

Pork with a high content of polyunsaturated fatty acids lowers LDL cholesterol in women¹⁻⁴

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ABSTRACT

Background: Animal products contribute significantly to the saturated fat and cholesterol content of the American diet. Contrary to dietary advice, consumers have not limited their consumption of animal products. Thus, an alternative approach might be to modify the fatty acid composition of animal products.

Objective: We tested the hypothesis that modified pork with a high content of polyunsaturated fatty acids (PUFAs) and a low content of saturated fatty acids (SFAs) would lower plasma LDL-cholesterol concentrations in women.

Design: Twenty women aged 19–24 y completed a crossover study with 2 diets. Nutritionally complete diets containing 42% of energy from fat differed only in the inclusion of either standard or modified pork. Venous blood samples were collected at weeks 0, 4, and 8.

Results: The diet containing modified pork significantly lowered total plasma ($P < 0.0076$) and LDL ($P < 0.0382$) cholesterol. The modified diet also resulted in an increase in the PUFA and a decrease in the SFA and monounsaturated fatty acid contents of the cholesteryl ester, free fatty acid, phospholipid, and triacylglycerol lipid classes in both plasma and erythrocytes. Plasma concentrations of glucose, insulin, triacylglycerol, and free fatty acids did not change significantly.

Conclusions: Consumption of pork with a high PUFA content resulted in a decrease in the subjects' total plasma and LDL cholesterol and shifted the fatty acid composition from SFAs to PUFAs in the plasma and erythrocytes. Modification of the fatty acid composition of animal foods will be a useful approach to lowering the saturated fat consumption of Americans. *Am J Clin Nutr* 2001;74:179–87.

KEY WORDS Designed foods, pork, fatty acids, polyunsaturated fatty acids, cholesterol, cardiovascular disease, low-density lipoproteins, LDL cholesterol, women

INTRODUCTION

Cardiovascular disease is the leading cause of death in the United States. More than 961 000 persons died from cardiovascular disease or stroke in 1998 and >58 million Americans have one or more types of cardiovascular disease, such as hypertension, coronary artery disease, stroke, and rheumatic heart disease (1). Some of the risk factors that can be modified to reduce the chances of developing cardiovascular disease are concentrations of LDL cho-

lesterol, HDL cholesterol, and triacylglycerol. One method used to control the concentrations of plasma lipids is to limit the amount of dietary fat and to control the specific fatty acids consumed.

The dietary energy currently consumed by Americans is composed of 33% fat, 15% protein, 50% carbohydrates, and 2% ethanol. The dietary energy from fat consists of 13% saturated fatty acids (SFAs), 13% monounsaturated fatty acids (MUFAs), and 7% polyunsaturated fatty acids (PUFAs). The average daily intake of cholesterol is 277 mg/d (2, 3). In the United States, 88% of the population consumes meat, poultry, or fish (3). Pork accounts for 29% and beef accounts for 50% of the total muscle foods consumed (3). All animal products collectively provide 56% of the total fat, 74% of the saturated fat, 70% of the protein, and 100% of the cholesterol consumed (4).

Manipulations of diets that decrease the total fat content or substitute unsaturated vegetable oils for saturated fats result in reductions in total plasma and LDL-cholesterol concentrations. These changes indicate that dietary reductions in saturated fats achieved by substituting MUFAs and PUFAs decrease concentrations of total, LDL, and HDL cholesterol (5–15).

We expect that consumers will continue to prefer to consume traditional food products, such as pork. Therefore, we evaluated the dietary effect of one variety of redesigned pork that contained higher amounts of PUFAs and lower amounts of SFAs on selected indexes of lipid metabolism in college-aged women.

SUBJECTS AND METHODS

Pork production

The Iowa State University Committee on Animal Care approved the procedures used for pork production at the Iowa State University

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TABLE 1
Composition of the diets fed to pigs¹

	Standard	Modified
Ingredient (g/kg dry matter)		
Ground shelled corn	858.4	462.0
Soybean meal ²	123.0	249.3
Soy oil ³	—	222.2
Solfo Flocc ⁴	—	46.7
Dicalcium phosphate	4.2	6.7
Calcium carbonate	9.2	7.8
Sodium chloride	2.7	2.8
Mineral-vitamin mix ⁵	2.5	2.5
Feed efficiency		
(g gain/kg feed)	308	468
(kg feed/kg gain)	3.25	2.14
Fat content (% of energy)	7	43

¹The standard diet contained standard pig rations. The modified diet contained rations modified to contain soy oil.

²Defatted, dehulled soybean meal provided 0.48 g crude protein/g soybean meal.

³Donated by Central Soya, Inc, Fort Wayne, IN, through the courtesy of D Strayer.

⁴Purchased from Fiber Sales and Development Corp, Urbana, OH.

⁵The commercial mixture contained the following per kg: 910 g calcium carbonate, 201.8 g magnesium oxide, 15.87 g copper sulfate, 503.1 mg iodine (ethylenediamine dihydroiodide), 103.2 g iron sulfate, 26.6 g manganese oxide, 266.66 mg sodium selenate, 44.44 g zinc oxide, 3.42 g vitamin A from retinyl acetate (650 000 IU/g), 1 g cholecalciferol (529 000 IU/g), 7.93 g vitamin E from *all-rac- α -tocopheryl* acetate, 291 mg menadione (K₃) sodium bisulfate complex, 38 269 mg choline, 14 108 mg niacin, 7054 mg D-calcium pantothenate, 1763 mg riboflavin, and 11 mg cyanocobalamin.

Swine Breeding Farm. Ten barrow pigs were divided into 2 groups. Each group was fed a different ration as listed in **Table 1**. The standard pig ration is typical of current farrow-to-finish farming operations in Iowa. The modified pig ration was supplemented with soybean oil so that 40% of the total energy in the ration was from soybean oil. Defatted, dehulled soybean meal was added to increase the dietary protein content to the amount recommended by the National Research Council (16). The ground, shelled corn was adjusted to attain the desired energy content. The pigs weighed 16 kg at the initiation of the regimen and were fed for 90 d until they reached \approx 110 kg. The pigs were then slaughtered at the US Department of Agriculture-inspected Iowa State University Meat Laboratory. From each group of pigs, boneless pork chops were saved and the remainder of the edible meat was ground and mixed to \approx 18% fat content and then frozen and stored until used to prepare the experimental meals for the human subjects. Lard was rendered with the inclusion of butylated hydroxyanisole and butylated hydroxytoluene (Alferi, Little Chute, WI) at 110 mg/kg lard. Lard was stored at 4°C until used to prepare the experimental meals.

Subject selection

The Iowa State University Human Subjects Committee approved the procedures for the human experiment, which was conducted in the metabolic unit of the Center for Designing Foods at Iowa State University. Twenty-four women aged 19–24 y were chosen after the assessment of multiple factors as listed in **Table 2**. The experimental regimen and the responsibilities of the subjects

were explained to and discussed with all subjects. Subjects signed informed consent statements. Experimental groups were assigned so that the following variables were matched: initial concentrations of total, HDL, and LDL cholesterol in serum; body mass index; and waist-to-hip ratio. Three subjects dropped out of the experiment during the first week and one subject was dismissed. A fifth subject completed 4 wk and then withdrew from the study. Subjects were instructed to eat only the food provided. Use of oral contraceptives was suspended 1 mo before the experiment and during the 8 wk of the experiment.

