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ORIGINAL ARTICLE

The Consumption of Food Products from Linseed-Fed Animals Maintains Erythrocyte Omega-3 Fatty Acids in Obese Humans

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Abstract Based on mechanistic and epidemiological data, we raise the question of the relationship between qualitative dietary polyunsaturated fatty acids (PUFA) changes and increase in obesity. In this double-blind trial, we studied the effects on 160 overweight volunteers (body mass index, BMI >30) of a 90 days experimental diet rich principally in animal fat with a low PUFA/saturated fatty acid (SFA) ratio but a low n-6/n-3 ratio, using animal products obtained from linseed-fed animals. The control diet provided less animal fat, a higher PUFA/SFA ratio and a higher n-6/n-3 ratio. Both diets excluded seafood. In the experimental group, we observed a significant increase in red blood cell (RBC) α-linolenic acid content and a slight increase in EPA and DHA derivatives, while in the control group we observed a significant reduction in EPA and DHA content. Between groups now, the difference in the three n-3 fatty acids changes in RBC was significant. This demonstrates that plasma EPA and DHA levels can be maintained without fish if products from linseed-fed animals are used. During the diets, we noted a significant reduction in weight, BMI and hip circumference within both groups of volunteers. However, no significant difference was observed between the control group and the experimental group. Interestingly, 150 days after the end of the trial (i.e., day 240), we noted a significant weight gain in the control group, whereas no significant weight gain was observed in the experimental group. This was also observed for the BMI and hip circumference. Moreover, significant differences in BMI ($P < 0.05$) and weight ($P = 0.05$) appeared between the two groups, showing in both cases a smaller increase in the experimental group. During the 90 days trial, we did not observe any differences between groups in terms of total cholesterol, HDL cholesterol, LDL cholesterol or triglycerides, suggesting that the saturate content and the P/S ratio are not as important as the n-6 and n-3 fatty acid composition.

Keywords n-3 Fatty acids · Linseed · Red blood cell · Fatty acid composition · Obese

Abbreviations

BMI	Body mass index
PUFA	Polyunsaturated fatty acids
FA	Fatty acid
SFA	Saturated fatty acid
LNA	Linoleic acid
ALA	α-Linolenic acid

Introduction

In only a few decades, obesity has become a major health concern, which also increases the risk of type 2 diabetes

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and cardiovascular disease. Obesity is a multifactorial disease. Excessive adipose tissue development is primarily due to an imbalance between caloric intake and caloric expenditure, this imbalance being related to changes in dietary habits and to more sedentary lifestyles. However, in addition to the energy imbalance, different aspects of lipid metabolism such as lipid synthesis, transport, storage and catabolism are also implied in obesity development. Amongst nutrients involved in this metabolism, all fatty acids play obviously a major role from a quantitative point of view, but polyunsaturated fatty acids (PUFA) may also play a qualitative role, which is less well established. Indeed, recent data show that PUFA from the n-6 or n-3 series do not play identical roles. While n-6 PUFA are now well known as activators of adipose tissue development [1] and their dietary excess is suspected to favor childhood adiposity [2] and adult adiposity [3–5], n-3 PUFA seem to play a more favorable role, acting against different mechanisms leading to obesity. Thus in humans, n-3 PUFA seem somewhat to limit metabolic syndrome and type 2 diabetes [6–8]. A negative correlation has been described between abdominal obesity and n-3 PUFA content of adipose tissue [9]. Moreover, n-3 fatty acid intake limits fat accumulation in mice [10] and rats [11].

The fatty acid (FA) composition of the diet has changed significantly since the 1960s, when obesity began to increase. The first change was the increase in the PUFA/(saturated fatty acid) SFA ratio, due to the increase in consumption of vegetable oils rich in PUFA rather than animal fat which are richer in SFA. The second change was the subsequent increase in n-6 PUFA content, while n-3 PUFA content decreased or remained constant. This is due to the very high levels of n-6 PUFA in the most common vegetable oils (soybean, corn, sunflower, etc.) used in human nutrition as well as in animal feeds. Consumption data show that the linoleic acid (LNA) to α-linolenic acid (ALA) ratio increased by approximately three times to reach a range of 12–25 in most of the western diets during this period [12, 13]. These dietary ratio values are consistent with other values in humans, measured in breast milk [14, 15] and in subcutaneous adipose tissue [16, 17].

