

Increased Beef Consumption Increases Apolipoprotein A-I but Not Serum Cholesterol of Mildly Hypercholesterolemic Men with Different Levels of Habitual Beef Intake

DANA R. SMITH,*¹ RANDALL WOOD,* STEPHEN TSENG,† AND STEPHEN B. SMITH‡
*Departments of *Biochemistry and Biophysics, †Medicine, and ‡Animal Science, Texas A&M University, College Station, Texas 77843*

The objective of this research was to compare the effects of a lean beef enriched in oleic acid to a beef that is typical of the commercial beef consumed in the United States. Ten mildly hypercholesterolemic men, ages 34–58 years old, were selected from the Texas A&M University faculty and staff. Subjects were randomly assigned to one of two diets for a 6-week duration followed by a crossover after a 4-week habitual diet washout period. Diets were consumed daily for a 6-week study period. Participants substituted lean beef obtained from Wagyu bullocks or commercial beef for the meat typically consumed. Total cholesterol, apolipoproteins A-I and B, triacylglycerols, and low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol were measured in serum samples collected weekly. Beef type had no effect on any measured variable. There were no significant differences between baseline HDL or LDL cholesterol concentrations after the consumption of the beef test diets. Apolipoprotein A-I, serum glucose, and uric acid concentrations were elevated by the additional dietary beef. Analysis of records of customary diets indicated that one group consumed 160 g of beef daily, whereas the other group consumed only 26 g of beef daily. Therefore, *post hoc* analyses tested the habitual beef intake \times treatment time interaction. LDL cholesterol concentration was markedly higher in the group with low habitual beef intake (180 vs 144 mg/dl), and HDL cholesterol was slightly higher (44 vs 40 mg/dl; post-test values) than for the group with high habitual beef intake, but there were no habitual intake \times time interactions for LDL or HDL cholesterol. Creatinine and blood urea nitrogen concentrations also were greater in the individuals habitually consuming less beef. This study had three important findings: i) a lean beef source enriched with oleic acid was no different from commercial beef

in its effect on lipoprotein fractions; ii) neither previous level of beef intake nor baseline LDL cholesterol concentration influenced the serum cholesterol response to added dietary beef, which was negative; and iii) apolipoprotein A-I, but not HDL or LDL cholesterol, was sensitive to the additional dietary beef.

[Exp Biol Med Vol. 227(4):266–275, 2002]

Key words: cholesterol; apolipoproteins; beef; fatty acids

Reports linking dietary fat to serum lipid levels have often been interpreted to mean that the general public, especially those at risk for coronary heart disease, should consume low fat diets containing little or no red meat. Researchers previously concluded that dietary saturated fatty acids elevate serum cholesterol concentrations, whereas polyunsaturated fatty acids reduce serum cholesterol concentrations, and monounsaturated fatty acids have little or no effect (1, 2). The major monounsaturated fatty acid in beef, oleic acid, and in the diet as a whole, has been studied in more detail and has been found to lower low density lipoprotein (LDL) cholesterol without affecting the beneficial high density lipoprotein (HDL) cholesterol (3–5). This effect is most convincing in studies in which natural foods were used to supplement diets with oleic acid (e.g., Refs. 5–7). In addition, saturated fatty acids have been found to have different effects. One of the major saturated fatty acids in beef, stearic acid, has been found to have no effect on or even to lower serum cholesterol (8, 9).

Monounsaturated fatty acids plus stearic acid represent 60% or more of the total fatty acids in beef (10, 11), therefore, the consumption of beef and its associated fat may not increase serum cholesterol. Lean beef has been shown to decrease (12) or have no effect (13) on serum cholesterol in free living individuals. We wished to test the effects of regular-fat beef on serum lipid levels of mildly hypercholesterolemic men. Furthermore, a leaner beef with a higher concentration of oleic acid was compared with typical com-

This work was supported by Briggs Ranch (Rice, TX) and by the Texas Agricultural Experiment Station.

¹ To whom requests for reprints should be addressed at Nutrition Consultant, 2914 Colton Place, College Station, TX 77845. E-mail: sbs2185@acs.tamu.edu

Received June 18, 2001.
Accepted December 3, 2001.

1535-3702/02/2274-0266\$15.00
Copyright © 2002 by the Society for Experimental Biology and Medicine

mercial beef to test the effects of calories from fat on serum cholesterol homeostasis.

Materials and Methods

This study was approved by the Texas A&M University Institutional Review Board for use of human subjects in research.

Subjects. Texas A&M University faculty and staff were recruited for this study. After screening 38 volunteers, 10 mildly hypercholesterolemic men, ages 34–58 years (average age of 47 ± 8 years), were selected. None of the participants had a history of coronary vascular disease or diabetes and none were on medication for hypercholesterolemia. The average body weight was 91 ± 11 kg, and all participants were normotensive. Screening criteria consisted of a battery of clinical chemistry tests, a physical examination including electrocardiogram, family health history, and personal conflicts (i.e., personal stressors). All participants were nonsmokers, and none were taking prescribed medication. All participants signed an informed consent, were free living, and were instructed to maintain routine activities and body weight (± 2.2 kg of entry weight). Exercise and physical activities were not restricted. Nine of the 10 men indicated that they were sedentary, whereas one individual participated in light exercise (not defined).

Experimental Design. Two groups of five men were randomly assigned to one of the two test beef diets for a 6-week duration followed by a crossover after a 4-week habitual diet washout period. The periods started January 29, 1998 and ended May 21, 1998, which allowed the university spring break and Easter holiday to fall within the habitual diet washout period.

Test Diets. Participants were instructed to incorporate the study beef into their diet with as little disruption as possible. Participants substituted an average of 98 g of lean or commercial beef for the meat they typically consumed daily for a 6-week study period. The beef was supplied in the form of 114 g of ground beef (four times per week) and 228 g of steaks (one time per week). Two weeks before the study, two Wagyu bullocks (which served as the source of the lean beef higher in oleic acid) weighing 400 kg each were butchered at the Texas A&M University Rosenthal Meat Science & Technology Center; this meat was graded USDA Low Choice. The commercial beef consisted of USDA Choice beef strip loins and USDA Choice beef rib-eye roasts purchased from a local meat purveyor. Both the lean and commercial beef were processed into ground beef and boneless steaks, and were individually vacuum packed and quick-frozen. The frozen beef for an entire diet period was delivered to the participants on or before the first day. No restrictions were placed on how the beef was to be prepared or the frequency of consumption.

