

Effects of dietary monounsaturated fatty acids on lipoprotein concentrations, compositions, and subfraction distributions and on VLDL apolipoprotein B kinetics: dose-dependent effects on LDL¹⁻³

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ABSTRACT

Background: Replacing dietary saturated fatty acids (SFAs) with monounsaturated fatty acids (MUFAs) lowers LDL cholesterol, but the underlying mechanisms remain unclear.

Objective: We assessed the effects of replacing dietary SFAs with MUFAs on concentrations and subclass distributions of VLDL, intermediate-density lipoprotein, LDL, and HDL and on VLDL apolipoprotein B kinetics.

Design: Thirty-five moderately hypercholesterolemic, middle-aged volunteers consumed for 6 wk, in random order, diets containing low (L-MUFA; 7.8% of energy from MUFAs), moderate (M-MUFA; 10.3% from MUFAs), or high (H-MUFA; 13.7% from MUFAs) amounts of MUFAs. Fasting blood samples were taken from all subjects after each intervention. VLDL apolipoprotein B kinetic studies were performed in a subgroup after the L-MUFA and H-MUFA diets.

Results: Plasma cholesterol concentrations decreased in a dose-dependent manner with increasing intakes of dietary MUFAs. This change was entirely accounted for by reduced LDL cholesterol (-0.20 and -0.49 mmol/L after the M-MUFA and H-MUFA diets, respectively, compared with the concentration after the L-MUFA diet; P for trend < 0.01). Plasma triacylglycerol and HDL cholesterol were not significantly affected by the dietary intervention, nor were the concentrations of VLDL₁ (S_r 60–400), VLDL₂ (S_r 20–60), or intermediate-density lipoprotein (S_r 12–20). Production and catabolic rates for VLDL₁ and VLDL₂ were also unaffected. HDL and LDL subclass distributions were not significantly altered, but as a consequence of the overall LDL lowering, concentrations of atherogenic LDL-III were 25% lower after the H-MUFA diet than after the L-MUFA diet ($P = 0.02$).

Conclusion: The effects of replacing dietary SFAs with MUFAs on lipoprotein metabolism appear to be almost exclusively limited to the LDL density class. *Am J Clin Nutr* 2003;78:47–56.

KEY WORDS Monounsaturated fatty acids, saturated fatty acids, LDL, VLDL, lipoprotein subfractions, cholesterol

INTRODUCTION

An elevated plasma concentration of LDL cholesterol is an important risk factor for cardiovascular disease (1, 2), and a large proportion of the Western population would probably benefit from cholesterol reduction (3, 4). Although pharmacologic interventions, such as statins, are very effective at reducing LDL-cholesterol

concentrations and cardiovascular disease, such treatments are expensive, and it is infeasible to provide these to the entire population at risk. Thus, to lower LDL-cholesterol concentrations in populations, nonpharmacologic interventions, such as dietary changes, are necessary. Replacement of dietary saturated fatty acids (SFAs) with monounsaturated fatty acids (MUFAs) lowers LDL cholesterol, without inducing the hypertriglycerolemia sometimes observed when SFAs are replaced by carbohydrates (5). Accordingly, this type of nutritional approach may provide a useful model on which to base dietary guidelines for populations.

Several studies showed that isoenergetic replacement of SFAs with MUFAs lowers LDL cholesterol (6–9). However, these studies generally focused on changes to the cholesterol content of LDL and HDL and did not consider effects on the concentration and composition of LDL particles as a whole or effects on the larger lipoprotein species [ie, VLDL and intermediate-density lipoprotein (IDL)]. Small, cholesterol-rich VLDLs (VLDL₂) are the major metabolic precursor particles for LDL, with LDL concentrations being positively related to the production rate of VLDL₂ but not of large, triacylglycerol-rich VLDL₁ (10). We therefore hypothesized that substituting MUFAs for SFAs would reduce the hepatic production rate of VLDL₂ and plasma concentrations of VLDL₂ and IDL (the lipoprotein particle intermediate between VLDL₂ and LDL) but would not influence the production or plasma concentration of VLDL₁.

Although the effects of substituting MUFAs for SFAs on LDL cholesterol and, to a lesser extent, HDL cholesterol have been widely studied, the influence of this dietary change on subfraction distributions of LDL and HDL is unclear. Because the respective atherogenic and atheroprotective characteristics of these lipoproteins depend on particle size as well as concentration (11, 12), this information is important when assessing the overall effects of replacing dietary SFAs with MUFAs on lipoprotein-associated cardiovascular disease risk.

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TABLE 1
Subject characteristics at baseline¹

	All (n = 35)	Men (n = 17)	Women (n = 18)
Age (y)	55.0 ± 5.6	56.8 ± 5.7	53.2 ± 5.1
BMI (kg/m ²)	26.3 ± 3.8	26.7 ± 2.5	26.0 ± 4.7
Plasma cholesterol (mmol/L)	6.12 ± 0.67	6.16 ± 0.65	6.08 ± 0.70
Plasma triacylglycerol (mmol/L)	1.54 ± 0.60	1.69 ± 0.50	1.41 ± 0.66
HDL cholesterol (mmol/L)	1.42 ± 0.42	1.15 ± 0.24	1.67 ± 0.40 ²

¹ $\bar{x} \pm SD$.

²Significantly different from men, $P < 0.01$.

The purpose of the present study, therefore, was to investigate the effects of diets containing low, moderate, and high amounts of MUFAs (with reciprocally high, moderate, and low amounts of SFAs) on the concentrations and compositions of VLDL₁, VLDL₂, IDL, and LDL and on the subfraction distributions of LDL and HDL. Three, rather than two, intervention diets were studied to determine whether LDL lowering occurred in a dose-dependent manner or whether a threshold level of SFA replacement was necessary to induce favorable lipoprotein changes. To determine whether altered production of VLDL₂ mediated the LDL-lowering effect, we studied VLDL₁ and VLDL₂ apolipoprotein (apo) B kinetics after the low- and high-MUFA dietary interventions. To ensure that any lipoprotein changes achieved were applicable at a population-wide level, dietary changes were introduced covertly by altering the fatty acid composition of foods widely consumed in the typical United Kingdom diet. Therefore, subjects did not have to radically alter their usual food intake during the dietary intervention periods. The subjects chosen for study were moderately hypercholesterolemic, middle-aged men and women. This group is at increased risk of cardiovascular disease but is unlikely to be offered lipid-lowering drug treatment; thus, this group is an ideal population to target for a dietary based lipid-lowering therapy. To obtain a more complete picture of dietary MUFA-induced changes to metabolism, concentrations of insulin, glucose, non-esterified fatty acids (NEFAs), and C-reactive protein were also measured at the end of each dietary intervention period.

SUBJECTS AND METHODS

Subjects

One hundred seventy-five persons were screened for participation in this study. Of these, 52 fulfilled our entry criteria and were enrolled in the study. The entry criteria were as follows: a plasma total cholesterol concentration in the range of 5.2–8.0 mmol/L; no history of hormonal, renal, hematologic, or hepatic dysfunction; no history of myocardial infarction; and no current treatment with lipid-lowering drugs. All subjects gave written informed consent, and the study was conducted with the approval of the Norwich District Ethics Committee. Two volunteers dropped out of the study because they were unable to comply with the intervention diets, 15 subjects dropped out for reasons unrelated to the study (eg, medical or domestic reasons, a change of job, or a move out of the area). Thirty-five subjects (17 men and 18 women) completed the study; their baseline characteristics are shown in **Table 1**.

