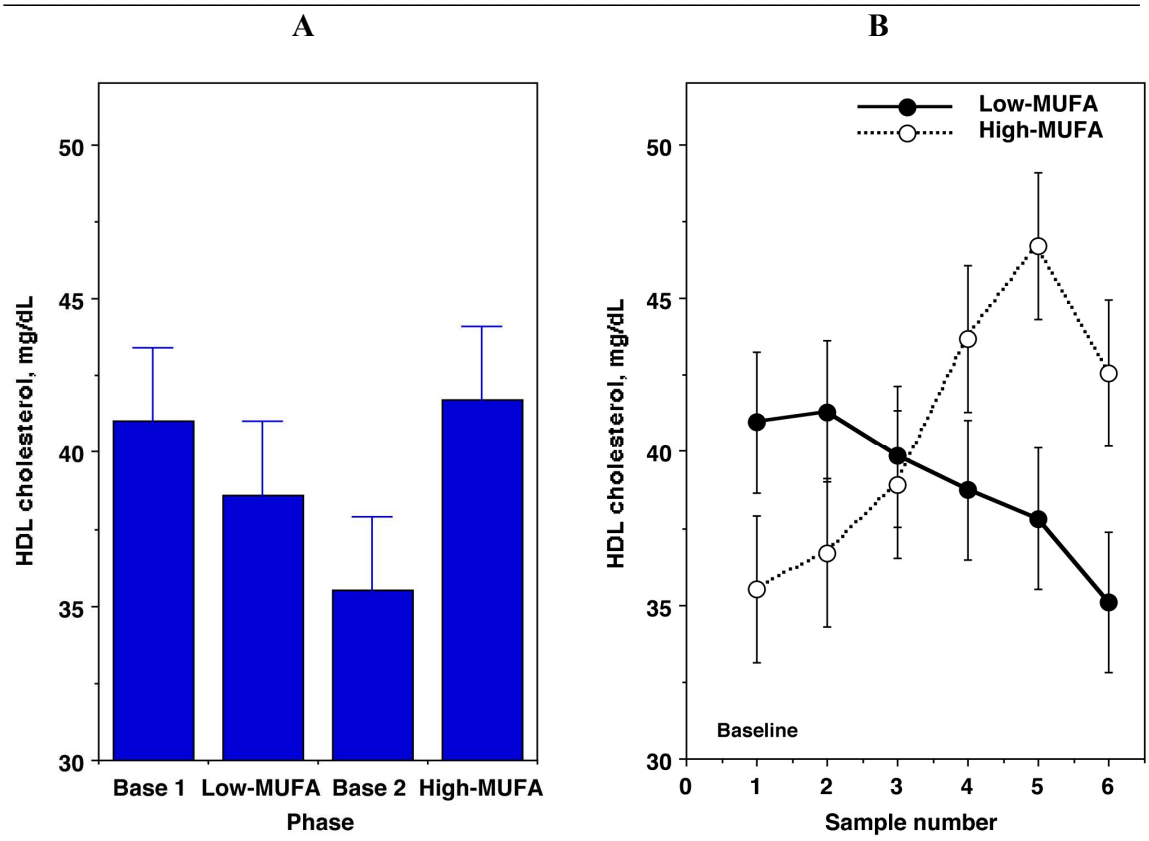


A: Treatment means pooled over sample number ($n = 10$; MUFA group, $P = 0.76$)
B: Treatment means at each sampling time:
Sample number, $P = 0.006$
MUFA group X Sample number, $P = 0.12$
Pooled SEM for each MUFA group is affixed to the means.

to affect an increase of LDL-C over baseline (**Figures 5B**).

There was no difference in plasma HDL cholesterol between MUFA groups (**Figure 6**), but there was a significant linear trend across the average of each treatment at each sampling (Sample number, $P = 0.006$). HDL-C sharply declined during the

Figure 6. Plasma HDL cholesterol of mildly hypercholesterolemic men rotated from hamburger containing fat trim high in saturated fatty acids (Low-MUFA) to hamburger containing fat trim high in monounsaturated fatty acids (High-MUFA).

