Dietary intake of palmitate and oleate has broad impact on systemic and tissue lipid profiles in humans\textsuperscript{1–3}

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ABSTRACT

Background: Epidemiologic evidence has suggested that diets with a high ratio of palmitic acid (PA) to oleic acid (OA) increase risk of cardiovascular disease (CVD).

Objective: To gain additional insights into the relative effect of dietary fatty acids and their metabolism on CVD risk, we sought to identify a metabolomic signature that tracks with diet-induced changes in blood lipid concentrations and whole-body fat oxidation.

Design: We applied comprehensive metabolomic profiling tools to biological specimens collected from 18 healthy adults enrolled in a crossover trial that compared a 3-wk high–palmitic acid (HPA) with a low–palmitic acid and high–oleic acid (HOA) diet.

Results: A principal components analysis of the data set including 329 variables measured in 15 subjects in the fasted state identified one factor, the principal components analysis factor in the fasted state (PCF1-Fasted), which was heavily weighted by the PA:OA ratio of serum and muscle lipids, that was affected by diet ($P < 0.0001$; HPA greater than HOA). One other factor, the additional principal components analysis factor in the fasted state (PCF2-Fasted), reflected a wide range of acylcarnitines and was affected by diet in women only ($P = 0.0198$; HPA greater than HOA). HOA lowered the ratio of serum low-density lipoprotein to high-density lipoprotein (LDL: HDL) in men and women, and adjustment for the PCF1-Fasted abolished the effect. In women only, adjustment for the PCF2-Fasted eliminated the HOA-diet effect on serum total- and LDL-cholesterol concentrations. The respiratory exchange ratio in the fasted state was lower with the HPA diet ($P = 0.04$), and the diet effect was eliminated after adjustment for the PCF1-Fasted. The messenger RNA expression of the cholesterol regulatory gene insulin-induced gene-1 was higher with the HOA diet ($P = 0.008$).

Conclusions: These results suggest that replacing dietary PA with OA reduces the blood LDL concentration and whole-body fat oxidation by modifying the saturation index of circulating and tissue lipids. In women, these effects are also associated with a higher production and accumulation of acylcarnitines, possibly reflecting a shift in fat catabolism. Am J Clin Nutr 2014;99:436–45.

INTRODUCTION

Western-style diets, which are rich in the SFA palmitic acid (PA\textsuperscript{4}; 16:0), have been linked to increased risk of cardiovascular disease (CVD), in part by promoting higher ratios of LDL cholesterol to HDL cholesterol in blood and tissues (1). In the Western diet, much of the fat is derived from animal products; therefore, these diets are also high in the MUFA, oleic acid (OA; 18:1 cis 9, 18:1 n–9, or o–9). PA partially inhibits acyl-CoA: cholesterol acyltransferase (ACAT) in the liver, which leads to a decreased cholesterol ester formation, increased sterol pool, and decreased expression of the LDL receptor in the liver (2). In contrast, OA is the preferred substrate for ACAT and has the opposite effect (2). Cellular cholesterol also regulates cholesterol synthesis and LDL uptake through the activation of the transcription factor sterol regulatory element binding protein (SREBP) (3). There are 2 major isoforms of this protein in liver, SREBP-2, which promotes cellular synthesis of cholesterol and its uptake via LDL, and SREBP-1c, which promotes the synthesis of long-chain fatty acids (FAs) and malonyl-CoA [the latter isoform inhibits fat oxidation (3)]. To be functionally active, all isoforms of SREBP must first be proteolytically processed in the Golgi apparatus (3). When cellular concentrations of cholesterol are high, insulin-induced gene-1 protein (Insig-1) binds to the protein SREBP cleavage-activating protein (Scap), thereby causing SREBP-2 to be retained in the endoplasmic reticulum where it remains biologically inactive as a transcription factor (3). Conversely, when cellular sterol and cholesterol

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\textsuperscript{4}Abbreviations used: ACAT, acyl-CoA:cholesterol acyltransferase; CRP, C-reactive protein; CVD, cardiovascular disease; FA, fatty acid; HOA, low palmitic acid and high oleic acid; HPA, high palmitic acid; Insig, insulin-induced gene; mRNA, messenger RNA; OA, oleic acid; PA, palmitic acid; PCA, principal components analysis; PCF1-Fasted, principal components analysis factor in the fasted state; PCF1-Fed, principal components analysis factor in the fed state; PCF2-Fasted, additional principal components analysis factor in the fasted state; POA, palmitoleic acid; REE, resting energy expenditure; RER, respiratory exchange ratio; SA, stearic acid; Scap, sterol regulatory element binding cleavage-activating protein; SREBP, sterol regulatory element binding protein.

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activator receptor coactivator Ppar

Ppar-α, SREBP-2 is activated and, in turn, upregulates a number of genes, including INSIG-1. However, the INSIG-1 protein is rapidly degraded unless sufficient cholesterol is present to stabilize the INSIG-1/Scap/SREBP-2 complex (3). Thus, the synthesis and degradation of INSIG-1 influences the activities of SREBP-2 and SREBP-1c, along with their actions to promote the biosynthesis and cellular uptake of cholesterol and inhibit fat oxidation. In aggregate, when dietary cholesterol intake is kept constant, long-chain saturated FAs, such as PA, suppress hepatic LDL-receptor activity and, indirectly, stimulate cholesterol synthesis, whereas unsaturated FAs have the opposite effect (2–4). Cellular concentrations of cholesterol, which are regulated in part by SREBP-2 activity and INSIG-1, also affect circulating concentrations of LDL (3). Debate is ongoing regarding what macronutrient should replace PA in an effort to reduce risk of CVD (1, 5). Lipidomics is a promising scientific approach for understanding how individual FAs may contribute to risk factors for CVD (6, 7).

