Fatty acid saturation of the diet and plasma lipid concentrations, lipoprotein particle concentrations, and cholesterol efflux capacity

Maria Teresa Montoya, Amelia Porres, Sagrario Serrano, Jean Charles Fruchart, Pedro Mata, Juan Antonio Gómez Gerique, and Graciela Rosa Castro

ABSTRACT

Background: The fatty acid content and saturation degree of the diet can modulate HDL composition and cholesterol efflux.

Objective: We studied the modifications in plasma lipoprotein particles and serum capacity to stimulate cholesterol efflux induced by different fatty acids.

Design: Seventeen women and 24 men followed in the same sequence 4 diets containing 35% of total energy as fat. The saturated fat diet contained 17% palm oil; the monounsaturated fat diet, 20.9% olive oil; the n–6 polyunsaturated fat diet, 12.5% sunflower oil; and the n–3 polyunsaturated fat diet, sunflower oil supplemented with 4–4.5 g fish oil/d. Each phase lasted 4–5 wk.

Results: In both sexes, apolipoprotein (apo) A-I concentrations were significantly lower with unsaturated fat diets than with the saturated fat diet, but concentrations of lipoproteins containing only apo A-I (Lp A-I) were lower only in the men. Concentrations of lipoproteins containing both apo A-I and apo A-II (Lp A-I:A-II) were lower with both polyunsaturated fat diets in the women but significantly higher in the men. Lp E concentrations were significantly higher with the 2 polyunsaturated fat diets. Lp E non–B particle concentrations were not modified in the men but were significantly higher in the women in both polyunsaturated fat phases. Lp C-III concentrations were higher with the saturated fat diet only in the men. The serum samples taken after the n–3 polyunsaturated fat phase were the most efficient for extracting cellular cholesterol in both sexes.

Conclusions: The monounsaturated and polyunsaturated fat diets were healthier, producing a better lipid profile.

KEY WORDS Diet, fatty acid, lipid, lipoprotein particle, cholesterol efflux, reverse cholesterol transport, HDL, LDL, saturated fat, polyunsaturated fat, monounsaturated fat, n–3 polyunsaturated fat, reverse cholesterol transport, HDL, LDL, saturated fat, polyunsaturated fat, monounsaturated fat

INTRODUCTION

The incidence of cardiovascular disease (CVD), the main cause of death and morbidity in developed countries (1), is closely related to diet, mainly to fat composition. The type of fat in the diet can modify the lipid profile, which is directly related to the growth of atheroma plaque (2, 3). In Spain, the mortality rate due to this disease is relatively low compared with that in other developed countries (4, 5). This finding could be explained by 2 facts: the Spanish diet is rich in vegetables, fruit, fish, and oils containing monounsaturated fatty acids (MUFAs), a regimen considered favorable for cardiovascular health (6), and Spaniards have elevated plasma concentrations of HDL (7).

However, in the dietetic studies performed until now, the results regarding changes in HDL with the administration of monounsaturated fats are controversial (2, 8, 9). Several epidemiologic studies showed an inverse correlation between serum concentrations of HDL cholesterol and the risk of CVD (10). This favorable role of HDL is mainly due to its participation in the metabolic reverse cholesterol transport, which allows the liver to eliminate excess cholesterol in peripheral tissues (11). Reverse cholesterol transport consists of several steps: efflux of cholesterol from cell membranes to acceptor particles, esterification of cellular cholesterol by phosphatidylcholine-sterol O-acyltransferase (lecithin-cholesterol acyltransferase), transfer of cholesterol esters to low-density or very-low-density particles with the contribution of the cholesterol ester transfer protein, and, finally, delivery of cholesterol esters to the liver (12).

The HDL fractions obtained by ultracentrifugation techniques (13) are a group of heterogeneous particles. Particles more homogeneous in structure and function are isolated according to apolipoprotein (apo) composition (14, 15). Particles that contain only apo A-I are termed Lp A-I; those containing apo A-I and apo A-II are termed Lp A-I:A-II. Several studies have shown important metabolic differences between these 2 kinds of particles (16–21). It is accepted that Lp A-I constitutes the antiatherogenic fraction of HDL, and serum concentrations of Lp A-I and Lp A-I:A-II are defined as a new CVD risk marker (22, 23).
TABLE 1
Mean daily intake of nutrients in each diet period

