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Postprandial effects of a lipid-rich meal in the rat are modulated by the degree of unsaturation of 18C fatty acids

Patrick Even[a,b], François Mariotti[a,b], Dominique Hermier[a,b,*]

[a]INRA, UMR914 Nutrition Physiology and Ingestive Behavior, F-75005 Paris, France
[b]AgroParisTech, UMR914 Nutrition Physiology and Ingestive Behavior, F-75005 Paris, France

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Abstract

The fatty acid composition of high-fat diets is known to influence the magnitude of postprandial events that increase the risk of metabolic syndrome. These variations in magnitude may be directly ascribed to differences in the channeling of lipids toward oxidation or storage. A study was designed to compare the effects of 4 dietary fats on postprandial energy expenditure and on some risk factors of the metabolic syndrome. To avoid usual confounding factors due to simultaneous variations in chain length and double-bounds number of fatty acids, dietary fats were chosen to provide mainly 18-carbon fatty acids with 0 (stearic acid [SA]), 1 (oleic acid [OA]), 2 (linoleic acid [LA]), or 3 (α-linolenic acid [ALA]) double bounds. They were given as single high-fat test meals to 4 different groups of male rats. The resting metabolic rate and the lipid and carbohydrate oxidation were measured from oxygen consumption and carbon dioxide production using indirect calorimetry 2 hours before and 6.5 hours after the test meal. Plasma glucose, triglyceride, and chylomicron concentrations were determined at 0, 1.5, and 4 hours after the test meal. Postprandial concentration of glucose and triglyceride did not vary with the nature of the test meals, whereas that of chylomicrons was the highest after the LA test meal and the lowest after the SA test meal. Postprandial increase in resting metabolic rate was the highest after the LA and OA test meals, and the lowest after the SA and ALA test meals. Compared with the 3 other diets, the ALA test meal enhanced lipid oxidation and decreased glucose oxidation during the early postprandial period (0.25-3.25 hours). This suggests that stearic acid may not induce all the adverse effects classically described for other saturated fatty acids and that α-linolenic acid may beneficially influence energy partitioning, especially during the early postprandial state.

1. Introduction

A large body of evidence shows that high dietary fat intake is associated with an increase in several risk factors of the metabolic syndrome, such as lipid storage and visceral obesity, insulin resistance, and dyslipidemia, which makes these patients more prone to type 2 diabetes mellitus and cardiovascular diseases [1]. Among dietary lipids, the differential effect of fatty acid classes on these long-term consequences (type 2 diabetes mellitus and cardiovascular diseases) is well documented, even if some of the underlying mechanisms remain unclear [2-6]. Briefly, saturated fatty acids (SFA) are considered to exhibit the most deleterious effects because they favor lipid storage, insulin resistance, and hypercholesterolemia. In contrast, when present in high-fat diet, monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), especially long-chain (LC) n-3 PUFA, are considered to have a lesser impact and even to be protective.

Interestingly, these long-term metabolic dysregulations, together with their pathologic consequences, may largely result from the short-lasting but repeated postprandial events resulting from lipid intake [7]. Indeed, it has been repeatedly reported in healthy humans and animals that a single high-fat bolus evokes a transient vascular endothelial dysfunction, oxidative stress, and low-grade inflammation, which are hallmarks of the metabolic syndrome [8]. In this context, the role of the fatty acid profile of this single fat bolus has received little attention. Endothelial reactivity is generally improved when the high-fat test meal contains PUFA rather than SFA or MUFA [9]. In contrast, postprandial plasma concentration of inflammatory cytokines may not be affected by the dietary fatty acid composition [10,11].

* Corresponding author. INRA, UMR914, Nutrition Physiology and Ingestive Behavior, AgroParisTech, F-75005 Paris, France. Tel.: +33 1 44 08 72 46; fax: +33 1 44 08 18 58.
E-mail address: dominique.hermier@agroparistech.fr (D. Hermier).