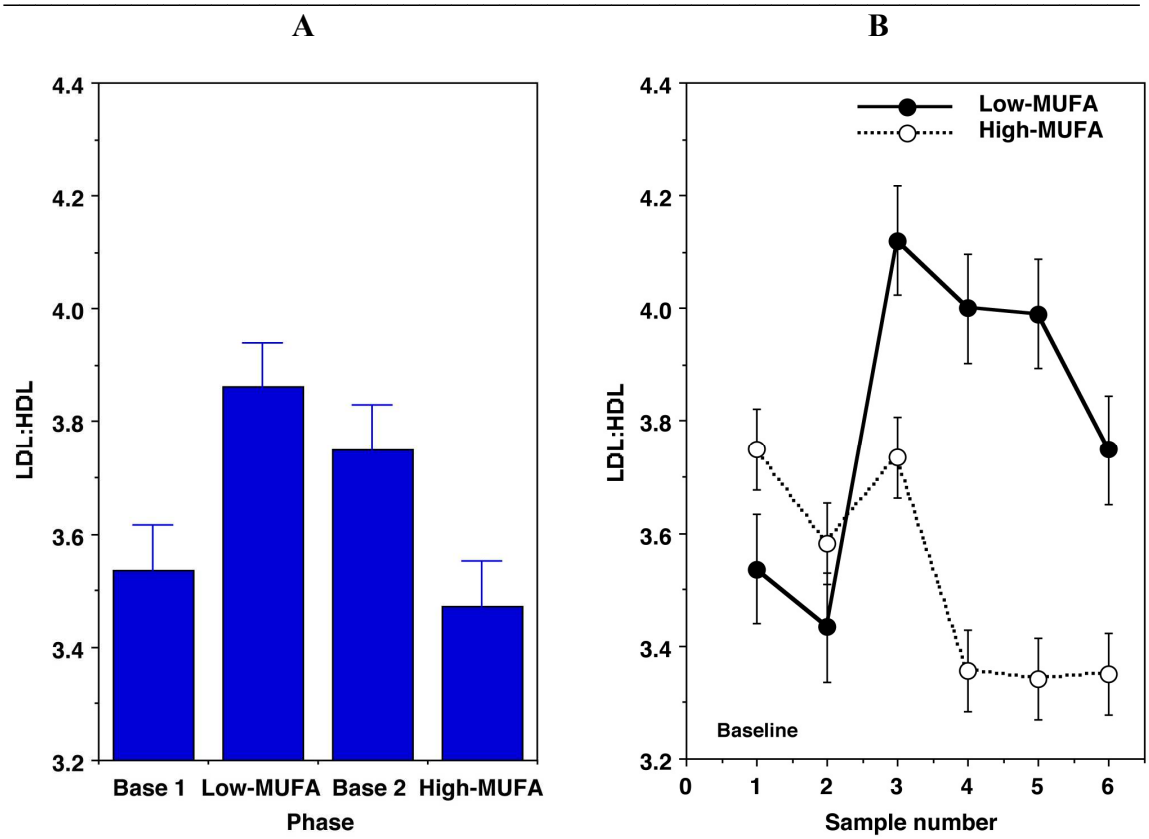


A: Treatment means pooled over sample number ($n = 10$; MUFA group, $P = 0.57$)
B: Treatment means at each sampling time:
Sample number, $P = 0.048$
MUFA group X Sample number, $P < 0.001$
Pooled SEM for each MUFA group is affixed to the means.

consumption of the low-MUFA ground beef and increased over the initial baseline values during consumption of the high-MUFA ground beef (MUFA group X Sample number, $P < 0.001$; **Figure 6B**).

Plasma LDL:HDL ratios were used to generate **Figure 7**. Treatment means pooled

Figure 7. Plasma LDL:HDL ratio of mildly hypercholesterolemic men rotated from hamburger containing fat trim high in saturated fatty acids (Low-MUFA) to hamburger containing fat trim high in monounsaturated fatty acids (High-MUFA).



A: Treatment means pooled over sample number ($n = 10$; MUFA group, $P = 0.29$)
B: Treatment means at each sampling time:
Sample number, $P = 0.006$
MUFA group X Sample number, $P < 0.001$
Pooled SEM for each MUFA group is affixed to the means.

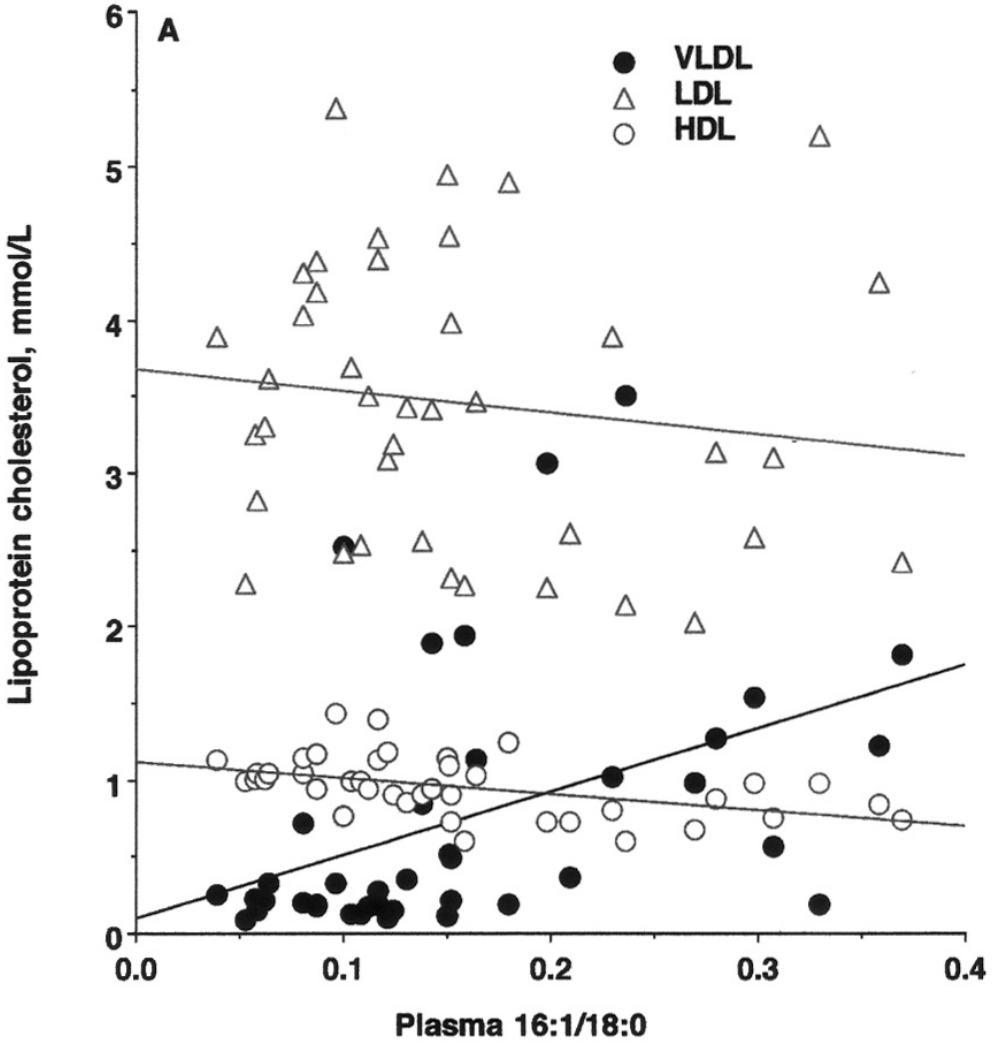
at each sample number was not significant between MUFA groups (**Figure 7A**, $P = 0.29$). However, the average of each treatment at each sampling showed a linear trend that was significant (Sample number, $P = 0.006$). In addition, there was a significant interaction among MUFA group X Sample number, with $P < 0.001$. The slope of the line for the low-MUFA group increased sharply opposing the downward slope in the high-MUFA treatment group (**Figure 7B**).

Table 8. Simple correlation coefficients between plasma fatty acids, fatty acid ratios and lipoprotein cholesterol measures for mildly hypercholesterolemic men fed hamburger containing fat trim low in monounsaturated fatty acids (high in saturated fatty acids) or fat trim high in monounsaturated fatty acids[1]

Fatty acid	TG	VLDL-C	LDL-C	HDL-C	LDL:HDL	LDL, *nm*
16:0	0.79***	0.53**	-0.13	-0.52**	0.34	-0.44*
16:1(n-7)	0.90***	0.73***	-0.13	-0.58**	0.45*	-0.28
18:0	-0.21	-0.28	-0.22	-0.01	-0.31	-0.40*
18:1(n-9)	0.51*	0.36	-0.34	-0.30	-0.21	-0.54**
18:2(n-6)	-0.54**	-0.68**	-0.10	0.31	-0.48*	0.05

[1]Data are from baseline and final samples. *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.