Blood Collection and Analysis. Weekly 12-ml samples of blood following a 14-hr fast were collected in Vacutubes containing clotting factor from all 10 participants

while they were consuming the test diets. In addition, baseline samples were collected for 2 consecutive weeks before beginning a test diet. Blood was collected from all participants at 0730 hr to 0830 hr on the same day.

Within 1 hr of collection, the serum was obtained by centrifugation. Aliquots were removed for immediate analyses and for future analyses of apolipoproteins and fatty acids. Aliquots for future analyses were stored at -20°C . To monitor the health of the participants, nonlipid analyses were performed weekly on fresh serum by a robotic centrifugal COBAS FARA analyzer (Roche Diagnostic Systems, Montclair, NJ). These analyses included total blood urea nitrogen, creatinine, glucose, lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, and total protein, albumin, phosphorous, uric acid, calcium, and magnesium. Most assays were conducted with reagents, calibrator standards, and reference standards purchased from Roche Diagnostic Systems and Sigma (St. Louis, MO). Immediately before the study began, the FARA analyzer was certified to be operating at manufacturer's specifications by a company technician.

Lipid and apolipoprotein measurements made with the FARA analyzer included total serum cholesterol, triacylglycerols, apolipoprotein A-I, and apolipoprotein B. Total cholesterol was analyzed using the multienzyme system described by Allain *et al.* (14). The HDL cholesterol was determined by the same assay after chylomicrons, very LDL (VLDL), and LDL cholesterol were precipitated with Dextran sulfate and magnesium sulfate (15) (Seragen, Indianapolis, IN). The LDL cholesterol values were calculated using the Friedewald, Levy, and Fredrickson (16) formula. The enzymatic method of Bucolo and David (17) was used to measure triacylglycerols. Apolipoproteins A-I and B were determined immunoturbidimetrically (18, 19) at the end of the study using a single lot of Roche reagents (Roche Diagnostic Systems, Nutley, NJ). Cholesterol and triglyceride determinations were made on fresh serum. Apolipoprotein determinations were made on frozen serum that was thawed only once. All serum analyses were run in duplicate. If duplicates for a specific analysis differed by more than 2%, then the analysis was repeated in triplicate.

Aliquots of serum (0.5–1.5 ml) and 1-g samples of the beef supplements were extracted by the Folch *et al.* (20) method aided by sonication. The chloroform solvent was removed under reduced pressure in a rotary evaporator, and traces of water were removed under vacuum. Aliquots of the total serum lipid were transesterified in acid-catalyzed anhydrous methanol. Methyl ester analyses were made on a 30 m \times 0.53 mm (ID) fused silica capillary column containing DB 225 liquid phase. The column temperature was programmed from 140° – 225°C at $3^{\circ}\text{C}/\text{min}$. The data were collected with an IBM model 9000 laboratory computer. Chromatography with standard reference fatty acids mixtures (Nu-Chek Prep, Elysian, MN) was used for fatty acid peak identification. All the procedures used to extract,

derivatize, and analyze the serum lipid methyl esters have been described previously in detail (21).

Statistical Analysis. Statistical analyses were performed using the SuperAnova program (Abacus Concepts, Berkeley, CA). Habitual consumption values for initial group assignments were compared by analysis of variance (ANOVA). Because analysis of habitual consumption indicated marked differences in beef intake between groups, the initial statistical model tested the beef type \times habitual consumption \times time interactions. Beef type had no significant effect on any measure and therefore was dropped from the model. Thereafter, habitual beef consumption group and time effects were tested by two-factor ANOVA. The model tested the consumption group and time main effects and the consumption group \times time interactions. Initial values were pooled and test values were pooled for the comparison of overall beef treatment effects by one-factor ANOVA. The data are expressed as means \pm SD.

Results

Beef and Serum Lipids. The commercial ground beef contained 17% total lipid. The Wagyu ground beef contained less extractable lipid, so Wagyu fat trim was added to raise the total lipid content of the ground beef to 16.6%. The commercial beef steaks contained 14.6% total lipid, whereas the Wagyu steaks contained only 2.3% total lipid. The fatty acid composition of the commercial and Wagyu ground beef consumed in the study is given in Figure 1A. Both beef sources contained the normally high levels of stearic, palmitic, and oleic acids. The Wagyu ground beef contained a higher percentage of oleic acid than did the commercial ground beef due to the higher percentage of monounsaturated fatty acids in the trimmable fat of the Wagyu beef (9). The Wagyu beef contained a higher percentage of linoleic acid than commercial beef (6.9% vs 2.6%; data not shown). However, averaged over the test period, the daily intake of linoleic acid was essentially identical between test beef types (2.3% vs 2.6%). Baseline serum fatty acid compositions were compared with serum fatty acid compositions during the consumption of the beef test diets (Fig. 1B). There were no significant differences between the serum fatty acid concentrations at baseline and with the consumption of the test diets.

Participant Characteristics and Food Intake Profiles. The age, height, weight, and food intake profiles (taken from dietary records) of the individual study participants are presented in Tables I and II. These data were grouped based on the *post hoc* separation of groups into a low-beef- and a high-beef-consuming group. Ages ranged from 33–58 years (mean age of 47 years). Height ranged from 170.2–185.4 cm (mean height of 179.1 cm), whereas body weight ranged from 73–111 kg (mean body weight of 91 kg). There were no differences between groups ($P > 0.63$). Body mass index, calculated from the data in Table I, was 28.4 ± 1.6 and 28.0 ± 3.8 for the low-beef and high-beef consuming groups ($P = 0.81$).

Analysis of the participants' 4-day diet records for their habitual diets indicated that mean protein, carbohydrate, and fat were 16%, 47%, and 35% of total kilocalories, respectively. Beef consumption represented 57% of total meat consumed, but the range was 0%–100%. The low-beef-intake group consumed an average of 26 g of beef/day, whereas the high-beef-intake group consumed an average of 160 g of beef/day. Only two participants consumed less than 30% of their energy intake as fat (Table II). There were no differences in the contribution of fatty acid classes (as a percentage of dietary calories) or cholesterol intake between the high- and low-beef-consuming groups (Table II).

