Available online at www.sciencedirect.com

SCIENCE DIRECT®

Journal of Nutritional Biochemistry 16 (2005) 375–382

Journal of Nutritional Biochemistry

Moderate intake of myristic acid in sn-2 position has beneficial lipidic effects and enhances DHA of cholesteryl esters in an interventional study☆

Henry Dabadie[a,*], Evelyne Peuchant[b], Mireille Bernard[a], Pascale LeRuyet[c], François Mendy[a]

[a]*Service de Nutrition, Hôpital Haut-Lévêque, 33600 Pessac, France*
[b]*Laboratoire de Biochimie, Université Victor Segalen, 33000 Bordeaux, France*
[c]*Lactalis Recherche et Developpment, 35240 Rétiers, France*

Abstract

Among the saturated fatty acids (SFA), myristic acid is known to be one of the most atherogenic when consumed at high levels. Our purpose was to compare the effects of two moderate intakes of myristic acid on plasma lipids in an interventional study. Twenty-five male monks without dyslipidemia were given two isocaloric diets for 5 weeks each. In diet 1, 30% of the calories came from fat (8% SFA, 0.6% myristic acid) and provided 200 mg cholesterol/day. Calories of diet 2 were 34% fat (11% SFA, 1.2% myristic acid) with the same levels of oleate, linoleate, α-linolenate and cholesterol. A baseline diet was provided before each diet.

In comparison with baseline, diets 1 and 2 induced a decrease in total cholesterol, LDL-cholesterol and triglycerides ($P<.001$); HDL-cholesterol was not modified and the apo A-I/apo B ratio increased ($P<.001$). Plasma triglycerides were lower after diet 2 than after diet 1 whereas HDL-cholesterol was higher ($P<.05$). In phospholipids, myristic acid, oleic acid, linoleic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) increased after diet 2 vs. baseline ($P<.01$) and diet 1 ($P<.05$). Both diets were associated with an increase in α-linolenate of cholesteryl esters ($P<.05$), but only diet 2 was associated with an increase in DHA of cholesteryl esters ($P<.05$). In diet 2, myristic acid intake was positively correlated with myristic acid of phospholipids, and α-linolenic acid intake was correlated with α-linolenic acid of cholesteryl esters.

Moderate intake (1.2% of total calories) of myristic acid has beneficial lipidic effects and enhances DHA of cholesteryl esters.

Keywords: Cholesteryl esters; Myristic acid; Saturated fatty acids; DHA Interventional Study

1. Introduction

Many epidemiologic studies have documented an association between fatty acid intake and the risk of coronary heart disease (CHD) [1–4]. In general, long-chain saturated fatty acids (SFA) (12–16 carbon atoms) tend to increase the risk of CHD, whereas monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) tend to decrease this risk, mainly α-linolenic acid [5,6]. These effects may be mediated in part by fatty acid-induced changes in serum lipid levels, especially LDL-cholesterol but also VLDL- and IDL-cholesterol (grouped together under the term *non-HDL-cholesterol*), oxidative status, hemostasis and blood pressure. The results of these studies were used to make specific dietary recommendations for the prevention and treatment of CHD which have led to a change in eating habits [7–9].

Recent feeding studies [10–16] have shown that individual SFA may affect the cholesterolemic response with different potencies. Myristic acid (C14:0) is more potent than lauric (C12:0) or palmitic (C16:0) acids in inducing increase in total cholesterol and LDL-cholesterol. In the Nurses' Health Study, Hu et al. [17] demonstrated that intake of stearic acid at 4.1% of total energy was associated with a 1.30 risk ($P=.009$) for CHD. Intakes of C12:0+C14:0 in five quintiles from 0.98% to 2.14% of total calories were associated with a U-shaped curve, with a lower CHD risk at 0.96 for an intake of 1.45%.

In many experimental diets myristic acid has been given in relatively high concentrations up to 3.4–4% of total energy (level in mother's milk where it is also in sn-2

Abbreviations: DHA, Docosahexaenoic acid; EPA, Eicosapentaenoic acid; SFA, Saturated fatty acid.

☆ Authors have agreed to submit the manuscript to this Journal, that no part of the work has been published before, except in abstract form, and that all human studies have been reviewed by the appropriate ethics committees.

* Corresponding author. Fax: +33 5 56 93 04 07.
E-mail address: henry.dabadie@thera.u-bordeaux2.fr (H. Dabadie).

0955-2863/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2005.01.010

position). Consequently, results have been obtained in a nonpertinent manner for utilization of myristic acid in medicine: 52% of total energy in Spady et al. [18], 17% in Tholstrup et al. [19], 11.3% in Zock et al. [20] or 10% in Temme et al. study [21]. Myristic acid came from trimyristine (a product of synthesis), or from coconut oil where it is usually in the external position of the triglyceride, and is associated with lauric acid in a ratio C12:0/C14:0 greater than 1.

Furthermore, Gillman et al. [22] have recently shown in the Framingham Heart Study that intakes of fat, saturated fat, and monounsaturated fat, but not polyunsaturated fat, were surprisingly associated with reduced risk of ischemic stroke in men. In addition, the initial results of the EVA Study [23] point out the lower risk of cognitive decline in the elderly (26% reduction) with a 1% increase in n-3 PUFA level, whereas a 1% increase in n-6 levels was associated with a higher risk (twofold greater). Intake of SFA increased this risk to a lesser extent.

The aim of our study was to test the effects of pertinent moderate intake of myristic acid (1.2% of total energy) on plasma lipids, lipoproteins and fatty acids of phospholipids and cholesteryl esters compared to a diet containing only half the amount of myristic acid but with 'physiological' intakes of oleic, linoleic and α-linolenic acids, at identical levels in both diets to avoid any bias.