Thus, based on both mechanistic and epidemiological data, we raise the question of the relationship between these qualitative changes in dietary PUFA and increase in obesity, and more precisely when the increase in the dietary PUFA/SFA ratio is always associated with a large increase in the dietary n-6/n-3 FA ratio.

In this double-blind trial, we studied the effects on volunteers of a 90 days experimental diet rich in animal fat with both low PUFA/SFA and low n-6/n-3 ratios, using principally animal products obtained from linseed-fed animals. The control diet provided less animal fat, a higher value for the PUFA/SFA ratio and a higher value for the n-6/n-3 ratio. The volunteers were overweight or obese (body mass index, BMI $\approx$ 31). The main results were: (1) Plasma EPA and DHA levels can be maintained without fish, if using products from linseed-fed terrestrial animals. (2) During the diets, we noted a significant reduction in weight, BMI, and hip circumference within both groups of volunteers, but no significant difference was observed between the control group and the experimental group. However, the experimental diet limited weight recovery after the end of the trial. (3) No comparative deleterious effect (on plasma lipid parameters) was observed between diets, suggesting that the n-6/n-3 ratio is as important as the SFA level.

Materials and Methods

Volunteers

One hundred and sixty volunteers with metabolic syndrome (78 men and 82 post-menopausal women) between 18 and 65 years old were selected from those responding to local press recruitment (293 volunteers were evaluated). Inclusion criteria were waist circumference $\geq$80 cm for women and $\geq$94 cm for men, plus at least two other risk factors: triglycerides (TG) $\geq$1.5 g/l; fasting plasma glucose (FPG) $\geq$1.0 g/l; HDL cholesterol $\leq$0.4 g/l for men and $\leq$0.5 g/l for women; systolic blood pressure (BP) $\geq$130 mmHg; diastolic BP $\geq$85 mmHg or treatment for hypertension. The average body weight, BMI and hip circumference were respectively, 88.5 $\pm$ 15.6 kg, 31.5 $\pm$ 4.3 kg/m^2 and 109 $\pm$ 7.6 cm. Exclusion criteria included diabetes, any treatment for hypercholesterolemia and any disease judged incompatible with the study by the investigator. During a visit, the purpose and method of the study was explained to the selected volunteers; written informed consent was obtained from those willing to participate and a prescription was given. Inclusion and exclusion criteria were checked during a medical visit. The proposed diets were then explained to the volunteers and some menu examples were given to help them to adhere to the nutritional advice given during an individual consultation with a dietitian. The diets were given to volunteers from day 0 to day 90. At day (D) 90, 137 volunteers remained in the trial: 72 in the experimental group and 65 in the control group. The others decided to stop the trial between D0 and D90. All statistical analyses at D90 were carried out with these 137 volunteers. At day 240, 98 volunteers (72%) came back for the last anthropometric measurements: 50 in the experimental group (69%) and 48 in the control group (74%).

Study Design

Subjects were randomly assigned to one of two different diets. As regards animal products, the experimental diet included products with a high level of n-3 originating from linseed-fed animals while the control group diet included standard animal products. The feeds of animals that supplied the experimental products (dairy products, meats and eggs) contained an average of 5% of extruded linseed and are described in details in other publications [18]. As regards plant products, the experimental group volunteers received 5 g/day of extruded linseed flour, while the control group volunteers received the same amount of extruded wheat/soybean (90/10) flour. Moreover, the experimental group's bread included 4% extruded linseed flour. In the experimental diet, flour plus bread represent 40% of the total ALA intake while animal products provide long-chain derivatives (EPA plus DHA), and account for 60% of ALA. The control group volunteers received margarine with a high n-6 content (rich in sunflower oil) instead of the butter that the experimental group received.

