HEART-HEALTHY DIETS, EVEN THOSE INCLUDING LEAN BEEF, CAN HELP MANAGE CHOLESTEROL

Beef in an Optimal Lean Diet study: effects on lipids, lipoproteins, and apolipoproteins
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Objective

Evaluate the LDL cholesterol–lowering effects of a DASH-like diet that contained lean beef and a moderate protein diet that contained lean beef compared with a healthy American control diet in individuals with elevated LDL-cholesterol concentrations.

Study Design and Setting

A 4-period randomized, crossover, controlled feeding design. Subjects were randomly assigned to consume each of the 4 diets: HAD (33% total fat, 12% SFA, 17% protein, and 20g beef/d), DASH (27% total fat ,6% SFA, 18% protein, and 28g beef/d), BOLD (28% total fat, 6% SFA, 19% protein, and 113 g beef/d), and BOLD+ (28% total fat, 6% SFA, 27% protein, and 153 g beef/d) for 5 weeks. A short compliance break (average of 1 week) separated the diet periods.

Participants

Thirty-six healthy men and women (30–65 years of age) with elevated LDL cholesterol concentrations (2.84–4.55 mmol/L) were recruited.

Additional inclusion criteria:

- BMI (in kg/m2) of 18.5–37
- Triglycerides concentration <3.95 mmol/L
- Blood pressure <140/90 mm Hg

Exclusion criteria:

- Use of cholesterol and lipid-lowering medications or supplements (psyllium, fish oil, soy lecithin, and phytoestrogens)
- Pregnancy or lactation
- Weight loss ≥10% of body weight within the 6 months before enrollment in the study
- Vegetarianism.

Results

- A decrease in total cholesterol (TC) and LDL cholesterol concentrations after consumption of the DASH, BOLD, & BOLD+ diets compared with after consumption of the HAD diet.
- Apolipoprotein A-I, C-III, and C-III bound to apolipoprotein A1 particles decreased after BOLD & BOLD+ diets compared with the HAD.
- There was a greater decrease in apolipoprotein B after consumption of the BOLD+ diet than after consumption of the HAD.
- LDL cholesterol and TC decreased after consumption of the DASH, BOLD, and BOLD+ diets when the baseline C-reactive protein (CRP) concentration was ,1 mg/L; LDL cholesterol and TC decreased when baseline CRP concentration was .1 mg/L with the BOLD and BOLD+ diets.

CONCLUSIONS

- Low-SFA, heart-healthy dietary patterns with increased lean beef consumption elicit favorable effects on cardiovascular disease lipid and lipoprotein risk factors that are comparable to those elicited by a DASH dietary pattern.
- These results, in conjunction with the beneficial effects on apolipoprotein CVD risk factors after consumption of the BOLD and BOLD+ diets, which were greater with the BOLD+ diet, provide support for including lean beef in a heart-healthy dietary pattern.

Beef in an Optimal Lean Diet study: effects on lipids, lipoproteins, and apolipoproteins[1–3]

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ABSTRACT

Background: A Step I diet with lean beef compared with lean white meat both decrease LDL cholesterol. To our knowledge, no studies have evaluated a low–saturated fatty acid (SFA) (<7% calories) diet that contains lean beef.

Objective: We studied the effect on LDL cholesterol of cholesterol-lowering diets with varying amounts of lean beef [ie, Dietary Approaches to Stop Hypertension (DASH): 28 g beef/d; Beef in an Optimal Lean Diet (BOLD): 113 g beef/d; and Beef in an Optimal Lean Diet plus additional protein (BOLD+): 153 g beef/d] compared with that of a healthy American diet (HAD).

Design: Thirty-six hypercholesterolemic participants (with LDL-cholesterol concentrations >2.8 mmol/L) were randomly assigned to consume each of the 4 diets (HAD: 33% total fat, 12% SFA, 17% protein, and 20 g beef/d), DASH (27% total fat, 6% SFA, 18% protein, and 28 g beef/d), BOLD (28% total fat, 6% SFA, 19% protein, and 113 g beef/d), and BOLD+ (28% total fat, 6% SFA, 27% protein, and 153 g beef/d) for 5 wk.

Results: There was a decrease in total cholesterol (TC) and LDL-cholesterol concentrations ($P < 0.05$) after consumption of the DASH (-0.49 ± 0.11 and -0.37 ± 0.09 mmol/L, respectively), BOLD (-0.48 ± 0.10 and -0.35 ± 0.9 mmol/L, respectively), and BOLD+ (-0.50 ± 0.10 and -0.345 ± 0.09 mmol/L, respectively) diets compared with after consumption of the HAD (-0.22 ± 0.10 and -0.14 ± 0.10 mmol/L, respectively). Apolipoprotein A-I, C-III, and C-III bound to apolipoprotein A1 particles decreased after BOLD and BOLD+ diets compared with after the HAD, and there was a greater decrease in apolipoprotein B after consumption of the BOLD+ diet than after consumption of the HAD ($P < 0.05$ for both). LDL cholesterol and TC decreased after consumption of the DASH, BOLD, and BOLD+ diets when the baseline C-reactive protein (CRP) concentration was <1 mg/L; LDL cholesterol and TC decreased when baseline CRP concentration was >1 mg/L with the BOLD and BOLD+ diets.

Conclusions: Low-SFA, heart-healthy dietary patterns that contain lean beef elicit favorable effects on cardiovascular disease (CVD) lipid and lipoprotein risk factors that are comparable to those elicited by a DASH dietary pattern. These results, in conjunction with the beneficial effects on apolipoprotein CVD risk factors after consumption of the BOLD and BOLD+ diets, which were greater with the BOLD+ diet, provide support for including lean beef in a heart-healthy dietary pattern. This trial was registered at clinicaltrials.gov as NCT00937898. *Am J Clin Nutr* 2012;95:9–16.

INTRODUCTION

The recommended approach for lowering LDL cholesterol, which is a primary target for CVD[4] risk reduction, is to reduce dietary SFA (<7% of energy), *trans* fatty acids (as low as possible), and cholesterol (<200 mg/d) (1). A dietary pattern that emphasizes fruit and vegetables, legumes, whole grains, nuts, and seeds is recommended (2). Skim and reduced-fat dairy products, moderate amounts of lean-protein sources, including meats, poultry, and eggs, and increased seafood (particularly fatty fish), as well as plant-based proteins also are recommended (2). It is not necessary to exclude lean beef, and the Adult Treatment Panel III Guidelines and the Dietary Guidelines for Americans indicate that lean red meat can be included in a heart-healthy dietary pattern that is low in SFA and cholesterol (1, 2). Beef is a popular food and a source of many nutrients, and, consequently, lean beef can be an important lean-protein food source to meet current food-based and nutrient recommendations.

Epidemiologic studies have reported mixed associations between red-meat consumption and CVD mortality (3), acute myocardial infarction, unstable angina, and metabolic syndrome (3–8). A recent report from the Nurses' Health Study showed that the replacement of one serving of unprocessed red meat with poultry or fish was associated with 19% and 24% reductions in coronary heart disease risk, respectively (5). Some of the discrepancies in the literature may reflect not differentiating higher-fat red meat from lean red meat (specifically beef) or not accounting for different meat processing and cooking methods (6) because some epidemiologic studies have not shown this

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[4] Abbreviations used: BOLD, Beef in an Optimal Lean Diet; BOLD+, Beef in an Optimal Lean Diet plus additional protein; CRP, C-reactive protein; CVD, cardiovascular disease; DASH, Dietary Approaches to Stop Hypertension; HAD, healthy American diet; HS, heparin supernatant fluid; SFA, saturated fatty acid; TC, total cholesterol.

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association (7, 8), especially when SFA was controlled (8) or when only red, and not processed, meat was included in the analysis (7).

Previous free-living and controlled-consumption studies reported comparable LDL cholesterol–lowering effects of a National Cholesterol Education Program Step I diet (total fat <30% and SFA <10% energy) (9) with lean beef or lean white meat (10–12). To our knowledge, no studies have examined the effects of an SFA-restricted (<7% calories), heart-healthy diet with lean beef. Red meat is limited in the DASH diet as a strategy to decrease SFA (13).

The cholesterol-lowering effects of a low-SFA diet with lean beef have not been rigorously evaluated. Thus, we conducted a well-controlled consumption study to evaluate the LDL cholesterol–lowering effects of a DASH-like diet that contained lean beef (BOLD diet: 28% total fat, 6% SFA, 54% carbohydrates, 19% protein, and 113 g beef/d, and a moderate protein diet that contained lean beef (BOLD+: 28% total fat, 6% SFA, 45% carbohydrates, 27% protein, and 153 g beef/d) compared with a healthy American control diet in individuals with elevated LDL-cholesterol concentrations. A DASH diet was included because it is the gold standard for contemporary dietary recommendations. Because Erlinger et al (14) showed that moderate elevations in CRP concentrations attenuated lipid-lowering responses to diet, we also examined this relation.

SUBJECTS AND METHODS

Subjects

Healthy men and women (30–65 y of age) with elevated LDL-cholesterol concentrations (2.84–4.55 mmol/L) were recruited. Additional inclusion criteria were as follows: BMI (in kg/m^2) of 18.5–37, triglycerides concentration <3.95 mmol/L, and blood pressure <140/90 mm Hg. Participants were allowed to take prescribed blood pressure–lowering medications and were eligible as long as their blood pressure was below the entry criteria. All participants were nonsmokers and free of established CVD, stroke, diabetes, liver, kidney, or autoimmune disease. Exclusion criteria included the use of cholesterol and lipid-lowering medications or supplements (psyllium, fish oil, soy lecithin, and phytoestrogens), pregnancy or lactation, weight loss ≥10% of body weight within the 6 mo before enrollment in the study, and vegetarianism. The Institutional Review Board at the Pennsylvania State University approved the experimental protocol, and all subjects provided written informed consent. This trial was registered at clinicaltrials.gov as NCT00937898.

Study design

The study used a 4-period randomized, crossover, controlled-feeding design. Subjects were randomly assigned to a treatment (diet) order; they consumed 4 diets (HAD and DASH, BOLD, and BOLD+ diets) for 5 wk each. A short compliance break (average of 1 wk) separated the diet periods. At the beginning of the study and at the end of each diet period, on 2 consecutive days, subjects completed a series of clinical and physical assessments (ie, blood draw, height, and weight measurements) at the General Clinical Research Center. The first participants began the study in September 2007; the last participant completed the study in March 2009.

Diets

The nutrient composition of experimental diets is presented in **Table 1**. Total energy was held constant for each participant throughout the 4-diet periods, and participants were monitored (daily weigh-ins) to ensure they remained weight stable. The 3 experimental diets (DASH, BOLD, and BOLD+ diets) contained similar amounts of total fat, SFA, PUFA, and cholesterol. The HAD was higher in total fat, SFA, MUFA, PUFA, and cholesterol and was lower in total fiber. BOLD and DASH diets were matched for macronutrient composition. The BOLD+ diet was higher in protein (27% of total energy) compared with the HAD (17%), DASH (18%), and BOLD (19%) diets and, thus, lower in carbohydrates (45% compared with 50–55%) (Table 1).

The HAD provided full-fat cheese and dairy products, more oil and butter, and refined grains. The DASH, BOLD, and BOLD+ diets provided low-fat or nonfat versions of these foods, less oil and butter, and more whole grains. All diets were rich in fruit, vegetables, and lean meats consistent with food-based dietary recommendations. Although matched for protein, the BOLD and DASH diets differed in their primary protein source; the BOLD diet included an average of 113 g lean beef/d. The BOLD+ diet contained 153 g lean beef/d, and the HAD and DASH diet contained 20 and 28 g lean beef/d, respectively.

The lean beef used in the study (primarily select-grade top round, chuck shoulder pot roast, and 95% lean ground beef) was purchased from The Pennsylvania State University Meats Laboratory. The meat was prepared via braising, grilling, or frying (95% lean ground beef only) and never over an open flame to prevent charring.

Menus were created for a 6-d diet cycle across a range of calorie amounts (1800–3600 kcal/d). Sample 1-d menus for each of the diets are shown in **Table 2**. All meals and snacks were prepared at the Metabolic Diet Study Center, The Pennsylvania State University. Participants ate one meal per day (Monday–Friday) in the Center, and their other meals were prepared and packed for off-site consumption. Adherence with the diets was monitored via daily and weekly compliance questionnaires.

Clinical assessments

Body weight was measured at each laboratory visit (in addition to daily weigh-ins at the diet center). All blood samples were collected after an overnight (10–12 h) fast according to a standardized protocol. Serum and plasma aliquots were stored at −80°C until the time of analysis.

Lipids, lipoproteins, and apolipoproteins

TC and triglycerides were measured by using enzymatic procedures with commercially available kits (cholesterol and triglyceride kits; Alfa Wassermann). HDL cholesterol was quantified according to the modified heparin-manganese precipitation procedure of Warnick and Albers (15). LDL cholesterol was determined by using Friedewald's equation as follows:

$$\text{LDL cholesterol} = \text{TC} - \text{HDL cholesterol} + (\text{triglycerides} \div 5) \quad (1)$$

These assays were conducted at the core endocrine laboratory at the MS Hershey Medical Center General Clinical Research

TABLE 1
BOLD study diets: energy, nutrient composition, and food-group servings[1]

	Diets			
	HAD	DASH	BOLD	BOLD+
Nutrient targets				
Calories	2097	2106	2100	2104
Protein (g; percentage of kcal)	17 (91.7)	18 (98.4)	19 (99.6)	27 (145.6)
Carbohydrates (g; percentage of kcal)	50 (268.1)	55 (298.3)	54 (287.4)	45 (243.7)
Fat (g; percentage of kcal)	33 (77.0)	27 (64.4)	28 (65.8)	28 (66.6)
Cholesterol (mg)	287	188	168	193
SFA (g; percentage of kcal)	12 (27.9)	6 (15.2)	6 (15.4)	6 (14.5)
PUFA (g; percentage of kcal)	7 (15.5)	8 (18.9)	7 (16.5)	7 (16.1)
MUFA (g; percentage of kcal)	11 (25.9)	9 (21.8)	11 (25.2)	12 (29.3)
Fiber (g)	24	36	32	38
Sodium (mg)	3243	2982.8	2712	3344
Potassium (mg)	3259	4247	3998	4417
Calcium (mg)	993	1140	936	1060
Magnesium (mg)	308	403	392	429
Food groups (servings/d)				
Fruit and juices (cups)	3.1	4.1	4.5	3.4
Vegetables (cups)	3.2	4.3	3.9	4.6
Grains (oz)	8.3	4.5	5.6	5.3
Low-fat dairy products (cups)	1.2	2.3	1.8	4.7
High-fat dairy products (cups)	0.7	0.1	0.0	0.0
Legumes, nuts, seeds, and other vegetable protein (oz)	0.6	2.1	1.3	4.2
Beef (oz)	0.7	1.0	4.0	5.4
Poultry, pork, and fish (oz)	3.7	3.7	1.0	1.0
Egg and egg-product substitutes (oz)	0.24	0.2	0.1	0.9
Fats and oils (g)	5.4	4.0	4.3	1.4

[1] On the basis of 2100 kcal/d. Average across a 6-d menu cycle. All values were determined by using Nutritionist Pro software (Axxya Systems LLC). BOLD, Beef in an Optimal Lean Diet; BOLD+, Beef in an Optimal Lean Diet plus additional protein; DASH, Dietary Approaches to Stop Hypertension; HAD, healthy American diet; SFA, saturated fatty acid.

Center (Hershey, PA). Apolipoprotein A1, B, and C-III were measured by using the immunoturbidimetric procedure of Riopponen et al (16) with corresponding monospecific polyclonal antisera. These assays were conducted at the Oklahoma Research Institute (Oklahoma City, OK) under the supervision of Petar Alaupovic.

Insulin and glucose

Insulin was measured by using a radioimmunoassay with ^{125}I-labeled human insulin and a human insulin antiserum (17). Glucose was determined with an immobilized enzyme biosensor for glucose (YSI 2300 STAT Plus Glucose & Lactate Analyzer; Yellow Springs Instruments) (18). These assays were conducted at the core endocrine laboratory at the MS Hershey Medical Center.

High-sensitivity CRP

CRP was measured with the use of latex-enhanced immunonephelometry (Quest Diagnostics).

Statistical analysis

Power calculations were conducted to estimate the required sample size on the basis of data from the original DASH study (19) in which LDL cholesterol was reduced by 9% after 8 wk consumption of the DASH diet compared with after consumption of the control diet. Analyses used the following assumptions: power was set at 0.8, α was set at 0.05, and 2-tailed tests were used. It was estimated that a sample size of 40 was sufficient to test the primary LDL-cholesterol hypothesis while allowing for a 10% dropout rate.

All statistical analyses were performed with SAS software (version 9.2; SAS Institute Inc). Two-sample t tests were used to determine significant differences between sexes at baseline for each outcome variable. The residuals for each variable were used to assess normality. Logarithmic transformations were used for nonnormally distributed variables (triglycerides). The mixed-models procedure (PROC MIXED) in the SAS software (version 9.2; SAS Institute Inc) was used to test the effects of diet and order on outcome variables. A doubly repeated measures ANCOVA (repeated for diet and day of blood draw) was used with age, weight, and baseline lipid concentrations as covariates for lipid and lipoprotein measurements. Repeated ANCOVA (repeated for diet) was used with age, weight, and baseline amounts for the remaining variables. The primary outcome was the change in LDL cholesterol after consumption of the BOLD and BOLD+ diets compared with after consumption of the HAD; Dunnett's post hoc test was used to determine whether these differences were significant ($P < 0.05$). Tukey-Kramer–adjusted P values were used to determine whether differences between the diets in secondary outcome variables were significant ($P < 0.05$). In the

TABLE 2
One-day DASH, BOLD, and BOLD+ menus[1]

	HAD	DASH	BOLD	BOLD+
Breakfast	Oatmeal packet Brown sugar Orange juice Milk English muffin Butter	Whole-grain cereal and milk Yogurt Banana Orange juice	Oatmeal packet Blueberries (frozen) Orange juice Milk Plain bagel and margarine	Whole-grain cereal and milk Orange juice Low-fat cottage cheese
Lunch	Tuna salad Pita bread Baby carrots Pretzels	Vegetarian chili Whole-wheat crackers Low-fat cheddar cheese Peaches, canned in juice	Meatballs and marinara sauce Sandwich roll and lettuce Broccoli, baby carrots, and ranch dressing Pretzels	Beef chili with shredded cheddar cheese Whole-wheat crackers Peaches, canned in juice
Dinner	Southwest fajita with chicken Flour tortillas Cheddar cheese (shredded) Lettuce Red bell pepper Sour cream	Turkey breast with mashed potatoes and gravy Broccoli Lettuce, cherry tomatoes, and flax tahini dressing Dinner roll with butter	Southwest fajita with beef Flour tortillas, cheddar cheese (shredded), lettuce, and red bell pepper	Pot roast with mashed potatoes and gravy Broccoli and edamame beans Lettuce, cherry tomatoes, and flax tahini dressing Dinner roll with margarine
Snack	Chocolate-chip cookies	Trail mix Grapes	Peanut butter Apple Celery sticks	Hummus Whole-wheat pita and baby carrots for dipping Trail mix

[1] BOLD, Beef in an Optimal Lean Diet; BOLD+, Beef in an Optimal Lean Diet plus additional protein; DASH, Dietary Approaches to Stop Hypertension; HAD, healthy American diet.

secondary analysis of CRP subgroups, P values were Bonferroni-adjusted for multiple comparisons; Tukey-Kramer–adjusted P values were not used to control for overadjustment because of erroneous comparisons.

RESULTS

Forty-two individuals were recruited for the study. During the study, one subject dropped out because of a job change and relocation, one subject dropped out because of an unrelated illness, and 4 subjects dropped out because of inability to adhere to the dietary protocol. A total of 36 subjects were included in the final analysis (**Figure 1**).

Baseline subject characteristics are presented in **Table 3**. Women had significantly higher serum TC (5.74 compared with 5.02 mmol/L; $P = 0.003$), LDL cholesterol (3.78 compared with 3.31 mmol/L; $P = 0.02$), and HDL cholesterol (1.45 compared with 1.16 mmol/L; $P = 0.01$) concentrations than did men. Men had significantly higher BMI (27.3 compared with 24.8; $P = 0.02$) and serum glucose (4.82 compared with 4.6 mmol/L; $P = 0.03$) concentrations compared with women. Despite these differences at baseline, no significant interactions of sex by outcome measure were shown. Subject adherence to the prescribed diets was 93% according to daily self-reporting forms. Body weight was maintained during the diet periods within 2.2 kg.

Lipids, lipoproteins, and apolipoproteins

TC, LDL cholesterol, and HDL cholesterol were significantly decreased after consumption of the DASH, BOLD, and BOLD+ diets but not the HAD diet ($P < 0.05$) (**Table 4**). Compared with the HAD, LDL cholesterol was significantly decreased by 5.5%, 4.7%, and 4.4% by the DASH, BOLD, and BOLD+ diets, respectively ($P < 0.05$; **Figure 2**). TC was decreased after consumption of the DASH, BOLD, and BOLD+ diets by 3.8%, 3.8%, and 4.6%, respectively, compared with after consumption of the HAD. There were no differences in any of the lipid and lipoprotein changes in the test diets (DASH, BOLD, and BOLD+) ($P > 0.1$).

Apolipoprotein A-I was significantly decreased after consumption of the BOLD and BOLD+ diets compared with after consumption of the HAD (130.6 and 130.1 compared with 135.9 mg/dL, respectively), with no significant differences after consumption of the DASH diet (133.7 mg/dL). Apolipoprotein B was significantly decreased after consumption of the BOLD+ diet than after consumption of the HAD (88.6 compared with 92.8 mg/dL). There were no significant differences in the A1:apolipoprotein B ratio for the DASH, BOLD, or BOLD+ diets compared with that for the HAD. Apolipoprotein C-III concentrations were significantly decreased after consumption of the BOLD+ diet (7.7 mg/dL) compared with after consumption of the HAD (8.5 mg/dL) and DASH (8.2 mg/dL) diets. Apolipoprotein C-III concentrations were also significantly decreased after consumption of the BOLD diet (7.9 mg/dL) but not after consumption of the DASH diet compared with after consumption of the HAD (**Table 5**). Apolipoprotein C-III HS concentrations were decreased after consumption of the BOLD (5.4 mg/dL) and BOLD+ (5.3 mg/dL) diets compared with after consumption of the HAD (5.8 mg/dL, $P < 0.05$). No significant differences were observed with heparin-precipitated apolipoprotein C-III concentrations or apolipoprotein C-III ratio (apolipoprotein C-III HS to heparin-precipitated apolipoprotein C-III) during this intervention.

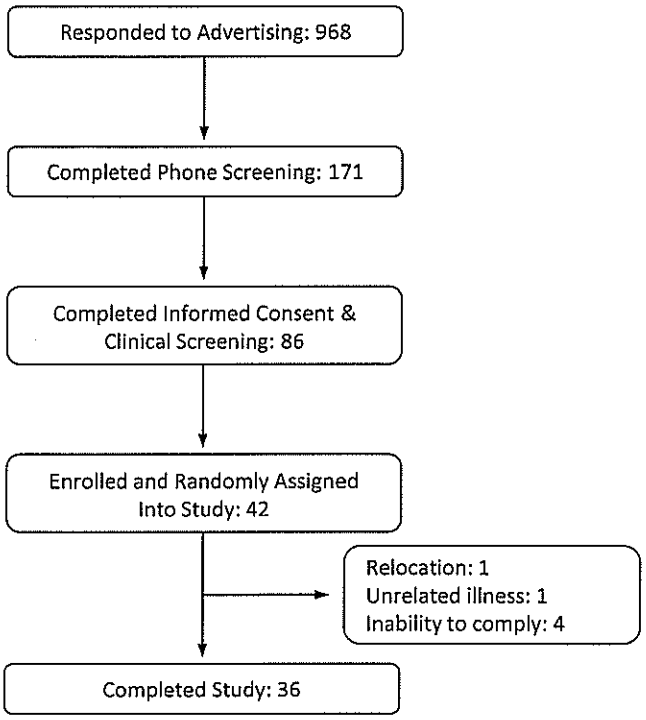

FIGURE 1. Recruitment flow diagram.

CRP

There were no differences in CRP after consumption of any of the dietary treatments (**Table 6**). Secondary analysis revealed that baseline CRP concentrations affected TC and the LDL-cholesterol responses of subjects to the dietary treatments (diet × baseline CRP; $P < 0.05$). Subjects with baseline CRP concentrations ≥1 mg/L experienced significant decreases in TC from baseline after consumption of the BOLD and BOLD+ diets. These changes were greater than after consumption of the HAD diet. In addition, the changes were greater after consumption of the BOLD diet than after consumption of the DASH diet. Similar effects of CRP were observed for LDL cholesterol; individuals with baseline CRP concentrations ≥1 mg/L had significant decreases in LDL cholesterol after consumption of the BOLD and BOLD+ diets, although these were not different from after consumption of the HAD ($P = 0.052$ and $P = 0.087$, respectively) or DASH ($P = 0.098$ and $P = 0.999$, respectively) diets (Table 6).

In comparison, in individuals with baseline CRP concentrations <1 mg/L, TC was significantly decreased after consumption of all 4 diets; the TC reduction was greatest for the DASH diet, and this was significantly different from that for the HAD but not different from that for the BOLD or BOLD+ diets. In the low-CRP subgroup (<1 mg/L), the experimental diets (not the HAD) significantly decreased LDL cholesterol; only differences between the HAD and DASH diet were significant (Table 6).

Glucose and insulin

Serum glucose and insulin concentrations were not different after consumption of the DASH (4.76 ± 0.05 mmol/L and 12.0 ± 0.06 μU/mL, respectively), BOLD (4.82 ± 0.05 mmol/L and 13.6 ± 0.5 μU/mL, respectively), or BOLD+ (4.92 ± 0.05 mmol/L and 13.6 ± 0.6 μU/mL, respectively) diets compared with after consumption of the HAD (4.82 ± 0.05 mmol/L and 12.6 ± 0.6 μU/mL, respectively).

DISCUSSION

Lipids, lipoproteins, and apolipoproteins

To our knowledge, this was the first controlled-consumption study that showed an increase in lean-beef consumption while controlling SFA (6% calories) in the context of a heart-healthy diet was associated with significant decreases in LDL cholesterol. In the current study, the BOLD and DASH diets decreased the LDL-cholesterol concentration by −0.41 mmol/L compared with similar decreases, compared with baseline, of −0.34 mg/dL and −0.30 mmol/L in the DASH (19) and OmniHeart (carbohydrate diet) (20) trials, respectively. In addition, the decrease in the LDL-cholesterol concentration for the BOLD+ and OmniHeart protein diets was similar at −0.42 mg/dL and −0.36 mmol/L, respectively. Decreases in LDL-cholesterol concentrations observed for the BOLD and BOLD+ diets were achieved with protein sources that differed from those used in the

TABLE 3
Baseline characteristics of study participants ($n = 36$)[1]

Characteristic	Men ($n = 15$)	Women ($n = 21$)	Combined
Age (y)	49 ± 1.8 (39-63)	50 ± 2.0 (45-97)	50 ± 1.4
BMI (kg/m^2)	27.3 ± 0.7 (19.4–35.5)	24.8 ± 0.5 (19.4–35.5)[2]	25.7 ± 0.5
TC (mmol/L)	5.02 ± 0.14 (3.98–6.16)	5.74 ± 0.22 (4.58–7.38)[2]	5.46 ± 0.12
LDL cholesterol (mmol/L)	3.31 ± 0.14 (2.46–2.46)	3.78 ± 0.12 (3.00–4.84)[2]	3.6 ± 0.1
HDL cholesterol (mmol/L)	1.16 ± 0.05 (0.91–1.55)	1.45 ± 0.08 (0.88–2.30)[2]	1.34 ± 0.06
Non–HDL cholesterol	3.87 ± 0.14 (3.04–5.05)	4.28 ± 0.13 (3.43–5.33)[2]	4.11 ± 0.19
TG (mmol/L)	1.18 ± 0.10 (0.55–1.88)	1.07 ± 0.06 (0.68–1.95)	1.12 ± 0.05
Glucose (mmol/L)	4.82 ± 0.10 (3.83–5.55)	4.60 ± 0.08 (4.05–5.38)[2]	4.7 ± 0.09
Insulin (IU/mL)	12 ± 1.9 (7-23)	13 ± 0.961 (9-17)	12 ± 0.97
CRP (mg/L)	1.43 ± 0.4 (5.4–0.3)	1.34 ± 0.3 (4.5–0.3)	1.4 ± 0.2

[1] All values are means ± SEMs; ranges in parentheses. Baseline values were measured before consuming any study food. CRP, C-reactive protein; TC, total cholesterol; TG, triglycerides.

[2] Two-sample t test was used to determine significant ($P < 0.05$) differences between sexes with SAS (version 9.2; SAS Institute Inc).

TABLE 4
Effect of diet on lipids and lipoproteins[1]

	HAD (n = 33)	DASH (n = 35)	BOLD (n = 34)	BOLD+ (n = 34)
TC (mmol/L)	5.25 ± 0.09[a]	4.98 ± 0.09[b]	4.99 ± 0.09[b]	4.96 ± 0.09[b]
TG (mmol/L)[2]	1.06 ± 0.06	1.08 ± 0.06	1.05 ± 0.07	1.00 ± 0.05
LDL cholesterol (mmol/L)	3.44 ± 0.08[a]	3.22 ± 0.07[b]	3.23 ± 0.07[b]	3.23 ± 0.07[b]
HDL cholesterol (mmol/L)	1.32 ± 0.05[a]	1.22 ± 0.04[b]	1.24 ± 0.04[b]	1.24 ± 0.04[b]
Non-HDL cholesterol (mmol/L)	3.89 ± 0.07[a]	3.71 ± 0.08[b]	3.70 ± 0.08[b]	3.66 ± 0.07[b]

[1] All values are means ± SEMs. The MIXED procedure (version 9.2; SAS Institute Inc) was used to test the effects of diet. Values in the same row with different superscript letters are significantly different (Dunnett-adjusted $P < 0.05$). BOLD, Beef in an Optimal Lean Diet; BOLD+, Beef in an Optimal Lean Diet plus additional protein; DASH, Dietary Approaches to Stop Hypertension; HAD, healthy American diet; TC, total cholesterol; TG, triglycerides.
[2] Raw values reported. Data were log transformed to achieve normality when testing for significant differences.

DASH (0.5 servings of beef, pork, or ham/d, and OmniHeart (0.9 servings of beef, pork, or ham/d studies. However, SFA concentrations were similar in the DASH, OmniHeart, and BOLD study diets (7%, 6%, and 6% of total calories, respectively). HDL cholesterol decreased during the DASH (1.2 mmol/L), BOLD (1.2 mmol/L), and BOLD+ (1.2 mmol/L) diets compared with during the HAD (1.3 mmol/L); these decreases were due to differences in total and saturated fat between diets (total fat: 33% compared with 28%; SFA: 12% compared with 6%). Thus, the protein source [with the exception of soy protein (21)] does not appear to modify the TC or LDL cholesterol response to a cholesterol-lowering diet.

The LDL-cholesterol decreases we observed extend the findings of studies conducted by Davidson et al (11) and Hunninghake et al (10) with free-living subjects who were instructed to substitute lean beef for chicken, fish, or pork in a National Cholesterol Education Program Step I diet (<30% total fat, <10% SFA, and <300 mg cholesterol/d). Subjects who consumed the BOLD diet experienced a greater decrease in TC (−9.3%) and LDL cholesterol (−10.1%) (compared with consumption of the HAD) from baseline than reported by Davidson et al (−1.0% and −1.7% respectively) and Hunninghake et al (−0.9% and −1.9%, respectively). These differences most likely were due to the lower SFA intake after consumption of the BOLD diet (6% SFA). In a controlled-consumption study conducted by Beauchesen-Rondeau et al (12) to evaluate the substitution of lean beef compared with chicken or white fish, the decrease in LDL cholesterol (−7.0%) was not as great as that (−10.1%) with the BOLD diet. Again, this result may reflect differences in the SFA content of the experimental diets (10% compared with 6%) compared with the control diets between studies [BOLD study: 6% compared with 12%; Beauchesen-Rondeau et al (12): 10% compared with 12%]. Despite the greater magnitude of the LDL-cholesterol decrease in our study than in some previous studies (10, 11), a key consistent finding (from our study and in the literature) is the equivalent decreases in LDL cholesterol with lean beef than with white meat when macronutrient profiles of diets are similar.

Compared with the HAD, the BOLD+ diet was the only treatment diet that significantly decreased apolipoprotein B. Compared with the HAD, both lean-beef diets significantly decreased apolipoprotein A1 and total apolipoprotein C-III. Smit et al (22), by using NHANES (phase 1) data, reported that the highest quartile of apolipoprotein B was associated with the highest intakes of beef. However, SFA was highly correlated with the protein source, which, thus, prevented conclusions from being drawn on the basis of individual predictors (ie, beef or SFA). Our results concurred with the apolipoprotein B findings from the OmniHeart trial in which the moderate protein diet yielded the greatest decrease in apolipoprotein B. Mensink et al (23) reported that an isocaloric substitution (1% of energy) of carbohydrates for MUFA and PUFA but not SFA decreased apolipoprotein B; however, isocaloric substitution of protein for carbohydrate also decreases apolipoprotein B; the extent of this effect has yet to be fully quantified.

Changes in total apolipoprotein C-III on lean-beef diets reflected decreases in apolipoprotein C-III HS, which represents the number of apolipoprotein C-III molecules bound to apolipoprotein A1–containing particles (Table 5). Kawakami and Yoshida (24) have suggested that apolipoprotein C-III bound to HDL inhibits the antiinflammatory properties of HDL. Although total apolipoprotein A1 was decreased in the BOLD and BOLD+ diets, the decrease in apolipoprotein C-III bound to apolipoprotein A1–containing particles suggested that the antiinflammatory capacity of the apolipoprotein A1–containing particles was improved.

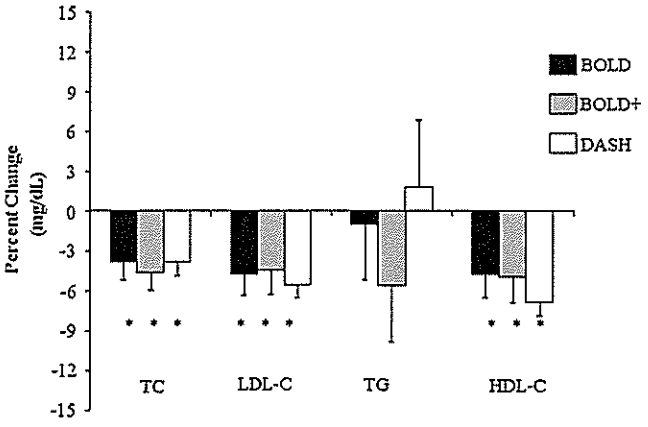

FIGURE 2. Change in lipids and lipoproteins. Mean percentage change (±SEM) from the HAD (HAD: n = 33; DASH: n = 35; BOLD: n = 34; and BOLD+: n = 34). The MIXED procedure in SAS software (version 9.2; SAS Institute Inc) was used to test the effects of diet. *Significantly different from the HAD, $P < 0.05$. BOLD, Beef in an Optimal Lean Diet; BOLD+, Beef in an Optimal Lean Diet plus additional protein; DASH, Dietary Approaches to Stop Hypertension; HAD, healthy American diet; TC, total cholesterol; TG, triglycerides.

TABLE 5
Effect of diet on apolipoproteins[1]

Apolipoprotein	HAD	DASH	BOLD	BOLD+
A-I (mg/dL)	135.9 ± 2.1[a]	133.73 ± 2.1[a,b]	130.6 ± 1.2[b]	130.11 ± 2.1[b]
B (mg/dL)	92.8 ± 1.5[a]	91.0 ± 1.5[a,b]	91.1 ± 1.5[a,b]	88.6 ± 1.5[b]
C-III (mg/dL)	8.5 ± 0.2[a]	8.21 ± 0.2[a,b]	7.94 ± 0.2[b,c]	7.71 ± 0.2[c]
C-III HP (mg/dL)	2.62 ± 0.1	2.6 ± 0.1	2.5 ± 0.1	2.43 ± 0.1
C-III HS (mg/dL)	5.83 ± 0.2[a]	5.59 ± 0.2[a,b]	5.4 ± 0.1[b]	5.30 ± 0.2[b]

[1] All values are means ± SEMs. The MIXED procedure (version 9.2; SAS Institute Inc) was used to test the effects of diet. Values in the same row with different superscript letters are significantly different (Tukey-adjusted $P < 0.05$). BOLD, Beef in an Optimal Lean Diet; BOLD+, Beef in an Optimal Lean Diet plus additional protein; DASH, Dietary Approaches to Stop Hypertension; HAD, healthy American diet; HP, heparin precipitated; HS, heparin supernatant fluid.

Baseline CRP concentrations appeared to influence TC and LDL cholesterol responses of subjects to the experimental diets. The link between inflammation and lipid and lipoprotein changes has been known for several decades (25, 26); however, the mechanism by which CRP modulates these responses is unknown. Previous studies have reported a blunted TC- and LDL cholesterol–lowering response to DASH (14) and Step I (27, 28) diets in individuals with increased baseline CRP. However, we observed a different effect in the high-CRP group for diets that contained lean beef. We observed a greater decrease in TC (compared with in the DASH diet) and LDL cholesterol (compared with zero) with consumption of BOLD and BOLD+ diets in subjects with high CRP concentrations. In subjects with high CRP, the BOLD and BOLD+ diets significantly decreased concentrations of TC (−0.53 and −0.54 mmol/L, respectively) and LDL cholesterol (−0.38 and −0.39 mmol/L respectively) similarly. The mechanisms that account for these different diet responses in subjects with high compared with low CRP are not clear. Additional studies are needed to determine whether diets that contain lean beef benefit individuals with high CRP to achieve a substantial cholesterol-lowering diet response.