Records of food consumption during the test period were not taken, so percentages of calories from each fatty acid class cannot be calculated. Daily intake of each fatty acid class contributed by the test beef sources is indicated in Table III. The test beef contributed 5.5 g (Wagyu) to 7.5 g (commercial) of fat to the diet daily, or at most 21% of the fat consumed in their customary diets (35 g/day). The lean, Wagyu beef provided less fat but a greater percentage of monounsaturated fatty acids than the commercial beef (47.9% vs 44.7%). The test beef contributed 73–78 mg/day cholesterol.

Serum Lipids. Beef type (lean versus commercial) had no effect on any variable measured, so the following discussion will focus on the effects of additional and customary beef intake. Total, LDL, and HDL cholesterol concentrations were unaffected by the consumption of the beef diets (Table IV; Fig. 2, A and B). LDL cholesterol concentration was markedly greater ($P = 0.001$) and HDL cholesterol was slightly higher ($P = 0.004$) in the group with low habitual beef intake than in the group with high habitual beef intake, but there were no habitual intake \times time interactions for LDL or HDL cholesterol. Apolipoprotein A-I was increased 15% ($P = 0.001$), whereas apolipoprotein B was unaffected ($P = 0.13$) by consumption of the additional test beef (Fig. 3, A and B). The apolipoprotein A-I:HDL cholesterol ratio was increased ($P = 0.003$) and the apolipoprotein B:LDL cholesterol ratio tended to be increased ($P = 0.08$) by the consumption of the test beefs. Both ratios were higher ($P = 0.001$) in those men habitually consuming more beef. Triglyceride concentrations also were greater ($P = 0.001$) in the high-beef-consuming group of men, but were unaffected ($P = 0.51$) by the test beef diets (Table IV).

Serum Values for Other Metabolites. Consumption of the beef diets significantly elevated serum glucose, creatinine, and uric acid concentrations over baseline values. Creatinine and blood urea nitrogen concentrations were greater in the individuals habitually consuming less beef (Table IV). Other serum variables were normal and are not reported.

Discussion

Participant Compliance. Only four blood samples were missing from the data set; these occurred due to travel.

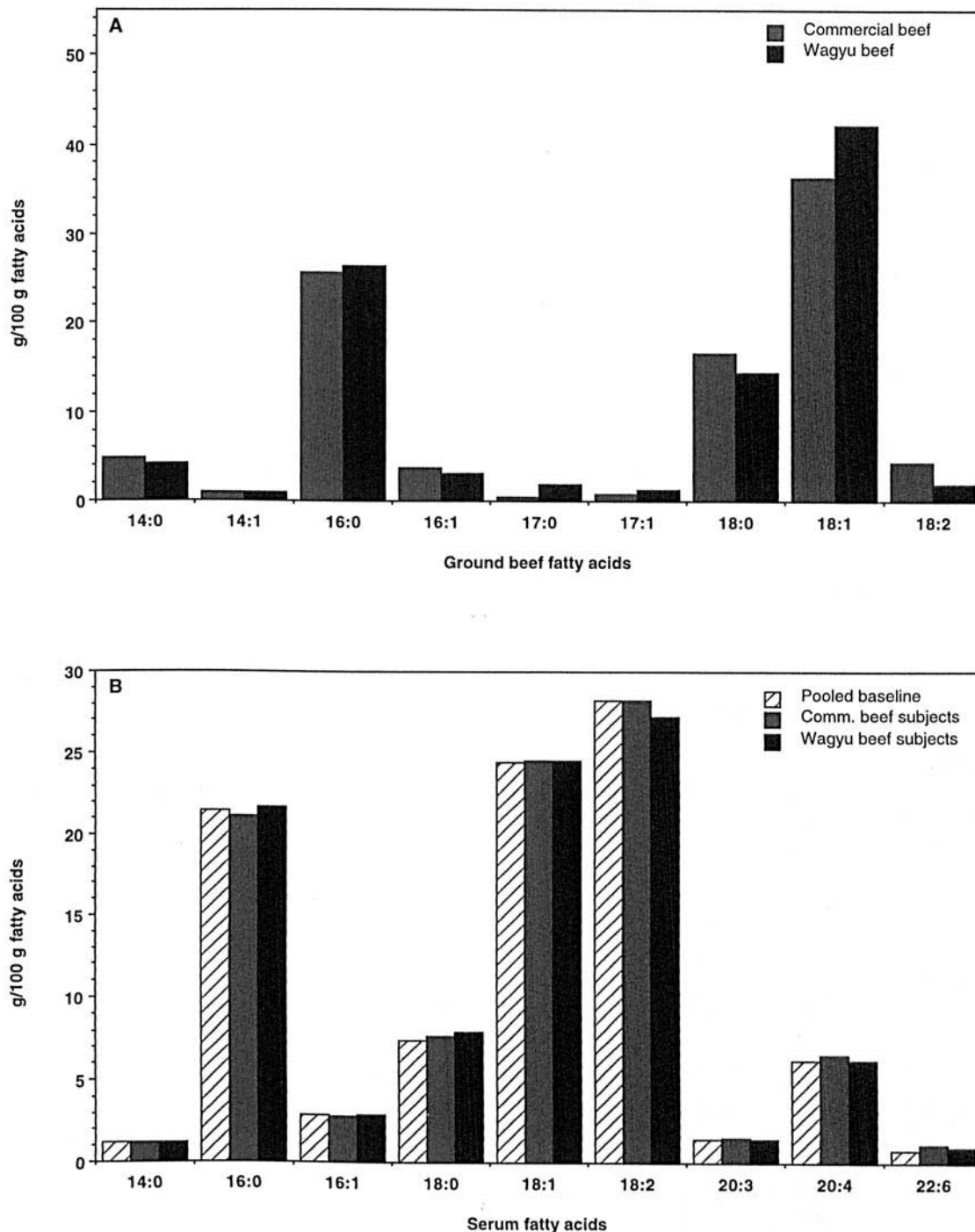


Figure 1. Fatty acid composition of ground beef (burger; A) formulated from commercial or lean (Wagyu) beef, and fatty acid composition of serum (B) from subjects at baseline or after consumption of commercial (Comm.) or Wagyu burger and whole steaks. Serum values are means averaged for four baseline and 12 test values. There were no significant differences in serum fatty acids before or after consumption of the test diets or between the test diets. Pooled SD for 14:0, 16:0, 16:1, 18:0, 18:1, 18:2, 20:3, 20:4, and 22:6 were 0.63, 2.9, 1.1, 0.91, 2.7, 5.0, 0.5, 1.5, and 0.38, respectively.