The macronutrient content of the two diets was similar. The overall composition and energy intake of the two diets did not differ. The proposed energy intake was 1,667 kcal for women and 1,931 kcal for men. Subjects were asked not to consume any fish or seafood products or products containing more than 5% fat. Products containing linseed or products with a high n-3 content were also forbidden. The only authorized oil was olive oil (10 g a day). Male and female diets only differed by the amount of carbohydrates and meat (+50 g and +40 g for men, respectively).

The fat spread was butter for the experimental group and margarine for the control group, which mainly explains the difference in the proportion of lipids of animal origin between the two diets. The recommended weekly intake of eggs was ten. Sugar (sucrose) was requested to be reduced or excluded from the diet in order to reduce total calorie intake and reduce weight.

Subjects undertook an adjustment period of 2 weeks during which they had to follow the prescribed diet, consuming their own usual products (usual meats, eggs, dairy products, and fat spread). Afterward, at day 0, experimental or control products were provided for the whole family. They were delivered every week to a local shop where subjects collected them, presenting their volunteer card. Compliance was verified by checking the collection of the products with the help of local shop managers. Each member of the family was given 1,050 ml of milk, 10 eggs, 250 g of fat spread, 210 g of hard cheese, 7 slices of ham, 50 g of diced bacon fat, 2 sausages, 2 loaves of bread, 2 pork chops plus occasionally beef, chicken or veal. Small quantity adaptations were necessary according to the number of persons in the family and the packaging size. Flour (linseed or wheat/soybean) were provided for the volunteers only and not for the whole family.

During the experimental period, from D0 to D90, the volunteers met a dietitian to receive nutritional advice at least once a month. Compliance with the diet was evaluated by weekly recording of consumption. From D90 to D240, the volunteers returned to a free diet with the reintroduction of fish and previously forbidden products and had no visits with any member of the dietitian's team.

Anthropometry and Assays

The following anthropometric and plasma lipids parameters were measured at the hospital at D0 and D90: body weight and BMI were measured by TANITA TBF 300 MA, and hip circumferences were measured with an automatic rubber band. Blood lipids (total cholesterol, HDL cholesterol and triglycerides) were analyzed with an enzymatic technique using Advia1650-Siemens, and LDL cholesterol was calculated by the Friedwald formula [19]. After the end of the trial (D90), volunteers were requested to come back 5 months later (D240) for additional anthropometric measurements only.

Lipid Extraction and Fatty Acid (FA) Analysis

RBC lipids were extracted from 0.5 ml of samples with a mixture of hexane/isopropanol (3:2 v/v), after acidification with 1 ml HCl 3 M [20]. Total lipid extracts were saponified for 30 min at 70 °C with 1 ml of 0.5 M NaOH in methanol and methylated with 1 ml BF_3 (14% w/v in methanol) for 15 min at 70 °C. Fatty acid methyl esters were extracted twice with pentane and analyzed by GC using an Agilent Technologies 6890N (Bios Analytic, Toulouse, France) with a split injector (20:1) at 250 °C and a bonded silica capillary column (BPX 70, 30 m × 0.25 mm; SGE, Villeneuve-St Georges, France) with a polar stationary phase of 70% cyanopropyl polysilphenylene-siloxane (0.25 µm film thickness). Helium was used as a carrier gas (average velocity 24 cm/s). The column temperature program started at 150 °C, increased by 2 °C/min to 220 °C and held at 220 °C for 10 min. The flame ionization detector temperature was 250 °C. Identification of FA methyl ester was based on retention times obtained for FA methyl esters prepared from FA standards.

Statistical Analysis

Analyses of covariance, with terms for the treatment group and the baseline value were used to compare least square means and to test for differences in baseline to endpoint changes between treatments. Paired *t*-tests are also performed within each treatment group. Different analyses

were performed using stratifications by sex and the value of each metabolic syndrome risk factor. All analyses were conducted using SAS® version 8.2. The study was designed as an intend-to-treat analysis. The difference was considered significant when $P < 0.05$ and a tendency when $0.05 < P < 0.10$.