The BOLD study has several strengths. We conducted a tightly controlled clinical study and achieved high levels of dietary adherence as verified by the daily monitoring forms. To our knowledge, the BOLD study is first study to examine the effects of increased lean-beef consumption in the context of current dietary recommendations. Finally, our study population was representative of a large portion of the US population [LDL-cholesterol concentrations in approximately the 35th percentile (1)], and thus, the findings have broad applicability. A limitation of our study was that we had only self-reported compliance measures and no biological measures of adherence to the lean beef and other test diets. In addition, we used a controlled-consumption study design and lean beef was preselected for, prepared for, and consumed by participants. In a free-living setting, individuals would be required to select lean cuts of beef from a wide variety of options in the marketplace and be mindful of preparation techniques and portion control. Although there are 29 cuts of beef that meet lean beef criteria, many grocery stores would feature the following cuts: top loin and top round steaks, top sirloin bottom round roast, and 95% lean ground beef. Adhering to the BOLD and BOLD+ diets that we prepared might be challenging for consumers, at least initially, and therefore, decreases in LDL-cholesterol concentrations would be less than expected. It is important for consumers to be mindful of the types of cut, preparation techniques, and portion control. For many individuals, this can be challenging, which would lessen the expected LDL cholesterol–lowering response.

In conclusion, the inclusion of lean beef (113 g/d) or the partial replacement of carbohydrates with protein (including lean beef) in a low-SFA, DASH-like diet significantly decreased TC and LDL cholesterol compared with in a HAD. These reductions were similar in magnitude to those observed for the DASH diet. The

TABLE 6
Effect of diet on CRP, TC, and LDL-cholesterol change[1]

	HAD	DASH	BOLD	BOLD+
CRP (mg/L)	1.14 ± 0.19	1.07 ± 0.15	1.0 ± 0.18	0.92 ± 0.09
TC-change (mmol/L)				
Baseline CRP				
<1 mg/L (n = 21)	−0.35 ± 0.13[a,2]	−0.73 ± 0.13[b,2]	−0.47 ± 0.13[a,b,2]	−0.49 ± 0.13[a,b,2]
≥1 mg/L (n = 15)	−0.08 ± 0.14[a]	−0.20 ± 0.14[a,b]	−0.53 ± 0.13[b,c,2]	−0.54 ± 0.14[b,2]
LDL-cholesterol change (mmol/L)				
Baseline CRP				
<1 mg/L (n = 21)	−0.19 ± 0.08[a]	−0.47 ± 0.10[b,2]	−0.29 ± 0.08[a,2]	−0.31 ± 0.10[a,2]
≥1 mg/L (n = 15)	−0.07 ± 0.13[a]	−0.21 ± 0.13[a]	−0.38 ± 0.13[a,2]	−0.39 ± 0.16[a,2]

[1] All values are means ± SEMs. CRP stratification was based on American Heart Association CRP cutoffs (29). Changes are from baseline. There was no significant effect of diet ($P < 0.05$) on CRP concentrations. Diet × baseline CRP, $P = 0.0008$ (LDL-cholesterol change) and $P = 0.0009$ (TC change). CRP was log transformed to achieve normality. Actual values of CRP are presented. Values in the same row with different superscript letters are significantly different, adjusted $P < 0.05$ (MIXED procedure, version 9.2; SAS Institute Inc). BOLD, Beef in an Optimal Lean Diet; BOLD+, Beef in an Optimal Lean Diet plus additional protein; CRP, C-reactive protein; DASH, Dietary Approaches to Stop Hypertension; HAD, healthy American diet; TC, total cholesterol.

[2] Significantly different from zero ($P < 0.05$) (MIXED procedure, version 9.2; SAS Institute Inc).

specific effects of the moderate protein, BOLD+ diet on apolipoprotein B and C-III merit additional study. These effects could reflect the increased lean-beef, total protein, or reduced carbohydrate content of the BOLD+ diet; additional research is needed to determine the role of each of these components in the BOLD+ diet on CVD risk. The results of the BOLD study provide convincing evidence that lean beef can be included in a heart-healthy diet that meets current dietary recommendations and reduces CVD risk.

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The authors' responsibilities were as follows—PMK-E, JPVH, SGW, and PJG: designed the research; MAR and AMH: conducted the research; PA: performed apolipoprotein analysis; MAR and TLG: performed the statistical analyses; MAR, AMH, SGW, JPVH, PJG, PA, and PMK-E: wrote the manuscript, and all authors: took responsibility for the final content of the manuscript. PMK-E and MAR received travel funds and honoraria from the Beef Checkoff Program for giving presentations on this research. MAR received travel funds from the Beef Checkoff Program for giving a presentation on this research. PMK-E, SGW and JPVH received funding from the Beef Checkoff Program for the research reported in this article. AMH, PA, PJG, and TLG had no conflicts of interest to declare. Financial supporters had no role in the design and conduct of the study, collection, analysis, and interpretation of data, or preparation, review, or approval of the manuscript.

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RED MEAT AND CARDIOVASCULAR DISEASE RISK

Total red meat intake of ≥0.5 servings/d does not negatively influence cardiovascular disease risk factors: a systemically searched meta-analysis of randomized controlled trials. O'Connor et al. The American Journal of Clinical Nutrition

OBJECTIVE

Assess the effects of consuming ≥0.5 or <0.5 servings of total red meat/d on cardiovascular disease (CVD) risk factors [blood total cholesterol (TC), LDL cholesterol, HDL cholesterol, triglycerides, ratio of TC to HDL cholesterol (TC:HDL), and systolic and diastolic blood pressures (SBP and DBP, respectively)].

STUDY DESIGN AND SETTING

A meta-analysis of randomized controlled trials (RCTs). Nine hundred and forty-five studies from PubMed, Cochrane Library and Scopus databases were independently screened. Studies were included if they used an RCT study design, subjects were aged ≥19 y, consumption of total red meat/d was ≥0.5 serving compared to <0.5 servings, and ≥1 CVD risk factor was reported as a dependent variable. A total of 24 qualified RCTs were extracted and included in the analysis.

RESULTS

There was a decrease from pre- to-post-intervention values of TC, LDL cholesterol, HDL cholesterol, TC:HDL, triglycerides, and DBP, but not SBP, ($P < 0.05$) in both groups.

There were no differences ($P > 0.05$) in post-intervention values between the groups who consumed ≥ or <0.5 servings of total red meat/d for any of the dependent variables.

- -0.01 mmol/L (-0.08, 0.06 mmol/L) for TC
- 0.02 mmol/L (-0.05, 0.08 mmol/L) for LDL cholesterol
- 0.03 mmol/L (-0.01, 0.07 mmol/L) for HDL cholesterol
- 0.04 mmol/L (-0.02, 0.10 mmol/L) for triglycerides
- -0.08 mm Hg (-0.26, 0.11 mm Hg) for TC:HDL
- -1.0 mm Hg (-2.4, 0.78 mmHg) for SBP
- 0.1 mm Hg (-1.2, 1.5 mm Hg) for DBP

CONCLUSIONS

- There was no indication that consumption of progressively higher red meat intake influenced CVD risk factors.
- Results are generalizable across a variety of populations, dietary patterns, and types of red meat.
- Further research is needed to reconcile the apparent disconnect between RCT and observation-based conclusions.

Total red meat intake of ≥0.5 servings/d does not negatively influence cardiovascular disease risk factors: a systemically searched meta-analysis of randomized controlled trials[1,2]

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ABSTRACT

Background: Observational associations between red meat intake and cardiovascular disease (CVD) are inconsistent. There are limited comprehensive analyses of randomized controlled trials (RCTs) that investigate the effects of red meat consumption on CVD risk factors.

Objective: The purpose of this systematically searched meta-analysis was to assess the effects of consuming ≥0.5 or <0.5 servings of total red meat/d on CVD risk factors [blood total cholesterol (TC), LDL cholesterol, HDL cholesterol, triglycerides, ratio of TC to HDL cholesterol (TC:HDL), and systolic and diastolic blood pressures (SBP and DBP, respectively)]. We hypothesized that the consumption of ≥0.5 servings of total red meat/d would have a negative effect on these CVD risk factors.

Design: Two researchers independently screened 945 studies from PubMed, Cochrane Library, and Scopus databases and extracted data from 24 qualified RCTs. Inclusion criteria were *1*) RCT, *2*) subjects aged ≥19 y, *3*) consumption of ≥0.5 or <0.5 total red meat servings/d [35 g (1.25 ounces)], and *4*) reporting ≥1 CVD risk factor. We performed an adjusted 2-factor nested ANOVA mixed-effects model procedure on the postintervention values of TC, LDL cholesterol, HDL cholesterol, TC:HDL cholesterol, triglycerides, SBP, and DBP; calculated overall effect sizes of change values; and used a repeated-measures ANOVA to assess pre- to postintervention changes.

Results: Red meat intake did not affect lipid-lipoprotein profiles or blood pressure values postintervention ($P > 0.05$) or changes over time [weighted mean difference (95% CI): −0.01 mmol/L (−0.08, 0.06 mmol/L), 0.02 mmol/L (−0.05, 0.08 mmol/L), 0.03 mmol/L (−0.01, 0.07 mmol/L), and 0.04 mmol/L (−0.02, 0.10 mmol/L); −0.08 mm Hg (−0.26, 0.11 mm Hg); and −1.0 mm Hg (−2.4, 0.78 mm Hg) and 0.1 mm Hg (−1.2, 1.5 mm Hg) for TC, LDL cholesterol, HDL cholesterol, triglycerides, TC:HDL cholesterol, SBP, and DBP, respectively]. Among all subjects, TC, LDL cholesterol, HDL cholesterol, TC:HDL cholesterol, triglycerides, and DBP, but not SBP, decreased over time ($P < 0.05$).

Conclusions: The results from this systematically searched meta-analysis of RCTs support the idea that the consumption of ≥0.5 servings of total red meat/d does not influence blood lipids and lipoproteins or blood pressures. *Am J Clin Nutr* 2017;105:57–69.

Keywords: dietary guidance, blood lipids, blood lipoproteins, blood pressure, animal flesh, meat products, diet, meat

INTRODUCTION

The effects of red meat consumption on cardiovascular disease (CVD)[3] are inconsistent throughout the literature. CVD has been the leading cause of death in the United States since the 1950s and is currently attributable to 610,000 US deaths each year (1). Historically, epidemiologic cohort data support associations between high red meat intake and CVD-related events (2, 3) and mortality (4–6). This notion is currently being challenged due to data collection methods that group red meat with processed meat and/or inconsistent nomenclature and classification of red meat throughout the literature (7, 8). Regardless of contradicting evidence, an observational study design is unable to show causality such as with a randomized controlled trial (RCT). There is a paucity of literature that systematically and comprehensively assesses the effects of total red meat consumption amounts on CVD risk with data from RCTs (9).

The purpose of this meta-analysis was to systematically search the literature to assess the effects of total red meat consumption on indexes of CVD risk. The search included studies with an RCT design that measured blood lipids, lipoproteins, and/or blood pressures. We hypothesized that the consumption of ≥0.5 servings of red meat/d (or ~3.5 servings/wk) would negatively affect blood lipids, lipoproteins, and blood pressures. Our hypothesis was based on a current prospective cohort analysis that estimated that 8.6% and 12.2% of CVD-related deaths in men and women, respectively, would be preventable if participants consumed <0.5 servings of total red meat/d (5).

[1] Supported by the Purdue University's Ingestive Behavior Research Center National Institutes of Health T32 training grant (LEO) and postdoctoral fellowship (JEK).

[2] Supplemental Tables 1 and 2 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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[3] Abbreviations used: CVD, cardiovascular disease; DASH, Dietary Approaches to Stop Hypertension; DBP, diastolic blood pressure; DGA, Dietary Guidelines for Americans; RCT, randomized controlled trial; SBP, systolic blood pressure; TC, total cholesterol; TC:HDL, ratio of total cholesterol to HDL cholesterol.

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TABLE 1
Description of PICOS criteria for a systematically searched meta-analysis assessing the effects of consuming ≥0.5 or <0.5 servings of total red meat/d on blood lipids, lipoproteins, and blood pressures[1]

Variable	Description
Population	Adults aged ≥19 y
Intervention	Groups who consumed ≥0.5 servings (35 g or 1.25 ounces) of total red meat/d
Comparator	Groups who consumed <0.5 servings of total red meat/d
Outcome	Changes in modifiable traditional cardiovascular disease risk factors, specifically blood lipids, lipoproteins, and blood pressures
Study design	Randomized controlled trials
Research question	What is the effect of consuming ≥0.5 servings of total red meat/d on blood lipids, lipoproteins, and blood pressure in adults?

[1] PICOS, Population, Intervention, Comparator Outcome, Study design.

METHODS

Search strategy and data extraction

We followed the same systematic search protocol as the 2015 Dietary Guidelines Advisory Committee from the Nutrition Evidence Library (10). The PICOS (Population, Intervention, Comparator, Outcome, Study design) criteria used to define our research question are listed in **Table 1**. Inclusion criteria consisted of the following: *1*) use of an RCT study design, *2*) subjects aged ≥19 y, *3*) an intervention group or phase with consumption of ≥0.5 servings of total red meat/d compared with a control group or phase with consumption of <0.5 servings of total red meat/d, and *4*) reporting of ≥1 CVD risk factor as a dependent variable [i.e., blood total cholesterol (TC), LDL cholesterol, HDL cholesterol, TC-to-HDL-cholesterol ratio (TC:HDL), triglycerides, systolic blood pressure (SBP), and diastolic blood pressure (DBP)]. Our meta-analysis followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (11).

The original search took place in May 2015 but was updated in May 2016. We identified studies via a computerized search of 3 databases: *1*) PubMed (http://www.ncbi.nlm.nih.gov/pubmed), *2*) Cochrane Library (http://www.cochranelibrary.com), and *3*) Scopus (http://www.scopus.com). We reviewed reference lists of the identified studies and found 10 additional potential studies. Search terms and results are identified in **Table 2**. All of the database searches were completed independently by the primary author (LEO) and the secondary author (JEK). A research librarian assisted both reviewers (see Acknowledgments) in database and search term selection to optimize the search process and to reduce the chance of bias.

We excluded 865 of 945 studies from our search for the following reasons: *1*) the study design was not an RCT, *2*) the population was <19 y of age or pregnant, *3*) the control and intervention diets did not differ in total red meat consumption amounts, or *4*) the researchers did not report the dependent variables of interest (see **Figure 1**). The primary and secondary authors independently read 80 potentially eligible studies to further assess inclusion criteria and to avoid selection bias. We contacted corresponding authors when clarification or unpublished data were needed. We excluded 56 of the 80 studies from the analysis for the following reasons: *1*) we were unable to determine the amount of red meat consumed, *2*) the control and intervention diets did not meet our requirements of ≥0.5 or <0.5 servings/d or ≥3.5 or <3.5 servings/wk of total red meat, or *3*) we were unable to obtain the dependent variables of interest in a usable data format. The primary and secondary authors independently extracted data from the final 24 studies including the following: *1*) author name, *2*) publication year, *3*) population size and description, *4*) intervention duration, *5*) protein source comparison consumed by the control group, and *6*) the amount of total red meat intake, dietary patterns, method of diet administration, assessment of dietary compliance, and pre- and postintervention values and net changes in blood TC, LDL cholesterol, HDL

TABLE 2
Search terms and results for a systematically searched meta-analysis assessing the effects of consuming ≥0.5 or <0.5 servings of total red meat/d on blood lipids, lipoproteins, and blood pressures[1]

Source	Search terms	Filters	Results yielded
PubMed database	("Meat"[MESH] OR "Meat Products"[MESH] OR "red meat" OR "beef" OR "pork") AND ("hypertension"[MESH] OR "Cholesterol, LDL"[MESH] OR "Cholesterol, HDL"[MESH] OR "Blood Pressure"[MESH] OR "lipoproteins"[MESH])	Humans, aged ≥19 y, English	332
Scopus database	Meat AND (blood pressure OR lipoprotein)	English, human, humans, source type journals, limit to article and conference paper; exclude physical sciences, social sciences, humanities, agriculture, immunology, chemistry, environmental sciences, neuroscience, chemical engineering, engineering, computer science, psychology, arts and humanities, mathematics, veterinary and multidisciplinary	426
Cochrane Central database	Meat AND (blood pressure OR lipoprotein)	Trials	177
Reference lists of identified studies	N/A	N/A	10
Total	—	—	945

[1] MESH, Medical Subject Heading; N/A, not applicable.

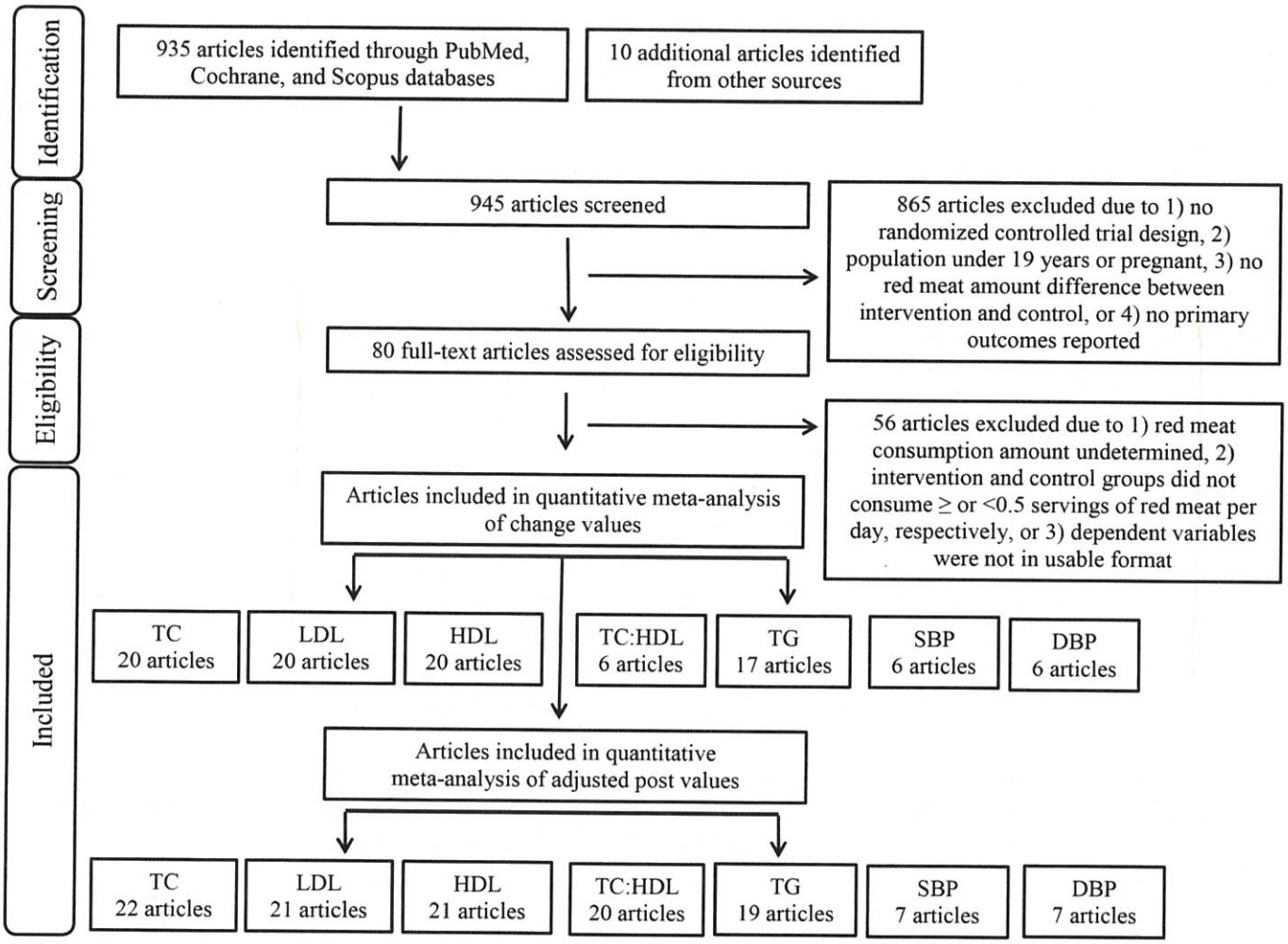

FIGURE 1 PRISMA flowchart. DBP, diastolic blood pressure; PRISMA, Preferred Reporting Items for Systematic Review and Meta-Analyses; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides.

cholesterol, TC:HDL, triglycerides, SBP, and DBP for both the control and intervention groups.

Definitions

For this meta-analysis, we used the 2015–2020 Dietary Guidelines for Americans (DGA) glossary definition of red meat (or "meat") and processed meat: "all forms of beef, pork, lamb, veal, goat, and non-bird games (e.g. venison, bison, elk)" and "preserved by smoking, curing, salting, and/or the addition of chemical preservatives," respectively (12). Unprocessed meat refers to meat that is preserved by refrigeration or freezing only (13). However, all meat available for purchase is processed to an extent (e.g., slaughtering and packaging) so the term "minimally processed" will be used in this meta-analysis to further describe the red meat consumed by research subjects. Blood TC, LDL cholesterol, HDL cholesterol, TC:HDL, triglycerides, SBP, and DBP are common modifiable biomarkers of CVD risk regularly assessed by physicians and therefore are the dependent variables assessed in this meta-analysis.

Calculations, bias assessment, and statistical analyses

We obtained or calculated the amount of red meat consumed by each group from the dietary data available in the study and contacted authors for clarification or raw data when needed. According to the American Heart Association, a serving size of cooked meat is 2–3 ounces (14); therefore, we considered 1 serving and 0.5 servings of red meat to be equivalent to 2.5 and 1.25 ounces, respectively. With the use of ProNutra software version 3.3 (Viocare, Inc.), we calculated that 1.25 ounces of red meat was equivalent to 35 g. The cutoff of 0.5 servings/d is supported by a 2012 prospective cohort analysis that estimated that 8.6% and 12.2% of CVD-related deaths in men and women, respectively, were preventable if subjects consumed <0.5 servings of total red meat/d (5).

We converted all blood lipid and lipoprotein data to mmol/L [TC, LDL-cholesterol, and HDL-cholesterol conversion: mg/dL ÷ 38.67; triglycerides conversion: mg/dL ÷ 88.57 (15)]. We extracted pre- and postintervention means, SDs, change values, and SDs of the change values from the studies when available. If not available, we calculated values, when appropriate, either from raw data obtained from the researchers or from information that was provided in the study and calculated change-value SDs by using a correlation factor representative of the change-value SDs that were available from the other studies (16). We evaluated the risk of selection, performance, and detection biases by using the modified Cochrane risk-of-bias assessment tool (17).

When discussing "studies" throughout this meta-analysis, we are referring to the entirety of each publication. Some studies contained >1 intervention or control group or phase. In this

case, these interventions are presented separately and treated as independent trials to account for within-study differences (18). Crossover trials were included in this meta-analysis; the present results and figures show crossover trial means and SDs incorporated into the data set as if they were parallel designs (19). This approach uses a correlational factor of 0 for all trial SDs. We recognize that this approach is conservative and causes crossover studies to be underweighted; therefore, we conducted secondary analyses to approximate a paired analysis for each variable by imputing missing SDs with the use of a correlational factor of 0.99 for all crossover design studies (20).

With the use of SAS version 9.4 (SAS Institute), we performed a repeated-measures ANOVA to assess pre- to postintervention changes in TC, LDL cholesterol, HDL cholesterol, TC:HDL, triglycerides, SBP, and DBP. We performed a 2-factor, nested ANOVA mixed-effects model procedure on the postintervention values of each dependent variable after adjustment for baseline values, age, sex, BMI, length of intervention, and whether energy restriction was or was not included in the protocol (21). These results are reported as adjusted least-squares means. We analyzed the change values by using STATA/IC 14 (StataCorp) and calculated the overall effect size by using the metaan function (intervention group or phase change value minus control group or phase change value). We used a random-effects model when heterogeneity was indicated by a significant chi-square test; otherwise, a fixed-effects model was used (22, 23). These results are reported as weighted mean differences and 95% CIs. Studies in **Figures 2–8** are organized in descending order from smallest to largest amounts of total red meat consumed per day by the intervention group or phase. Significance was set at $P < 0.05$. A statistical consultant approved all calculations and analyses (see Acknowledgments).

We performed traditional sensitivity analyses by removing 1 study or trial at a time and reconducting the analyses. We performed additional sensitivity analyses by removing clusters of

	≥0.5 servings of red meat/day			<0.5 servings of red meat/day					
Study	mean (mmol/L)	SD	n	mean (mmol/L)	SD	n	weight (%)	mean difference (mmol/L), random effects [95% CI]	
Grieger, 2014 (24)	-0.10	0.74	37	0.10	0.40	43	3.70	-0.20	[-0.47, 0.07]
Aadland, 2015 (25)	-0.38	0.70	19	-0.37	0.54	20	2.23	-0.01	[-0.40, 0.38]
Nowson, 2009 (26)	0.34	1.00	46	0.38	1.25	49	1.78	-0.05	[-0.50, 0.40]
Liao, 2007 (27)	-0.47	0.22	15	-0.61	0.16	15	7.07	0.15	[0.01, 0.29]
Sayer, 2015 (28)	-0.68	0.41	19	-0.36	0.38	19	4.06	-0.33	[-0.58, -0.07]
Mahon, 2007 CHICK (29)	-0.59	0.87	14	-0.49	1.54	15	0.51	-0.10	[-1.00, 0.80]
Mahon, 2007 CARB (29)	-0.59	0.87	14	-1.14	2.91	14	0.17	0.54	[-1.05, 2.13]
Mahon, 2007 CON (29)	-0.59	0.87	14	-0.16	2.10	11	0.25	-0.44	[-1.75, 0.87]
Flynn, 1981 F1 (30)	-0.13	0.86	31	-0.03	1.05	31	1.66	-0.10	[-0.57, 0.37]
Flynn, 1981 F2 (30)	-0.03	0.68	24	0.18	0.69	24	2.23	-0.21	[-0.60, 0.18]
Flynn, 1981 M1 (30)	-0.10	0.51	38	-0.16	0.77	38	3.39	0.05	[-0.24, 0.34]
Flynn, 1981 M2 (30)	-0.10	0.74	36	0.21	0.68	36	2.85	-0.31	[-0.64, 0.02]
Flynn, 1982 F1 BEEF (31)	-0.10	1.06	12	-0.13	1.09	12	0.56	0.03	[-0.83, 0.89]
Flynn, 1982 F1 PORK (31)	-0.21	0.49	12	-0.13	1.09	12	0.90	-0.08	[-0.75, 0.59]
Flynn, 1982 F2 BEEF (31)	0.13	0.76	17	0.26	0.76	17	1.45	-0.13	[-0.64, 0.38]
Flynn, 1982 F2 PORK (31)	-0.34	0.62	17	0.26	0.76	17	1.66	-0.60	[-1.06, -0.12]
Flynn, 1982 M1 BEEF (31)	-0.18	0.53	21	0.03	0.62	21	2.62	-0.21	[-0.56, 0.14]
Flynn, 1982 M1 PORK (31)	-0.03	0.38	21	0.03	0.62	21	3.10	-0.05	[-0.36, 0.26]
Flynn, 1982 M2 BEEF (31)	-0.16	0.77	26	0.13	0.76	26	2.07	-0.28	[-0.69, 0.13]
Flynn, 1982 M2 PORK (31)	-0.13	0.65	26	0.13	0.76	26	2.23	-0.26	[-0.65, 0.13]
de Mello, 2006 CHICK (32)	0.03	0.68	17	-0.26	0.57	17	1.92	0.29	[-0.14, 0.72]
de Mello, 2006 LVLP (32)	0.03	0.68	17	-0.28	0.56	17	2.07	0.31	[-0.10, 0.72]
Ashton, 2000 (33)	-0.14	0.58	42	-0.37	0.61	42	4.06	0.23	[-0.03, 0.49]
Davidson, 1999 (34)	-0.08	0.27	89	-0.13	0.31	102	8.89	0.05	[-0.03, 0.13]
Wolmarans, 1999 (35)	-0.29	0.63	39	-0.45	0.55	39	4.06	0.16	[-0.10, 0.42]
O'Brien, 1980 HC G1 (36)	0.44	0.87	15	0.00	0.57	15	1.36	0.44	[-0.09, 0.97]
O'Brien, 1980 LC G1 (36)	-0.13	0.81	15	-0.08	0.57	15	1.45	-0.05	[-0.56, 0.46]
O'Brien, 1980 HC G2 (36)	0.41	0.43	14	0.49	0.31	14	3.70	-0.08	[-0.35, 0.19]
O'Brien, 1980 LC G2 (36)	-0.18	0.28	14	-0.47	0.30	14	4.89	0.28	[0.06, 0.50]
Foerster, 2014 (37)	-0.10	0.49	20	0.00	0.58	20	2.85	-0.10	[-0.43, 0.23]
Hodgson, 2006 (38)	-0.10	0.52	29	-0.10	0.47	31	4.06	0.00	[-0.26, 0.26]
Gascon, 1996 (39)	-0.34	0.39	14	-0.14	0.44	14	3.10	-0.20	[-0.51, 0.11]
Wolmarans, 1991 (40)	0.12	0.73	28	-0.14	0.66	28	2.41	0.26	[-0.11, 0.63]
Haub, 2005 (41)	0.30	0.30	11	-0.06	0.70	10	1.66	0.36	[-0.11, 0.83]
Beauchesne-Rondeau, 2003 F (42)	-0.50	0.52	18	-0.30	0.52	18	2.85	-0.20	[-0.53, 0.13]
Beauchesne-Rondeau, 2003 P (42)	-0.50	0.52	18	-0.50	0.52	18	2.85	0.00	[-0.33, 0.33]
Sinclair, 1987 SF (43)	-1.05	0.82	10	-1.03	0.44	10	1.20	-0.02	[-0.59, 0.55]
Sinclair, 1987 TF (43)	-1.05	0.82	10	-0.89	0.52	11	1.13	-0.16	[-0.75, 0.43]
Sinclair, 1987 VEG (43)	-1.05	0.82	10	-1.10	0.49	7	1.01	0.05	[-0.58, 0.68]
						Overall	100.00	-0.01	[-0.08, 0.06]

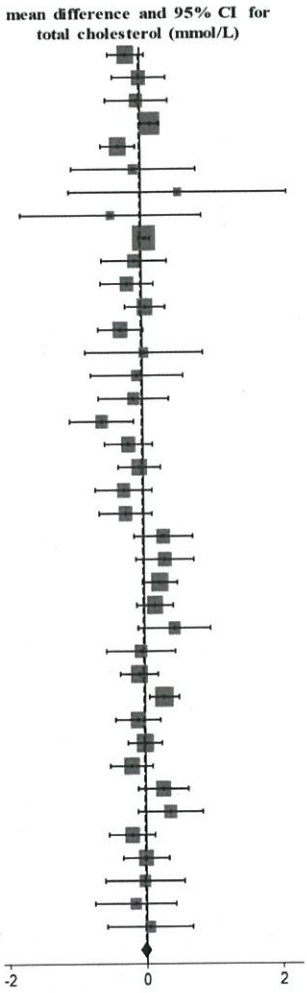

FIGURE 2 Random-effects model meta-analysis for changes in total blood cholesterol concentrations from randomized controlled trials comparing ≥0.5 or <0.5 servings of total red meat/d. Heterogeneity: $\tau^2 = 0.011$, $\chi^2 = 1.48$, df = 38 ($P = 0.028$), $I^2 = 32\%$. Data are shown in descending order from smallest to largest amounts of red meat consumed by the intervention group or phase. CARB, carbohydrate control diet; CHICK, chicken control diet; CON, habitual control diet; F, lean fish control diet; F1, first female group; F1 BEEF, first female group consuming beef diet; F1 PORK, first female group consuming pork diet; F2, second female group; F2 BEEF, second female group consuming beef diet; F2 PORK, second female group consuming pork diet; HC G1, first group consuming high-cholesterol diet; HC G2, second group consuming high-cholesterol diet; LC G1, first group consuming low-cholesterol diet; LC G2, second group consuming low-cholesterol diet; LVLP, lactovegetarian low-protein control diet; M1, first male group; M1 BEEF, first male group consuming beef diet; M1 PORK, first male group consuming pork diet; M2, second male group; M2 BEEF, second male group consuming beef diet; M2 PORK, second male group consuming pork diet; P, poultry control diet; SF, southern fish control diet; TF, tropical fish control diet; VEG, vegetarian control diet.

studies containing design features that had the potential to confound results, including weight-loss diets (27, 29), heart-healthy diets (25, 26, 28, 34, 35, 39, 42, 43), diseased populations [hypertensive (26, 28, 38), hypercholesterolemic (34, 35, 42), and/or diabetic (32)], studies that resulted in significant weight loss (25, 27–29, 35), inclusion of processed meat (45), studies that did not specify the degree of meat processing (24, 25, 27, 32, 36, 40, 43, 46, 47), and studies that used different amounts of protein intake in the control and intervention group or phase (29, 32, 38, 43). We also performed post hoc analyses by dividing the studies into specific quantities of red meat consumption [1.0–1.9 servings of red meat/d (24–29), 2.0–2.9 servings of red meat/d (30–37), or ≥3.0 servings of red meat/d (38–43)] and re-conducted the analyses in STATA.

RESULTS

Study features and subject characteristics

Twenty-four studies were included in the statistical analyses (see Figure 1); some contained >1 control group or phase (29–32, 36, 42, 43) and are reported as separate studies. Details of each study are shown in **Table 3**. The median total red meat servings per day in the control and intervention groups were 0 servings/d (range: 0–0.4 servings/d or 0–30 g/d) and 2 servings/d (or 140 g/d; range 1.0–7.1 servings/d or 68–500 g), respectively. Two of the selected studies included a weight-loss diet (27, 29), 8 studies included a heart-healthy dietary pattern (25, 26, 28, 34, 35, 39, 42, 43), the subjects self-selected their diet similar to their habitual intake in 9 studies (24, 30, 31, 36–38, 46, 40, 41), and 5

	≥0.5 servings of red meat/day			<0.5 servings of red meat/day					
Study	mean (mmol/L)	SD	n	mean (mmol/L)	SD	n	weight (%)	mean difference (mmol/L), random effects [95% CI]	
Grieger, 2014 (24)	-0.10	0.58	37	0.20	0.63	43	3.63	-0.30	[-0.57, -0.03]
Aadland, 2015 (25)	-0.26	0.61	19	-0.22	0.49	20	2.49	-0.04	[-0.39, 0.31]
Nowson, 2009 (26)	0.28	0.75	46	0.30	1.15	49	2.10	-0.01	[-0.40, 0.38]
Liao, 2007 (27)	-0.23	0.18	15	-0.40	0.04	15	9.21	0.17	[0.07, 0.27]
Sayer, 2015 (28)	-0.46	0.23	19	-0.32	0.64	19	2.99	-0.14	[-0.45, 0.17]
Mahon, 2007 CHICK (29)	-0.44	0.49	14	-0.41	1.35	15	0.70	-0.03	[-0.76, 0.70]
Mahon, 2007 CARB (29)	-0.44	0.49	14	-0.52	1.67	14	0.45	0.08	[-0.84, 1.00]
Mahon, 2007 CON (29)	-0.44	0.49	14	-0.26	1.42	11	0.48	-0.18	[-1.06, 0.70]
Flynn, 1981 F1 (30)	0.18	1.74	31	-0.14	1.97	31	0.45	0.32	[-0.60, 1.24]
Flynn, 1981 F2 (30)	0.00	1.38	24	0.28	1.40	24	0.61	-0.28	[-1.06, 0.50]
Flynn, 1981 M1 (30)	-0.01	0.93	38	-0.09	1.25	38	1.44	0.08	[-0.41, 0.57]
Flynn, 1981 M2 (30)	0.02	1.39	36	0.29	1.18	36	1.04	-0.27	[-0.86, 0.32]
Flynn, 1982 F1 BEEF (31)	0.14	1.48	12	-0.13	1.84	12	0.22	0.27	[-1.06, 1.60]
Flynn, 1982 F1 PORK (31)	-0.41	0.82	12	-0.13	1.84	12	0.30	-0.28	[-1.42, 0.86]
Flynn, 1982 F2 BEEF (31)	0.07	1.48	17	0.34	1.53	17	0.37	-0.27	[-1.29, 0.75]
Flynn, 1982 F2 PORK (31)	-0.44	1.23	17	0.34	1.53	17	0.43	-0.78	[-1.72, 0.16]
Flynn, 1982 M1 BEEF (31)	-0.07	0.95	21	0.17	1.14	21	0.92	-0.24	[-0.87, 0.39]
Flynn, 1982 M1 PORK (31)	-0.12	0.69	21	0.17	1.14	21	1.10	-0.29	[-0.86, 0.28]
Flynn, 1982 M2 BEEF (31)	0.06	1.46	26	0.22	1.32	26	0.64	-0.17	[-0.93, 0.59]
Flynn, 1982 M2 PORK (31)	-0.24	1.15	26	0.22	1.32	26	0.82	-0.47	[-1.14, 0.20]
de Mello, 2006 CHICK (32)	0.47	0.91	17	0.22	0.87	17	0.98	0.25	[-0.36, 0.86]
de Mello, 2006 LVLP (32)	0.47	0.91	17	0.13	0.84	17	1.04	0.34	[-0.25, 0.93]
Ashton, 2000 (33)	-0.12	0.84	42	-0.20	0.85	42	2.49	0.08	[-0.27, 0.43]
Davidson, 1999 (34)	-0.08	0.41	89	-0.13	0.44	102	8.38	0.05	[-0.07, 0.17]
Wolmarans, 1999 (35)	0.12	0.86	39	-0.17	0.80	39	2.29	0.29	[-0.08, 0.66]
O'Brien, 1980 HC G1 (36)	0.05	0.07	15	0.08	0.08	15	10.76	-0.03	[-0.09, 0.03]
O'Brien, 1980 LC G1 (36)	0.08	0.07	15	-0.10	0.08	15	10.76	0.18	[0.12, 0.24]
O'Brien, 1980 HC G2 (36)	0.03	0.09	14	0.08	0.12	14	10.02	-0.05	[-0.13, 0.03]
O'Brien, 1980 LC G2 (36)	-0.05	0.10	14	0.00	0.12	14	10.02	-0.05	[-0.13, 0.03]
Hodgson, 2006 (38)	0.00	0.72	29	-0.20	0.72	31	2.29	0.20	[-0.17, 0.57]
Gascon, 1996 (39)	-0.26	0.46	14	-0.06	0.45	14	2.73	-0.20	[-0.53, 0.13]
Wolmarans, 1991 (40)	0.12	0.86	28	-0.17	0.80	28	1.79	0.29	[-0.14, 0.72]
Haub, 2005 (41)	0.30	0.48	11	-0.08	0.76	10	1.18	0.38	[-0.17, 0.93]
Wiebe, 1984 (44)	-0.36	0.72	8	-0.36	0.70	8	0.78	0.00	[-0.69, 0.69]
Beauchesne-Rondeau, 2003 F (42)	-0.30	0.71	18	-0.20	0.81	18	1.44	-0.10	[-0.59, 0.39]
Beauchesne-Rondeau, 2003 P (42)	-0.30	0.71	18	-0.40	0.71	18	1.54	0.10	[-0.37, 0.57]
Sinclair, 1987 SF (43)	-0.58	1.29	8	-0.67	0.57	10	0.41	0.09	[-0.87, 1.05]
Sinclair, 1987 TF (43)	-0.58	1.29	8	-0.56	0.62	8	0.38	-0.02	[-1.00, 0.98]
Sinclair, 1987 VEG (43)	-0.58	1.29	8	-0.53	0.68	7	0.37	-0.05	[-1.07, 0.97]
Overall							**100.00**	**0.02**	**[-0.05, 0.08]**

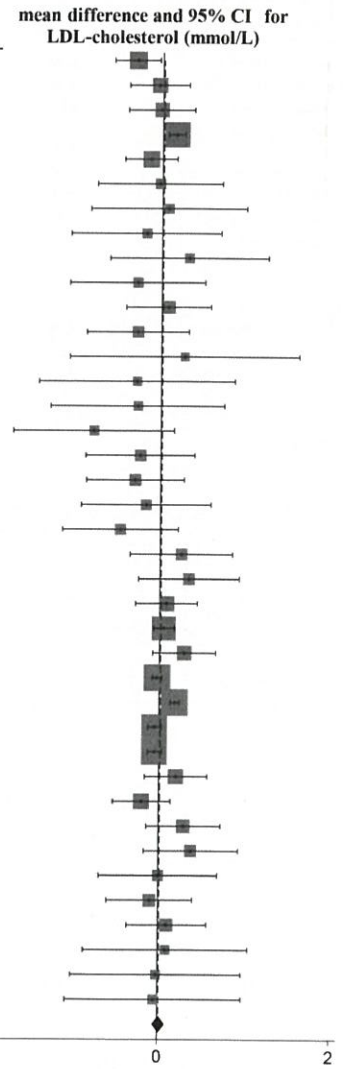

FIGURE 3 Random-effects model meta-analysis for changes in blood LDL-cholesterol concentrations from randomized controlled trials comparing ≥0.5 or <0.5 servings of total red meat/d. Heterogeneity: $\tau^2 = 0.011$, $\chi^2 = 6.62$, df = 38 ($P = 0.001$), $I^2 = 85\%$. Data are shown in descending order from smallest to largest amounts of red meat consumed by the intervention group or phase. CARB, carbohydrate control diet; CHICK, chicken control diet; CON, habitual control diet; F, lean fish control diet; F1, first female group; F1 BEEF, first female group consuming beef diet; F1 PORK, first female group consuming pork diet; F2, second female group; F2 BEEF, second female group consuming beef diet; F2 PORK, second female group consuming pork diet; HC G1, first group consuming high-cholesterol diet; HC G2, second group consuming high-cholesterol diet; LC G1, first group consuming low-cholesterol diet; LC G2, second group consuming low-cholesterol diet; LVLP, lactovegetarian low-protein control diet; M1, first male group; M1 BEEF, first male group consuming beef diet; M1 PORK, first male group consuming pork diet; M2, second male group; M2 BEEF, second male group consuming beef diet; M2 PORK, second male group consuming pork diet; P, poultry control diet; SF, southern fish control diet; TF, tropical fish control diet; VEG, vegetarian control diet.