The deposition of FAs within specific lipid species will be a function of both intake and metabolism (8). The oxidation compared with storage of FA may affect cellular function and alter risks for clinically significant insulin resistance and the metabolic syndrome (9, 10). We reported that, in young, nonobese, human subjects, a diet high in both PA and OA lowered fat oxidation (11), especially in women (12). Estrogen may regulate genes that promote fat oxidation, such as peroxisome proliferation activator receptor (Ppar)-δ, Ppar-α, pyruvate dehydrogenase kinase 4 (Pdk4), and peroxisome proliferation activator receptor coactivator−1α (Pgoc-1α) (13, 14). Thus, in women, the impact of dietary FA intake on muscle gene expression might depend on the estrogen concentration.

In light of the foregoing findings, in this study, we elected to analyze the impact of sex on diet-induced changes in the muscle expression of lipid-regulatory genes. To this end, we studied women in the luteal phase of their menstrual cycle, when estrogen (and also progesterone) secretion is high. We hypothesized that the replacement of dietary PA with OA would influence clinically important risk factors such as blood lipoprotein profiles by altering the FA composition of cellular lipids, which would, in turn, influence skeletal muscle expression of genes involved in lipid metabolism.

SUBJECTS AND METHODS

Subjects, screening, diet, and overall design

Procedures followed for this study were in accordance with the ethical standards of the institutional committees associated with the University of Vermont General Clinical Research Center, which approved the study, and in accordance with the Helsinki Declaration of 1975 as revised in 1983. The first volunteer began this study on 30 August 2007; therefore, the study was not registered as a clinical trial. Healthy men (n = 9) and women (n = 9) aged 18–40 years with BMI (in kg/m²) >18 and <30 constituted the cohort for all results in this article. However, with the low–palmitic acid and high–oleic acid (HOA) diet, in 2 men, the serum concentration of triacylglycerols was below the concentration of detectability (20 mg/dL). Therefore, LDL was calculated for 16 subjects. For this cohort, all subjects were white.

One white man was Hispanic, and one white man listed his ethnicity as unknown. Exclusion criteria included regular aerobic exercise training, dyslipidemia (15), evidence of type 2 diabetes or insulin resistance (16, 17), and a vegetarian diet or habitual fat intake <25% of dietary energy. Women were enrolled if they did not receive hormonal forms of contraception and manifested normal ovulation based on a urine luteinizing hormone test and serum concentrations of estradiol and progesterone.

The prestudy dietary intake was assessed by a 24-h dietary recall by using the multiple pass method with standard diet-assessment software (The Food Processor SQL Version 10.6.3; ESHA Research), but as part of a new study, we have screened 5 women and 6 men of similar age as the subjects reported in this articles by using the Automated Self-administered 24-h Dietary Recall tool (ASA24; National Cancer Institute). With the use of data from these surveys, it appeared that the habitual intake of our volunteers was ~37% of kilocalories from total fat, 14.5% of kilocalories from saturated fat, and 12% of kilocalories from monounsaturated fat, which was consistent with the usual American diet (18, 19). After screening, all subjects ingested a low-fat and low-PA baseline-control diet for 7 days (protein: 19.7% of kilocalories; carbohydrate: 51.6% of kilocalories; fat: 28.4% of kilocalories; PA: 5.3% of kilocalories; OA: 15.9% of kilocalories). This diet was patterned after the Therapeutic Lifestyles Diet (15). On the morning of day 8 of the baseline-control diet, in the fasted state, blood and muscle tissue were collected at 0700 (20), and 3 h after a breakfast (one-third of daily kilocalories), a muscle biopsy and blood collection were repeated. The subject then participated in a crossover study of 3-week diet periods, which consisted of a diet that resembled the habitual diet and had high palmitic acid (HFA) (fat: 40.4% of kilocalories; PA: 16.0% of kilocalories; OA: 16.2% of kilocalories; linoleic acid: 5.0% of kilocalories) or an HOA diet (fat: 40.1% of kilocalories; OA: 24.4% of kilocalories; OA: 28.8% of kilocalories; linoleic acid: 6.4% of kilocalories) (on the basis of an actual analysis at Covance Laboratories) (Table 1). The FA composition of the HFA diet reasonably approximated that which is typical of many residents of North America (21, 22) as also was confirmed by our prestudy diet history. The FA composition of the HOA diet resembled that of olive oil (23). Diets were described in random order and separated by a 1-week period of the baseline-control diet. Blood and muscle were also collected in both fasted and fed states at the end of the HFA and HOA diets but not at the end of the second baseline-control diet (washout period of 7 days) (8, 20). In women, postexperimental diet evaluations took place in the luteal phase of the cycle before menstruation.

All food and drink, except water, were provided by the General Clinical Research Center, and the body energy balance was maintained throughout the study as previously described (20, 24). Except for added fat as vegetable oil, the low-fat foods we used were identical for HFA and HOA diets, but the amounts of individual foods at a given meal varied slightly to meet goals for the FA content. In the 3-d rotating menu, we used zero-fat dairy products, and the only meats were lean chicken and turkey. Thus, FAs were mainly provided by vegetable oil blends appropriate to each diet (Natural Oils International Inc). These oil blends, at room temperature, were mixed with food that had been warmed; thus, these oils were not used for cooking as occurred in some other studies (25). The oil blend for the control diet consisted of...
palm oil (36.9%), high oleic sunflower oil (19.3%), and hazelnut oil (43.8%). The HPA oil blend consisted of palm oil (89%), peanut oil (6.75%), and olive oil (4.25%), and the HOA blend consisted only of hazelnut oil. Except for virgin olive oil, which was used only in the HPA diet, all natural oils were first extracted (eg, by centrifugation) and refined [alkaline refining (crude oil treated with alkali to separate impurities from triglycerides) and filtering to remove impurities such as seed fragments]. HOA and HPA diets had identical low glycemic loads (10.7; average of the 3 days; 26, 27). Because of slight variation in amounts of individual food items (per kcal) in HPA and HOA diets, there also were the following small differences in cholesterol contents of diets plus the accompanying oil: 1) control diet: day 1, 115 mg/2000 kcal; day 2, 74 mg/2000 kcal; 2) HPA diet: day 1, 47 mg/2000 kcal; day 2, 62 mg/2000 kcal; day 3, 56 mg/2000 kcal; and 3) HOA diet: day 1, 55 mg/2000 kcal; day 2, 63 mg/2000 kcal; and day 3, 62 mg/2000 kcal (ProNutra 3.2; Viocare Technologies Inc).