Results

Effects of Changes in Animal Feeding on Animal Products Composition and on FA Composition of Volunteers Diets

The main lipid sources used in the volunteers' diets are shown in Table 1. In the two diets, lipids provided 33% of the total caloric intake. Animal products used in the experimental diet originated from animals fed 5% extruded linseed, which provides C18:3n-3 (ALA) to the animals. In the control diet, animal products came from standard French breeding farms. This variable fraction represents 77% of the volunteers' lipid intake. The diets given to the volunteers are described in Tables 2 and 3. The real consumption of each volunteer is not known very precisely as meals were taken at home. The two diets were iso-caloric and iso-lipidic. In addition to the animal lipids presented above, margarine was used in the control diet. This margarine is a commercial product, the composition of which is close to the composition of French lipid intakes (Table 1). The experimental diet was rich in animal lipids (76% of total lipids). It provided high levels of n-3 PUFA, (not only ALA but also long-chain derivatives EPA and DHA) and low levels of n-6 PUFA. 40% of ALA is provided by linseed flour plus bread, in the experimental diet. Compared with the experimental diet, the control diet was richer in plant lipids (animal fats provided only 49% of lipids) and was richer in total PUFA (17.3 vs. 9.1 g/day in the experimental diet). Moreover, LNA content was much higher in the control diet (16.0 vs. 5.5 g/day in the experimental diet). As a result, the following ratios were very different between the two diets: PUFA/SFA values were 0.7 in the control diet versus 0.3 in the experimental diet, and C18:2n-6/C18:3n-3 values were 22.9 in the control diet versus 2.3 in the experimental diet.

Change in Volunteers' RBC FA Composition

The two diets produced clear changes in the content and ratios of numerous FAs in the RBC (Table 4). For n-3 PUFA, we observed in the volunteers receiving the experimental diet (animal products from linseed-fed animals plus a small amount of linseed flour and bread) a significant increase in ALA content between day 0 and day 90 and a significant increase in the total n-3 fatty acids. In the control group of volunteers, no effect was observed as regards ALA content, but the EPA and DHA content and total n-3 fatty acids decreased significantly. Between groups, the differences in the ALA, EPA and DHA changes (D90-D0) were significant.

Table 1 Fatty acid (FA) composition (% of total FA) of main lipid sources given to volunteers

FA composition (% of total FA)	Standard Margarine[a]	Standard dairy products[b]	Dairy products from "linseed industry"[b]	Standard eggs	Eggs from "linseed industry"	Standard pork[c]	Pork from "linseed industry"[c]
C14:0	1.7	11.6 ± 0.1	10.4 ± 0.3	0.4 ± 0.0	0.3 ± 0.0	1.3 ± 0.1	1.3 ± 0.2
C16:0	9.7	33.3 ± 0.9	26.0 ± 1.5	25.3 ± 0.8	21.0 ± 0.8	24.9 ± 1.0	24.6 ± 1.2
C18:0	8.2	8.7 ± 0.3	11.0 ± 0.4	7.7 ± 0.2	7.8 ± 0.4	13.5 ± 1.4	14.3 ± 1.3
C18:1n-9	21.1	17.4 ± 0.5	20.4 ± 0.6	46.7 ± 2.7	45.9 ± 2.5	43.3 ± 3.1	39.7 ± 3.1
C18:2n-6	51.5	1.5 ± 0.1	1.8 ± 0.0	12.0 ± 2.6	12.7 ± 1.3	10.0 ± 1.9	10.7 ± 2.1
C20:4n-6		0.1 ± 0.0	0.1 ± 0.0	1.5 ± 0.2	0.8 ± 0.1	1.1 ± 0.9	1.5 ± 1.2
∑n-6 PUFA	51.5	1.6 ± 0.1	2.0 ± 0.1	13.6 ± 2.6	13.6 ± 1.4	11.5 ± 2.6	12.6 ± 3.0
C18:3n-3	0.2	0.3 ± 0.0	0.9 ± 0.3	0.5 ± 0.2	6.5 ± 0.8	0.6 ± 0.1	2.3 ± 0.9
C20:5n-3					0.2 ± 0.0	0.1 ± 0.1	0.3 ± 0.3
C22:5n-3		0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.2	0.6 ± 0.4
C22:6n-3				0.7 ± 0.1	1.3 ± 0.2	0.1 ± 0.1	0.2 ± 0.1
∑n-3 PUFA	0.2	0.4 ± 0.1	1.2 ± 0.4	1.5 ± 0.3	8.2 ± 0.7	1.2 ± 0.4	3.5 ± 0.9
C18:2n-6/C18:3n-3	257.5	5.2 ± 1.1	2.1 ± 0.6	24.4 ± 4.9	2.0 ± 0.1	16.5 ± 4.5	5.6 ± 2.9