The low serum glucose levels indicated that the participants had fasted prior to the blood sampling. These observations suggest participant compliance with study guidelines.

Group 2 participants habitually consumed an average of 115 g/day of meat, and only 26 g/day of beef. During the test periods, they consumed an average of 98 g/day of beef (averaged over the consumption of ground beef and steak), which was a dramatic increase in their consumption of beef.

Participants collectively lost 4 kg during the first 6-week study period and 3 kg during the second 6-week study period, indicating that the test beef was substituted for some portion of the participants' customary diets. Group 1 participants consumed the lean (Wagyu) beef during the first period, whereas Group 2 participants consumed the fatter commercial beef during the first period, but there was no difference between groups in weight loss ($P > 0.25$). Others

Table I. Age, Height, Weight, and Food Intake Profiles of Participant's Customary Diets as Determined from 4-Day Diet Records

Participant	Age (year)	Height (cm)	Weight (kg)	Average daily intake				
				Calories (kcal)	Protein (%)	CHO ^a (%)	Beef (g)	Meat (g)
Group 1 (high beef)								
1	34	175.2	86.4	2112	21	40	196	258
4	46	182.9	100.0	2000	22	36	238	298
5	43	175.2	90.9	2363	14	50	174	180
6	58	177.8	90.9	2053	14	51	122	164
10	51	180.3	84.1	2693	15	48	70	161
Group 2 (low beef)								
2	48	177.8	75.0	1726	15	46	32	32
3	57	182.9	90.9	2255	15	41	28	228
7	33	185.4	104.5	2174	11	68	0	82
8	48	182.9	113.4	2400	15	47	56	125
9	50	170.2	73.6	2187	19	47	14	109
Group 1 mean	46	178.3	90.4	2244	17	45	160	212
SD	9	3.3	6.1	286	4	7	65	62
Group 2 mean	47	179.8	91.1	2148	15	50	26	115
SD	9	6.1	16.9	252	3	10	21	72
P values	0.89	0.63	0.93	0.59	0.34	0.41	0.002	0.05

^a Abbreviations: CHO, carbohydrates; SD, standard deviation.

Table II. Percentage of Fat Calories by Class of Fatty Acids and Quantity of Cholesterol Consumed by Participants Consuming Their Customary Diets

Participant	Fat		Percentage of dietary calories from ^a :			Cholesterol (mg/day)
	%	g/day	Saturated fatty acids	Monounsaturated fatty acids	Polyunsaturated fatty acids	
Group 1 (high beef)						
1	39	93	15.6	12.9	7.8	680
4	42	76	14.7	18.5	5.5	300
5	36	121	13.0	12.2	6.1	472
6	34	97	9.9	12.6	8.2	139
10	26	88	9.1	9.1	6.5	246
Group 2 (low beef)						
2	39	79	9.4	16.8	10.1	355
3	44	51	14.5	16.3	9.7	320
7	21	103	6.5	7.8	4.8	230
8	38	77	16.3	10.6	8.7	330
9	33	74	10.6	12.9	5.6	340
Group 1 mean	35.4	86.2	12.5	13.0	6.8	367
SD	6.1	4.3	2.8	3.4	1.1	212
Group 2 mean	35.0	85.6	11.4	12.9	7.9	315
SD	8.7	27.0	3.9	3.8	2.4	49
P values	0.93	0.96	0.66	0.93	0.44	0.60

^a The sum of the saturated, monounsaturated, and polyunsaturated fatty acid diet calories do not equal the total fat calories because some food tables do not give a breakdown of fatty acids on some foods. SD, standard deviation.

(e.g., 22) have indicated that consumption of diets low in carbohydrates and high in protein elicited weight losses. However, the participants of this study were instructed to substitute the test beef for their customary meat sources and not other dietary components, so their intake of carbohydrate and protein should not have changed over the test periods.

Diet Effect on Serum Fatty Acid Values. There were no significant differences between the serum lipid fatty acid composition after consumption of the test beef diets and baseline (habitual) diet compositions. Nor was

there any similarity in the fatty acid composition of the beef and the serum fatty acid composition of the participants. Linoleic acid (18:2 *n*-6) is a minor fatty acid of beef lipids but is the major serum lipid fatty acid. In humans, there is not a strong relationship between dietary and plasma fatty acids (23–27). We did observe a nonsignificant depression in linoleic acid in those subjects consuming the Wagyu beef. The Wagyu ground beef contained a lesser percentage of linoleic acid (although the steaks contained a higher percentage). This was not of significance in this study because

Table III. Grams of Fat by Class of Fatty Acids and Quantity of Cholesterol Consumed by Participants Provided by the Test Beef

Participant	Fat (g/day ^a)	Percentage of fat from:			Cholesterol (mg/day ^c)
		Saturated fatty acids	Monounsaturated fatty acids	Polyunsaturated fatty acids ^b	
Commercial beef					
Ground beef	11.1	48.1	42.0	4.4	53
Steaks	4.8	45.5	49.9	2.6	25
Daily average	15.9	47.6	44.7	3.9	78
Wagyu beef					
Ground beef	10.8	47.7	47.6	1.9	52
Steaks	0.7	42.7	48.8	6.9	21
Daily average	11.5	47.6	47.9	2.2	73

^a Total grams of fat from each dietary source was based on the consumption of 446 g/week of ground beef and 228 g/week of whole steak. Ground beef and whole steak from the commercial source contained 17% and 14.6% fat, respectively. Ground beef and whole steak from the Wagyu bullocks contained 16.6% and 2.3% fat, respectively.

^b Linoleic acid (18:2 *n*-6) was the only measurable polyunsaturated fatty acid in the test beef.