Subjects ate breakfast in the General Clinical Research Center on Sunday through Friday, but most subjects chose to eat their 2 remaining meals each day at home. Each meal was packaged and ready to be reheated by using either an oven or microwave. Because the foods themselves were practically devoid of fat, subjects also were given containers of oil to add to each meal but only after the food had been reheated. Subjects also were given instructions regarding convenient ways to add the oils to various food items on the menu. Each day, subjects completed and signed a questionnaire in which they attested to their having eaten all of the food (and food oil) and to not having consumed any food or drink, except water, that was not on the menu. On Sunday, volunteers completed questionnaires pertaining to Saturday as well as Friday. All food and oil containers were inspected each day to be sure all food and oil were consumed. Any food or dietary oil left over in containers was weighed, and data were used to construct a modified food intake for that day. Subjects were given instructions to use spatulas provided to help scrape all oil from its container but ultimately were instructed to lick the container to finally empty it. Occasionally, there was evidence of incomplete food or oil consumption, but any consistent noncompliance was grounds for removal from the study. Only one subject quit the study (on day 3) because of a dislike of a specific food. Completely following the diet was a dichotomous issue, and subjects were queried each day about this issue. The average number of days when food was returned with HPA and HOA diets was 1.33 and 1.67 d, respectively, and the average daily consumption of oil for the HPA and HOA diets as a percentage of the total oil administered was 99.9% (127.8 g/d) and 99.2% (127.6 g/d), respectively.

### Body composition

On the first day of the baseline diet and at the end of HPA and HOA diets, fat mass and fat-free mass were assessed by using dual-energy X-ray absorptiometry (GE Lunar Prodigy Densitometer, version 5.6; GE Corp).

### Indirect calorimetry

On the 20th day of each experimental diet period (HPA and HOA), after an evening meal at 1800 (one-third of the daily energy intake), we carried out indirect calorimetry overnight in both fed and fasted states as previously described in detail (11). The measurements of oxygen consumption and CO₂ production (Vmax SPECTRA 29; Sensor Medics Corp) were used at 1740, 1900, 2000, 2100, 2200, 2300, 2400, 0100, 0300, and 0500. Urine urea nitrogen was measured during the 12-h interval, and protein oxidation was estimated for both fed (1720–0120) and fasting (0120–0520) periods (28). Resting energy expenditure (REE) and substrate utilization were calculated according to standard procedures by using urine urea nitrogen measurements as estimates of protein oxidation (28–30) and Weir’s equation (31). Urine urea nitrogen was measured by the Fletcher Allen Health Center clinical laboratory by using a urease quinolinium dye method (Vitros 250 Chemistry System; Ortho-Clinical Diagnostics). The respiratory exchange ratio (RER), which is an index of the respiratory quotient and rates of fat and carbohydrate oxidation, was estimated for both fed and fasted states from the average value for each period (28, 32).

### Blood concentrations of total cholesterol, LDL, HDL, triacylglycerols, C-reactive protein, estradiol, progesterone, and testosterone

Serum concentrations of total cholesterol, HDL cholesterol, and triacylglycerols were measured at the Clinical Chemistry Laboratory at Fletcher Allen Health Care, which is an affiliate of the University of Vermont, by using a colorimetric method (Vitros 5.1 FS Chemistry System; Ortho-Clinical Diagnostics), and LDL cholesterol was calculated. The serum C-reactive protein (CRP) concentration (fasted state) was measured at the Laboratory for Clinical Biochemistry Research at the University of Vermont by using nephelometry (CardioPhase hsCRP Assay, BNÖ II System; Siemens Healthcare Diagnostics Inc). Serum concentrations of estrogen and progesterone in women and testosterone in men were measured by the clinical laboratory of Fletcher Allen Health Care by using competitive, chemiluminescent immunoassays.
Metabolite assays

As part of our metabolomic analysis, we analyzed 329 total variables during the fasted state; this analysis included the following classes of compounds 1) serum and muscle phospholipids (total and FA composition of phosphatidylcholine, phosphatidylethanolamine, and cardiolipin); 2) FA composition of muscle diacylglycerol and triacylglycerol; 3) individual serum and muscle acylcarnitine species; 4) serum ceramide species; 5) serum and muscle amino acids; 6) serum concentrations of glucose, β-hydroxybutyrate, total and individual nonesterified FAs, total FAs (protein bound plus nonesterified), insulin, total ketones, adiponectin, and leptin; 7) muscle organic acids (citrate, fumarate, lactate, malate, pyruvate, succinate, and 2-ketoglutarate); and 8) urinary concentrations of creatinine, organic acids (citric, fumaric, lactic, malic, pyruvic, succinic, glutaric, 2-ketoglutaric, 3-hydroxy-3-methylglutaric, ethylmalonic, homovanillic acid, methylmalonic, methysuccinic, orotic, adipic, and suberic), and acylglycines (hexanoyl-, isobutyryl-, butyryl-, and isovaleryl-).