2. Subjects and methods

2.1. Subjects

Twenty-five male members of a Benedictine monastery (Belloc Abbey) located in the Southwest of France were recruited for the study and all of them completed each diet phase. They were between 35 and 88 years of age (average, 61 years), weighed 57–87 kg (average, 72 kg), and their body mass index ranged from 32 to 18 kg/m^2 (average, 25 kg/m^2). None had a history of atherosclerotic disease and their responses to a medical questionnaire appeared to indicate that they were healthy. They were all nonsmokers. None had known dyslipidemia according to NCEP before the study or was taking any lipid-lowering drug or medication affecting lipid metabolism. Most of them performed moderate amounts of physical activity (e.g., work in the fields, regular walking). Physical exercise and general life style were kept constant during the study. Anthropometric measurements and blood pressure determination were obtained in each subject at baseline and at the end of the two dietary periods. The protocol and aim of the study were fully explained to the subjects who gave their written consent. The research protocol was approved by the hospital ethics committee.

2.2. Diets

Two different test diets were given to 25 monks for 5 weeks, each separated by 4 weeks of the subject's usual diet. Both interventional diets provided roughly 2200 kcal and 15% of the energy contribution was from proteins, 12% from oleic acid, 6% from linoleic acid and 1% from α-linolenic acid. The diets were also quite similar in cholesterol content (approximately 200 mg/day). Intakes of fat, SFA and myristic acid were different in the two interventional diets. Thirty percent of the calories in diet 1 were provided by fat (8% SFA, 0.6% myristic acid) whereas 34% of the calories came from fat (11% SFA, 1.2% myristic acid) in diet 2. The higher SFA content of diet 2 was mainly related to the increase in myristic acid (twofold) (Table 1). The usual large consumption of milk and cheese (30 g/day) of the monks provided SFA, whereas MUFA and PUFA came from sunflower margarine and rapeseed oil. Half-skimmed milk (500 ml/day), 180 g/day of bread and 30 g/day of sugar were served in diet 1 instead of full cream milk (500 ml/day), 150 g/day of bread and 20 g/day of sugar in diet 2. In these experimental conditions (Table 2), myristic acid content of full cream milk was 3.6 vs. 1.8 g/kg in half-skimmed milk (2 times more), full cream palmitic acid content was 9.1 vs. 7.5 g/kg in half-skimmed (1.2 times more), and full cream stearic acid content was 4.2 vs. 2 g/kg in half-skimmed milk (2 times more). The PUFA/SFA ratio was 1 and 0.75 in diets 1 and 2, respectively. The linoleic/α-linolenic ratio (LA/ALA ratio) was roughly 7.6 in both experimental diets. Except for carbohydrates which were lower in diet 2 vs. diet 1 (51% and 55%, respectively) and vitamin A which was higher in diet 2, intakes in other nutrients were similar (Table 1). The composition of the interventional diets was evaluated using software (Bilnut program) that had previously been tested. The menus were designed in accordance with the group's eating habits. Food was prepared by a local caterer during weekdays and by a monk at the kitchen of the abbey in the weekend. As a measure of compliance, 7-day intake was analyzed once at the end of each diet period to compare the amount of food actually consumed with the amount served.

The baseline diet, which was the subjects' habitual diet (Table 1), provided 2400 kcal, 14% from proteins, 51% from carbohydrates and 35% from fat (13% SFA, 1.4% myristic acid, 12% MUFA and 10% PUFA; PUFA/SFA ratio, 0.76, and LA/ALA ratio, 68.3). This habitual diet was consumed before the first intervention diet and for 4 weeks between diets 1 and 2.

2.3. Plasma lipid and fatty acid analysis

Fasting blood samples were obtained from each subject at a baseline visit before initiation of each interventional period and at completion of the two 5-week nutritional interventions. Blood was collected into EDTA, placed immediately on ice, and the plasma was separated. Total lipids were extracted from plasma with 5 ml of hexane/isopropanol (3:2, v/v). Total cholesterol, HDL-cholesterol and triacylglycerols were analyzed enzymatically using a multiparameter automated analyzer (Baxter, Paris). LDL-cholesterol was calculated using the Friedewald equation.

Table 1
Daily intakes of principal nutrients in subjects receiving baseline diet and the two interventional diets

	Baseline diet	Diet 1	Diet 2
Energy (kcal)	2400±173	2202±85	2198±128
Fat, g (%)	92.1±4.0 (34.5)	73.2±2.0 (29.9)	83.2±3.0 (34.0)[a]
Saturated fat, g (%)	34.7±0.9 (13.0)	19.3±0.6 (7.9)	26.1±0.7 (10.7)[b]
Myristic acid, g (%)	3.7±0.4 (1.4)	1.6±0.3 (0.6)	2.9±0.5 (1.2)[d]
Palmitic acid, g (%)	14.4±0.7 (5.4)	8.7±0.8 (3.5)	12.2±0.7 (5.0)[c]
Stearic acid, g (%)	5.5±0.5 (2.2)	4.2±0.4 (1.7)	5.6±0.5 (2.3)[b]
Monounsaturated fat, g (%)	30.7±2.0 (11.5)	34.0±1.1 (13.9)	37.0±1.2 (15.1)
Oleic acid, g (%)	20.4±1.0 (7.7)	27.6±0.8 (11.3)	30.6±1.5 (12.5)
Polyunsaturated fat, g (%)	26.7±1.9 (10.0)	19.9±1.5 (8.1)	20.1±1.8 (8.2)
Linoleic acid, g (%)	20.5±0.2 (7.6)	15.5±0.1 (6.3)	15.7±0.2 (6.4)
Linolenic acid, g (%)	0.3±0.1 (0.1)	2.0±0.1 (0.8)	2.1±0.1 (0.9)
P/S ratio	0.76	1	0.75
Cholesterol (mg)	210±15.2	173±8.1	206±10.7
Carbohydrate, g (%)	309.6±20.1 (51.6)	303.8±11.6 (55.2)	281.3±20.3 (51.2)[e]
Protein, g (%)	83.4±2.2 (13.9)	82.0±1.9 (14.9)	81.3±3.3 (14.8)
Fiber (g)	20.7±4.0	21.7±2.0	20.2±1.8
Alcohol (g)	10.1±8.5	10.0±8.3	11.3±9.0
Calcium (mg)	1116.8±75.2	1109.5±64.6	1087.6±93.8
Vitamin A (μg RE)	601.2±22.5	594.4±32.8	694.7±59.9[a]
Vitamin C (mg)	115±7.2	119±9.2	120±2.6
Vitamin E (mg)	17.7±0.5	18.2±0.3	18.0±0.2