^c Cholesterol intake (mg/day) from the test beef was calculated as (g fat/day × 120 mg cholesterol/100 g fat) + (60 mg cholesterol/100 g serving × g beef/day; Refs. 38, 39).

Table IV. Serum Lipid and Metabolite Values of Mildly Hypercholesterolemic Men Habitually Consuming Low (26 g/day) or High (160 g/day) Amounts of Beef

Item	Low-beef group		High-beef group		P values		SE
	Initial value	Test value	Initial value	Test value	Group	Time	
Total cholesterol ^a	241.1	252.9	229.0	232.7	0.008	0.47	33.9
HDL cholesterol	41.7	44.1	41.7	40.2	0.004	0.71	0.5
LDL cholesterol	172.5	179.5	148.8	144.4	0.001	0.57	2.6
Apolipoprotein A-I	173.1	199.0	175.3	203.8	0.38	0.001	2.5
Apolipoprotein B	155.8	177.1	154.1	173.1	0.62	0.13	3.4
ApoA-I:HDL cholesterol ratio	4.19	4.57	4.38	5.17	0.001	0.003	0.16
ApoB:LDL cholesterol ratio	0.90	0.98	1.05	1.26	0.001	0.08	0.05
Triacylglycerols	134.1	146.5	192.5	240.3	0.001	0.51	8.0
Glucose	81.2	90.9	83.5	91.1	0.74	0.001	1.1
Creatinine	1.18	1.26	1.17	1.18	0.01	0.004	0.01
Blood urea nitrogen	14.9	17.3	14.0	14.7	0.006	0.67	0.37
Uric acid	5.38	6.15	5.56	6.04	0.84	0.001	0.08

^a All concentrations are milligrams per decaliter.

there was no effect of beef type on any of the lipoprotein cholesterol fractions.

Diet Effect on Nonlipid Serum Values. The reason for the significant elevation of serum glucose levels when consuming the beef diets is not obvious. Shiue *et al.* (22) demonstrated recently that individuals consuming an additional seven portions of beef weekly had greater serum glucose than individuals consuming the American Heart Association pyramid diet. Those consuming the additional beef also had higher concentrations of circulating alanine and glutamine, which could serve as substrate for elevated gluconeogenesis. Also, beef protein has been suggested to be 10 times more effective in stimulating glucagon release than glucose is in suppressing glucagon levels (28). This could trigger increased serum glucose levels via gluconeogenesis as the liver catabolized amino acids in excess of dietary requirements.

Diet Effect on Serum Lipid Values. There was a highly significant increase in apolipoprotein A-I, a major

component of HDL cholesterol, with the consumption of both beef diets. The elevated apolipoprotein A-I concentration and apolipoprotein A-I:HDL cholesterol ratio in mildly hypercholesterolemic men consuming beef might seem unexpected considering guidelines that suggest that consumption of animal fat and beef should be limited (29–31). However, earlier studies demonstrated that consumption of regular beef (32, 33) had no effect on cholesterol in men, whereas lean beef (7% lipid) reduced serum cholesterol (12) in healthy men and women.

For Group 2 (low-beef-consuming) participants, the addition of five servings of beef weekly to the diet represented a profound change in dietary habits. During the 4-day period in which records were kept, most Group 2 individuals consumed beef only once. The test beef would have changed the intake many nutrients, especially the B vitamins. We assumed that Group 1 participants replaced their customary beef with the test beef, which should not have caused a substantial change in the intake of other nutrients. Because

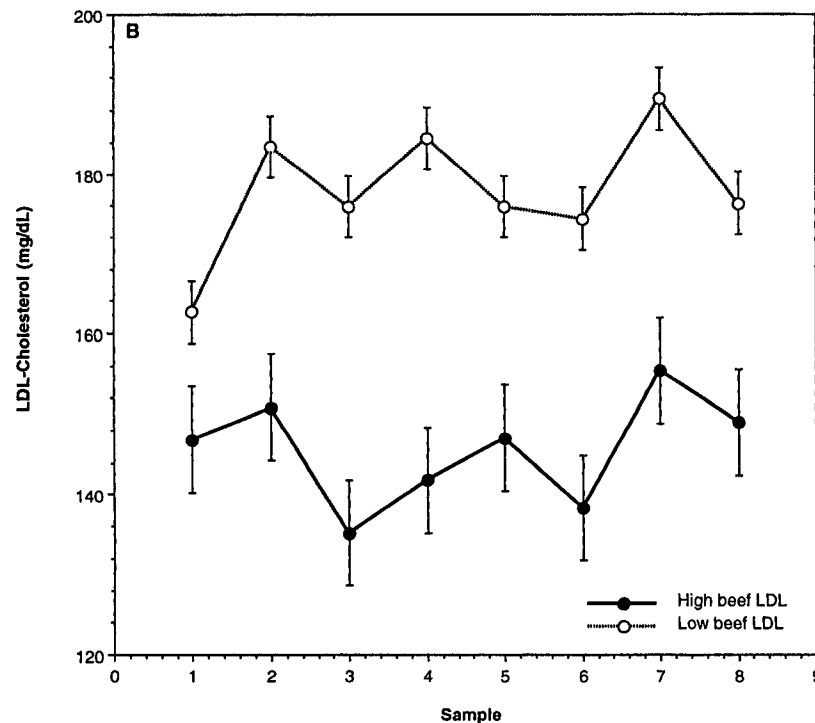
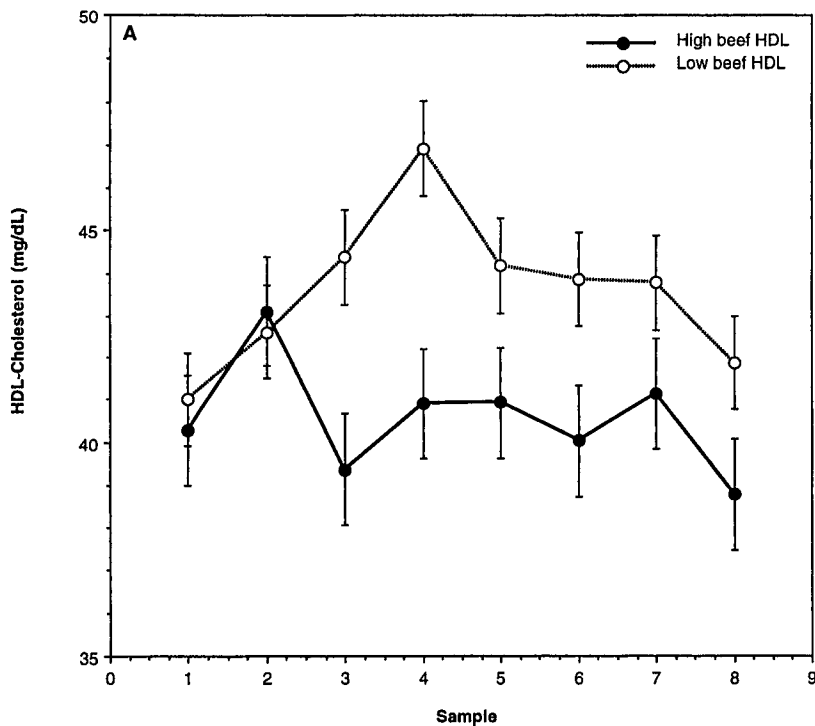