<table>
<thead>
<tr>
<th></th>
<th>SFA diet</th>
<th>MUFA diet</th>
<th>n-6 PUFA diet</th>
<th>n-3 PUFA diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (MJ) Men (n = 24)</td>
<td>10.2 ± 1.4</td>
<td>10.1 ± 1.3</td>
<td>10.2 ± 1.4</td>
<td>10.0 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>7.5 ± 1.0</td>
<td>7.4 ± 0.9</td>
<td>7.6 ± 1.1</td>
<td>7.6 ± 1.1</td>
</tr>
<tr>
<td>Protein (% of energy)</td>
<td>14.5</td>
<td>15</td>
<td>14.8</td>
<td>15.1</td>
</tr>
<tr>
<td>Carbohydrates (% of energy)</td>
<td>49.5</td>
<td>49.8</td>
<td>49.6</td>
<td>49.8</td>
</tr>
<tr>
<td>Fatty acids (% of energy)</td>
<td>35.5</td>
<td>35.2</td>
<td>35.6</td>
<td>35.1</td>
</tr>
<tr>
<td>SFAs (% of total fatty acids)</td>
<td>17.3</td>
<td>9.2</td>
<td>9.5</td>
<td>9.2</td>
</tr>
<tr>
<td>16:0</td>
<td>11.7</td>
<td>5.3</td>
<td>5</td>
<td>5.1</td>
</tr>
<tr>
<td>18:0</td>
<td>2.9</td>
<td>2.3</td>
<td>2.8</td>
<td>2.2</td>
</tr>
<tr>
<td>MUFAs (% of total fatty acids)</td>
<td>13.6</td>
<td>20.9</td>
<td>12</td>
<td>11.8</td>
</tr>
<tr>
<td>18:1</td>
<td>13.2</td>
<td>20.4</td>
<td>11.6</td>
<td>11.3</td>
</tr>
<tr>
<td>PUFAs (% of total fatty acids)</td>
<td>3.6</td>
<td>4</td>
<td>12.5</td>
<td>12.7</td>
</tr>
<tr>
<td>18:2</td>
<td>3.2</td>
<td>3.7</td>
<td>12.2</td>
<td>10.6</td>
</tr>
<tr>
<td>20:5</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>0.6</td>
</tr>
<tr>
<td>22:6</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>0.8</td>
</tr>
<tr>
<td>n-3 Fatty acids (% of total fatty acids)</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Cholesterol (mg/d)</td>
<td>276</td>
<td>264</td>
<td>260</td>
<td>259</td>
</tr>
<tr>
<td>Fiber (g/d)</td>
<td>24.5</td>
<td>24.7</td>
<td>24.8</td>
<td>25.3</td>
</tr>
</tbody>
</table>

1Values are based on chemical analysis of duplicate diets. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.
2± SD.

Other minor particles contain apo C-III and apo E (Lp C-III non-B and Lp E non-B). The importance of these particles, the role of which is still not completely elucidated, has been suggested by data from case-control studies that implicated apo C-III and apo E ratios (eg, Lp C-III non-B/Lp C-III:B and Lp E non-B/Lp E:B) as potential CVD risk markers (24, 25). Another type of particle containing apo E as the only apolipoprotein (γLp E) has been proposed as an element responsible for promoting cholesterol efflux, mainly when there is a shortage of the main acceptor particles (26).

It has been suggested that eating foods rich in fatty acids of different saturation grades modifies the composition and structure of HDL by changing the acyl chain content of HDL phospholipids (27). Furthermore, several studies have confirmed that the concentration of Lp A-I and Lp A-I:AI particles changes when the fatty acid composition of the diet is modified (28, 29). On the other hand, the results of several studies of the effect of dietary fatty acid composition on serum capacity to promote cellular cholesterol efflux are still controversial (30–33).

In the present study, a long-term diet rich in vegetables, fruit, fish, and oils was administered to a healthy population. Different diets were created by varying the fatty acid saturation grade to study the effect on the plasma lipid and lipoprotein particle profile. This report is a post hoc analysis, and we describe the changes in the sera capacity to induce efflux of cholesterol from cells in culture.

SUBJECTS AND METHODS

The study design, population, and diets were described in previous publications (34).

Study population

Forty-one healthy volunteers gave their written consent to take part in the study. The population comprised 17 nuns (10 postmenopausal) and 24 priests, with a mean (±SD) age of 45.8 ± 9.5 and 45.1 ± 16.2 y, respectively, and a body mass index (in kg/m²) of 24.8 ± 4.3 (26.6 ± 4.6 in the men, 23.1 ± 3 in the women). The subjects were free of clinical evidence of any chronic illness or obesity and had no family history of premature cardiovascular disease. Five of the men were smokers. The men had a baseline alcohol intake of <30 g/d. All of the women were nonsmokers consumed no alcohol. The diets were prepared and consumed in the subjects’ communities. During the study period the subjects maintained their regular physical activity and lifestyle. This protocol was approved by the Human Studies Committee of the Jiménez Díaz Foundation.

Diets and study design

The participants were successively and in the same sequence fed 4 diets with the same total energy content: a saturated fatty acid (SFA) diet with a high palm oil content (17.3% of total energy); a MUFA diet with a predominance of oleic acid (18:1; 20.9% of total energy); an n-6 polyunsaturated fatty acid (PUFA) diet with a high percentage of sunflower oil (12.8% of total energy), containing mainly linoleic acid (18:2n-6); and another PUFA diet (n-3) enriched with fish oil (4.4.5 g/d; 1.6% of total energy). The energy composition of the diets was calculated by using food-composition tables. All menus were prepared with conventional food items. Special emphasis was placed on using foods that were not substantially different from those habitually consumed by the communities.