Experimental design

The subjects were free-living throughout the entire study. All subjects were requested to maintain a dietary log of their self-selected diets for 7 d before the start of the experimental feeding. On Monday through Friday of each experimental week, the subjects consumed all meals in the metabolic unit. After the Friday evening meal, the subjects were given meals for Saturday and Sunday in microwave-safe containers. The experimental design was a balanced crossover design with 2 diets (4 groups). Each combination was represented in the 4 groups. At week 0, the subjects were divided into 2 groups and fed complete diets that contained either the standard pork and lard or the modified pork and lard with a high PUFA content. After 4 wk, each group was divided to yield 4 subgroups. Two subgroups continued to be fed the original diet (standard or modified) and the 2 remaining subgroups were crossed over to the other diet. All experimental regimens were continued for an additional 4 wk.

Experimental diets

Dietary composition was calculated by using NUTRITION-IST 4 (version 2.0; N-Squared Computing, Salem, OR). The calculated composition of the standard diet was used to develop 7 menus, one for each day of the week. The same menus were used throughout all 8 wk of the experiment. The menus consisted of common foods and met all recommended dietary allowances (17). The lard and meats from the standard and modified pork were the only sources of fat used in the recipes developed. The calculated diets contained \approx 8368 kJ (2000 kcal)/d. For each diet, an extra plate of every meal was prepared and

TABLE 2
General characteristics of the women selected as experimental subjects

Factor	Desired range	Mean value ¹
General health status	Good to excellent	—
Food allergies	None	—
Medications	None	—
Menses	Normal cycle	—
Smoking status	Nonsmoker	—
Family medical history	Absence of heart disease	—
Blood chemistry values	Normal	—
Body fat content (%) ²	18.2–33.8	25.7 \pm 0.9
Body mass index (kg/m ²)	18.6–26.6	22.1 \pm 0.4
Waist-to-hip ratio	0.67–0.77	0.73 \pm 0.01
Total cholesterol (mmol/L)	3.336–4.965	4.161 \pm 0.084
LDL cholesterol (mmol/L)	1.681–3.543	2.539 \pm 0.096
HDL cholesterol (mmol/L)	0.828–1.603	1.112 \pm 0.044
Triacylglycerol (mmol/L)	0.598–1.885	1.106 \pm 0.076
Glucose (mmol/L)	3.830–5.218	4.732 \pm 0.054

¹ $\bar{x} \pm$ SEM; $n = 24$.

²As assessed by total-body electrical conductivity.

combined into a daily composite sample. The daily collections of food were frozen and saved for proximate analysis and determination of cholesterol content and fatty acid composition. The subjects were allowed to drink ≤ 710 mL (24 oz) of an energy-free beverage daily and to chew ≤ 5 sticks of sugarless gum daily. Water consumption was allowed ad libitum. All extra beverages and sticks of gum were recorded and averaged 42.63 kJ (10.19 ± 2.99 kcal)/d.

Anthropometric measurements

Height, weight, total-body electrical conductivity (EM-SCAN, Springfield, IL) (18–20), and waist-to-hip ratios (21) were measured at weeks 0, 4, and 8. Body weights were recorded weekly to ensure that the subjects maintained a constant weight. For the 6 subjects who lost weight over 2 consecutive weeks, extra muffins containing the appropriate lard were given daily until completion of the experiment to increase energy intake.

Preparation of plasma, platelets, erythrocytes, and lipoproteins

At weeks 0, 4, and 8, the subjects fasted after the Wednesday evening meal. All blood was collected on Thursday mornings. Clinical blood chemistry measurements (Metropolitan Reference Laboratories, St Louis) were made in serum. Complete blood counts were measured in EDTA-treated whole blood (Metropolitan Reference Laboratories). Additional EDTA-treated blood samples were collected for analysis of plasma lipids, lipoproteins, and plasma metabolites. Erythrocytes, platelets, and plasma were separated in clear polypropylene 15-mL centrifuge tubes as described by Garcia et al (22). The platelet-poor plasma samples were transferred to new polypropylene test tubes and 3.0 mL was saved for lipoprotein separation as outlined below. The remaining plasma, erythrocyte, and platelet samples were layered with nitrogen gas and frozen at -80°C until analyzed.

Lipoproteins were fractionated by the sequential flotation method of Havel et al (23) and Lindgren (24). The saved 3 mL plasma was placed into thick-wall, polycarbonate (13×64 mm) ultracentrifuge tubes. Coomassie blue (20 μL containing 50 g/L) was added to a reference sample. The samples then were layered with the appropriate density solutions and placed into a Beckman Ti 50.3 rotor and centrifuged for 18 or 22 h at $109000 \times g$ and 18°C (L8-M or L3–50; Beckman, Palo Alto, CA). Densities used for separation were 950–1006 g/L for VLDL, 1006–1063 g/L for LDL, 1063–1125 g/L for HDL₂, and 1125–1210 g/L for HDL₃. After separation, the lipoprotein fractions were collected and placed into graduated cryogenic storage vials (Perfecto Scientific, Atascadero, CA), layered with nitrogen gas, and frozen at -80°C until analyzed.

Analytic procedures

Composite daily food samples were weighed and homogenized at 4°C in a blender equipped with a temperature control coil (Waring Commercial 4 Liter Laboratory Blender and 4 L 2610T coil; Waring, New Hartford, CT) connected to a cryogenic cooler (model 2050; Caron, Marietta, OH). Two weighed aliquots were collected, one for proximate analysis and a second for determination of fatty acid composition. The first weighed aliquot was frozen, lyophilized (Virtis, Gardiner, NY), and stored in a desiccator at 4°C until used for proximate analysis. The samples were digested in 20 mL of 18 mol H_2SO_4 /L and 27 mmol SeO_2 /L at 420°C for 1 h in a Kjeldahl digester (Tecator, Höganäs, Sweden). Nitrogen in the digested food sample

was assayed by the method of Chaney and Marbach (25). The nitrogen concentration was multiplied by 6.25 to yield total protein in grams. Fat was extracted from a weighed portion of the lyophilized samples by the method of Folch et al (26) with chloroform:methanol (2:1, by vol). Optima grade solvents (Fisher Scientific, Chicago) were used for all chemical analyses. The lipid extracts were filtered through sodium sulfate on filter paper. The solvent was evaporated from a weighed beaker on a steam table. The lipid content was determined gravimetrically and the carbohydrate content was calculated by difference.

The second weighed aliquot of the homogenized food composite sample was used to determine fatty acid composition. Lipids were extracted by the method of Folch et al (26) with minor modifications. First, 250 μL of 3.7 mmol heptadecanoic acid/L ethanol was added to each sample as an internal standard. Samples were saponified with 6 mL 5% KOH in 95% ethanol for 1 h at 80°C . Nonsaponifiable lipids were extracted 3 times with 5 mL petroleum ether and saved for cholesterol analysis. The remaining saponifiable lipids were acidified with 2 mL of 12 mol HCl/L, and the fatty acids were extracted 3 times with 5 mL petroleum ether. The fatty acid methyl esters (FAMES) were prepared as described by Morrison and Smith (27). FAMES were analyzed by gas chromatography on a gas chromatograph (model 6890; Hewlett-Packard, Palo Alto, CA) fitted with a highly polar, polyethylene glycol DB-WAX capillary column (J & W, Folsom, CA) that was $30.0 \text{ m} \times 250 \mu\text{m} \times 0.25 \mu\text{m}$. The injector and detector temperatures were 250°C . The oven temperature was 200°C . Helium velocity was 35 cm/s and the split ratio was 1:50. The order of elution of FAMES on the gas chromatography column was confirmed by positive chemical ionization mass spectrometry with a Fisons Trio 1000 gas chromatograph–mass spectrometer (Thermoquest, San Jose, CA).