Values are means±S.D. RE, retinol equivalents; P-S, ratio of polyunsaturated to SFA.
[a] Increase of 15% with diet 2 vs. diet 1.
[b] Increase of 35% with diet 2 vs. diet 1.
[c] Increase of 45% with diet 2 vs. diet 1.
[d] Increase of 100% with diet 2 vs. diet 1.
[e] Decrease of 7.5% with diet 2 vs. diet 1.

Apolipoprotein (apo) A-I and apo B were measured in serum using a nephelometry method (the BNA system of Behring, Paris).

Plasma fatty acid composition of the phospholipids and cholesteryl esters was determined from 2 ml of the lipid extract after transformation into isopropyl esters. The isopropyl esters were separated by gas chromatography (Carlo Erba 6000 G, Fisons Instruments, France) using a 25-m Carbowax capillary column (internal diameter, 0.32 mm). Column conditions were 180°C for 5 min, increasing by 7.5°C/min to 220°C for 30 min. The injector was at 60°C and the flame ionization detector was at 250°C. Helium was used as the carrier gas (flow rate, 2 ml/min). The peaks were identified by comparison with reference fatty acid esters (Sigma) and peak areas were measured with an automatic integrator (DP 700, Fisons Instruments, France). The results of each fatty acid were expressed as percentage of total fatty acids.

Fatty acid analyses of half-skimmed and full cream milk were performed according to the same extraction method and the results were expressed in grams per kilogram.

Table 2
Comparison in fatty acid composition of half-skimmed milk and full cream milk

	Half-skimmed milk	Full cream milk
SFA	12.0	22.0
Lauric acid (12:0)	0.7	1.3
Myristic acid (14:0)	1.8	3.6
Pentadecanoic acid (15:0)	0.2	0.4
Palmitic acid (16:0)	7.5	9.1
Margaric acid (17:0)	0.1	0.2
Stearic acid (18:0)	2.0	4.2
MUFA	3.6	10.1
Palmitoleic acid (16:1)	0.2	0.6
Oleic acid (18:1)	3.4	8.6
PUFA	0.6	1.0
Linoleic acid (18:2)	0.4	0.8
Linolenic acid (18:3)	0.2	0.2

Values are expressed in grams per kilogram.

Table 3
Plasma lipids, lipoproteins and apolipoproteins after the two interventional diets

Parameters	Baseline diet	Diet 1	Baseline diet	Diet 2
Total cholesterol (1)	220±38	193±31[d]	213±38	191±31[c]
Triglycerides (1)	99±52	85±41[b]	101±43	77±34[d,A]
HDL-cholesterol (1)	44±10	45±10	46±10	48±11[A]
LDL-cholesterol (1)	153±34	132±31[d]	146±38	129±30[b]
Total-C/HDL-C	5.14±1.80	4.56±1.54[d]	4.85±1.65	4.27±1.28[b]
LDL-C/HDL-C	3.72±1.31	3.10±1.26[c]	3.37±1.33	2.91±1.10[b]
Apo A-I/Apo B	1.56±0.50	1.66±0.53[c]	1.48±0.50	1.73±0.54[c]

Values are expressed in mg/dl±S.D.
Lipidic parameters of experimental diets (diets 1 and 2) were compared to those of baseline diets consumed prior to experimental diets.
Means with different lowercase superscript letters are significantly different from baseline diet; means with different uppercase superscript letters are significantly different from diet 1. [a,A]$P<.05$; [b,B]$P<.005$; [c,C]$P<.001$; [d,D]$P<.0001$.

Table 4
Plasma fatty acid profiles of the phospholipids after the two interventional diets

Fatty acids	Baseline diet	Diet 1	Baseline diet	Diet 2
Myristic acid (14:0)	1.01±0.25	0.93±0.21	1.10±0.27	1.30±0.33[b,E]
Palmitic acid (16:0)	27.48±2.54	26.42±1.73[a]	27.23±2.79	26.54±3.01
Stearic acid (18:0)	16.06±4.12	10.84±2.05[d]	16.34±1.47	16.65±2.21
Oleic acid (18:1)	14.10±5.15	17.62±2.27[c]	16.49±1.88	20.83±2.51[e,E]
Linoleic acid (18:2)	30.11±4.57	32.51±2.58[c]	29.24±3.19	34.92±4.57[e,C]
Arachidonic acid (20:4)	6.50±1.17	5.80±2.04	6.10±1.61	6.30±1.45
Linolenic acid (18:3)	0.43±0.14	0.86±0.25[e]	0.53±0.38	0.75±0.23[A]
EPA (20:5)	0.56±0.13	0.73±0.29[b]	0.60±0.33	1.00±0.28[a,D]
DHA (22:6)	2.70±0.65	2.40±0.47	2.50±0.64	2.80±0.58[a,B]
ARA/EPA	12.00±2.60	8.90±4.00[c]	12.40±7.00	6.90±3.00[d,A]
PUFA/SFA	0.81±0.02	1.12±0.05[e]	0.90±0.08	1.10±0.07[c]

Values are expressed in % total fatty acids±S.D.
Means with different lowercase superscript letters are significantly different from baseline diet; means with different uppercase superscript letters are significantly different from diet 1. [a,A]$P<.05$; [b,B]$P<.01$; [c,C]$P<.005$; [d,D]$P<.0005$; [e,E]$P<.0001$.