Figure 8. VLDL, LDL and HDL cholesterol concentrations as a function of the plasma 16:1/18:0 ratio.



Data are from baseline and final samples, for all participants.
VLDL: $y = 4.10x + 0.10$, $R^2 = 0.18$
LDL: $y = -1.39x + 3.68$, $R^2 = 0.02$
HDL: $y = -1.05x + 1.13$, $R^2 = 0.23$

Lipoprotein concentrations as a function of plasma 16:1/18:0 ratio

The plasma palmitoleic (16:1)/stearic acid (18:0) ratio ranged from a minimum of 0.04 to a maximum of 0.37 and similarly VLDL-C concentrations ranged from 0.08 to 3.5 mmol/L (**Figure 8**). The relationship between the palmitoleic/stearic acid ratio and VLDL-C was significant ($R^2 = 0.18$).

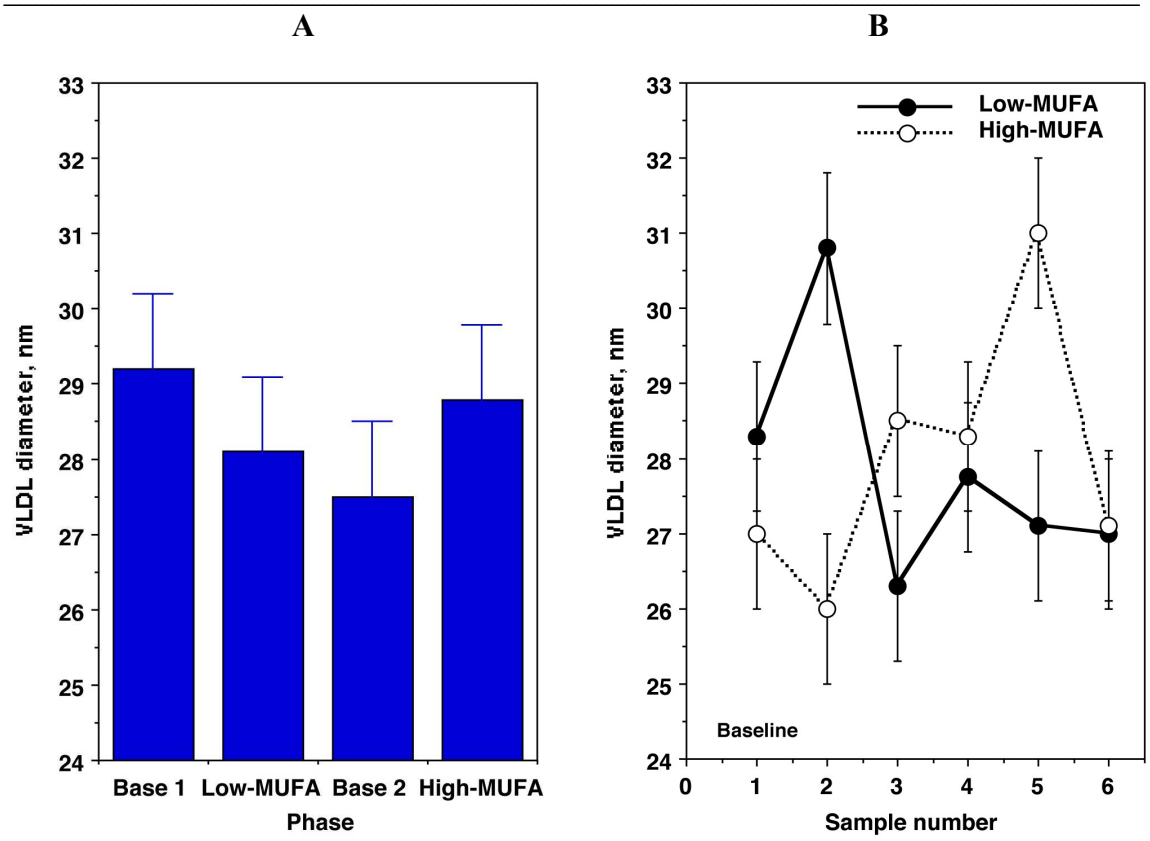
Plasma palmitic, palmitoleic, and oleic acid were positively correlated with plasma triacylglycerol and VLDL-C concentrations (**Table 8**). The highest correlation was between palmitoleic acid and triacylglycerols ($r = 0.90$). Palmitic and palmitoleic acid were negatively correlated with HDL-C and positively correlated with the LDL:HDL ratio (all $r \geq 0.34$). Linoleic acid was negatively associated with triacylglycerols, VLDL-C, and the LDL:HDL ratio.

Lipoprotein particle diameters

There was no significant difference in the treatment means for VLDL diameter in response to the MUFA group treatment (**Figure 9A**). VLDL diameters were not significant for trends by comparing the average of each MUFA group at each sampling, nor was there a significant interaction for MUFA group X Sample number ($P = 0.33$, **Figure 9B**).