Study	≥0.5 servings of red meat/day mean (mmol/L)	SD	n	<0.5 servings of red meat/day mean (mmol/L)	SD	n	weight (%)	mean difference (mmol/L), random effects [95% CI]
Grieger, 2014 (24)	0.10	0.94	37.00	0.10	1.01	43.00	0.75	0.00 [-0.43, 0.43]
Aadland, 2015 (25)	0.03	0.52	46.00	-0.02	0.50	49.00	2.10	0.06 [-0.14, 0.26]
Nowson, 2009 (26)	-0.24	0.22	19.00	-0.09	0.18	20.00	3.02	-0.15 [-0.27, -0.03]
Liao, 2007 (27)	-0.05	0.02	15.00	-0.02	0.09	15.00	3.86	-0.03 [-0.07, 0.01]
Sayer, 2015 (28)	-0.16	0.02	19.00	-0.08	0.01	19.00	3.96	-0.08 [-0.10, -0.06]
Mahon, 2007 CHICK (29)	-0.05	0.08	14.00	0.00	0.17	15.00	3.26	-0.05 [-0.15, 0.05]
Mahon, 2007 CARB (29)	-0.05	0.08	14.00	-0.31	0.19	14.00	3.02	0.26 [0.14, 0.38]
Mahon, 2007 CON (29)	-0.05	0.08	14.00	0.08	0.15	11.00	3.26	-0.13 [-0.23, -0.03]
Flynn, 1981 F1 (30)	-0.18	0.30	31.00	0.00	0.32	31.00	2.54	-0.18 [-0.34, -0.02]
Flynn, 1981 F2 (30)	0.05	0.41	24.00	-0.23	0.36	24.00	1.91	0.28 [0.06, 0.50]
Flynn, 1981 M1 (30)	-0.16	0.15	38.00	0.00	0.19	38.00	3.49	-0.16 [-0.24, -0.08]
Flynn, 1981 M2 (30)	-0.05	0.20	36.00	-0.08	0.17	36.00	3.49	0.03 [-0.05, 0.11]
Flynn, 1982 F1 BEEF (31)	-0.21	0.21	12.00	0.00	0.19	12.00	2.54	-0.21 [-0.37, -0.05]
Flynn, 1982 F1 PORK (31)	0.18	0.17	12.00	0.00	0.19	12.00	2.77	0.18 [0.04, 0.32]
Flynn, 1982 F2 BEEF (31)	0.13	0.40	17.00	-0.28	0.37	17.00	1.59	0.41 [0.16, 0.67]
Flynn, 1982 F2 PORK (31)	0.10	0.40	17.00	-0.28	0.37	17.00	1.59	0.39 [0.14, 0.65]
Flynn, 1982 M1 BEEF (31)	-0.16	0.16	21.00	-0.08	0.22	21.00	3.02	-0.08 [-0.20, 0.04]
Flynn, 1982 M1 PORK (31)	0.10	0.10	21.00	-0.08	0.22	21.00	3.26	0.18 [0.08, 0.27]
Flynn, 1982 M2 BEEF (31)	-0.08	0.20	26.00	-0.10	0.15	26.00	3.26	0.03 [-0.07, 0.13]
Flynn, 1982 M2 PORK (31)	0.08	0.19	26.00	-0.10	0.15	26.00	3.26	0.18 [0.08, 0.28]
de Mello, 2006 CHICK (32)	-0.03	0.34	17.00	-0.03	0.32	17.00	1.91	0.00 [-0.22, 0.22]
de Mello, 2006 LVLP (32)	-0.03	0.34	17.00	-0.03	0.30	17.00	1.91	0.00 [-0.22, 0.22]
Ashton, 2000 (33)	0.07	0.53	42.00	-0.01	0.48	42.00	1.91	0.08 [-0.14, 0.30]
Davidson, 1999 (34)	0.03	0.44	89.00	0.02	0.47	102.00	2.77	0.01 [-0.13, 0.15]
Wolmarans, 1999 (35)	0.00	0.13	39.00	-0.03	0.14	39.00	3.70	0.03 [-0.03, 0.09]
O'Brien, 1980 HC G1 (36)	0.05	0.07	15.00	0.08	0.08	15.00	3.70	-0.03 [-0.09, 0.03]
O'Brien, 1980 LC G1 (36)	0.08	0.07	15.00	-0.10	0.08	15.00	3.70	0.18 [0.12, 0.24]
O'Brien, 1980 HC G2 (36)	0.03	0.09	14.00	0.08	0.12	14.00	3.49	-0.05 [-0.13, 0.03]
O'Brien, 1980 LC G2 (36)	-0.05	0.10	14.00	0.00	0.12	14.00	3.49	-0.05 [-0.13, 0.03]
Hodgson, 2006 (38)	-0.01	0.67	29.00	-0.02	0.65	31.00	1.11	0.01 [-0.32, 0.34]
Gascon, 1996 (39)	-0.09	0.35	14.00	-0.06	0.46	14.00	1.21	-0.03 [-0.34, 0.28]
Wolmarans, 1991 (40)	-0.01	0.54	28.00	0.09	0.53	28.00	1.45	-0.10 [-0.37, 0.17]
Haub, 2005 (41)	0.08	0.10	11.00	-0.10	0.10	10.00	3.49	0.18 [0.10, 0.26]
Wiebe, 1984 (44)	-0.13	0.11	8.00	-0.28	0.09	8.00	3.26	0.16 [0.06, 0.26]
Beauchesne-Rondeau, 2003 F (42)	-0.01	0.23	18.00	0.02	0.30	18.00	2.31	-0.03 [-0.21, 0.15]
Beauchesne-Rondeau, 2003 P (42)	-0.01	0.23	18.00	0.05	0.26	18.00	2.54	-0.06 [-0.22, 0.10]
Sinclair, 1987 SF (43)	-0.29	0.33	8.00	-0.33	0.52	10.00	0.87	0.04 [-0.35, 0.43]
Sinclair, 1987 TF (43)	-0.29	0.33	8.00	-0.32	0.73	8.00	0.50	0.03 [-0.52, 0.58]
Sinclair, 1987 VEG (43)	-0.29	0.33	8.00	-0.46	0.51	7.00	0.69	0.17 [-0.28, 0.62]
Overall							**100.00**	**0.03 [-0.01, 0.07]**

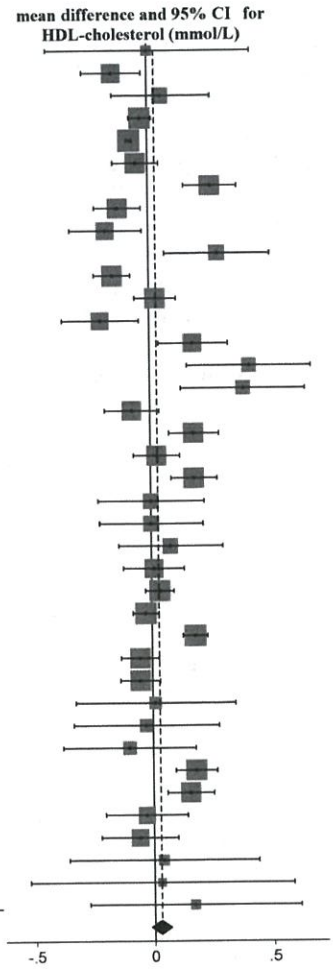

FIGURE 4 Random-effects model meta-analysis for changes in blood HDL-cholesterol concentrations from randomized controlled trials comparing ≥0.5 or <0.5 servings of total red meat/d. Heterogeneity: $\tau^2 = 0.011$, $\chi^2 = 6.62$, df = 38 ($P = 0.001$), $I^2 = 85\%$. Data are shown in descending order from smallest to largest amounts of red meat consumed by the intervention group or phase. CARB, carbohydrate control diet; CHICK, chicken control diet; CON, habitual control diet; F, lean fish control diet; F1, first female group; F1 BEEF, first female group consuming beef diet; F1 PORK, first female group consuming pork diet; F2, second female group; F2 BEEF, second female group consuming beef diet; F2 PORK, second female group consuming pork diet; HC G1, first group consuming high-cholesterol diet; HC G2, second group consuming high-cholesterol diet; LC G1, first group consuming low-cholesterol diet; LC G2, second group consuming low-cholesterol diet; LVLP, lactovegetarian low-protein control diet; M1, first male group; M1 BEEF, first male group consuming beef diet; M1 PORK, first male group consuming pork diet; M2, second male group; M2 BEEF, second male group consuming beef diet; M2 PORK, second male group consuming pork diet; P, poultry control diet; SF, southern fish control diet; TF, tropical fish control diet; VEG, vegetarian control diet.

of the selected studies were unclear about the diet other than the predominant protein source (32, 33, 44, 45, 47). Only minimally processed meats were consumed in 15 studies (25, 26, 28, 29, 30, 31, 33–35, 37–39, 41, 42, 44), highly processed meats were consumed in 1 study (45), and the extent of meat processing was unclear in the remaining 8 studies (24, 27, 32, 36, 40, 43, 46, 47). Intervention lengths varied from 2 to 32 wk.

Quality and bias of selected studies

Due to clear reporting of randomization methods, we deemed 5 studies at low risk of selection bias (24, 25, 29, 38, 46). Researchers disclosed allocation concealment methods in 2 studies (24, 25), but the rest were unclear about allocation methods. Three studies were at low risk of performance bias [2 investigator-blinded studies (34, 38) and 1 double-blind study (45)] but the rest did not report blinding. Detection bias was unclear in all of the studies except for 3 that were blinded for outcome assessment (25, 34, 38) (see **Supplemental Table 1**). In 16 articles, the researchers provided food to the subjects (mainly protein-rich foods) (24, 26–29, 31, 33, 37–45), but the rest did not provide food or did not specify if they provided food to the subjects. Researchers assessed dietary compliance in numerous ways, which are shown in Table 3, including dietary counseling, interviews, or questionnaires (24–27, 33–35, 37, 39, 41, 43, 46); food records, logs, or menus (26, 28–32, 34–36, 38, 40, 43, 46); and/or urinary markers such as urinary 3-methyl histidine (45), urinary electrolyte excretion (26), and 24-h urinary urea nitrogen output (28, 32). Most studies showed the use of >1 of these methods of dietary compliance.

Results of statistical analyses

There was a decrease from pre- to postintervention values of TC, LDL cholesterol, HDL cholesterol, TC:HDL, triglycerides,

Study	≥0.5 servings of red meat/day mean (mmol/L)	SD	n	<0.5 servings of red meat/day mean (mmol/L)	SD	n	weight (%)	mean difference (mmol/L), random effects [95% CI]	
Aadland, 2015 (25)	0.24	0.39	19	-0.1	0.4	20	10.48	0.34	[0.06, 0.60]
Sayer, 2015 (28)	-0.11	0.48	19	-0.06	0.37	19	10.14	-0.50	[-0.32, 0.22]
Mahon, 2007 CHICK (29)	-0.50	0.10	14	-0.30	0.30	15	12.03	-0.20	[-0.36, -0.04]
Mahon, 2007 CARB (29)	-0.50	0.10	14	0.10	0.30	14	12.03	-0.60	[-0.76, -0.44]
Mahon, 2007 CON (29)	-0.50	0.10	14	-0.30	0.20	11	12.29	-0.20	[-0.34, -0.06]
Davidson, 1999 (34)	-0.20	0.25	89	-0.20	0.26	102	12.90	0.00	[-0.08, 0.08]
Haub, 2005 (41)	-0.07	0.40	11	0.33	0.60	10	7.55	-0.40	[-0.83, 0.03]
Beauchesne-Rondeau, 2003 F (42)	-0.40	0.29	18	-0.49	0.31	18	11.45	0.09	[-0.11, 0.29
Beauchesne-Rondeau, 2003 P (42)	-0.40	0.29	18	-0.71	0.37	18	11.13	0.31	[0.09, 0.53]
Overall							**100.00**	**-0.08**	**[-0.26, 0.11]**

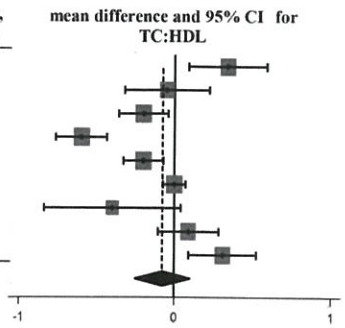

FIGURE 5 Random-effects model meta-analysis for changes in blood TC:HDL from randomized controlled trials comparing ≥0.5 or <0.5 servings of total red meat/d. Heterogeneity: $\tau^2 = 0.064$, $\chi^2 = 9.93$, df = 8 ($P = 0.001$), $I^2 = 90\%$. Data are shown in descending order from smallest to largest amounts of red meat consumed by the intervention group or phase. CARB, carbohydrate control diet; CHICK, chicken control diet; CON, habitual control diet; F, lean fish control diet; P, poultry control diet; TC:HDL, ratio of total cholesterol to HDL cholesterol.

and DBP but not SBP in both groups (repeated-measures ANOVA). The results showed no differences in postintervention values between the groups who consumed ≥0.5 or <0.5 servings of total red meat/d for any of the dependent variables (2-factor nested ANOVA mixed-effects model; $P > 0.05$ for all variables; see **Table 4**). Our analysis of the change values suggested no difference in responses over time between the groups who consumed ≥0.5 or <0.5 servings of total red meat/d in TC, LDL cholesterol, HDL cholesterol, TC:HDL, triglycerides, SBP, or DBP (fixed- or random-effects model; see Figures 2–8). There was no indication that consumption of progressively higher red meat amounts influenced these CVD risk factors (see Figures

Study	≥0.5 servings of red meat/day mean (mmol/L)	SD	n	<0.5 servings of red meat/day mean (mmol/L)	SD	n	weight (%)	mean difference (mmol/L), random effects [95% CI]	
Grieger, 2014 (24)	-0.10	0.48	37	-0.10	0.51	43	3.64	0.00	[-0.22, 0.22]
Aadland, 2015 (25)	0.01	0.45	46	0.09	0.55	49	3.92	-0.08	[-0.28, 0.12]
Nowson, 2009 (26)	0.10	0.20	19	-0.17	0.27	20	4.84	0.29	[0.15, 0.43]
Liao, 2007 (27)	-0.25	0.13	15	-0.11	0.13	15	5.43	-0.14	[-0.24, -0.04]
Sayer, 2015 (28)	-0.11	0.19	19	-0.11	0.13	19	5.43	0.01	[-0.09, 0.11]
Mahon, 2007 CHICK (29)	-0.28	0.26	14	-0.26	0.32	15	3.64	-0.02	[-0.24, 0.20]
Mahon, 2007 CARB (29)	-0.11	0.61	14	-0.26	0.32	14	2.14	0.15	[-0.20, 0.50]
Mahon, 2007 CON (29)	-0.02	0.43	14	-0.26	0.32	11	2.68	0.24	[-0.05, 0.53]
Flynn, 1981 F1 (30)	-0.27	1.44	31	0.26	1.33	31	0.76	-0.53	[-1.22, 0.16]
Flynn, 1981 F2 (30)	-0.17	0.44	24	0.29	1.09	24	1.42	-0.46	[-0.93, 0.01]
Flynn, 1981 M1 (30)	0.14	0.18	38	-0.14	0.17	38	5.69	0.27	[0.19, 0.34]
Flynn, 1981 M2 (30)	-0.16	0.67	36	-0.02	0.67	36	2.49	-0.14	[-0.45, 0.17]
Flynn, 1982 F1 BEEF (31)	-0.09	0.45	12	0.00	0.51	12	1.86	-0.09	[-0.48, 0.30]
Flynn, 1982 F1 PORK (31)	0.05	0.15	12	0.00	0.51	12	2.68	0.05	[-0.24, 0.34]
Flynn, 1982 F2 BEEF (31)	-0.16	0.51	17	0.44	1.36	17	0.76	-0.60	[-1.29, 0.09]
Flynn, 1982 F2 PORK (31)	0.00	0.15	17	0.44	1.36	17	0.84	-0.44	[-1.09, 0.21]
Flynn, 1982 M1 BEEF (31)	0.09	0.19	21	-0.15	0.19	21	5.14	0.24	[0.12, 0.36]
Flynn, 1982 M1 PORK (31)	-0.01	0.13	21	-0.15	0.19	21	5.43	0.14	[0.04, 0.24]
Flynn, 1982 M2 BEEF (31)	-0.29	0.90	26	0.02	0.14	26	2.14	-0.32	[-0.67, 0.03]
Flynn, 1982 M2 PORK (31)	0.08	0.13	26	0.02	0.14	26	5.69	0.06	[-0.02, 0.14]
Ashton, 2000 (33)	-0.19	0.96	42	-0.34	0.96	42	1.73	0.15	[-0.26, 0.56]
Davidson, 1999 (34)	-0.03	0.59	89	-0.03	0.52	102	4.53	0.00	[-0.16, 0.16]
Wolmarans, 1999 (35)	0.03	0.39	39	-0.08	0.32	39	4.53	0.11	[-0.05, 0.27]
Foerster, 2014 (37)	0.10	0.60	20	0.20	0.72	20	1.73	-0.10	[-0.51, 0.31]
Hodgson, 2006 (38)	-0.05	0.95	29	0.01	0.64	31	1.73	-0.06	[-0.47, 0.35]
Gascon, 1996 (39)	-0.01	0.21	14	-0.09	0.17	14	4.84	0.08	[-0.06, 0.22]
Haub, 2005 (41)	-0.16	0.50	11	0.27	0.60	10	1.42	-0.43	[-0.90, 0.04]
Beauchesne-Rondeau, 2003 F (42)	-0.30	0.33	18	-0.30	0.33	18	3.64	0.00	[-0.22, 0.22]
Beauchesne-Rondeau, 2003 P (42)	-0.30	0.33	18	-0.40	0.33	18	3.64	0.10	[-0.12, 0.32]
Sinclair, 1987 SF (43)	0.31	0.59	10	0.04	0.10	10	1.99	0.27	[-0.10, 0.64]
Sinclair, 1987 TF (43)	0.31	0.59	10	0.07	0.25	11	1.86	0.24	[-0.15, 0.63]
Sinclair, 1987 VEG (43)	0.31	0.59	10	0.19	0.25	7	1.73	0.12	[-0.29, 0.53]
Overall							**100.00**	**0.04**	**[-0.02, 0.10]**

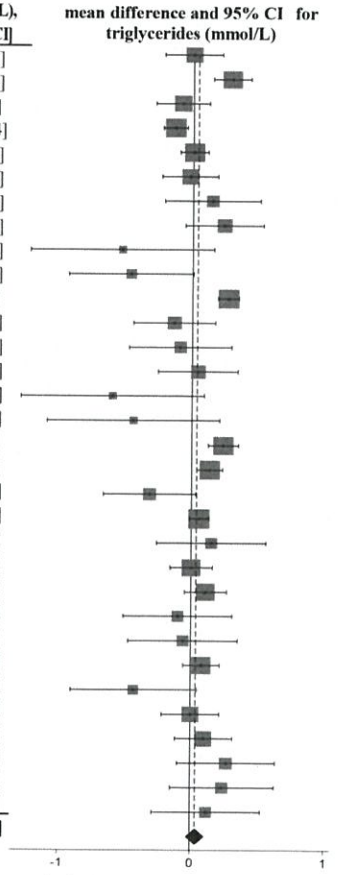

FIGURE 6 Random-effects model meta-analysis for changes in blood triglyceride concentrations from randomized controlled trials comparing ≥0.5 or <0.5 servings of total red meat/d. Heterogeneity: $\tau^2 = 0.017$, $\chi^2 = 3.16$, df = 31 ($P = 0.001$), $I^2 = 68\%$. Data are shown in descending order from smallest to largest amounts of red meat consumed by the intervention group or phase. CARB, carbohydrate control diet; CHICK, chicken control diet; CON, habitual control diet; F, lean fish control diet; F1, first female group; F1 BEEF, first female group consuming beef diet; F1 PORK, first female group consuming pork diet; F2, second female group; F2 BEEF, second female group consuming beef diet; F2 PORK, second female group consuming pork diet; M1, first male group; M1 BEEF, first male group consuming beef diet; M1 PORK, first male group consuming pork diet; M2, second male group; M2 BEEF, second male group consuming beef diet; M2 PORK, second male group consuming pork diet; P, poultry control diet; SF, southern fish control diet; TF, tropical fish control diet; VEG, vegetarian control diet.

	≥0.5 servings of red meat/day			<0.5 servings of red meat/day					
Study	mean (mm Hg)	SD	n	mean (mm Hg)	SD	n	weight (%)	mean difference (mmol/L), fixed effects [95% CI]	
Grieger, 2014 (24)	0.0	13.3	37.0	-2.0	14.3	43.0	8.9	2.0	[-4.1, 8.0]
Nowson, 2009 (26)	-5.6	8.8	46.0	-2.7	7.0	49.0	31.6	-2.9	[-6.1, 0.32]
Liao, 2007 (27)	1.6	11.7	15.0	2.9	6.1	15.0	7.3	-1.3	[-8.0, 5.4]
Sayer, 2015 (28)	-8.0	8.9	19.0	-6.2	7.4	19.0	12.1	-1.8	[-7.0, 3.0]
Wolmarans, 1999 (35)	0.4	6.8	39.0	-1.2	8.6	39.0	27.5	1.7	[-1.8, 5.1]
Hodgson, 2006 (38)	-1.9	9.8	29.0	1.6	10.3	31.0	12.6	-3.5	[-8.6, 1.6]
Overall							**100.0**	**-1.0**	**[-2.4, 0.8]**

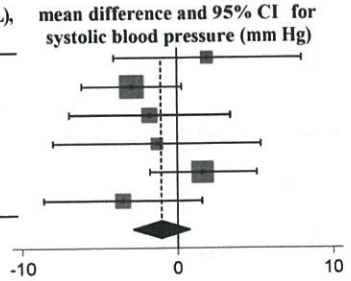

FIGURE 7 Fixed-effects model meta-analysis for changes in systolic blood pressure from randomized controlled trials comparing ≥0.5 or <0.5 servings of total red meat/d. Heterogeneity: $\tau^2 = 0.662$, $\chi^2 = 4.42$, df = 5 ($P = 0.346$), $I^2 = 11\%$. Data are shown in descending order from smallest to largest amounts of red meat consumed by the intervention group or phase.

2–8; the amount of red meat consumed progressively increases from top to bottom of each figure). Results from imputing SDs of crossover designs with 0.99 as the correlational factor did not differ from the original results with the use of 0 as the correlational factor.

More than 99% of the traditional sensitivity analyses showed no significant change in results. No cluster sensitivity analyses significantly changed results when we removed studies that included weight-loss diets, heart-healthy diets, significant weight loss, diseased populations, consumption of processed red meats or no specification of the degree of meat processing, and studies that used different amounts of protein intake in the control and intervention group/phase. Post hoc analyses of red meat consumption amounts showed no differences in change values between the control and intervention group, whether consuming 1.0–1.9, 2.0–2.9, or ≥3.0 servings of red meat/d, with the exception that HDL cholesterol was higher when ≥3.0 servings of red meat/d was consumed (weighted mean difference: 0.10; 95% CI: 0.05, 0.16). (See **Supplemental Table 2** for results of all sensitivity analyses.)

DISCUSSION

To the best of our knowledge, this is the first systematically searched meta-analysis to assess the consumption of ≥0.5 servings of total red meat/d on blood lipids, lipoproteins, and blood pressures by using data from RCTs. This serving size is consistent with the dietary patterns recommended by the 2010–2015 DGA and the Scientific Report of the 2015 Dietary Guidelines Advisory Committee. Our results indicate that the consumption of ≥0.5 servings of total red meat/d does not influence these clinically relevant and commonly measured modifiable CVD risk factors. These results do not support our hypothesis, which was based on a 2012 observational cohort study that estimated that the consumption of ≥0.5 servings of total red meat/d would increase CVD mortality (5). Our results align with a previous meta-analysis of 8 studies, which concluded that changes in blood lipids and lipoproteins did not differ when lean, unprocessed beef was consumed compared with poultry or fish (9). Our meta-analysis of 24 studies is more generalizable because it was inclusive of a variety of red meat types and also assessed blood pressure. It is important to emphasize that our conclusions do not support a cardioprotective effect of higher red meat consumption, such as is shown with fatty fish (48), but that the consumption of ≥0.5 servings of total red meat/d does not affect changes in blood lipids, lipoproteins, and blood pressures.

Although the median daily total red meat intake in the intervention group or phase was 2 servings, almost double what the average American consumes [~1.2 servings/d (49)], the range was large (1.0–7.1 servings/d). There is no visual threshold of total red meat consumption that indicates an apparent negative effect on blood lipids, lipoproteins, and blood pressures, as shown by the nondescript dispersal of the data in Figures 2–8. Although we used the cutoff of 0.5 servings of total red meat/d (5), we performed post hoc analyses to test if the studies with lower red meat consumption were washing out the effects of higher red meat consumption. The highest category of red meat consumption (>3 servings of red meat/d) showed no negative effects on blood lipid and lipoprotein concentrations and blood pressures and resulted in higher HDL concentrations. Because substituting protein for carbohydrate and adopting a "heart healthy" diet are shown to improve blood lipid and lipoprotein

	≥0.5 servings of red meat/day			<0.5 servings of red meat/day					
Study	mean (mm Hg)	SD	n	mean (mm Hg)	SD	n	weight (%)	mean difference (mmol/L), fixed effects [95% CI]	
Grieger, 2014 (24)	37.0	-1.0	37.0	-1.0	11.0	43.0	15.5	0.0	[-3.8, 3.8]
Nowson, 2009 (26)	46.0	-4.1	46.0	-2.9	4.9	49.0	25.3	-1.2	[-3.4, 1.0]
Liao, 2007 (27)	15.0	-5.0	15.0	-0.2	8.5	15.0	6.7	-4.8	[-11.6, 2.0]
Sayer, 2015 (28)	19.0	-4.9	19.0	-6.0	5.0	19.0	18.0	1.1	[-2.2, 4.4]
Wolmarans, 1999 (35)	39.0	2.2	39.0	-1.2	6.9	39.0	20.9	3.4	[0.5, 6.2]
Hodgson, 2006 (38)	29.0	-0.9	29.0	0.8	7.6	31.0	13.6	-1.7	[-5.9, 2.5]
Overall							**100.0**	**0.1**	**[-1.2, 1.5]**

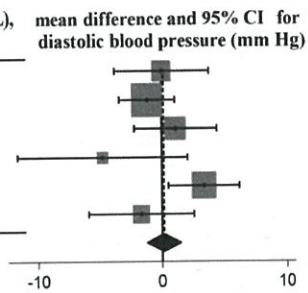

FIGURE 8 Fixed-effects model meta-analysis for changes in diastolic blood pressure from randomized controlled trials comparing ≥0.5 or <0.5 servings of total red meat/d. Heterogeneity: $\tau^2 = 0.662$, $\chi^2 = 4.42$, df = 5 ($P = 0.097$), $I^2 = 46\%$. Data are shown in descending order from smallest to largest amounts of red meat consumed by the intervention group or phase.

TABLE 3
Study design of randomized controlled trials assessing the effects of consuming ≥0.5 or <0.5 servings of total red meat/d on blood lipids, lipoproteins, and blood pressures[1]

First author, year (ref)	Total red meat servings in intervention (g/d); type of red meat; degree of meat processing	Total red meat servings in control (g/d); comparison protein source	Dietary pattern; diet administration method; dietary compliance assessment	Intervention length in weeks; study design	Population size and description; mean age in years; mean BMI in kg/m^2
Grieger, 2014 (24)	1.0 (68); beef, lamb, ham, pork; N/A	0; fatty fish	Habitual diet; protein source and some other foods provided; verbal motivation and interviews every 2 wk	8; 2-arm parallel	80 generally healthy men and women; 69.6; 26.4
Prescott, 1988 (45)	1.0 (72); meat supplement containing sausage, beef, lamb, pork; processed	0; nonmeat supplement	N/A; 2 meals/d and protein source provided; urinary 3-methyl histidine	12; 2-arm parallel	64 generally healthy men and women; N/A; N/A
Aadland, 2015 (25)	1.1 (77); pork and lean beef; minimally processed	0; lean seafood (cod, pollock, saithe, and scallops)	Norwegian nutritional recommendations; some food provided; daily oral questionnaire and regular weigh-ins	20; 2-phase crossover	20 generally healthy men and women; 50.6; 25.6
Nowson, 2009 (26)	1.2 (86); raw lean beef, lamb, veal, or combination; minimally processed	≤0.4 (28.6); combination control	DASH; protein source and some other food provided with dietary counseling; 24-h urinary electrolyte excretion and food records	14; 2-arm parallel	95 normal-hypertensive postmenopausal women; 59.2; 29.6
Liao, 2007 (27)	1.5 (105); N/A; N/A	0; soy	Weight-loss diet (1200 kcal); partial food provided for soy group but none for intervention group with dietary counseling and education; N/A	8; 2-arm parallel	30 generally healthy men and women; 33.4; 29.8
Navas-Carretero, 2009 (46)	1.6 (113); red meat; N/A	0.3 (22.3); oily fish	Habitual diet; daily 24-h dietary recalls and monthly 72-h detailed intake report; daily menu forms and weekly interviews	8; 2-phase crossover	25 iron-deficient women; 18–30; 22.1
Sayer, 2015 (28)	1.7 (121); pork tenderloin, uncured ham, beef tenderloin; minimally processed	0.2 (10.7); chicken or fish	DASH; protein source provided; daily food logs and 24-h urinary urea nitrogen	6; 2-phase crossover	19 prehypertensive or hypertensive men and women; 61; 31.2
Mahon, 2007 (29)	1.7 (121); cooked beef tenderloin; minimally processed	0; chicken, carbohydrate, or habitual control	Weight-loss diet (1250 kcal); protein source provided with dietary counseling, written instructions, menus, and shopping lists; biweekly dietary counseling sessions	9; 4-arm parallel	43 generally healthy postmenopausal women; 58; 29.6
Flynn, 1981 (30)	2.0 (140); raw beef; minimally processed	0; fish or poultry	Habitual diet; protein source provided; daily food logs and 4-d food records	12; 2-phase crossover	129 generally healthy men and women; N/A; N/A
Flynn, 1982 (31)	2.0 (140); raw beef or pork; minimally processed	0; fish or poultry	Habitual diet; protein source provided; 4-d food records	12; 2-phase crossover	76 generally healthy men and women; N/A; N/A
de Mello, 2006 (32)	2.0 (141); beef; N/A	0; chicken or lactoovo low-protein	N/A; no food provided; 2-d weighed food records and 24-h urinary urea nitrogen output	4; 2-phase crossover	17 men with type 2 diabetes and macroalbuminuria; 59; 26.2
Ashton, 2000 (33)	2.1 (150); lean, raw red meat; minimally processed	0; tofu	N/A; tofu provided, dietary counseling for meat selection; N/A	4; 2-phase crossover	42 generally healthy men; 45.8; 26.2
Davidson, 1999 (34)	2.2 (159); lean beef, veal, pork, or lamb; minimally processed	21.9; lean white meat (poultry and fish)	National Cholesterol Education Program Step I diet; no food provided, dietary counseling; food logs	32; 2-arm parallel	165 hypercholesterolemic men and women; 55.8; 27.3

(Continued)

TABLE 3 (*Continued*)

First author, year (ref)	Total red meat servings in intervention (g/d); type of red meat; degree of meat processing	Total red meat servings in control (g/d); comparison protein source	Dietary pattern; diet administration method; dietary compliance assessment	Intervention length in weeks; study design	Population size and description; mean age in years; mean BMI in kg/m^2
Wolmarans, 1999 (35)	2.4 (165); lean beef and lean mutton; minimally processed	0; chicken or fish	Prudent; pre and post 7-d weighed food records; postquestionnaires to assess compliance	6; 2-phase crossover	39 hypercholesterolemic men and women; 33.4; 24.4
O'Brien, 1980 (36)	2.4 (170); beef, pork, or lamb; N/A	0; fish or poultry with varying dietary cholesterol prescriptions	Varying cholesterol prescriptions but otherwise habitual diet; N/A; diet records	6; 4-phase crossover	29 generally healthy men; 43; N/A
Horrocks, 1999 (47)	2.9 (200); pork; N/A	0; chicken	N/A; N/A; N/A	4; 2-phase crossover	20 generally healthy women; N/A; N/A
Foerster, 2014 (37)	2.9 (200); fresh pork cutlet and beef steak; minimally processed	<0.4 (30); whole-grain products	Habitual diet; protein sources provided; regular check-ins with research staff	10; 2-phase crossover	20 generally healthy men and women; 40.1; 24.4
Hodgson, 2006 (38)	3.1 (215); lean, raw red meat; minimally processed	0; plant protein	Habitual diet; protein source provided with dietary counseling; pre and post 3-d weighed food diary	8; 2-arm parallel	60 hypertensive men and women; 58.7; 27.7
Gascon, 1996 (39)	3.3 (230); lean beef, pork, veal; minimally processed	0; lean white fish	American Heart Association prudent diet; partial meals provided; verbal interview every 2 d	~4 (1 menstrual cycle); 2-phase crossover	14 generally healthy women; 22.4; 22
Wolmarans, 1991 (40)	3.5 (246); beef and mutton; N/A	0; fatty fish	Habitual diet; N/A ; pre and post 7-d dietary records	6; 2-phase crossover	28 generally healthy men and women; 33.8; N/A
Haub, 2005 (41)	3.5 (248); cube steak, ground beef, beef tips; minimally processed	0; plant protein	Habitual diet; protein source provided; routine interviews to assess compliance	12; 2-arm parallel	21 generally healthy men; 65.0; 28.2
Wiebe, 1984 (44)	3.6 (250); frozen beef patties; minimally processed	0; plant protein	Controlled but not specified; food provided; N/A	3; 2-phase crossover	8 generally healthy men; 20.9, 21.7
Beauchesne-Rondeau, 2003 (42)	5.4 (380); lean ground beef, exterior round, sirloin top; minimally processed	0; lean fish or poultry	American Heart Association diet; partial food provided; N/A	3; 3-phase crossover	17 hypercholesterolemic men; 50.1; 26.5
Sinclair, 1987 (43)	7.1 (500); kangaroo; N/A	0; southern fatty fish, tropical fatty fish, or plant protein	Low-fat (<7% of total energy); protein source provided with dietary counseling; daily food records	2; 4-phase crossover	13 generally healthy men and women; 31.3; 21.2

[1] DASH, Dietary Approaches to Stop Hypertension; lactoovo, lacto-ovovegetarian; N/A, not applicable; post, postintervention; pre, preintervention.