Because serum phospholipids were not measured in the fed state, only 277 variables comprised individual variables analyzed as part of the metabolomic analysis in the fed state. The FA composition of serum and muscle phospholipids and muscle diacylglycerol and triacylglycerol was analyzed by using recently described methods (thin-layer chromatography using the EM plate 5715–7; EMD Chemicals Inc) (20, 33). Standard radioimmunoassay kits and a Wallac Wizard 1470–010 automatic γ counter were used for assays of leptin and adiponectin (Linco RIA kits; Linco Research Inc). β-Hydroxybutyrate and nonesterified FAs were measured by using standard methods with kits (Wako). Ceramides were extracted and analyzed on the basis of the methods of Merrill et al (34). Nonesterified FAs (free) and total FAs (free plus esterified) in serum were assessed by the transesterification to their methyl esters, extraction in ethyl acetate, conversion to trimethyl silyl esters, and analysis by using capillary gas chromatography–mass spectrometry as described previously (35, 36). Muscle and serum concentrations of acylcarnitines and amino acids were measured by using direct-injection electrospray tandem mass spectrometry (37). Muscle organic acids were quantified as previously described (38).

Muscle gene-expression studies

On the basis of our a priori hypotheses regarding genes affecting lipid metabolism, we measured the expression of the following genes involved in the regulation of FA oxidation during the fed state (only) for HPA and HOA diets: PGC-1α; INSG-1; PDK4; hydroxyacetyl-CoA dehydrogenase (HADH); acyl-CoA dehydrogenase, medium chain [ACADM; also known as medium chain acyl-CoA dehydrogenase (MCAD)]; and stearoyl-CoA desaturase 1 (SCD1).

A real-time quantitative polymerase chain reaction was performed by using an ABI PRISM 7000 Sequence Detection System instrument and software (PE Applied Biosystems Inc) with prevalidated 5′-6-FAM (6-carboxyfluorescein)–labeled TaqMan probes (ABI). The expression of messenger RNA (mRNA) was normalized for comparison by determining cytoplasmic β-actin concentrations by using a multiplex real-time quantitative polymerase chain reaction. Relative gene expression was determined by using the ΔΔCt difference method.

Statistics

All data are expressed as means ± SEMs. Because of the number of statistical comparisons, the approach of Benjamini and Hochberg (39) was used to compute the false discovery rate within families of hypotheses to adjust the nominal P ≤ 0.05 for statistical significance. Analyses were performed with SAS software (version 9.2; SAS Institute Inc). Because sex-specific responses to diets were anticipated a priori (12), men and women were analyzed both as a group and separately. This study used a crossover design; subjects were randomly assigned into 2 groups that differed with respect to the treatment order, with the trial lasting for 2 treatment periods. Diet effects were analyzed by using a repeated-measures ANOVA specified for a crossover study, including sequence and treatment effects, with the baseline value as a covariate, when available. Because of missing data on some variables, maximum-likelihood–based procedures were used that allowed for the use of all available data without imputation. A ranked transformation followed by repeated-measures ANOVA (40) were used to examine diet differences in CRP that did not conform to normality assumptions of the parametric test. Diet differences in the PA:OA ratio in muscle phosphatidylcholine were examined by using Wilcoxon’s signed-rank test. All correlations reported are Spearman’s rank correlations.

A principal components analysis (PCA) was used to reduce the dimensionality of metabolite data in both fasted and fed states and to aid in the explanation of the highest variance within the overall data set. PCA-component scores were limited to subjects who had a complete data set for all metabolomic variables in either the fasted or fed condition. In the fasted state, 2 subjects had complete data on the HPA diet but missing data on the HOA diet, whereas one subject had complete data on the HOA diet but missing data on the HPA diet. In the fed state, an additional 2 subjects had complete data on the HPA diet but missing data on the HOA diet, whereas one additional subject had complete data on the HPA diet but missing data on the HOA diet. In the fed state, an additional 2 subjects had complete data on the HPA diet but missing data on the HOA diet, whereas one additional subject had complete data on the HPA diet but missing data on the HOA diet. An ANCOVA with the use of PCA factors as well as correlations of differences in PCA factors on each diet were completed on the basis of all data that were available (n = 15 of 18 subjects) without imputation. An orthogonal rotation was used to aid in the interpretation of components. Components with an eigenvalue ≥1.0 were retained, and component scores for each subject were calculated by using standardized scoring coefficients (which consisted of a weighted sum of values of standardized variables weighted by the component loading calculated for each individual variable). Diet differences in component scores were examined by using repeated-measures ANOVA methods previously described. In addition, select components were included as time-varying covariates in the analysis to examine the relation between dependent variables of interest and component scores.

RESULTS

Body composition, physical fitness, and physical activity

The FA composition of experimental diets did not affect body weight, BMI, or body composition (Table 2).
Demographic and metabolic characteristics

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<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
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<tr>
<td></td>
<td>Screening</td>
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<td>Age (y)</td>
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<td>Body weight (kg)</td>
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<td>LDL (mmol/L)</td>
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<tr>
<td>HDL (mmol/L)</td>
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<td>LDL:HDL ratio</td>
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<tr>
<td>Triacylglycerols (mmol/L)</td>
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<td>0.53 ± 0.08</td>
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</table>

All values are means ± SEMs. Results were based on data from 9 men and 9 women, except for LDL, which was based on 7 men and 9 women. The HOA-diet lipid composition resembled the Mediterranean diet. The HPA-diet lipid composition resembled the Western diet. *Diet effect (HPA compared with HOA), P = 0.05 (repeated-measures ANOVA specified for a crossover study, including sequence and treatment effects, with the baseline value as a covariate, when available). HOA, low palmitic acid and high oleic acid; HPA, high palmitic acid.