The erythrocyte fraction was prepared for analysis by adding 1 mL of 9 g NaCl/L to the 6 mL of the erythrocyte fraction, which was layered with nitrogen gas and sonicated for 5 min. Next, 25 μL of 7.4 mmol heptadecanoate/L ethanol was added to the 1.0 mL of suspended platelet samples as the internal standard. Then 50 μL of a mix of internal standards in chloroform—which contained 2.4 mmol triheptadecanoic/L, 3.1 mmol cholesteryl heptadecanoate/L, 2.6 mmol L- α -phosphatidylcholine diheptadecanoyl/L, and 7.4 mmol heptadecanoate/L—was added to 0.5 mL plasma or to 7 mL sonicated erythrocytes. The lipids were extracted with 2 mL hexane:isopropanol (4:1, by vol) (28), followed by the addition of 1.0 mL of a saturated sodium chloride solution. The samples were centrifuged at $1000 \times g$ for 10 min at room temperature. The supernatant fluid was pipetted into a clean tube, the bottom layer was extracted twice more with 2.0 mL hexane, and all fractions of supernatant fluid were combined. The plasma and erythrocyte lipid fractions of supernatant fluid were separated further into cholesterol esters, triacylglycerol, free fatty acids, and phospholipids by a modification of the method by Hamilton and Comai (29), which uses sequential elution of lipid through silica solid-phase extraction cartridges (Alltech, Deerfield, IL). All lipid fractions were evaporated to dryness at 37°C under nitrogen gas and were then saponified by adding 1 mL 15% KOH in methanol, layered with nitrogen gas, and heated at 80°C for 1 h. After the tubes cooled, 2 mL H_2O was added, followed by 1 mL of a saturated sodium chloride solution. Nonsaponifiable lipids were extracted 3 times with 2 mL hexane and discarded. Congo red pH paper was added to the remaining bottom layer, followed by the addition of 1 mL of 12 mol HCl/L.

TABLE 3
Energy, macronutrient, and fatty acid consumption with the self-selected and experimental diets¹

	Self-selected: calculated ² (n = 20)	Standard		Modified: measured ³ (n = 49)
		Calculated ² (n = 7)	Measured ³ (n = 50)	
Energy (kJ)	8508 ± 47 ^{a,b}	8147 ± 79 ^a	9158 ± 30 ^{c,d}	8979 ± 30 ^{b,c}
Protein (% of energy)	14.4 ± 0.5 ^a	17.1 ± 0.9 ^b	14.5 ± 0.3 ^a	14.3 ± 0.3 ^a
Carbohydrates (% of energy)	53.9 ± 0.9 ^a	53.0 ± 1.6 ^a	43.4 ± 0.6 ^b	43.9 ± 0.6 ^b
Fat (% of energy)	31.8 ± 1.0 ^a	30.0 ± 1.7 ^a	42.1 ± 0.6 ^b	41.8 ± 0.6 ^b
Fatty acids (% by wt of total fatty acids) ⁵				
SFA	40.2 ± 0.8 ^a	41.8 ± 1.3 ^a	39.9 ± 0.5 ^a	25.0 ± 0.5 ^b
MUFA	41.2 ± 5.5 ^a	44.7 ± 2.1 ^a	44.8 ± 0.8 ^a	34.0 ± 0.8 ^b
PUFA	18.5 ± 1.2 ^a	13.5 ± 1.9 ^b	15.3 ± 0.7 ^{a,b}	41.0 ± 0.7 ^c
Cholesterol (mg/d)	203.7 ± 23 ^a	163.2 ± 39 ^a	325.0 ± 14.4 ^b	302.1 ± 14.6 ^b

¹ $\bar{x} \pm \text{SEM}$ of the number of samples indicated per group. Means within a row with different superscript letters are significantly different, $P < 0.05$ (ANOVA followed by test of least significant difference). SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

²Calculated by using NUTRITIONIST 4 (N-Squared Computing, Salem, OR).

³Measured values were from composite daily food samples. Fifty-six composite daily food samples were collected from both the standard and the modified diets. During processing, 6 were lost from the standard group and 7 from the modified group.

⁴Calculated from measured consumption of the analyzed food samples.

⁵Multivariate ANOVA comparisons were made between the entire fatty acid profiles of the standard and modified diets. Calculated and measured fatty acid profiles of the standard diet were significantly different, $P < 0.003$. The measured fatty acid profile of the modified diet was significantly different than that of the standard diet, $P < 0.0001$.

The fatty acids were extracted 3 times with 2 mL hexane. The supernatant fluid was collected and dried under nitrogen gas, methyl esters were prepared, and the samples were analyzed by gas chromatography as described above.

Cholesterol concentrations were determined enzymatically with Sigma kit 352 (Sigma, St Louis) from the nonsaponifiable fractions of the homogenized food samples. To solubilize the food sample, 1 mL of 10 g Triton X-100/L in 2-propanol was added to each sample, standard, or blank. The samples were dried under nitrogen gas and resuspended in 10 μL 2-propanol. Blanks, standards, and samples were prepared as directed by the instructions in the kit. One milliliter cholesterol reagent was added, samples were incubated, and absorbance was read at 500 nm. Cholesterol concentrations were determined directly in plasma and lipoprotein fractions with kit 352. Triacylglycerols were quantified with kit 334-UV (Sigma). Glucose was measured with kit Glucose (HK) 16-UV (Sigma). Free fatty acids were quantified by the method of Shimizu et al (30). Insulin concentrations were measured by radioimmunoassay with the coated tube ¹²⁵I RIA kit (ICN Biomedicals, Costa Mesa, CA).

Statistical analysis

The 8-wk data were evaluated by a 2×2 factorial analysis of variance (ANOVA) with the general linear model (GLM) procedure for unequal group size (31). Tested at week 8 were the effects of treatment, time, and the interaction between diets consumed during weeks 0–4 and those consumed during weeks 4–8 on the result at week 8. Results at weeks 0, 4, and 8 were evaluated (SAS, version 6.12; SAS Institute Inc, Cary, NC) by ANOVA with the GLM procedure for unequal group size. Individual differences within weeks 0, 4, and 8 were evaluated by Dunnett's test. For each lipid class, multivariate analysis of variance (MANOVA) was used to test for significant differences between the entire fatty acid profiles of the standard and modified diets for plasma, erythrocytes, and platelets (32). Wilks' lambda from the MANOVA is the equivalent of the univariate ANOVA F test of the H_0 hypothesis (no treatment effect) (32). MANOVA levels ≤ 0.05 were considered significant.