TABLE 4
Analysis of postintervention values of consuming ≥0.5 or <0.5 servings of total red meat/d in randomized controlled trials[1]

Dependent variable[2]	Number of studies included	≥0.5 servings of total red meat/d	<0.5 servings of total red meat/d	*P*
Total cholesterol, mmol/L	22	4.93 ± 0.11	4.88 ± 0.10	0.57
LDL cholesterol, mmol/L	21	3.18 ± 0.08	3.13 ± 0.07	0.52
HDL cholesterol, mmol/L	21	1.30 ± 0.04	1.27 ± 0.03	0.41
Triglycerides, mmol/L	20	1.23 ± 0.05	1.21 ± 0.05	0.83
TC:HDL	19	3.93± 0.07	3.98 ± 0.07	0.46
Systolic blood pressure, mm Hg	7	121± 10	122 ± 11	0.51
Diastolic blood pressure, mm Hg	7	64 ± 4	63 ± 5	0.55

[1] Unless otherwise indicated, values are least-squares means ± SEs adjusted for baseline values, age, sex, BMI, length of intervention, and whether energy restriction was or was not included in the protocol. A 2-factor nested ANOVA showed no differences between post values of consuming ≥0.5 or <0.5 servings of total red meat/d. Total cholesterol, LDL-cholesterol, and HDL-cholesterol conversion: mmol/L × 38.67 = mg/dL; triglyceride conversion: mmol/L × 88.57 = mg/dL. TC:HDL, ratio of total cholesterol to HDL cholesterol.

[2] A repeated-measures ANOVA showed that all dependent variables changed over time except for systolic blood pressure ($P < 0.05$).

concentrations and blood pressure (50–53), we performed cluster sensitivity analyses to assess studies without these characteristics. This did not influence our conclusion that consuming ≥0.5 servings of red meat/d does not affect changes in blood lipid and lipoprotein concentrations and blood pressures. Therefore, this meta-analysis compared protein sources rather than macronutrient compositions within the context of a variety of diets.

The Mediterranean-style and the DASH (Dietary Approaches to Stop Hypertension) dietary patterns are "heart healthy" diets that include <0.5 servings of red meat/d. The Mediterranean-style dietary pattern is predominantly modeled on observational cohort studies (54–57) and 1 large-scale RCT (58) that indicate a lower incidence of CVD-related events, mortality, and lower CVD risk with the consumption of this dietary pattern. However, these studies reported red meat consumption of >0.5 servings of red meat/d [range: ~2–3.5 servings/1000 kcal; see Figure D1.59 in the Scientific Report of the 2015 Dietary Guidelines Advisory Committee (59) for a graphic summary of these studies, with the exception of our reference 57]. Therefore, it is unclear what studies are supportive of this recommendation for red meat in the context of a Mediterranean-style diet. The DASH diet, by design, limits red meat consumption to <0.5 servings/d (60). However, current RCTs showed that the DASH diet has equivalent effectiveness to reduce blood lipids, lipoproteins, and blood pressures when it contains >0.5 servings of red meat/d [1.6 or 2.2 servings of beef (61, 62) or 1.7 servings of pork (28) daily]. Collectively, these studies suggest that the consumption of >0.5 servings of red meat/d in the context of these recommended dietary patterns does not hinder improvements in CVD risk factors.

The conflicting literature creates ambiguous conclusions in dietary guidance pertaining to red meat consumption amounts. The Scientific Report of the 2015 Dietary Guidelines Advisory Committee concluded that "lean meats" can be incorporated into a healthy diet in relatively small amounts, but there is no specificity to the type or amount of lean meat. Communication to the general public from the 2015–2020 DGA combines red meat with the "meat, eggs, and poultry" recommendation rather than its own food group (12), as done in previous DGAs (63). Dietary recommendations based on the 2010–2015 DGA, with support from the 2015 Advisory Report, suggest that red meat consumption should be limited to ~0.5–0.7 servings/d or ~3.5–5 servings/wk (59, 63); this varies because the serving size range is 2–3 ounces. The Dietary Guidelines Advisory Committee search process has strict criteria that limit the inclusion of data from available RCTs (64), so this conclusion is based predominantly on epidemiologic associations (63). This restricts the conclusions to be mainly based on associative conclusions of morbidity and mortality rather than cause and effect of disease risk, both of which need to be considered in determining dietary guidance and public policy.

A strength of this systematically searched meta-analysis is the use of RCT designs, which allows our conclusions to be based on the principle of causation. These RCTs assessed the effects of consumption of total red meat on CVD risk factors for relatively short periods of time (2–32 wk). In contrast, epidemiologic studies have assessed the association between total red meat consumption and CVD-related morbidity and mortality that typically require years or decades of follow-up and are not suitable to determine causality. Thus, results from RCTs support that the consumption of red meat does not influence CVD risk factors, whereas epidemiologic studies support that the consumption of red meat is associated with higher incidences of CVD-related morbidity and mortality. Future efforts and research by academic, industry, and government leaders are needed to improve the scientific foundation and communication to the public about the effects of red meat on diet quality and human health by including evidence from both types of study designs.

Another strength of this meta-analysis is the high external validity because we did not restrict our search to certain dietary patterns, populations, or types of red meat (65). Although this created heterogeneity among data within each blood lipid and lipoprotein variable (indicated by the I^2 scores; see Figures 2–6), the extensive sensitivity analyses did not affect overall findings when potential modifiers were excluded. Data from other CVD risk factors, such as endothelial cell function and inflammation, were not collected for this meta-analysis. These factors can progress to CVD when traditional risk factors are unchanged (66) and therefore may be a limitation of this analysis. We did not exclude studies based on the criteria used by the Dietary Guidelines Advisory Committee (64) and recognize that a meta-analysis is only as strong as the empirical evidence included. We raise concern about the unclear bias reporting, which was

common in the studies included in this meta-analysis, and urge researchers to comprehensively report study design characteristics. We are also aware that there are other potential human and environmental health risks associated with higher red meat intake, which are beyond the scope of this review, and include but are not limited to cancer (67) and environmental sustainability (68, 69).

In conclusion, the results from this systematically searched meta-analysis of RCTs support that the consumption of ≥0.5 compared with <0.5 servings of total red meat/d does not influence blood lipids, lipoproteins, and/or blood pressures, which are clinically relevant CVD risk factors. These results are generalizable across a variety of populations, dietary patterns, and types of red meat. These results are inconsistent with much of the observational evidence related to red meat consumption and CVD, which prompts the need for future research to reconcile the apparent disconnect between RCT and observation-based conclusions.

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The authors' responsibilities were as follows—LEO, JEK, and WWC: designed the research; LEO and JEK: conducted the research; LEO: analyzed the data; and LEO and WWC: wrote the manuscript and have primary responsibility for the final content. During the time this manuscript was being developed and written, WWC received research support from American Egg Board–Egg Nutrition Center, Beef Checkoff, Coca-Cola Foundation, National Dairy Council, National Institutes of Health, Pork Checkoff, and USDA and had a consulting arrangement with Coca-Cola Company. None of these organizations provided support to conduct this meta-analysis. WWC also served on the 2015 Dietary Guidelines Advisory Committee and was a member of the Advisory Council on Nutrition and Healthy Food Choices, Foundation for Food and Agriculture Research. JEK received support from the American Egg Board–Egg Nutrition Center. LEO reported no conflicts of interest.

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HEART-HEALTHY, HIGHER PROTEIN DIETS WITH LEAN BEEF HELP IMPROVE BLOOD PRESSURE & VASCULAR HEALTH

Effects of a DASH-like diet containing lean beef on vascular health
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Objective

Study the effect of DASH-like diets that provided different amounts of protein from lean beef on blood pressure, endothelial function and vascular reactivity versus a healthy American diet (HAD).

Study Design and Setting

A 4-period, randomized, crossover, controlled feeding design. Subjects were randomly assigned to a treatment (diet) order, and consumed each diet (HAD: 33% total fat, 12% SFA, 17% protein, 20g beef/d; DASH: 27% total fat, 6% SFA, 18% protein, 28g beef/d; BOLD: 28% total fat, 6% SFA, 19% protein, 113g beef/d; and BOLD+: 28% total fat, 6% SFA, 27% protein, 153g beef/d) for 5 weeks. The diet periods were separated by a brief compliance break (average 1 week).

Participants

Thirty-six nonsmoking normo- (SBP, 116±3.6mmHg) or pre- (BP <140/90mmHg) hypertensive men and women (30–65 years) with moderately elevated low-density lipoprotein cholesterol (110–176mgdl-1) were recruited.

Additional inclusion criteria:

- Body mass index (18.5–37kgm-2)
- Fasting triglycerides <350mgdl-1

Exclusion criteria:

- Established CVD
- Stroke
- Diabetes
- Liver, kidney or autoimmune disease
- The use of cholesterol/lipid-lowering medication or supplements (psyllium, fish oil, soy lecithin and phytoestrogens)
- Being pregnant or lactating
- Experiencing weight loss of ≥10% of body weight within the 6 months before enrolling in the study
- Vegetarianism

Results

- Systolic blood pressure (SBP) was significantly reduced subjects on the BOLD+ diet (111.4±1.9mmHg) versus HAD (115.7±1.9).
- Augmentation index, a marker of arterial status, was significantly reduced in participants on the BOLD diet (-4.1%).
- A moderate protein DASH-like diet including lean beef decreased SBP in normotensive individuals.
- The inclusion of lean beef in a heart healthy diet also reduced peripheral vascular constriction.

CONCLUSIONS

These results suggest that total protein and not type of protein is important for eliciting reductions in systolic blood pressure.

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ORIGINAL ARTICLE

Effects of a DASH-like diet containing lean beef on vascular health

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A DASH (dietary approaches to stop hypertension) dietary pattern rich in fruits and vegetables and low-fat dairy products with increased dietary protein provided primarily from plant protein sources decreases blood pressure. No studies, however, have evaluated the effects of a DASH-like diet with increased dietary protein from lean beef on blood pressure and vascular health. The aim of this study was to study the effect of DASH-like diets that provided different amounts of protein from lean beef (DASH 28 g beef per day; beef in an optimal lean diet (BOLD) 113 g beef per day; beef in an optimal lean diet plus additional protein (BOLD+) 153 g beef per day) on blood pressure, endothelial function and vascular reactivity versus a healthy American diet (HAD). Using a randomized, crossover study design, 36 normotensive participants (systolic blood pressure (SBP), 116 ± 3.6 mm Hg) were fed four isocaloric diets,: HAD (33% total fat, 12% saturated fatty acids (SFA), 17% protein (PRO), 20 g beef per day), DASH (27% total fat, 6% SFA, 18% PRO, 28 g beef per day), BOLD (28% total fat, 6% SFA, 19% PRO, 113 g beef per day) and BOLD+ (28% total fat, 6% SFA, 27% PRO, 153 g beef per day), for 5 weeks. SBP decreased ($P<0.05$) in subjects on the BOLD+ diet (111.4 ± 1.9 mm Hg) versus HAD (115.7 ± 1.9). There were no significant effects of the DASH and BOLD diets on SBP. Augmentation index (AI) was significantly reduced in participants on the BOLD diet (−4.1%). There were no significant effects of the dietary treatments on diastolic blood pressure or endothelial function (as measured by peripheral arterial tonometry). A moderate protein DASH-like diet including lean beef decreased SBP in normotensive individuals. The inclusion of lean beef in a heart healthy diet also reduced peripheral vascular constriction.

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INTRODUCTION

Atherosclerotic cardiovascular disease (CVD) is a multifactorial disease. Estimates indicate that more than 82 million American adults (1 in 3) have one or more types of CVD.[1,2] Many individuals in all ethnic populations have multiple risk factors for CVD, and the number of risk factors in individuals without diagnosed CVD is increasing.[1] The major risk factors include smoking status, elevated body weight, total cholesterol, low-density lipoprotein cholesterol, blood pressure (BP) and fasting glucose.[1] Approximately 60% of Caucasian adults and 80% of African American adults have at least one risk factor. DASH (dietary approaches to stop hypertension) is the 'gold standard' dietary pattern recommended by the American Heart Association,[3] American Society of Hypertension[2] and 2010 Dietary Guidelines for Americans[4] for reducing many of these major CVD risk factors including abnormal lipids and lipoproteins, high BP, overweight/obesity and elevated blood glucose levels.[5,6] The DASH dietary pattern is reduced in saturated fatty acids (SFA), with emphasis on dietary carbohydrate from fruits, vegetables and whole grains; multiple minerals (potassium, magnesium and calcium) and fibre are also increased.

In addition to the major risk factors for CVD, there are other risk factors including those related to vascular health (endothelial function, vascular reactivity and so on). Our understanding of how diet affects vascular health is still evolving and more information is needed.

Individuals are often advised to avoid or restrict beef because it is a source of saturated fat in the diet. However, many Americans enjoy beef, commonly choosing cuts deemed lean by United States Department of Agriculture (USDA), and report better adherence to dietary advice that includes some lean beef.[7] In addition, beef's contribution to SFA in the American diet is often overstated in that it is not one of the top five contributors of SFA for Americans.[4] In the BOLD Study, we showed that the inclusion of lean beef (4.0 or 5.4 oz per day) in a DASH-like diet decreased total cholesterol and low-density lipoprotein cholesterol similarly to the DASH diet.[8] The DASH diet guidelines suggest reducing red meat as a strategy for controlling saturated fat;[9] however, little is known about the effects on vascular health when lean beef is incorporated in a DASH diet.

In the present study conducted with normotensive individuals, we evaluated the effects of a traditional DASH diet as well as a DASH-like diet containing lean beef (beef in an optimal lean diet (BOLD); 113 g beef per day) and a moderate protein diet containing lean beef (BOLD+; 153 g beef per day) compared with a healthy American diet (HAD) as the control on vascular health, a secondary end point in the BOLD Study.

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SUBJECTS AND METHODS

Subjects

The methods used for this study have been described in detail previously.[8] Nonsmoking normo- or pre-hypertensive (BP $<140/90$ mm Hg) men and women (30–65 years) with moderately elevated low-density lipoprotein cholesterol (110–176 mg dl^{-1}) were recruited.[8] Additional inclusion criteria were body mass index (18.5–37 kg m^{-2}) and fasting triglycerides <350 mg dl^{-1}. Participants taking prescribed BP-lowering medication were eligible as long as their BP was below the exclusion criteria (one participant on BP medication was enrolled in the trial but excluded from the vascular health analyses). Exclusion criteria were: established CVD, stroke, diabetes, liver, kidney or autoimmune disease, the use of cholesterol/lipid-lowering medication or supplements (psyllium, fish oil, soy lecithin and phytoestrogens), being pregnant or lactating, experiencing weight loss of $\geqslant 10\%$ of body weight within the 6 months before enrolling in the study and vegetarianism. The Institutional Review Board at The Pennsylvania State University approved the experimental protocol, and all subjects provided written informed consent. This study is registered at ClinicalTrials.gov NCT00937898.

Study design

The study employed a four-period, randomized, crossover, controlled-feeding design. Subjects were randomly assigned to a treatment (diet) order, and consumed each diet (HAD, DASH, BOLD and BOLD+) for 5 weeks. The diet periods were separated by a brief compliance break (average 1 week). On two consecutive days at the beginning of the study (baseline) and at the end of each diet period, participants completed a series of clinical and physical assessments (blood draw, height and weight) at the General Clinical Research Center of The Pennsylvania State University. The initial participants were enrolled in the study in September 2007; the final participants completed the study in March 2009.

Diets

The composition of the experimental diets is presented in Table 1. The Harris–Benedict equation was used to estimate each participant's energy needs, participants were monitored (daily weigh-ins) to verify they remained weight stable and calorie adjustments were made in 100 kcal increment to assure that weight remained stable for the duration of the study. All diets were rich in fruits, vegetables and lean meats consistent with food-based dietary recommendations. The three experimental diets (DASH, BOLD and BOLD+) contained similar amounts of total fat, SFA, monounsaturated fatty acids, polyunsaturated fatty acids and cholesterol. The HAD was higher in total fat, SFA, monounsaturated fatty acids, polyunsaturated fatty acids and cholesterol, and was lower in total fibre. The BOLD and DASH diets were matched for macronutrient composition. The BOLD+ diet was higher in protein (27% of total energy; 19% plant, 26% dairy, 42% lean beef and 12% other animal sources) as compared with the HAD (17%; 13% plant, 26% dairy, 12% lean beef and 49% other animal sources), DASH (18%; 20% plant, 31% dairy, 9% lean beef and 40% other animal sources) and BOLD (19%; 13% plant, 23% dairy, 53% lean beef and 11% other animal sources) diets, and lower in carbohydrate (45 vs 50–54%) (Table 1). A description of the food groups (and respective servings) fed has been published previously.[8]

Although matched for protein, the BOLD and DASH diets differed in the quantity of lean beef (Table 1). Select grade top round, chuck shoulder pot roast and 95% lean ground beef were used in the study. The meat was prepared via braising, grilling or frying (95% lean ground beef only), and was never cooked over an open flame in order to prevent charring.

A 6-day menu cycle was used throughout the study (1800–3600 kcal per day). All meals and snacks were prepared at the Metabolic Diet Study Center at Pennsylvania State University. Participants ate one meal per day (Monday–Friday) in the Metabolic Diet Study Center and their other meals were prepared and packed for off-site consumption. On weekends when the Study Center was closed, participants received a cooler that contained all of their meals and snacks for 2 days. Compliance with the prepared diets was monitored via self-report to document whether study foods/meals were omitted and/or replaced. Participants limited caffeinated beverages to 8 oz per day and alcoholic beverages to <2 servings per week. Participants were allowed to continue their current exercise regimen but were instructed not to increase or decrease duration or intensity during the study.

Table 1. BOLD Study diets: energy (based on 2100 kcal meal plans) and nutrient composition (% of energy)[a,b,c]

Nutrient targets, kcal % (g)	*Diets*			
	HAD	*DASH*	*BOLD*	*BOLD+*
Calories	2097	2106	2100	2104
Protein (g)	17 (91.7)	18 (98.4)	19 (99.6)	27 (145.6)
Carbohydrate (g)	50 (268.1)	55 (298.3)	54 (287.4)	45 (243.7)
Fat (g)	33 (77.0)	27 (64.4)	28 (65.8)	28 (66.6)
Cholesterol (mg)	287	188	168	193
SFA (g)	12 (27.9)	6 (15.2)	6 (15.4)	6 (14.5)
PUFA (g)	7 (15.5)	8 (18.9)	7 (16.5)	7 (16.1)
MUFA (g)	11 (25.9)	9 (21.8)	11 (25.2)	12 (29.3)
Fibre (g)	24	36	32	38
Micronutrients				
Sodium (mg)	3243	2983	2712	3344
Potassium (mg)	3259	4247	3998	4417
Calcium (mg)	993	1140	936	1060
Magnesium (mg)	308	403	392	429
Lean beef, g/day	20	28	113	153

Abbreviations: BOLD, beef in an optimal lean diet; BOLD+, beef in an optimal lean diet plus additional protein; DASH, dietary approaches to stop hypertension diet; HAD, healthy American diet; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids. [a]Based on 2100 kcal per day. [b]Average across a 6-day menu cycle. [c]All values were determined using NUTRTIONST PRO (Axxya Systems LLC, Stafford, TX, USA).

Clinical assessments

Body weight was measured each weekday in the Metabolic Diet Study Center before eating that day's meal and at each laboratory visit. Blood samples were collected after a 10–12-h fast. Serum and plasma aliquots were stored at −80 C until time of analysis.

Vascular health

Measures of vascular health were secondary end points in the BOLD Study. BP was assessed using a single measurement at the beginning of the study and at the end of each diet period before the baseline period of the endothelial function test (Dinamap Pro 100, Critikon, Milwaukee, WI, USA). Participants were seated with arm at heart level and appropriate cuff sizes were used. After a 12-h fast, EndoPAT2000 (Itamar Medical, Ltd, Caesarea, Israel) was used to measure relative changes in pulse wave amplitude before vs after occlusion.[10] The EndoPAT technique is validated as a measure of endothelial function.[10,11] Two flexible probes were placed on the index fingers of the right (ischaemic) and left (control) hands, and a counter pressure was applied to both fingers continuously throughout the test. A BP cuff was placed on the right forearm and pulse amplitude was measured during baseline (5 min), occlusion (5 min) and reactive hyperaemia (5 min). Reactive hyperaemia index (RHI) was calculated as the ratio of the average pulse wave amplitude during hyperaemia (60 to 120 s of the post-occlusion period) to the average pulse wave amplitude during baseline in the occluded hand divided by the same values in the control hand and then multiplied by a baseline correction factor. We also calculated the Framingham RHI (FRHI) as described previously.[12,13]

The EndoPAT device was used to generate the augmentation index (AI). The EndoPAT-generated AI measurement is determined from the baseline resting pulse wave. In stiff arteries, the pulse wave travels rapidly to the periphery where it encounters resistance at the peripheral arterioles, and the reflected wave augments central BP. Thus, higher AI indicates greater arterial stiffness. Proprietary software automatically identifies inflection points distinguishing the systolic peak and the reflected peak for the calculation of this ratio and converts it into a percentage ($p1 - p2/p1 \times 100$).[14] EndoPAT-derived AI measures correlate well with AI measures from other devices.[15] AI can be adjusted to a heart rate of 75 beats per min to correct for the independent effect of heart rate on AI measurement; both AI and AI at 75 beats per min are reported.

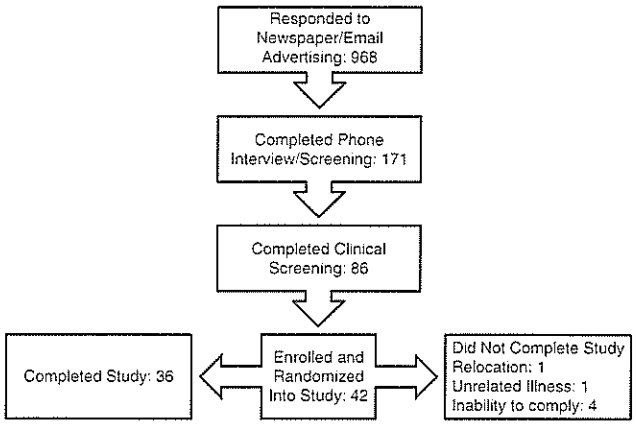

Figure 1. Recruitment flow diagram.

Table 2. Baseline characteristics of study participants ($n = 36$)[a]

Characteristic	*Males (n = 15)*	*Females (n = 21)*
Age (years)	49 ± 1.8 (39–63)	50 ± 2.0 (45–97)
BMI (kg m^{-2})	27.3 ± 0.7 (19.4–35.5)	24.8 ± 0.5 (19.4–35.5)[b]
SBP (mm Hg)	124 ± 2.6 (111–143)[b]	112.4 ± 3.2 (94–150)
DBP (mm Hg)	72 ± 2.0 (60–85)	66 ± 2.6 (45–97)
RHI	1.98 ± 0.18 (1.4–3.6)	2.33 ± 0.13 (1.1–3.0)
FRHI	0.43 ± 0.12 (0.2–1.4)	0.77 ± 0.10 (0.01–1.4)[b]
AI	7.33 ± 5.1 (−20.4–53.2)	18.0 ± 5.08 (−17.6–49.4)

Abbreviations: AI, augmentation index; BMI, body mass index; BPM, beats per min; DBP, diastolic blood pressure; FRHI, Framingham reactive hyperaemia index; RHI, reactive hyperaemia index; SBP, systolic blood pressure. [a]Mean ± s.e.m. (range). Baseline values were measured before consuming any study food. [b]Two-sample *t*-test was used to determine significant ($P < 0.05$) differences between genders (SAS version 9.2; SAS Institute Inc., Cary, NC, USA).

Statistical analysis

All statistical analyses were performed using SAS (Version 9.2; Statistical Analyses System, Cary, NC, USA). Two sample *t*-tests were used to determine significant differences between genders at baseline for each outcome variable. The residuals for each variable were used to assess normality. Logarithmic transformations were used for non-normally distributed variables (AI and RHI). The mixed models procedure (PROC MIXED) was used to test the effects of diet and order on the outcome variables. A repeated analysis of covariance (repeated for diet) was used with age, weight and baseline values as covariates. Tukey–Kramer adjusted *P*-values were used to determine whether the differences between the diets for outcome variables were significant ($P < 0.05$).

RESULTS

Figure 1 presents information about the number of subjects who responded to the study advertisements ($n = 968$); completed the phone interview/screening ($n = 171$); completed the clinical screening ($n = 86$); and enrolled in the study ($n = 42$). The vast majority of potential subjects were excluded based on the inclusion and exclusion criteria of the study after they completed the screening processes. Some individuals who met the study eligibility criteria elected to not participate because of the requirements imposed by the study. During the study, one subject dropped out because of a job change and relocation, one to an unrelated illness and four because of an inability to adhere to the dietary protocol (the latter occurred within the first week of the study). One participant was on BP-lowering medication for the duration of the study and was excluded in the analysis. There were 36 subjects included in the final analysis (Figure 1).

Baseline subject characteristics are presented in Table 2. Females had a significantly higher FRHI (0.79 vs 0.43; $P = 0.04$) compared with males. Males had a significantly greater body mass index (27.3 vs 24.8 kg m^{-2}; $P = 0.02$) compared with females (Table 2). There were no gender differences in response to any of the dietary treatments. Subject adherence to the prescribed diets was 93% according to daily self-reporting forms. Body weight was maintained during the diet periods within ± 2.2 kg. The metabolic status (lipids, glucose, insulin and C-reactive protein) of these subjects has been described previously.[8]

Blood pressure

Systolic blood pressure (SBP) was significantly reduced following the BOLD+ diet compared with the HAD ($P < 0.01$); no other significant reductions in SBP were observed. There were no significant changes in DBP on the DASH, BOLD or BOLD+ diets.

Endothelial function and vascular stiffness

The RHI and FRHI scores did not differ among diets (Table 3). AI significantly decreased following the BOLD diet compared with the HAD, DASH and BOLD+ diets (Table 3).

A significant interaction was observed between subject age and diet for AI. To further explore this interaction, subjects were grouped according to whether their age was a risk factor for CVD (females ⩾55 years and males ⩾45 years).[16] This secondary analysis revealed that AI was significantly reduced on the BOLD diet in younger participants, but not older individuals.

DISCUSSION

The BOLD Study is the first controlled clinical trial to show that a moderate protein diet (based on the DASH eating plan) that emphasized lean beef (113 g per day) as the main protein source reduced SBP in normotensive individuals when compared with a healthy control diet that was lower in protein and higher in carbohydrate and saturated fat. The BOLD+ diet contained 10% more calories from protein and reduced SBP by 4.2 mm Hg versus HAD, whereas the BOLD and DASH diet elicited nonsignificant reductions of 1.6 and 2.8 mm Hg, respectively.

In the OmniHeart trial, the high-protein diet that had a comparable macronutrient profile to the BOLD+ diet resulted in a −9.5 mm Hg reduction in SBP from baseline.[17] The difference in the magnitude of BP reductions in the present study and the protein diet evaluated in the OmniHeart trial could be due, in part, to the participants studied. Specifically, participants in the BOLD Study were normotensive (mean baseline SBP, 116 ± 3.6 mm Hg), whereas participants in the OmniHeart trial were pre-hypertensive (mean baseline SBP, 131.3 ± 10.8 mm Hg). In the BOLD+ diet, as in the higher protein OmniHeart diet, increases in total protein (from either animal or plant protein) suggest that the BP reductions reflect a total protein effect or the synchronous reduction in carbohydrates.

Compared with the original DASH trial (which lowered SBP by −3.5 mm Hg),[18] the BOLD and DASH diets yielded similar, yet nonsignificant changes in SBP versus HAD (−1.9 and −2.8 mm Hg, respectively). The minor differences in response to the DASH diet and the similarly designed BOLD diet in our study may be because of the normotensive status of the study population compared with the pre-hypertensive/hypertensive participants in the DASH trial. Individuals with hypertension have greater reductions in BP following a heart healthy diet,[19] as well as after weight loss[20] compared with their normotensive counterparts. In addition, our study had far fewer subjects ($n = 36$) than the DASH trial ($n = 459$), and this could explain the lack of statistical significance for the SBP change observed. The

Table 3. Effect of diet on blood pressure, endothelial function and vascular reactivity*

	HAD	*DASH*	*BOLD*	*BOLD+*
Weight, kg	74.1 ± 2.3	73.8 ± 2.3	73.7 ± 2.3	74.1 ± 2.3
SBP, mm Hg	115.7 ± 1.9[a]	112.9 ± 1.9[a]	114.0 ± 1.9[a]	111.4 ± 1.9[b]
DBP, mm Hg	69.8 ± 1.5	69.1 ± 1.5	69.4 ± 1.5	69.1 ± 1.5
FRHI	0.65 ± 0.05	0.66 ± 0.05	0.64 ± 0.05	0.62 ± 0.05
RHI	2.21 ± 0.09	2.19 ± 0.10	2.31 ± 0.09	2.13 ± 0.11
Heart rate, BPM	58.01 ± 0.74	58.30 ± 0.74	60.03 ± 0.74	59.30 ± 0.75
AI**	14.47 ± 3.6[a]	13.56 ± 3.3[a]	10.37 ± 3.0[b]	13.48 ± 3.0[a]
AI @75 BPM	2.84 ± 2.2	3.87 ± 2.2	1.23 ± 2.2	4.61 ± 2.2
Older, AI**	21.97 ± 5.0	22.71 ± 2.4	19.62 ± 4.1	17.72 ± 4.1
Younger, AI**	6.08 ± 4.5[a]	3.86 ± 3.7[a]	0.03 ± 26[b]	8.48 ± 4.4[a]

Abbreviations: AI, augmentation index; BOLD, beef in an optimal lean diet; BOLD+, beef in an optimal lean diet plus additional protein; BPM, beats per min; DASH, dietary approaches to stop hypertension diet; DBP, diastolic blood pressure; FRHI, Framingham reactive hyperaemia index; HAD, healthy American diet; RHI, reactive hyperaemia index; SBP, systolic blood pressure. The MIXED procedure (version 9.2; SAS Institute Inc., Cary, NC, USA) was used to test the effects of diet. Values in the same row with different superscripts (a, b) are significantly different, adjusted $P<0.05$. *All values are mean ± s.e.m. **Raw values reported. Data were log transformed to achieve normality when testing for significant differences. Older females ⩾55 years and males ⩾45 years.

differences in the response observed in the BOLD Study may also be due in part to differences in total and saturated fat between the control diet used in the initial DASH study (total fat = 37%; SFA = 16% of total energy) and the HAD (total fat = 33%; SFA = 12% of total energy).[21]

In addition to the aforementioned protein effect (or carbohydrate reduction), potassium, magnesium, sodium and calcium are minerals of importance with respect to their role in modulating BP.[22] Sodium and calcium intakes were similar for the HAD and BOLD+ diets (Table 1). Potassium and magnesium levels were lower in the HAD compared with the BOLD+ diet. The BOLD+ diet provided similar amounts of potassium and magnesium compared with the original DASH study (4415 and 480 mg per day, respectively),[18] and potassium levels were similar to those recommended by the American Society of Hypertension (4700 mg per day).[2] A 2006 Cochrane review on magnesium supplementation for the treatment of high BP did not find evidence to support a causal relationship.[23] A systematic review by Dickinson *et al.*[24] found that magnesium supplementation (0.2–1.0 g per day) reduced SBP by 1.3 mm Hg, but this was nonsignificant. Therefore, we believe that it is unlikely that the 121 mg per day difference in magnesium between the HAD and BOLD+ diet significantly contributed to the reduction in SBP. We hasten to add, however, that in a diet that is also increased in other nutrients that are shown to lower BP, a small increase in magnesium may contribute to a BP-lowering effect.

It is also unlikely that dietary fibre is responsible for the reduction in SBP. A meta-analysis of 25 randomized controlled trials found no effect of dietary fibre intake on SBP in normotensive individuals.[25] Thus, the 14 g per day increase in dietary fibre between the HAD and BOLD+ diet likely did not influence SBP in normotensive subjects in the BOLD Study. Although the separate effect of fibre and select minerals does not fully explain the BP-lowering effects, there is most likely an effect of the synergy of these changes, as seen in the original DASH trial. Taking this into account, we still propose that the primary explanation for the changes in SBP in our normotensive study population was most likely because of the increase in total protein (from a variety of protein sources, including lean beef) that also led to a slight decrease in carbohydrate. Further studies are needed to resolve this question.

On the BOLD diet, AI was significantly reduced compared with the HAD, despite no significant changes in DBP or SBP. In addition, AI was not significantly correlated with DBP ($R=0.063$, $P=0.4$) or SBP ($R=0.15$, $P=0.06$). However, there is conflicting evidence regarding the relationship between peripheral BP and AI.[26–28] Hamburg *et al.*[13] hypothesized that the discrepancy in the relationship between peripheral arterial tonometry measures, like AI, and systemic BP may be because of the limited effect of systemic BP on the distal microcirculation. This might be the case in the BOLD Study as significant improvements in AI were observed in the BOLD but not BOLD+ diets, whereas SBP was reduced in the BOLD+ but not BOLD or DASH diets.

AI and age are also related.[29] Our results are consistent with this finding as a secondary analysis revealed a significant reduction in AI following the BOLD diet only in younger participants (males <45 years and females <55 years). This suggests that the arterial stiffness associated with ageing[30] was not modified by the BOLD diet. Thus, dietary interventions designed to improve vascular reactivity may need to be initiated earlier in life to have a significant effect.

The mechanism accounting for the significant reduction in AI following the BOLD diet compared with the HAD, DAS and BOLD+ diets is unclear. This is one of the first controlled-feeding studies to measure the impact of different macronutrient composition (as well as protein sources) on AI. The improvements in AI observed for the BOLD diet underscore the need to better understand the effects of diet and protein (that is, quantity and source) on vascular elasticity.

The endothelial function results of the current study (as assessed by EndoPat) agree with those of a 35-day controlled-feeding intervention conducted by Vega-López *et al.*[31] who found no effect of increasing the dietary lysine/arginine ratio (0.7 to 1.4; a common measure of the animal/plant protein ratio) of a low SFA diet (<7% total calories) on flow-mediated dilation or peripheral arterial tonometry (precursor to RHI measurement provided by EndoPat 2000). Al-Solaiman *et al.*[32] and Hodson *et al.*[33] also found no changes in endothelial function in healthy individuals following the DASH diet.

There were three potential limitations of the vascular health end points in the BOLD Study. One potential limitation was that BP was only measured once at baseline and at each end point visit. A minimum of two measurements taken 1 min apart is the preferred method to reduce measurement error. In addition, the null finding for RHI and FRHI may have been influenced by the menstrual phase that was not controlled for in the present study; however, we also did not observe any significant changes in RHI or FRHI in male subjects. Finally, in this study, macronutrient intakes and other nutrient guidelines were set based on percent of total calories or in the context of the base diet of 2100 calories. Although the calorie levels for individuals were adjusted up or down based on the energy needs required to maintain a participant's weight, nutrients such as fibre, potassium, sodium and magnesium were also increased or decreased but not always

in an exact proportional manner. Because it is not known whether meeting the exact nutrient targets established in the DASH study for different calorie levels is important, this could be one possible reason why a statistically significant BP reduction was not observed for the DASH and BOLD diets in the present study.

The BOLD diet was the only diet that significantly reduced AI, and the BOLD + diet was the only diet that significantly reduced SBP. These findings suggest that heart-healthy diets containing different amounts of macronutrients including those contained in lean beef (that is, primarily protein) can positively affect vascular health, although via potentially different mechanisms. It has been suggested previously that increased dietary plant protein (versus animal protein) may be responsible for the protein-associated reductions in BP.[34] However, the present findings show that a variety of protein sources including lean beef can also be used to increase total dietary protein in a heart-healthy diet as a strategy to reduce SBP in normotensive individuals. Thus, increasing total dietary protein (or decreasing dietary carbohydrate) in combination with a diet rich in fruits, vegetables, fibre and low-fat dairy appears to play an important role in reducing SBP.

We had previously shown[8] that DASH, BOLD and BOLD + each lowered cholesterol similarly compared with HAD and that these diets had no effect on fasting glucose and insulin levels. Thus, it is unlikely that the differential effects of DASH, BOLD and BOLD + on vascular status are mediated by changes in lipids, glucose or insulin.

Further controlled clinical trials are needed to elucidate the role and mechanism(s) of action of both protein sources and quantity on BP and vascular health in normotensive and hypertensive individuals.

What is known about this topic

- The DASH dietary pattern has been shown to reduce blood pressure and other risk factors for cardiovascular disease.
- A higher protein version of the DASH diet has been shown to elicit reductions in blood pressure in hypertensive individuals.
- Epidemiologic studies suggest that plant protein may be an important effector of blood pressure change.

What this study adds

- A DASH-like diet containing lean beef can improve vascular elasticity in individuals where age is not a risk factor for cardiovascular disease.
- A DASH-like diet containing lean beef reduces systolic blood pressure in normotensive individuals.
- These results suggest that total protein and not type of protein is important for eliciting reductions in systolic blood pressure.

CONFLICT OF INTEREST

PMK-E received travel funds and an honorarium from the Beef Checkoff Program for giving presentations on this research. MAR received travel funds and honoraria from the Beef Checkoff Program for giving presentations on this research. PMK-E, SGW and JPVH received grant funds from the Beef Checkoff Program. The other authors declare no conflict of interest.

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DISCLAIMER

Scientists affiliated with the Beef Checkoff Program did not have any input in the study design and implementation, as well as analysis and interpretation of the data nor were they involved in writing the manuscript.

AUTHOR CONTRIBUTIONS

PMK-E, JPVH, SGW, JSU and PJG designed the research; MAR and AMH conducted the research; MAR and TLG performed the statistical analyses; and MAR, AMH, SGW, JPVH, PJG and PMK-E wrote the manuscript. MAR and PMK-E had primary responsibility for final content. All authors have read and approved the final manuscript.

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BEEF IS AS EFFECTIVE AS CHICKEN AS PART OF A HEALTHY DIET TO MANAGE CHOLESTEROL

A meta-analysis of randomized controlled trials that compare the lipid effects of beef versus poultry and/or fish consumption.
Maki et al. Journal of Clinical Lipidology, 2012

Objective

Evaluate the effects of beef, independent of other red and processed meats, compared with poultry and/or fish consumption, on lipoprotein lipids.

Study Design and Setting

A meta-analysis of randomized, controlled, clinical trials (RCTs). RCTs published from 1950 to 2010 were considered for inclusion. Studies were included if they reported fasting lipoprotein lipid changes after beef and poultry/fish consumption by subjects free of chronic disease. A total of 124 RCTs were identified, and 8 studies involving 406 subjects met the pre-specified entry criteria and were included in the analysis.