The total energy provided by each of the diets was adjusted to the participants’ needs. Diet composition was similar: 15% of energy as protein, 50% of energy as carbohydrate, and 35% of energy as fat. The menus of each diet were repeated weekly. The proportional fatty acid content of the diets was adjusted by varying the culinary fat used. Butter and palm oil were used in the SFA diet, virgin olive oil in the MUFA diet, and sunflower oil in the PUFA diets. Changes were also made in foods with high fat contents (eg, milk, cheese, and nuts). During the n-3 PUFA period, fish oil was added daily to the sunflower oil.

Duplicate samples of all meals from randomly selected individuals (one man and one woman) were collected every day during 1 wk of each diet phase and stored at –20°C (Table 1). The
fatty acid composition of the dietary oils used in this study was
determined by capillary gas-liquid chromatography at the Food
Analysis Laboratory of the Spanish Ministry of Agriculture (35).
Palm oil consisted mainly of (% by wt) 37% palmitic acid (16:0),
43.6% oleic acid, and 11.7% linoleic acid. The major fatty acids
in olive oil were palmitic acid (9.2%), oleic acid (80%),
and linoleic acid (4.7%). The sunflower oil contained 6.8% palmitic
acid, 29% oleic acid, and 56% linoleic acid.

Each period lasted 5 wk, except the SFA diet phase, which
lasted 4 wk. During the last 2 wk of each period, a physical exam-
ination was made of each subject during which blood pressure and
weight were recorded. Physical activity and diet were recorded in
a personal log, and blood was drawn to measure serum concentra-
tions of the main indexes of lipid metabolism (lipoproteins and
lipoprotein particles) and to measure cholesterol efflux.

Blood sampling and biochemical determinations

After the subjects had fasted for 12–14 h overnight, blood
samples were obtained by venous puncture, collected in tubes
(Vacutainer; Terumo Europe, Leuven, Belgium) containing a coag-
ulation activator, and taken to the laboratory within 1 h. The tubes
were centrifuged at 1500 × g for 15 min at room temperature,
and the sera obtained were used for the following determinations.

Lipid and lipoprotein measurements were performed by using
a combined ultracentrifugation-precipitation method recom-
pended by the American Lipid Research Clinics (36). After pre-
cipitation-centrifugation of lipoproteins containing apo B with
phosphotungstic acid and magnesium chloride (Boehringer
Mannheim, Mannheim, Germany), cholesterol and phospholipid
HDL were analyzed (37). Lipids in total serum and in the frac-
tions were measured by using automated enzymatic methods
(38, 39) (CHOD-PAP and GPO-PAP; Boehringer Mannheim).
Phospholipids (Wako Chemical GmbH, Neuss, Germany) were
measured in an RA-XT 100 autoanalyzer (Technicon, Tarrytown,
NY), modifying the sample-reagent relation for each lipoprotein
fraction to obtain good photometric accuracy. Apo B and A-I
concentrations were measured by immunoturbidimetric methods
(Boehringer Mannheim).

In all determinations the intra- and interassay CVs were
always <5%. To confirm diet compliance, the fatty acid composi-
tion of the cholesterol ester and phospholipid fractions of
plasma LDL was assessed at the end of each period by using
thin-layer chromatography (40).

The serum concentration of lipoprotein particles was measured
by using electroimmunodiffusion in agarose gels (Hydragel; Sebia,
Issy-les-Moulineaux, France) (25, 41, 42) containing the corre-
sponding monospecific antibody. Apo A-I, apo B, Lp A-I, Lp C-III,
and Lp E were quantified in total sera, and Lp E non-B and Lp C-III
non-B concentrations were quantified after precipi-
tation of lipoproteins containing apo B. The Lp A-I:A-II concentra-
tion was calculated by subtracting the Lp A-I concentration
from apo A-I (measured by using electroimmunodiffusion).

Cell culture, lipid radiolabeling, and efflux assay

Cellular cholesterol efflux was determined by using rat Fu5AH
hepatoma cells, following the procedure previously described by
De la Llera Moya et al (43). Briefly, the cells were maintained in
MEM (Gibco BRL, Gaithersburg, MD) supplemented with 5% celf serum (Gibco BRL). Fu5AH cells (40–50 × 10⁶ cells/L) were
plated on 3.5-cm multwell plates (Linbro; Polylabo, Strasbourg,
France). Two days after plating, cellular cholesterol was labeled
during a 48-h incubation with [³H]cholesterol (NEN; Perkin-
Elmer Life Sciences, Brussels) at a concentration of 37 kBq/well.
To allow equilibration of the label, the cells were rinsed and incu-
bated for 24 h in MEM containing 0.5% bovine serum albumin
(Sigma-Aldrich, St Louis). To measure cholesterol efflux, the cells
were washed with phosphate-buffered saline and incubated at
37°C for 4 h, with the serum from each subject diluted to 2.5% in
MEM. At the end of the efflux period, the medium was removed
and centrifuged. Cell monolayers were washed 3 times with phos-
phate-buffered saline and harvested with 0.5 mL 0.1 mol NaOH/L.
Finally, radioactivity was measured in both medium and cells,
allowing the total radioactivity content in each well to be deter-
dined. Cholesterol efflux, expressed as a percentage, was calcu-
lated as the amount of label recovered in the medium divided by
the total label in each well. Three wells of cells were incubated
with 50 g HDL₃/L as a positive control and another 3 with MEM
as a negative control for each assay. The same HDL pool was used
in all experiments for method standardization, and the mean efflux
produced was 20 ± 0.9%.