Plasma leptin and adiponectin concentrations and serum sex-hormone concentrations

In the combined group of men and women, the HPA diet caused a higher plasma adiponectin concentration during the fed state (10,354 ± 832 compared with 9827 ± 740 ng/mL; P = 0.04); however, there were no diet differences in the fasted state. There were no significant differences between diets when men and women were analyzed separately. The diet condition did not affect plasma concentration of leptin in either fasted or fed state.

Serum concentrations of estradiol and progesterone were not affected by the diet condition in women; in men, serum testosterone was 13% lower with the HOA diet (485 ± 6 ng/dL) than with the HPA diet (559 ± 28 ng/dL) (P < 0.01).

FA oxidation

In the fasted state, the RER was significantly lower with the HPA diet (P = 0.04), which implied a higher rate of fat oxidation with the diet (Figure 1A). We also examined the rate of FA oxidation for the respective fasted and fed measurements expressed as either grams per minute or the fraction of the REE, but there was no significant diet-group effect. However, as noted in Subjects and Methods, we made multiple measurements of substrate oxidation and REE starting 1 h after the evening meal at 1800. Variations in the rate of meal ingestion, gastric emptying, and intestinal motility might alter the time course of fat oxidation. Thus, we examined the diet-group effect on the RER and the rate of FA oxidation as a percentage of the REE at the very end of the fasting period (0500) and at the time when the RER peaked for each subject (which reflected the peak absorption and oxidation of carbohydrate), but there were no significant diet differences (Figure 1B).

There was no significant sex effect on the RER in either the fed or fasted state on either diet. There also was no difference between men and women in the rate of fat oxidation expressed as a ratio to REE on either diet or during fasted or fed states. We also explored whether the diets differentially affected FA oxidation when men and women were considered separately. There was no interaction for sex and diet group for various estimates of FA oxidation, but as noted, we extended an a priori hypothesis that men and women would respond differently. However, there were no significant diet-group effects on FA oxidation for men or women separately.

Serum lipid concentrations

In men and women combined (n = 16), relative to the HPA diet, the HOA diet lowered serum concentrations of LDL (P < 0.01) and the LDL:HDL ratio (P = 0.03) (Figure 2, A and B). Likewise, total cholesterol was 13% higher with the HPA diet.

![Figure 1](https://academic.oup.com/ajcn/article-abstract/99/3/436/4577328/40)
DIETARY PALMITATE/OLEATE AND LIPID METABOLISM

Serum CRP concentration

Of 54 total samples that comprised baseline, HPA, and HOA diets, one-half of serum CRP values were at the lowest concentration of detectability (0.17 mg/L) (14 control, 10 HOA, and 3 HPA samples); this result precluded the use of normal distribution statistics or describing means and SEMs. In men and women considered together, the serum concentration of CRP was lower during the HOA diet (2.2 mg/L). However, in men, there was no significant difference in CRP between diets, in part because of an exceptionally high value in an otherwise healthy woman consuming the HOA diet (2.2 mg/L). In women, there was much more overlap between the 2 diets. Thus, the PA:OA ratio in serum phosphatidylcholine usefully distinguished the 2 dietary conditions in each subject.

A PCA was used to reduce the dimensionality of data sets that contained all metabolites measured for subjects in the fasted and fed states, separately, including metabolites measured in serum and plasma, muscle, and urine, into a smaller number of orthogonal principal components. The first 31 principal components resulting from the PCA in both fasted and fed states were retained for further analysis. For metabolites measured in the fasted state, the first 2 components [PCA factor in the fasted state (PCF1-Fasted) and an additional PCA factor in the fasted state (PCF2-Fasted)] accounted for 10% and 9%, respectively, of the overall variance, whereas the fed component of interest [PCA factor in the fed state (PCF1-Fed)] accounted for 10% of the overall variance. In both men (P < 0.0001) and women (P < 0.0001), diets significantly changed the mean component score for 1 factor in the fasted state (PCF1-Fasted). In the fed state, again, 1 factor (PCF1-Fed) was affected by the diet in both men (P < 0.0001) and women (P < 0.0001). Variables that contributed a high loading score (>0.4) for the PCF1-Fasted and PCF1-Fed mostly reflected PA and OA contents, which had respective positive and negative loading scores, but serum

Metabolomics (lipidomics) and PCA

Our previous article (8) summarized data that indicated that HPA and HOA diets had their predicted effects on the PA:OA ratio of various serum and muscle lipids, respectively. As an indication of the strength of dietary effects (and implicitly, subject compliance with ingestion of diets), in every subject (men and women), the HOA diet induced a decrease in the PA:OA ratio in serum and muscle phosphatidylcholine, which is a marker of membrane phospholipid composition (Figure 3, A and B). For serum phosphatidylcholine, only 1 of 18 subjects who consumed the HOA diet exhibited a PA:OA ratio that was higher than the lowest value with the HPA diet; for muscle phosphatidylcholine, there was much more overlap between the 2 diets. Thus, the PA:OA ratio in serum phosphatidylcholine usefully distinguished the 2 dietary conditions in each subject.