Results

Relative to the baseline diet, mean ± standard error changes (in mg/dL) after beef versus poultry/fish consumption, respectively, were:

- -8.1 ± 2.8 vs. -6.2 ± 3.1 for total cholesterol (P = .630)
- -8.2 ± 4.2 vs. -8.9 ± 4.4 for low-density lipoprotein cholesterol (P = .905)
- -2.3 ± 1.0 vs. -1.9 ± 0.8 for high-density lipoprotein cholesterol (P = .762)
- -8.1 ± 3.6 vs. -12.9 ± 4.0 mg/dL for triacylglycerols (P = .367)

CONCLUSIONS

- Changes in the fasting lipid profile were not significantly different with beef consumption compared with those with poultry and/or fish consumption.
- Inclusion of lean beef in the diet increases the variety of available food choices, which may improve long-term adherence with dietary recommendations for lipid management.

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A meta-analysis of randomized controlled trials that compare the lipid effects of beef versus poultry and/or fish consumption

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KEYWORDS:
Beef;
Coronary heart disease;
Lipids;
Meta-analysis

BACKGROUND: Limited consumption of red meat, including beef, is one of many often-suggested strategies to reduce the risk of coronary heart disease (CHD). However, the role that beef consumption specifically plays in promoting adverse changes in the cardiovascular risk factor profile is unclear.

OBJECTIVE: A meta-analysis of randomized, controlled, clinical trials (RCTs) was conducted to evaluate the effects of beef, independent of other red and processed meats, compared with poultry and/or fish consumption, on lipoprotein lipids.

METHODS: RCTs published from 1950 to 2010 were considered for inclusion. Studies were included if they reported fasting lipoprotein lipid changes after beef and poultry/fish consumption by subjects free of chronic disease. A total of 124 RCTs were identified, and 8 studies involving 406 subjects met the prespecified entry criteria and were included in the analysis.

RESULTS: Relative to the baseline diet, mean ± standard error changes (in mg/dL) after beef versus poultry/fish consumption, respectively, were −8.1 ± 2.8 vs. −6.2 ± 3.1 for total cholesterol (P = .630), −8.2 ± 4.2 vs. −8.9 ± 4.4 for low-density lipoprotein cholesterol (P = .905), −2.3 ± 1.0 vs. −1.9 ± 0.8 for high-density lipoprotein cholesterol (P = .762), and −8.1 ± 3.6 vs. −12.9 ± 4.0 mg/dL for triacylglycerols (P = .367).

CONCLUSION: Changes in the fasting lipid profile were not significantly different with beef consumption compared with those with poultry and/or fish consumption. Inclusion of lean beef in the diet increases the variety of available food choices, which may improve long-term adherence with dietary recommendations for lipid management.

The role of red meat consumption in promoting adverse changes in the cardiovascular risk factor profile is unclear. Red meat (i.e., fresh beef, lamb, pork, veal) represents a significant dietary source of saturated fatty acids (SFA) and cholesterol, which have been shown in controlled feeding studies to increase serum cholesterol concentrations.[1,2] However, results from observational studies have not universally supported an association between red meat intake and the risk of coronary heart disease (CHD).[3,4] Beef is the most prevalent source of red meat in the U.S. diet (66%), but few epidemiologic studies have investigated

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Table 1 Design and subject characteristics of randomized controlled trials of beef consumption

Author, year	Design	Background diet	Type of beef consumed (quantity per day)*,‡,§	Comparator food (quantity per day)*,‡,§	Duration (weeks)		Baseline cholesterol†	Sex (n)	Age (mean ± SD or range, yr)
					Treatment	Washout			
Beauchesne-Rondeau et al 2003[17]	Crossover	AHA diet (2000)	Lean (380 g)	Lean chicken and ground turkey (405 g) or lean fish (495 g)	3.7	6	High	Males (17)	50 ± 13
Flynn et al 1981[18]	Crossover	Self-selected	Higher fat (20%) (minimum 140 g/d)	Lean chicken and turkey, and lean fish (minimum 140 g/d)	12	0	Borderline high	Males (74); Females (55)	23–70
Flynn et al 1982[19]	Crossover	Self-selected	Higher fat (20%) (minimum 140 g/d)	Lean chicken and turkey, and lean fish (minimum 140 g/d)	12	6 (after 2nd dietary treatment)	Borderline high	Males (47); Females (29)	32–62
Leaf and Hatcher 2009[20]	Crossover	25% fat or 40% fat	Higher fat (15%) (~276 g)	Lean, omega-3 fatty acid rich fish (~276 g)	12	0	Borderline high	Males (6); Females (4)	46 ± 9
Mahon et al 2007[21]	Parallel	Energy-restricted diet (prescribed low fat)	Lean (250 kcal of beef)	Lean chicken (250 kcal of chicken)	9	na	Borderline high	Females (54)	58 ± 2
Melanson et al 2003[22]	Parallel	Energy-restricted diet (prescribed low fat)	Lean (nr/~20% total kcal as protein)	Lean chicken (nr/~20% total kcal as protein)	12	na	Near/ above optimal	Females (61)	43 ± 8
Scott et al 1991[23]	Parallel	AHA step 1	Lean (226 g)	Lean chicken (113 g) and lean fish (113 g)	4	na	Borderline high	Males (46)	25–55
Scott et al 1994[24]	Parallel	NCEP/AHA step 1	Lean (85 g)	Lean chicken (85 g)	5	na	High	Males (38)	20–55

AHA, American Heart Association; NCEP, National Cholesterol Education Program; LDL-C, low-density lipoprotein cholesterol; na, not applicable; nr, not reported; TC, total cholesterol.

*Total amount of protein consumed per day identical between dietary treatments.

†Subjects were classified according to National Cholesterol Education Program Adult Treatment Panel III Guidelines based on the mean baseline values for TC and/or LDL-C.

‡Estimates based on food menus.

§"Lean" descriptor based on USDA food labeling guidelines, "Seafood or game meat products that contain less than 10 g total fat, 4.5 g or less saturated fat, and less than 95 mg cholesterol per reference amount customarily consumed per eating occasion and per 100 g."

Table 2 Beef vs poultry and/or fish: differences in mean responses, overall and by subgroup

	Number of studies (treatment conditions)	Weighted average difference (mg/dL) beef–poultry/fish	95% confidence interval for weighted average difference
Total cholesterol	8 (14)	−2.5	−5.6, 0.5
Excluding Leaf and Hatcher 2009*	7 (13)	−3.2	−6.3, −0.2
Sex			
Men	5 (8)	−1.8	−6.3, 2.7
Women	4 (6)	−5.0	−7.3, −2.6
Type of beef			
Lean	5 (5)	1.71	−2.08, 5.51
Other (not specified as lean)	3 (3)	−5.48	−7.92, −3.04
Length of study			
<9 weeks test period	4 (4)	4.8	1.2, 8.4
≥9 weeks test period	4 (10)	−5.4	−6.9, −4.0
Study design			
Crossover design	4 (10)	−4.9	−7.3, −2.5
Parallel design	4 (4)	2.1	−2.5, 6.6
LDL-C	6 (6)	1.8	−1.0, 4.5
Excluding Leaf and Hatcher 2009*	5 (5)	2.3	−0.3, 4.9
Sex			
Men	3 (3)	1.8	−1.6, 5.2
Women	2 (2)	3.1	−1.9, 8.1
Type of beef			
Lean	5 (5)	2.33	−2.74, 4.94
Other (not specified as lean)	1		
Length of study			
<9 weeks test period	4 (4)	1.0	−2.4, 4.5
≥9 weeks test period	2 (2)	3.1	−1.9, 8.1
Study design			
Crossover design	2 (2)	−0.2	−9.7, 9.4
Parallel design	4 (4)	2.0	−1.2, 5.1
HDL-C	8 (14)	0.4	−2.2, 3.0
Excluding Leaf and Hatcher 2009*	7 (13)	0.2	−2.5, 2.9
Sex			
Men	5 (7)	−0.4	−3.7, 2.9
Women	4 (6)	0.9	−4.6, 6.4
Type of beef			
Lean	5 (5)	0.53	−2.09, 3.14
Other (not specified as lean)	3 (3)	0.34	−3.80, 4.48
Length of study			
<9 weeks test period	4 (4)	1.3	−2.1, 4.7
≥9 weeks test period	4 (10)	0.0	−3.6, 3.6
Study design			
Crossover design	4 (10)	0.0	−3.5, 3.5
Parallel design	4 (4)	1.6	0.1, 3.1
TAG	8 (14)	−2.6	−9.9, 4.7
Excluding Leaf and Hatcher 2009*	7 (13)	−3.6	−10.9, 3.7
Sex			
Men	5 (7)	7.4	3.6, 11.2
Women	4 (6)	−17.2	−27.8, −6.7
Type of beef			
Lean	5 (5)	4.62	−2.56, 11.79
Other (not specified as lean)	3 (3)	−6.64	−17.85, 4.56
Length of study			
<9 weeks test period	4 (4)	10.0	4.5, 15.5
≥9 weeks test period	4 (10)	−7.8	−17.5, 1.8

(*continued on next page*)

Table 2 (*continued*)

	Number of studies (treatment conditions)	Weighted average difference (mg/dL) beef–poultry/fish	95% confidence interval for weighted average difference
Study design			
Crossover design	4 (10)	−5.0	−14.9, 4.9
Parallel design	4 (4)	3.2	−6.5, 13.0

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TAG, triacylglycerol.
*Subanalyses excluding the Leaf and Hatcher[20] data were conducted to identify whether the use of a cholesterol-free lead-in diet and the use of a fish comparator high in long-chain omega-3 fatty acids materially altered the overall results of the meta-analysis.

specifically the relationship between risk of CHD and beef consumption.[4,5]

The lipid effects of specific foods, such as beef, on CHD risk cannot be easily predicted on the basis of total SFA content because individual SFAs differ in their effects on the blood lipid profile.[6–8] Approximately one-third of the SFAs in beef is stearic acid, which does not increase blood cholesterol concentrations.[9] Furthermore, results from a recent meta-analysis suggest that dietary SFA intake is not clearly associated with increased risk of CHD.[10]

The U.S. Dietary Guidelines for Americans, National Cholesterol Education Program guidelines, and other dietary recommendations for heart healthy eating from national professional organizations encourage Americans to limit their intake of meat.[11,12] This recommendation is based in part on prospective cohort studies, which implicate that a "Western" diet, characterized by greater intakes of red meats, processed meats, high-fat dairy products, refined grains, and sweets, is a significant contributor to a greater risk for CHD compared with a dietary pattern high in nuts, fruits, vegetables, legumes, fish, poultry, and whole grains.[13,14]

Given that approximately 50% of beef's fatty acids are monounsaturated, and approximately 30% of the SFA content in beef is stearic acid, both of which have neutral-to-favorable effects on atherogenic blood lipid concentrations,[15,16] we hypothesized that beef consumption would not contribute to an increase in atherogenic lipoprotein lipids compared with consumption of poultry and/or fish. Thus, for the purpose of this review and analysis, we identified peer-reviewed RCTs that investigated the effects of beef, independent of other red or processed meats, as compared to poultry or fish, on the fasting lipoprotein lipid profile in healthy individuals or those with chronic disease risk factors. A meta-analysis of these RCTs was conducted to evaluate the effects of beef consumption compared with poultry and/or fish consumption on fasting lipoprotein lipid concentrations.

Methods

Search strategy

Randomized controlled trials published between 1950 and 2010 were identified through a search of the PubMed, OVID, Embase, and Agricola databases and the Cochrane library. Bibliographies of relevant publications were also searched. The following keywords and search terms were used: beef, red meat, cow/cattle, and high-protein diet. The following Medical Subject Headings were also used: cardiovascular disease, meat, cattle, and cattle diseases.

Included studies met all of the following criteria:

- beef-containing diets were evaluated in comparison to poultry and/or fish-containing diets;
- peer-reviewed RCTs available in English and published since 1950;
- provided lipoprotein lipid data (at least total cholesterol [TC] and high-density lipoprotein cholesterol [HDL-C]);
- were conducted in healthy adult subjects, or in adults with chronic disease risk factors such as body mass index $\geq$25 kg/m^2, TC $\geq$200 mg/dL, or low-density lipoprotein cholesterol (LDL-C) $\geq$130 mg/dL, but otherwise free of chronic disease; and
- beef consumed was whole, conventionally raised beef, that is, not isolated beef tallow or beef from exclusively grass-fed animals.

Studies were excluded if:

- results specific for beef, independent of other red meat (veal, pork, lamb) or processed meats (cold cuts, bacon, sausage, etc.), were not provided;
- cattle were not grain-finished/conventionally raised (eg, were grass-fed); or
- the beef consumed was modified/experimentally prepared and therefore not representative of that commercially available.

Two levels of study screening were used. Level 1 screening was performed on abstracts and citations downloaded from the literature searches noted previously. At Level 1 screening, any study with one or more definite exclusion criteria was rejected. If an abstract was not available or if an abstract was not sufficient to determine eligibility, the full paper was retrieved for review. In Level 2 screening, accepted abstracts had full papers retrieved and were reviewed for meeting all of the prespecified criteria as described.

A total of 113 abstracts were identified from the electronic search; additional publications were identified from relevant bibliographies yielding a total of 124

publications. The primary reason for exclusion from the evidence base was failure to examine the lipid effects of beef independent of other red or processed meats.

Data extraction and statistical analyses

Qualitative information (eg, baseline diet description, study population characteristics) and quantitative data (eg, pre- and post-intervention cholesterol values) were extracted from each study that met the criteria for inclusion. Cholesterol data provided in SI format were converted to mg/dL by multiplying by 38.7 and triacylglycerol (TAG) data were converted by the use of a factor of 88.5. All extracted data were validated independently by two reviewers.

Random effects models were used to generate weighted averages and 95% confidence intervals within dietary condition (ie, beef and poultry/fish) and for changes from baseline in lipid variables. Two-sided P-values $< .05$ were considered statistically significant. Heterogeneity in statistical models was assessed using Cochran's Q. I^2 statistics were calculated to estimate the percentage of variation attributable to heterogeneity across studies. Subanalyses were planned *a priori* and were performed on the following variables: sex, study duration, design (crossover vs parallel), and background diet (habitual vs therapeutic). These analyses were conducted to examine relevant patterns of associations by study factor and to identify potential sources of heterogeneity. In addition, *post-hoc* sensitivity analyses were conducted to assess the influence of beef type (lean vs other, ie, not specified as lean) and to evaluate the influence of excluding one study due to an atypical design. This trial used fish high in long-chain omega-3 fatty acids as the comparator to the beef treatment and utilized a cholesterol-free lead-in diet. The possible presence of publication bias in the primary models was assessed visually by examining a funnel plot measuring the standard error as a function of effect size, as well as performing Egger's regression method and the Duval and Tweedie imputation method. Comprehensive Meta-Analysis software version 2 (Englewood, NJ) was used for the analyses.

Results

Study characteristics

A group of 8 randomized controlled trials utilizing crossover (n = 4)[17–20] or parallel (n = 4)[21–24] designs and including a total of 406 individuals was included in the meta-analysis (Table 1). The sample sizes of the 8 studies ranged from 10 to 129 participants with mean ages between 20 and 73 years. In the majority of studies (n = 5), subjects at baseline were described as borderline hypercholesterolemic (TC = 200-239 mg/dL; LDL-C = 130-159 mg/dL)[18–21,23]; in one study the subjects were described as having optimal or near-optimal lipid values.[22] The treatment diets included 30–226 g/d of beef, poultry, and/or fish. Baseline diets included self-selected (n = 2),[18,19] American Heart Association (n = 3),[17,23,24] or other customized dietary plans (n = 3).[20–22] Five of the 8 studies reported the use of lean beef as defined by USDA food labeling guidelines as seafood or game meat products that contain less than 10 g total fat, 4.5 g or less saturated fat, and less than 95 mg cholesterol per reference amount customarily consumed per eating occasion and per 100 g,[17,21–25] and the length of dietary treatment ranged from 3.7 to 12 weeks.

Changes in lipoprotein lipids

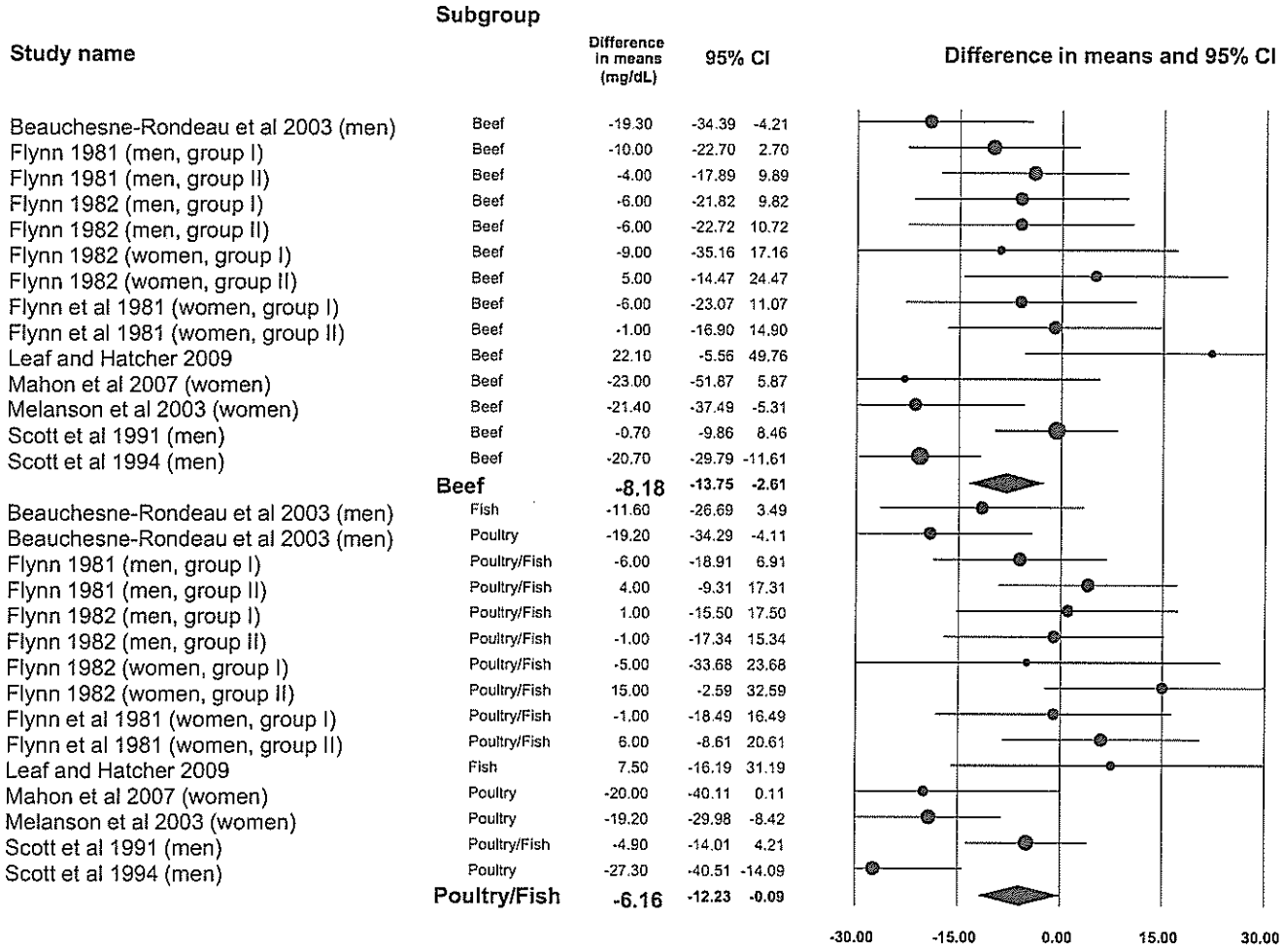
The beef and poultry and/or fish dietary treatments produced weighted mean reductions from baseline in TC, LDL-C, HDL-C, and TAG (Table 2, Figs. 1–4). Mean ± SEM changes (in mg/dL) following beef versus poultry/fish consumption, respectively, were −8.2 ± 2.8 versus −6.2 ± 3.1 for TC ($P = .630$, beef vs poultry/fish), −8.2 ± 4.2 vs −8.9 ± 4.4 for LDL-C ($P = .905$, beef vs poultry/fish), −2.3 ± 1.0 vs −1.9 ± 0.8 for HDL-C ($P = .762$, beef vs poultry/fish), and −8.1 ± 3.6 vs. −12.9 ± 4.0 for TAG ($P = .367$, beef vs poultry/fish; Figs. 1–4).

On-treatment comparisons were also calculated but were not significantly different from each other. The mean and 95% confidence interval (95% CI) values for the weighted averages of the individual study differences (beef minus poultry/fish) in mg/dL were −2.5 (−5.6, 0.5); $P = .107$ for TC; 1.8 (−1.0, 4.5); $P = .206$ for LDL-C; 0.4 (−2.2, 3.0); $P = .765$ for HDL-C; and −2.6 (−9.9, 4.7); $P = .481$ for TAG.

Statistically significant heterogeneity (i.e., $P < .10$) was observed in all within-group models except for beef and TAG ($P = .157$; Fig. 4). In addition, significant heterogeneity was present in models comparing on-treatment differences between beef and poultry/fish. Subgroup analyses revealed that participant sex, study duration, beef type (lean vs other), and study design were factors that may have contributed to the heterogeneity in lipid responses. These results, however, were inconclusive because of the small numbers of studies (Table 2). In the subgroup analyses, greater TC and TAG decreases were observed with the beef treatment in women versus men, and greater TC decreases were observed with the beef treatment in studies with a treatment duration ≥9 weeks, and studies using a crossover design. The number of available studies was insufficient to conduct a subanalysis based on type of background diet.

Despite the uncertainty about the precise fat content in the beef provided in some of the studies, a subset analysis for lean versus other (not specified as lean) beef was conducted. Studies using higher fat beef showed larger reductions in total cholesterol compared to poultry/fish (Table 2).

The non-beef treatment in the Leaf and Hatcher[20] study included oily fish high in long-chain omega-3 fatty acids



Weighted average of individual study differences (beef vs. poultry/fish): -2.5 mg/dL (-5.6, 0.5 mg/dL); $p = 0.107$

Figure 1 The dietary treatment effects on TC from each study included in the meta-analysis are shown in mg/dL. The circle sizes are proportional to the weights used in the meta-analysis, and the lines indicate 95% CIs. The diamond represents the summary measure (mean and 95% CI) for the lipoprotein lipid impacts of beef or poultry/fish consumption. I^2 (beef) = 44.0%, P = .039; I^2 (poultry and/or fish) = 58.5%, P = .002.

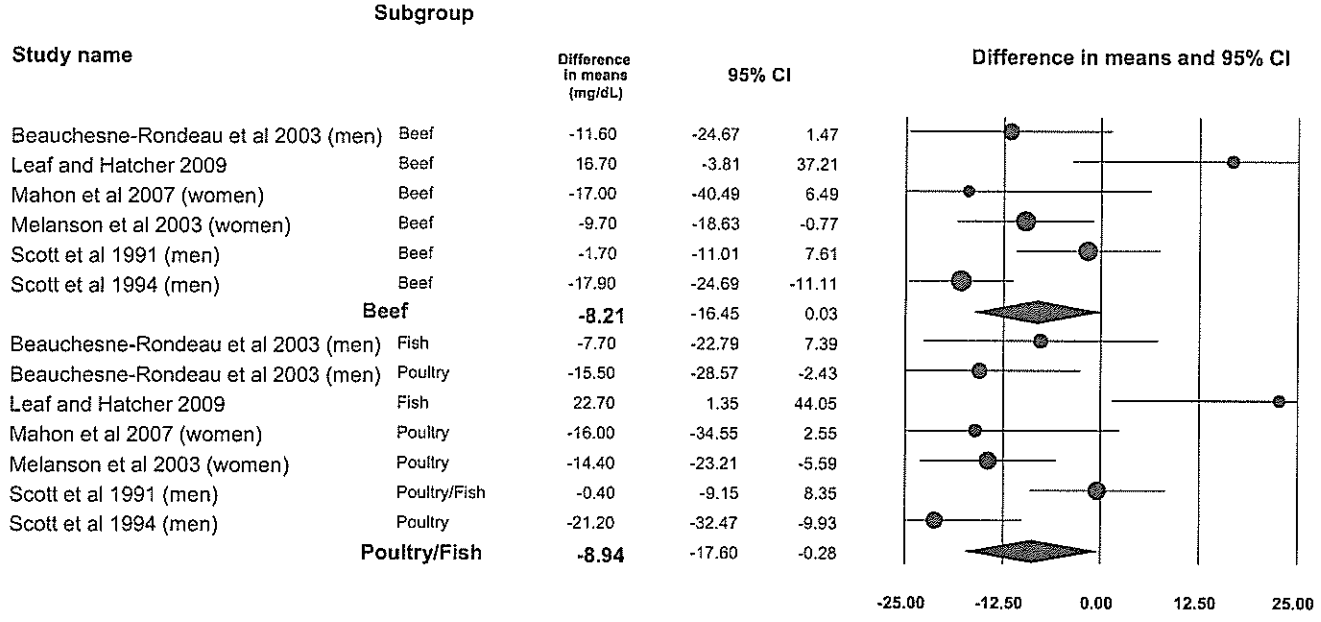
(estimated intake of 2.5 g/d) and used a cholesterol-free lead-in diet; therefore, a sensitivity analysis was conducted excluding this trial to assess the degree to which it may have influenced the results. This sensitivity analysis demonstrated that beef consumption was associated with significantly ($P < .05$) greater reductions in TC relative to fish and/or poultry in the remaining trials, whereas the statistical significance of the results for other lipoprotein lipids were unchanged compared with the primary analysis (Table 2).

Publication bias

Funnel plots for the meta-analysis models did not generally suggest evidence of publication bias (data not shown). The only model that suggested slight publication bias was beef and TAG, however, when data groups for the Flynn et al studies were combined,[18,19] the suggestion of potential publication bias disappeared.

Discussion

Results of the present meta-analysis of eight randomized controlled trials suggest that the impact of beef consumption on the lipoprotein lipid profile of humans is similar to that of fish and/or poultry. The finding that beef intake did not raise atherogenic lipoprotein lipid concentrations relative to poultry and/or fish was not surprising in light of the fatty acid profile of beef. Approximately 50% of the fatty acids in conventional (grain-finished) beef are monounsaturated fatty acids, primarily oleic acid.[26] Data from controlled feeding trials indicate that oleic acid has a modest cholesterol-lowering effect compared with saturated fatty acids or carbohydrate.[16,27] Approximately 45% of the fatty acids in beef are saturated fatty acids. However, roughly 30% of these are stearic acid.[26] Approximately 19% of stearic acid is converted to oleic acid *in vivo* and stearic acid has effects on total and LDL-C similar to those of oleic acid.[28,29]



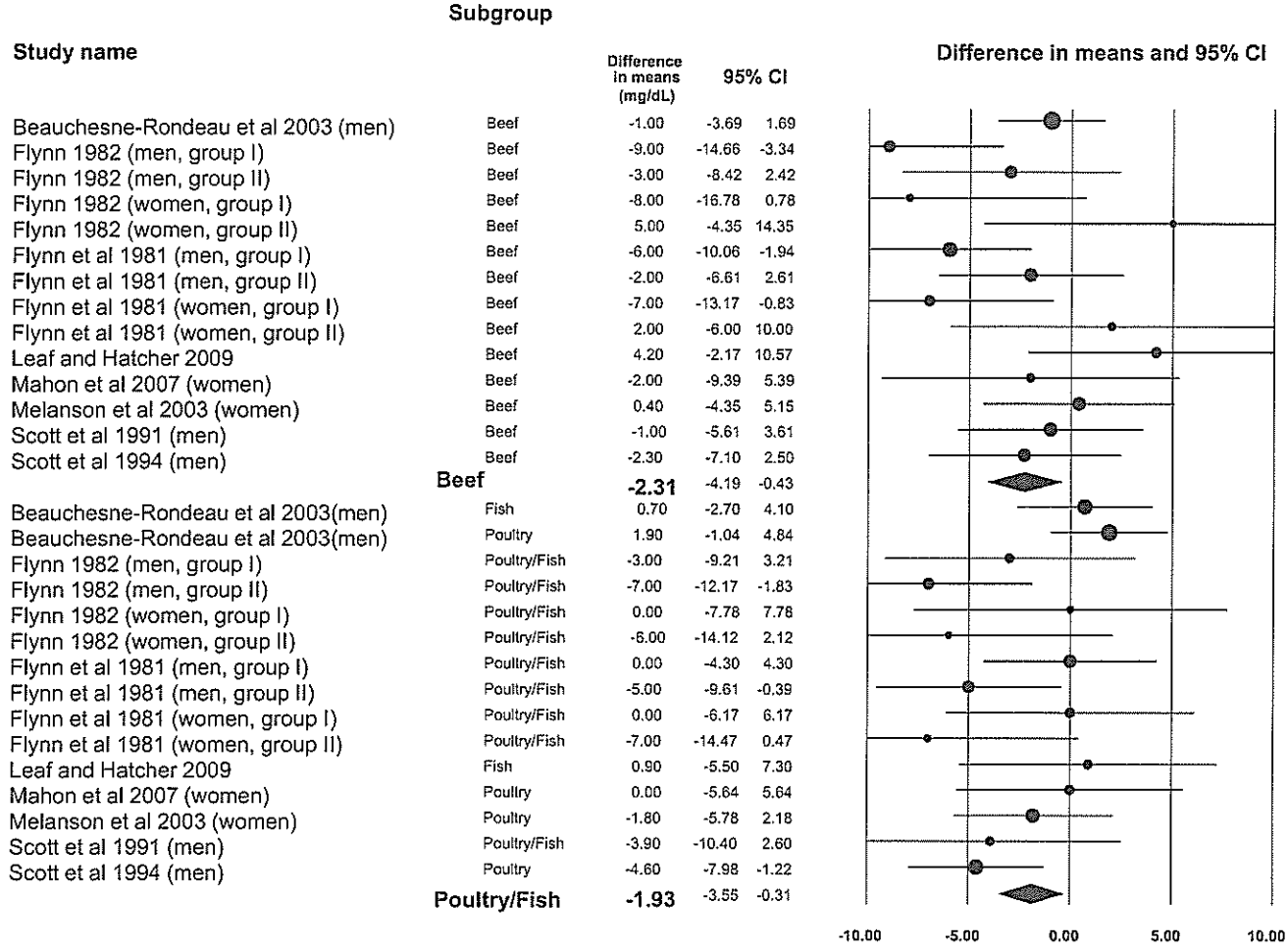
Weighted average of individual study differences (beef vs. poultry/fish): 1.8 mg/dL (-1.0, 4.5 mg/dL); $p = 0.206$

Figure 2 The dietary treatment effects on LDL-C from each study included in the meta-analysis are shown in mg/dL. The circle sizes are proportional to the weights used in the meta-analysis, and the lines indicate 95% CIs. The diamond represents the summary measure (mean and 95% CI) for the lipoprotein lipid impacts of beef or poultry/fish consumption. I^2 (beef) = 42.0%, $P = .01$; I^2 (poultry and/or fish) = 36.9%, $P = .003$.

Data from a recent analysis of U.S. National Health and Nutrition Examination Survey (NHANES) food survey data indicate that among adults (19−50 years), mean daily beef consumption equates to 49.3 g (1.76 ounces).[30] The amount of beef in five of the eight studies analyzed in this meta-analysis exceeded average amounts consumed on a daily basis in the United States. Despite this relatively high level of beef consumption in a majority of the studies, the changes in lipoprotein lipid profile were neither detrimental (TC and LDL-C were slightly reduced) nor significantly different from changes observed following similar intakes of poultry and/or fish. These findings are consistent with those from studies demonstrating that protein as a substitute for carbohydrate in the diet can result in favorable lipoprotein lipid changes.[31,32]

Results from this meta-analysis are in alignment with those from previously published cohort studies and clinical trials of red meat, independent of processed meat. Wagemakers et al[33] evaluated the atherogenic lipid profile over a 10-year period in a British cohort of 517 men and 635 women. Red meat intake (beef, lamb, pork veal, and mutton) ranged from 0 to 224 g/d in men and 0 to 231 g/d in women. No significant differences in CHD risk markers, including TC, LDL-C, and HDL-C concentrations; systolic or diastolic blood pressure; body mass index; or waist circumference, were noted among the study participants consuming lower vs. higher intakes of red meat per day. Similarly, in a long-term randomized study of 191 hypercholesterolemic men and women, those who consumed 80% of their meat in the form of either unprocessed lean red meat (lean beef, veal, or pork) or unprocessed lean white meat (poultry or fish) had nearly identical mean concentrations of TC and LDL-C after 36 weeks.[34] These results were confirmed when the subjects in the two groups crossed over to the opposite condition for an additional 36 weeks.[35] There were also no significant differences in TAG or HDL-C concentrations observed between the two conditions.[34,35]

In a recent meta-analysis by Micha et al[3] that included 1,218,380 individuals, and 23,889 CHD, 2280 stroke, and 10,797 diabetes mellitus cases, the authors observed no association between unprocessed red meat (beef, hamburgers, lamb, pork, or game) intake and risk for CHD (relative risk [RR] = 1.0 per 100 g serving per day; 95% CI, 0.81−1.23), stroke, or diabetes. They also evaluated processed meat consumption (bacon, salami, sausages, hot dogs, processed deli or luncheon meats including some poultry), which was associated with a 42% greater risk of CHD (RR = 1.42 per 50 g serving per day; 95% CI, 1.07−1.89) and 19% greater risk of diabetes mellitus (RR = 1.19 per 50 g serving per day; 95% CI, 1.11−1.27). In contrast, Bernstein et al[4] assessed the associations between protein sources and CHD risk in the Nurses' Health Study and found that red meat consumption was associated with a modest but significant increase in CHD risk (RR = 1.16 per serving per day; 95% CI, 1.09−1.23) in multivariate analysis, and this relationship persisted when red meat intake, excluding processed meat consumption, was evaluated (RR = 1.19 per serving per day; 95% CI, 1.07−1.32).

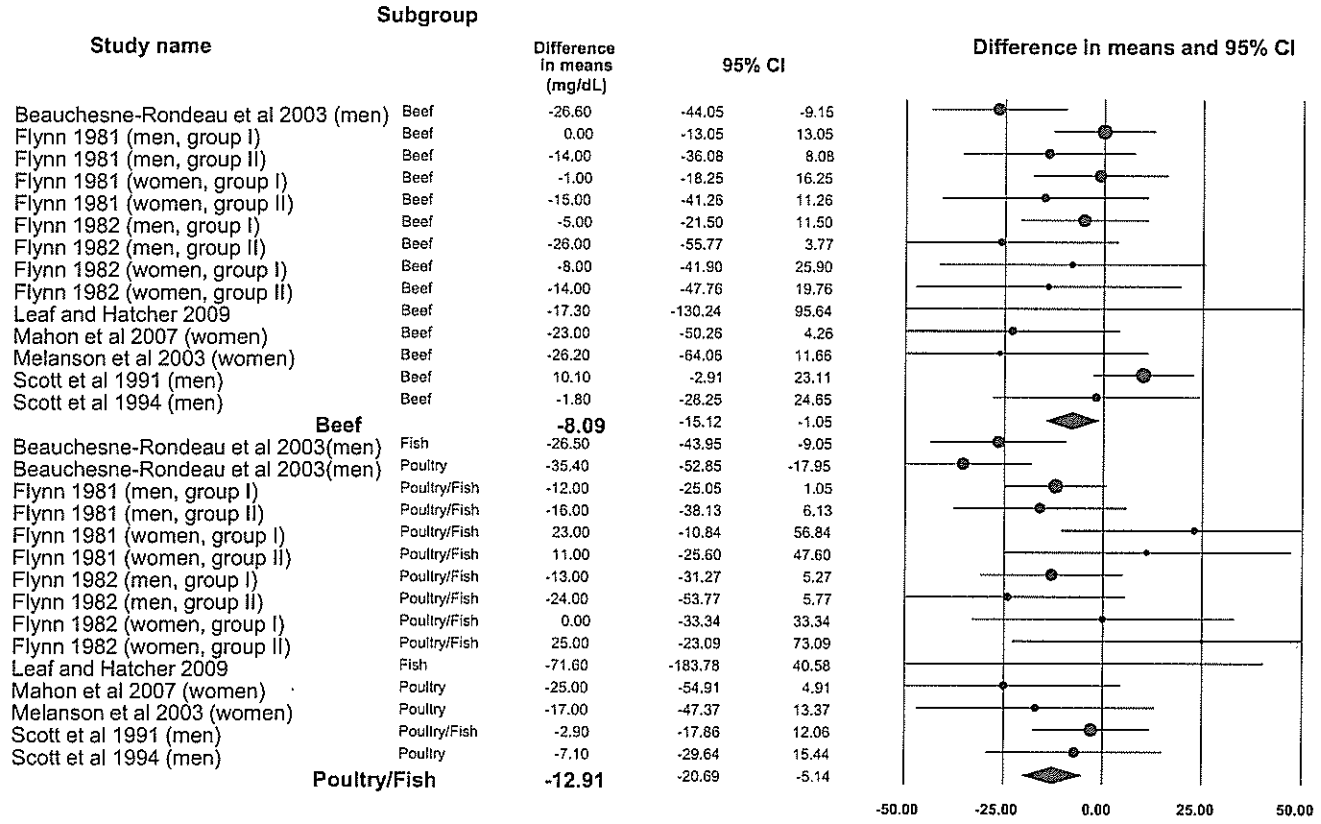


Weighted average of individual study differences (beef vs. poultry/fish): 0.4 mg/dL (-2.2, 3.0 mg/dL); $p = 0.765$

Figure 3 The dietary treatment effects on HDL-C from each study included in the meta-analysis are shown in mg/dL. The circle sizes are proportional to the weights used in the meta-analysis, and the lines indicate 95% CIs. The diamond represents the summary measure (mean and 95% CI) for the lipoprotein lipid impacts of beef or poultry/fish consumption. I^2 (beef) = 66.9%, $P = .05$; I^2 (poultry and/or fish) = 69.3%, $P = .075$.

Not all studies have evaluated dietary intakes of processed and unprocessed meats separately,[36] which may confound results from some epidemiological studies, and limit the ability to generalize findings to beef. Furthermore, both red and processed meat consumption correlate with other dietary and behavioral factors such as lower fiber intake, greater smoking prevalence and lower physical activity, all of which may contribute to increased CHD risk. Thus, the potential for confounding (and residual confounding) of the relationship between red meat intake and CHD risk by other unmeasured (and imperfectly measured) factors cannot be excluded. Examination of the impact of individual foods, including red meat (and beef specifically), on the risk of major disease in the context of a healthy eating pattern has been identified as a priority area for future research,[6] and would further clarify the nature of the relationship between red meat and/or beef and CHD risk.