RESULTS

Food intake and composition and body composition

The calculated composition of the subjects' self-selected diets for the 7 d immediately preceding the experiment was not significantly different from the calculated composition of the standard diet for energy, carbohydrates, total fat, SFAs, MUFAs, and cholesterol (**Table 3**). Self-selected protein consumption, however, was significantly lower than the calculated value for the standard diet. For both experimental diets, there were significant differences between calculated and measured protein, carbohydrate, fat, and cholesterol concentrations. In addition, by MANOVA, the calculated and measured fatty acid profiles of the standard diet were significantly different. The measured standard and modified diets did not differ significantly in energy, protein, carbohydrate, fat, or cholesterol content. As expected, however, striking and significant differences were observed in the measured fatty acid profiles of the standard and modified diets: SFA content, 39.9% compared with 25.0%, respectively; MUFA content, 44.8% compared with 34.0%, respectively; and PUFA content, 15.3% compared with 41.0%, respectively. As shown in **Table 4**, the modified diet contained lower amounts of SFAs (14:0, 16:0, and 18:0) and MUFAs (16:1, 18:1n-9, and 18:1n-7) and higher amounts of PUFAs (18:2n-6 and 18:3n-3) than did the standard diet ($P < 0.0001$). The ratio of PUFAs to SFAs for the pork products from the pigs fed the standard diet was 0.54 for ground pork, 0.18 for pork chops, and 0.24 for lard. The ratio of PUFAs to SFAs for the pork products from pigs fed the modified diet was 1.84 for ground pork, 1.41 for pork chops, and 2.08 for lard. Body fat content, BMI, and waist-to-hip ratios of the subjects were not significantly different from baseline at weeks 0, 4, and 8 regardless of the diet fed.

Plasma cholesterol concentrations

Total plasma cholesterol, LDL-cholesterol, HDL₂-cholesterol, and HDL₃-cholesterol concentrations were not significantly different among the 2 experimental groups at week 0 (**Table 5**). The



TABLE 4
Fatty acid analysis of the experimental diets¹

Fatty acid	Standard (n = 56)	Modified (n = 56)
<i>% by wt of total fatty acids</i>		
14:0	1.73 ± 0.06	1.11 ± 0.06 ²
16:0	24.68 ± 0.09	15.33 ± 0.29 ²
16:1	2.22 ± 0.02	0.96 ± 0.02 ²
18:0	12.81 ± 0.06	7.44 ± 0.15 ²
18:1n-9	38.89 ± 0.16	28.65 ± 0.64 ²
18:1n-7	2.94 ± 0.16	2.04 ± 0.04 ²
18:2n-6	13.42 ± 0.15	36.43 ± 0.66 ²
18:3n-3	1.13 ± 0.04	4.00 ± 0.09 ²

¹ $\bar{x} \pm$ SEM of the number of samples indicated per group. Fatty acids <1% are not listed. The entire fatty acid profiles of the standard and modified diets were significantly different, $P < 0.0001$ (multivariate ANOVA).

²Significantly different from standard diet, $P < 0.05$ (ANOVA).

2×2 factorial ANOVA of the week 8 data indicated that the diet from weeks 0–4 had no significant effects on the results at week 8 (column A). Also, the week 8 results did not depend on the previous dietary treatment of weeks 0–4 (column A \times B). For total and LDL-cholesterol concentrations, only the dietary regimen of weeks 4–8 had significant effects at week 8 (column B). Therefore, the 4 groups at week 8 were collapsed into 2 groups that represented the dietary regimen of only weeks 4–8. Significant decreases in total plasma cholesterol and LDL-cholesterol concentrations were noted at week 8 in subjects fed the modified diet. Concentrations of HDL₂ and HDL₃ cholesterol did not change significantly during the course of the dietary treatments. The ratios of total to LDL cholesterol and of LDL to HDL cholesterol were not significantly different at weeks 0, 4, and 8.

Fatty acid profiles of plasma lipid classes

No significant differences in the fatty acid profiles of the plasma cholesteryl esters, free fatty acids, phospholipids, and triacylglycerols were observed at week 0 (Table 6). The 2×2 factorial ANOVA of the week 8 data indicated that the diet from weeks 0–4 had no significant effect on the results at week 8 (column A). The week 8 results also did not depend on the previous dietary treatment of weeks 0–4 (column A \times B). In all plasma lipid classes, only the dietary regimen from weeks 4–8 had signi-

ficant effects on the final fatty acid profiles at week 8 (column B). Therefore, the 4 groups at week 8 were collapsed into 2 groups that represented the dietary regimen of only weeks 4–8.

As tested by MANOVA, SFAs and MUFAs were significantly lower and PUFAs were significantly higher in all plasma lipid classes at week 4 in the subjects fed the modified diet than in those fed the standard diet. These same significant differences were observed at week 8.

Fatty acid profiles of erythrocytes

The fatty acid profiles of the erythrocyte cholesteryl esters, free fatty acids, phospholipids, and triacylglycerols were not significantly different at week 0, as shown in Table 7 (MANOVA). The 2×2 factorial ANOVA of the week 8 data indicated that the diet from weeks 0–4 had no significant effects on the results at week 8 (column A). The week 8 results also did not depend on the previous dietary treatment of weeks 0–4 (column A \times B). The dietary regimen from weeks 4–8 had significant effects on the final fatty acid profiles at week 8 when significant dietary effects were observed (column B). PUFAs in the erythrocyte free fatty acid fraction were significantly higher at weeks 4 and 8 in the subjects fed the modified diet than in subjects fed the standard diet. Major changes in fatty acid patterns were observed mostly in the erythrocyte triacylglycerol fraction at week 8; in this fraction, MUFAs were significantly lower and PUFAs were significantly higher in the subjects fed the modified diet than in those fed the standard diet.

Fatty acid profiles of platelets

The fatty acid profile of platelet total lipids was not significantly different at week 0, as shown in Table 8 (MANOVA). The 2×2 factorial ANOVA of the week 8 data indicated that the diet from weeks 0–4 had no significant effects on the results at week 8 (column A). The dietary regimen from weeks 4–8 had a significant effect on the final fatty acid profile at week 8 only for a very modest change in PUFAs (column B). A significant carryover effect of the SFA differences from weeks 0–4 on weeks 4–8 was evident (column A \times B). The platelet PUFA content was slightly higher at week 8, but not strikingly so, in the subjects fed the modified diet than in those fed the standard diet.

Plasma metabolites and insulin

Plasma metabolite and insulin concentrations were measured in 20 subjects, who were split into groups fed either the standard

TABLE 5
Cholesterol concentrations in plasma and lipoprotein fractions in women fed the standard and modified diets¹

	Week 0		Week 4		Week 8		A	B	A \times B
	Standard (n = 9)	Modified (n = 11)	Standard (n = 9)	Modified (n = 11)	Standard (n = 10)	Modified (n = 9)			
	<i>mmol/L</i>		<i>mmol/L</i>		<i>mmol/L</i>				
Total	3.853 ± 0.181	3.784 ± 0.154	3.791 ± 0.189	3.480 ± 0.177	4.012 ± 0.159	3.386 ± 0.086 ²	NS	<0.0076	NS
LDL	2.145 ± 0.231	2.348 ± 0.149	1.946 ± 0.186	1.829 ± 0.124	2.335 ± 0.142	1.784 ± 0.169 ²	NS	<0.0382	NS
HDL ₂	1.346 ± 0.100	1.108 ± 0.082	1.378 ± 0.097	1.209 ± 0.080	1.352 ± 0.052	1.158 ± 0.094	NS	<0.0405	NS
HDL ₃	0.022 ± 0.004	0.018 ± 0.004	0.021 ± 0.006	0.014 ± 0.004	0.032 ± 0.005	0.032 ± 0.002	NS	NS	<0.0390

¹ $\bar{x} \pm$ SEM of the number of observations indicated per group. A is the probability of an overall effect of dietary treatment of weeks 0–4 on the result at week 8. B is the probability of an overall effect of the dietary treatment of weeks 4–8 on the result at week 8. A \times B is the probability that the result of the dietary treatment of weeks 4–8 depended on the diet from weeks 0–4. Because weeks 0–4 had no significant effects on the results at week 8 and the week-8 results did not depend on prior dietary treatment at weeks 0–4, the 4 groups at week 8 were collapsed into 2 groups that represented the dietary regimen of only weeks 4–8. See Methods for details of the experimental design.