Figure 2. HDL (A) and LDL (B) cholesterol in subjects with high habitual beef intake (High beef) or low habitual beef intake (Low beef). Data are means of five individuals per intake group, pooled over two test periods (commercial and Wagyu beef). Average SE for HDL and LDL cholesterol are attached to the lines. Low-beef intake participants had greater overall HDL ($P = 0.004$) and LDL ($P = 0.001$) cholesterol concentrations than high-beef intake participants.

apolipoproteins and lipoprotein cholesterol fractions did not respond differently between groups, we conclude that some factor besides alterations in the intake of nutrients or micronutrients was responsible for the elevated apolipoprotein A-I caused by the test beef.

We previously demonstrated that enriching the diets of normocholesterolemic, middle-aged men with linoleic acid significantly depressed serum apolipoprotein A-I concentrations (23, 24). Chan *et al.* (34) had demonstrated that dietary

α -linolenic acid (18:3 *n*-3) reduced apolipoprotein A-I in normolipidemic men. The participants in this study consumed as much as 10% of the dietary calories as total polyunsaturated fatty acids, so dilution of dietary linoleic and/or α -linolenic by the added beef may have elicited the observed increases in apolipoprotein A-I concentrations. As in the present study, changes in apolipoprotein A-I concentrations with increased dietary α -linolenic acid were not accompanied by parallel changes in HDL cholesterol, leading

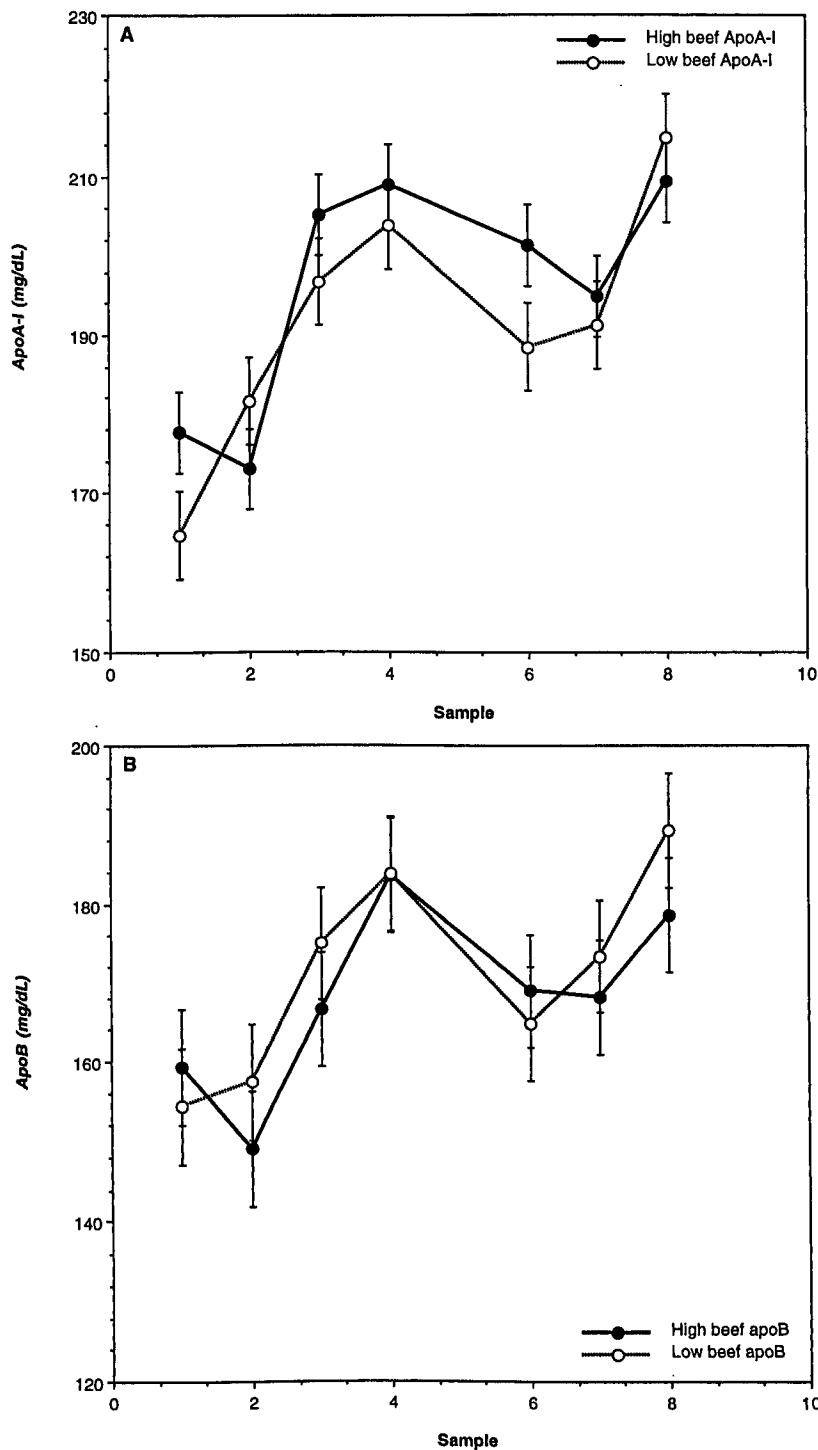


Figure 3. Apolipoprotein A-I (Apo A-I; A) and apolipoprotein B (Apo B; B) concentrations (milligrams per decaliter) in subjects with high habitual beef intake (High beef) or low habitual beef intake (Low beef). Data are means of five individuals per intake group pooled over two test periods (commercial and Wagyu beef). Average SE for apolipoprotein A-I and Apo B are attached to the lines. The test beef increased ($P = 0.001$) apolipoprotein A-I concentrations (pooled over beef types), but not apolipoprotein B concentrations ($P = 0.13$).