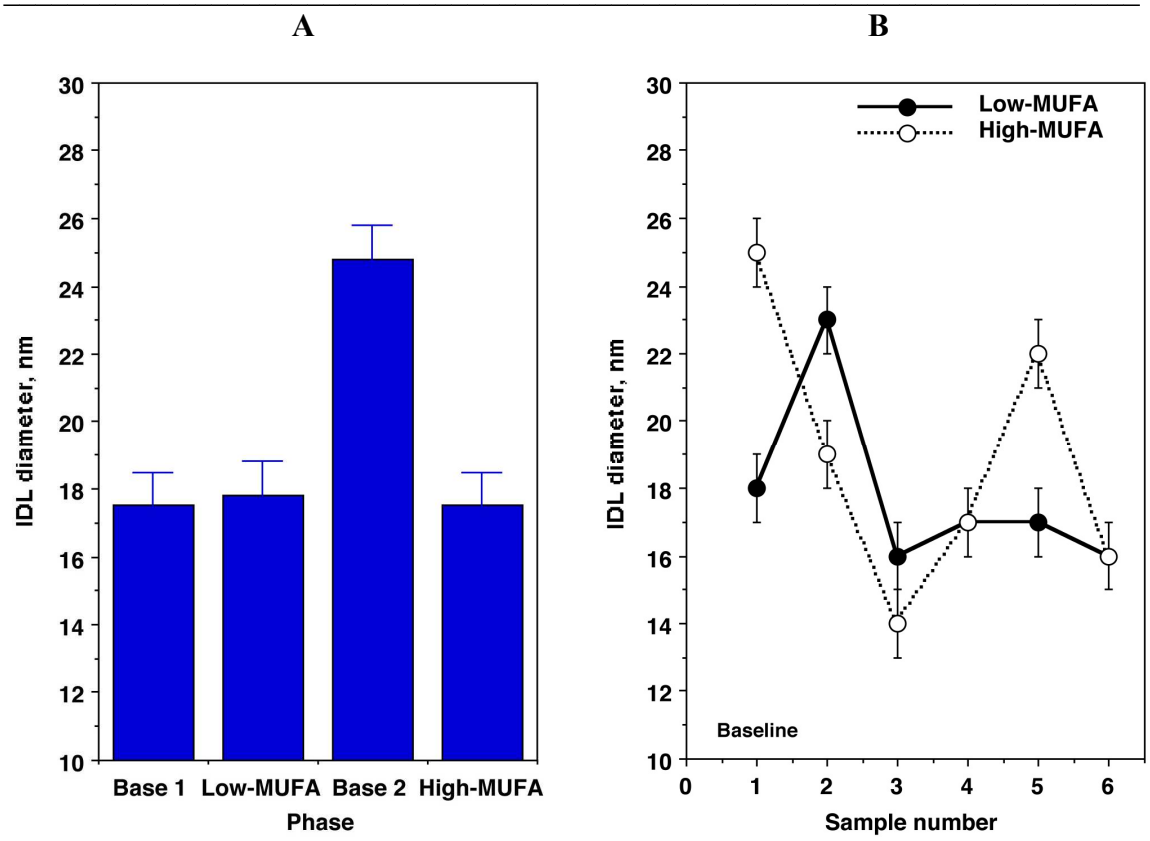
IDL particle diameters were not different comparing pooled treatment means. The treatment means at each sampling was not significantly different and there was no

Figure 9. VLDL diameters of mildly hypercholesterolemic men rotated from hamburger containing fat trim high in saturated fatty acids (Low-MUFA) to hamburger containing fat trim high in monounsaturated fatty acids (High-MUFA).



A: Treatment means pooled over sample number (n = 10; MUFA group, P = 0.94)
B: Treatment means at each sampling time:
Sample number, P = 0.89
MUFA group X Sample number, P = 0.33
Pooled SEM for each MUFA group is affixed to the means

Figure 10. IDL diameters of mildly hypercholesterolemic men rotated from hamburger containing fat trim high in saturated fatty acids (Low-MUFA) to hamburger containing fat trim high in monounsaturated fatty acids (High-MUFA).



A: Treatment means pooled over sample number (n = 10; MUFA group, P = 0.77)
B: Treatment means at each sampling time:
Sample number, P = 0.23
MUFA group X Sample number, P = 0.51
Pooled SEM for each MUFA group is affixed to the means

significant interaction for MUFA group X Sample number (**Figure 10**).

Pooled MUFA group means over sample number were not significantly different for LDL diameters (**Figure 11**). There was no significant difference for treatment means at each sampling time, but LDL particle diameter indicated a decrease in particle diameter over time while participants were consuming low-MUFA treatments. High-MUFA groups showed no change in particle diameter size, when analyzing pooled treatment means (**Figure 11**).

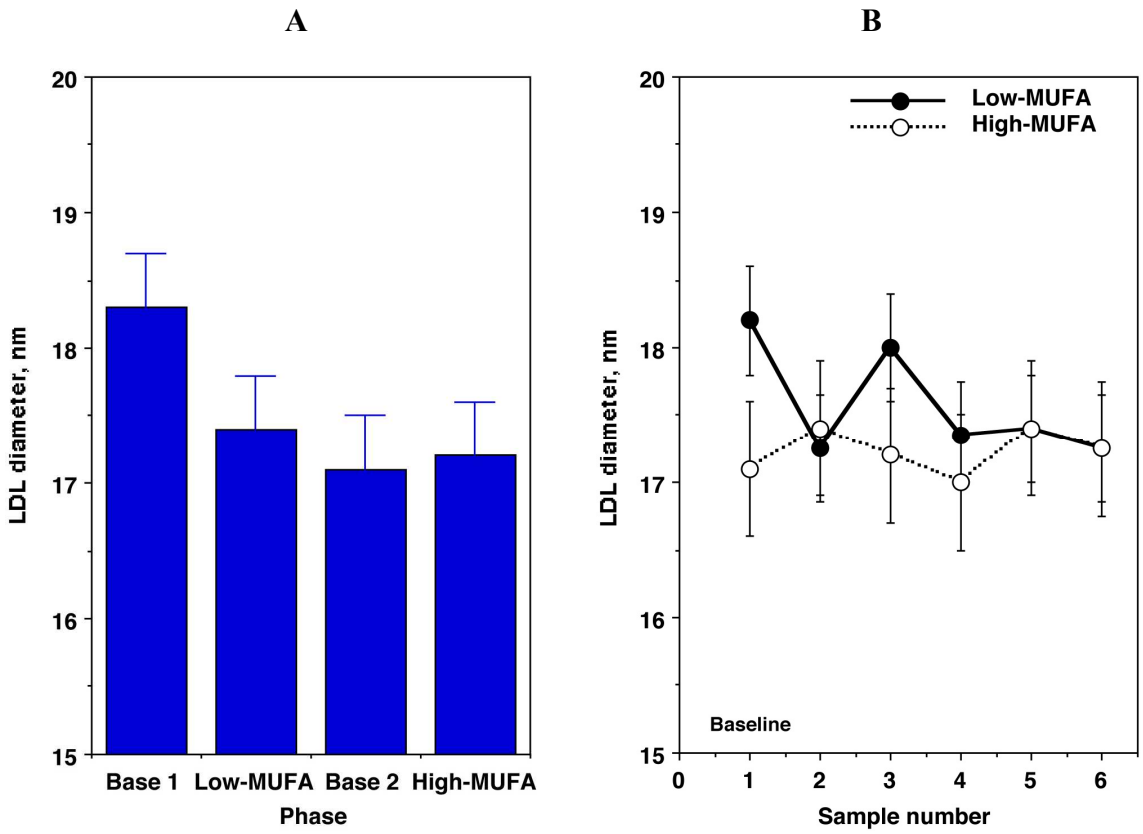
Mean LDL particle diameters were 19.7 ± 0.6 nm at baseline (**Table 3**), and LDL particle diameters ranged from 13.4 ± 0.4 nm at the 10^{th} percentile to 25.4 ± 0.7 nm at the 90^{th} percentile of the overall particle population diameter distribution (**Table 3, Figure 12**). Percentage baseline particle diameter was significantly different between the low-MUFA and high-MUFA treatment periods beyond the 50^{th} percentile of the LDL particle population (**Figure 12**), reflecting a decrease in particle diameter caused by the low-MUFA hamburger (to 18.1 ± 0.7 nm; **Tables 3 & 7**). LDL particle diameters did not increase significantly during the 3-wk washout period (18.3 ± 0.2 nm; **Figure 12**) or during the high-MUFA hamburger phase (18.4 ± 0.3 nm; **Table 7**).