A PCA was used to reduce the dimensionality of data sets that contained all metabolites measured for subjects in the fasted and fed states, separately, including metabolites measured in serum and plasma, muscle, and urine, into a smaller number of orthogonal principal components. The first 31 principal components resulting from the PCA in both fasted and fed states were retained for further analysis. For metabolites measured in the fasted state, the first 2 components [PCA factor in the fasted state (PCF1-Fasted) and an additional PCA factor in the fasted state (PCF2-Fasted)] accounted for 10% and 9%, respectively, of the overall variance, whereas the fed component of interest [PCA factor in the fed state (PCF1-Fed)] accounted for 10% of the overall variance. In both men (P < 0.0001) and women (P < 0.0001), diets significantly changed the mean component score for 1 factor in the fasted state (PCF1-Fasted). In the fed state, again, 1 factor (PCF1-Fed) was affected by the diet in both men (P < 0.0001) and women (P < 0.0001). Variables that contributed a high loading score (>0.4) for the PCF1-Fasted and PCF1-Fed mostly reflected PA and OA contents, which had respective positive and negative loading scores, but serum

FIGURE 3. A: Individual changes in the PA/OA ratio in serum PC during HPA and HOA diets (n = 18) (diet effect, P < 0.001; Wilcoxon’s test). B: Individual changes in the PA/OA ratio in muscle PC (n = 16) (diet effect, P < 0.001; Wilcoxon’s test). HOA, low palmitic acid and high oleic acid; HPA, high palmitic acid; PA/OA, palmitic acid/oleic acid; PC, phosphatidylcholine.
ceramide species also strongly contributed to higher PCF1-Fasted and PCF1-Fed scores.

The PCF2-Fasted was affected by the diet \( (P = 0.02) \) in women only (HPA greater than HOA); the PCF2-Fasted mainly reflected a wide range of saturated and unsaturated, long- and medium-chain acylcarnitines in muscle.

In the combined cohort of men and women, the diet-induced change in the PCF1-Fasted (HOA – HPA) correlated positively with the change in total cholesterol \( (r = 0.811, P < 0.001, n = 15) \), LDL \( (r = 0.835, P < 0.001, n = 13) \) (Figure 2C), and the LDL: HDL ratio \( (r = 0.670, P = 0.012, n = 13) \) (Figure 2D). These results meant that a relatively lower PA:OA ratio in blood and muscle lipids with the HOA diet was associated with a lower total cholesterol, LDL, and LDL: HDL ratio with this diet. When separated by sex, the relation between changes in the PCF1-Fasted and total cholesterol was maintained in men \( (r = 0.759, P = 0.029, n = 8) \) but not women \( (P = 0.12, n = 7) \). The change in the PCF1-Fasted correlated positively with the change in LDL in women \( (r = 0.865, P = 0.012, n = 7) \) but not in men \( (n = 6) \). When the PCF1-Fasted was included in the ANOVA as an additional covariate along with the estimate of the LDL: HDL ratio with the baseline diet, the diet-related differences in the LDL: HDL ratio were no longer significant in both men and women, which implied that the degree of saturation of serum and muscle lipids mediated diet effects on the LDL: HDL ratio. In women, although the change in the PCF2-Fasted did not correlate with the change in total cholesterol, LDL, or the LDL: HDL, after adjustment for the PCF2-Fasted, serum total and LDL cholesterol concentrations were not lower with the HOA diet. Because the PCF2-Fasted was heavily weighted by mitochondrial-derived acylcarnitine metabolites, this finding suggested a strong connection between FA catabolism and lipoprotein metabolism.

In men and women considered together or separately, the change in PCF1-Fed did not correlate with the change in serum lipids. In the fasting state \( (P < 0.01) \), and the POA:PA ratio was also 35% higher in the fed state \( (P = 0.01) \) but not in the fed state. As noted, the mRNA expression of SCD1 measured in the fed state was not different between HPA compared HOA diets (Figure 4). Thus, the relative unsaturation of skeletal muscle lipids seemed to be governed by diet and not by SCD1.

DISCUSSION

CVD is one of the most prevalent and costly medical problems confronting modernized societies, and elevated blood concentration of LDL is an important risk factor for CVD \( (1, 41) \). The pharmaceutical treatment of hypercholesterolemia with the statin class of drugs as well as new technological advancements in the

Figure 4. Relative mean (±SEM) effects of HOA compared with HPA diets on genes that regulate fatty acid oxidation in skeletal muscle in the fed state. Genes: \( ACADM \), acyl-CoA dehydrogenase, medium chain (also known as medium-chain acyl-CoA dehydrogenase or \( MCAD \)); \( HADH \), hydroxyacyl-CoA dehydrogenase; \( INSIG-1 \), insulin induced gene 1 (women: \( 2.19 \pm 0.72 \); men: \( 1.40 \pm 0.16 \); \( PDK4 \), pyruvate dehydrogenase kinase 4; \( PGC-1α \), peroxisome proliferation activator receptor coactivator—1α; SCD1, stearoyl-CoA desaturase 1. *Diet effect (repeated-measures ANOVA specified for a crossover study, including sequence and treatment effects, with the baseline value as a covariate), \( P < 0.05 \). HOA, low palmitic acid and high oleic acid; HPA, high palmitic acid; REL., relative.
management of myocardial infarction have reduced both the incidence of CVD and mortality from it. However, the institution of statin therapy is often based on the serum concentration of LDL, and thus, it is important to understand how the dietary fat composition alters this screening test (20). The prevention of abnormal serum lipid concentrations and systemic inflammation is important to reduce CVD and is intimately linked to correcting poor dietary habits, especially a high PA intake, and secondary perturbations in systemic lipid metabolism. Consistent with our previous studies (12, 20) and those of other authors (42, 43), we showed that an HOA diet compared with an HPA diet significantly lowered LDL in men and women by 19% and 15%, respectively (Table 2). These decrements in LDL were linked to the FA composition of the diet and its attendant effects on tissue FA concentrations, which may have independent effects on metabolic health (2, 22). Because the respective lowering and enhancing activity of hepatic LDL-receptor activity by PA and OA appears to be minimized by low dietary cholesterol (2, 4), our observations, which were made by using a low-cholesterol diet, may mean that the HOA diet would be even more advantageous in individuals with a higher cholesterol intake. As in a recent study in pigs (44), we observed a lower serum concentration of CRP with the HOA diet, although values were generally very low with both diets in these nonobese, young adults.