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The level of postprandial deleterious events resulting from lipid intake has been related to the duration and magnitude of the postprandial hypertriglyceridemia, which are directly influenced by the dietary fatty acid profile. Indeed, in healthy humans, when compared with an SFA-rich test meal, postprandial triglyceride (TG) and chylomicron (CM) concentrations were lower after test meals rich in either oleic acid (18:1 n-9) [12,13] or LC n-3 PUFA [14,15], but was the same [14,15] or even higher [16] after a test meal rich in linoleic acid (18:2 n-6). Besides, when compared with 18:1 n-9, postprandial hypertriglyceridemia was attenuated after a test meal rich in stearic acid (18:0) [17].

Differential effects of the nature of dietary fatty acids on the postprandial hypertriglyceridemia may be directly ascribed to differences in their metabolic fate and more precisely to the extent of their postprandial oxidation. Studies using radioisotope tracer techniques in rats or humans showed that fatty acid oxidation decreased with increased chain length and saturation degree of dietary fat [18-23]. When considering specifically fatty acids transported in CM, fractional oxidation rate of 18:1 n-9 was greater than that of 16:0 [24]. However, oxidation ranking between 18:1 n-9, 18:n-6, and 18:3 n-3 was somehow inconsistent, even when compared during the first hours after tracer administration [18,21,22,25].

The use of tracers provides very useful data on the differential use of various fatty acids as substrates by the body, but does not allow understanding how specific fatty acids influence the energy metabolism of nutrients, which necessitates the use of indirect calorimetry. Regarding the influence of the chain length on energy expenditure, when compared with a single test meal rich in 18 carbons mostly, postprandial lipid oxidation was higher after a test meal containing only SFA with 8 to 10 carbons [26] and was identical [27], even lower [28], after a test meal rich in SFA from dairy fat (12-16 carbons) [28]. Regarding the number of double bounds, postprandial increase in energy expenditure after a test meal rich in 18:1 n-9 was the same as that after a test meal rich in α-linolenic acid (18:3 n-3) [27] or was higher than after test meals rich in 18:2 n-6 or 18:3 n-3 [29]. Besides, in both these studies, postprandial changes in lipid or carbohydrate oxidation were not significantly affected by the dietary fatty acid pattern.

To our knowledge, postprandial events, especially those related to hyperlipidemia, have not been investigated in relation with the metabolic use of the dietary fatty acids provided. Besides, investigations of postprandial events usually compare SFA provided by animal fats and rich in palmitic acid (16:0) and shorter fatty acids on the one side with MUFA and PUFA rich in 18:1 n-9, 18:2 n-6, or n-3 LC-PUFA (20-22 carbons) on the other side. This variability in length and unsaturation may explain part of the discrepancies between studies regarding hyperlipidemia or substrate oxidation and does not allow concluding faithfully on the respective contribution of chain length and double-bound number. The present study was therefore designed to compare, in the rat, the metabolic use of 18-carbon fatty acids with 0 to 3 double bounds provided as a single high-fat bolus in relation to the magnitude of some postprandial metabolic events.

2. Experimental methods

2.1. Experimental procedure

2.1.1. Animals

The *Principles of Laboratory Animal Care* (National Institutes of Health publication no. 85-23, revised 1985) and French guidelines concerning the care and use of laboratory animals were followed. Two groups of 36 Wistar-Hanover male rats (Harlan, Gannat, France), weighing 250 to 275 g on arrival, were housed in colony cages with wire floor and wood litter (4-5 rats per cage) in a controlled environment (23°C ± 1°C, 12-hour/12-hour light-dark cycle with the light going on at 6:00 AM). They had free access to food and tap water.