Understanding the relationship between beef consumption and the lipid profile is important for those involved in counseling patients on diets for the management of dyslipidemia. A greater range of food choices is likely to improve the acceptability of such diets to patients.[37] Results from a subset analysis comparing the effects of lean versus other beef (not specified as lean) did not suggest a more favorable effect of lean beef on the lipid profile. In fact, there was a trend toward greater total and LDL-C reduction with the beef not specified as lean. However, because of the small number of studies that used lean beef in the present analysis, caution is warranted in the interpretation of these findings. Recommendations from health authorities, including those from the National Cholesterol Education Program and the Dietary Guidelines for Americans emphasize selection of lean cuts of meat.[11,12] The widespread availability of lean beef may provide additional flexibility for individuals following saturated fat-restricted diets and the results of the present analysis suggest that consumption of lean beef or poultry/fish results in comparable levels of blood lipids.



Weighted average of individual study differences (beef vs. poultry/fish): -2.6 mg/dL (-9.9, 4.7 mg/dL); $p = 0.481$

Figure 4 The dietary treatment effects on TAG from each study included in the meta-analysis are shown in mg/dL. The circle sizes are proportional to the weights used in the meta-analysis, and the lines indicate 95% CIs. The diamond represents the summary measure (mean and 95% CI) for the lipoprotein lipid impacts of beef or poultry/fish consumption. I^2 (beef) = 27.9%, $P = .157$; I^2 (poultry and/or fish) = 36.2%, $P = .08$.

Some limitations of the current meta-analysis should be noted. Importantly, relatively few studies were available that evaluated the lipid effects of beef alone, which limited the number of meaningful subset analyses that could be conducted. Further investigation of the effects of lean versus non-lean beef consumption, as well as possible interactions with other dietary factors deserve additional investigation. Another limitation is that the results from the individual studies showed significant heterogeneity for TC, HDL-C, LDL-C, and TAG. Taking into consideration the differences between the trials in clinical and design components, this is not unexpected. Despite the small number of studies and the heterogeneity of results among trials, the results from the present analysis are consistent with findings from studies that have compared the lipid effects of a mix of red meat types with poultry or poultry and fish.[34,35,38] Nevertheless, differences in surrogate markers, including total and LDL-C concentrations, have not always been associated with the expected differences in atherosclerosis development or CHD event risk.[39–41] Thus, data from dietary intervention studies on surrogate markers for CHD risk must be interpreted in light of available evidence from other sources.[42]

Conclusion

In conclusion, the results from this meta-analysis suggest that the impact of beef consumption on the lipoprotein lipid profile of humans is similar to that of fish and/or poultry. These findings add to the evidence suggesting that moderate consumption of lean beef, as part of a balanced diet, may be considered when recommending diets for the management of blood lipids.

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Beef Association. Dr. McNeill is an employee of National Cattlemen's Beef Association.

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TODAY'S BEEF IS MUCH LEANER AND LOWER IN SATURATED FAT THAN 30 YEARS AGO

The evolution of lean beef: Identifying lean beef in today's U.S. marketplace
McNeill et al. Meat Science, 2012

INTRODUCTION

While lean beef has always been a popular, nutrient-rich source of high-quality protein that can help Americans meet their nutrient needs, today's beef is leaner than ever.

Lean beef is more widely available in the U.S. today because of many changes during the past 40 years in cattle breeding and management practices and retail trimming, many of which were driven by changing dietary recommendations and consumer preferences.

BEEF PRODUCTION PRACTICES

Changes in cattle breeding and fat trimming methods have resulted in increased availability of leaner beef. Today, more than two-thirds (69%) of beef sold at retail, including popular cuts like Sirloin steak, Tenderloin and 95% lean Ground Beef, meet the government guidelines for lean.

Less than 10% of saturated fat and total fat in the diet comes from beef, and the total and saturated fat content from trimmed steak has declined throughout the past 50 years. For example, the total fat content for a completely trimmed Sirloin steak has declined 34% from 1963 to 2015, and the saturated fat content has declined 17% between 1990 and 2010.

CHANGES IN CONSUMER PREFERENCES

The public is aware of longstanding current nutrition recommendations advising them to "go lean with protein" and they recognize this can be accomplished by choosing lean meats and "trimming excess fat off meats". To ensure a beef supply that meets consumer expectations for leaner cuts, there has been an 80% decrease in external fat on retail beef cuts throughout the past 26 years.

IMPACT ON HEART HEALTH

Research shows lean beef can be good for heart health. Evidence from clinical trials indicates that lean beef is equally as effective as lean white meat at lowering LDL cholesterol when included as part of a well-balanced, low-saturated fat diet.

Contributing to its heart-health benefits, half of the fatty acids in beef are monounsaturated (the same type of heart-healthy fat found in olive oil) and nearly one-third of the saturated fat is stearic acid, a fatty acid that has been shown to have neutral effects on cholesterol.

CONCLUSIONS

Beef is a popular, nutrient-dense food and the availability of at least 29 lean cuts of beef in the U.S. marketplace can help consumers meet their cardiovascular health goals.

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Review

The evolution of lean beef: Identifying lean beef in today's U.S. marketplace

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ABSTRACT

Changes in cattle breeding and management coupled with extensive trimming of visible fat from retail cuts have resulted in the wide-spread availability of lean beef to U.S. consumers. Despite these changes, there is limited awareness regarding the reduced total fat content and the favorable fatty acid profile of beef. Relative to the calories it contributes, the impact of beef on the nutritional quality of the American diet via its contribution of protein and certain key micronutrients is often under appreciated. The following discussion documents the progressive reduction in fat content of U.S. beef during the past 30 years, highlights ongoing efforts to update United States Department of Agriculture nutrient data for beef, and summarizes findings from randomized controlled trials of beef and plasma lipid outcomes. Beef is a popular, nutrient-dense food and the availability of at least 29 lean cuts of beef in the U.S. marketplace can help consumers meet their cardiovascular health goals.

Contents

1. Introduction

For more than three decades, beginning with the 1977 Dietary Goals for the United States, government-issued dietary guidance has emphasized the need for Americans to decrease their intake of total fat, saturated fat, and cholesterol while increasing the amount of polyunsaturated fat and, more recently, monounsaturated fatty acids (Dietary Guidelines Advisory Committee, 2010; U.S. Department of Agriculture and U.S. Department of Health and Human Services, 2010). Taste is likely the most common reason that Americans consume beef, but total and saturated fat content of beef may be among the reasons Americans choose to eat less beef in their diet. Recent U.S. survey data indicate that 63% of consumers are trying to consume less animal fat (International Food Information Council Foundation, 2009), and 41% of consumers are estimated to have decreased their consumption of beef between 2002 and 2008

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(American Dietetic Association, 2008). Another consumer survey of 2000 U.S. adults found that 53% cited red meat as being the "least healthy" protein among red meat, chicken/poultry, fish/seafood or pork (Mintel Oxygen, 2008). Survey findings also suggest a higher proportion of U.S. dietitians regard beef as a greater source of saturated fat than pork, poultry, or dairy products (unpublished data, 2007), even though dairy products are the largest contributor to saturated fat intake in the American diet (Dietary Guidelines Advisory Committee, 2010). In order to help today's consumers make educated dietary decisions, it is important that nutrition professionals have access to the latest evidence from clinical trials and the most up to date nutrient composition data for beef. This review will document the progressive reduction in fat content of U.S. beef during the past 30 years, highlight ongoing efforts to update United States Department of Agriculture (USDA) nutrient reference data for beef, and summarize findings from randomized controlled trials of beef intake and plasma lipid outcomes.

2. Beef production and trimming practices contribute to leaner U.S. beef

The availability of leaner beef in the U.S. is due to a collective effort over three decades throughout the entire U.S. beef production and merchandising chain. A similar experience is reported in the production of red meat in the U.K with just over 20 years of change in animal husbandry resulting in 30% reduced carcass fat for pork, 15% for beef and 10% for lamb (Higgs, 2000). The following discussion will detail changes in breeding and management along with trimming practices of processors, retailers, and food service operators that has led to an estimated 44% reduction in available total fat (from 13% to 7%) and a 29% reduction in saturated fat per capita (from 13% to 9%) contributed by beef as calculated from food disappearance data (Hiza and Bente, 2007).

2.1. Decreased carcass fat through change in U.S. breeding and management

Improvements of per unit production efficiency in a quest for more sustainable profitability along with greater consumer focus have been cattle industry drivers for the past four decades. Beginning in the 1970s and continuing in earnest well until the 1980s, U.S. cattlemen imported a significant number of cattle of various breeds from Europe (Field, 2007). This influx of Continental breeds changed U.S. beef cattle population significantly and, when coupled with other innovations available to the beef industry, resulted in several important outcomes:

1. Fed cattle could be taken to heavier finished weights with improved carcass cutability.
2. Efficiencies of production could be gained from incorporating technologies such as growth promotants that increased lean yield per animal.
3. The availability of high speed computing made national cattle genetic evaluation both possible and practical.
4. Utilization of both additive and non-additive genetic effects via focused selection strategies and planned crossbreeding systems optimized the production of beef that was acceptable in both flavor and leanness.

(Field, 2007)

The characterization of British and Continental breeds of cattle in the Beef Germplasm Evaluation project at the ARS Meat Animal Research Center demonstrates that British breeds excel in producing carcasses with a high percentage of superior USDA quality grades (enhanced palatability) while Continental breeds provide superior cutability (leanness) as compared to British breeds (Table 1). In an effort to take advantage of these unique genetic differences, the mainstream cattle producer created cattle that were approximately half British and half Continental in genetic composition in response to market signals to reduce trimmable fat from the carcass while retaining appropriate levels of marbling (Doherty et al., 1999; Field, 2007).

Genetic evaluation innovations allowed seedstock producers to more precisely focus selection pressure on multiple traits of economic importance while providing their customers herd bulls that were more specifically characterized for their ability to transmit superior genetic merit particularly in regard to the primary carcass value influencing traits of carcass weight, marbling score, ribeye area, and backfat thickness. Seedstock producers of both British and Continental cattle were able to affect the genetic trend within their respective breeds for these traits (Tables 2 and 3). These genetic trends show that breeders were able to increase carcass weight, marbling, and muscularity while reducing or holding constant carcass fat thickness. The interaction of breed and diet also influenced the deposition of individual fatty acid classes. For example, divergent effects on saturated fatty acid deposition in response to annual vs. perennial grass feeding is reported for two common U.S. cattle breeds, Angus and Simmental (Itoh et al., 1999). Successful reduction in total and saturated fat through combined improvements in beef breeding and management practices are evident from the current nutrient data for beef from the USDA National Nutrient Database for Standard Reference. Specifically, the total fat content for a completely trimmed sirloin steak, all grade average, has declined 34% from 1963 to 2010 and the saturated fat content has declined 17% (Watt and Merrill, 1963; USDA, 2010; Fig. 1).

These trends have improved the value of beef carcasses by enhancing both palatability and leanness. Growth enhancement technologies also improve lean yield per head and reduce cost of gain (Field, 2007). U.S. cattle feeders have incorporated the use of growth enhancement technologies to the point that in 1999, more than 96% of cattle upon entering U.S. feed yards were implanted at least once (National Animal Health Monitoring System, 2000).

Taken in total, the U.S. fed cattle population performance in USDA Quality and Yield grade has altered significantly because the production sector responded to market signals. The series of National Beef Quality Audits funded by the Beef Checkoff delivered a consistent message to reduce subcutaneous fat while assuring appropriate levels of marbling to maintain beef palatability (Boleman et al., 1998; Garcia, et al., 2008; Lorenzen, et al., 1993; and McKenna, et al., 2002). Degree

Table 1
Percent USDA quality grade and yield grade performance from Meat Animal Research Center Germplasm Evaluation Project[a].

	British breeds [d] (%)	Continental breeds [e] (%)
USDA yield grade[b]		
1	4.5	22.4
2	29.2	47.4
3	43.4	26.9
4	22.9	3.3
USDA quality grade[c]		
Prime	2.1	0.3
Choice	84.0	57.3
Select	13.9	42.1
Standard	0.0	0.3

[a] Wheeler et al., 2006.
[b] Estimates percent of carcass weight converted to boneless, closely trimmed retail products. Yield grade 1 has the highest percent cutability while a YG 5 would have poor cutability.
[c] Estimates palatability based on assessments of intramuscular fat and maturity. Prime and Choice have the most desirable palatability while Standard (carcasses not presented to be quality graded) typically have poorer palatability and tenderness.
[d] Angus, Hereford and Red Angus.
[e] Charolais, Gelbvieh, Limousin, and Simmental.

Table 2
Genetic trend in Expected Progeny Differences (EPD) for Carcass traits of British breed cattle[a].

Year	Carcass weight EPD[b]			Marbling EPD			Ribeye area EPD			Backfat thickness EPD		
	A[c]	H[d]	RA[e]	A	H	RA	A	H	RA	A	H	
1975	0	NA	−9	0	NA	−.06	.01	NA	.04	0	NA	−.01
1980	0	NA	−5	.01	NA	−.05	−.01	NA	.02	0	NA	−.01
1985	0	NA	4	.04	NA	−.06	−.03	NA	−.01	−.003	NA	−.01
1990	3	NA	11	.10	−.02	−.06	−.04	−.08	−.02	−.003	−.03	−.02
1995	4	NA	19	.15	−.01	−.05	−.06	−.02	−.05	0	−.03	−.01
2000	7	NA	25	.21	−.01	−.02	−.02	.05	−.05	.003	−.02	−.01
2005	11	NA	32	.34	−.01	.03	.03	.14	.01	.007	−.01	−.01
2009	14	NA	36	.43	−.04	.07	.07	.22	.07	.012	.002	0.0

[a] Source: National Sire Evaluation Databases of the American Angus Association, American Hereford Association and the Red Angus Association of America.
[b] Expected progeny differences.
[c] Angus, [d] Herford, [e] Red Angus.

of marbling is the primary determination of quality grade. Marbling is determined by the amount and distribution of marbling in the ribeye muscle at the cut surface after the carcass has been ribbed between the 12th and 13th ribs. Yield grade estimates the amount of boneless, closely trimmed retail cuts available from the high-value parts of the carcass with Yield Grade 1 being the highest yielding carcass. According to the Agricultural Marketing Service of USDA, in 1974, 75% of the fed cattle in the U.S. were the highest quality grades (USDA Choice or Prime) with only 30% categorized as USDA Yield Grade 1 and 2. By 1996, 61% of fed cattle met the standards for USDA Yield Grade 1 and 2 with 55% graded as USDA Choice or Prime thus rebalancing the grade mix. In response to strong signals for improved eating quality, the industry shifted the grade distribution by 2010 such that approximately 55% and 64% of beef carcasses qualified as USDA Yield Grade 1 and 2 and USDA Prime and Choice, respectively (Table 4).

2.2. Decreased carcass fat through change in butchery practice

The first U.S. Dietary Guidelines for Americans were issued in 1980. Included in the 1980 guidelines were recommendations to "choose lean meat, fish, poultry, dry beans and peas as your protein sources" and "trim excess fat off meats" in an effort "to avoid too much fat, saturated fat, and cholesterol." (USDA and U.S. Department of Health and Human Services, 1980) These recommendations increased consumer demand for leaner beef cuts and increased trimming of visible fat at the retail level. In the 1980s, most beef in the U.S. retail meat case had 1.3 cm (0.5 in.) of external fat (Cross et al., 1986). The need to meet consumer demand by providing retail cuts with less visible fat was confirmed by the results of the National Consumer Retail Beef Study (NCRBS). Conducted in 1983, the NCRBS examined the interaction of quality grade, price, and external fat trim. The results indicated that consumers were less willing to purchase beef cuts with excess external fat, regardless of grade, and would be willing to pay a slightly higher price per pound for closely trimmed cuts as consumers considered cut with 0.8 cm or less external fat to be more healthful (Cross et al., 1986). Results indicated that lesser grade beef cuts were perceived as more healthful with U.S. Good grade cuts, identified as "Select" in the NCRBS, rated high by consumers for leanness. Interestingly, results of the NCRBS were used as the impetus to change the name of the U.S. Good grade to U.S. Select as "Good" was seen as communicating a negative image to consumers but "Select" was positively associated with leanness. U.S. Select grade beef contains slight marbling and is derived primarily from Yield Grade 2 or higher carcasses. By 1988 the average external fat thickness for all retail beef cuts had been trimmed to an overall mean of 0.31 cm (Savell et al., 1991). More recent data from 2005 show that the external fat on retail beef cuts averages 0.24 cm, virtually devoid of external fat, marking an 81% decrease in external fat on retail cuts in 26 years (Savell, et al., 2005). Furthermore, national consumer studies report that 77% of consumers prefer to trim visible fat from beef before consuming (Cattlemen's Beef Board and National Cattlemen's Beef Association, 2010). Through the combination of changes in beef breeding and management and availability of near zero external fat through trimming, 63% of U.S. fresh whole muscle beef cuts, including 15 of the top 20 most popular currently sold at retail, meet Food and Drug Administration (FDA) guidelines for lean, having less than 10 g of total fat, 4.5 g or less of saturated fat and less than 95 mg of cholesterol per serving and per 100 g (FDA, 2008). In total, there are at least 29 fresh cuts of cooked beef that meet the FDA definition of lean (Fig. 2).

2.3. Communicating lean beef availability

Despite the widespread availability of lean beef cuts, survey research has found that, on average, U.S. registered dietitians believe there are only about seven cuts of lean beef, and more than half believe there are only three to five lean beef cuts available to consumers (unpublished data, 2007). Communicating the availability of lean beef has likely been hindered, at least in part, by infrequent updates of the nutrient composition data for various beef cuts in the USDA National Nutrient Database for Standard Release. For example, in 2006, sampling of retail cuts from the chuck, rib, loin, or round identified 11 total cuts that averaged 35% less external fat than was reported in the USDA nutrient database (Mason, et al., 2009).

Table 3
Genetic trend for carcass trait Expected Progeny Differences (EPD) of continental cattle[a].

Year	Carcass weight EPD			Marbling EPD			Ribeye area EPD			Backfat thickness EPD		
	S[b]	C[c]	L[d]	S	C	L	S	C	L	S	C	L
1991	−4.0	4.6	−7.4	−.03	0.0	−.07	−.12	.03	.24	0.0	−.003	−.15
1995	−2.9	6.0	−3.0	.01	.01	−.07	−.08	.06	.29	0.0	−.003	−.14
2000	−2.8	9.0	3.2	.06	.01	−.08	−.02	.09	.35	0.0	−.002	−.08
2005	−2.4	11.9	10.1	.10	.01	−.06	.04	.14	.39	.01	−.001	−.06
2009	−1.7	14.1	19.4	.15	.01	−.04	.10	.18	.49	.01	−.001	−.04

[a] Sources: National Genetic Evaluation Databases of the American Simmental Association, American International Charolais Association, and the North American Limousin Foundation.
[b] Simmental, [c] Charolais, [d] Limousin.

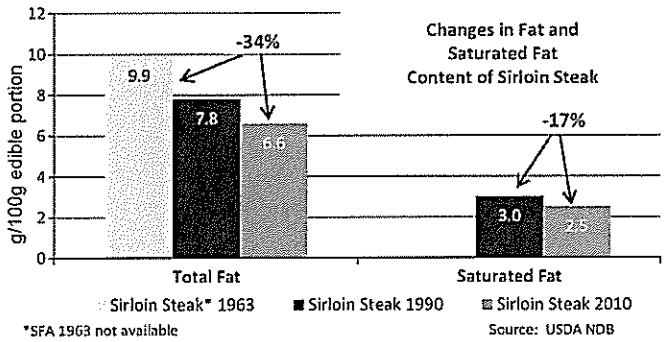

Fig. 1. Total fat and saturated fatty acid (SFA) content of sirloin steak, completely trimmed of external fat, as reported by Watt and Merrill (1963) and the USDA National Nutrient Database (1990; 2010). Data reported for 100 g of choice grade, cooked via broiling.

Inaccurate estimation of the total and saturated fat content of beef available in the marketplace can impact national food intake survey data that links to national nutrient databases. Outdated national nutrient data for beef is not a problem unique to the U.S. In a recent comparison of selected nutrients in beef according to food composition databases from various countries, Wyness et al. (2011) noted a range of 3.6–10.4 g total fat per 100 g of raw, lean, beef. Wyness et al. (2011) listed variable time periods of analyses, with some being conducted more recently than others or with newer methods, as one of the reasons for this range.

Through a nutrition research grant from The Beef Checkoff, USDA is leading a Beef Nutrient Database Improvement Initiative to update the nutrient composition data of beef retail cuts. The first revision of beef nutrient data from this initiative has resulted in significant updates to the nutrient information in the USDA Nutrient Database and the release of the USDA Nutrient Data Set for Retail Beef Cuts, Release 1.0 (Patterson et al., 2009). This recently released data set represents the collaborative effort of the USDA Nutrient Data Laboratory, The Beef Checkoff program, and various U.S. universities in three different studies designed to update or expand the data for beef cuts including the *1/8 Inch Study*, the *Beef Value Cuts Study (BVC)*, and the *Beef Nutrient Database Improvement Study Phase 1 (NDI Phase 1)*. The *1/8 Inch Study* determined the physical characteristics and nutrient composition of 13 raw and cooked retail cuts with fat trim levels representative of current retail cuts and provided analytical data not previously available in the USDA Nutrient Database (Patterson et al., 2009). The *BVC study* provided nutrient information for a new line of retail roasts and steaks including the top blade steak (Infraspinatus), shoulder top and center steaks (Triceps brachii), shounder tender (Teres major), tip center (Rectus femoris), tip side (Vastus lateralis) and bottom round (Biceps femoris). Finally, the *NDI Phase I* study focused on providing nutrient data for all retail cuts from the beef chuck that lacked data in the USDA Nutrient Database. Whereas the USDA Nutrient Data Set for Retail Beef Cuts, Release 1.0 is designed to provide retailers easier access to the most current and accurate beef nutrient data for "on-pack" nutrition labeling, it is also a resource for consumers and health professionals to quickly and easily determine the complete nutrient profile of 10 commonly consumed beef cuts. Release 1.0 is the first of continuously planned updates. Also included among recent database updates is the USDA Ground Beef Calculator, an on-line nutrient composition tool recently developed to aid consumers, researchers, and health professionals obtain accurate data for ground/minced beef (USDA, 2009). Estimates suggest that 42% of beef consumed in the U.S. is purchased ground at retail (Davis and Lin, 2005). Whereas, 95% lean ground beef meets the FDA definition of lean (FDA, 2008), ground beef is unique in that a wide range of products ranging from 5 to 30% fat are available in most retail stores and are *voluntarily* labeled with either the percentage lean or fat content. Perhaps one of the greatest opportunities to communicate the nutrient composition of ground beef comes from the recently finalized rule for Nutrition Labeling of Single Ingredient Products and Ground or Chopped Meat and Poultry Products, by USDA Food Safety and Inspection Service (USDA FSIS, 2010). This rule *mandates* nutrition information on pack for all ground or chopped single-ingredient meat and poultry products and on-pack or at point of purchase nutrition labeling of major cuts of single-ingredient, raw meat and poultry. The rule also allows "Percent Lean" (% lean) use on labels of ground or chopped products that do not meet regulatory criteria for "low-fat" provided that a statement of fat percentage (or % fat) is also displayed next to % lean in the same font size on the label. Compliance with this rule is required by January 2012. In the meantime, the USDA Ground Beef Calculator can help consumers and health professionals alike to decode the fat/lean content of various ground beef offerings and allow continued calculation of nutrients not required by labeling regulation at any fat level between 5 and 30%

Table 4
Grade performance of U.S. fed cattle (1996–2010)[a].

	1996	2000	2005	2010
USDA yield grade[a] (%)				
1	12.7	10.9	10.5	11.9
2	48.2	45.6	40.4	40.0
3	37.2	41.0	39.9	39.9
4	1.5	2.1	8.0	7.3
5	0.2	0.2	1.0	0.8
USDA quality grade[b] (%)				
Prime	2.1	3.2	2.9	3.1
Choice	53.2	52.4	52.9	60.9
Select	32.7	36.0	36.5	29.8
Standard and no roll	12.0	8.4	7.8	8.2

[a] Source: Meat Grading and Certification Branch, USDA, 2011.
[b] Estimates percent of carcass weight converted to boneless, closely trimmed retail products. Yield grade 1 has the highest percent cutability while a YG 5 would have poor cutability.
[b] Estimates palatability based on assessments of intramuscular fat and maturity. Prime and Choice have the most desirable palatability while Standard and no roll (carcasses not presented to be quality graded) typically have poorer palatability and tenderness.

3. Beef consumption and cardiovascular health endpoints

The recommendation to restrict beef consumption is most often rooted in the assumption that beef is over-consumed and that the fatty acid profile is counterproductive to optimal health (Hu, et al., 1999). However, in a recent analysis of U.S. National Health and Nutrition Examination Survey (NHANES) food survey data adults (19–50 years) total beef and lean beef consumption equated to 49.3 g and 45.5 g, respectively of the daily 142–198 g total meat and meat equivalents (i.e. beans and nuts) recommended by the USDA "MyPyramid" food plan for adults (Zanovec et al., 2010). These data indicate that beef is moderately consumed despite its popularity with consumers. Analysis of NHANES data has also found that, in healthy women age 50 and older, those who adhered most closely to a dietary pattern with beef as a primary source of protein had the lowest probability of being overweight or obese, a greater likelihood of normal systolic blood pressure, and an overall diet that conformed most closely with the 2005 U.S. Dietary Guidelines for Americans (Lopez et al., 2008). For U.S. children, NHANES data indicate that those 4–8 years consume 22.7 g total beef and 20.8 g lean beef and those 9–13 years consume 37 g total beef with 34 g as lean beef contributing only 9.8–13.9% of total protein, again suggesting modest consumption of beef (O'Neil et al., 2011). Based on these data, beef intake in the average American diet appears well within the recommendations made by the 2010 Dietary Guidelines Advisory Committee (DGAC).

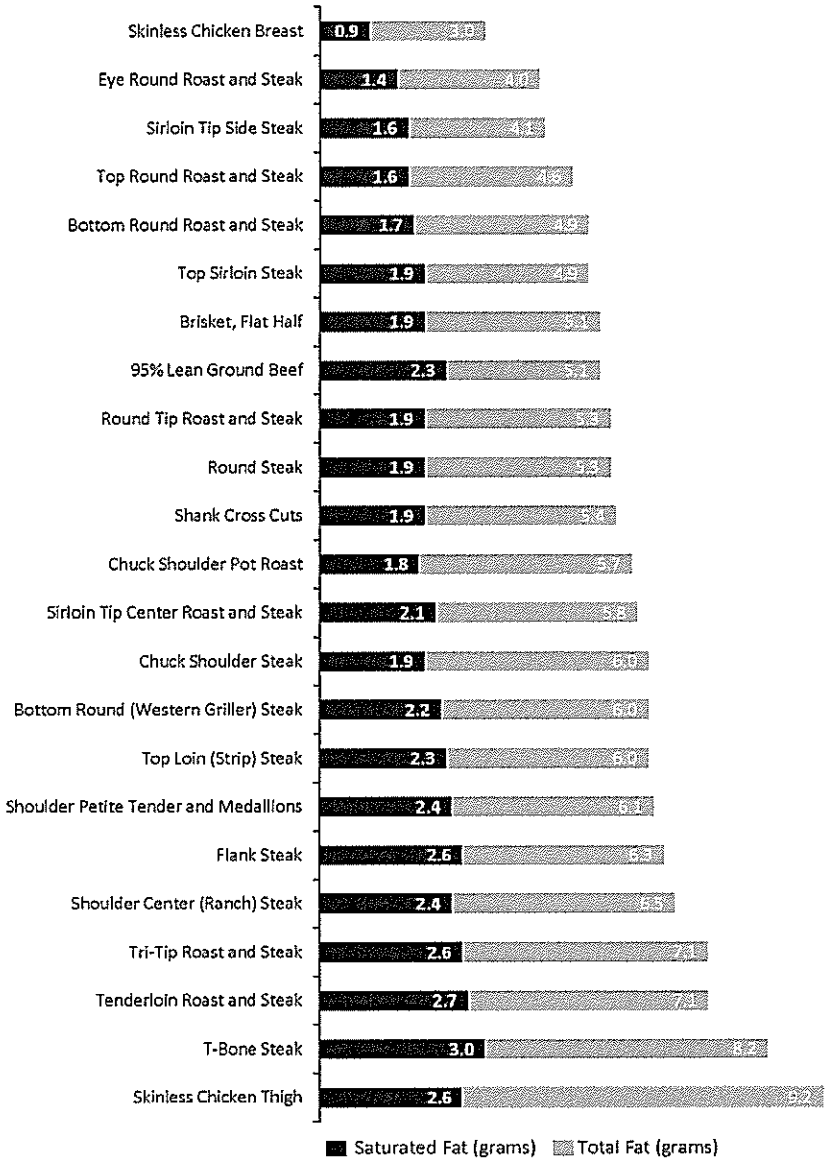

Fig. 2. Cuts of meat that meet FDA guidelines for lean. Roast and steak cuts are combined for illustration. All data based on USDA Nutrient Database for Standard Reference, Release 23 (USDA, 2010). Cooked, lean only, all visible fat removed based on "all grades" designation, if available (aggregate of USDA Select and Choice based on retail volume), otherwise average of USDA Select and Choice. Copyright courtesy of the Beef Checkoff.

In addition, consistent evidence from clinical trials indicates that the inclusion of lean beef in a well-balanced diet designed to manage cardiovascular risk is equally as effective as including lean white meat for low-density lipoprotein cholesterol (LDL) reduction (Table 5). In fact, a systematic review of red meat studies provides supportive evidence that, when included as part of a diet low in saturated fat (≤10%), fresh red meat from both grass-fed and grain-finished animals is associated with reductions in LDL in both healthy and mildly hypercholesterolemic individuals (Li et al., 2005).

These results are not surprising when the fatty acid profile of beef is considered. Regardless of feeding regime roughly fifty percent of the fatty acids in U.S. beef are monounsaturated (USDA, 2009a), and nearly one-third of the saturated fat in beef is stearic acid, a fatty acid that has been shown to have a neutral effect on LDL cholesterol (DGAC, 2010). Reports from the U.S. Institute of Medicine (IOM, 2005), the 2010 DGAC and, most recently, Food and Agriculture Organization (2010) all recognize the neutral effect of stearic acid on LDL-cholesterol. Whereas reducing dietary saturated fat has generally been thought to improve cardiovascular health, a recent meta-analysis of prospective cohort studies found that saturated fat was not associated with an increased risk of heart disease (pooled relative risk estimate 1.07 between intake quartiles) (Siri-Tarino et al., 2010). Research also suggests that *trans* fatty acid intake, a diet with a high glycemic index, and high dietary salt are more significant risk factors for heart disease than dietary saturated fat (Danaei et al., 2009). In fact, the higher sodium content of processed red meats is a likely contributor to recent observations that processed meats, but not fresh cuts, are associated with an increased risk for cardiovascular disease (Micha et al., 2010).

4. Contribution of beef to nutrient adequacy

Looking beyond fat, beef significantly contributes to the overall nutrient intake of Americans. As noted by the 2010 DGAC meat, including beef, is commonly recognized as an important source of high-quality protein and highly bioavailable iron (DGAC, 2010). U.S. dietary survey data indicate that fresh beef is the number one source of protein, vitamin B_{12}, and zinc (Cotton et al., 2004) in the American

Table 5
Beef versus other lean animal protein and LDL outcomes in individuals at increased risk for heart disease.

Study	Study type/duration	Study population	Test diets	Intervention	Results
Beauchesne-Rondeau et al., 2003 (Canada)	Cross-over 3 × 26-day test periods separated by 6-week washouts	Mild hyperC[a] and overweight men/n = 18	<30% of calories from fat; <10% SFA[b]; 250 mg chol[c]/day. Beef, chicken or fish provided as prepared lunch or dinner. Breakfast self-selected.	425 g/day (average) lean beef (sirloin strip), fish (lean white), or chicken (skinless breast or ground turkey).	All diets significantly ↓ plasma LDL-C[d] 5-9%. Beef – 7–8%; no significant difference between diets.
Leaf and Hatcher, 2009 (U.S.A)	Cross-over 3 × 28-day test periods, no washout.	HyperC and overweight men and women/n = 10	Cholesterol-free Diet 25 – 24% calories from fat; 8.1% SFA, 0 mg chol/day from liquid formula; Cholesterol-free Diet 40 – 40% calories from fat; 0 mg chol/day; White Fish Diet 25–24% calories from fat; 8.1% SFA; 50 mg/1000 kcal; White Fish Diet 40 – 40% calories from fat; 12.3% SFA; 50 mg chol/1000 kcal; Ground Beef Diet 25 – 24% calories from fat; 10.8% SFA; 65 mg chol/1000 kcal; Ground Beef Diet 40 – 40% calories from fat; 15.2% SFA; 65 mg chol/1000 kcal. All meals prepared by research staff, 2 meals eaten under supervision/day.	276 g/1000 kcal lean fish or ground beef (15% fat).	Cholesterol free diet significantly lowered plasma LDL-C 12% compared to fish or beef. No significant difference in LDL-C following fish or beef combined with either level of fat intake.
Mahon et al. 2007 (U.S.A.)	RCT[e] 9 weeks	HyperC, overweight, post-menopausal women/n = 54	1000 kcal lacto-ovo vegetarian weight-loss diet with 250 kcal added as various protein or CHO[f]/fat. Diets ≤30% of calories from fat. Test article provided, other foods self-purchased and prepared.	Additional 250 kcal as lean beef (tenderloin), lean chicken, or non-meat CHO/fat	All diets significantly ↓ plasma LDL-C ~ 12%, no significant difference between diets.
Melanson et al. 2003 (U.S.A)	RCT 10 weeks	Mild hyperC, obese, women/ n = 61	Hypocaloric (−500 kcal/d) compared to usual, 24.5% calories from fat; 6.4% SFA; 125 mg chol/day. All foods self-purchased and prepared.	Primary protein source (70 g/d) as lean beef (sirloin, top round, 94% ground) or lean chicken (skinless breast, thighs, ground chicken)	Plasma LDL-C ↓ 7–11% from baseline, no significant difference between lean beef vs. lean chicken.
Scott et al. 1994 (U.S.A)	RCT 13 weeks	HyperC[1] men/n = 38	Stabilization diet: 40% calories from fat; 18% SFA; 400 mg chol/day; Test diet: 30% of calories total fat; <10% as SFA; 250 mg chol/day. All foods other than low calorie, free choice provided.	Stabilization: 45 g/d beef (cut not specified) or chicken (cut not specified) during 5 week period Test: 85 g/d lean beef (strip loin steak) or lean chicken during 5 week test period	Significant 9–11% ↓in plasma LDL-C in response to either lean beef or chicken. No significant difference between protein sources.
Scott et al. 1991 (U.S.A.)	RCT 11 weeks	Mild hyperC men/n = 46	Stabilization diet: 35% calories from fat; 8% SFA; 222 mg chol/day Test diet: 29% of calories from fat; 6% as SFA; 228 mg chol/day. All foods other than low calorie, free choice provided.	Stabilization: 226 g/d beef (13.6% fat, cut not specified) for 4 weeks Test: 226 g/d lean beef (top round, top loin steak) or lean chicken (breast) and fish (red snapper) during 4 week test	No effect of either lean beef or chicken/fish during test period on plasma LDL-C.
Roussell et al., 2010 (U.S.A.)	Cross-over/4 × 35-day test periods separated by 14 day washout	Mild hyper C men and women/n = 37	Average American Diet (33% calories from fat; 11.3% SFA); DASH[g] Diet (28% calories from fat; 6.5% SFA); BOLD[h] Diet (28% calories from fat; 6.4% SFA); BOLD + Diet (28% calories rom fat; 6.1% SFA).	Average American Diet: 28 g lean beef /day(cuts not specified);DASH Diet: 50 g lean beef/day;BOLD Diet – 126 g lean beef/day; BOLD + Diet: 177 g lean beef/day	All diets resulted in a significant, ~8%↓in plasma LDL-C compared with the Average American Diet

[a] Hyper C = Hypercholesterolemic – mild defined as plasma cholesterol >200 mg/d but <240 mg/dl.
[b] SFA = Saturated fat as a percent of total calories.
[c] chol = dietary cholesterol.
[d] LDL-C = low-density lipoprotein cholesterol.
[e] RCT = randomized, controlled trial.
[f] CHO = carbohydrate.
[g] DASH = Dietary approaches to stop hypertension.
[h] BOLD = Beef in an optimal lean diet.

diet and a leading source of selenium, iron, and monounsaturated fatty acids (Zanovec et al., 2010). On average, in a 85-g cooked serving, the 29 lean cuts of beef (Fig. 2) contribute 8% of calories (154 cal) to a 2000 calorie diet, 50% of the daily value for protein, 45%–62% U.S. Recommended Dietary Allowance (RDA; adult under 50 years male–female, respectively) for zinc, 91% of the adult RDA for vitamin B_{12}, 52% of selenium, 21% of phosphorus, 31–36% of niacin, 31% of vitamin B_6, 27–12% of iron, and 13–15% of riboflavin (USDA, 2010).

5. Conclusion

Lean beef cuts are widely available in the U.S. marketplace as the result of progressive changes over the past 30 years in cattle breeding and management practices and retail trimming. Numerous updated nutrient data tools are available from USDA and The Beef Checkoff to enable consumers and health professionals to confidently identify the best beef choices to meet nutritional needs. Beef is a popular, nutrient-dense protein source and lean beef can help consumers meet their cardiovascular health goals.

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A MEDITERRANEAN-STYLE EATING PATTERN WITH LEAN UNPROCESSED RED MEAT HAS CARDIOMETABOLIC BENEFITS

For adults who are overweight/obese in a randomized crossover controlled feeding trial
O'Connor et al. American Journal of Clinical Nutrition, 2017

OBJECTIVE

To assess the effects of consuming a Mediterranean pattern with different amounts of red meat on CMD risk factors.