the authors to suggest that α -linolenic acid increased the cholesterol-apolipoprotein ratio in HDL particles. Also, Osada *et al.* (35) demonstrated that a high oleic acid diet upregulated apolipoprotein A-I gene expression in rat liver, suggesting that the addition of beef to their customary diets may have had a direct effect (rather than merely diluted polyunsaturated fatty acids) on plasma apolipoprotein A-I concentrations.

The Chan *et al.* (34) study also demonstrated that dietary α -linolenic acid reduced LDL cholesterol and apoli-

poprotein B concentrations proportionately, whereas in the present investigation, apolipoprotein B concentration and the apolipoprotein B:LDL cholesterol ratio seemed to be elevated by the test beef, although the LDL cholesterol concentration was completely unaffected. We cannot explain this dichotomy. The reductions in body weight that we observed may have increased the hematocrit, leading to increased concentration of the apolipoproteins, but the elevation in apolipoprotein A-I concentration occurred within 1 week after the subjects were placed on the test diets. This

immediate effect would seem to rule out increased hematocrit or some other variable related to loss in body weight as causative for the elevation in apolipoprotein concentrations.

Beef Types. Finally, some mention needs to be made about our selection of beef types. For several years, we have attempted to increase the oleic concentration in beef to provide a potentially more healthful product, but we have met with only limited success (e.g., Refs. 10 and 36). However, Japanese Black, or Wagyu, cattle have inherently higher concentrations of monounsaturated fatty acids in their lean and adipose tissues (11, 37). Thus, our choice of young Wagyu bulls (bullocks) was founded on the basis that meat from these animals should contain more oleic acid than commercial beef. Although we could not test this statistically in the current investigation, mean values for ground beef indicated that this was indeed the case. Moreover, by using bullocks, we were able to provide a leaner beef than is obtained from typical slaughter-weight steers (from which most whole cuts of beef are derived). It is clear from these data that a combination of steaks plus ground beef with reduced total fat did not reduce serum cholesterol fractions. This casts doubt on the wisdom of attempting to modify beef fatty acid composition or even reducing the total fat content of beef as a means of providing a more healthful protein source, especially in light of the fact that the commercial beef did not cause an elevation in serum cholesterol.

Conclusions. It seems contradictory that LDL cholesterol concentrations would be higher in the group of men with low habitual intake of beef. However, these individuals may have been limiting their habitual meat intake in response to concern over their lipid profiles. If so, addition of beef to their diets certainly did not further increase their LDL cholesterol concentrations. This investigation is consistent with previous studies that demonstrated that maintaining or even increasing beef fat consumption has no effect on serum LDL cholesterol in men (32, 33). The current study extends previous investigations in its demonstration of greater apolipoprotein A-I in response to additional dietary beef.