HDL particle size was not affected by hamburger type (MUFA group), but was slightly lower overall during the second phase (**Figure 13;** $P = 0.13$). There was a trend that showed a sinusoidal tendency, but was not significant for treatment by sampling number (Figure 13B; $P = 0.74$). There was no significance in the interaction of MUFA group by sampling number (MUFA group X Sample number, $P = 0.47$).

Lipoprotein diameters as a function of plasma 16:1/18:0 ratio

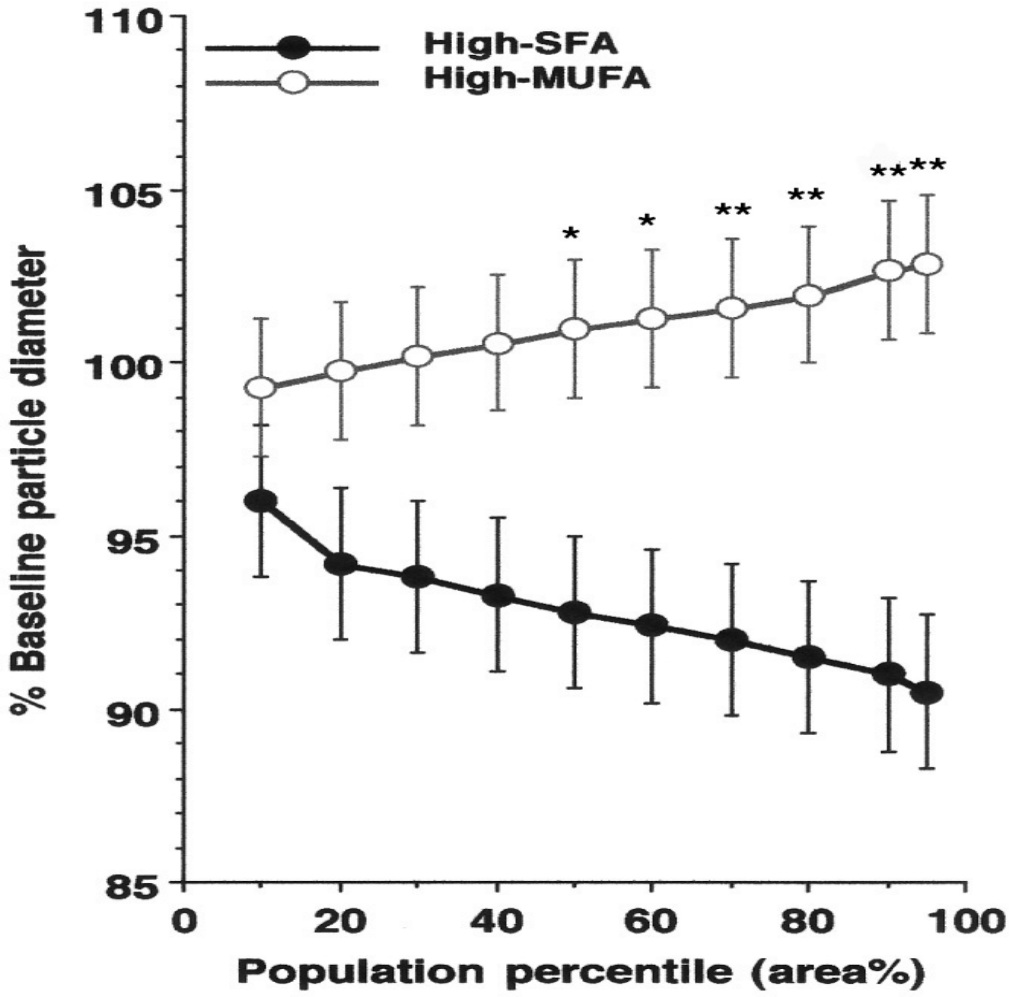
The plasma palmitoleic (16:1)/stearic acid (18:0) ratio ranged from a minimum of 0.04 to a maximum of 0.37 in **Figure 14**, repeating observations represented in **Figure 8**. In addition, VLDL diameters ranged from 15.3 to 47.5 nm (**Figure 14**). There were significant correlations between the palmitoleic/stearic acid ratio and VLDL particle diameter ($R^2 = 0.08$). Palmitic, stearic and oleic acid were negatively correlated with LDL particle diameters (**Table 8**).

Figure 11. LDL diameters of mildly hypercholesterolemic men rotated from hamburger containing fat trim high in saturated fatty acids (Low-MUFA) to hamburger containing fat trim high in monounsaturated fatty acids (High-MUFA).