Statistical analyses

Statistical analyses were performed with the use of the SAS
statistical software (version 6.12; SAS Institute, Inc, Cary, NC).
To investigate whether the changes observed with the different
diets were dependent on sex, we initially performed a two-way
analysis of variance for the main variable (cholesterol efflux), with
the use of diet as a fixed effect and subject as a random effect and
including a fixed sex effect and fixed sex-by-diet interaction
(nesting the random subject effect within sex). Because in this
analysis we obtained a strong sex-by-diet interaction (P < 0.0001),
the analyses of the different variables related to efflux were per-
dormed for each sex independently, by using a two-way analysis
of variance with diet as a fixed effect and subject as a random
effect and comparing means after each diet by using Tukey’s test
in the model.

For a multiple regression analysis with the change in efflux
as a dependent variable and the changes in lipid and lipoprotein
particle variables as independent variables, we first ran a model
including both sexes and tested for interactions between sex
and the independent (predictor) variables. Because the results
of this analysis showed no significant sex-by-treatment interac-
tion, we performed only one multiple regression analysis,
including both sexes.

RESULTS

Diet composition and compliance

Diet composition, fatty acid profile, and cholesterol content
data are shown in Table 1. The protein, carbohydrate, and fat
contents of the 4 diets were nearly identical.

The fatty acid composition of the cholesterol ester and the phos-
pholipid fractions of plasma LDL was assessed at the end of each
period to confirm diet compliance. The results of this analysis are
shown in Table 2. The palmitic acid content of both fractions was
slightly but significantly higher after consumption of the SFA diet
than after the 3 other diets. With the MUFA diet, the oleic acid con-
tent was significantly higher than with the other diets. During both
PUFA diet periods, the linoleic acid content was significantly
higher than during the SFA and MUFA phases; in addition, the
long-chain PUFA content [eicosapentaenoic acid (20:5n–3) and
TABLE 2
Effect of diets on fatty acid composition of serum phospholipids (PLs) and cholesterol esters (CEs) 