2.1.2. Diets

Upon arrival, rats were fed a standard pelleted chow (SAFE, Augy, France) for 2 days; then they were fed a semisynthetic maintenance powdered chow prepared under strict laboratory conditions by UPAE (Experimental Food Preparation Unit, INRA, Jouy-en-Josas, France) and of which the calculated composition (in weight) was 10.8% protein, 69.8% carbohydrate (including 5% cellulose), 4.0% lipid, 10.7% water, 1.2% vitamins, and 3.5% minerals. Energy content of this maintenance diet was 14.6 kJ/g. The experimental diets consisted (in weight) of 13.9% protein (provided by 18% total milk protein containing 77% protein), 46.4% carbohydrate (39.8% corn starch, 6.5% sucrose, 5% cellulose), 26.0% lipid, 9.2% water, 1.2% vitamins, (including 0.23% choline), and 3.5% minerals. Total exceeds 100% (100.2%) because some values have been approximated for consistency (eg, exact protein content is 13.86% instead of 13.9%). Energy content of the experimental diets was 19.6 kJ/g. Lipids accounted for 50% energy and were provided by 1 of the following 4 fats: shea butter, rich in stearic acid (18:0, 45%) (Soetenaey, Fécamp, France) (SA diet); oleic sunflower oil, rich in oleic acid (18:1 n-9, 79%) (gift from Bernadette Deplanque, University Paris XI, Orsay, France) (OA diet); sunflower oil, rich in linoleic acid (18:2 n-6, 63%) (gift from Lesieur, Asnière, France) (LA diet); or linseed oil, rich in α-linolenic acid (18:3 n-3, 56%) (gift from Vandeputte, Mouscron, Belgium) (ALA diet) (Table 1). Palmitic acid (18:0) accounted for only 3% to 6% of total fatty acids.

2.1.3. Experimental design

During the adaptation period on the standard powered diet, all rats were accustomed to be gently restrained in a cloth and to drink from a syringe, in about 1 minute, 6 g of a mixture consisting of 3 g of the maintenance powered chow diluted in 3 mL of water.

After 10 days on the maintenance diet, 4 groups of 9 rats (corresponding to each of the 4 experimental diets; mean

Table 1
Fatty acid composition (percentage of total fatty acids in experimental oil) of the experimental diets

	SA	OA	LA	ALA
C14:0	0.02	0.03	0.06	0.03
C15:0	0.01	0.01	0.01	0.02
C16:0	3.29	3.38	5.81	5.04
C16:1 n-9	0.02	0.03	0.02	0.02
C16:1 n-7	0.03	0.07	0.07	0.06
C17:0	0.08	0.03	0.04	0.07
C18:0	44.50	3.58	3.88	4.35
C18:1 n-9	43.92	79.12	24.72	17.36
C18:1 n-7	0.43	0.58	0.65	0.70
C18:2 n-6	5.68	11.61	63.46	15.52
C18:3 n-6	0.02	0.01	0.01	0.01
C18:3 n-3	0.10	0.09	0.14	56.40
C20:0	1.50	0.30	0.26	0.16
C20:1 n-9	0.26	0.25	0.16	0.13
C22:0	0.15	0.91	0.71	0.13

weight, 305 ± 10 g) were fasted overnight. The next morning, they were given 2 g/100 g body weight (ie, 6 g approximately) of a test meal made of one of the experimental diets diluted in water (1:1weight) and providing 19.6 kJ/100 g body weight. Such a test meal corresponded to the maximal food ingested spontaneously by a rat (300 g body weight) in a single meal and accounted for about 20% of total spontaneous daily intake [30]. Blood was drawn from a tail vein before (0.3 mL) and then 90 and 240 minutes (1 mL) after the test meal, and was dropped into prechilled tubes containing 70 μL (per milliliter of blood) of a solution of EDTA (0.7% [wt/vol]) and aprotinin (0.014% [wt/vol]). Plasma was separated by centrifugation (1700*g*, 20 minutes, 4°C), and aliquots were stored at −20°C.

The response of energy metabolism to feeding was measured by use of open-circuit indirect calorimetry, as described previously [31,32]. Four other groups of 9 rats (mean weight, 310 ± 4 g) were placed at 6:00 PM in individual respiration chambers and fasted, with free access to water; and energy expenditure was recorded for the following 22 hours. The morning after (10:00 AM), the rats were given one of the same test meals as above (3 g powder and 3 g water per rat; ie, 58.9 kJ).