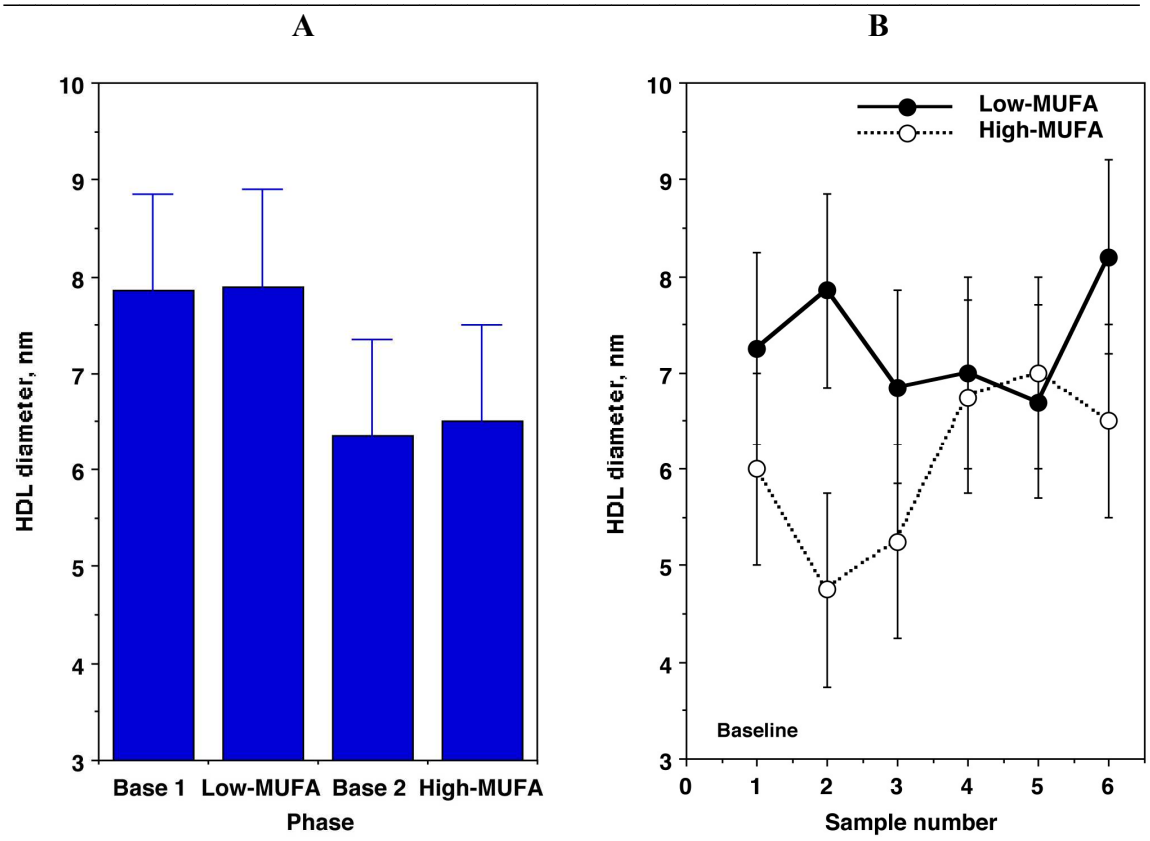
A: Treatment means pooled over sample number (n = 10; MUFA group, P = 0.44)
B: Treatment means at each sampling time:
Sample number, P = 0.51
MUFA group X Sample number, P = 0.31
Pooled SEM for each MUFA group is affixed to the means.

Figure 12. Changes from baseline values for LDL particle diameter percentiles for men rotated from hamburger high in saturated fatty acids (High-SFA; low MUFA) to hamburger enriched in monounsaturated fatty acids (High-MUFA).



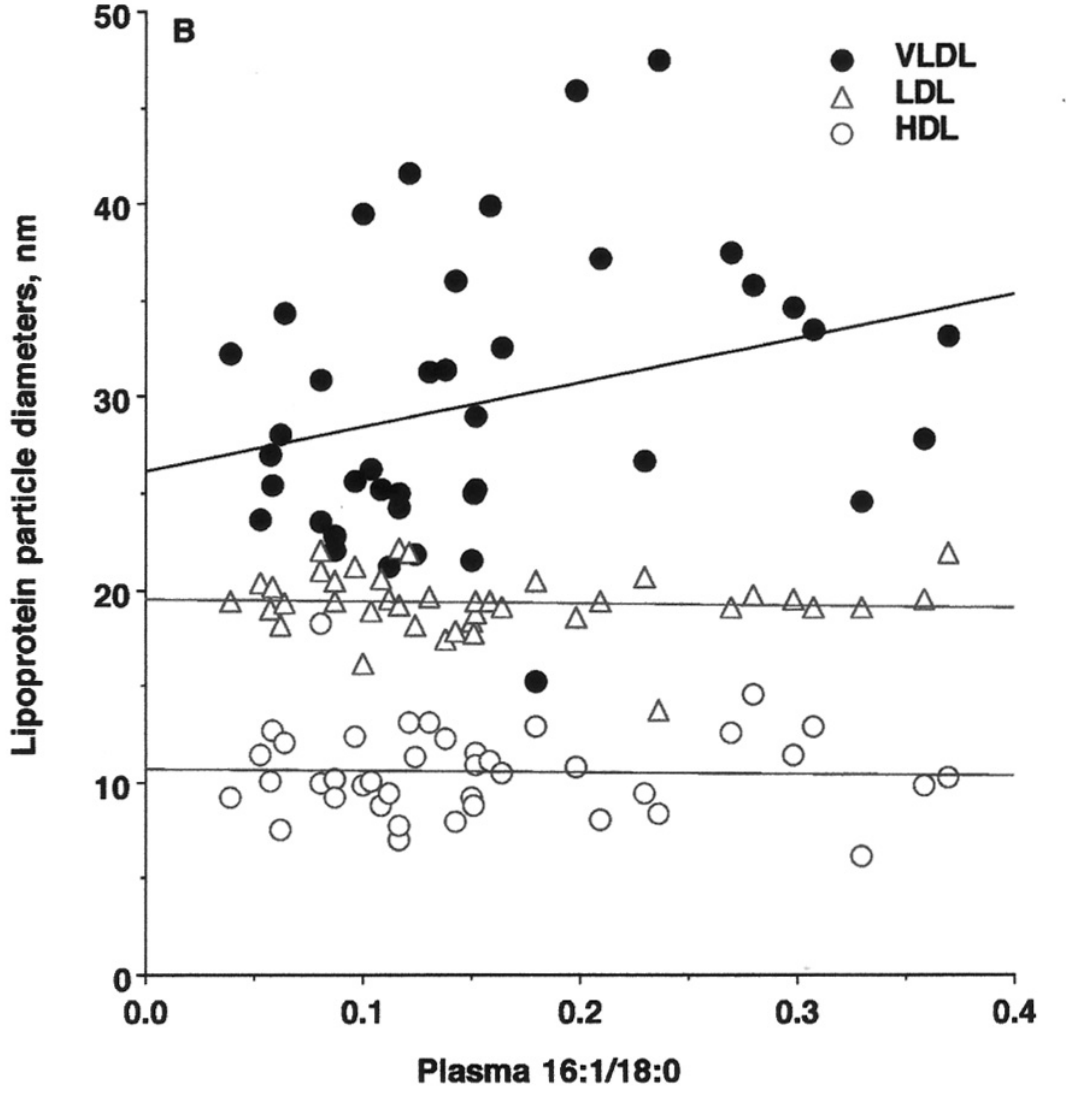
Baseline LDL particle diameters were:
19.1 ± 0.7 nm [prior to low MUFA (high SFA) group sampling]
18.3 ± 0.2 nm [prior to high MUFA phase]
Data are population percentiles for 10 men for each MUFA group.
Diameters at each decile were compared by a paired *t*-test.
Pooled SEM are affixed to the symbols.
*$P \leq 0.05$
**$P \leq 0.01$