STUDY DESIGN & SETTING

An investigator-blinded, randomized, crossover, controlled feeding trial. Subjects were provided a Mediterranean Pattern for two 5-week interventions, separated by 4-weeks of self-selected eating. The Mediterranean Patterns contained ~500 g (17.6 oz.) (Med-Red) and ~200 g (7.05 oz.) (Med-Control) of lean unprocessed beef/pork/wk.

PARTICIPANTS

Overweight or obese (BMI: 25-37 kg/m2) adults (30-69 yrs.) were recruited.

Additional inclusion criteria:

- Total cholesterol <120.7 mg/dl
- LDL cholesterol <73.8 mg/dl
- Triglycerides <81.1 mg/dl
- Fasting glucose <109.9 mg/dl
- Systolic blood pressure <160 mm Hg
- Diastolic blood pressure <100 mm Hg
- Body mass <140 kg
- No acute illness
- Non-smoker
- Normal liver and kidney functions
- Non-diabetic
- Weigh stable (±4.5 kg)
- Consistent physical activity levels for 3 months prior to starting the study
- Stable medication use for six months prior to and throughout the study

RESULTS

- Total cholesterol decreased, but greater reductions occurred with Med-Red compared to Med-Control (-7.2 ± 0.1 and -1.8 ± 0 mg/dl, respectively).
- LDL decreased with Med-Red, but was unchanged with Med-Control (-5.4 ± 0.1 and -1.8 ± 0.1 mg/dl, respectively).
- HDL concentrations decreased non-differentially (-1.8 ± 0.0 mg/dl).
- Triglycerides, total cholesterol: HDL, glucose and insulin did not change with either Med-Red or Med-Control.
- All blood pressure parameters improved, except during sleep, independent of red meat intake amount.

CONCLUSIONS

- Adults who are overweight/obese can consume typical U.S. intake quantities of red meat, as lean and unprocessed beef and pork, when adopting a Mediterranean Pattern to improve cardiometabolic disease risk factors.
- Unprocessed and/or lean red meat consumption does not increase the risk of developing cardiovascular disease or impair associated risk factors.

A Mediterranean-style eating pattern with lean, unprocessed red meat has cardiometabolic benefits for adults who are overweight or obese in a randomized, crossover, controlled feeding trial

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ABSTRACT

Background: A Mediterranean-style eating pattern (Mediterranean Pattern) is often described as being low in red meat. Research shows that lean, unprocessed red meat can be incorporated into healthy eating patterns to improve cardiometabolic disease (CMD) risk factors.

Objective: We assessed the effects of consuming different amounts of lean, unprocessed red meat in a Mediterranean Pattern on CMD risk factors. We hypothesized that consuming a Mediterranean Pattern would improve CMD risk factors and that red meat intake would not influence these improvements.

Design: In an investigator-blinded, randomized, crossover, controlled feeding trial, 41 subjects [mean ± SD age: 46 ± 2 y; mean ± SD body mass index (kg/m^2): 30.5 ± 0.6] were provided with a Mediterranean Pattern for two 5-wk interventions separated by 4 wk of self-selected eating. The Mediterranean Patterns contained ~500 g [typical US intake (Med-Red)] and ~200 g [commonly recommended intake in heart-healthy eating patterns (Med-Control)] of lean, unprocessed beef or pork per week. Red meat intake was compensated by poultry and other protein-rich foods. Baseline and postintervention outcomes included fasting blood pressure, serum lipids, lipoproteins, glucose, insulin, and ambulatory blood pressure. The presented results were adjusted for age, sex, and body mass at each time point ($P < 0.05$).

Results: Total cholesterol decreased, but greater reductions occurred with Med-Red than with Med-Control (−0.4 ± 0.1 and −0.2 ± 0.1 mmol/L, respectively, intervention × time = 0.045]. Low-density lipoprotein decreased with Med-Red but was unchanged with Med-Control [−0.3 ± 0.1 and −0.1 ± 0.1 mmol/L, respectively, intervention × time = 0.038], whereas high-density lipoprotein (HDL) concentrations decreased nondifferentially [−0.1 ± 0.0 mmol/L]. Triglycerides, total cholesterol:HDL, glucose, and insulin did not change with either Med-Red or Med-Control. All blood pressure parameters improved, except during sleep, independent of the red meat intake amount.

Conclusions: Adults who are overweight or moderately obese may improve multiple cardiometabolic disease risk factors by adopting a Mediterranean-style eating pattern with or without reductions in red meat intake when red meats are lean and unprocessed. This trial was registered at clinicaltrials.gov as NCT02573129. *Am J Clin Nutr* 2018;108:1–8.

Keywords: beef, pork, healthy eating pattern, blood lipids, blood lipoproteins, blood pressure

INTRODUCTION

The historically low chronic disease rates in Mediterranean countries are often attributed to eating habits. In the 1960s, a Mediterranean-style eating pattern (Mediterranean Pattern) was first recognized in a small cohort of coastal Greek olive farmers who had lower rates of cardiovascular disease than six other world regions (1). Their eating pattern was predominantly plant-based, notably low in red meat, and olive oil was the main source of fat (2). The health-promoting properties of a Mediterranean Pattern, including reduced risk of developing cardiovascular disease and type 2 diabetes, are supported by recent and larger studies (3–7). These recent studies, including the Prevención con Dieta Mediterránea (PREDIMED) (5) and Seguimiento Universidad de Navarra (SUN) cohorts (8), were largely conducted on

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Supplemental Tables 1–4 and Supplemental Figure 1 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: ApoB, apolipoprotein B; CMD, cardiometabolic disease; CRP, C-reactive protein; DASH, Dietary Approaches to Stop Hypertension; DGAC, Dietary Guidelines Advisory Committee; LS, least squares; Med-Control, Mediterranean-style eating pattern with ~200 g of lean, unprocessed red meat per week; Mediterranean Pattern, Mediterranean-style eating pattern; Med-Red, Mediterranean-style eating pattern with ~500 g of lean, unprocessed red meat per week; PREDIMED, Prevención con Dieta Mediterránea; total-C, total cholesterol.

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Spaniards who had higher red meat intakes (~700–1200 g/wk) (9) than the historic Greek olive farmers (~245 g/wk) (10). These studies are mostly observational in nature and were not designed to directly compare consuming Mediterranean Patterns with different amounts of red meat intake on cardiometabolic disease risk factors (CMD).

Conclusions about the cardiometabolic risks of consuming red meat are historically inconsistent. The supporting literature base consists largely of observational cohort studies in which "red meat" is often ill-defined and grouped with processed meat as one intake category (11). This leads to inconsistent conclusions about the associations between red meat consumption and CMD (11). More recent observational research which assesses unprocessed red meat independently of processed meat shows little or no association between unprocessed red meat consumption and CMD (11, 12). In agreement, a compilation of randomized controlled trial data shows that total red meat, but mostly unprocessed beef and pork, consumption has no negative effect on cardiovascular disease risk factors (13). Nevertheless, US residents are encouraged to lower their red meat intake (14, 15).

The foundation for the recommendation to lower red meat intake in the context of a Mediterranean Pattern is unclear. US residents typically consume less red meat (11, 16) than what was reported in the large Mediterranean Pattern studies showing cardiometabolic benefits mentioned previously (5, 8). The primary objective of this controlled feeding trial was to assess the effects of consuming a Mediterranean Pattern with different amounts of red meat on CMD risk factors. We compared a Mediterranean Pattern with ~500 g lean, unprocessed red meat/wk (Med-Red) and a Mediterranean Pattern with ~200 g lean, unprocessed red meat/wk (Med-Control) because these are the amounts that are typically consumed by US residents (11, 16) and commonly recommended in heart-healthy eating patterns (17, 18), respectively. We hypothesized that the amount of red meat consumed would not influence Mediterranean Pattern-induced improvements in CMD risk factors of adults who are overweight or obese.

METHODS

Experimental design

This experimental design was a 16-wk randomized, crossover, investigator-blinded, controlled feeding study. Subjects consumed a Mediterranean Pattern for two 5-wk controlled feeding interventions separated by at least 4 wk of a self-selected and unrestricted eating pattern (washout). Dietary intake, body mass and composition, and CMD risk factors [including total cholesterol (total-C), LDL cholesterol, HDL cholesterol, total-C:HDL cholesterol, total apolipoprotein B (ApoB), triglycerides, glucose, insulin, HOMA-IR, C-reactive protein (CRP), fasting blood pressure, ambulatory blood pressure, and the Framingham Heart Study 10-y cardiovascular disease risk and vascular age] were measured at both baselines and during the last week of each Mediterranean Pattern intervention. Randomization was completed using an online randomization plan generator (http://www.randomization.com/). The trial was registered at clinicaltrials.gov as NCT02573129.

Subjects

Subjects who were overweight or obese [BMI (kg/m^2) 25–37], aged 30–69 y [representing middle-aged adults and adulthood life stage groups of the Dietary Reference Intakes (19)], and not already following a Mediterranean Pattern [as indicated by a score of <5 on the 14-item Mediterranean Diet Assessment Tool (20)] were recruited from the Greater Lafayette, IN area. Subject inclusion criteria were total-C <6.70 mmol/L, LDL cholesterol <4.10 mmol/L, triglycerides <4.5 mmol/L, fasting glucose <6.1 mmol/L, systolic blood pressure <160 mm Hg, diastolic blood pressure <100 mm Hg, body mass <140 kg, no acute illness, nonsmokers, normal liver and kidney functions, and nondiabetic. Subjects were required to be weight stable (±4.5 kg), to have consistent physical activity levels for 3 mo prior to starting the study, and to have stable medication use for 6 mo prior to and throughout the study. A physician reviewed each individual's screening measurements to ensure that they met the study inclusion criteria and to approve them for participation.

Assessment of self-selected eating pattern

Before being randomized into the study, subjects completed the Mediterranean Diet Assessment Tool (20) to confirm that they were not already consuming a Mediterranean Pattern. Subjects were instructed to consume their self-selected unrestricted eating patterns (recorded with 3-d food logs) both during the baseline testing weeks and throughout the washout.

Mediterranean Pattern

Menus were developed using Pronutra software (Viocare, Inc.) and followed the PREDIMED protocol (21) to achieve the desired Mediterranean Pattern. The menus were verified using the Mediterranean Diet Assessment Tool (20). Daily macronutrient intakes were targeted at 40% of total energy as carbohydrate, 22% protein, and 40% fat. Daily fat intakes were targeted at 7% of total energy as saturated fat and 20% monounsaturated fat. Med-Red and Med-Control differed predominantly in the amounts of red meat and poultry provided. Further adjustments were required to match the energy and macronutrients of the Med-Red and Med-Control menus, which was achieved by manipulation of mainly dairy, egg, and grain consumption. Fish and legume intake were similar in both Mediterranean Patterns in order to achieve the desired eating pattern per the PREDIMED protocol. Sodium, potassium, magnesium, and calcium intakes were targeted to be within ±15% between the Med-Red and Med-Control menus, and were calculated using the Linear Index Model (22). Each subject's energy requirement was estimated using sex-specific equations published by the Institute of Medicine (19), and menus were designed to maintain subjects' baseline 1 body mass. Subjects were given the option to consume 150 mL of self-selected dry red wine daily.

All foods were prepared and provided to subjects during the two Mediterranean Pattern interventions by the NIH-supported Indiana Clinical Research Center Bionutrition Facility at Purdue University. The red meats and poultry provided were beef or pork tenderloins and chicken or turkey breasts (white meat with the skin removed prior to cooking). The meats were consumed in mixed heterogeneous dishes. All red meat and poultry provided was lean [<10 g total fat, <5 g saturated fat, and <95 mg cholesterol (23)]. All red meats and poultry

underwent no further preservation processing beyond refrigeration or freezing (24), i.e., no smoking, curing, salting, or the addition of chemical preservatives (14). While meat processing terms vary, we use the term "unprocessed" throughout the article to be consistent with previous literature on this topic (11). Subjects weighed in and met with study staff weekly to monitor body mass and promote compliance, respectively. Subjects completed daily (and returned weekly) menu check-off lists to track self-reported deviations from the provided Mediterranean Pattern. Dietary intake and compliance were measured from the menu check-off lists of 3 d during the last week of each intervention.

Body mass and composition

Body mass and composition (percentage body fat and fat-free mass) were measured at during both baselines periods and during the last week of each intervention via the BOD POD Gold Standard Body Composition Tracking System (COSMED USA, Inc.).

Cardiometabolic disease risk factors

Cardiometabolic disease risk factors were measured for all subjects ($n = 41$) during both baseline periods and during the last week of each intervention. Fasting blood samples were collected from an antecubital vein into serum separator tubes and centrifuged for 15 min at 3.0 g and 4°C. Fresh serum was then shipped to Mid America Clinical Laboratories to determine total-C, HDL cholesterol, triglycerides, and glucose concentrations via enzymatic colorimetry using oxidase methods on a COBAS Integra 400 Plus Analyzer (Roche Diagnostics Ltd). LDL cholesterol was calculated using the following equation: LDL cholesterol = total-C − [HDL cholesterol + (triglycerides/5)]. The remaining serum was divided into samples, stored at −80°C, then thawed after all subjects had completed both interventions for analyses of insulin, total ApoB, and CRP concentrations. Fasting serum ApoB and CRP were measured via enzymatic colorimetry via oxidase methods on a COBAS Integra 400 Plus analyzer. Fasting serum insulin was measured via an electrochemiluminescence immunoassay on COBAS e411 analyzer (Roche Diagnostics Ltd).

Ambulatory and fasting blood pressures were measured during both baseline periods and during the last week of each intervention. Subjects wore an ambulatory blood pressure monitor for 48 h (Oscar2, Suntech Medical, Inc.). Blood pressure measurements were taken at 30 min intervals during the day (0800–2100) and at 90 min intervals through the night (2230–0730). Data were excluded from the analysis if >20% of scheduled measurements were invalid. Fasting blood pressures were measured in a quiet, dimly lit room. Measurements were taken after subjects sat upright for 15 min of rest (HEM-780, Omron Healthcare, Inc.). Two measurements were recorded (a third if the values differed by ≥3 mm Hg) and were averaged.

Cardiometabolic disease risk prediction

Predictions of long-term cardiovascular disease risk and vascular age were calculated using the Framingham Heart Study 10-y cardiovascular disease risk lipid equation (25).

Ethics

The study protocol and all study documents were approved by the Purdue University Biomedical Institutional Review Board (protocol #1501015662). All subjects provided written informed consent and received a monetary stipend.

Statistics

Power calculations (G*Power version 3.1.9.2, Heinrich-Heine-Universität Düsseldorf) indicated that 40 subjects would provide >95% power to detect changes in fasting serum total-C and fasting systolic blood pressure, as achieved in a similar randomized crossover trial assessing the effects of consuming lean, unprocessed pork as opposed to chicken or fish in a Dietary Approaches to Stop Hypertension (DASH) eating pattern ($\alpha = 0.05$) (26). We hypothesized that the inclusion of unprocessed red meat in a Mediterranean Pattern would not influence changes in these variables. Power calculation indicated that 40 subjects would provide >85% power to detect a differential response between Med-Red and Med-Control that was equal to half of the standard deviation of the response (effect size = 0.5).

All data were double entered independently and cross-checked for accuracy by the study manager (LEO). Data from 41 subjects who completed both interventions were analyzed via a doubly repeated-measures ANOVA using the PROC MIXED command in SAS version 9.4 (SAS Institute). This analysis measured: *1*) main effects of time (baseline compared with postintervention measurements; one-tailed), *2*) interaction of time and intervention (Med-Red changes compared with Med-Control changes; two-tailed), *3*) changes over time within Med-Red and within Med-Control (intervention-specific effect indicated by intervention × time P value < 0.05; one-tailed), *4*) comparison of Med-Red and Med-Control baseline measurements (intervention × time sliced by time; two-tailed), *5*) comparison of Med-Red and Med-Control preintervention measurements (intervention × time sliced by time; two-tailed), and *6*) comparison of baseline 1 and baseline 2 measurements (trial × time interaction sliced by time; two-tailed) to determine if subjects' baseline 1 health status was re-established at baseline 2. These analyses were repeated using baseline and intervention alcoholic drink-equivalents per day as covariates. The PROC MIXED command in SAS uses maximum likelihood to account for missing data in dependent variables (27). The number of observations available at each time point for all outcome variables are listed in **Supplemental Tables 1** and **2**. All cardiometabolic outcomes of interest were controlled for age, sex, and body mass at each time point, and body mass and composition were controlled for age and sex. Results are presented as adjusted least squares (LS) means ± SEM, and P values are Tukey-Kramer adjusted for multiple comparisons ($P < 0.05$).

WWC has full access to all the data from this study and takes responsibility for its integrity and analysis. Summaries of LS means ± SEM ($n = 41$), raw means ± SD ($n = 41$), and sex-specific raw means ± SD for females and males are presented in **Supplemental Tables 1–4**, respectively. Primary deidentified data, analytical methods, and study materials are available upon request.

TABLE 1
Subject characteristics at baseline 1[1]

Outcome	Baseline 1
Age, y	46 ± 2
BMI, kg/m^2	30.5 ± 0.6
Total cholesterol, mmol/L	4.97 ± 0.13
LDL cholesterol, mmol/L	3.08 ± 0.10
HDL cholesterol, mmol/L	1.27 ± 0.05
Triglycerides, mmol/L	1.3 ± 0.1
Glucose, mmol/L	5.5 ± 0.1
Insulin, pmol/L	86.1 ± 8.3
Systolic/diastolic blood pressure, mm Hg	118 ± 2/80 ± 1
14-point Mediterranean Diet Assessment Tool (20)	4 ± 0

[1] Values are means ± SEMs. There were no differences between baseline 1 and baseline 2 measurements ($n = 41$). Conversion factors are available at: http://www.amamanualofstyle.com/page/si-conversion-calculator.

TABLE 2
Prescribed daily dietary intakes of the Mediterranean-style eating pattern menus[1]

	Med-Red	Med-Control
Energy, kcal	2601 ± 428	2573 ± 405[†]
Protein, %en	18 ± 0	19 ± 1[†]
Carbohydrate, %en	42 ± 1	42 ± 2
Fat, %en	40 ± 1	40 ± 1
Monounsaturated fat, %en	22 ± 1	21 ± 1[†]
Polyunsaturated fat, %en	8 ± 0	9 ± 1[†]
Saturated fat, %en	7 ± 0	8 ± 0[†]
Sodium, mg	2645 ± 354	2604 ± 317
Potassium, mg	4859 ± 624	4330 ± 653[†]
Magnesium, mg	490 ± 96	483 ± 74

[1] Intakes were averaged across a 7-d menu cycle. Results are presented as unadjusted means ± SDs ($n = 41$). [†] Difference between Med-Red and Med-Control indicated by a paired t-test, $P < 0.05$. %en, percentage of total energy; Med-Control, Mediterranean-style eating pattern with ~200 g lean, unprocessed red meat/wk; Med-Red, Mediterranean-style eating pattern with ~500 g lean, unprocessed red meat/wk.

RESULTS

Subject characteristics

Fifty individuals were randomized into the study, but 18% (9) dropped out during week 1 of the first intervention. The remaining 41 subjects (28 women and 13 men) completed both interventions (see **Supplemental Figure 1**). Baseline 1 values of mean age, BMI, and fasting serum total-C, LDL cholesterol, HDL cholesterol, triglycerides, glucose, insulin concentrations, and fasting blood pressures are shown in **Table 1**.

Dietary intakes

Subjects were not consuming a Mediterranean Pattern at the start of the study, as indicated by a mean score of 4 ± 0 on the 14-item Mediterranean Diet Assessment tool (20). Self-reported dietary intake results from 3-d food logs did not differ between baseline 1 and baseline 2, confirming that subjects resumed their self-selected unrestricted eating patterns during the washout.

Mediterranean Diet Assessment Tool scores (20) increased ≥200%, as indicated by scores of 12 and 13 for the Med-Red and Med-Control menus, respectively. The Med-Red menu received one point less than Med-Control for the preferential use of red meat over poultry. The Med-Red and Med-Control menus had comparable daily energy contents, and intervention-specific macronutrient distributions were within ±1% (see **Table 2**). Daily or weekly servings of the US Dietary Guidelines for Americans designated food groups are shown in **Table 3** for representative Med-Red and Med-Control 7-d menu cycles. Mean self-reported compliance to the provided Med-Red and Med-Control menus were both ≥95%. Eleven subjects during Med-Red and 14 subjects during Med-Control consumed less than one 150-mL serving of wine/wk and were classified as non-wine drinkers. Among wine drinkers, 90 ± 3 mL of wine was consumed per day, on average, in both Med-Red ($n = 15$) and Med-Control ($n = 12$).

Body mass and composition

Chronologically, body mass at baseline 1 and baseline 2 did not differ. Body mass decreased more with Med-Red than Med-Control (−1.6 ± 0.5 vs. −1.0 ± 0.5 kg, intervention × time = 0.023), but postintervention values did not differ. Body fat percentage did not change with Med-Red or Med-Control.

Cardiometabolic disease risk factors

Chronologically, measurements of CMD risk factors at baseline 1 and 2 did not differ. Med-Red decreased total-C 3% more than Med-Control. LDL cholesterol and ApoB decreased by 8% and 6%, respectively, with Med-Red, but did not change with Med-Control (see **Figure 1**). Total-C:HDL cholesterol, triglycerides, CRP, glucose, insulin, and HOMA-IR score did not change with Med-Red or Med-Control. Fasting and ambulatory blood pressure parameters improved with both Mediterranean Patterns, except during sleep, independent of red meat intake amount (see **Figure 2**). There were no differences between postintervention values of Med-Red and Med-Control for any CMD risk factors. Our results showed no difference between males and females in Mediterranean Pattern-induced cardiometabolic changes, independent of red meat intake amount. When considering baseline and intervention drink-equivalents as a covariate, there were still greater reductions in total-C with Med-Red, and reductions in LDL cholesterol with Med-Red but no changes with Med-Control, but the overall time effect and intervention-specific effects on ApoB diminished. Adjusted means ± SEMs and unadjusted means ± SDs for all CMD risk factors are available in Supplemental Tables 1 and 2, respectively. Sex-specific unadjusted means ± SDs are available in Supplemental Tables 3 and 4.

Cardiovascular disease risk prediction

Framingham Heart Study 10-y cardiovascular disease risk decreased by 1% and vascular age increased by 2–3 y with a Mediterranean Pattern, independent of red meat intake amount.

DISCUSSION

Simultaneously adopting a Mediterranean Pattern and reducing red meat intake is commonly recommended to decrease CMD risk (14, 15). Our results show that adopting a Mediterranean

TABLE 3
Prescribed daily and weekly food group servings for the median energy intake level[1]

	Med-Red	Med-Control
Servings of fruit/d,[2] *n*	4	4
Servings of vegetables/d,[3] *n*	7	8
Dark green vegetables	1	2
Red and orange vegetables	1	1
Legumes	1	1
Starchy vegetables	1	1
Other vegetables	3	3
Servings of grains/d,[4] *n*	4	5
Whole grains	4	4
Refined grains	0	1
Protein-rich foods/wk,[5] g		
Red meat	476	196
Poultry	112	420
Seafood	336	336
Whole eggs	2	3
Nuts, seed, soy[6]	560	616
Servings of dairy/d,[7] *n*	3	2
Olive oil/wk,[8] g	247	247
14-point Mediterranean Diet Assessment Tool Score (20)	12	13

[1]Food group servings presented for representative 2492 kcal Med-Red and Med-Control diets averaged across a 7-d menu cycle. Med-Control, Mediterranean-style eating pattern with ~200 g lean, unprocessed red meat/wk; Med-Red, Mediterranean-style eating pattern with ~500 g lean, unprocessed red meat/wk.
[2]Half a cup or 1 medium fresh fruit.
[3]Half a cup of fresh or 1 cup of cooked vegetables.
[4]28 g = half a cup or 1 oz.
[5]28 g = 1 oz; cooked weights.
[6]28 g = 1 tbsp of nut butter, 0.5 oz of nuts or seeds, or ~1 oz-equivalent.
[7]1 cup of milk or yogurt.
[8]4.5 g = 1 tsp.

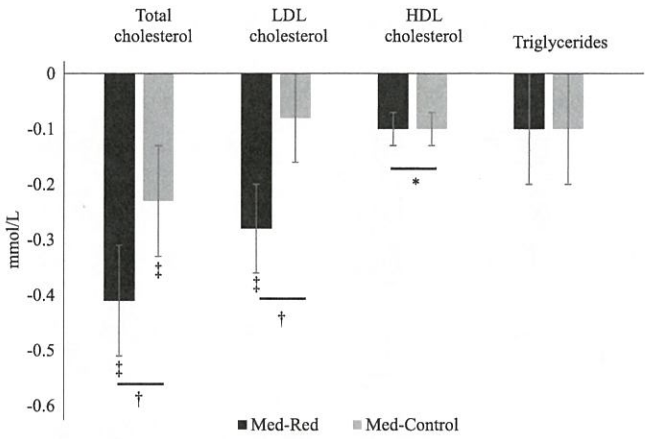

FIGURE 1 Changes in lipids and lipoproteins after consuming a Med-Red or Med-Control diet for 5 wk. Results are presented as LS means ± SEMs ($n = 41$). Data were analyzed using a doubly repeated-measures ANOVA adjusted for age, sex, and body mass at each time point. *Nondifferential change over time. †Differential response between Med-Red and Med-Control when intervention × time P value < 0.05. ‡Intervention-specific change over time indicated by intervention × time $P < 0.05$. ApoB results followed a similar pattern as LDL cholesterol and are available in Supplemental Tables 1 and 2. Conversion factors are available at: http://www.amamanualofstyle.com/page/si-conversion-calculator. ApoB, apolipoprotein B; LS, least squares; Med-Control, Mediterranean-style eating pattern with ~200 g lean, unprocessed red meat/wk; Med-Red, Mediterranean-style eating pattern with ~500 g lean, unprocessed red meat/wk.

Pattern with or without reducing red meat intake improves CMD risk factors if the red meat is lean and unprocessed. Our results support previous findings that consuming lean, unprocessed red meat [~120 g pork (26), ≤153 g beef (28–30), or ~86 g lean beef, veal, or lamb (31)/d] does not hinder the effectiveness of a DASH pattern to improve CMD risk factors in the absence of clinically meaningful body mass reductions.

The American Heart Association and the American College of Cardiology declare inconsistent effects of consuming a Mediterranean Pattern on blood lipid and lipoprotein concentrations (32). The randomized controlled trials referenced by these societies are largely dietary counseling interventions and have inadequate control groups (33–35). Our study provided a novel opportunity to assess the effects of a Mediterranean Pattern in a tightly controlled crossover trial. Adopting a Mediterranean Pattern improved overall CMD risk factor profiles. However, reductions in LDL cholesterol and ApoB concentrations were largely attributable to Med-Red because there were no changes in these outcomes with Med-Control. Our results indicate that variations in Mediterranean Pattern compositions (36), such as meat source, may help explain inconsistent effects described by the American Heart Association and the American College of Cardiology (32). Further, meat source in our study did not affect Mediterranean Pattern-induced improvements in predictions of long-term cardiovascular disease risk (Framingham Heart Study 10-y cardiovascular disease risk and vascular age). These results are consistent with evidence that a Mediterranean Pattern decreases the risk of coronary heart disease, stroke, and total mortality (37), but changes in atherosclerosis-promoting lipid and lipoprotein concentrations may not be the mechanism (38, 39).

This study was not designed to identify mechanisms by which lean, unprocessed red meat consumption might differentially affect atherosclerosis-promoting lipids and lipoprotein concentrations. One speculation is that the greater body mass loss with Med-Red may be a mediating factor. Despite randomization of trial order, the baseline Med-Red body mass was quantitatively, but not statistically, 0.7 kg higher than the baseline Med-Control body mass. It is perhaps noteworthy that participants lost 0.6 kg more during Med-Red than during Med-Control, which was a statistically significant difference. Both of these body mass changes were modest (Med-Red: −1.8%; Med-Control: −1.1%), body masses were not different at the end of the interventions, and there were no differential changes in absolute or relative fat or fat-free masses. We controlled for body weight at each time point in our statistical model, and body mass was not a significant covariate for total-C ($P = 0.321$) or LDL cholesterol ($P = 0.125$), but was for ApoB ($P = 0.035$). The combination of the small magnitude of difference between Med-Red and Med-Control body mass changes (clinical relevancy of 0.6 kg difference) and the lack of significance in our statistical model suggests that the differential effects in total-C, LDL cholesterol, and ApoB are not because of differences in body mass. However, an impact of changes in body mass on changes in LDL cholesterol cannot be ruled out.

Meta-analyses of prospective cohort studies show that each 100-g serving unprocessed red meat/d increases the risk of developing type 2 diabetes by 19% (11, 40), but there is a paucity of experimental evidence to support this. Our Mediterranean

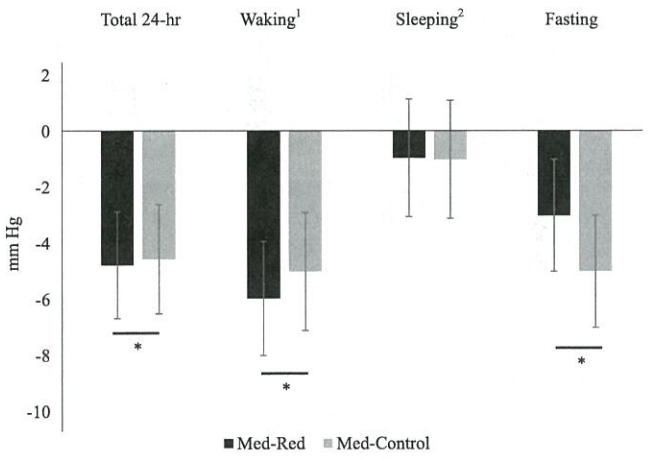

FIGURE 2 Changes in systolic blood pressures from consuming a Med-Red or Med-Control diet for 5 wk. Results are presented as LS means ± SEMs ($n = 41$). Data were analyzed using a doubly repeated-measures ANOVA adjusted for age, sex, and body mass at each time point. *Change over time. [1]Waking blood pressure: 0800–2100. [2]Sleeping blood pressure: 2230–0730. Diastolic blood pressure results followed similar patterns and are available in Supplemental Tables 1 and 2. LS, least squares; Med-Control, Mediterranean-style eating pattern with ~200 g lean, unprocessed red meat/wk; Med-Red, Mediterranean-style eating pattern with ~500 g lean, unprocessed red meat/wk.

Pattern study and the weight maintenance DASH Pattern studies previously mentioned (26, 29) showed no effect of these eating patterns on fasting glucose, insulin, or HOMA-IR, independent of red meat intake. One study compared the effects of energy-restricted DASH Patterns substituting plant protein with beef (12, 139, or 196 g lean unprocessed beef) combined with exercise on metabolic syndrome outcomes. The researchers concluded that weight loss was the primary modifier of metabolic improvements, independent of protein source (30). These studies support that Med and DASH Patterns are typically not effective at improving metabolic markers in the absence of weight loss or exercise (41–44). These eating patterns, particularly over the short term, are not suitable to assess the effects of red meat intake on changes in glycemic control. Future randomized controlled trials are warranted to assess the effects of lean, unprocessed red meat consumption on type 2 diabetes risk factors in eating patterns known to improve these outcomes.

There are different ways of quantifying the effectiveness of a nutrition intervention on CMD outcomes. Most commonly, researchers compare changes between groups or the differences between groups at the end of each intervention. In our study, 40 subjects provided >95% power to detect changes in fasting serum total-C and systolic blood pressure, and >85% power to detect a differential response between Med-Red and Med-Control. It is noteworthy that the postintervention values did not differ between Med-Red and Med-Control for any of the CMD risk factors measured, including those that showed differential changes (total-C, LDL cholesterol, and ApoB). The end of intervention values show that meat source did not influence Mediterranean Pattern–induced cardiometabolic responses. These results are consistent with previous studies that showed no postintervention differences in CMD risk factors between traditional DASH Patterns and DASH Patterns with higher red meat intake and similar macronutrient distributions (26, 28, 29).

Our randomized controlled trial is strengthened by a low dropout rate (<18%) and a successful washout period (baseline 1 measures were re-established at baseline 2), but is not without limitations. The self-reported >95% menu compliance was not objectively confirmed. Our results are not generalizable to all cuts of beef and pork because only tenderloins were provided to subjects. Future studies should include various types of lean, unprocessed red meat in a feasibility study to follow up on our findings. We were unable to supply or encourage consumption of red wine owing to university regulations, but slight differences in wine intake between the Med-Red and Med-Control groups did not influence the results. Although unintentional, 98% of our sample population was Caucasian. Future research is needed to assess whether race and/or ethnicity influences responses.

The 2000-kcal Mediterranean Pattern proposed by the Dietary Guidelines Advisory Committee (DGAC) contains ~300 g red meat/wk (45). The supporting literature base is largely prospective cohort studies that assess associations between red meat consumption and chronic disease in the context of a Western-style eating pattern (40, 46–48). Unhealthy lifestyle behaviors are correlated with red meat intake in this population which confounds the positive associations between red meat and chronic disease risk (49). The Mediterranean Pattern studies identified by the DGAC show low chronic disease risk with red and processed meat consumption up to ~1200 g/wk for a 2000-kcal diet (9). Our results, as well as the Mediterranean Pattern studies identified in the report, do not support red meat reductions in the context of a Mediterranean Pattern. Further, the DGAC did not assess the health effects of unprocessed red meat independent of processed meats (which includes red meat and poultry). There is building evidence that unprocessed red meat consumption has little to no influence on cardiometabolic disease risk compared with processed meats (11, 12). Future DGACs need not only to consider the amount of red meat included in a Mediterranean Pattern, but also to be cognizant of the leanness and degree of meat processing.

In conclusion, adults who are overweight or obese can consume typical US intake quantities of red meat (~70 g/d) as lean and unprocessed beef and pork when adopting a Mediterranean Pattern to improve cardiometabolic disease risk factors. Our results support previous observational and experimental evidence which shows that unprocessed and/or lean red meat consumption does not increase the risk of developing cardiovascular disease (11) or impair associated risk factors (13).

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Research. DP-J's relationships over the past 2 y include grant funding to support other research projects unrelated to the research presented in this article and/or travel reimbursements or honoraria from American Egg Board–Egg Nutrition Center, Leprino Foods, National Dairy Council, National Cattlemen's Beef Association, and US Dairy Export Council. DP-J also participates on scientific advisory panels. LEO and AJW declare no disclosures. None of the other authors report a conflict of interest related to research presented in this article.

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BEEF WISE STUDY: BEEF'S ROLE IN WEIGHT IMPROVEMENT, SATISFACTION AND ENERGY

Equivalent reductions in body weight during the Beef WISE Study
Sayer et al. Obesity Science & Practice, 2017- Vol 3

OBJECTIVE

To determine the impact of consuming lean beef as part of a high protein (HP) weight-reducing diet on changes in body weight, body composition, and cardiometabolic health.

STUDY DESIGN AND SETTING

A 16-week randomized, equivalence trial. Subjects were randomly assigned to consume either a HP diet with ≥ 4 weekly servings of lean beef (B) or a HP diet restricted in all red meats (NB).

PARTICIPANTS

Overweight or obese (BMI ≥ 27.0 kg/m2) men and women (18-50 yrs.) were recruited.

Additional inclusion criteria:

- Weight stable (±3 kg in previous 3 months)
- Able to progress to 70 min per day of moderate intensity exercise

Exclusion criteria:

- Pregnant or trying to become pregnant
- Diabetes
- LDL cholesterol > 160 mg/dL
- Triglycerides > 400 mg/dL
- Untreated or unstable hypothyroidism
- Medication use that could cause weight loss or gain
- Vegetarian or vegan
- Current eating disorder

RESULTS

- Body weight was reduced by 7.8 ± 5.9% in B and 7.7 ± 5.5% in NB.
- Fat mass was reduced in both groups (B: 8.0 ± 0.6 kg, NB: 8.6 ± 0.6 kg).
- Lean mass was not reduced in either group.
- Improvements in markers of cardiometabolic health (total cholesterol, low-density lipoprotein cholesterol, triglycerides, blood pressure) were not different between B and NB.

CONCLUSIONS

- Consuming lean beef within the context of a HP weight-reducing diet resulted in equivalent reductions in body weight and no difference in improvements of body composition and cardiometabolic health compared to a HP that was restricted in red meats.
- Results of the study demonstrate that HP diets, either rich or restricted in red meat intakes, are effective for decreasing body weight (especially body fat) and improving cardiometabolic health.

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ORIGINAL ARTICLE

Equivalent reductions in body weight during the Beef WISE Study: beef's role in weight improvement, satisfaction and energy

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Summary

Objective

The objective of this randomized equivalence trial was to determine the impact of consuming lean beef as part of a high protein (HP) weight-reducing diet on changes in body weight, body composition and cardiometabolic health.

Methods

A total of 120 adults (99 female) with overweight or obesity (BMI: 35.7 ± 7.0 kg m^{-2}) were randomly assigned to consume either a HP diet with ≥4 weekly servings of lean beef (B; $n = 60$) or a HP diet restricted in all red meats (NB; $n = 60$) during a 16-week weight loss intervention.

Results

Body weight was reduced by 7.8 ± 5.9% in B and 7.7 ± 5.5% in NB ($p < 0.01$ for both). Changes in percent body weight were equivalent between B and NB (mean difference: 0.06%, 90% confidence interval: (−1.7, 1.8)). Fat mass was reduced in both groups ($p < 0.01$; B: 8.0 ± 0.6 kg, NB: 8.6 ± 0.6 kg), while lean mass was not reduced in either group. Improvements in markers of cardiometabolic health (total cholesterol, low-density lipoprotein cholesterol, triglycerides and blood pressure) were not different between B and NB.

Conclusion

Results of this study demonstrate that HP diets – either rich or restricted in red meat intakes – are effective for decreasing body weight and improving body composition and cardiometabolic health.

Keywords: Body composition, lean body mass, obesity, red meats, weight loss.

Introduction

While there are many available options for achieving weight loss (1), higher protein (HP) diets have garnered considerable attention within the general populous and scientific community due to potential beneficial effects on satiety, postprandial thermogenesis, resting energy expenditure, body composition and certain cardiometabolic risk factors (2). Further, evidence from multiple systematic reviews and meta-analyses support modestly greater effects of HP compared to lower protein diets on weight/fat loss, lean mass retention, triglycerides and/or blood pressure (3–5). Although at least one meta-analysis found no beneficial (or detrimental) effect of HP diets on these outcomes (6).