2.2. Analyses

2.2.1. Plasma biochemical analyses

Blood glucose concentrations were measured using an Accu-Chek Glucometer (Roche Diagnostics, Meylan, France). Plasma insulin concentration was determined by the mean of a solid-phase 2-site enzyme immunoassay kit (Mercodia Rat Insulin ELISA; Biovalley, Mane-la-Vallèe, France).

Plasma TG concentration was determined using a standard enzymatic assay (Triglycerides PAP 150; BioMerieux, Marcy-l'Etoile, France) [33].

Chylomicrons were separated from 0.5 mL plasma layered by 2.0 mL of a 9-g/L NaCl solution and then centrifuged for 35 minutes at 40 790*g* at room temperature in a Sorvall Super Speed T21 high-speed centrifuge (Thermo Fisher Scientific, Villebon, France) using a Sorvall SL-50T rotor. Triglycerides, total cholesterol (TC), and phospholipids (PL) were then determined in CM with the corresponding enzymatic kits provided by BioMerieux for TG and TC (Cholesterol RTU) and by BioDirect (La Villeneuve, France) for PL [34,35].

Interleukin (IL)-1β, IL-6, tumor necrosis factor-α (TNFα), plasminogen activator inhibitor–1 (PAI-1), and monocyte chemoattractant protein–1 (MCP-1) were assayed by a Linco kit (Clinisciences, Montrouge, France) using multiplex technology with a Bioplex device (Biorad, Marnes-la-Coquette, France).

2.2.2. Energy expenditure

Measured parameters were oxygen consumption, carbon dioxide production, and spontaneous activity. As previously described [31,32], a specific procedure (modelization/filtering) allowed computing of the changes in oxygen consumption and carbon dioxide production specifically induced by the bouts of spontaneous activity. Therefore, by subtraction, it was then possible to analyze the thermogenic response to feeding from changes in resting metabolic rate (RMR), respiratory quotient (RQ), resting glucose oxidation (rGox), and resting lipid oxidation (rLox) in these free-moving rat. The changes induced by the test meals on RMR, rGox, and rLox were computed during 7 hours by difference with the premeal fasting values (computed during 2 hours) and used to compute the thermic effect of the meal (ΔRMR) and the changes induced on Gox and Lox (ΔGox and ΔLox). Protein oxidation has been discarded because, on one hand, it made only a small part of total energy expenditure (8%-10% of total metabolism with a diet containing 14% energy as protein) and, on the other hand, it cannot be measured on a short-term basis according changes in oxygen consumption and carbon dioxide production. Therefore, it is necessary to assume that, because protein content in the diet is the same, protein oxidation was also the same in both groups. The Gox and Lox equations were not corrected for protein oxidation, which overestimated the corresponding values by about 5%. From this point, there are 2 solutions to apply to the Gox and Lox equations: either selecting a theoretical value for protein oxidation (in this study, let us say 10%) or simply ignoring any correction, which is what we did. In fact, one or the other method would change the absolute Gox and Lox values (by about 5%), but exactly in the same way in all groups, therefore creating no bias in the between-group comparison. Because 18-carbon fatty acids accounted for more than 90% in the 4 experimental diets, the average chain lengths of the fatty acids in the different fat sources were the same (17.92-17.97 carbons). Besides, the average values for the level of saturation differed by no more than 1.5 double bounds, corresponding to an average difference of 0.015 at the RQ level and a difference of less than 1% at the energy content level. For these reasons, the equations for Gox and Lox calculation were not corrected for chain length or unsaturation degree.