Figure 13. HDL diameters of mildly hypercholesterolemic men rotated from hamburger containing fat trim high in saturated fatty acids (Low-MUFA) to hamburger containing fat trim high in monounsaturated fatty acids (High-MUFA).



A: Treatment means pooled over sample number (n = 10; MUFA group, P = 0.13)
B: Treatment means at each sampling time:
Sample number, P = 0.74
MUFA group X Sample number, P = 0.47
Pooled SEM for each MUFA group is affixed to the means.

Figure 14. Particle diameters as a function of the plasma 16:1/18:0 ratio.



Data are from baseline and final samples, for all participants.
VLDL: $y = 23.15x + 26.18$, $R^2 = 0.08$
LDL: $y = -1.08x + 19.58$, $R^2 = 0.04$
HDL: $y = -0.94x + 10.74$, $R^2 = 0.001$

CHAPTER V

SUMMARY AND CONCLUSIONS

Relative impetus

The primary cause of death in America is cardiovascular disease (CVD), according to the United States Department of Health and Human Services – National Center for Health Statistics. Diet influences the risk of CVD, although the exact components of a healthy diet are debatable and persistently being pursued by science. On the other hand, it is evident how costly CVD has become; the estimated direct and indirect cost of CVD for 2006 was $403.1 billion per annum [(33)]. Recommendations by the Nutrition Committee of the American Heart Association coupled with recommendations from the National Cholesterol Education Program (**NCEP**) Adult Treatment Panel III, redirected an adamant low-fat diet ruling to include diets that met 40% of dietary energy through fat intake. They set the Dietary Recommended Intake (**DRI**) to include 20% MUFA, < 7% saturated SFA, and 10% PUFA and concluded these DRI were as healthy for the heart as low-fat diets[(16)]. This recommendation has since been revised to reduce the intake of TFA to as low as possible. This set of recommendations initialized a wave of research to determine the nutrient density of typically higher fat foods such as dairy, nuts, and dietary oils such as olive oil, which is rich in the MUFA, oleic acid[(17)].

Dietary consumption of beef has not been thoroughly evaluated with regards to deliver its chief component of MUFA, oleic acid. This is the major theme in this

dissertation, which was conducted to determine if there was a relationship between oleic acid in beef-containing diets and indicators of CVD risk.

Participants in clinical trials under free-living conditions

It is of interest to note that during consumption of their habitual diets, total fat constituted approximately 35% of total dietary energy, with 11% from SFA, 11% from MUFA, and 5% from PUFA. The diet records indicated that the hamburger patties were added onto the habitual diets, rather than replacing a portion of the meat of their habitual diets, so the participants consumed an additional 40 g/patty during the test phases (45 – 46% total dietary energy from fat). Participants consumed as much as 2.5 g more TFA, 12 g more SFA, and 15.5 g less MUFA each week during the low-MUFA hamburger phase than they consumed during the high-MUFA hamburger phase.

Public interpretation and access to beef of variable nutrient constituents

Beef or beef products that vary widely in fatty acid composition have not yet been evaluated with regards to their effects on risk factors for CVD, perhaps because the fatty acid composition of beef was considered to be constant. Our survey of retail ground beef indicated that the MUFA:SFA tested in this study were reflective of the variation present in the available food supply. Of the ground beef types evaluated, most contained approximately 20% total fat, but the chub pack ground beef contained considerably more fat (28%) than the other ground beef types. Both the chub pack and ground chuck

ground beef from local retail outlets contained more total TFA than the low-MUFA hamburger used in this study, even though the low-MUFA hamburger contained more total fat. These data indicate that habitual consumption of the relatively inexpensive high-fat, chub pack ground beef potentially could cause some of the same effects caused by the low-MUFA test hamburger.

Plasma palmitoleic acid and apparent hepatic SCD1 activity

Warensjo *et al.* [34] evaluated the relationship between serum fatty acids and risk for CVD mortality and total mortality in 1,885 men from the Uppsala Longitudinal Study of Adult Men. They reported that, of the individual serum fatty acids, the greatest mortality risk was associated with palmitoleic acid, followed closely by palmitic acid. The serum concentration of linoleic acid was inversely related with CVD and total mortality. Warensjo *et al.* [34] concluded that serum palmitoleic acid and the palmitoleic:palmitic served as indices of hepatic stearoyl-CoA desaturase-1 (SCD1) activity, and that elevated hepatic SCD1 activity was positively associated with CVD mortality. In the current investigation, palmitoleic acid was the plasma fatty acid most highly correlated with changes in triacylglycerols, VLDL-C and HDL-C, followed by palmitic acid. The highest plasma palmitoleic acid concentration was observed at the end of the low-MUFA phase and the lowest after the high-MUFA phase, even though low-MUFA hamburger consumption delivered 29.5 g of palmitoleic acid in the 5-wk feeding period, which was much less than the 43.5 g provided by 5 wk of high-MUFA hamburger consumption. Clearly, the concentration of palmitoleic acid in the test hamburger cannot explain the

variation in plasma palmitoleic acid. Therefore, the low-MUFA ground beef may have stimulated hepatic SCD1 activity, which was reversed by consumption of the high-MUFA ground beef.