<table>
<thead>
<tr>
<th>Fatty acids and lipid fraction</th>
<th>SFA diet</th>
<th>MUFA diet</th>
<th>n-6 PUFA diet</th>
<th>n-3 PUFA diet</th>
<th>% by wt of total fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:0 PL</td>
<td>0.6 ± 0.4 ( ^a )</td>
<td>0.5 ± 0.3 ( ^a )</td>
<td>0.5 ± 0.4( ^b )</td>
<td>0.6 ± 0.5 ( ^b )</td>
<td></td>
</tr>
<tr>
<td>12:0 CE</td>
<td>0.6 ± 0.5</td>
<td>0.5 ± 0.4</td>
<td>0.5 ± 0.5</td>
<td>0.7 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>14:0 PL</td>
<td>0.9 ± 0.3 ( ^a )</td>
<td>0.8 ± 0.4( ^b )</td>
<td>0.8 ± 0.3 ( ^b )</td>
<td>0.9 ± 0.3 ( ^b )</td>
<td></td>
</tr>
<tr>
<td>14:0 CE</td>
<td>1.5 ± 0.5</td>
<td>1.6 ± 0.6</td>
<td>1.5 ± 0.6</td>
<td>1.5 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>16:0 PL</td>
<td>30.0 ± 1.9 ( ^a )</td>
<td>27.3 ± 1.4 ( ^a )</td>
<td>27.1 ± 2.1 ( ^a )</td>
<td>26.9 ± 1.8 ( ^a )</td>
<td></td>
</tr>
<tr>
<td>16:0 CE</td>
<td>11.7 ± 1.8 ( ^b )</td>
<td>11.0 ± 1.8 ( ^b )</td>
<td>9.8 ± 1.5 ( ^b )</td>
<td>15.1 ± 1.5 ( ^b )</td>
<td></td>
</tr>
<tr>
<td>18:0 PL</td>
<td>13.5 ± 2.2 ( ^a )</td>
<td>14.4 ± 1.1 ( ^a )</td>
<td>16.1 ± 1.5 ( ^c )</td>
<td>16.0 ± 1.3 ( ^c )</td>
<td></td>
</tr>
<tr>
<td>18:0 CE</td>
<td>1.2 ± 0.5</td>
<td>1.0 ± 0.4</td>
<td>1.0 ± 0.5</td>
<td>1.2 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>18:1 PL</td>
<td>11.1 ± 1.2 ( ^a )</td>
<td>15.3 ± 1.5 ( ^b )</td>
<td>8.0 ± 1.5 ( ^b )</td>
<td>7.6 ± 1.1 ( ^b )</td>
<td></td>
</tr>
<tr>
<td>18:1 CE</td>
<td>20.1 ± 2.1 ( ^a )</td>
<td>27.1 ± 2.9 ( ^a )</td>
<td>13.5 ± 1.8 ( ^a )</td>
<td>13.7 ± 1.3 ( ^a )</td>
<td></td>
</tr>
<tr>
<td>18:2 PL</td>
<td>19.8 ± 1.8 ( ^a )</td>
<td>17.3 ± 1.9 ( ^a )</td>
<td>25.0 ± 2.3 ( ^a )</td>
<td>23.0 ± 2.7 ( ^a )</td>
<td></td>
</tr>
<tr>
<td>18:2 CE</td>
<td>52.5 ± 3.2 ( ^b )</td>
<td>45.4 ± 3.0 ( ^b )</td>
<td>62.9 ± 2.6 ( ^b )</td>
<td>60.7 ± 2.9 ( ^b )</td>
<td></td>
</tr>
<tr>
<td>20:3 PL</td>
<td>4.0 ± 0.7 ( ^a )</td>
<td>4.1 ± 0.7 ( ^a )</td>
<td>4.2 ± 0.9 ( ^a )</td>
<td>3.4 ± 0.8 ( ^a )</td>
<td></td>
</tr>
<tr>
<td>20:3 CE</td>
<td>0.7 ± 0.2b</td>
<td>0.8 ± 0.2b</td>
<td>0.7 ± 0.2b</td>
<td>0.5 ± 0.2b</td>
<td></td>
</tr>
<tr>
<td>20:4 PL</td>
<td>8.3 ± 1.2 ( ^a )</td>
<td>8.7 ± 1.2 ( ^a )</td>
<td>8.4 ± 1.5 ( ^a )</td>
<td>6.8 ± 1.0 ( ^a )</td>
<td></td>
</tr>
<tr>
<td>20:4 CE</td>
<td>6.8 ± 1.7 ( ^a )</td>
<td>7.0 ± 1.9 ( ^a )</td>
<td>6.6 ± 1.8 ( ^a )</td>
<td>5.3 ± 1.2 ( ^a )</td>
<td></td>
</tr>
<tr>
<td>20:5 PL</td>
<td>1.1 ± 0.4 ( ^a )</td>
<td>1.3 ± 0.3 ( ^a )</td>
<td>0.7 ± 0.3 ( ^a )</td>
<td>2.8 ± 1.0 ( ^a )</td>
<td></td>
</tr>
<tr>
<td>20:5 CE</td>
<td>1.2 ± 0.5 ( ^a )</td>
<td>1.4 ± 0.6 ( ^a )</td>
<td>0.8 ± 0.3 ( ^a )</td>
<td>3.1 ± 1.2 ( ^a )</td>
<td></td>
</tr>
<tr>
<td>22:6 PL</td>
<td>5.6 ± 1.3 ( ^a )</td>
<td>5.8 ± 1.1 ( ^a )</td>
<td>5.2 ± 1.3 ( ^a )</td>
<td>7.0 ± 1.4 ( ^a )</td>
<td></td>
</tr>
<tr>
<td>22:6 CE</td>
<td>1.1 ± 0.2 ( ^a )</td>
<td>1.3 ± 0.1 ( ^a )</td>
<td>1.2 ± 0.6 ( ^a )</td>
<td>1.5 ± 0.4 ( ^a )</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a,b} x \pm SD; n = 41. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. Values in the same row with different superscript letters are significantly different, \( \text{P} < 0.01 \).

Cholesterol efflux

The serum capacity to induce cholesterol efflux was significantly higher during the n−3 PUFA phase than during the other phases in the men. In the women, this effect was observed during both PUFA phases (Figure 1).

Lipoprotein particles

Serum Lp A-I concentrations were significantly lower with the MUFA and both PUFA diets than with the SFA diet, but only in the men (Table 4). As expected, in all phases the men’s serum Lp A-I concentrations were lower than those observed in the women. Lp A-I:A-II concentrations were significantly lower with the 2 PUFA diets than with the SFA and MUFA diets in the women. In the men, Lp A-I:A-II concentrations were significantly higher with the n−6 PUFA diet than with all the other diets.

Serum Lp E concentrations were significantly higher during both PUFA phases than during the SFA and MUFA phases only in the women. Lp E non-B particle concentrations were not significantly different in the different phases in the men, but were higher in the women with the n−3 PUFA diet than with the other diets. Serum concentrations of both types of lipoprotein particles with apo C-III did not differ significantly by diet in the women; in the men they were higher with the SFA diet than with the n−3 PUFA diet (Lp C-III) and higher with the SFA diet than with the MUFA and n−6 PUFA (Lp C-III non-B).

**Figure 1.** Mean (±SEM) cellular cholesterol efflux from rat Fu5AH hepatoma cells treated with serum collected from 17 women and 24 men at the end of each diet phase: a saturated fatty acid (SFA) diet with a high palm oil content, a monounsaturated fatty acid (MUFA) diet with a high oleic acid content, an n−6 polyunsaturated fatty acid (PUFA) diet with a high percentage of sunflower oil, and an n−3 PUFA diet enriched with fish oil. The serum from each subject was diluted to 2.5% in MEM and the cells were incubated at 37°C for 4 h. Results of cholesterol efflux are percentage of total radioactivity. Bars within each sex with different superscript letters are significantly different, \( \text{P} < 0.05 \) (ANOVA with post hoc Tukey’s test).