2.2.3. Statistical analyses

All values are presented as means ± SEM. All postprandial repeated measurements were analyzed using mixed models with meal type and time after meal as independent, fixed factors (SAS, Cary, NC). When appropriate, different covariance structures for time-repeated measurements were tested; and the one to retain was selected on objective criteria based on fitting statistics. When there was a significant meal effect or meal × time interaction, preplanned contrasts were built to compare different meal effects in the overall postprandial period (ie, postmeal values excluding the baseline value). Analysis of the calorimetric data showed that ΔGox and ΔLox curves differed markedly between the first (0-3.25 hours) and the second (3.5-6.5 hours) halves of the postprandial period ("Results"). Post hoc testing with ad hoc contrasts was therefore performed, first, to compare the values between the first and second half of the postprandial period irrespective of the meal type and, second, when there was a significant meal × period interaction, to compare the effect of the meal type within the first and the second half of the postprandial period. When there was a significant meal effect or meal × time interaction, multiple post hoc comparisons were done with the Tukey-Kramer adjustment. Statistical significance was set at the 5% level.

3. Results

3.1. Plasma parameters

Compared with fasting values, plasma glucose and TG concentrations increased significantly in response to the high-fat test meals (Table 2); but the increase was not affected by the nature of the meal. At 4 hours, glycemia remained elevated and similar to values at 1 hour 30 minutes, whereas plasma TG concentration values did not markedly differ from premeal values. In contrast, plasma CM concentration and composition were influenced by both meal type and time after meal (Table 2). Plasma concentrations of total CM-lipids and the separate CM-lipid components (TG, TC, PL) were approximately 2-fold higher at 1 hour 30 minutes than at 4 hours, in accordance with the highest TG concentration at 1 hour 30 minutes. The nature of meal fatty acids had a significant impact on plasma concentration of TC and PL in CM, and closely tended to significance for CM-TG (P = .0570) and CM-lipids (P = .0538). The concentrations of CM–total lipids and their individual lipid components were significantly higher after the LA meal (rich in 18:2 n-6) than after the SA diet (rich in 18:0), whereas the responses to OA and ALA were numerically intermediary (but not significantly different from LA or SA groups). Four hours after the test meal, plasma concentrations of TNFα were less than the lower limit of quantification of the method. Plasma concentrations of IL-1β, IL-6, MCP-1, and PAI-1 were not significantly affected by the nature the dietary fatty acids (Table 3).

3.2. Energy expenditure

Fasting premeal RMR, RQ, rGox, and rLox values were similar in all groups (data not shown). The RMR, RQ, rGox, and rLox values varied significantly with time after the test meal (P < .001) (Figs. 1 and 2, Table 4). When postprandial changes were computed over 6.5 hours, only ΔRMR was affected by the nature of dietary fatty acids (Table 4). Indeed,

Table 2
Plasma concentration of glucose, TG, and CM in response to a lipid-rich test meal

Plasma parameter	Time after test meal (min)	SA	OA	LA	ALA	Diet	Time	Interaction
Glucose (g/L)	0	0.84 ± 0.04	0.71 ± 0.08	0.77 ± 0.08	0.78 ± 0.03	.4422	<.0001	.8974
	90	1.32 ± 0.05	1.20 ± 0.06	1.24 ± 0.04	1.22 ± 0.05			
	240	1.26 ± 0.07	1.28 ± 0.07	1.29 ± 0.05	1.28 ± 0.06			
TG (g/L)	0	1.22 ± 0.09	0.99 ± 0.14	1.04 ± 0.13	1.06 ± 0.10	.6076	<.0001	.9729
	90	1.51 ± 0.17	1.64 ± 0.14	1.64 ± 0.23	1.69 ± 0.21			
	240	1.31 ± 0.16	1.09 ± 0.16	1.13 ± 0.21	1.21 ± 0.19			
CM-TG (mg/L)	90	304 ± 53	491 ± 83	753 ± 23	593 ± 129	.0570	.0024	.6140
	240	184 ± 68	239 ± 41	414 ± 149	205 ± 39			
	Post hoc analysis of meal effect	bc	ac	a	ac			
CM-TC (mg/L)	90	22 ± 5	68 ± 16	61 ± 20	30 ± 5	.0086	.0072	.6703
	240	14 ± 4	33 ± 16	32 ± 10	11 ± 3			
	Post hoc analysis of meal effect	b	a	a	b			
CM-PL (mg/L)	90	63 ± 9	145 ± 30	193 ± 58	133 ± 28	.0333	.0022	.6022
	240	40 ± 14	78 ± 23	91 ± 31	47 ± 9			
	Post hoc analysis of meal effect	bc	ac	a	ac			
CM–total lipid (mg/L)	90	388 ± 66	704 ± 127	1007 ± 304	756 ± 161	.0538	.0023	.7079
	240	237 ± 86	349 ± 77	537 ± 189	264 ± 49			
	Post hoc analysis of meal effect	bc	ac	a	ac			