2.4. Statistical analysis

The Wilcoxon signed-rank test was used for comparisons of plasma lipid and fatty acid levels and P values <.05 were considered to be significant. Results were expressed as mean±S.D. Means of separate measurements for each lipid and lipoprotein variable during the baseline and the two interventional diets were calculated in each subject. Differences between baseline and interventional study values and between diet 1 and 2 values were tested by one-factor repeated-measures analysis of variance. Pearson's correlation coefficient was used as a measure of the associations between fatty acid intake and fatty acids of cholesteryl esters after the two interventional diets.

3. Results

In comparison with the baseline diet consumed prior to diet 1, diet 1 induced a decrease in plasma total cholesterol ($P<.0001$), triglycerides ($P<.005$) and LDL-cholesterol ($P<.0001$). Plasma HDL-cholesterol was not significantly modified by diet 1. The total-C/HDL-C and LDL-C/HDL-C ratios were significantly lower ($P<.0001$ and $P<.001$, respectively) and the apo A-I/apo B ratio was higher ($P<.001$) (Table 3).

In comparison with the baseline diet consumed prior to diet 2, diet 2 was associated with a significant decrease in plasma total cholesterol ($P<.001$), triglycerides ($P<.0001$) and LDL-cholesterol ($P<.005$), no change in HDL-cholesterol, a significant decrease in the total-C/HDL-C and LDL-C/HDL-C ratios ($P<.005$), and an increase in the apo A-I/ apo B ratio ($P<.001$). Plasma triglycerides were significantly lower after diet 2 than after diet 1 ($P<.05$), whereas HDL-cholesterol was higher ($P<.05$) (Table 3). Plasma total cholesterol, LDL, HDL, triglycerides, total-C/HDL-C, LDL-C/HDL-C and apo A-I/apo B ratios did not differ at the end of each baseline diet period.

After diet 1, there was a decrease in the palmitic and stearic acid content of the plasma phospholipids in comparison with baseline results ($P<.05$ and $P<.0005$, respectively), whereas the percentage of oleic acid, linoleic acid, α-linolenic acid and eicosapentaenoic acid (EPA) moieties increased ($P<.005$, $P<.005$, $P<.0001$ and $P<.01$, respectively). On the other hand, myristic acid, arachidonic acid (ARA) and docosahexaenoic acid (DHA) levels were unchanged. The ARA/EPA ratio decreased ($P<.005$) and the PUFA/SFA ratio increased ($P<.0001$) (Table 4).

Diet 2 was associated with an increase in myristic acid, oleic acid, linoleic acid, EPA and DHA ($P<.01$, $P<.0001$, $P<.0001$, $P<.05$ and $P<.05$, respectively), whereas pal-

Table 5
Plasma fatty acids of the cholesteryl esters after the two interventional diets

Fatty acids	Baseline diet	Diet 1	Baseline diet	Diet 2
Palmitic acid (16:0)	26.58±7.40	22.72±6.73[a]	25.08±7.90	25.91±8.21
Stearic acid (18:0)	13.43±7.61	10.14±4.81	13.58±6.01	13.09±6.60
Oleic acid (18:1)	23.78±7.33	28.47±7.79	26.14±9.33	28.28±7.06
Linoleic acid (18:2)	29.63±9.93	30.54±9.76	23.82±8.39	26.78±9.93
Arachidonic acid (20:4)	1.49±1.00	1.93±0.97[a]	1.83±1.01	2.03±1.04
Linolenic acid (18:3)	1.45±0.69	2.15±1.02[a]	1.50±0.83	1.96±1.03[a]
DHA (22:6)	2.67±1.26	2.34±1.53	3.11±1.40	3.76±1.73[A]
PUFA/SFA	0.81±0.26	1.21±0.33	0.78±0.28	0.91±0.30

Values are expressed in % total fatty acids±S.D.
Means with different lowercase superscript letters are significantly different from baseline diet; means with different uppercase superscript letters are significantly different from diet 1. [a,A]$P<.05$.

Table 6
Correlation between linoleic and linolenic intakes with linoleic and linolenic acids of cholesteryl esters

Fatty acids	Diet 1	Diet 2
Linoleic acid	$r=-.50$, $P<.04$	$r=-.13$, $P=.61$
Linolenic acid	$r=.36$, $P=.16$	$r=.64$, $P<.005$

mitic, stearic, arachidonic and α-linolenic acids were unchanged (Table 4). The ARA/EPA ratio decreased ($P<.0005$) and the PUFA/SFA ratio was increased ($P<.005$).

In comparison with diet 1, diet 2 was associated with an increase in myristic acid, oleic acid, linoleic acid, EPA and DHA ($P<.0001$, $P<.0001$, $P<.005$, $P<.0005$ and $P<.01$, respectively) and with a decrease in α-linolenic acid ($P<.05$), and in the ARA/EPA ratio ($P<.05$) (Table 4). There was no significant difference in any of the plasma fatty acid levels nor the ARA/EPA and PUFA/SFA ratios determined after the two baseline periods.

After diet 1, there was a decrease in the palmitic acid ($P<.05$) and an increase in the arachidonic and α-linolenic acids contained in the plasma cholesteryl esters ($P<.05$) (Table 5) in comparison with the baseline diet. Diet 2 was associated only with an increase in α-linolenic acid ($P<.05$) compared to the baseline diet results. In comparison with diet 1, diet 2 was associated with an increase in DHA of cholesteryl esters ($P<.05$) (Table 5).