Serum concentrations of apo A-I measured in the SFA phase were significantly higher than with the diets rich in MUFA or n−3 PUFA in the women; in the men, apo A-I concentrations were significantly lower only with the PUFA-rich diets. In the women, apo B concentrations were significantly lower with the diets rich in PUFA than with the other diets. In the men, apo B concentrations were lower with the MUFA and n−3 PUFA diets than with the other diets.

**Table 2**

Effect of diets on fatty acid composition of serum phospholipids (PLs) and cholesterol esters (CEs). Values in the same row with different superscript letters are significantly different, \( \text{P} < 0.01 \).

**Figure 1.** Mean (±SEM) cellular cholesterol efflux from rat Fu5AH hepatoma cells treated with serum collected from 17 women and 24 men at the end of each diet phase: a saturated fatty acid (SFA) diet with a high palm oil content, a monounsaturated fatty acid (MUFA) diet with a high oleic acid content, an n−6 polyunsaturated fatty acid (PUFA) diet with a high percentage of sunflower oil, and an n−3 PUFA diet enriched with fish oil. The serum from each subject was diluted to 2.5% in MEM and the cells were incubated at 37°C for 4 h. Results of cholesterol efflux are percentage of total radioactivity. Bars within each sex with different superscript letters are significantly different, \( \text{P} < 0.05 \) (ANOVA with post hoc Tukey’s test).

**Lipid and lipoprotein profile**

Lipid and lipoprotein particle profiles (Table 3) were analyzed for each sex independently because the cholesterol efflux analysis showed a significant interaction between sex and the responses obtained in each diet period. Serum concentrations of total and LDL cholesterol were significantly lower in both sexes after all of the unsaturated fat diets than after the SFA diet. The addition of n−3 PUFA did not cause further significant modifications in the women, but the men had a lower LDL concentration with the n−3 PUFA diet than with the other diets. Triacylglycerol concentrations in the men were significantly lower with the PUFA diets than with the SFA and MUFA diets, but this result was not seen only with the MUFA diet in the women. HDL-cholesterol concentrations did not differ among diet phases in either sex. In the women, but not in the men, the serum HDL phospholipid concentration was significantly lower after the n−3 PUFA diet than after the SFA diet.
TABLE 3
Lipid and lipoprotein distribution at the end of each diet period†

<table>
<thead>
<tr>
<th></th>
<th>Women (n = 17)</th>
<th></th>
<th>Men (n = 24)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SFA diet</td>
<td>MUFA diet</td>
<td>PUFA diet</td>
<td>SFA diet</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>6.09 ± 0.95a</td>
<td>5.59 ± 0.73b</td>
<td>4.97 ± 0.7c</td>
<td>5.00 ± 0.65c</td>
</tr>
<tr>
<td>(mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triacylglycerols</td>
<td>0.95 ± 0.34b</td>
<td>0.99 ± 0.33b</td>
<td>0.84 ± 0.31a</td>
<td>0.85 ± 0.21a</td>
</tr>
<tr>
<td>(mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLDL cholesterol</td>
<td>0.18 ± 0.12a</td>
<td>0.23 ± 0.13b</td>
<td>0.19 ± 0.12a</td>
<td>0.16 ± 0.09a</td>
</tr>
<tr>
<td>(mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>4.25 ± 0.85a</td>
<td>3.82 ± 0.65b</td>
<td>3.29 ± 0.61c</td>
<td>3.28 ± 0.63c</td>
</tr>
<tr>
<td>(mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>1.49 ± 0.29a</td>
<td>1.49 ± 0.29c</td>
<td>1.40 ± 0.29</td>
<td>1.42 ± 0.29</td>
</tr>
<tr>
<td>(mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-PL (g/L)</td>
<td>1.52 ± 0.19a</td>
<td>1.49 ± 0.22b</td>
<td>1.43 ± 0.23b</td>
<td>1.42 ± 0.21b</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apo B (g/L)</td>
<td>1.04 ± 0.23a</td>
<td>1.02 ± 0.17b</td>
<td>0.92 ± 0.16b</td>
<td>0.91 ± 0.18b</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apo A-I (g/L)</td>
<td>1.59 ± 0.23b</td>
<td>1.51 ± 0.23b</td>
<td>1.54 ± 0.22b</td>
<td>1.49 ± 0.20b</td>
</tr>
</tbody>
</table>

†± SD. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; Apo, apolipoprotein. Values in the same row with different superscript letters are significantly different at the P value indicated.