Results are means ± SEM of 7 to 9 rats in each group. Postprandial meal effects were based on between-meal contrasts (collapsing values at 1 hour 30 minutes and 4 hours). Corresponding entries not sharing a same letter differ significantly at P < .05. There was no significant postprandial meal effect for plasma glucose and TG concentration.

Table 3
Plasma concentration (in picograms per milliliter) of inflammatory cytokines and PAI-1 240 minutes after a lipid-rich test meal

Cytokine	SA	OA	LA	ALA	*P* ANOVA
IL-1β	43.3 ± 11.3	20.8 ± 3.7	24.4 ± 5.3	33.6 ± 8.3	.2135
IL-6	406 ± 101	584 ± 157	453 ± 116	493 ± 111	.7666
PAI-1	322 ± 71	265 ± 56	273 ± 41	390 ± 66	.4419
MCP-1	300 ± 61	201 ± 32	190 ± 34	181 ± 30	.1768

Results are means ± SEM of 7 to 9 rats in each group.

ΔRMR increased more after the LA test meal than after the SA and ALA test meals, indicating that thermogenesis was enhanced in response to a fat meal rich in 18:2 n-6 (LA). The ΔRMR also tended to be greater after the OA test meal than after the SA and ALA test meals (P = .0721 and .0545, respectively), suggesting a propensity toward an enhanced thermogenesis in response to a fat meal rich in 18:1 n-9. On average, ΔRMR amounted to 6.7% to 10.2% of the ingested calories, which is in agreement with the well-known reduced thermogenic effect of high-fat meals. The ΔGox and ΔLox showed a 2-phase evolution with time. They reached a maximum and minimum, respectively, then plateaued until 3.50 hours after the meal, before returning to fasting values after 6.50 hours (Fig. 2).

Statistical analysis of the postprandial period effects showed that ΔRMR, ΔGox, and ΔLox did vary significantly between the first (0-3.25 hours) and the second (3.5-6.5 hours) postprandial periods, irrespective of the meal test type (Table 4). Besides, ΔGox, ΔLox, and the resulting ΔRQ showed a significant interaction with period (Table 4). This allowed a subsequent post hoc analysis of the meal-type effect for these 3 parameters (Fig. 3).

In the early postprandial period (first 3.5 hours after the test meal), the changes in substrate oxidation reflected the metabolic shift from the fasting state, during which energy expenditure was mainly fueled by lipid adipose stores (two thirds from lipids vs one third from carbohydrates, as calculated from absolute values), to the postprandial state, during which carbohydrates were preferentially oxidized. Indeed, their contribution to energy expenditure, as calculated from absolute values, increased to reach two thirds between 3 and 4 hours after the meal, whereas that of lipids decreased to one third. Change in Lox was numerically smaller with the ALA diet than with the 3 other diets, but the difference was significant with the LA diet only (Fig. 3B). Likewise, ΔGox increased less in response to the ALA diet compared with the 3 other diets (Fig. 3A). Accordingly, ΔRQ was lower with the ALA diet than with the SA and LA diets (Fig. 3C).

In the late postprandial period (between 3.5 and 6.5 hours after the test meal), lipid and carbohydrate oxidation was only marginally affected by the nature of dietary fatty acids (Fig. 3A, B). The only significant difference was a slower return to fasting value of Lox with the ALA diet than with the LA diet.