Stepwise univariate regression showed that myristic acid intake in diet 2 was positively correlated with myristic acid of phospholipids (not shown). Linoleic acid intake in diet 1, but not in diet 2, was correlated with linoleic acid of cholesteryl esters, whereas α-linolenic acid intake in diet 2, but not in diet 1, was correlated with α-linolenic acid of cholesteryl esters (Table 6).

4. Discussion

New interest has focused recently on the cholesterolemic effect of individual fatty acids in humans rather than on classes of fatty acids [17,24–26]. There is no consensus on this subject. For Yu et al. [25] stearic acid is neutral, and for Hu et al. [17] the relative risk (RR) of CHD varies according to quintiles of intakes of SFA intakes. This RR increases from 1 to 1.15 for intakes of C12:0 plus C14:0 from 0.98% to 2.14% of total energy with a lower CHD risk at 0.96 for an intake of 1.45%. For stearic acid the RR increases from 1 to 1.24 for intakes of 2.61% to 4.91% of total energy [17].

More recently, Müller et al. [24] in a study including three other groups investigated a group of 28 men on a diet providing 34.8% of total energy from fat, with 2.3% from myristic acid provided by butter (C12:0/C14:0 ratio=0.3 and C14:0 >70% in sn-2 position). Regression analysis in this group confirmed that myristic acid was the most hypercholesterolemic fatty acid and indicated that *trans*-fatty acids are less hypercholesterolemic than the SFA myristic and palmitic acids. However, precise analysis of the diet shows that stearic acid provided 3% total energy and that myristic and stearic acids therefore gave an RR of CHD greater than 1 in Hu et al.'s [17] study. Similarly, palmitic acid, which provides 7.9% of total energy, is situated in the zone where it could induce hypercholesterolemia despite the contribution of 6% total energy by linoleic acid. This is especially so since the diet provides more than 300 mg/day of cholesterol [27,28]. This diet studied by Müller et al. [24] provides 420 mg/day of cholesterol, 5.4% total energy from linoleic, 0.8% from α-linolenic and 8% from oleic acid. This contribution of oleic acid is far below the usual recommendations and leads to an oleic/linoleic ratio of 1.5, which is also lower than recommended levels.

We therefore decided to reexamine this issue in conditions closer to medical reality with myristic acid intake below 2% of total energy. Stearic, palmitic acid and cholesterol intakes were unable to introduce a bias in the interpretation of the results, and oleic, linoleic and α-linolenic acid intakes ensured the best possible maintenance. Therefore, the level of palmitic acid varied from 3.5% to 5% of total energy, that is, a non-hypercholesterolemic contribution to a diet providing less than 300 mg/day of cholesterol and 6% total energy of linoleic acid. The level of stearic acid varied from 1.7% to 2.3%, that is, below the lowest quintile of Hu et al.'s [17] study where the RR of CHD is 1. Since intakes of oleic, linoleic and α-linolenic acids were 12%, 6% and 1% of total energy, respectively, and cholesterol intake was below 250 mg/day, our study was situated within the safety limits recognized at present.

Therefore, the main difference between the two interventional studies was the contribution of myristic acid in the sn-2 position: 0.6% of total energy in diet 1 and 1.2% in diet 2. As shown, intake of myristic acid in diet 2 vs. diet 1 was increased to 100%, whereas intakes of palmitic and stearic acids were only increased to 45% and 35%, respectively (Table 1). These differences could be explained by fat content in full cream vs. half-skimmed milk where increase of myristic acid was the highest in comparison with palmitic and stearic acids. The estimated increase in total cholesterol (i.e., 2.38 mg/dl) induced by difference of palmitic acid in experimental diets, according to recent meta-analysis [29], is probably counteracted by this physiological nutritional status where palmitic and stearic acid intakes are in the lowest quintiles of Hu's study and linoleic acid intake represents around 6% of total energy. Given the results of Hu et al. [17], both diets are situated in a zone of the RR of CHD which is 1 or slightly below (U-shaped curve with RR=0.96 for 1.45% total energy in the form C12:0+C14:0, which corresponds to diet 2 in our study). To our knowledge, myristic acid in sn-2 position has not yet been studied in conditions as close to clinical reality within the framework of such a controlled balanced diet. It is well known that myristic acid from milk is mainly in the sn-2 position that is the physiological form for absorption.

Diet 1 was quite similar to the American dietary guidelines for higher-risk individuals (previous Step 2 diet)

[30], whereas diet 2 was in upper limit of guidelines for the general population (previous Step 1) that advocate limitation of fat intake ≤30% of total energy, SFA <10% of energy and cholesterol to <300 mg/day [9]. Compared with baseline diet (fat and SFA intakes of 34.5% and 13%, respectively), diets 1 and 2 were associated with a decrease in total cholesterol, LDL-cholesterol and triglycerides, and with an increase in apo A-I/apo B ratio, whereas HDL-cholesterol was unchanged. These beneficial effects on plasma lipids and lipoproteins with such intakes of total fat and SFA were similar to those reported in various types of studies [1,31,32].

Many questions are raised by comparing diets 1 and 2. If one accepts the conclusions of Gaggiula and Mustad [33], Clarke et al. [34], Howell et al. [35] and Verschuren et al. [36], the better results should have been obtained with diet 1 and not diet 2. The MUFA and PUFA intakes are identical in both diets, while the PUFA/SFA ratio is 1 in diet 1 and 0.75 in diet 2. The predictive equations of Keys et al. [37,38], Mensink and Katan [26], Yu et al. [25], Clarke et al. [34], Howell et al. [35] and Müller et al. [24] point to the superiority of diet 1 over diet 2. The main difference between the diets is that there is twice as much myristic acid in diet 2. There can be no bias from differences in stearic acid (diet 2=+33% vs. diet 1), palmitic acid (diet 2=+40% vs. diet 1), lauric acid (mainly in sn-1 or sn-3 position so oxidized very early) or cholesterol (diet 2=+19% vs. diet 1), which would have led to an unfavorable effect on diet 2. Moreover, the decrease in carbohydrates in diet 2 (51.2%) vs. diet 1 (55.2%) should have worsened the lipid results. Yet at these levels which are pertinent in clinical terms and are widely accepted, the present findings are in contradiction with the various theories based on explanatory equations and on certain studies performed at levels not encountered in daily life.