Study design

Each volunteer underwent three 6-wk dietary interventions in random order, during which they consumed diets containing

low (L-MUFA), moderate (M-MUFA), or high (H-MUFA) proportions of MUFAs but the same total amount of fat, with a washout period between diets of ≥ 8 wk. The subjects were blinded to the study diets, as were the scientists who undertook the sample analyses. Before the dietary interventions, the volunteers completed a 7-d diary of their food intake, and their energy intake from this diary was used to prescribe their fat intake during the 6-wk intervention periods. With their habitual diets, the subjects consumed $32.4 \pm 6.7\%$ ($\bar{x} \pm SD$) of energy from fat, with $9.3 \pm 2.0\%$ of energy from MUFAs.

To adjust the fatty acid composition of the intervention diets, “test fats” containing low (25% *cis* MUFAs, 50% SFAs, 21% *cis* PUFAs, and 4% *trans*), moderate (35% *cis* MUFAs, 37% SFAs, 24% *cis* PUFAs, and 4% *trans*), and high (51% *cis* MUFAs, 24% SFAs, 21% *cis* PUFAs, and 4% *trans*) proportions of MUFAs were specially manufactured by Anglia Oil Ltd (Hull, United Kingdom) and incorporated into spreads (Chemtech International Ltd, Reading, United Kingdom) and ready-made baked products (ie, cakes, cookies, and scones). During each 6-wk intervention period, the volunteers were permitted to eat the following: the test fat provided in the form of margarine or spread, the test fat in a selection of baked products, a selection of commercially available, meat-containing or vegetarian, low-fat ready meals (containing < 10 g fat/serving), and any “fat-free” foods containing < 1 g fat/portion (eg, most cereals, fruit, vegetables, rice, low-fat soups, and skim milk). Subjects were also permitted to eat lean white meats (eg, chicken breast without skin, lean pork, and lean ham).

Each subject was given a daily target for test fatty acid intake based on his or her usual dietary intake; this target was designed to provide 30% of energy from fat. For ease, this was quantified in terms of lipid units (each containing 4.8 g fat), and food portions were designed to contain integer multiples of lipid units. For example, a subject might be prescribed 16 lipid units/d, of which approximately one-half would be provided as the test spread and approximately one-half as baked goods. To assist the subjects and aid compliance, we provided them with a list of fat-free foods that they could eat freely and with a list of foods to avoid. The subjects were also provided with low-fat ready meals, some fat-free foods (eg, breakfast cereals, packet and canned soups, skim milk, salad dressings) to eat during the intervention periods, and a recipe booklet describing meals that could be prepared with the test fats. Dietary compliance was ensured through weekly meetings with the subjects during which the intervention foods for the following week were provided and the level of consumption of the intervention foods was assessed.

Measurements made during dietary interventions

To ensure that the subjects remained weight-stable during the intervention periods, we measured their body masses at the

Institute of Food Research at the start and the end of each intervention period, and the subjects measured their own body masses in their homes, at first daily and later weekly. During the third week of each 6-wk intervention, the subjects recorded their food intake for 5 d. All food diaries were analyzed by using FOODBASE 2000 (The Institute of Brain Chemistry, University of North London, London), which calculated macronutrient intakes and provided detailed information on the intakes (as percentages of total energy) of saturated, monounsaturated, polyunsaturated, and trans fatty acids.

At the end of each dietary intervention period, the subjects returned to the Institute of Food Research after an overnight fast, and blood samples were collected in tubes containing EDTA, lithium heparin, or citrate. Plasma was separated from whole blood by low-speed centrifugation at $2000 \times g$ and room temperature for 10 min within 30 min of sampling. Fresh (ie, unfrozen) plasma from the EDTA-coated tubes was sent to Glasgow by overnight courier for lipid and lipoprotein analyses. The remaining plasma (ie, from the tubes containing EDTA, lithium heparin, or citrate) was divided into aliquots, immediately frozen on solid carbon dioxide, and stored at -70°C for future analysis.

Apolipoprotein B turnover studies

A subgroup of 17 subjects (9 men and 8 women) underwent apo B turnover studies to determine the production and catabolic rates of VLDL₁ and VLDL₂ at the end of the L-MUFA and H-MUFA diets (ie, during the last 2 d of each intervention period). The subjects arrived at the Metabolic Suite after an overnight fast. While the subjects were in the fasted state, a blood sample was collected in an EDTA-coated tube, and then a bolus injection of 7 mg L-[5,5,5-²H₃]leucine (CK Gas Products, Berkshire, United Kingdom)/kg body wt (up to a maximum body wt of 78.5 kg; the dose was fixed at 550 mg for subjects weighing > 78.5 kg) was administered. Further blood samples (collected in EDTA-coated tubes) were taken at multiple time points for 10 h after the injection (2, 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 210, 240, 300, 360, 480, and 600 min after injection). During this 10-h period, the subjects continued to fast but were allowed to consume nonenergetic drinks. The subjects were then given a fat-free meal (containing < 3 g total fat), and a further blood sample was taken 12 h after the injection. Further blood samples were obtained in the fasted state 24 and 48 h after the injection. Plasma was separated from whole blood by low-speed centrifugation at $2000 \times g$ and room temperature for 10 min within 30 min of sampling and was sent by overnight courier to Glasgow for analysis.

Analytic methods for fasting samples

Concentrations of triacylglycerol and of total, VLDL, LDL, and HDL cholesterol in plasma collected in EDTA-coated tubes were measured according to the Manual of Laboratory Operations: Lipid Research Clinics Program (13) in a laboratory accredited by the Centers for Disease Control and Prevention. In a separate procedure, VLDL₁ (*S_r* 60–400), VLDL₂ (*S_r* 20–60), and IDL (*S_r* 12–20) were isolated from plasma collected in EDTA-coated tubes by a cumulative gradient ultracentrifugation technique that was previously described (14, 15), and LDL (density = 1019–1063 g/L) was isolated from plasma collected in EDTA-coated tubes by sequential flotation ultracentrifugation as described previously (16). Each lipoprotein fraction sample was assayed for cholesterol, free cholesterol, triacylglycerol, and phospholipid with the use of enzymatic colorimetric methods and commercially available kits (Roche Diagnostics GmbH, Mannheim, Germany; Wako Chemicals

USA, Inc, Richmond, VA) on an automated Clinical Chemistry analyzer (models 704 and 717; Hitachi Ltd, Tokyo). Protein was measured by using a modified Lowry assay (17). Cholesteryl ester concentrations were determined by multiplying the difference between total cholesterol and free cholesterol concentrations (in mg/dL) by 1.68 to account for the mass of the esters. Lipoprotein concentrations were calculated by summing the concentrations (in mg/dL) of these components (ie, triacylglycerol, free cholesterol, cholesteryl ester, phospholipid, and protein), and the contribution of each component to the total mass was expressed as a percentage. LDL subfraction distribution was determined by using the method of Griffin et al (18), with LDL-I, LDL-II, and LDL-III characterized by densities of 1025–1034, 1034–1044, and 1044–1060 g/L, respectively. Concentrations of HDL₂ and HDL₃ lipoproteins in cholesterol were measured by using the dextran sulfate–magnesium chloride dual-precipitation method described by Warnick et al (19).

Concentrations of lipoprotein(a), apo B, and apo A-I and of glucose and NEFAs in plasma collected in EDTA-coated tubes were measured with the use of immunoturbidimetric and enzymatic colorimetric methods, respectively, and commercially available kits (Bio-Stat Diagnostics, Stockport, United Kingdom; Roche Diagnostics GmbH; Wako Chemicals USA, Inc) on an automated Clinical Chemistry analyzer (model 704; Hitachi Ltd). Insulin in plasma collected in lithium heparin-coated tubes was analyzed with the use of an in-house immunoradiometric assay, radiolabelled mouse monoclonal anti-insulin, and solid-phase guinea pig anti-insulin (both antibodies were supplied by the Scottish Antibody Production Unit, Edinburgh) (20). C-reactive protein in plasma collected in EDTA-coated tubes was measured with the use of a high-sensitivity, two-site enzyme-linked immunoassay and a peroxidase-conjugated rabbit antihuman C-reactive protein antibody (DK2600; Dako, Glostrup, Denmark) and a polyclonal anti-C-reactive protein capture antibody (21). The fatty acid compositions of spreads, commercially available low-fat meals, and plasma samples were determined by using the method described in Brown et al (22).