TABLE 4
Lipoprotein particle distribution at the end of each diet period†

<table>
<thead>
<tr>
<th></th>
<th>Women (n = 17)</th>
<th></th>
<th>Men (n = 24)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SFA diet</td>
<td>MUFA diet</td>
<td>PUFA diet</td>
<td>SFA diet</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apo A-I</td>
<td>1.12 ± 0.18a</td>
<td>1.08 ± 0.18b</td>
<td>1.01 ± 0.19b</td>
<td>0.99 ± 0.16b</td>
</tr>
<tr>
<td>Lp A-I</td>
<td>0.50 ± 0.08</td>
<td>0.49 ± 0.11</td>
<td>0.48 ± 0.09</td>
<td>0.49 ± 0.08</td>
</tr>
<tr>
<td>Lp A-I-A-II</td>
<td>0.62 ± 0.15a</td>
<td>0.58 ± 0.17b</td>
<td>0.52 ± 0.18b</td>
<td>0.50 ± 0.15b</td>
</tr>
<tr>
<td>Lp E</td>
<td>0.047 ± 0.023a</td>
<td>0.046 ± 0.026a</td>
<td>0.054 ± 0.025b</td>
<td>0.061 ± 0.031c</td>
</tr>
<tr>
<td>Lp E non-B</td>
<td>0.04 ± 0.02a</td>
<td>0.04 ± 0.02b</td>
<td>0.04 ± 0.03a</td>
<td>0.05 ± 0.03b</td>
</tr>
<tr>
<td>Lp C-III</td>
<td>0.020 ± 0.004</td>
<td>0.020 ± 0.004</td>
<td>0.020 ± 0.005</td>
<td>0.020 ± 0.005</td>
</tr>
<tr>
<td>Lp C-III non-B</td>
<td>0.02 ± 0.003</td>
<td>0.02 ± 0.003</td>
<td>0.02 ± 0.003</td>
<td>0.02 ± 0.003</td>
</tr>
<tr>
<td>Lp A-I-A-II</td>
<td>0.012 ± 0.003a</td>
<td>0.009 ± 0.004a</td>
<td>0.018 ± 0.007b</td>
<td>0.011 ± 0.004a</td>
</tr>
</tbody>
</table>

†± SD. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; apo, apolipoprotein; Lp, lipoprotein. Values in the same row with different superscript letters are significantly different at the P value indicated.
CHOLESTEROL EFFLUX AND DIETARY FATTY ACID SATURATION

TABLE 5
Differences in efflux between the saturated fatty acid–rich diet and the other diets for men and women combined.

<table>
<thead>
<tr>
<th>Multiple regression analysis</th>
<th>Coefficient</th>
<th>Adjusted $R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lp A-I/Lp A-II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUFA diet</td>
<td>0.35</td>
<td>0.096</td>
</tr>
<tr>
<td>n−6 PUFA diet</td>
<td>0.08</td>
<td>0.25</td>
</tr>
<tr>
<td>n−3 PUFA diet</td>
<td>0.41</td>
<td>0.23</td>
</tr>
<tr>
<td>Lp A-I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUFA diet</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>n−6 PUFA diet</td>
<td>0.47</td>
<td>0.38</td>
</tr>
<tr>
<td>n−3 PUFA diet</td>
<td>0.70</td>
<td>0.34</td>
</tr>
</tbody>
</table>

$r$ values were obtained by sequential entry of variables in the multiple regression equation. Only variables that increased the $r$ value significantly were considered. Lp, lipoprotein; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

There were no significant differences in the Lp E non-B/Lp E:B and Lp C-III non-B/Lp C-III:B coefficients by diet. In the multiple regression analysis, the variables associated with change in efflux were Lp A-I/Lp A-I-A-II and Lp A-I (Table 5). We found no significant correlations between the changes in the lipid composition of HDL and serum capacity to promote the efflux of cellular cholesterol.

DISCUSSION

The great differences in total cardioischemic mortality rates among developed countries whose citizens have similar lipid profiles and cholesterol concentrations indicate that other risk factors, such as diet, associated with the traditions of each country are decisive for CVD. The low mortality rate from CVD in Spain compared with that in other European countries is explained in part by the current Mediterranean diet, which contains large amounts of fruit, fish, and olive oil, foods able to reduce the effect of some risk factors (44). After the Seven Countries Study ascribed an important beneficial effect to the Mediterranean diet, it was proposed as a model (45, 46).

Despite several studies claiming to identify which diet is the healthiest and most advisable for the general population, the results are still controversial. Consumption of SFAs produces an increase in the total serum cholesterol concentration, mainly of the low-density atherogenic fraction; consumption of PUFAs decreases total serum cholesterol and LDL cholesterol concentrations in an efficient manner, although PUFAs also decrease the HDL-cholesterol fraction. Furthermore, MUFAs are responsible for a possible cardioprotective effect by diminishing LDL without modifying HDL cholesterol.