Compared to diet 1, diet 2 was associated an increase in HDL-cholesterol. This effect of myristic acid is already known but at levels much greater than 1.2% of total energy. Total cholesterol, LDL-cholesterol, total-C/HDL-C, LDL-C/HDL-C and apo A-I/apo B ratios were not significantly different, but triglycerides were significantly lower in diet 2 vs. diet 1 ($P<.05$). The results obtained were almost the opposite of what was expected. Indeed, based on Mensink et al. [29], diet 2 with a 2.8% energy higher level of total saturates than diet 1 should have produced an estimated increase in total cholesterol and LDL-cholesterol of 3.89 and 3.46 mg/dl, respectively. In addition, in a large study of 103 subjects, Ginsberg et al. [39] have shown a significant increase in total cholesterol, LDL-C and HDL-C on a Step I diet (30% energy total fat, 9.5% energy SFA) vs. a Step II diet (26% energy total fat, 6% energy SFA) in which the difference in SFA was 3.5% energy. Compared to baseline diet (where myristic acid intake was 1.4% of total energy), diet 2 was associated with a decrease in total cholesterol, LDL-cholesterol and triglycerides (the contribution in carbohydrates was equal), whereas HDL-cholesterol was not significantly different.

Within the framework of combined physiological intakes, there would seem to be a synergistic effect between myristic acid and α-linolenic acid in sn-2 position. This is confirmed by the levels of fatty acids in plasma phospholipids (Table 4). Compared to diet 1, diet 2 was associated with an increase in myristic, oleic, linoleic acids, EPA and DHA, and with a decrease in α-linolenic acid. Thus, the ARA/EPA ratio decreased without any significant change in arachidonic acid. With regard to the cholesteryl esters, there are two major findings. First, there was a correlation between the dietary intake of α-linolenic acid in sn-2 position and the level of α-linolenic acid in cholesteryl esters with diet 2 ($r=.64$, $P<.005$) but not with diet 1 ($r=.36$, $P=.16$). Second, the inverse correlation between the dietary intake of linoleic acid and the level of linoleic acid in cholesteryl esters which was observed in diet 1 ($r=-.50$, $P<.04$) was no longer present in diet 2 ($r=-.13$, $P<.61$). One of us has already obtained similar results in a study of 37 premature infants [40]. The correlation coefficient was $r=.704$ ($P=.0001$) between the dietary intake of α-linolenic acid in sn-2 position in the present diet and the level of α-linolenic acid in cholesteryl esters analyzed at the 37th week of postconception age. At the same time, the correlation between linoleic acid in the diet and linoleic acid in cholesteryl esters decreased to $r=.169$ ($P=.31$). In the premature infants, the level of α-linolenic acid in sn-2 position of the diet was 0.8% of total energy, the level of myristic acid was 1.8% (3.4% in human milk), the level of linoleic acid was 5% and the level of oleic acid was 12%. Apart from the higher level of myristic acid in the study by Babin compared to the present study, the intakes in unsaturated fatty acids were identical.

One of these explanations could be a synergistic effect between myristic acid in sn-2 position and α-linolenic acid in sn-2 position at the level of the LCAT. There were no differences other than the intake of myristic acid in sn-2 position between diets 1 and 2. A favorable level of α-linolenic acid in plasma cholesteryl esters (>0.60%) has been found to be a reliable biomarker to assess the risk of CHD [3] and stroke [41]. Moreover, Bemelmans et al. [42] found that there was a strong inverse association between the cholesteryl ester LA/ALA ratio and cholesteryl ester α-linolenic acid ($r=-.95$, $P<.01$).

The second major finding is the significant increase in the DHA level in diet 2 vs. diet 1, reaching 3.76% of total fatty acids ($P<.05$). This clearly raises the question of a potentially favorable effect of myristic acid in sn-2 position on the elongation and/or desaturation of α-linolenic acid, which is already protected from oxidation by its sn-2 position on the dietary triglyceride. High DHA levels in erythrocyte phosphatidylethanolamines have already been obtained in a study on premature infants with a very similar mixture of fatty acids (myristic acid in sn-2 position: 1.8% of total energy, linoleic acid: 5%, α-linolenic acid in sn-2 position: 0.8%, oleic acid: 12%) [43]. Recently, it has been shown [44] that myristic acid increases Δ6-desaturase acti-

vity in cultured rat hepatocytes. This improvement seems to be reproducible for three substrates of Δ6-desaturase, that is, oleic acid, linoleic acid and α-linolenic acid, and it is dose-dependent in the range 0.1–0.5 mmol/l myristic acid concentration. Two main mechanisms of this data could be argued: (1) myristic acid is one of the most potent SFA inducer of peroxisomal fatty acid oxidation in hepatocytes and it may control Δ6-desaturase via PPARα, since Δ6-desaturase emerges as a PPARα target gene; (2) myristic acid could increase the overall level of cellular protein myristoylation, which may govern Δ6-desaturase expression and/or activity. Considering the enzyme activity, direct acylation of Δ6-desaturase by myristic acid could also occur [44].