Analytic methods for turnover samples

VLDL₁ and VLDL₂ were isolated from plasma as outlined above, and apo B was isolated from both VLDL subfractions by the addition of an equal volume of isopropanol (23). After exhaustive delipidation with ethanol:ether, which was followed by an ether wash, the apo B remained as a fine white pellet. The amino acids contained in the apo B pellets were released by hydrolysis at 110°C for 24 h in the presence of 1.0 mL of a 6-mol HCl/L solution and were then concentrated to dryness in a centrifugal evaporator (Gyrovap; VA Howe, Banbury, United Kingdom). Proteins were precipitated from 1 mL plasma with 10% (by vol) trichloroacetic acid, and amino acids were prepared by cation-exchange chromatography with Dowex AG-50W-X8 resin (H⁺ form, 50–100 mesh; Biorad, Richmond, CA). The amino acids bound to the resin were desorbed by a 4-mol NH₄OH/L solution and evaporated to dryness in a centrifugal evaporator. The samples were derivatized by using acetonitrile (Perbio Science, Chester, United Kingdom) and *N-tert*-butyldimethylsilyl-*N*-methyl-trifluoroacetamide (Fluka Chemicals Ltd, Epsom, United Kingdom). Analysis of the [²H₃]leucine enrichment (ie, the ratio of tracer to tracee) was carried out by using a gas chromatograph–mass spectrometer (MD 800; VG Masslab, Manchester, United Kingdom) as described previously (10, 15).

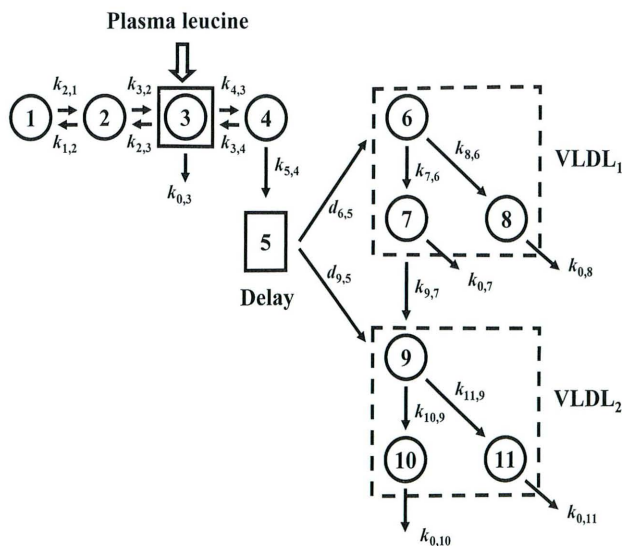


FIGURE 1. Multicompartmental model of apolipoprotein B metabolism in VLDL₁ and VLDL₂. Plasma leucine (compartment 3) receives the L-[5,5,5-²H₃]leucine tracer and distributes it to the body protein pools (compartments 1 and 2) and to an intracellular precursor pool for apolipoprotein B synthesis (compartment 4). After a delay (compartment 5), the tracer appears in VLDL₁ via compartment 6 and VLDL₂ via compartment 9 ($d_{6,5}$ and $d_{9,5}$ describe the proportion of hepatically synthesized apolipoprotein B directly appearing in VLDL₁ and VLDL₂, respectively). Compartments 6, 7, 9, and 10 form a delipidation chain, whereas movement from compartment 6 to compartment 8 and from compartment 9 to compartment 11 represents remnant formation. Variable dependencies were as follows: $k_{2,1} = 0.1 \times k_{1,2}$, $k_{3,4} = k_{4,3}$, $k_{3,2} = k_{2,3}$, $k_{3,4} = k_{4,3}$, $k_{8,6} = k_{11,9}$, $k_{0,8} = k_{0,11}$, and $k_{7,6} = k_{9,7}$.

The apo B content of VLDL₁ and VLDL₂ was calculated as the difference between the total protein and the isopropanol-soluble protein as measured by the Lowry assay (17). Apo B plasma pool sizes in each lipoprotein fraction were calculated as the apo B concentration multiplied by the plasma volume, which was estimated as being 4% of body weight. These were corrected for centrifugal losses (24), and the leucine content was calculated from the amino acid composition of apo B (25).

Kinetic analysis and multicompartmental modeling

The changes over time in the ratio of tracer to tracee in plasma and in apo B in VLDL₁ and VLDL₂, together with the measured apo B pool sizes in each lipoprotein fraction, were used to derive kinetic variables by using the SIMULATION ANALYSIS AND MODELING program II (version 1.1.1 for WINDOWS; SAAM Institute, Seattle). The multicompartmental model was described in detail previously (10, 15) and is illustrated in **Figure 1**. Steady-state production rates (in mg/d) and transport rates (or flux, in mg/d) of apo B from one lipoprotein fraction to another were obtained by the application of the model to the observed data. The fractional transfer rate for VLDL₁ to VLDL₂ was calculated as the transport rate from compartments 7 to 9 divided by the VLDL₁ apo B mass (combined masses of compartments 6, 7, and 8). The fractional transfer rate for VLDL₂ to IDL was calculated as the output from compartment 10 divided by the VLDL₂ apo B mass (combined masses of compartments 9, 10, and 11). Fractional rates of direct catabolism for VLDL₁ were derived by dividing the sum of outputs from compartments 7 and 8 (in mg/d) by the

VLDL₁ apo B mass, and the rates for VLDL₂ were derived by dividing the output from compartment 11 by the VLDL₂ apo B mass. Production rates were calculated as the product of the pool size in a lipoprotein fraction and its fractional catabolic rate. This was calculated as the sum of the fractional transfer rate and the fractional rate of direct catabolism.

The model has the following features. Plasma leucine is represented by a compartment (compartment 3) in rapid equilibrium with an intracellular compartment (compartment 4) that is the immediate source of leucine for apo B synthesis. Compartments 1 and 2 are required to account for the uptake and subsequent slow release of leucine by body protein pools that have a slow turnover. Compartment 5 is a delay component that was usually set at 0.5 h but could be adjusted if required. The same delay was used for both turnovers (L-MUFA and H-MUFA) in a given subject. Direct input of apo B occurs in VLDL₁ (compartment 6) and VLDL₂ (compartment 9). Compartments 6, 7, 9, and 10 form a delipidation chain, a feature found in most models of apo B metabolism, and compartments 8 and 11 represent “remnant populations” of particles that are cleared relatively slowly from each density interval. Physiologically plausible constraints were introduced to reduce the number of unknown variables to give the model a priori identifiability. These constraints were $k_{2,1} = 0.1 \times k_{1,2}$, $k_{3,4} = k_{4,3}$, $k_{8,6} = k_{11,9}$, $k_{7,6} = k_{9,7}$, and $k_{0,8} = k_{0,11}$.

Statistical analyses

Statistical analyses were performed by using SPSS for WINDOWS (version 10.0; SPSS Inc, Chicago). Differences between diets were assessed by using one-way repeated-measures analysis of variance, and linear trend analysis was used to determine whether variables changed in a dose-dependent manner with increasing dietary MUFA intake when all 3 diets were compared. Differences between the L-MUFA and H-MUFA diets were assessed by using paired *t* tests. Differences in baseline characteristics between the men and the women were assessed by using unpaired *t* tests. Data having a distribution that was significantly different from normal were logarithmically transformed before statistical analysis. Homeostasis model assessment (fasting insulin \times fasting glucose/22.5) was used as a validated surrogate measure of insulin sensitivity (26). Significance was accepted at $P < 0.05$, and data are presented as means \pm SEMs unless otherwise stated.