[a] Margarine was chosen because of its high similarity with the mean FA acid profile of vegetable oils consumed in France

[b] Lipids from butter, milk and cheeses

[c] Lipids from cooked meats (bacon pieces, sausage and ham) and fresh meat

Table 2 Estimation of the consumption of products and fatty acids (g/volunteer/day) by volunteers

	Control group		Experimental group	
	Products	Fatty acids	Products	Fatty acids
Margarine	25	20.0	0	0.0
Butter*	0	0.0	25	20.0
Semi-skimmed milk*	155	2.6	155	2.6
Cheese*	32	9.1	32	9.1
Eggs*	82	9.1	82	8.2
Cooked meats[a]*		9.9		9.9
Pork*		1.2		1.2
Bread*	100	0.5	100	1.0
Linseed meal	0	0.0	10	1.4
Soya/wheatmeal[b]	10	0.3	0	0.0
Olive oil	10	9.4	10	9.4
Other animal lipids[c]		1.6		1.6
Other vegetable oils[d]		4.8		4.8
Fish[e]	0	0.0	0	0.0
Total		68.5		69.2
Animal lipids (% of total lipids)		49		76

[a] Bacon pieces, sausage and ham

[b] Wheatmeal (90%) and soybean meal (10%)

[c] Beef, lamb, chicken (no qualitative difference between both groups)

[d] Fatty acids from industrial products, starchy vegetables and fruits (no qualitative difference between both groups)

[e] Consumption of fish and seafood products was prohibited during the experiment

* Products in the standard group were standard products, while products in the experimental group came from (1) animals (beef, chicken and pork) fed a linseed-enriched diet (5% of cooked linseed) or (2) linseed-enriched bread (5% of cooked linseed)

The n-6 PUFA content increased between D0 and D90 in the RBC of the control group, the increase being significant for LNA and for total n-6 fatty acids, but not significant for arachidonic acid (C20:4n-6). In the experimental group, we did not observe any significant change in the n-6 fatty acid content of the RBC. Between groups, the difference in the LNA and total n-6 fatty acid changes (D90–D0) was significant.

We noted also that the LNA/ALA ratio increased significantly in the control group and decreased significantly in the experimental group. The difference in changes between groups was also significant. This was equally true for the Σn-6/Σn-3 ratio.

More generally, the total PUFA content increased significantly in the control group and did not change in the experimental group. Furthermore, an increase in stearic acid content was noted in the experimental group, whereas oleic acid content was reduced in both groups.

Changes in Anthropometric Parameters

From D0 to D90, i.e., over the period of the diets, we noted a significant reduction in weight, BMI, and hip circumference in both groups of volunteers (Table 5). However, no significant difference was observed between the control group and the experimental group. Interestingly, from D90 to D240, i.e., after the end of the trial, we noted a significant weight gain in the control group, whereas no significant weight gain was observed in the experimental group. This was equally true for the BMI and hip circumference, which both increased solely in the control group. Between the two groups, from D90 to D240 we showed a significant difference for the BMI ($P < 0.05$), and a difference at the limit of significance for weight ($P < 0.06$), showing in both cases a smaller increase in the experimental group.

Changes in Plasma Lipid Parameters

These parameters were studied at D0 and D90. In Table 6, we first observed within each of the two groups a general deterioration in all the parameters, with an increase in LDL cholesterol in both groups and an increase in TG in the experimental group. However, the main result was that no significant difference between the groups was noted in total cholesterol, HDL cholesterol, LDL cholesterol or triglycerides over the period from D0 to D90. These parameters were not measured at D240. This is an interesting and novel result when considering the dietary characteristics

Table 3 Estimation of the mean fatty acid consumption by volunteers (g/volunteer/day)