The numerous nutritional studies performed in the last 30 years with nonphysiological levels of myristic acid (greater than 3.4% of total energy), in a non-sn-2 position on the triacylglycerol or in complex nutritional conditions (e.g., insufficient supply of linoleic acid and/or α-linolenic acid and/or oleic acid; excessive supply of palmitic and stearic acids) have no doubt led to overload effects and even to negative synergies. This has globally contributed to giving myristic acid and unfavorable nutritional image.

On the other hand, in fundamental biochemical terms, apart from its role as energy provider, myristic acid has been assigned a very important physiological role in fatty acylation of signaling protein [45]. Myristoyl CoA binds to an *N*-myristoyl transferase (NMT), the peptidic substrate binds to NMT and the myristate is transferred to the N-terminal glycine of the peptide target. Finally, the myristoyl peptide is released. In the cell, N-myristoylation is a cotranslational process that occurs while the nascent polypeptide chain is still attached to ribosome. In this way, many proteins are myristoylated, such as protein kinases and phosphatases, guanidine nucleotide-binding proteins, membrane and cytoskeletal-bound structural proteins (MARCKS), NADH cytochrome B5, NO synthase, and Δ6-desaturase.

Moreover, the endogenous synthesis of myristic acid is not as clear as that of palmitic acid. It requires the presence of a "chain-cutting" enzymatic factor, thioesterase II, which hydrolyses the thioester group binding the growing chain to the 4′-phosphopantein of the fatty acid synthase. Unlike the thioesterase I of the liver, it is not associated covalently with the fatty acid synthase and mainly occurs in the mammary gland [46]. Therefore, myristic acid may be obtained only by a break in the chain of fatty acid synthesis, which leads to palmitic acid, or by moderate peroxisomal β-oxidation. These two processes are unusual because peroxisomal β-oxidation usually occurs with very long-chain fatty acids. Myristic acid synthesis therefore seems to be regulated and difficult to evidence, except in the case of milk secretion. In addition, except when it is present in sn-2 position on triglyceride, which is easily released by the pancreatic lipase [47], myristic acid is the most quickly oxidized fatty acid with free α-linolenic acid [48,49].

It would therefore seem that to maintain various physiological reactions and regulations, the dietary supply of a certain amount of myristic acid in sn-2 position is necessary. In the adults of this study, 0.6% of total energy provided by myristic acid in sn-2 position was clearly insufficient, while 1.2% of total energy gave excellent results on lipid status. Incremental doses of myristic acid from 1.2% to 3.4% (intake of mother's milk) now need to be studied in the same conditions in order to establish in man a dose–effect curve. This would probably be a U-shaped curve as in the hamster [50].

Acknowledgments

This study was funded by grants from Centre de Recherche et d'Information Nutritionnelles and from Conseil Régional d'Aquitaine, France.

We are indebted to the participants from Belloc Abbey for their continuing outstanding level of cooperation.

References

[1] Keys A. Seven countries: a multivariate analysis of death and coronary heart disease. Cambridge (Mass): Harvard University Press; 1980.
[2] Hjermann I, et al. Effect of diet and smoking intervention on the incidence of coronary heart disease. Report from the Oslo study group of a randomized trial in healthy men. Lancet 1981;2:1303–10.
[3] Sandker GW. Serum cholesteryl ester fatty acids and their relation with serum lipids in elderly men in Crete and the Netherlands. Eur J Clin Nutr 1992;47:201–8.
[4] Voskuil DW, et al. Intake and sources of a-linolenic acid in Dutch elderly men. Eur J Clin Nutr 1996;50:784–7.
[5] Ascherio A, et al. Dietary fat and risk of coronary heart disease in men: cohort follow up study in the United States. BMJ 1996;313:84–90.
[6] de Lorgeril M, et al. Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. Lancet 1994;343: 454–9.
[7] The Expert Panel. Summary of the second report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel II). JAMA 1993;269:3015–23.
[8] Krauss RM, et al. Dietary guidelines for healthy American adults. Circulation 1996;94:1795–800.
[9] Krauss RM, et al. AHA dietary guidelines. Circulation 2000;102: 2284–306.
[10] Aro A, et al. Stearic acid, *trans* fatty acids, and dairy fat: effects on serum and lipoprotein lipids, apoproteins, lipoprotein(a), and lipid transfer proteins in healthy subjects. Am J Clin Nutr 1997;65:1419–26.
[11] Kris-Etherton PM, Yu S. Individual fatty acid effects on plasma lipids and lipoproteins: human studies. Am J Clin Nutr 1997;65:823–30.
[12] Temme EHM, Mensink RP, Hornstra G. Comparison of the effects of diets enriched in lauric, palmitic, or oleic acids on serum lipids and lipoproteins in healthy women and men. Am J Clin Nutr 1996;63: 897–903.
[13] Tholstrup T, et al. Effect on blood lipids, coagulation, and fibrinolysis of a fat high in myristic acid and a fat high in palmitic acid. Am J Clin Nutr 1994;60:919–25.
[14] Emken EA. Metabolism of dietary stearic acid relative to other fatty acids in human subjects. Am J Clin Nutr 1994;60:1023S–8S.
[15] Denke MA, Grundy SM. Comparison of effects of lauric acid and palmitic acid on plasma lipids and lipoproteins. Am J Clin Nutr 1992; 56:895–8.