The widespread interest in HP diets has led to research to determine the importance of protein source/type (e.g. animal vs. vegetable ((7,8)), soy (9), milk/dairy (10), red meats (8,11–13)) on weight loss and/or cardiometabolic outcomes. In particular, red meat (beef, pork, veal, lamb and mutton) has been the subject of substantial scientific debate (14–16). Recommendations to limit or restrict red meat consumption (17,18) are common due to positive associations between its consumption and cardiovascular diseases (19), type 2 diabetes (20) and cancer (21,22) in observational studies. According to the

2015 Dietary Guidelines for Americans, eating patterns that include lower intake of red meats are associated with reduced risk of obesity (18). However, findings from randomized controlled trials largely find no detrimental impact of lean red meat consumption on markers of cardiometabolic health during weight loss (8,23) and weight maintenance (11–13). Red meat is a major contributor to overall protein intake and represents 58% of all meat consumption in the United States (24). Therefore, its exclusion from the diet represents a potential barrier to the long-term adoption of HP diets.

Previous randomized clinical studies demonstrated that lean beef (11,12) and pork (13) can be effectively incorporated into dietary patterns for improving cardiometabolic health during weight maintenance conditions. At least two randomized clinical trials found that including red meat in energy-restricted diets does not negatively influence weight loss or improvements in cardiometabolic health (8,23). However, previous weight loss intervention trials were limited by relatively small sample sizes, absence of a HP control diet with no red meat (8,23) and the manipulation of multiple dietary components (23). Therefore, this randomized equivalence trial in 120 adults with overweight/obesity was conducted to determine the impact of consuming two HP diets (1: ≥4 weekly servings of lean beef [B] vs. 2: no red meat consumption [NB]) on weight loss, body composition and cardiometabolic health during a 16-week weight loss intervention. It was hypothesized that B and NB would result in equivalent weight loss (primary aim) and that beneficial changes in body composition and cardiometabolic health would not differ between groups.

Methods

Participants

One-hundred twenty individuals (99 female, 21 male) were recruited from the Denver, CO metropolitan area to participate in a behavioural weight loss study at the University of Colorado Anschutz Health and Wellness Center (AHWC; Figure 1). Inclusion criteria for the study were: male or female; age 18–50 years; BMI ≥ 27.0 kg m^{-2}; weight stable (± 3 kg in previous 3 months); able to progress to 70 min day^{-1} of moderate intensity exercise; willing to comply with all study procedures including attendance to 16 weekly classes and three study visits. Individuals were excluded from the study if: pregnant or trying to become pregnant; diagnosis of diabetes; LDL cholesterol >160 mg dL^{-1}; triglycerides >400 mg dL^{-1}; untreated or unstable hypothyroidism; medication use that could cause weight loss or gain; following vegetarian or vegan diet; current eating disorder; any medical condition for which consuming a HP diet and/or engaging in 70 min of exercise daily would be inadvisable. All participants provided written informed consent and received a monetary stipend. The consent form and all study procedures and documents were approved for use by the Colorado Multiple Institutional Review Board. Of the 120 participants who provided consent for the

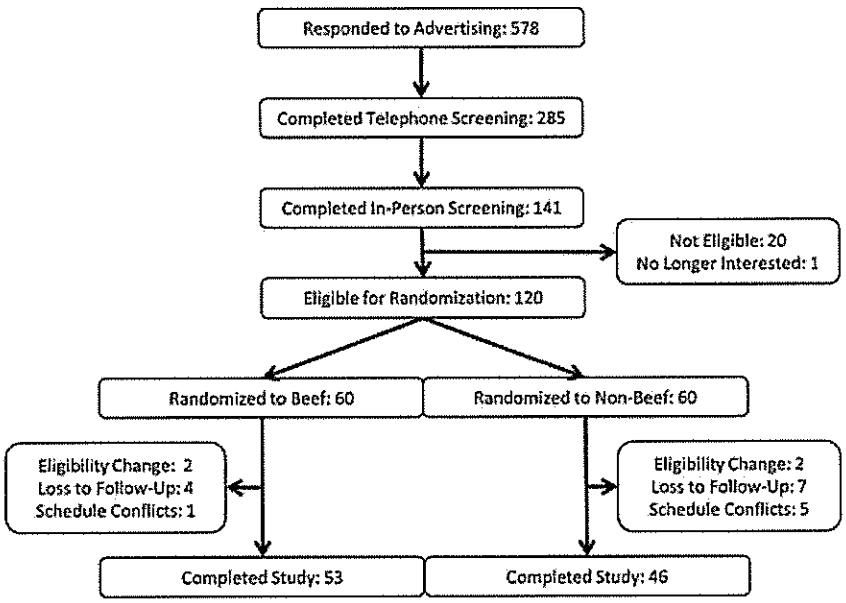

Figure 1 Participant recruitment diagram.

study, 99 individuals (83 female, 16 male) completed the 16-week intervention for a retention rate of 82.5% (Figure 1).

Experimental design

All participants participated in the *State of Slim* (SOS) weight management program, which is a 16-week group-based, lifestyle modification program (25). The program consisted of weekly classes of 20 participants that were stratified by diet assignment. A copy of the SOS book and access to the online SOS community were provided to all participants. Membership to the AHWC fitness facility was also provided to participants for the duration of their participation in the study. Participants were randomly assigned to one of two experimental diets; a HP diet with instructions to consume ≥4 weekly servings of lean beef as the only source of red meat (B) or a HP diet with instructions not to consume any red meat for the duration of the study (NB).

Body weight, body composition and indices of cardiometabolic health were measured at baseline and after completing the weight loss intervention (week 16). The study was registered on ClinicalTrials.gov (NCT02627105) and included a 2-month follow-up period following the 16-week weight loss intervention. Because the primary objective of the current study was to assess the equivalence of B and NB for weight loss during the active intervention, results from the follow-up period are not reported here.

Diet intervention

The SOS diet plan is HP, low in fat and emphasizes non-starchy (i.e. vegetable) and whole-grain carbohydrates (Table 1). The diet is plan is structured into three distinct phases with phase-specific food choices from which participants can chose to eat in defined portions rather than counting calories. The SOS diet plan utilizes five 'Diet Rules' through all three phases of the diet to encourage weight loss: (i) Eat 6 times per day; (ii) Have breakfast within 1 h of waking; (iii) Don't count calories, measure portions; (iv) Have the right carbohydrate and protein mix at every meal (one carbohydrate and one protein at every meal, vegetables as only carbohydrate source at three meals); and (v) Eat a healthy fat twice a day.

Participants completed daily food logs throughout the 16-week weight loss intervention. However, the logs were not designed or intended as a measure total energy consumption or macronutrient distribution. Rather, the logs were used as a self-monitoring tool to enhance weight loss (26) and as an indicator of beef consumption during the study. Self-reported energy intake and macronutrient distribution were not tracked during the study because a principal aspect of the SOS program is to focus on portion sizes rather than counting calories (Diet Rule #3 above). A detailed food log would therefore be inconsistent with the goals and structure of the program. Further, self-reported measures of food intake are highly unreliable, and their suitability for use in clinical research has been questioned (27).

Protein foods throughout the entire SOS program are lean and minimally processed (i.e. lean meat and poultry, fish, egg whites and fat-free dairy). Lean beef is included in all three phases of the published diet plan. Prescribed food lists and portion sizes for the SOS diet plan (as published in the SOS book) are presented in Tables 2–4. All participants in the study were provided with a SOS book and instructed to select foods from the list with additional group-specific instructions. Specifically, participants randomly assigned to B were instructed to consume ≥4 weekly servings of lean beef from the options included in the food lists. Participants assigned to NB were instructed not to consume any red meats (beef, pork, veal, lamb and mutton) for the duration of the study. Consuming four weekly servings of lean beef would result in ~20 ounces or 567 g (recommended portion size is 4–6 ounces) of total red meat consumption per week, which is comparable with total mean red meat consumption for US adults aged 20–49 years (80 grams per day) (24). Recommended sources of non-beef dietary protein and total recommended protein consumption were the same between B and NB.

Table 1 recommended energy, macronutrient distribution and fibre intake of the published *State of Slim* diet plan*

Nutrient	Phase 1 (Weeks 1–2)	Phase 2 (Weeks 3–8)	Phase 3 (Weeks 9–16)
Energy (kcals d^{-1})	1,644	1,754	1,834
Carbohydrate (%)	26	28	32
Protein (%)	50	45	40
Fat (%)	24	27	28
Fibre (g d^{-1})	16	25	28

*Nutrition Data System for Research (NDSR) was used to calculate approximate recommended energy, carbohydrate, protein, fat and fibre intakes during each phase of the SOS diet. The data for NDSR calculations were derived from phase-specific food lists, recommended portion sizes and sample menus published in the SOS book (25). Group-specific diet analyses (B vs. NB) were not completed as part of the study, but recommendations for total energy, carbohydrate, protein, fat and fibre intakes were the same for B and NB.

Table 2 *State of Slim* diet plan, Phase 1 (weeks 1–2)*

The leanest proteins (have one at every meal and snack)		
Meat and poultry		
	Beef, ground, extra lean	4–6 oz
	Beef, lean cuts	4–6 oz
	Chicken breast, without skin	4–6 oz
	Turkey breast, without skin	4–6 oz
	Turkey breast, lean ground	4–6 oz
Fish		
	Cod	4–6 oz
	Mahi mahi	4–6 oz
	Salmon†	4–6 oz
	Snapper	4–6 oz
	Tilapia	4–6 oz
	Tuna	4–6 oz
	White fish	4–6 oz
Egg and high-protein dairy		
	Cottage cheese, fat-free	8 oz
	Egg whites	1 cup or 5–6 whites
	Greek yogurt, nonfat plain	8 oz
Other		
	Protein powder	1 scoop
Reignite carbohydrates (have one at a maximum of three meals and snacks		
Grains		
	Oats, steel-cut	¼ cup dry
	Oats, old-fashioned rolled	½ cup dry
Dairy and dairy substitutes		
	Almond milk, unsweetened	1 cup
	Fat-free milk	1 cup
Starchy vegetables		
	Pumpkin	1 cup mashed
Vegetable carbohydrates (only carbohydrate at three meals or snacks a day, unlimited portions)		
Artichoke	Asparagus	Beets
Broccoli	Brussels sprouts	Cabbage and Chinese cabbage
Carrots	Cauliflower	Celery
Cucumbers	Dark leafy greens	Eggplant
Fennel	Green beans	Mushrooms
Onions and scallions	Parsnips	Peppers, sweet and hot
Salad greens, all varieties	Summer squash	Tomato and tomato sauce
Turnips and rutabagas	Zucchini	
Only the healthiest fats (include fats in two meals or snacks per day)		
Nuts		
	Almonds	15–18
	Walnuts	8–9 halves
Oils		
	Canola oil	1 tbsp
	Olive oil	1 tbsp

*This list is unedited from the published food list in the *State of Slim* book. All participants in the study were provided with a copy of the book and instructed to choose foods and portions from list and given additional group-specific dietary instructions (B: ≥4 weekly servings of lean beef but no other sources of red meat, NB: no red meats).
†Salmon also counts as 1 fat.
Abbreviations: oz, ounces; tbsp, tablespoon.

Anthropometric measurements

Body weight was measured using a digital platform scale (PS-6600 ST, Befour, Inc., Saukville, WI, USA) at baseline and week 16 in a fasted-state with the participant wearing light clothing and after voiding. Height was measured using a stadiometer at baseline. Body mass index (BMI; kg m^{-2}) was calculated using these measurements. Body

Table 3 *State of Slim* diet plan, phase 2 (weeks 3–8)*

The leanest proteins (have one at every meal and snack)		
Meat and poultry		
	Beef, ground, extra lean	4–6 oz
	Beef, lean cuts	4–6 oz
	Buffalo, lean cuts†	**4–6 oz**
	Canadian bacon	**4 oz**
	Chicken breast, without skin	4–6 oz
	Ostrich, lean cuts	**4–6 oz**
	Pork tenderloin	**4–6 oz**
	Turkey breast, without skin	4–6 oz
	Turkey breast, lean ground	4–6 oz
	Venison, lean cuts	**4–6 oz**
Fish		
	Cod	4–6 oz
	Crab	**4–6 oz**
	Lobster	**4–6 oz**
	Mahi mahi	4–6 oz
	Salmon‡	4–6 oz
	Scallops	**4–6 oz**
	Shrimp	**4–6 oz**
	Snapper	4–6 oz
	Tilapia	4–6 oz
	Tuna	4–6 oz
	White fish	4–6 oz
Egg and high-protein dairy		
	Cottage cheese, fat-free	8 oz
	Eggs, whole	**1, plus 3–4 whites**
	Egg whites	1 cup or 5–6 whites
	Greek yogurt, nonfat plain	8 oz
Other		
	Protein powder	1 scoop
Rebuild carbohydrates (have one at a maximum of three meals and snacks		
Fruit		
	Apple	**1 medium**
	Berries	**1 cup**
	Grapefruit	**1 medium**
Breads		
	Ezekiel Bread	**1 slice**
	Whole grain pita or tortilla	**1**
Grains		
	Barley	**½ cup cooked**
	Brown or wild rice	**½ cup cooked**
	Oats, steel-cut	¼ cup dry
	Oats, old-fashioned rolled	½ cup dry
	Quinoa	**½ cup cooked**
	Rice cakes	**2**
Dairy and dairy substitutes		
	Almond milk, unsweetened	1 cup
	Fat-free milk	1 cup
	Fat-free or part-skim ricotta cheese	**½ cup**
	Reduced-fat string cheese	**1–2 pieces**
Beans and starchy vegetables		
	Beans	**½ cup whole; ⅓ cup fat-free refried**
	Pumpkin	1 cup mashed
	Sweet potato	**4 oz, ½ cup mashed**
	Winter squash	**4 oz, ½ cup mashed**

Continues

Table 3. *Continued*

The leanest proteins (have one at every meal and snack)		
Vegetable carbohydrates (only carbohydrate at three meals or snacks a day, unlimited portions)		
Artichoke	Asparagus	Beets
Broccoli	Brussels sprouts	Cabbage and Chinese cabbage
Carrots	Cauliflower	Celery
Cucumbers	Dark leafy greens	Eggplant
Fennel	Green beans	Mushrooms
Onions and scallions	Parsnips	Peppers, sweet and hot
Salad greens, all varieties	Summer squash	Tomato and tomato sauce
Turnips and rutabagas	Zucchini	
Only the healthiest fats (include fats in two meals or snacks per day)		
Nuts		
	Almonds	15–18
	Pistachios	**25**
	Walnuts	8–9 halves
Oils		
	Canola oil	1 tbsp
	Olive oil	1 tbsp
Other		
	Avocado	**⅓ medium**
	Olives	**10 small or 5 medium/large**

*This list is unedited from the published food list in the *State of Slim* book. All participants in the study were provided with a copy of the book and instructed to choose foods and portions from list and given additional group-specific dietary instructions (B: ≥ 4 weekly servings of lean beef but no other sources of red meat, NB: no red meats).

†Boldface foods added in Phase 2.

‡Salmon also counts as 1 fat.

Abbreviations: oz, ounces; tbsp, tablespoon.

composition (fat and lean mass) was measured using dual x-ray absorptiometry (Discovery QDR DXA System, APEX software version 4.5, Hologic, Inc., Marlborough, MA, 01752, USA). Waist circumference (WC) was measured at the midpoint between the lowest rib and iliac crest in accordance with recommendations from the World Health Organization (28).

Cardiometabolic health

Two blood samples were obtained from an antecubital vein by a trained phlebotomist after an overnight fast at baseline and week 16. One sample was processed to obtain plasma, and analyses for glucose, total cholesterol, low-density lipoprotein cholesterol (LDL; calculated), high-density lipoprotein cholesterol (HDL) and triglycerides were completed by the UC Health Clinical Laboratory within 24 h of collection. A whole-blood sample was sent to the UC Health Clinical Laboratory and analysed for haemoglobin A1c (HbA1c) within 72 h of collection. Blood pressure (BP) was measured at the left upper arm by trained research staff using a manual sphygmomanometer after the participant rested quietly in a seated position for ≥5 min with his/her legs uncrossed and back and arms supported.

Statistical analysis

Study data were collected and managed using REDCap electronic data capture tools hosted at University of Colorado – Anschutz Medical Campus. REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies, providing: (i) an intuitive interface for validated data entry; (ii) audit trails for tracking data manipulation and export procedures; (iii) automated export procedures for seamless data downloads to common statistical packages; and (iv) procedures for importing data from external sources (29).

This randomized equivalence clinical trial was powered on the intent-to-treat (ITT) analysis using two one-sided *t*-tests (TOST) to establish equivalence for percent weight loss (primary outcome) between the B and NB groups. An interval of −2.5 to 2.5% of the between-group mean difference in percent weight loss over 16 weeks was considered clinically equivalent in this study. When delivered as a fee-based program at the AHWC, average percent weight loss during the SOS program is 10.4 ± 4.6%. With these parameters and 60 participants per arm, a statistical power calculation indicated there was 81% power at 5% significance to establish clinical

Table 4 *State of Slim* diet plan, phase 2 (weeks 9–16)*

The leanest proteins (have one at every meal and snack)		
Meat and poultry		
	Beef, ground, extra lean	4–6 oz
	Beef, lean cuts	4–6 oz
	Buffalo, lean cuts	4–6 oz
	Canadian bacon	4 oz
	Chicken breast, without skin	4–6 oz
	Filet mignon†	**4–6 oz**
	Lean deli meat	**4–6 oz**
	Lean ham	**4–6 oz**
	New York strip steak	**4–6 oz**
	Ostrich, lean cuts	4–6 oz
	Pork tenderloin	4–6 oz
	Turkey bacon	**4 slices**
	Turkey breast, without skin	4–6 oz
	Turkey breast, lean ground	4–6 oz
	Turkey sausage	**½–1 cup or 2 patties**
	Venison, lean cuts	4–6 oz
Fish		
	Cod	4–6 oz
	Crab	4–6 oz
	Lobster	4–6 oz
	Mahi mahi	4–6 oz
	Salmon‡	4–6 oz
	Scallops	4–6 oz
	Sea bass	**6–8 oz**
	Shrimp	4–6 oz
	Snapper	4–6 oz
	Tilapia	4–6 oz
	Trout	**6–8 oz**
	Tuna	4–6 oz
	White fish	4–6 oz
Egg and high-protein dairy		
	Cottage cheese, fat-free	8 oz
	Eggs, whole	1, plus 3–4 whites
	Egg whites	1 cup or 5–6 whites
	Greek yogurt, nonfat plain	8 oz
	Greek yogurt, nonfat or low-fat, flavoured	**8 oz**
Other		
	Protein bars	**1 bar**
	Protein powder	1 scoop
Reinforce carbohydrates (have one at a maximum of three meals and snacks		
Fruit		
	Apple	1 medium
	Apricots	**3 fruit or 1 cup**
	Banana	**1 fruit or 1 cup**
	Berries	1 cup
	Cherries	**1 cup**
	Dried cherries	**1 ½ tbsp**
	Grapes	**1 cup**
	Grapefruit	1 medium
	Kiwifruit	**1 fruit or 1 cup**
	Mango	**1 cup**
	Orange	**1 fruit or 1 cup**
	Peach	**1 fruit or 1 cup**
	Pear	**1 fruit or 1 cup**
	Plum	**1 fruit or 1 cup**
Breads		
	English muffin	**1**

Continues

Table 4. *Continued*

The leanest proteins (have one at every meal and snack)		
	Ezekiel Bread	1 slice
	Whole grain bagel thins	**1**
	Whole grain bread	**1 slice**
	Whole grain pita or tortilla	1
Grains		
	Barley	½ cup cooked
	Brown or wild rice	½ cup cooked
	Cereal, high-fibre, low sugar	**1 cup**
	Oats, steel-cut	¼ cup dry
	Oats, old-fashioned rolled	½ cup dry
	Quinoa	½ cup cooked
	Rice cakes	2
	Whole grain couscous	**½–1 cup cooked**
	Whole grain pasta	**½–1 cup cooked**
Dairy and dairy substitutes		
	Almond milk, unsweetened	1 cup
	Fat-free milk	1 cup
	Fat-free or part-skim ricotta cheese	½ cup
	Low-fat or reduced-fat cheeses	**¼ c grated or 1 oz**
	Nonfat or low-fat regular yogurt, fruit-flavoured or plain	**6–8 oz**
	Reduced-fat string cheese	1–2 pieces
Beans and starchy vegetables		
	Beans	½ cup whole; ⅓ cup fat-free refried
	Baked potato	**1 medium, 6–8 oz**
	Corn	**1 cup or 1 medium ear**
	Edamame	**½ cup shelled**
	Peas	**1 cup**
	Pumpkin	1 cup mashed
	Sweet potato	4 oz, ½ cup mashed
	Winter squash	4 oz, ½ cup mashed
Vegetable carbohydrates (only carbohydrate at three meals or snacks a day, unlimited portions)		
Artichoke	Asparagus	Beets
Broccoli	Brussels sprouts	Cabbage and Chinese cabbage
Carrots	Cauliflower	Celery
Cucumbers	Dark leafy greens	Eggplant
Fennel	Green beans	Mushrooms
Onions and scallions	Parsnips	Peppers, sweet and hot
Salad greens, all varieties	Summer squash	Tomato and tomato sauce
Turnips and rutabagas	Zucchini	
Only the healthiest fats (include fats in two meals or snacks per day)		
Nuts		
	Almond butter	**1 tbsp**
	Almonds	15–18
	Peanut butter	**1 tbsp**
	Pistachios	25
	Walnuts	8–9 halves
Oils		
	Canola oil	1 tbsp
	Olive oil	1 tbsp
Other		
	Avocado	⅓ medium
	Hummus	**¼ cup**
	Olives	10 small or 5 medium/large

*This list is unedited from the published food list in the *State of Slim* book. All participants in the study were provided with a copy of the book and instructed to choose foods and portions from list and given additional group-specific dietary instructions (B: ≥4 weekly servings of lean beef but no other sources of red meat, NB: no red meats).

†Boldface foods added in Phase 3.

‡Salmon also counts as 1 fat.

Abbreviations: oz, ounces; tbsp, tablespoon.

equivalence between two treatments using TOST or the between group difference in least square means (LSMEANS) plus a 90% confidence interval (CI). This power analysis assumed no expected difference between groups.

Demographic, baseline clinical and lab data were summarized by treatment groups (B vs. NB) using descriptive statistics and reported as mean ± SD. Imbalance in these data was examined using Student's *t*-tests. Any significantly imbalanced confounding variables from the analysis of baseline data were adjusted using a linear regression model. Any participants with one or more observations after intervention were analysed, and baseline-observation-carried-forward (BOCF) was used for missing data points at week 16. Equivalence was assessed a 90% CI of the mean between-group difference in % weight loss between two groups, which is equivalent to using TOST. Changes in body weight are reported as % body weight loss (mean ± SD).

Changes in body composition and cardiometabolic health were secondary outcomes, and *a priori* statistical power calculations were not completed to determine equivalence in these outcomes. In order to assess the between- and within-group differences, a linear mixed effects model was used to test effects of time (baseline vs. week 16), group (B vs. NB) and their interaction term on changes in body composition (fat mass, lean mass and WC) and cardiometabolic health (glucose, total cholesterol, LDL, HDL, triglycerides, HbA1c and BP). Changes in body composition and cardiometabolic health are reported as LSMEANS ±SE, and $\alpha = 0.05$ was used to determine statistical significance.

Results

Participant characteristics

The mean age of participants was 37.6 ± 8.1 years with BMI of 35.7 ± 7.0 kg m^{-2} at baseline. Indices of cardiometabolic health were within normal reference ranges (Table 5). Participants randomly assigned to B were 3 years younger than participants assigned to NB (36.0 ± 8.3 years vs. 39.3 ± 7.8 years, $p = 0.026$). Compared to those who completed the study, participants who withdrew from the study were younger (33.3 ± 8.1 years vs. 38.5 ± 7.9 years, $p = 0.010$) and had lower fasting total (147.1 ± 23.3 mg dL^{-1} vs. 171.5 ± 34.4 mg dL^{-1}, $p = 0.0037$) and LDL (82.1 ± 20 mg dL^{-1} vs. 103.3 ± 28.7 mg dL^{-1}, $p = 0.0027$) cholesterol concentrations at baseline. More participants withdrew from NB ($n = 14$) than B ($n = 7$, Figure 1), but differences in attrition were not statistically significant ($p = 0.22$).

Table 5 Baseline participant characteristics ($n = 120$)†

Parameter	All	Beef	Non-beef
Age (year)	37.6 ± 8.1	36.0 ± 8.3	39.3 ± 7.8*
Body weight (kg)	101.1 ± 22.8	100.8 ± 21.9	101.5 ± 24.0
BMI (kg m^{-2})	35.7 ± 7.0	35.9 ± 6.8	35.4 ± 7.1
Glucose (mg dL^{-1})	94.0 ± 9.7	94.0 ± 10.4	94.1 ± 9.0
Total cholesterol (mg dL^{-1})	167.6 ± 34.0	168.6 ± 35.6	166.6 ± 32.5
LDL (mg dL^{-1})	99.9 ± 28.5	101.4 ± 30.5	98.3 ± 26.5
HDL (mg dL^{-1})	46.6 ± 10.1	45.4 ± 9.2	47.9 ± 10.8
Triglycerides (mg dL^{-1})	103.6 ± 50.3	107.2 ± 49.0	100.0 ± 51.8
Haemoglobin A1c (%)	5.5 ± 0.4	5.4 ± 0.4	5.5 ± 0.4
Systolic BP (mm Hg)	116.5 ± 11.1	115.5 ± 10.3	117.4 ± 11.8
Diastolic BP (mm Hg)	76.4 ± 8.4	75.6 ± 8.6	77.2 ± 8.1

†All values are mean ± SD.
*Indicates significant difference ($p < 0.05$) between beef and non-beef by unpaired *t*-test (SAS Proc Ttest).
BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol.

Beef intake

Participants were instructed to complete daily food logs as a self-monitoring tool and an indicator of beef consumption during the 16-week intervention. Food log completion was the highest during Phase 1 (B: 6.5 ± 1.8 days of week 2, NB: 6.3 ± 1.8 days of week 2), but decreased over the course of the intervention ([Phase 2: B: 4.8 ± 3.2 days of week 8, NB: 4.6 ± 3.3 days of week 8], [Phase 3: B: 3.1 ± 3.5 days of week 16, NB: 4.0 ± 3.5 days of week 16]).

Participants assigned to B reported consuming 5.6 ± 2.0 weekly servings of lean beef during Phase 1 (week 2), 4.65 ± 1.7 weekly servings of lean beef during Phase 2 (week 8), and 5.75 ± 1.8 weekly servings of lean beef during Phase 3 (week 16) of the SOS diet, and reported no additional sources of red meat during any phase of the SOS diet. No participants assigned to NB reported beef or red meat consumption during any phase of the SOS diet.

Weight loss and body composition

Percent weight loss was equivalent in B and NB (B: 7.8 ± 5.9% vs. NB: 7.7 ± 5.5%, Figure 2). Total body mass and fat mass were significantly reduced at week 16 compared to baseline in B and NB with no differences between groups (Figure 3). Total lean mass was not different at the conclusion of the intervention compared to baseline (Figure 3). Waist circumference was reduced at the end of the intervention in both groups, but the reduction was greater in NB compared to B (10.6 ± 1.0 cm vs. 7.0 ± 1.0 cm, $p = 0.034$). However, reductions in trunk fat measured by DXA were not different between B (4.4 ± 0.4 kg) and NB (4.7 ± 0.4 kg; $p = 0.55$).

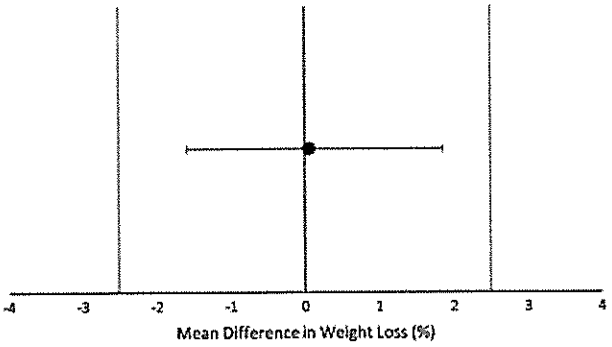

Figure 2 Mean difference in weight loss between Beef and Non-beef groups. Equivalence was assessed using a 90% CI of the mean between-group difference in % weight loss between two groups. An interval of −2.5% to 2.5% (vertical bars) of the between-group mean difference in percent weight loss over 16 weeks was considered clinically equivalent. Changes in body weight were equivalent between Beef and Non-beef groups.

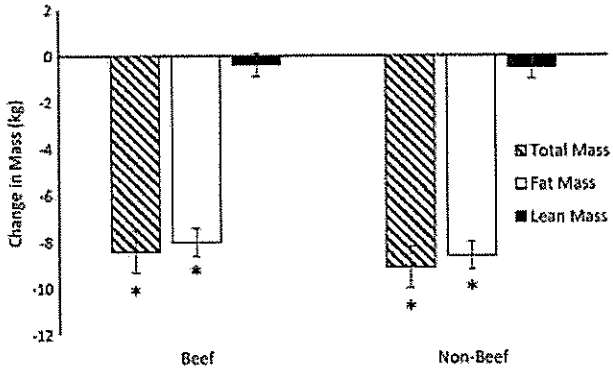

Figure 3 Changes in total, fat and lean mass. Linear mixed models (SAS, Proc Mixed) were used to assess changes in total, fat and lean between groups (Beef vs. Non-beef) and over time (Baseline vs. Week 16). Significant reductions in total and fat mass were observed that did not differ between Beef and Non-beef. Lean mass at Week 16 was not significantly different from Baseline in either group. Change in mass presented as LSMEANS ± SE from linear mixed model and * indicates a significant change from Baseline.

Cardiometabolic health

In both B and NB, total cholesterol, LDL, triglycerides, systolic BP and diastolic BP were reduced at week 16 compared to baseline with no differences between groups (Table 6). High-density lipoprotein cholesterol, glucose and HbA1c did not change over 16 weeks (Table 6).

Discussion

Consistent with the hypotheses, the B and NB diets produced equivalent reductions in body weight and improvements in body composition and cardiometabolic health. While there was a greater decrease in WC in the NB group, no differences in amount of trunk fat assessed by DXA were observed. Findings from the current study indicate that lean beef can be effectively incorporated into a HP diet for weight loss and improving body composition and cardiometabolic health. Weight loss in the current study (~8%) was slightly lower than the average weight loss for SOS when delivered as a fee-based program through the AHWC (~10%). This discrepancy in weight loss between SOS when delivered in research vs. commercial settings likely occurred due to the use of ITT analysis with BOCF for non-completers in the current study. In addition, participants in the current study received the SOS program free-of-charge, and it is possible that paying for the program – as in the commercial program at the AHWC – could enhance motivation and result in greater weight loss.

A relative retention of lean mass during weight loss is a commonly cited benefit of high vs. standard protein diets (2) that is supported by results from meta-analyses in young, middle-aged (5) and older adults (3). While there was no standard protein group for comparison, the virtually complete retention of lean mass observed in the current study deserves mention. Approximately 95% of changes in total mass were due to changes in fat mass, and lean mass was not significantly reduced by the weight loss intervention in either group. Future research comparing the SOS weight loss program with a HP diet vs. a standard protein diet is warranted in light of past observations that ~25% of typically observed reductions in total body mass are due to a loss of lean mass (3,30).

The upper age limit for the current study was 50 years, and the average age of participants was 38 years. The impact of the SOS weight loss program on lean mass retention should be tested in older adults, particularly those with or at risk for sarcopenic obesity. Weight loss is often discouraged in these individuals due to justifiable concerns regarding frailty, disability and loss of independence related to skeletal muscle loss (30). Thus, effective weight loss interventions that preferentially reduce body fat would substantially influence strategies for the prevention and treatment of sarcopenic obesity.

Results of the current study add further support to other evidence from randomized clinical trials demonstrating that consuming lean, minimally processed red meats does not adversely affect weight loss (8,23) or improvements in indices of cardiometabolic health when consumed as part of 'healthy' dietary patterns (11–13,31). The current study builds upon findings from past research by investigating the impact of lean beef consumption within the context of a HP diet for weight loss in a large randomized equivalence trial. Achieving ≥5% weight loss is widely recognized to elicit health

Table 6 Parameters of cardiometabolic health*

Parameter	Group	Baseline	Week 16	Difference†	*P*-value
Glucose (mg dL^{-1})	Beef	94 (1)	92 (1)	2 (1)	0.065
	Non-beef	94 (1)	93 (1)	1(1)	0.272
	Difference‡	0 (2)	−1 (2)	1 (1)	0.645
Cholesterol (mg dL^{-1})	Beef	169 (4)	156 (4)	12 (3)	<.001
	Non-beef	167 (4)	153 (4)	14 (3)	<.001
	Difference	2 (6)	3 (6)	−1 (4)	0.711
LDL (mg dL^{-1})	Beef	101 (3)	93 (4)	8 (2)	<.001
	Non-beef	98 (4)	89 (4)	9 (2)	<.001
	Difference	3 (5)	4 (5)	−1 (3)	0.851
HDL (mg dL^{-1})	Beef	45 (1)	46 (1)	0 (1)	0.576
	Non-beef	48 (1)	47 (1)	1 (1)	0.328
	Difference	−3 (2)	−1 (2)	−1 (1)	0.273
Triglycerides (mg dL^{-1})	Beef	107 (1)	85 (6)	22 (5)	<.001
	Non-beef	100 (6)	82 (6)	18 (5)	<.001
	Difference	7 (9)	4 (9)	3 (7)	0.628
Haemoglobin A1c (%)	Beef	5.39 (0.05)	5.33 (0.05)	0.06 (0.03)	0.089
	Non-beef	5.52 (0.05)	5.50 (0.05)	0.03 (0.04)	0.453
	Difference	−0.13 (0.07)	−0.17 (0.07)	0.03 (0.05)	0.548
Systolic BP (mm Hg)	Beef	116 (2)	111 (2)	5 (1)	<.001
	Non-beef	117 (2)	109 (2)	8 (1)	<.001
	Difference	−2 (2)	1 (2)	−3 (2)	0.097
Diastolic BP (mm Hg)	Beef	76 (1)	72 (1)	3 (1)	<.001
	Non-beef	77 (1)	72 (1)	5 (1)	<.001
	Difference	−2 (2)	0 (2)	−1 (1)	0.327

*Values are LSMEANS (SE) and rounded to the nearest whole number (except haemoglobin A1c).

†Within group changes calculated as Baseline − Week 16 (positive numbers indicate reduction in parameter). Differences may not exactly reflect values for Baseline and Week 16 due to rounding.

‡Between group differences calculated as Beef − Non-beef. Differences may not exactly reflect values for Beef and Non-Beef due to rounding. A linear mixed effects model (SAS, Proc Mixed) was used to test effects of time (Baseline vs. Week 16), group (Beef vs. Non-beef), and their interaction term on changes in glucose, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, haemoglobin A1c and blood pressure. Total cholesterol, LDL-cholesterol and triglycerides were reduced at Week 16 vs. Baseline but there were no differences between Beef and Non-beef.

BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol.

benefits (32–34), and the current study demonstrates that regularly consuming lean beef for 16 weeks does not influence weight loss or the resultant improvements in cardiometabolic health. These findings are consistent with those of Ziegler *et al.* (23) and Hill *et al.* (8) indicating that weight loss improved cardiac vagal function and metabolic syndrome criteria, respectively, independent of red meat consumption. A recent meta-analysis of randomized controlled trials also concluded that consuming ≥0.5 daily servings of red meat does not influence blood lipids/lipoproteins or BP compared to consuming <0.5 servings of red meat/d (35). Participants randomized to B in the current study were instructed to consume ≥4 weekly servings of lean beef, which is ~0.6 servings of red meat/d and further corroborates the results of the meta-analysis.

The overall participant retention rate was 82.5% for the current study, but dropout rates differed by diet assignment. However, numerical differences in retention rates between B and NB were not confirmed statistically. Fifty-three of 60 participants (88%) randomly assigned to B completed the study compared to 46 of 60 (77%) of those assigned to NB. Greater perceived diet/nutritional deprivation has been associated with poorer dietary adherence (36,37). The SOS diet plan includes lean beef along with other protein sources that are low in fat and saturated fat (25), and both groups followed the same SOS plan except for the NB group was instructed to abstain from consuming beef. It is possible that the inclusion of lean beef in the published diet plan coupled with the broad popularity of beef (24) led to greater feelings of deprivation and diet inflexibility in the NB group leading to a greater dropout rate.

A major strength of the current study is the use of a randomized equivalence trial design (38). Previous work by our group indicated that the equivalence design was more conservative (least likely to show group-level differences) than several alternative methods including

linear mixed models, multiple imputation, ANCOVA and independent *t*-tests (39). The use of a popular and evidence-based weight loss program (SOS) (25) represents an additional strength of the current study. Participants assigned to B received the SOS program with very limited alterations (non-beef sources of red meat were excluded) and is therefore available to the general public through the published book and/or participation in the commercial, fee-based SOS program.

The current study is limited in some aspects including the lack of a standard defined protein control group, which would allow more definitive conclusions regarding the impact of the HP diets on study outcomes, especially the observed lack of changes in lean mass. Although no influence of sex was observed for any study outcomes, the majority of participants in the current study were women and results may not fully extrapolate to men. Last, the current study was of relatively short duration, and the results should not be extrapolated beyond the constraints of the study design (i.e. 16 weeks, majority of participants as women, age limited to 18–50 years). Future studies of longer duration and in diversified populations are required to fully evaluate the effectiveness of consuming red meat during weight loss and for long-term weight loss maintenance.

In conclusion, consuming lean beef within the context of a HP weight-reducing diet resulted in equivalent reductions in body weight and no difference in improvements of body composition and cardiometabolic health compared to a HP that was restricted in red meats. Results of this study demonstrate that HP diets – either rich or restricted in red meat intakes – are effective for decreasing body weight (especially body fat) and improving cardiometabolic health.

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Author Contributions

J.O.H., H.R.W. and J.C.P. conceived the research project; R.D.S. and K.J.S. conducted the research; R.D.S. and Z. P. performed the statistical analyses; R.D.S. drafted the manuscript, and K.J.S., J.O.H., H.R.W., J.C.P. and Z.P. provided critical feedback and edits to the manuscript. All authors take responsibility for the final content of the manuscript.

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