	Control group (standard products)	Experimental group (products from "linseed industry")
Total FA	68.5	69.2
C4 to C12	2.8	4.3
C14:0	1.6	3.4
C16:0	12.9	14.8
C18:0	5.5	6.4
∑Saturated FA (SFA)	24.3	30.9
C16:1n-7	0.6	0.7
C18:1n-9 cis	24.4	25.0
Others C18:1 (*cis*/*trans*)	0.2	1.9
∑Monounsaturated FA (MUFA)	26.9	29.1
C18:2n-6	16.0	5.5
C20:4n-6	0.21	0.17
∑n-6 PUFA	16.2	5.7
C18:3n-3	0.7	2.4
C20:5n-3	0.01	0.07
C22:5n-3	0.03	0.07
C22:6n-3	0.07	0.13
∑n-3 PUFA	0.8	2.7
∑Polyunsaturated FA (PUFA)	17.3	9.1
PUFA/SFA	0.7	0.3
C18:2n-6/C18:3n-3	22.9	2.3

other than the n-3 fatty acids. Indeed, the high level of SFA (44% fatty acid = 14.6% energy) in the experimental diet did not lead to any deleterious effect on plasma lipid parameters, as compared with the control diet (35% fatty acid = 12% energy). Moreover, the experimental diet was also low in n-6 fatty acids (8.2 vs. 23.6% fatty acid), low in total PUFA (13.1 vs. 25.2% fatty acid) and none of these characteristics caused any deleterious effect on the plasma lipid parameters.

Discussion

The fatty acid content of RBC is considered to be a tissue marker for dietary fatty acid composition [21, 22]. The first result of this study is the increase in the ALA content of RBC in the experimental group, whereas it did not vary in the control group. This increase in ALA is mainly due to the dietary animal products obtained from linseed-fed animals but also from linseed flour and bread included in the experimental diet. All together the ALA supply was about 2.4 g/day, which is a little higher than the French recommended intake [23]. We noted in the control group that ALA intake corresponded to the French average consumption [13, 17] and that this intake was sufficient to maintain its levels in the RBC during the study. More interesting is the effect of the diet on EPA and DHA content in the RBC. These contents decreased significantly

Table 4 Red blood cell fatty acid composition in volunteers (% of total FA)

	Control group			Experimental group	
	Day 0	Day 90		Day 0	Day 90
C16:0	20.2 ± 1.4	20.0 ± 1.8		20.0 ± 1.6	20.3 ± 1.7
C18:0	10.5 ± 2.9	11.5 ± 3.5		10.5 ± 3.4	12.1 ± 3.5**
∑Saturated FA (SFA)	32.5 ± 2.9	33.2 ± 4.1		32.2 ± 3.7	34.2 ± 4.0**
C18:1n-9	23.5 ± 2.55	21.0 ± 4.3***		22.8 ± 2.8	21.8 ± 3.3*
∑Monounsaturated FA (MUFA)	29.7 ± 3.2	25.9 ± 4.4***	##	28.5 ± 3.5	27.1 ± 3.8*
C18:2n-6	20.9 ± 4.0	24.0 ± 5.5***	###	22.2 ± 5.0	20.6 ± 5.2
C20:4n-6	8.9 ± 2.1	9.5 ± 2.8		9.0 ± 2.2	9.4 ± 2.4
∑n-6 PUFA	32.7 ± 3.3	36.5 ± 5.1***	###	34.1 ± 3.7	32.9 ± 4.3
C18:3n-3	0.43 ± 0.2	0.46 ± 0.7	#	0.42 ± 0.1	0.68 ± 0.4***
C20:5n-3	0.62 ± 0.2	0.40 ± 0.2***	###	0.73 ± 0.3	0.77 ± 0.3
C22:6n-3	2.6 ± 0.8	2.3 ± 0.8*	#	2.6 ± 0.8	2.7 ± 0.9
∑n-3 PUFA	4.6 ± 1.2	4.0 ± 0.9***		4.7 ± 1.2	5.3 ± 1.3**
C18:2n-6/C18:3n-3	55.4 ± 19.9	77.0 ± 28.7***	###	58.0 ± 19.8	35.6 ± 14.1***
∑n-6 PUFA/∑n-3 PUFA	7.6 ± 2.0	9.8 ± 3.6***	###	7.6 ± 2.1	6.6 ± 2.0**