²Significantly different from standard diet, $P \leq 0.05$ (ANOVA followed by Dunnett's test).

TABLE 6
Fatty acid profiles of platelet-poor plasma lipid classes from women fed the standard and modified diets¹

Lipid class	Week 0		Week 4		Week 8		A	B	A × B
	Standard (n = 9)	Modified (n = 11)	Standard (n = 9)	Modified (n = 11)	Standard (n = 10)	Modified (n = 9)			
	% by wt of total fatty acids		% by wt of total fatty acids		% by wt of total fatty acids				
Cholesteryl esters									
SFA	11.1 ± 0.4	11.1 ± 0.4	11.7 ± 0.5	11.0 ± 0.3	12.2 ± 0.4	10.3 ± 0.6 ²	NS	<0.0164	NS
MUFA	18.5 ± 0.6	19.4 ± 0.9	20.5 ± 0.3	13.5 ± 0.6 ²	21.1 ± 1.1	12.8 ± 0.5 ²	NS	<0.0001	NS
PUFA	70.4 ± 0.7	69.5 ± 1.0	67.9 ± 0.7	75.5 ± 0.6 ²	66.7 ± 1.1	76.9 ± 1.0 ²	NS	<0.0001	NS
<i>P</i> ³	NS		<0.0001		<0.0001		NS	<0.0001	NS
Free fatty acids									
SFA	43.1 ± 1.7	43.9 ± 2.1	41.4 ± 1.5	37.0 ± 0.6 ²	42.8 ± 2.1	36.2 ± 1.6 ²	NS	<0.0387	NS
MUFA	33.1 ± 1.5	32.4 ± 1.1	37.6 ± 1.2	33.6 ± 1.1 ²	36.6 ± 1.5	31.0 ± 1.6 ²	NS	<0.0274	NS
PUFA	23.9 ± 0.8	23.7 ± 1.6	21.0 ± 0.7	29.4 ± 1.3 ²	20.6 ± 1.1	32.7 ± 2.2 ²	NS	<0.0002	NS
<i>P</i> ³	NS		<0.0002		<0.0008		NS	<0.0008	NS
Phospholipids									
SFA	49.9 ± 0.7	48.0 ± 0.8	49.5 ± 0.8	51.0 ± 0.8	49.1 ± 0.3	50.8 ± 0.7 ²	NS	<0.0159	NS
MUFA	12.0 ± 0.7	12.5 ± 0.5	12.0 ± 0.6	9.2 ± 0.4 ²	13.1 ± 0.5	8.1 ± 0.8 ²	NS	<0.0002	NS
PUFA	38.1 ± 1.0	39.5 ± 0.7	38.4 ± 0.5	39.7 ± 0.6	37.9 ± 0.5	41.0 ± 0.6 ²	NS	<0.0022	NS
<i>P</i> ³	NS		<0.0013		<0.0010		NS	<0.0010	NS
Triacylglycerols									
SFA	32.1 ± 1.1	33.5 ± 1.2	33.2 ± 0.8	29.7 ± 0.5 ²	32.3 ± 0.5	30.0 ± 1.0	NS	<0.0484	NS
MUFA	47.2 ± 1.1	47.8 ± 1.1	49.7 ± 0.8	41.9 ± 1.0 ²	51.2 ± 0.9	42.0 ± 1.4 ²	NS	<0.0001	NS
PUFA	20.6 ± 0.9	18.8 ± 1.1	17.1 ± 0.7	28.4 ± 1.1 ²	16.5 ± 0.9	28.0 ± 1.1 ²	NS	<0.0001	NS
<i>P</i> ³	NS		<0.0001		<0.0001		NS	<0.0001	NS

¹ $\bar{x} \pm$ SEM of the number of observations indicated per group. A is the probability of an overall effect of dietary treatment of weeks 0–4 on the result at week 8. B is the probability of an overall effect of the dietary treatment of weeks 4–8 on the result at week 8. A × B is the probability that the result of the dietary treatment of weeks 4–8 depended on the diet from weeks 0–4. Because weeks 0–4 had no significant effects on the results at week 8 and the week-8 results did not depend on prior dietary treatment at weeks 0–4, the 4 groups at week 8 were collapsed into 2 groups that represented the dietary regimen of only weeks 4–8. See Methods for details of the experimental design. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

²Significantly different from standard diet, $P < 0.05$ (ANOVA followed by Dunnett's test).

³Multivariate ANOVA was used to test the significant differences between the complete fatty acid profiles within each time period as described in Methods. At week 8, multivariate ANOVA was also used to test the effect of the dietary treatment from week 0–4 (A) on the complete fatty acid profiles at week 8 and to determine whether prior dietary treatment from week 0–4 influenced the effect of dietary treatment from week 4–8 (A × B).

or the modified diet. At week 0, the plasma triacylglycerol concentration was 1.0348 ± 0.1197 mmol/L ($\bar{x} \pm$ SEM), the free fatty acid concentration was 0.7080 ± 0.0440 mmol/L, the glucose concentration was 3.9528 ± 0.0820 mmol/L, and the insulin concentration was 98.7367 ± 5.9371 mmol/L. Plasma triacylglycerol, free fatty acid, glucose, and insulin concentrations did not change significantly in any group during the 8-wk experimental period.

DISCUSSION

Persons at risk of cardiovascular disease should decrease their plasma concentrations of total and LDL cholesterol. Reducing dietary fat, especially SFAs, is an important recommendation for meeting this goal (5–15). It is expected, however, that consumers will continue to choose traditional food products, such as pork. In our experiment, pork products with a high PUFA content were substituted for standard pork in the diet. Our modification of the swine feed ingredients to increase the PUFA concentration of the feed resulted in pork and lard with a higher amount of PUFAs and lower amounts of SFAs and MUFAs than in standard pork. Inclusion of the modified pork products in complete diets resulted in a diet with a high content of PUFAs and a low content of SFAs and MUFAs (Tables 3 and 4). Others have also shown that the fatty acid composition of swine tissues can be easily changed by modifying swine feed ingredients (33–36).

When calculating the composition of the experimental diets, we assumed, on the basis of the research of Marshall and Judd (37), that the calculated composition of the diets would be comparable with the analyzed composition. However, the measured protein, carbohydrate, and fat contents of the standard diet were significantly different from the calculated contents (Table 3). The energy measured as fat in the standard diet was much higher than the calculated fat for the standard diet. Analyzed percentages of SFAs, MUFAs, and PUFAs were similar to the calculated percentages. There were no significant differences in the measured protein, carbohydrate, and fat contents of the modified and standard diets because these diets were the same except for the source of pork and lard.