RESULTS

Dietary intervention

All 3 diets were well tolerated by the subjects. The nutrient intakes assessed from 5-d weighed food intakes during the 3 diet periods are shown in **Table 2**. The interventions successfully resulted in a stepwise substitution of dietary *cis* MUFAs for SFAs. There was also a small but significant difference between the 3 diet periods in dietary *cis* PUFAs ($\approx 1\%$ of energy), and a small but significant decrease in the intake of *trans* fatty acids (0.3% of energy) was observed across the 3 diet periods (from low to high MUFAs). Energy intake did not differ significantly between the 3 diet periods, nor did total fat intake. However, there were small ($\leq 1.2\%$) but significant differences between the 3 diet periods in the percentage of energy derived from fat. The differences between the diet periods in reported dietary fatty acid intake was

TABLE 2

Dietary intakes and plasma fatty acid compositions of subjects who consumed diets containing low (L), moderate (M), and high (H) amounts of monounsaturated fatty acids (MUFAs)¹

	L-MUFA diet	M-MUFA diet	H-MUFA diet	<i>P</i> for trend ²
Dietary intakes				
Energy (kcal/d)	2397 ± 66	2474 ± 79	2490 ± 87	NS
SFAs (% of energy)	14.7 ± 0.3	11.2 ± 0.2	7.3 ± 0.1	<0.01
12:0	1.3 ± 0.03	1.0 ± 0.04	0.7 ± 0.01	<0.01
14:0	0.8 ± 0.02	0.6 ± 0.02	0.4 ± 0.01	<0.01
16:0	10.6 ± 0.2	7.6 ± 0.3	4.5 ± 0.1	<0.01
18:0	1.5 ± 0.04	1.3 ± 0.05	1.3 ± 0.04	<0.01
<i>cis</i> MUFAs (% of energy)	7.8 ± 0.2	10.3 ± 0.2	13.7 ± 0.2	<0.01
<i>cis</i> PUFAs (% of energy)	6.1 ± 0.1	7.2 ± 0.1	6.8 ± 0.1	<0.01
<i>trans</i> FAs (% of energy)	1.2 ± 0.03	0.9 ± 0.02	0.9 ± 0.02	<0.01
SFA:MUFA	1.88 ± 0.01	1.09 ± 0.01	0.53 ± 0.003	<0.01
Total fat (g/d)	69.1 ± 2.4	69.5 ± 2.3	68.2 ± 2.5	NS
Fat (% of energy)	33.1 ± 0.6	32.7 ± 0.6	31.9 ± 0.5	0.01
Carbohydrate (% of energy)	52.2 ± 0.6	52.5 ± 0.7	53.2 ± 0.8	NS
Protein (% of energy)	12.4 ± 0.4	12.2 ± 0.3	12.3 ± 0.4	NS
Alcohol (g/d)	6.5 ± 1.5	8.2 ± 2.3	8.6 ± 1.9	NS
Plasma fatty acids				
MUFAs (%)	20.9 ± 0.5	22.3 ± 0.5	25.2 ± 0.4	<0.01
SFAs (%)	35.1 ± 0.5	33.9 ± 0.5	32.7 ± 0.6	<0.01
SFA:MUFA	1.71 ± 0.05	1.54 ± 0.04	1.31 ± 0.03	<0.01

¹ $\bar{x} \pm \text{SEM}$; *n* = 35. SFA, saturated fatty acid; PUFAs, polyunsaturated fatty acids; FAs, fatty acids.

²ANOVA.

reflected in plasma fatty acid compositions, with the ratio of SFAs to MUFAs decreasing significantly with increasing intakes of dietary MUFAs. The subjects' body masses at the start of each dietary intervention period did not differ significantly between the 3 diet periods (76.0 ± 2.1, 74.8 ± 2.1, and 75.9 ± 2.1 kg for the L-MUFA, M-MUFA, and H-MUFA diet periods, respectively) and did not change significantly during any of the intervention periods (−0.7 ± 0.2, −0.4 ± 0.3, and −0.7 ± 0.2 kg for the L-MUFA, M-MUFA, and H-MUFA diet periods, respectively).

Fasting plasma lipid and apolipoprotein profile

The fasting lipid profiles of the subjects after consumption of the 3 diets are shown in **Table 3**. Total cholesterol decreased in a dose-dependent manner with increasing intakes of dietary MUFAs: changing from the L-MUFA diet to the H-MUFA diet reduced total cholesterol concentrations by 7.4%. These reductions were entirely accounted for by reduced LDL cholesterol (11.7% change between the L-MUFA and H-MUFA diet periods); increasing dietary MUFAs did not significantly influence con-

centrations of VLDL cholesterol, HDL cholesterol, lipoprotein(a), and triacylglycerol. In accordance with the LDL-lowering effect, increasing dietary MUFAs produced a dose-dependent reduction in plasma apo B concentrations—a 6.4% reduction between the L-MUFA and H-MUFA diets. Apo A-I was not significantly influenced by the MUFA content of the diet; this is consistent with the fact that HDL cholesterol was also not influenced by dietary MUFAs. The men and the women responded similarly to the dietary interventions, experiencing reductions in LDL cholesterol of 11% and 13%, respectively, when changing from the L-MUFA to the H-MUFA diet.

Fasting lipoprotein concentrations and compositions

VLDL₁, VLDL₂, IDL, and LDL concentrations in the subjects after consumption of the 3 diets are shown in **Figure 2**. There were no significant differences in VLDL₁, VLDL₂, or IDL concentrations (in mg/dL) between the 3 diet periods, but LDL concentrations decreased in a dose-dependent manner with increasing intakes of dietary MUFAs. Changing from the L-MUFA to the

TABLE 3

Lipid and apolipoprotein concentrations of subjects in the fasted state who consumed diets containing low (L), moderate (M), and high (H) amounts of monounsaturated fatty acids (MUFAs)¹

	L-MUFA diet	M-MUFA diet	H-MUFA diet	<i>P</i> for trend ²
Total cholesterol (mmol/L)	6.10 ± 0.14	5.96 ± 0.14	5.65 ± 0.12	<0.01
Triacylglycerol (mmol/L)	1.79 ± 0.13	1.76 ± 0.12	1.87 ± 0.14	NS
VLDL cholesterol (mmol/L)	0.55 ± 0.07	0.59 ± 0.07	0.63 ± 0.07	NS
LDL cholesterol (mmol/L)	4.20 ± 0.13	4.00 ± 0.14	3.71 ± 0.10	<0.01
HDL cholesterol (mmol/L)	1.35 ± 0.07	1.37 ± 0.08	1.33 ± 0.07	NS
Apo A-I (mg/dL)	132.5 ± 4.7	135.2 ± 5.3	133.1 ± 4.4	NS
Apo B (mg/dL)	120.8 ± 3.1	116.9 ± 3.4	113.1 ± 3.1	<0.01
Lp(a) (mg/dL)	31.4 ± 5.4	33.8 ± 5.9	31.7 ± 5.2	NS

¹ $\bar{x} \pm \text{SEM}$; *n* = 35. Apo, apolipoprotein; Lp(a), lipoprotein(a).

²ANOVA.

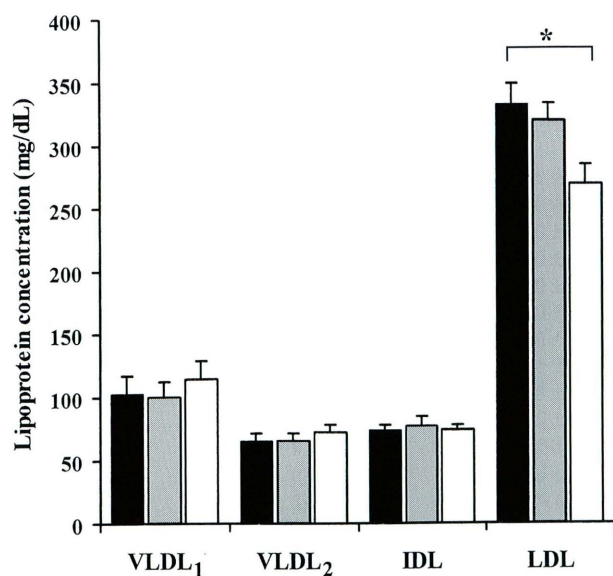


FIGURE 2. Mean (\pm SEM) concentrations of VLDL₁, VLDL₂, intermediate-density lipoprotein (IDL), and LDL in subjects ($n = 35$) who consumed diets containing low (■), moderate (▒), and high (□) amounts of monounsaturated fatty acids. * P for trend < 0.01 (ANOVA).