Significant intra-group difference: $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***)

Significant inter-group difference: $P < 0.05$ (#), $P < 0.01$ (##), $P < 0.001$ (###)

Table 5 Change in anthropometric parameters in volunteers

	Control group		Experimental group	
	Day 90–Day 0	Day 240–Day 90	Day 90–Day 0	Day 240–Day 90
Weight (kg)	−3.5 ± 3.0*	+1.7 ± 2.6**	−2.9 ± 2.6*	+0.4 ± 3.0†
BMI (kg/m^2)	−1.3 ± 1.0*	+0.6 ± 0.9**	−1.0 ± 0.9*	+0.1 ± 1.1#
Hip circumference (cm)	−2.5 ± 3.4*	+0.9 ± 2.7*	−2.0 ± 2.6*	0 ± 2.8

Significant intra-group difference: $P < 0.05$ (*), $P < 0.01$ (**)

Significant inter-group difference: $P < 0.06$ (†), $P < 0.05$ (#)

Table 6 Plasma lipid parameters (mmol/l) in volunteers

	Control group		Experimental group	
	Day 0	Day 90	Day 0	Day 90
Triglycerides	1.44 ± 0.59	1.48 ± 0.92	1.46 ± 0.76	1.62 ± 0.86*
Total cholesterol	5.47 ± 0.99	5.58 ± 0.94†	5.67 ± 1.09	5.76 ± 0.99
HDL cholesterol	1.36 ± 0.33	1.31 ± 0.30†	1.41 ± 0.32	1.32 ± 0.28**
LDL cholesterol	3.47 ± 0.82	3.63 ± 0.82**	3.57 ± 0.85	3.72 ± 0.88*

Significant intra-group difference: $P < 0.06$ (†), $P < 0.05$ (*), $P < 0.01$ (**)

No significant inter-group difference

in the control group, whereas they were maintained in the experimental group. Maintaining the EPA and DHA levels in the RBC in the experimental group, without consuming fish and other seafood during the trial, constitutes the major result of this study. This demonstrate the efficiency of the terrestrial animal vector for providing EPA and DHA to humans, when the animals receive small amounts of the precursor ALA alone (linseed-fed animals). This can be mainly explained by the direct intake of EPA + DHA contained in the animal products of the experimental diet (Table 3), since these derivatives are synthesized from ALA by the linseed-fed animals during their growth. In addition, it is likely that low conversion of ALA to EPA + DHA also took place in the volunteers during the trial, but conversion in humans is known to be very low to EPA, and extremely low (less than 0.5% ALA) to DHA [24](for a review on ALA conversion and supplementation). Moreover, for these reasons, ALA provided by flour and bread in the experimental diet, could account only modestly for EPA synthesis and not for DHA synthesis, in volunteers.

Before the trial, the volunteers consumed a varied diet including fish and other seafood, and were recruited in a marine area (large fishing harbor) where a high consumption of n-3 fatty acids from fish and other seafood was described in a previous study [25]. Although fish and other seafood remain the main source of n-3 derivatives (EPA + DHA) for the population, the supplies of fish are limited whereas people have been encouraged to increase their intake [26]. We show here that improving the feeds of terrestrial animals by incorporating linseed is quantitatively capable of maintaining the EPA and DHA levels in the RBC of a population. In an earlier study, only EPA was increased by a similar experimental diet [18], but this previous study was performed with healthy volunteers exhibiting a high level of DHA in the RBC (4.8%), whereas the present study was performed with obese subjects having only 2.6% DHA in the RBC. This low level of DHA observed here also suggests a low DHA consumption by obese volunteers or a reduced conversion of α-linolenic precursor or even a higher catabolism (β-oxidation) of this fatty acid, and raises the question of larger requirement of n-3 to maintain tissues in obese. Finally, we showed here a decrease in EPA and DHA derivatives in the control group, suggesting (at least in the absence of fish consumption) that the average ALA intake in France is not sufficient to maintain correct EPA + DHA levels by ALA conversion alone, especially with a high dietary LNA/ALA ratio .