Because of the nature of the groups used, our study was designed as a parallel study (with every individual receiving the same diet at the same time), which may have allowed environmental factors to influence the results. Nevertheless, the data obtained in our study after administration of diets with different fatty acid saturations agree with those of the literature, showing that the lipoprotein profile after consumption of a diet with a high unsaturated fat content is hemodynamically healthier than profiles obtained after diets containing saturated fat because the unsaturated fat diet lowers total cholesterol and the atherogenic LDL fraction (8, 9). Unlike results in other studies, no substantial changes were seen in HDL-cholesterol concentrations in the men after a PUFA diet. However, apo A-I concentrations decreased with unsaturated fat (MUFA and PUFA) diets, agreeing with studies that showed an increase in the catabolism of these proteins with unsaturated fats (47). Thus, in the men, the serum Lp A-I particle concentrations were lower after consumption of diets with high MUFA and PUFA (n−3 and n−6) contents than after consumption of the SFA diet, as was observed in other studies (28, 29).

In the women, the concentrations of HDL particles containing apo E (Lp E non-B) were higher with the n−3 PUFA diet.

The Lp E non-B/Lp E:B and Lp C-III non-B/Lp C-III:B coefficients, recently described as important CVD markers, differed significantly by diet in the present study, but when the mean ratio values were compared with those from other European populations (France and Northern Ireland) with a higher CVD mortality rate, we observed that the values for the Spanish population were higher (25).

In our study, the sera obtained after the administration of diets with a high n−6 PUFA content supplemented by fish revealed a major extraction of cell cholesterol in both sexes. Furthermore, in the women, the sera obtained after the consumption of diets with a high n−6 PUFA content also showed significantly more cholesterol efflux than did sera drawn during the SFA and MUFA phases. Although several studies (30–33, 48) recently investigated the effect of dietary fat type on cholesterol efflux, the results do not agree, and this important issue remains to be clarified.

Gillette et al (32) performed a detailed study in primates of the possible effects of diets differing in degree of saturation. They concluded that all diets produce HDL of the same fluidity and size with an identical capacity to promote cellular cholesterol efflux. Besides using L-cell fibroblasts, this study also used the cell line used in our assays with similar incubation periods. The diets used in that study and our own are very similar, but the n−3 PUFA diet used by Gillotte et al contained a substantial percentage of SFA, which could have masked the effect found with our PUFA diet with a high fish content. The number of participants in our study was much higher (41 compared with 6 subjects), and this could explain the statistically significant differences we found.

Another study, performed in a different Spanish population (33) and using the same methodology and cell line, compared the effect of the low-fat diets recommended by the National Cholesterol Education Program (49) with a diet with a high MUFA content. There were no significant differences in cholesterol efflux, which could be attributed to the addition of MUFAs.

A study by Sakr et al (48), also using the same cell line but using postprandial plasma samples obtained after a single fat load, showed that a diet with a monounsaturated fat overload conferred a greater capacity to extract cellular cholesterol than did diets with a high SFA or PUFA content. Those results are probably the consequence of an acute effect, whereas our work shows the effect of modifying dietary fat saturation over a longer period.

In a study with a design quite similar to ours, Solá et al (30) previously showed that a diet with a high monounsaturated fat content produced smaller-sized HDL particles with greater fluidity and capacity to promote cholesterol efflux in fibroblasts than did the saturated and polyunsaturated fat diets. The study used the n−3 PUFA, SFA, and MUFA diets and showed that the n−3 PUFA diet has the lowest efficacy in cholesterol extraction. Although initially the results seemed contrary to ours, important differences could explain these discrepancies: this group performed 24-h incubations of fibroblast cultures with one isolated fraction of the serum, HDL$_x$, thus excluding particles that play an important role in the exit of cellular cholesterol, pre-β-HDL (16),...

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Lp A-IV (50, 51), γLp E (23), and other plasma components that could modulate the action of these particles [eg, phosphatidylcholine-steryl O-acetyltransferase and cholesteryl ester transfer protein (11, 52, 53)]. Our assay, which used a dilution of whole serum and an incubation time of 4 h, is much closer to physiological conditions.

The same group used monocyte-derived macrophages and fibroblasts to compare the effect of a linoleic acid–rich diet with another diet enriched in oleic oil, without finding any differences in fluidity, size, or the cholesterol efflux capacity of the HDL, obtained after both diets (31). In our results, the modifications in the cholesterol efflux capacity were not correlated with the modifications produced in HDL lipid concentrations but rather with the changes observed in some lipoprotein particle concentrations: Lp A-I and Lp A-I/Lp A-I:A-II. Our results suggest that a diet with an adequate ratio of monounsaturated (oleic acid) and polyunsaturated (especially fish oil) fatty acids and a low proportion of saturated fats is hemodynamically healthiest, producing a significant decrease in atherogenic lipoprotein particles. Furthermore, unsaturated fats induce an increase in serum capacity to promote cholesterol efflux from cells in culture, the first step in the antiatherogenic pathway of reverse cholesterol transport. Our study could explain the influence of diet on the complex mechanism of reverse cholesterol transport in lipid metabolism as well as its repercussions on inhibiting the formation or promoting the regression of atheromatous plaque.

We thank Alberto García (Logitest, Madrid) for his assistance in the statistical analysis, José R Garcia Hierro and the Analytical Laboratory of the Spanish Ministry of Agriculture for the analysis of the diets, Catherine de Geitere and Bernard Delfly for technical assistance, and the participants of the study for their enthusiasm.

REFERENCES