M-MUFA diet decreased LDL concentrations by 3.7%, whereas changing from the L-MUFA to the H-MUFA diet decreased LDL concentrations by 18.7%.

The percentages of phospholipids, protein, free cholesterol, cholesteryl esters, and triacylglycerol in VLDL₁ were not significantly affected by the MUFA content of the diet (data not shown), but percentages of cholesteryl esters in VLDL₂ and IDL particles decreased significantly with increasing intakes of dietary MUFAs [VLDL₂: $24.8 \pm 0.8\%$, $23.3 \pm 1.0\%$, and $22.0 \pm 0.7\%$ for the L-MUFA, M-MUFA, and H-MUFA diet periods, respectively (P for trend < 0.01); IDL: $39.6 \pm 0.7\%$, $39.1 \pm 0.8\%$, and $36.9 \pm 1.0\%$ for the L-MUFA, M-MUFA, and H-MUFA diet periods, respectively (P for trend = 0.01)]. VLDL₂ particles became significantly more triacylglycerol enriched as dietary MUFA intake increased ($34.4 \pm 0.8\%$, $35.8 \pm 1.0\%$, and $36.2 \pm 0.9\%$ for the L-MUFA, M-MUFA, and H-MUFA diet periods, respectively; P for trend = 0.04). The composition of LDL particles was not significantly influenced by dietary MUFA intake (data not shown). The ratio of cholesteryl esters to triacylglycerol in VLDL₂ and IDL particles decreased significantly with increasing intakes of dietary MUFAs, which implies that increasing MUFAs produced VLDL₂ and IDL particles that were relatively depleted in cholesteryl esters and relatively enriched in triacylglycerol [VLDL₂: 0.76 ± 0.5 , 0.70 ± 0.05 , and 0.63 ± 0.03 for the L-MUFA, M-MUFA, and H-MUFA diet periods, respectively (P for trend < 0.01); IDL: 3.99 ± 0.37 , 3.31 ± 0.27 , and 3.20 ± 0.27 for the L-MUFA, M-MUFA, and H-MUFA diet periods, respectively (P for trend = 0.02)]. However, it should be noted that absolute concentrations of cholesteryl esters in these particles were not significantly affected by dietary MUFA content (VLDL₂: 16.7 ± 1.7 , 15.4 ± 1.6 , and 16.7 ± 1.6 mg/dL for the L-MUFA, M-MUFA, and H-MUFA diet periods, respectively; IDL: 29.5 ± 1.7 , 30.1 ± 2.5 , and 27.6 ± 1.8 mg/dL for the L-MUFA, M-MUFA, and H-MUFA diet periods, respectively). The ratio of cholesteryl esters to

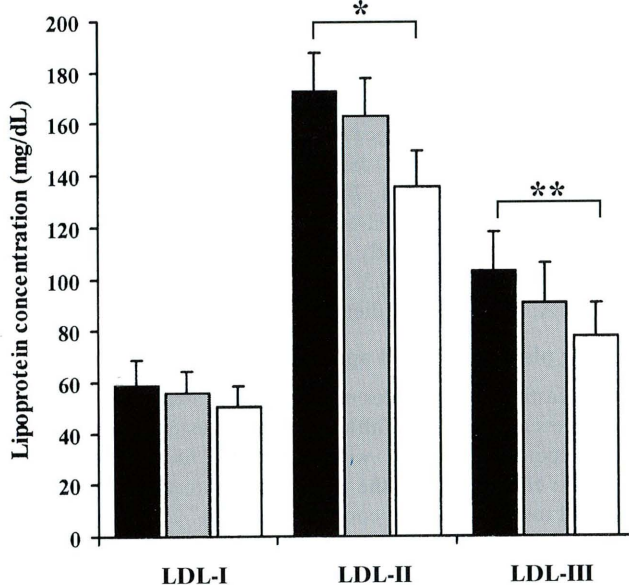
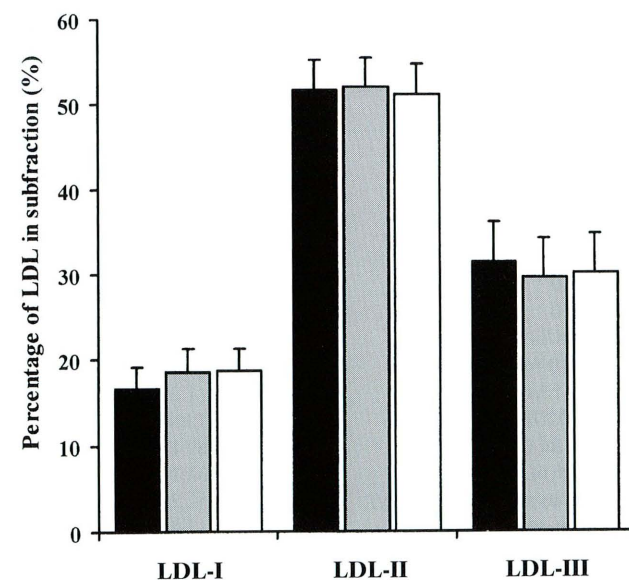


FIGURE 3. Mean (\pm SEM) percentages of total LDL contained in LDL-I, LDL-II, and LDL-III subfractions (top) and mean (\pm SEM) absolute concentrations of LDL-I, LDL-II, and LDL-III lipoproteins (bottom) in subjects ($n = 32$) who consumed diets containing low (■), moderate (▒), and high (□) amounts of monounsaturated fatty acids. * P for trend = 0.04 (ANOVA); ** P for trend = 0.02 (ANOVA).

triacylglycerol in VLDL₁ and LDL particles was not significantly affected by dietary MUFA content (data not shown).

LDL and HDL subfraction distribution

There was no significant effect of dietary MUFAs on LDL subfraction distribution (Figure 3, top panel). However, because increasing dietary MUFAs decreased LDL concentrations, concentrations of LDL-II and LDL-III lipoproteins decreased significantly (by 21.5% and 24.5%, respectively) as dietary MUFA