The difference in calculated compared with measured values for energy from fat for the standard diet may have been the result of the choices that the NUTRITIONIST IV program gave for the cuts of meat and the actual composition of the meat used to make the ground pork. When the pork was processed, sufficient pork chops were saved to complete the feeding study. Then, all remaining edible muscle meats were combined and ground. This was not a choice in the NUTRITIONIST IV database. For the purposes of calculating and planning, we assumed that the composition of the ground pork was comparable with that of ground shoulder meat. The small number of pigs used for each diet group may not have been representative of the larger number of

TABLE 7

Fatty acid profiles of erythrocyte lipid classes from women fed the standard and modified diets¹

Lipid class	Week 0		Week 4		Week 8		A	B	A × B
	Standard (n = 8)	Modified (n = 11)	Standard (n = 8)	Modified (n = 11)	Standard (n = 10)	Modified (n = 9)			
	% by wt of total fatty acids		% by wt of total fatty acids		% by wt of total fatty acids				
Cholesteryl esters									
SFA	20.7 ± 6.6	49.7 ± 10.6 ²	13.5 ± 3.2	21.3 ± 1.8 ²	19.3 ± 2.3	20.0 ± 1.5	NS	NS	NS
MUFA	10.2 ± 3.0	9.5 ± 2.8	20.3 ± 1.6	10.2 ± 1.5 ²	17.9 ± 2.1	12.9 ± 0.8	NS	NS	NS
PUFA	69.2 ± 9.4	40.8 ± 9.3	66.2 ± 2.5	68.5 ± 1.9	62.8 ± 1.4	67.0 ± 2.3	NS	NS	NS
P ³	NS		<0.0018		NS		<0.0471	NS	NS
Free fatty acids									
SFA	75.3 ± 3.2	73.2 ± 2.6	60.4 ± 2.2	56.3 ± 1.9	57.9 ± 2.5	54.5 ± 1.8	NS	NS	NS
MUFA	13.1 ± 1.8	14.6 ± 1.6	22.4 ± 1.7	22.8 ± 1.1	24.4 ± 1.4	22.8 ± 1.4	NS	NS	NS
PUFA	11.7 ± 1.4	12.2 ± 1.2	17.2 ± 1.1	20.9 ± 1.0 ²	17.7 ± 1.4	22.7 ± 1.4 ²	NS	<0.0228	NS
P ³	NS		<0.0392		NS		NS	NS	NS
Phospholipids									
SFA	49.0 ± 1.1	48.6 ± 1.1	45.1 ± 0.8	45.8 ± 0.6	45.3 ± 1.0	45.9 ± 0.9	NS	NS	NS
MUFA	18.0 ± 0.5	17.6 ± 0.6	18.6 ± 0.3	16.4 ± 0.4 ²	18.2 ± 0.4	16.3 ± 0.2 ²	NS	<0.0019	NS
PUFA	32.9 ± 0.9	33.8 ± 0.7	36.3 ± 0.7	37.7 ± 0.5	36.5 ± 0.8	37.8 ± 0.9	NS	NS	NS
P ³	NS		<0.0082		<0.0044		NS	<0.0044	NS
Triacylglycerols									
SFA	69.5 ± 7.7	55.2 ± 8.3	36.7 ± 1.8	50.8 ± 7.5	35.8 ± 2.6	28.8 ± 3.8	NS	NS	NS
MUFA	20.9 ± 5.2	24.5 ± 4.9	40.8 ± 1.3	26.5 ± 4.1 ²	40.1 ± 1.2	34.3 ± 1.8 ²	NS	<0.0129	NS
PUFA	9.6 ± 3.6	11.3 ± 3.1	22.5 ± 2.0	22.7 ± 3.5	24.0 ± 2.7	36.9 ± 2.8 ²	NS	<0.0045	NS
P ³	NS		<0.0010		<0.0040		NS	<0.0040	NS

¹ $\bar{x} \pm$ SEM of the number of observations indicated per group. A is the probability of an overall effect of dietary treatment of weeks 0–4 on the result at week 8. B is the probability of an overall effect of the dietary treatment of weeks 4–8 on the result at week 8. A × B is the probability that the result of the dietary treatment of weeks 4–8 depended on the diet from weeks 0–4. Because weeks 0–4 had no significant effects on the results at week 8 and the week-8 results did not depend on prior dietary treatment at weeks 0–4, the 4 groups at week 8 were collapsed into 2 groups that represented the dietary regimen of only weeks 4–8. See Methods for details of the experimental design. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

²Significantly different from standard diet, $P < 0.05$ (ANOVA followed by Dunnett's test).

³Multivariate ANOVA was used to test the significant differences between the complete fatty acid profiles within each time period as described in Methods. At week 8, multivariate ANOVA was also used to test the effect of the dietary treatment from week 0–4 (A) on the complete fatty acid profiles at week 8 and to determine whether prior dietary treatment from week 0–4 influenced the effect of dietary treatment from week 4–8 (A × B).

TABLE 8

Fatty acid profiles of platelet total lipids from women fed the standard and modified diets¹

Total lipid	Week 0		Week 4		Week 8		A	B	A × B
	Standard (n = 9)	Modified (n = 11)	Standard (n = 9)	Modified (n = 11)	Standard (n = 10)	Modified (n = 9)			
	% by wt of total fatty acids		% by wt of total fatty acids		% by wt of total fatty acids				
SFA	60.5 ± 3.6	55.1 ± 1.1	48.8 ± 0.8	49.8 ± 1.0	49.6 ± 0.7	48.5 ± 0.7	NS	NS	<0.0373
MUFA	11.2 ± 1.5	15.0 ± 1.3	18.6 ± 0.5	16.5 ± 0.7 ²	17.9 ± 0.7	16.5 ± 0.5	NS	NS	NS
PUFA	28.2 ± 2.9	29.9 ± 1.1	32.6 ± 0.7	33.7 ± 0.6	32.5 ± 0.6	35.0 ± 0.4 ²	NS	<0.0020	NS
P ³	NS		<0.0445		<0.0096		NS	<0.0096	NS

¹ $\bar{x} \pm$ SEM of the number of observations indicated per group. A is the probability of an overall effect of dietary treatment of weeks 0–4 on the result at week 8. B is the probability of an overall effect of the dietary treatment of weeks 4–8 on the result at week 8. A × B is the probability that the result of the dietary treatment of weeks 4–8 depended on the diet from weeks 0–4. Because weeks 0–4 had no significant effects on the results at week 8 and the week-8 results did not depend on prior dietary treatment at weeks 0–4, the 4 groups at week 8 were collapsed into 2 groups that represented the dietary regimen of only weeks 4–8. See Methods for details of the experimental design. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

²Significantly different from standard diet, $P < 0.05$ (ANOVA followed by Dunnett's test).

³Multivariate ANOVA was used to test the significant differences between the complete fatty acid profiles within each time period as described in Methods. At week 8, multivariate ANOVA was also used to test the effect of the dietary treatment from week 0–4 (A) on the complete fatty acid profiles at week 8 and to determine whether prior dietary treatment from week 0–4 influenced the effect of dietary treatment from week 4–8 (A × B).

samples inherent in the database. The dietary fatty acid analysis of the modified diet indicated a significant shift from SFAs and MUFAs to PUFAs (Tables 3 and 4), which reflects the fatty acid content of soybean oil. Our experience clearly shows that it is important to verify diet composition by actual analysis.

Our data show that when the modified pork was included as part of a complete diet, plasma total and LDL-cholesterol concentrations decreased compared with values in subjects who consumed the standard diet (Table 5). These changes likely reflect events at the cellular level. Exogenous SFAs increase serum LDL-cholesterol concentrations by decreasing the number of LDL receptors (38–44). A decrease in the number of LDL receptors decreases the fractional catabolic rate of LDL cholesterol and therefore increases the circulating LDL-cholesterol concentration. VLDL remnants also attach to LDL receptors. Therefore, if the number of LDL receptors decreases, more VLDL remnants will remain in circulation to be converted into intermediate-density lipoprotein and LDL. When dietary SFAs were replaced with PUFAs by feeding the subjects the modified diet, the total and LDL-cholesterol concentrations probably decreased because the receptor repression by SFAs was removed. Alternatively, LDL production may have been suppressed by the high dietary PUFA content.