Ntambi and coworkers previously demonstrated that VLDL-triacylglycerols were virtually undetectable in mice with a disruption in the SCD1 gene[35]. In livers of SCD1 knockout mice, the concentration of palmitoleic was reduced nearly 50%. Sampath *et al.* [36] reported that Δ9 desaturation of saturated fats such as stearic acid by SCD1 was an essential step in mediating their ability to induce hepatic lipogenesis. Enoch *et al.* [37] demonstrated that palmitoyl-CoA and stearoyl-CoA have similar substrate properties for SCD1, and that oleoyl-CoA inhibits SCD1, in rat hepatocytes. The low-MUFA hamburger provided 15.7 g of SCD1 substrates (palmitic and stearic acid) and 15 g of potentially SCD1 inhibitory oleic acid. In contrast, the high-MUFA hamburger provided 16% less (13.2 g) SCD1 substrate and 15% more (17.2 g) inhibitory oleic acid. The marked changes in plasma palmitoleic acid in this study suggest that hepatic SCD1 activity is sensitive to the composition of ground beef available in retail markets, a proposition that requires direct testing.

LDL particle diameters

LDL particle diameters were reduced by the low-MUFA hamburger, and diameters remained depressed even after the 3-wk washout period as well as after consumption of the high-MUFA hamburger. Similarly, plasma palmitic acid was elevated by the low-MUFA diet, and remained elevated thereafter. Differences in LDL particle diameter

represent specific metabolic changes that increase the atherogenicity of LDL[19]. Small, dense LDL particles are recognized as a risk factor for CVD, as this form of LDL is more susceptible to oxidative damage[21] and promotes vascular inflammation[18]. The persistent, high circulating concentrations of palmitic acid following consumption of the low-MUFA hamburger may have depressed LDL clearance. This would have caused the reduced LDL particle diameters we observed following the low-MUFA phase which persisted through the washout period and the high-MUFA phase. This is supported by the negative correlation between plasma palmitic acid and LDL particle diameters. The observation that LDL particle diameters were not affected by the high-MUFA hamburger suggests that the additional oleic acid in the high-MUFA hamburger was unable to offset the depression in LDL diameter caused by the palmitic acid.

We previously established the effects of the consumption of low-MUFA (high-SFA) hamburger (17% fat; MUFA:SFA = 0.83 – 0.96) on lipoprotein cholesterol metabolism in free-living men[13]. Low-MUFA hamburger increased the apoB:LDL-cholesterol ratio, suggesting that LDL particles became smaller and more dense. This was confirmed by the results of the current study, and indicates that reduction of LDL particle diameters is a consistent effect of low-MUFA hamburger. A previous study[37] concluded that, relative to a high SFA, habitual diet, consumption of oils enriched in MUFA or polyunsaturated fatty acids reduced LDL diameter. However, these changes were less than 0.36 nm and the diet highest in MUFA (olive oil) actually increased LDL particle diameter by 0.13 nm[38]. Krause[39] previously reported that in approximately 70% of men (LDL subclass pattern A), reduction in LDL-C in response to low-fat diets

is the result of depletion of the cholesterol content of LDL particles; this is accompanied by a shift to smaller LDL particles. Wang *et al.* [29] later confirmed that high carbohydrate diets reduce LDL particle diameters in hamsters.

These earlier studies suggest that, in response to a high-fat diet enriched in SFA (and lower in carbohydrate), LDL particle diameters should have increased during the first phase of this study. However, the change in percentage energy from carbohydrates between the habitual (approximately 45%) and test hamburger phases (38 – 39%) in this study would not be considered to constitute a shift from a high carbohydrate to a low carbohydrate diet; nor would any of these diets be considered as low-fat diets (35 – 46% energy from fat). Instead, some component(s) of the low-MUFA ground beef interacted with the increase in total fat intake to reduce LDL particle diameter. Potential candidates are 18:1*trans*-10 and *trans*-vaccenic acid, as *trans*-fatty acids have been shown to have adverse effects on measures of CVD[40]. *trans*-Vaccenic acid has been shown to increase the LDL/HDL ratio in hamsters[41], although the effects of *trans*-fatty acids on LDL particle diameters has not been reported.

General considerations

Ground beef and hamburger from fast-food outlets are the most common sources of MUFA for adults[42], so production practices that can increase the concentration of oleic acid, or conversely, increase SFA and TFA in beef may differentially affect risk factors for CVD. Cattle with a genetic predisposition to deposit MUFA in their lean and fat tissues, such as Wagyu cattle[14,15] can be used to produce beef products that are

especially enriched with oleic acid and lower in SFA and TFA, and feeding practices can further enhance the composition of beef fat. This indicates that ground beef or hamburger products can be produced that are naturally enriched with oleic acid, and conversely that certain production practices can impair the nutritional quality of beef fat.

Finally, we cannot discern from this study design whether the high-MUFA hamburger reversed the effects of the hamburger with high SFA, or whether the subjects gradually adapted to the elevated intake of total fat. It is clear, however, that the high-MUFA hamburger did not exacerbate any of the effects of the low-MUFA hamburger and can be viewed as at least neutral in its effects on HDL-C and triacylglycerols.

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VITA

Thaddeus Hunter Adams

Department of Animal Science
Texas A&M University
College Station, Texas 77843-2471

(979) 845-1541
thadams@tamu.edu

Education

Texas A&M University	Texas A&M University	Texas A&M University
College Station, TX	College Station, TX	College Station, TX
Ph.D. in Nutrition	M.S. in Nutrition	B.S. in Biomedical Science
Aug 2012	Dec 2007	Dec 1995

Experience

Graduate Research Assistant, Texas A&M University	Dec 2002–Aug 2012
Graduate Teaching Assistant, Texas A&M University	Jan 2006–Sep 2007
Laboratory, Teaching & Field Assistant, Cornell University	May 2000–Dec 2001
Graduate Research Assistant, Texas A&M University	May 1996–May 1999
Laboratory Technician, Texas A&M University	Aug 1995–May 1996

International Experience

Graduate Research Fellow National Institutes of Health Mexico International Research Training in Environmental Health Universidad Nacional Autonóma de México, México City	Summer 1998
Aggies For Christ to the Orient, Thailand, Philippines, Malaysia	Summer 1992
Brazos County 4-H Ambassador, Australia & New Zealand	Summer 1990

Publications

Adams TH, Walzem RL, Smith DR, Tseng S, and Smith SB (2010) Hamburger high in total, saturated and trans-fatty acids decreases HDL cholesterol and LDL particle diameter, and increases TAG, in mildly hypercholesterolaemic men. *Br J of Nutr* **103**, 91-98.

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