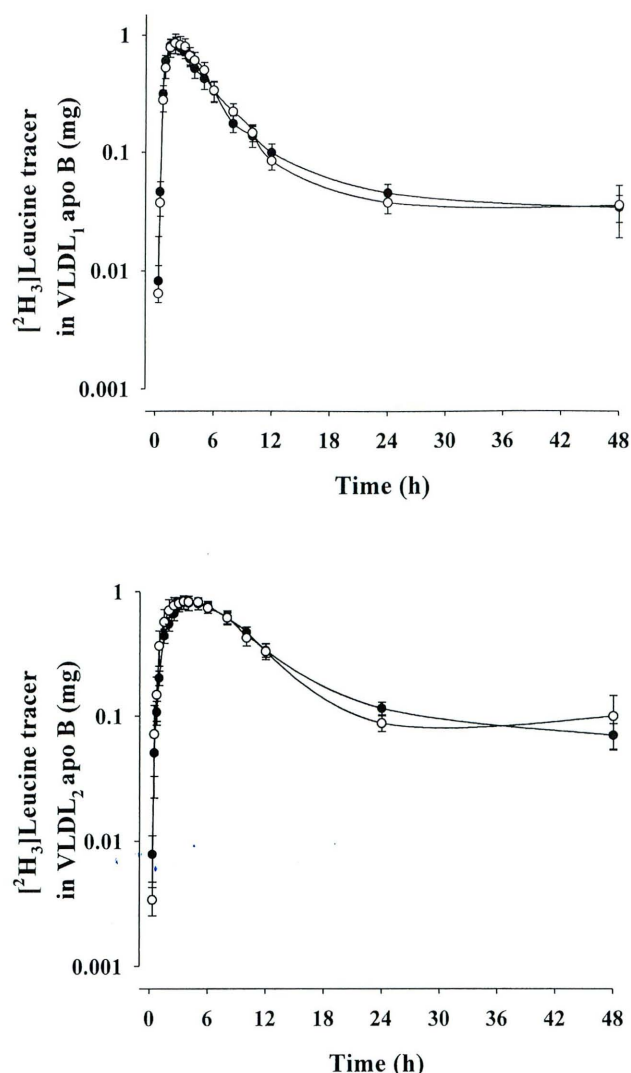


FIGURE 4. Mean (\pm SEM) incorporation of L-[5,5,5- $^2\text{H}_3$]leucine into VLDL₁ and VLDL₂ apolipoprotein B (apo B) during the 48 h after injection of the tracer in subjects ($n = 16$) who consumed diets containing low (\bullet) and high (\circ) amounts of monounsaturated fatty acids.

content increased (Figure 3, bottom panel). There were no significant differences between the 3 diet periods in HDL₂- and HDL₃-cholesterol concentrations (HDL₂: 0.22 ± 0.06 , 0.20 ± 0.06 , and 0.19 ± 0.04 mmol/L for the L-MUFA, M-MUFA, and H-MUFA diet periods, respectively; HDL₃: 1.09 ± 0.05 , 1.15 ± 0.04 , and 1.11 ± 0.05 mmol/L for the L-MUFA, M-MUFA, and H-MUFA diet periods, respectively).

VLDL₁ and VLDL₂ apolipoprotein B kinetics

After preliminary examination of the findings, we decided to exclude the results from one subject from the final analyses. This subject experienced a 5-fold change in VLDL₁ apo B pool size between the L-MUFA (165 mg) and H-MUFA diets (809 mg)—a change that differed markedly from that of any of the other subjects, skewed the data considerably, and was not a plausible consequence of the dietary intervention. Thus, the data presented below are from analyses performed without the data from this subject (ie, $n = 16$). Tracer enrichment of VLDL₁ and VLDL₂ apo B during the hours

TABLE 4

VLDL₁ and VLDL₂ apolipoprotein (apo) B kinetics in subjects who consumed diets containing low (L) and high (H) amounts of monounsaturated fatty acids (MUFAs)[†]

	L-MUFA diet	H-MUFA diet
VLDL₁		
Apo B pool size (mg)	119 ± 22	117 ± 18
Production (mg/d)	626 ± 112	737 ± 146
Fractional rate of:		
Direct catabolism (pools/d)	1.0 ± 0.5	0.8 ± 0.4
Transfer to VLDL ₂ (pools/d)	6.1 ± 1.1	5.9 ± 0.8
VLDL₂		
Apo B pool size (mg)	202 ± 17	206 ± 18
Production (mg/d)	171 ± 25	190 ± 25
Fractional rate of:		
Direct catabolism (pools/d)	0.5 ± 0.2	0.2 ± 0.05
Transfer to IDL (pools/d)	3.3 ± 0.4	3.7 ± 0.5

[†] $\bar{x} \pm$ SEM; $n = 16$. IDL, intermediate-density lipoprotein. There were no significant differences between the 2 diet periods (paired t test).

after the [$^2\text{H}_3$]leucine injection is shown in **Figure 4**. The mean apo B pool sizes for VLDL₁ and VLDL₂ and the rate constants generated from multicompartmental modeling for the production and fractional catabolism of these lipoproteins are shown in **Table 4**. There were no significant differences between the L-MUFA and H-MUFA diets in any of the kinetic variables.

Insulin, glucose, and fatty acid concentrations

Increasing dietary MUFAs significantly increased fasting insulin concentrations and insulin resistance estimated from homeostasis model assessment and significantly decreased fasting NEFA concentrations. Plasma glucose and C-reactive protein concentrations were not significantly affected by dietary MUFA content (**Table 5**).

DISCUSSION

This study showed that increasing the isoenergetic replacement of dietary SFAs with MUFAs reduced LDL cholesterol in a dose-dependent manner in middle-aged subjects with moderately elevated plasma cholesterol. The 7.4% decrease in total cholesterol (11.7% decrease in LDL cholesterol) achieved when the subjects changed from the L-MUFA to the H-MUFA diet is likely to be of clinical importance because it is generally held that every 1% reduction in plasma cholesterol confers a 2% reduction in cardiovascular disease risk (27). Thus, the cholesterol reduction achieved in this study would have induced a reduction in risk of $\approx 15\%$. Moreover, these LDL reductions were achieved by covert dietary manipulation, with foods typically consumed in a “Western,” United Kingdom diet and without the incorporation of additional visible oils into the subjects’ diets. Thus, with the cooperation of food manufacturers (ie, by using high-MUFA rather than high-SFA oils in the manufacture of processed foods), this largely “invisible” dietary change could be achieved at a population level in countries such as the United Kingdom and the United States because it would not require major changes to individual eating habits. Adding visible high-MUFA oils, such as olive oil, to the H-MUFA diet would have made it possible to increase the dietary MUFA content further and presumably reduce LDL concentrations to an even greater extent. However, this approach would have required a much more overt change to dietary habits because liquid oils represent a relatively small proportion of the total fat

TABLE 5

Plasma concentrations of insulin, glucose, nonesterified fatty acids (NEFAs), and C-reactive protein (CRP) and homeostasis model assessment (HOMA) scores in subjects who consumed diets containing low (L), moderate (M), and high (H) amounts of monounsaturated fatty acids (MUFAs)¹

	L-MUFA diet	M-MUFA diet	H-MUFA diet	<i>P</i> for trend ²
Insulin (μU/mL)	6.2 ± 0.5	7.3 ± 0.6	7.6 ± 0.6	<0.01
Glucose (mmol/L)	4.6 ± 0.1	4.7 ± 0.1	4.7 ± 0.1	NS
HOMA	1.28 ± 0.10	1.55 ± 0.14	1.59 ± 0.13	0.02
NEFAs (mmol/L)	0.33 ± 0.02	0.33 ± 0.02	0.27 ± 0.02	0.01
CRP (mg/L)	1.82 ± 0.32	2.02 ± 0.26	1.65 ± 0.29	NS

¹ $\bar{x} \pm \text{SEM}$; *n* = 35.

²ANOVA.

intake in the usual Western diet, and thus the magnitude of any lipoprotein changes observed would have been less applicable to the population at large.

It is unclear from this study design whether the LDL lowering achieved by the dietary interventions was a consequence of reducing the SFA intake, increasing the MUFA intake, or a combination of the 2 factors. However, the reductions in total cholesterol achieved in changing from the L-MUFA to the M-MUFA and H-MUFA diets (0.14 and 0.45 mmol/L, respectively; 0.20 and 0.49 mmol/L, respectively, for LDL cholesterol) were comparable to the cholesterol reductions predicted by the classic equation of Keys et al (28) (0.24 and 0.47 mmol/L, respectively). In this equation, a neutral effect of MUFAs on plasma cholesterol concentrations is assumed, and only the hypercholesterolemic effects of SFAs and the hypocholesterolemic effects of PUFAs are considered. Thus, the cholesterol lowering achieved in the present study may have been the consequence of removing cholesterol-increasing SFAs from the diet, rather than of a specific cholesterol-lowering effect of MUFAs. However, more recent reports suggest that dietary MUFAs do have an independent hypocholesterolemic effect (29); thus, increasing dietary MUFAs per se may have contributed to the cholesterol-lowering observed in the present study, although the relative importance of this effect in the present study is impossible to quantify.