4. Discussion

The present study aimed to characterize, in the rat, the influence of the nature of some dietary fatty acids on the magnitude of various postprandial events after a single high-fat test meal. Indeed, discrepancies of the literature probably result, at least partly, from confounding factors due to the experimental comparison of dietary lipids differing in their chain length and unsaturation degree simultaneously. In the present study, lipids of the experimental test meals consisted mainly of 18C fatty acids. Thus, dietary fatty acids exhibited the same chain length and differed only in their unsaturation degree (0-3 double bounds). Under these experimental

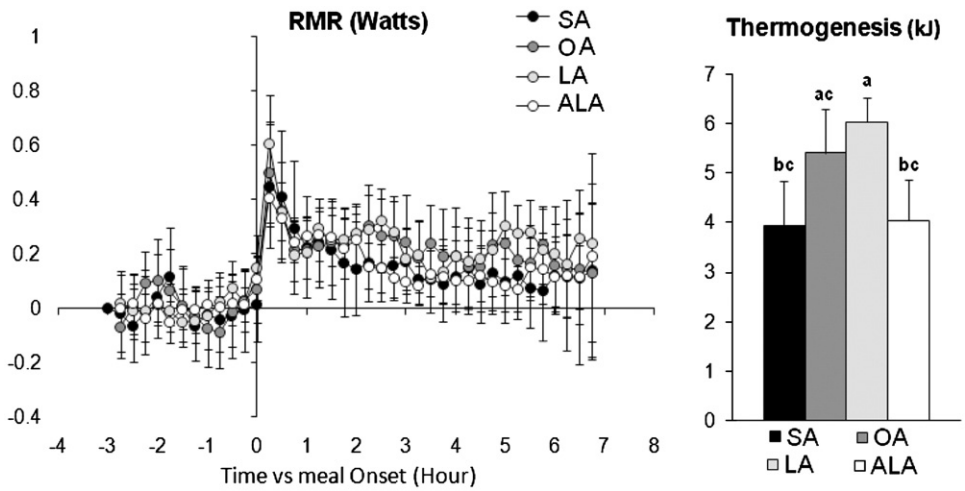

Fig. 1. Changes in RMR induced by the test meals. Postprandial changes in RMR (watts, means ± SEM) were measured relative to the average 2-hour premeal fasting rate and therefore named *ΔRMR* (left). Area under curve (kilojoules, means ± SE) represents the cumulated values of ΔRMR (meal-induced thermogenesis) over 6.5 hours (right). Values not sharing the same superscript differed significantly at $P < .05$.