As regards n-6 fatty acids, we observed an increase in LNA content in the RBC of the control group, which reflects the control diet, characterized by standard animal products and sunflower-margarine. This increase inside the control group explains the significant difference observed for LNA between the two groups, since no significant change in LNA content was observed inside the experimental group. Although the dietary LNA content was three times lower in the experimental group than in the control group, it was sufficient to maintain the LNA and arachidonic acid levels in the RBC.

One of the goals of this work was: to evaluate the effect of the experimental diet, rich in n-3 fatty acids, on various anthropometric parameters of obesity. The results showed clearly that the volunteers from both groups lost weight and that no significant difference of weight was observed between the two groups over the period of the diets. This is probably due to the fact that both diets were normo-caloric; in other words probably hypo-caloric (for obese individuals) when compared with the diet consumed before the trial. This suggests that the reduction in caloric intake may have masked any qualitative effects relating to the diet's fatty acid composition. However, the results obtained at day 240 (in only 72% of the day 90 population) showed a weight gain in the control group, whereas no weight gain was determined in the experimental group. This interesting result suggests that the experimental diet, provided between day 0 and day 90, was able to limit the weight gain recovery. The changes in BMI and hip circumference confirmed the effect on weight, between day 90 and day 240, within the groups. Between groups, the BMI was significantly lower at day 240 in the experimental group and weight tended to be lower in the experimental group than in the control group. Our results suggest that n-3 fatty acids may have several metabolic effects. More precisely, we can suppose that the enrichment of the tissues in n-3 fatty acids improves various metabolic syndrome parameters, which is consistent with the literature [10, 11, 27–29], and reduces the weight gain recovery phenomenon. One can also suggest a different energy expenditure, implying membrane processes, which are known to be modulated by membrane fatty acid composition [30, 31]. A very recent study showed that plasma n-3 PUFA were negatively associated with obesity [32]. Finally, we can also suppose that the high n-6 fatty acid levels in the control diet could also explain the difference between groups, since LNA is considered to be very adipogenic [1, 4, 5]. Moreover, the weight gain observed in the control group is in good agreement with the work by Dayton et al. [3] showing an even stronger effect but with longer exposure to the same type of diet rich in LNA. In any case, the n-6/n-3 balance seems to be involved, whereas the proportion of saturated fatty acids does not appear to be a determinant of weight gain, since the experimental diet was the richest in saturated fatty acids. Lastly, observing the inhibiting effect on weight gain recovery in the long term after the end of the trial is a new and very interesting observation, since this long-term period is frequently subject to dramatic weight recovery as is often described in the literature in humans [33]. However, the effects reported here on anthropometric parameters at D240 can be considered only as preliminary results for further investigations.

Finally, we studied the plasma lipid parameters in the volunteers. Surprisingly, the plasma lipid parameters tended to worsen within each group although volunteers lost weight. In the literature, weight loss generally improves plasma lipid parameters [34–37] but this occurs after caloric restriction, which is not the case in the present study. However, our results suggest at least that content and duration of both diets are unable to stop the lipid parameter evolution in obese persons. Interestingly, between groups, we observed no significant difference in total cholesterol, HDL cholesterol, LDL cholesterol or triglycerides during the trial (D0–D90). In other words, the experimental diet was much richer in saturated fatty acids, but did not alter the plasma lipid parameters during the 90 days as compared with the control group. This suggests that the level where saturated fatty acids are deleterious remains to be determined and probably should be re-evaluated [38], depending on the diet composition in n-3 and n-6 PUFA. If we consider the PUFA/SFA ratio, the experimental diet exhibited the lower value, without any deleterious effects on the plasma lipid parameters (as compared with the control diet), which is in good agreement with a previous work with different values of the PUFA/SFA ratio in humans [39]. This suggests that the PUFA/SFA ratio is less relevant than the balanced composition of the experimental diet in both n-6 and n-3 fatty acids, as compared with the excess of n-6 fatty acid in the control diet. Finally, the results in terms of plasma lipid parameters also suggest that a diet containing 76% animal lipids (experimental diet) does not appear to be more deleterious than a diet containing 49% animal lipids only (control diet).

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