The data reported here (Table 5) confirm similar findings of other authors who directly replaced SFAs in the diet with PUFAs. Meta-analyses of many published studies indicate that dietary SFAs increase serum cholesterol and dietary PUFAs decrease serum cholesterol (45–48). MUFAs generally do not have a significant effect on serum cholesterol. High amounts of dietary cholesterol alone increase serum total cholesterol (45). In our experiment the changes in LDL-cholesterol concentrations were parallel to the changes in serum total cholesterol concentrations, as was reported by others (45–48).

MUFAs and PUFAs are reported to decrease total cholesterol, LDL cholesterol, and HDL cholesterol by several mechanisms, including decreased LDL apolipoprotein B production rates and increased HDL apolipoprotein A-I catabolism in monkeys (49, 50). In addition, PUFAs are reported to increase LDL apolipoprotein B catabolism (49). PUFAs may also decrease total and LDL cholesterol by modifying the oxidation-reduction or phosphorylation state of a nuclear transcription protein that governs the synthesis of fatty acid synthase, acetyl-CoA carboxylase, or stearoyl-CoA desaturase (51, 52).

By week 4, changes in the fatty acid profiles were observed within all the lipid classes (Table 6) before changes in cholesterol concentrations were (Table 5). At week 8, the fatty acid profiles of the plasma cholesteryl ester, free fatty acid, phospholipid, and triacylglycerol fractions reflected the influence of the diet fed during weeks 4–8 regardless of the diet fed during weeks 0–4 (Table 6). This indicates that a 4-wk feeding period was long enough to observe changes in plasma lipid fatty acid composition. In all of these plasma lipid fractions, the modified pork diet resulted in a fatty acid profile with a higher amount of PUFAs. The changes in plasma fatty acids in all lipid classes were more dramatic than those in erythrocytes (Table 7) or platelets (Table 8).

Because lipids in platelets are predominantly phospholipids (53), we analyzed the total fatty acid profile in the platelets. The changes in fatty acid profiles enabled us to assess adherence to the experimental diets (54, 55). Plasma phospholipid fatty acid changes due to dietary intakes of different concentrations of fatty acids are reported to reflect the same changes in liver and bile phospholipid fatty acid in piglets (56). The dramatic and more rapid changes in fatty acid

profiles within the plasma lipid classes may indicate that changes in liver occur more rapidly than do changes within erythrocytes and platelets and that changes within the liver are more reflective of dietary manipulations than are changes in either erythrocytes or platelets. Serum cholesteryl esters and triacylglycerols need ≈ 2 wk to equilibrate (54). Erythrocyte membranes require 4–6 wk to equilibrate but can indicate significant changes after 8 d (54). Platelets have a half-life of 4 d. Therefore, the fatty acid profile of platelets might be a good indicator of dietary fatty acid intake after 4 wk (54). We did not confirm this observation. Our data indicate that plasma fatty acids in the lipid classes measured were good indicators of the type of dietary fatty acid ingested.

In summary, modification of the fatty acid composition of pork and lard to increase the concentration of PUFAs and incorporation of the resultant pork and lard into a complete diet for college-aged women resulted in a significant decrease in plasma total and LDL cholesterol. There was also a significant change from SFAs and MUFAs to PUFAs in plasma and erythrocytes. Pork designed to contain a higher PUFA content did not significantly change the subjects' plasma glucose, insulin, triacylglycerol, or free fatty acid concentrations. The decreases in plasma total cholesterol, LDL cholesterol, and SFA contents were most likely a response to the decreased dietary intake of SFAs. This project shows that modification of the fatty acid composition of traditional animal foods, such as pork, will be a useful approach to lowering the amount of saturated fat consumed by Americans. 

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REFERENCES

1. American Heart Association. 1998 Heart and stroke statistical update. Dallas: American Heart Association, 1997.
2. McDowell MA, Briefel RR, Alaimo K, et al. Energy and macronutrient intakes of persons ages 2 months and over in the United States: third National Health and Nutrition Examination Survey, phase 1, 1988–91. *Adv Data* 1994;255.
3. Enns CW, Goldman JD, Cook A. Trends in food and nutrient intakes by adults: NFCS 1977–78, CSFII 1989–91, and CSFII 1994–95. *Fam Econ Nutr Rev* 1997;10:1–11.
4. Rhee KS. Fatty acids in meats and meat products. In: Chow CK, ed. *Fatty acids in foods and their health implications*. New York: Marcel Dekker, 1992:65–93.
5. Berry EM, Eisenberg S, Haratz D, et al. Effects of diets rich in monounsaturated fatty acids on plasma lipoproteins—the Jerusalem Nutrition Study: high MUFAs vs high PUFAs. *Am J Clin Nutr* 1991; 53:899–907.
6. Kasim SE, Martino S, Kim PN, et al. Dietary and anthropometric determinants of plasma lipoproteins during a long-term low-fat diet in healthy women. *Am J Clin Nutr* 1993;57:146–53.
7. Ginsberg HN, Karmally W, Barr SL, Johnson C, Holleron S, Ramakrishnan R. Effects of increasing dietary polyunsaturated fatty acids. *Arterioscler Thromb* 1994;14:892–901.
8. Judd JT, Oh SY, Hennig B, Dupont J, Marshall M. Effects of low fat diets differing in degree of fat unsaturation on plasma lipids, lipoproteins and apolipoproteins. *J Am Coll Nutr* 1988;7:223–34.
9. Mensink RP, Katan MB. Effect of a diet enriched with MUFA or PUFA fatty acids on levels of LD and HD lipoprotein cholesterol in healthy women and men. *N Engl J Med* 1989;32:436–41.
10. Marshall MW, Judd JT, Matusik EJ, Church J, Canary JJ. Effects of low fat diets varying in P/S ratio on nutrient intakes, fecal excretion, blood chemistry profiles and fatty acids of adult men. *J Am Coll Nutr* 1986;5:263–79.