The main finding of the present study was that the changes to lipoprotein concentrations elicited by substituting dietary MUFAs for SFAs were predominantly limited to LDL cholesterol. Increasing the MUFA content of the diet did not significantly reduce concentrations of VLDL₁, VLDL₂, IDL, or lipoprotein(a) or fasting triacylglycerol concentrations. Plasma concentrations of HDL cholesterol, apo A-I, and the HDL subfractions HDL₂ and HDL₃ were also not significantly influenced by dietary MUFA content. Likewise, LDL subfraction distributions did not differ significantly between the 3 diet periods. Plasma apo B concentrations were, however, significantly reduced by increasing dietary MUFA concentrations; this is consistent with the LDL-lowering effect because plasma apo B concentrations provide a measure of the number of circulating LDL particles.

The lack of significant change in HDL and apo A-I concentrations in the present study is consistent with reports from previous studies indicating no change or a slight (often nonsignificant) decrease in HDL cholesterol when dietary SFAs are replaced by MUFAs (6–9). The neutral effect of this dietary intervention on HDL is of practical importance when considering hypolipidemic therapies, because the alternative LDL-lowering approach of replacing SFAs with carbohydrates often induces a parallel reduction in the HDL cholesterol concentration (30). It is perhaps unsurprising that the present dietary intervention had a neutral

effect on HDL-cholesterol concentrations, as well as on the subclass distributions of HDL and LDL, because the concentrations and kinetics of triacylglycerol-rich VLDL₁ particles were not significantly affected by the fatty acid composition of the diet. It is now well established that HDL-cholesterol concentrations and HDL and LDL subclass distributions are governed, in large part, by the metabolism of large triacylglycerol-rich lipoproteins; thus, in the absence of changes to VLDL₁, perturbations of these lipoprotein variables would not be expected to occur (10, 31). However, although increasing dietary MUFAs did not significantly alter the LDL subclass profile, the overall LDL-lowering effect of this intervention resulted in a significant (≈25%) reduction in atherogenic LDL-III when changing from the L-MUFA to the H-MUFA diet (as well as a concurrent reduction in LDL-II). LDL-III concentrations after consumption of the L-MUFA diet (103.0 ± 15.0 mg/dL) were at a level that conferred elevated atherosclerotic risk (32), so the reduction elicited by the present dietary intervention is likely to make a clinically important difference in the cardiovascular disease risk profile.

Of particular interest was the fact that replacing SFAs with MUFAs did not significantly influence VLDL₂ concentrations or kinetics. Thus, our original hypothesis that at least part of the LDL-lowering effect of replacing dietary SFAs with MUFAs would be due to reduced production of VLDL₂—because this is the major metabolic precursor particle for LDL (10)—was disproved. Together with our finding that concentrations of IDL (the intermediate lipoprotein particle in the VLDL₂-to-LDL delipidation chain) were also not significantly affected by increasing dietary MUFAs, these data suggest that decreased LDL production was not the primary mechanism underpinning the LDL-lowering effect. Thus, an increase in LDL clearance—probably mediated by up-regulation of LDL receptor activity—seems likely to have been responsible for the highly specific LDL-lowering effect seen with the current intervention. Animal and tissue culture studies showed both that SFAs decrease (33–35) and that MUFAs increase (33, 35, 36) the activity of the LDL receptor. Thus, the isoenergetic replacement of SFAs with MUFAs may well increase LDL receptor activity (and therefore reduce plasma LDL concentrations) both by reducing SFA inhibition of receptor activity and by facilitating MUFA up-regulation of activity. However, studies in humans with dietary fatty acid changes that are achievable in practice have not been undertaken, and further study is needed to confirm whether, in fact, this mechanism also operates in whole-body human interventions.

Although isoenergetic replacement of SFAs with MUFAs did not significantly alter the concentrations of any lipoprotein other than LDL, this dietary change did significantly alter the composition of VLDL₂ and IDL, resulting in particles that were relatively

rich in triacylglycerol and relatively poor in cholesteryl esters. However, because absolute cholesteryl ester concentrations in these lipoprotein particles were not significantly reduced as the dietary MUFA content increased, it seems unlikely that these compositional changes would have been a major contributor to the observed reduction in LDL concentrations. The relative cholesteryl ester decrement in VLDL₂ and IDL may have been the consequence of reduced cholesteryl ester transfer protein (CETP) activity with consumption of the high-MUFA diet because other studies have shown that CETP is reduced when SFAs are replaced with MUFAs or carbohydrates (37). However, changes in CETP would be expected to induce changes in VLDL₁, as well as VLDL₂, but such changes were not seen. It is, therefore, not clear whether altered CETP activity is consistent with the changes observed in the present study. Although it has been reported that diet-induced changes in LDL cholesterol are significantly related to changes in CETP concentration (37), the direction of causality in this relation is not known. It has been reported that the abundance of CETP messenger RNA in adipose tissue is regulated by the intracellular cholesterol content (38); thus, any changes in CETP that may have occurred in the present study may well have been a consequence rather than a cause of the cholesterol-lowering effect.

The present data suggest that replacing dietary SFAs with MUFAs may increase insulin resistance because fasting insulin concentrations and homeostasis model assessment scores were significantly higher after consumption of the M-MUFA and H-MUFA diets than after consumption of the L-MUFA diet. However, because plasma NEFA concentrations decreased significantly with increasing dietary MUFA content, a change associated with increased insulin sensitivity, the significance of this finding is unclear. This finding was somewhat unexpected, because a high proportion of SFAs in plasma and cell membranes was previously associated with insulin resistance (39). Although we used relatively crude methods to assess insulin sensitivity, the implications of this potentially adverse consequence of replacing dietary SFAs with MUFAs should be considered carefully. Roche et al (40) found that postprandial insulin concentrations were higher after consumption of a high-MUFA diet than after consumption of a high-SFA diet, which supports the suggestion that increasing dietary MUFAs may increase insulin resistance. However, the present findings contrast with a recent report suggesting that replacing dietary SFAs with MUFAs increases insulin sensitivity, at least when total dietary fat intake is <37% (41). Note, however, that the intervention period in the study by Vessby et al (41) was 90 d (ie, twice as long as that in the present study), and it is possible that the increased insulin resistance observed with increasing intakes of dietary MUFAs in the present study was a transient effect that would not have persisted in the long term. This warrants further investigation because if the replacement of dietary SFAs with MUFAs is associated with adverse, as well as beneficial, effects, the net change in risk associated with this dietary change needs to be carefully assessed before general recommendations can be made.

In conclusion, this study showed that the effects of replacing dietary SFAs with MUFAs on lipoprotein metabolism are almost exclusively limited to LDL. This highly specific effect occurred in a dose-dependent manner, and increased LDL clearance appeared to be the most likely mediator of this change. In addition, replacing dietary SFAs with MUFAs reduced concentrations of atherogenic LDL-III particles by ≈25%. However, increasing dietary MUFAs also

appeared to increase insulin resistance. Further study may be necessary to ascertain the net benefits of replacing dietary SFAs with MUFAs. ■

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The experimental work was undertaken by JMRG, JCB, DMW, JC, DB, and DAH. JCB and DMW recruited subjects and designed the dietary intervention. JMRG and MJC (Glasgow) and JCB (Norwich) managed the project. JMRG, JCB, and MJC analyzed the data. JMRG wrote the first draft of the manuscript. JCS and CJP had the initial idea for the study and obtained funding. CJP oversaw the project. No author had any financial or personal interest in the UK Food Standards Agency.