An inferential statistical analysis, in this case the PCA, provided insight into the mechanistic pathway for effects of these particular dietary oils on serum concentrations of total and LDL cholesterol. We propose that pervasive changes in the FA composition of intracellular lipids induced by our 2 experimental diets caused changes in LDL metabolism, possibly by affecting the ACAT reaction in the liver and, secondarily, changes in the expression of the LDL receptor (2, 4). We speculate that these diets altered the liver FA composition in a corresponding fashion to how they affected the FA composition of skeletal muscle and blood lipids. We are not aware of any human studies that used similar diets to study the FA composition of the liver. Studies of the liver in pigs (45), rats (46), and mice (47, 48) have suggested that the PA and OA composition of neutral or total lipids, but not phospholipids, are directly affected by PA and OA intake, but polar lipids were unaffected (45).

Aside from the well-known effects of saturated FAs, including PA, on circulating LDL concentrations, growing in vitro evidence has supported the concept that PA and OA may have distinct and opposing effects on inflammation and insulin resistance (which would also affect serum lipid concentrations) (8, 10, 49–52). Intracellular pools of acyl-CoAs or acylcarnitines also might ultimately influence or be indicative of biochemical pathways, which indirectly alter cholesterol metabolism. We showed that the HOA-diet-induced change (HOA−HPA) in the concentration of acylcarnitines in women was a significant mediator of the respective diet-induced difference in total cholesterol and LDL; this observation was generally congruent with our previous data that suggested that the serum ratio of medium- to long-chain acylcarnitines in women in the fed state inversely correlated with insulin sensitivity (8). Low micromolar concentrations of medium-chain acylcarnitines stimulated the activity of the stress-sensitive transcription factor nuclear factor-κB in a murine monocytic cell line (53). In accordance with the potential translational relevance to cell-based studies of metabolic stress, in our study, the HOA diet appeared to lower the circulating CRP concentration (19, 54). The dietary FA composition may induce subtle alterations in inflammatory pathways even in low-risk people, which may affect CVD risk and the circulating concentration of LDL (8).

This study showed that markedly lowering the PA content of the diet and replacing PA with OA (the HOA diet) was associated with a lower rate of total FA oxidation. Although we could not readily explain the apparent discrepancy with our previous study with regard to FA oxidation (11, 12), there were 2 differences in the designs of the 2 studies, namely that, in the current study, we used solid-food diets and a crossover design. In addition, in contrast with our previous study (12), all women manifested normal ovulation. Our goal was to conduct our studies in women with the background of postluteal concentrations of estradiol and progesterone in women. However, without specific studies that used the pharmacologic suppression of sex-hormone secretion, we could not rule out that, eg, a high serum concentration of estradiol may have obscured the effects of the dietary FA composition on FA oxidation.

The HOA diet resulted in a significantly higher muscle mRNA expression of INSIG-1. Because the INSIG-1 transcript is strongly responsive to insulin, this outcome could have reflected enhanced insulin sensitivity with the HOA diet (8, 55). Carrobbio et al (55) recently reported that INSIG-1 mRNA expression was abnormally decreased in morbidly obese, insulin-resistant human subjects. INSIG-1 expression is influenced by SREBP activity and vice versa. Although the functional importance of this finding remains uncertain at this stage, INSIG-1 represents an intriguing candidate that could link changes in the dietary OA and PA content and FA metabolism (both oxidation and acylcarnitine production) to the regulation of blood LDL and total cholesterol concentrations (2, 3).

In conclusion, this investigation used a novel lipidomics approach to show that shifting from an HPA to HOA diet resulted in system-wide changes in the lipid composition along with favorable effects on CVD risk factors in humans. Effects of the diets on blood lipid concentrations were consistent with the results of several previous studies in our laboratory (12, 20) and by other researchers (42, 43), which were likely attributable to the strict nature of our experimental design and a high level of subject compliance. Although previous studies have suggested that factors other than the FA composition contribute to the benefits of a low-SFA diet (1, 56), our PCA identified the PA:OA ratio of blood and muscle lipids as the main factor that mediated diet-induced changes in serum cholesterol and LDL in the current study (2). Our results also suggest that lower FA oxidation and a diminished formation of incompletely oxidized acyl-CoAs (as reflected by acylcarnitine profiling) might contribute to lower serum LDL, especially in women. Finally, the inhibitory effects of the HOA diet on mRNA expression of INSIG-1 warrant an additional analysis of the Insig-1/Scap/SREBP complex as a potential regulatory site linking the dietary OA and PA content to cholesterol homeostasis, fat oxidation, and perhaps, indirectly, insulin sensitivity (2, 3, 55).