11. Laine DC, Snodgrass CM, Dawson EA, Ener MA, Kuba K, Frantz ID Jr. Lightly hydrogenated soy oil versus other vegetable oils as a lipid-lowering dietary constituent. *Am J Clin Nutr* 1982;35:683-90.
12. Grundy SM, Nix D, Whelan MF, Franklin L. Comparison of three cholesterol-lowering diets in normolipidemic men. *JAMA* 1986; 256:2351-5.
13. Mattson FH, Grundy SM. Comparison of effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. *J Lipid Res* 1985;26:194-202.
14. Cheung MC, Lichtenstein AH, Schaefer EJ. Effects of a diet restricted in saturated fatty acids and cholesterol on the composition of apolipoprotein A-1-containing lipoprotein particles in the fasting and fed states. *Am J Clin Nutr* 1994;60:911-8.
15. Nestel PJ. Polyunsaturated fatty acids (n-3, n-6). *Am J Clin Nutr* 1987;45:1161-7.
16. National Research Council. Nutrient requirements of swine. 9th ed. Washington, DC: National Academy Press, 1988.
17. National Research Council. Recommended dietary allowances. 10th ed. Washington, DC: National Academy Press, 1989.
18. Segal KR, Gutin B, Presta E, Wang J, Van Itallie TB. Estimation of human body composition by electrical impedance methods: a comparative study. *J Appl Physiol* 1985;58:1565-71.
19. Cochran WJ, Wong WW, Fioritto ML, Sheng HP, Klein PD, Klish WJ. Total body water estimated by measuring total-body electrical conductivity. *Am J Clin Nutr* 1988;48:946-50.
20. Van Loan M, Mayclin P. A new TOBEC instrument and procedure for the assessment of body composition: use of Fourier coefficients to predict lean body mass and total body water. *Am J Clin Nutr* 1987;45:131-7.
21. Ostlund RE, Staten M, Kohrt WM, Schultz J, Malley M. The ratio of waist-to-hip circumference, plasma insulin level, and glucose intolerance as independent predictors of the HDL₂ cholesterol level in older adults. *N Engl J Med* 1990;322:229-34.
22. Garcia PA, Hanson KB, Kies C, Oh SY, Story JA, Dupont J. Studies of women eating diets with different fatty acid composition. Part I. Plasma lipoproteins and steroid excretion. *J Am Coll Nutr* 1991; 10:315-21.
23. Havel RJ, Eder HA, Bragdon JH. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J Clin Invest* 1955;34:1345-53.
24. Lindgren FT. Preparative ultracentrifugal laboratory procedures and suggestions for lipoprotein analysis. In: Perkins E, ed. *Analysis of lipids and lipoproteins*. Champaign, IL: Association of Official Analytical Chemists, 1975:204-24.
25. Chaney AL, Marbach EP. Modified reagents for determination of urea and ammonia. *Clin Chem* 1962;8:130-2.
26. Folch J, Lees M, Stanley GHS. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 1957;226:497-509.
27. Morrison WR, Smith LM. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. *J Lipid Res* 1964;5:600-8.
28. Radin NS. Preparation of lipid extracts. In: Lowenstein JM, ed. *Methods Enzymol* 1981;72:5-7.
29. Hamilton JG, Comai K. Rapid separation of neutral lipids, free fatty acids and polar lipids using prepacked silica sep-pack columns. *Lipids* 1988;23:1146-9.
30. Shimizu S, Inoue K, Tani Y, Yamada H. Enzymatic microdetermination of serum free fatty acids. *Anal Biochem* 1979;98:341-5.
31. Snedecor GW, Cochran WG. *Statistical methods*. Ames, IA: Iowa State University Press, 1989:299-303.
32. Johnson RA, Wichern DW. *Applied multivariate statistical analysis*. Englewood Cliffs, NJ: Prentice Hall, 1992:241-60.
33. Rhee KS, Davidson TL, Knabe DA, Cross HR, Ziprin YA. Effect of dietary high oleic sunflower oil on pork carcass traits and fatty acid profiles of raw tissues. *Meat Sci* 1988;24:249-60.
34. Miller MF, Shackelford SD, Hayden KD, Reagan JO. Determination of the alteration in fatty acid profiles, sensory characteristics and carcass traits of swine fed elevated levels of monounsaturated fats in the diet. *J Anim Sci* 1990;68:1624-31.
35. Skelley GC, Borgman RF, Handlin DL, et al. Influence of diet on quality, fatty acids and acceptability of pork. *J Anim Sci* 1975;41: 1298-304.
36. Zollitsch W, Wetscherek W, Lettnew F. Use of differently processed full-fat soybeans in a diet for pig fattening. *Anim Feed Sci* 1993;41: 237-46.
37. Marshall MW, Judd J. Calculated vs. analyzed composition for four fat diets. *J Am Diet Assoc* 1982;80:537-49.
38. Grundy SM. Monounsaturated fatty acids, plasma cholesterol, and coronary heart disease. *Am J Clin Nutr* 1987;45:1168-75.
39. Spady DK, Dietschy JM. Interaction of dietary cholesterol and triacylglycerides in the regulation of hepatic low density lipoprotein transport in the hamster. *J Clin Invest* 1988;81:300-9.
40. Grundy SM, Denke MA. Dietary influences on serum lipids and lipoproteins. *J Lipid Res* 1990;31:1149-72.
41. Woollett LA, Spady DK, Dietschy JM. Saturated and unsaturated fatty acids independently regulate low density lipoprotein receptor activity and production rate. *J Lipid Res* 1992;33:77-88.
42. Mustad VA, Etherton TD, Cooper AD, et al. Reducing saturated fat intake is associated with increased levels of LDL receptors on mononuclear cells in healthy men and women. *J Lipid Res* 1997; 38:459-68.
43. Grundy SM. Cholesterol and coronary heart disease. *JAMA* 1986; 256:2849-58.
44. Dietschy JM. Theoretical considerations of what regulates low-density-lipoprotein and high-density-lipoprotein cholesterol. *Am J Clin Nutr* 1997;65(suppl):1581S-9S.
45. Hegsted DM, Ausman LM, Johnson JA, Dallal GE. Dietary fat and serum lipids: an evaluation of the experimental data. *Am J Clin Nutr* 1993;57:875-83.
46. Mensink RP, Katan MB. Effect of dietary fatty acids on serum lipids and lipoproteins: a meta-analysis of 27 trials. *Arterioscler Thromb* 1992;12:911-9.
47. Howell WH, McNamara DJ, Tosca MA, Smith BT, Gaines JA. Plasma lipid and lipoprotein responses to dietary fat and cholesterol: a meta-analysis. *Am J Clin Nutr* 1997;65:1747-64.
48. Kris-Etherton PM, Yu S. Individual fatty acid effects on plasma lipids and lipoproteins: human studies. *Am J Clin Nutr* 1997;65(suppl): 1628S-44S.
49. Brousseau ME, Stucchi AF, Vespa DB, Schaefer EJ, Nicolosi RJ. A diet enriched in monounsaturated fats decreases low density lipoprotein concentrations in cynomolgus monkeys by a different mechanism than does a diet enriched in polyunsaturated fats. *J Nutr* 1993;123:2049-58.
50. Brousseau ME, Ordovas JM, Osada J, et al. Dietary monounsaturated and polyunsaturated fatty acids are comparable in their effects on hepatic apolipoprotein mRNA abundance and liver lipid concentrations when substituted for saturated fatty acids in cynomolgus monkeys. *J Nutr* 1995;125:425-36.
51. Clark SD, Jump DB. Dietary polyunsaturated fatty acid regulation of gene transcription. *Annu Rev Nutr* 1994;14:83-98.
52. Clark SD, Jump DB. Polyunsaturated fatty acid regulation of hepatic gene transcription. *Lipids* 1996;31:S7-11.
53. Maupin B. Survival and preservation. In: Maupin B. *Blood platelets in man and animals*. Vol 1. Oxford, United Kingdom: Pergamon Press, 1969:89-125.
54. Sarkkinen ES, Agren JJ, Ahola I, Ovaskainen ML, Uusitupa MIJ. Fatty acid composition of serum cholesterol esters, and erythrocyte and platelet membranes as indicators of long term adherence to fat-modified diets. *Am J Clin Nutr* 1994;59:364-70.
55. Glatz JFC, Soffers AEMF, Katan MB. Fatty acid composition of serum cholesteryl esters and erythrocyte membranes as indicators of linoleic acid intake in man. *Am J Clin Nutr* 1989;49:269-76.
56. Rioux FM, Innis SM, Dyer R, MacKinnon M. Diet-induced changes in liver and bile but not brain fatty acids can be predicted from differences in plasma phospholipid fatty acids in formula- and milk-fed piglets. *J Nutr* 1997;127:370-7.