[16] Bonanome A, Grundy SM. Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. N Engl J Med 1988;318:1244–8.
[17] Hu FB, et al. Dietary saturated fats and their food sources in relation to the risk of coronary heart disease in women. Am J Clin Nutr 1999;70:1001–8.
[18] Spady DK, Woollett LA, Dietschy JM. Regulation of plasma LDL-cholesterol levels by dietary cholesterol and fatty acids. Annu Rev Nutr 1993;13:355–81.
[19] Tholstrup T, et al. Fat high in stearic acid favorably affects blood lipids and factor VII coagulant activity in comparison with high in palmitic acid or high in myristic and lauric acids. Am J Clin Nutr 1994;59:371–7.
[20] Zock P, de Vries J, Katan M. Impact of myristic acid versus palmitic acid on plasma lipids and lipoprotein levels in healthy women and men. Arterioscler Thromb 1994;14:567–75.
[21] Temme EHM, Mensink RP, Hornstra G. Effects of medium chain fatty acids (MCFA), myristic acid, and oleic acid on serum lipoproteins in healthy subjects. J Lipid Res 1997;38:1746–54.
[22] Gillman MW, et al. Inverse association of dietary fat with development of ischemic stroke in men. JAMA 1997;278:2145–50.
[23] Heude B, et al. Cognitive decline and fatty acid composition of erythrocyte membrane in the EVA Study. 1st Congress of International Academy on Nutrition and Aging, 2001, Paris, 29–30 June, [abstract].
[24] Müller H, Kirkhus B, Pedersen JI. Serum cholesterol predictive equations with special emphasis on *trans* and saturated fatty acids. An analysis from designed controlled studies. Lipids 2001;36:783–91.
[25] 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.
[26] 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.
[27] Clandinin MT, Cook SL, Konard SD, French MA. The effect of palmitic acid on lipoprotein cholesterol levels. Int J Food Sci Nutr 2000;51:S61–71.
[28] Ramamoorthy L, Gupta SV, Khosla P. Effects of exchanging 4% energy between dietary stearic and palmitic acid on hamster plasma lipoprotein metabolism. Int J Food Sci Nutr 2000;51:S51–9.
[29] Mensink RP, Zock PL, Kester AD, Katan MB. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. Am J Clin Nutr 2003;77:1146–55.
[30] Schaefer EJ, et al. Individual variability in lipoprotein cholesterol response to national cholesterol education program step 2 diets. Am J Clin Nutr 1997;65:823–30.
[31] Grundy SM. Comparison of monounsaturated fatty acids and carbohydrates for lowering plasma cholesterol. N Engl J Med 1986;314:745–8.
[32] Hu FB, et al. Dietary fat intake and the risk of coronary heart disease in women. N Engl J Med 1997;337:1491–9.
[33] Caggiula AW, Mustad VA. Effects of dietary fat and fatty acids on coronary artery disease risk and total and lipoprotein cholesterol concentrations: epidemiologic studies. Am J Clin Nutr 1997;65:1597S–610S.
[34] Clarke R, et al. Dietary lipids and blood cholesterol: quantitative meta-analysis of metabolic ward studies. BMJ 1997;314:112–7.
[35] Howell WH, et al. Plasma lipid and lipoprotein responses to dietary fat and cholesterol: a meta-analysis. Am J Clin Nutr 1997;65:1747–64.
[36] Verschuren WMM, et al. Serum total cholesterol and long-term coronary heart disease mortality in different cultures: twenty-five year follow-up of the seven countries study. JAMA 1995;274:131–6.
[37] Keys A, Anderson JT, Grande F. Serum cholesterol response to changes in the diet. II. The effects of cholesterol in the diet. Metabolism 1965;14:759–65.
[38] Keys A, Parlin RW. Serum cholesterol response to changes in dietary lipids. Am J Clin Nutr 1966;19:175–81.
[39] Ginsberg HN, et al. Effects of reducing dietary saturated fatty acids on plasma lipids and lipoproteins in healthy subjects, the Delta Study Protocol I. Arterioscler Thromb Vasc Biol 1998;18:441–9.
[40] Babin F, Rodriguez A, Sarda P, Vandeputte B, Mendy F, Descomps B. Alpha-linolenic acid in cholesteryl esters: a marker of alpha-linolenic acid intake in newborns. Eur J Clin Nutr 2000;54:840–3.
[41] Simon JA, Fong J, Bernert JT, Browner WS. Serum fatty acids and the risk of stroke. Stroke 1995;26:778–82.
[42] Bemelmans WJE, Meskiet FAJ, Feskens EJM, de Vries JHM, Broer J, May JF, et al. Associations of alpha-linolenic acid and linoleic acid with risk factors for coronary heart disease. Eur J Clin Nutr 2000;54:865–71.
[43] Billeaud C, Bouglé D, Sarda P, Combe N, Mazette S, Babin F, et al. Effects of preterm infant formula supplementation with α-linolenic acid with linoleate/α-linolenate ratio of 6: a multicentric study. Eur J Clin Nutr 1997;51:520–6.
[44] Jan S, Guillou H, D'Andrea S, Daval S, Bouriel M, Rioux V, et al. Myristic acid increases Δ6-desaturase activity in cultured rat hepatocytes. Reprod Nutr Dev 2004;44:131–40.
[45] Resh MD. Fatty acylation of proteins: new insights in membrane targeting of myristoylated and palmitoylated proteins. Biochim Biophys Acta 1999;1451:1–16.
[46] Smith S. Medium chain fatty acyl-*s*-4′-phosphopanthein-fatty acid synthase thioester hydrolase from lactating mammary gland of rat. Methods Enzymol 1981;71:188–200.
[47] Bracco U. Effects of triglyceride structure on fat absorption. Am J Clin Nutr 1994;60:1002S–9S.
[48] Wang S, Koo SI. Plasma clearance and hepatic utilization of stearic, myristic and hepatic utilization of stearic, myristic, and linoleic introduced via chylomicrons in rats. Lipids 1993;28:697–708.
[49] Hughes TA, et al. Comparative lipoprotein metabolism of myristate, palmitate, and stearate in normolipidemic men. Metabolism 1996;9:1108–18.
[50] Loison C, Mendy F, Sérougne C, Lutton C. Dietary myristic acid modifies the HDL-cholesterol concentration and liver scavenger receptor BI expression in the hamster. Br J Nutr 2002;87:1–13.