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research; JYB: analyzed data; CLK, JYB, TRK, and DMM: wrote the manu-
script; CLK: had primary responsibility for the final content of the manu-
script; and all authors: read and approved the final manuscript. None of the
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REFERENCES
1. Astrup A, Dyerberg J, Elwood P, Hermansen K, Hu FB, Jakobsen MU, 
Kok FJ, Krauss RM, Leercer JM, Legrand P, et al. The role of reducing
intakes of saturated fat in the prevention of cardiovascular disease:
2. Dietschy JM. Dietary fatty acids and the regulation of plasma low density
4. Nicolosi RJ. Dietary fat saturation effects on low-density-lipoprotein
concentrations and metabolism in various animal models. Am J Clin
Nutr 1997;65:1617S–27S.
5. Ramsden CE, Zamora D, Leelarthaepin B, Majchrzak-Hong SF, Faurot
KJ, Liu J. Potential role of protein in signaling of lipics in microsomes
and serum acylcarnitine profile in human subjects. Obesity (Silver
plasma lipid concentrations and serum acylcarnitine profile in human subjects. 
Obesity (Silver Spring) 2011;19:305–11.
7. Kontush A, Chapman MJ. Lipidomics as a tool for the study of lipoprotein
8. Kien CL, Bunn JY, Poynter ME, Stevens R, Bain J, Ikayeva O, 
analysis of the relationship between dietary fatty acid composition and
9. Muoio DM, Newgard CB. Obesity-related derangements in metabolic
10. Kien CL. Dietary interventions for metabolic syndrome: role of mod-
11. Kien CL, Bunn JY, Ugrasbal F. Increasing dietary palmitic acid decreases
12. Kien CL, Bunn JY. Gender alters the effects of palmitate and oleate on
fat oxidation and energy expenditure. Obesity (Silver Spring) 2008;16:
29–33.
13. D’Eon TM, Souza SC, Aronovitz M, Obin MS, Fried SK, Greenberg AS.
Estrogen regulation of adiposity and fat partitioning. Evidence of
 genomic and non-genomic regulation of lipogenic and oxidative
Ezaki O. Varioicetome in mice decreases lipid metabolism-related
gene expression in adipose tissue and skeletal muscle with increased
Cholesterol in Adults. Executive Summary of the Third Report of The
National Cholesterol Education Program (NCEP) Expert Panel on
Detection, Evaluation, and Treatment of High Blood Cholesterol In
16. American Diabetes Association. Diagnosis and classification of di-
17. Stern SE, Williams K, Ferrimmoni E, Debronzo RA, Bogardus C, Stern MP.
Identification of individuals with insulin resistance using routine
18. Roger VL, Go AS, Lloyd-Jones DM, Adams RJ, Berry JD, Brown TM, 
Carnethon MR, Dai S, de Simone G, Ford ES, et al. Heart disease and
stroke statistics—2011 update: a report from the American Heart As-
19. Lakoski SG, Cushman M, Criqui M, Rundek T, Blumenthal RS, 
D’Agostino RB Jr, Herrington DM. Gender and C-reactive protein in
dmission to the Multicenter Study of Atherosclerosis (MESA) cohort.
Am Heart J 2006;152:593–8.
Short-term effects of dietary fatty acids on muscle lipid composition and
21. Mustad VA, Jonnalagadda SS, Smukto SA, Pelkman CL, Rolls BJ,
Behr SR, Pearson TA, Kris-Etherton PM. Comparative lipid and lip-
oprotein responses to solid-food diets and defined liquid-formula di-
22. Berglund L, Lefevre M, Ginsberg HN, Kris-Etherton PM, Elmer PJ,
Comparison of monounsaturated fat with carbohydrates as a re-
placement for saturated fat in subjects with a high metabolic risk
profile: studies in the fasting and postprandial states. Am J Clin Nutr
Nutritional biochemistry and metabolism with clinical applications.
24. Kien CL, Bunn JY, Tompkins CL, Dumas JA, Crain KI, Ebenstein DB,
Koves TR, Muoio DM. Substituting dietary monounsaturated fat for
saturated fat is associated with increased daily physical activity and
resting energy expenditure and with changes in mood. Am J Clin
25. Goodman SG, Ng TK, Lee VK, Nesarathum K. Diets high in palmitic acid
(16:0), laurie and myristic acids (12:0 + 14:0), or oleic acid (18:1) do not
alter postprandial or fasting plasma homocysteine and inflamma-
adding glycemic index values to 24-hour recalls. Nutrition 2011;27:
59–64.
27. Foster-Powell K, Holt SH, Brand-Miller JC. International table of glycemic
28. Roux LR, Hammel KD, Jensen MD. Effects of isoeogenic, low-fat
diets on energy metabolism in lean and obese women. Am J Clin Nutr
29. Bray GA. Energy balance and obesity in man. Amsterdam, Nether-
30. Frayn KN. Calculation of substrate oxidation rates in vivo from gase-
31. Weir JB. New methods for calculating metabolic rate with special
32. Black AE, Prentice AM, Coward WA. Use of food quotients to predict
respiratory quotients for the doubly-labeled water method of measuring
33. Clore JN, Harris PA, Li J, Azzam A, Gill R, Zueler W, Rizzo WB,
Blackard WG. Changes in phosphatidylcholine fatty acid composition
are associated with altered skeletal muscle insulin responsiveness
34. Merrill AH Jr, Sullards MC, Allegood JC, Kelly S, Wang E. Sphin-
golipidomics: high-throughput, structure-specific, and quantitative analysis of
phospholipids by liquid chromatography tandem mass spectrometry.
35. Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF, 
alpha-keto acid-related metabolic signature that differentiates obese and
lean humans and contributes to insulin resistance. Cell Metab 2009;9:
311–26.
36. Lepage G, Roy CC. Direct transesterification of all classes of lipids in
Z, Newgard CB, Muoio DM. PPARgama coactivator-1alpha-lipid
mmodelling of skeletal myocytes mimics exercise training and
280:33588–98.
38. Jensen MV, Joseph JW, Ilkayeva O, Burgess S, Lu D, Ronnebaum SM,
Blackard WG. Changes in phosphatidylcholine fatty acid composition
are associated with altered skeletal muscle insulin responsiveness
golipidomics: high-throughput, structure-specific, and quantitative analysis of
phospholipids by liquid chromatography tandem mass spectrometry.
40. Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF, 
alpha-keto acid-related metabolic signature that differentiates obese and
lean humans and contributes to insulin resistance. Cell Metab 2009;9:
311–26.