REFERENCES

1. Pekkanen J, Linn S, Heiss G, et al. Ten-year mortality from cardiovascular disease in relation to cholesterol level among men with and without preexisting cardiovascular disease. *N Engl J Med* 1990;322:1700–7.
2. Castelli WP, Anderson K, Wilson PW, Levy D. Lipids and risk of coronary heart disease. The Framingham Study. *Ann Epidemiol* 1992; 2:23–8.
3. Grundy SM. Primary prevention of coronary heart disease: role of cholesterol control in the United States. *J Intern Med* 1997;241: 295–306.
4. Stamler J, Wentworth D, Neaton JD. Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? Findings in 356,222 primary screenees of the Multiple Risk Factor Intervention Trial (MRFIT). *JAMA* 1986;256: 2823–8.
5. 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.
6. Howard BV, Hannah JS, Heiser CC, et al. Polyunsaturated fatty acids result in greater cholesterol lowering and less triacylglycerol elevation than do monounsaturated fatty acids in a dose-response comparison in a multiracial study group. *Am J Clin Nutr* 1995;62: 392–402.
7. Mensink RP, Katan MB. Effect of a diet enriched with monounsaturated or polyunsaturated fatty acids on levels of low-density and high-density lipoprotein cholesterol in healthy women and men. *N Engl J Med* 1989;321:436–41.
8. Hodson L, Skeaff CM, Chisholm WA. The effect of replacing dietary saturated fat with polyunsaturated or monounsaturated fat on plasma lipids in free-living young adults. *Eur J Clin Nutr* 2001;55:908–15.
9. Williams CM, Francis-Knapper JA, Webb D, et al. Cholesterol reduction using manufactured foods high in monounsaturated fatty acids: a randomized crossover study. *Br J Nutr* 1999;81:439–46.
10. Packard CJ, Demant T, Stewart JP, et al. Apolipoprotein B metabolism and the distribution of VLDL and LDL subfractions. *J Lipid Res* 2000;41:305–18.
11. Griffin BA. Lipoprotein atherogenicity: an overview of current mechanisms. *Proc Nutr Soc* 1999;58:163–9.
12. Tailleux A, Fruchart JC. HDL heterogeneity and atherosclerosis. *Crit Rev Clin Lab Sci* 1996;33:163–201.
13. National Heart and Lung Institute. Manual of laboratory operations: Lipid Research Clinics Program. Bethesda, MD: US Department of Health, Education, and Welfare, 1975. [DHEW publication no. (NIM) 75.]
14. Lindgren FT, Jensen LC, Hatch FT. The isolation and quantitative analysis of serum lipoproteins. In: Nelson GJ, ed. Blood lipids and lipoproteins: quantitation, composition and metabolism. New York: Wiley-Interscience, 1972:181–274.
15. Demant T, Packard CJ, Demmelmair H, et al. Sensitive methods to study human apolipoprotein B metabolism using stable isotope-labeled amino acids. *Am J Physiol* 1996;270:E1022–36.

16. 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.
17. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951;193:265-75.
18. Griffin BA, Caslake MJ, Yip B, Tait GW, Packard CJ, Shepherd J. Rapid isolation of low density lipoprotein (LDL) subfractions from plasma by density gradient ultracentrifugation. *Atherosclerosis* 1990;83:59-67.
19. Warnick GR, Benderson J, Albers JJ. Dextran sulfate-Mg²⁺ precipitation procedure for quantitation of high-density-lipoprotein cholesterol. *Clin Chem* 1982;28:1379-88.
20. Dorrian CA. Human proinsulin and insulin: antibody production, assay development and clinical application. PhD thesis. University of Glasgow, Glasgow, United Kingdom, 1989.
21. Packard CJ, O'Reilly DS, Caslake MJ, et al. Lipoprotein-associated phospholipase A2 as an independent predictor of coronary heart disease. West of Scotland Coronary Prevention Study Group. *N Engl J Med* 2000;343:1148-55.
22. Brown JC, Hughes DA, Stanley JC. Use of argentation TLC with GC to resolve C18:1 fatty acid isomers in test and commercial spreads. *Biochem Soc Trans* 1998;26:S176.
23. Egusa G, Brady DW, Grundy SM, Howard BV. Isopropanol precipitation method for the determination of apolipoprotein B specific activity and plasma concentrations during metabolic studies of very low density lipoprotein and low density lipoprotein apolipoprotein B. *J Lipid Res* 1983;24:1261-7.
24. Gaw A, Packard CJ, Lindsay GM, et al. Overproduction of small very low density lipoproteins (S_v 20-60) in moderate hypercholesterolemia: relationships between apolipoprotein B kinetics and plasma lipoproteins. *J Lipid Res* 1995;36:158-71.
25. Hardman DA, Kane JP. Isolation and characterization of apolipoprotein B-48. *Methods Enzymol* 1986;128:262-72.
26. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412-9.
27. Carleton RA, Dwyer J, Finberg L, et al. Report of the Expert Panel on Population Strategies for Blood Cholesterol Reduction. A statement from the National Cholesterol Education Program, National Heart, Lung, and Blood Institute, National Institutes of Health. *Circulation* 1991;83:2154-232.
28. Keys A, Anderson JT, Grande F. Serum cholesterol response to changes in the diet. IV. Particular saturated fatty acids in the diet. *Metabolism* 1965;14:776-87.
29. Yu S, Derr J, Etherton TD, Kris-Etherton PM. Plasma cholesterol-predictive equations demonstrate that stearic acid is neutral and monounsaturated fatty acids are hypocholesterolemic. *Am J Clin Nutr* 1995;61:1129-39.
30. Katan MB. Effect of low-fat diets on plasma high-density lipoprotein concentrations. *Am J Clin Nutr* 1998;67(suppl):573S-6S.
31. Cohn JS. Postprandial lipemia: emerging evidence for atherogenicity of remnant lipoproteins. *Can J Cardiol* 1998;14:18B-27B.
32. Griffin BA, Freeman DJ, Tait GW, et al. Role of plasma triglyceride in the regulation of plasma low density lipoprotein (LDL) subfractions: relative contribution of small, dense LDL to coronary heart disease risk. *Atherosclerosis* 1994;106:241-53.
33. Daumerie CM, Woollett LA, Dietschy JM. Fatty acids regulate hepatic low density lipoprotein receptor activity through redistribution of intracellular cholesterol pools. *Proc Natl Acad Sci U S A* 1992;89:10797-801.
34. 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.
35. Kurushima H, Hayashi K, Shingu T, et al. Opposite effects on cholesterol metabolism and their mechanisms induced by dietary oleic acid and palmitic acid in hamsters. *Biochim Biophys Acta* 1995;1258:251-6.
36. Rumsey SC, Galeano NF, Lipschitz B, Deckelbaum RJ. Oleate and other long chain fatty acids stimulate low density lipoprotein receptor activity by enhancing acyl coenzyme A:cholesterol acyltransferase activity and altering intracellular regulatory cholesterol pools in cultured cells. *J Biol Chem* 1995;270:10008-16.
37. Jansen S, Lopez-Miranda J, Castro P, et al. Low-fat and high-monounsaturated fatty acid diets decrease plasma cholesterol ester transfer protein concentrations in young, healthy, normolipemic men. *Am J Clin Nutr* 2000;72:36-41.
38. Radeau T, Lau P, Robb M, McDonnell M, Ailhaud G, McPherson R. Cholesteryl ester transfer protein (CETP) mRNA abundance in human adipose tissue: relationship to cell size and membrane cholesterol content. *J Lipid Res* 1995;36:2552-61.
39. Vessby B. Dietary fat and insulin action in humans. *Br J Nutr* 2000;83:S91-6.
40. Roche HM, Zampelas A, Knapper JM, et al. Effect of long-term olive oil dietary intervention on postprandial triacylglycerol and factor VII metabolism. *Am J Clin Nutr* 1998;68:552-60.
41. Vessby B, Unsitupa M, Hermansen K, et al. Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: the KANWU Study. *Diabetologia* 2001;44:312-9.