1. Hegsted DM, McGandy RB, Myers ML, Stare FJ. Quantitative effects of dietary fat on serum cholesterol in man. *Am J Clin Nutr* **17**:281-295, 1965.
2. Keys A, Anderson JT, Grande F. Serum cholesterol response to diet. *Metabolism* **14**:747-787, 1965.
3. Baggio G, Pagnan A, Muraca M, Martini S, Opportuno A, Bonanome A, Amrosio GB, Ferrari S, Guarini P, Piccolo D, Manzato E, Corrocher R, Crepaldi G. Olive-oil-enriched diet: effect on serum lipoprotein levels and biliary cholesterol saturation. *Am J Clin Nutr* **47**:960-964, 1988.
4. Grundy SM, Florentin L, Nix D, Whelan MF. Comparison of monounsaturated fatty acids and carbohydrates for reducing raised levels of plasma cholesterol in man. *Am J Clin Nutr* **47**:965-969, 1988.
5. Mensink RP, Katan MB. Effect of a diet enriched with monounsaturated or polyunsaturated fatty acids on levels of low-density and high-density lipoprotein cholesterol in healthy women and men. *N Engl J Med* **321**:436-441, 1989.
6. Berry EM, Eisenberg S, Haratz D, Friedlander Y, Norman Y, Kaufmann NA, Stein Y. Effects of diets rich in monounsaturated fatty acids on plasma lipoproteins: the Jerusalem Nutrition Study: high MUFAs vs high PUFAs. *Am J Clin Nutr* **53**:899-907, 1991.
7. Berry EM, Eisenberg S, Friedlander Y, Haratz D, Kaufmann NA, Norman Y, Stein Y. Effects of diets rich in monounsaturated fatty acids on plasma lipoproteins: the Jerusalem Nutrition Study: II Monounsaturated fatty acids vs carbohydrates. *Am J Clin Nutr* **56**:394-403, 1992.
8. Bonanome A, Grundy SM. Effects of dietary stearic acid on plasma cholesterol and lipoprotein levels. *N Engl J Med* **318**:1233-1248, 1988.
9. Grande F, Anderson JT, Keys A. Comparison of effects of palmitic and stearic acids in the diet on serum cholesterol in man. *Am J Clin Nutr* **23**:1184-1193, 1970.
10. St. John LC, Young CR, Knabe DA, Schelling GT, Grundy SM, Smith SB. Fatty acid profiles and sensory and carcass traits of tissues from steers and swine fed an elevated monounsaturated fat diet. *J Anim Sci* **64**:1441-1447, 1987.
11. Sturdivant CA, Lunt DK, Smith C, Smith SB. Fatty acid composition of subcutaneous and intramuscular adipose tissues and M. longissimus dorsi of Wagyu cattle. *Meat Sci* **32**:449-458, 1992.
12. O'Dea K, Traianedes K, Chisholm K, Leyden H, Sinclair AJ. Cholesterol-lowering effect of a low-fat diet containing lean beef is reversed by the addition of beef fat. *Am J Clin Nutr* **52**:491-494, 1990.
13. Scott LW, Kimball KT, Wittels EH, Dunn KJ, Brauchi DJ, Pownall HJ, Herd JA, Smith BR, Savell JW, Papadopoulos LS, Collins SA, Cross HR, Gotto AM. Effects of a lean beef diet and of a chicken and fish diet on lipoprotein profiles. *Nutr Metab Cardiovasc Dis* **1**:25-30, 1991.
14. Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. *Clin Chem* **20**:470-475, 1974.
15. Kostner GM. Enzymatic determination of cholesterol in high-density lipoprotein fractions prepared by polyanion precipitation. *Clin Chem* **22**:695, 1976.
16. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* **18**:499-502, 1972.
17. Bucolo G, David H. Quantitative determination of serum triglycerides by the use of enzymes. *Clin Chem* **19**:476-482, 1973.
18. Rifai N, King ME. Immunoturbidimetric assays of apolipoproteins A₁, A₂, and B in serum. *Clin Chem* **32**:957-961, 1986.
19. Siedel J, Schiefer S, Rosseneu S, Bergeaud M, De Keersgieter W, Pautz B, Vinaimont N, Ziegenhorn J. Immunoturbidimetric method for routine determinations of apolipoproteins A₁, A₂, and B in normo- and hyperlipidemic sera compared with immunonephelometry. *Clin Chem* **34**:1821-1825, 1988.
20. Folch J, Lees M, Sloane SH. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* **226**:497-509, 1957.
21. Wood R. Sample preparation, derivatization and analysis. In: Perkins EB, Ed. *Analyses of Fats, Oils and Lipoproteins*. Champaign, IL: AOCS Press, pp236-269, 1991.
22. Shiue HJ, Sather C, Layman, DK. Reduced carbohydrate/protein ratio enhances metabolic changes associated with weight loss diet. *FASEB J* **15**:A301, 2001.
23. Wood R, Kubena K, O'Brien B, Tseng S, Martin G. Effect of butter, mono- and polyunsaturated fatty acid-enriched butter, trans fatty acid margarine, and zero trans fatty acid margarine on serum lipids and lipoproteins in healthy men. *J Lipid Res* **34**:1-11, 1993.
24. Wood R, Kubena K, Tseng S, Martin G, Crook R. Effect of palm oil, margarine, butter, and sunflower oil on the serum lipids and lipoproteins of normocholesterolemic middle-aged men. *J Nutr Biochem* **4**:286-297, 1993.
25. Shore VG, Butterfield G, Krauss RM. Effects of varying the dietary ratio of polyunsaturated to saturated fats on plasma lipids and lipo-

- proteins. In: Perkins EG, Visek WJ, Eds. *Dietary Fats and Health*. Champaign, IL: AOCS Press, pp667–678, 1983.
26. Flynn MA, Nolph GB, Sun GY, Navidi M, Krause G. Effects of cholesterol and fat modification of self-selected diets on serum lipids and their specific fatty acids in normocholesterolemic and hypercholesterolemic humans. *J Am Coll Nutr* **10**:93–106, 1991.
 27. Nestel P, Clifton P, Noakes M. Effects of increasing dietary palmitoleic acid compared with palmitic and oleic acids on plasma lipids of hypercholesterolemic men. *J Lipid Res* **35**:656–662, 1994.
 28. Westphal SA, Gannon MC, Nuttall, F. Metabolic response to glucose ingested with various amounts of protein. *Am J Clin Nutr* **52**:267–272, 1990.
 29. American Heart Association's Position Statement. Dietary guidelines for healthy American adults. *Circulation* **77**:721A–724A, 1988.
 30. Expert Panel. Summary of the second report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II). *J Am Med Assoc* **269**:3015–3023, 1993.
 31. USDA Food Guide Pyramid. Stock No. 001-000-04587-3, U.S. Government printing office. Washington, D.C., 1992.
 32. O'Brien BC, Reiser R. Human plasma lipid responses to red meat, poultry, fish, and eggs. *Am J Clin Nutr* **33**:2573–2580, 1980.
 33. Reiser R, Probstfield JL, Silvers A, Scott LW, Shorney ML, Wood RD, O'Brien BC, Gotto AM Jr, Insull W Jr. Plasma lipid and lipoprotein response of humans to beef fat, coconut oil and safflower oil. *Am J Clin Nutr* **42**:190–197, 1985.
 34. Chan JK, Bruce VM, McDonald BE. Dietary α -linolenic acid is as effective as oleic acid and linoleic acid in lowering blood cholesterol in normolipidemic men. *Am J Clin Nutr* **53**:1230–1234, 1991.
 35. Osada J, Fernandez-Sanchez A, Diaz-Morillo, JL, Aylagas H, Miro-Obradors, MJ, Ordovas, JM, Palacios-Alaiz E. Hepatic expression of apolipoprotein A-I gene in rats is upregulated by monounsaturated fatty acid diet. *Biochem Biophys Res Commun* **180**:162–168, 1991.
 36. Chang JHP, Lunt DK, Smith SB. Fatty acid composition and fatty acid elongase and stearoyl-CoA desaturase activities in tissues of steers fed high oleate sunflower seed. *J Nutr* **122**:2074–2080, 1992.
 37. Zembayashi M, Nishimura K, Lunt DK, Smith SB. Effect of breed type and sex on the fatty acid composition of subcutaneous and intramuscular lipids of finishing steers and heifers. *J Anim Sci* **73**:3325–3332, 1995.
 38. Hoelscher LM, Savell JW, Smith SB, Cross HR. Subcellular distribution of cholesterol within muscle and adipose tissue of beef loin steaks. *J Food Sci* **53**:718–722, 1988.
 39. Sweeten MK, Cross HR, Smith GC, Smith SB. Subcellular distribution and composition of lipids in muscle and adipose tissue. *J Food Sci* **55**:43–45, 1990.