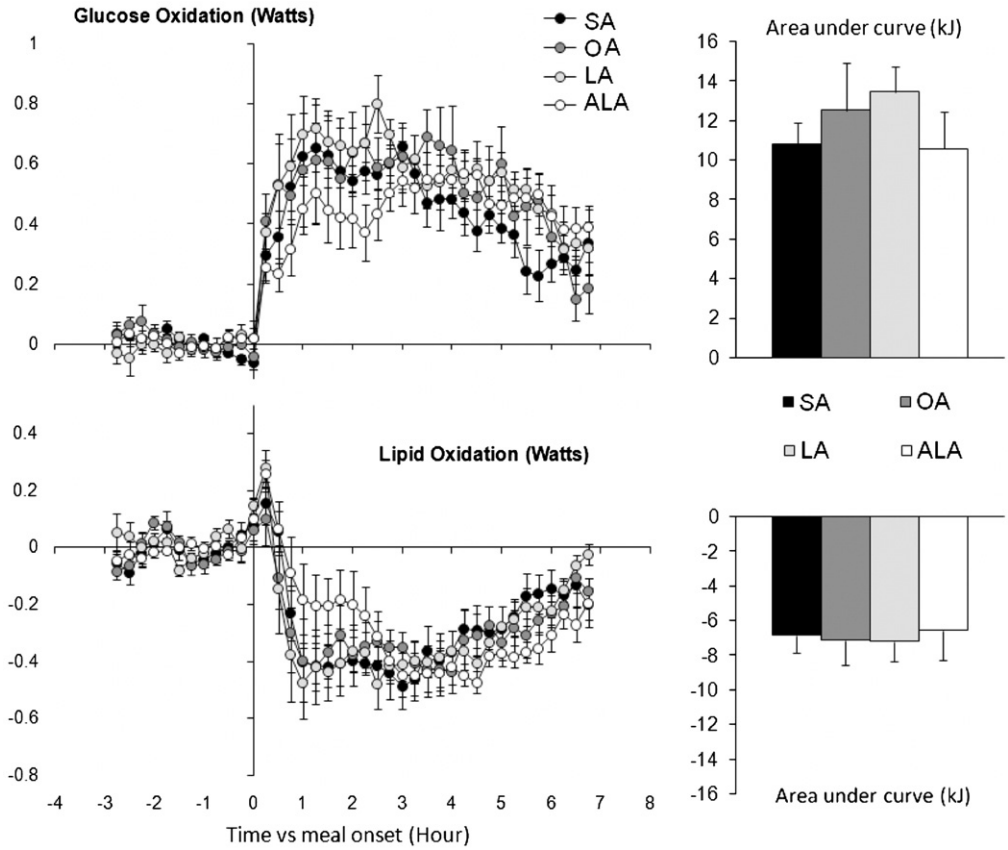

Fig. 2. Changes in Gox and Lox induced by ingestion of the test meals. Postprandial changes in rGox and rLox (watts, means ± SEM) were measured relative to the average 2-hour premeal fasting rate and therefore named *ΔGox* and *ΔLox*. Areas under curve (kilojoules, means ± SE) represent the cumulated values of ΔGox and ΔLox over 6.5 hours (right).

conditions, postprandial plasma concentrations of glucose, TG, and inflammatory cytokines did not vary with the degree of unsaturation of dietary fatty acids (Table 2). Regarding cytokines, this is consistent with previous studies performed in similar experimental conditions showing that plasma concentrations of inflammatory cytokines varied only marginally after a single fat bolus and were not influenced by the nature of dietary lipids in healthy or mildly diabetic subjects [10,11,36].

Under these acute experimental conditions, glucose homeostasis was not affected by the nature of dietary fatty acids, despite the fact that we reported some variations in

Table 4
Statistical analyses of energy expenditure at 6.50 hours after test meal

	ΔRMR (W)	ΔGox (W)	ΔLox (W)	ΔRQ
SA	0.159 ± 0.014^{bc}	0.441 ± 0.018^{ab}	-0.281 ± 0.018	0.060 ± 0.002
OA	0.221 ± 0.011^{ac}	0.481 ± 0.023^{ab}	-0.292 ± 0.018	0.057 ± 0.002
LA	0.245 ± 0.013^{a}	0.546 ± 0.019^{a}	-0.300 ± 0.019	0.065 ± 0.002
ALA	0.160 ± 0.012^{bc}	0.404 ± 0.015^{b}	$-0.276 \pm +0.019$	0.056 ± 0.002
Effects (*P* value)				
Meal type	.0250	.1654	.9751	.5673
Time	<.0001	<.0001	<.0001	<.0001
Meal type × time interaction	.8382	.0163	.1280	.1135
Period	<.0001	.0072	.0482	.3915
Meal type × period interaction	.5039	<.0001	<.0001	<.0001

Results are mean ± SEM of 6 to 8 rats in each group. Postprandial changes in RMR, rGox, rLox, and RQ (means ± SEM) were measured relative to the average 2-hour premeal fasting rate and therefore expressed as ΔRMR, ΔGox, ΔLox, and ΔRQ. Values not sharing the same letter in the same row differed significantly at $P < .05$. *Time-related effects* refer to the complete postprandial duration over 6.50 hours. *Period-related effects* refer to the 2 postprandial periods (0-3.25 and 3.50-6.50 hours).

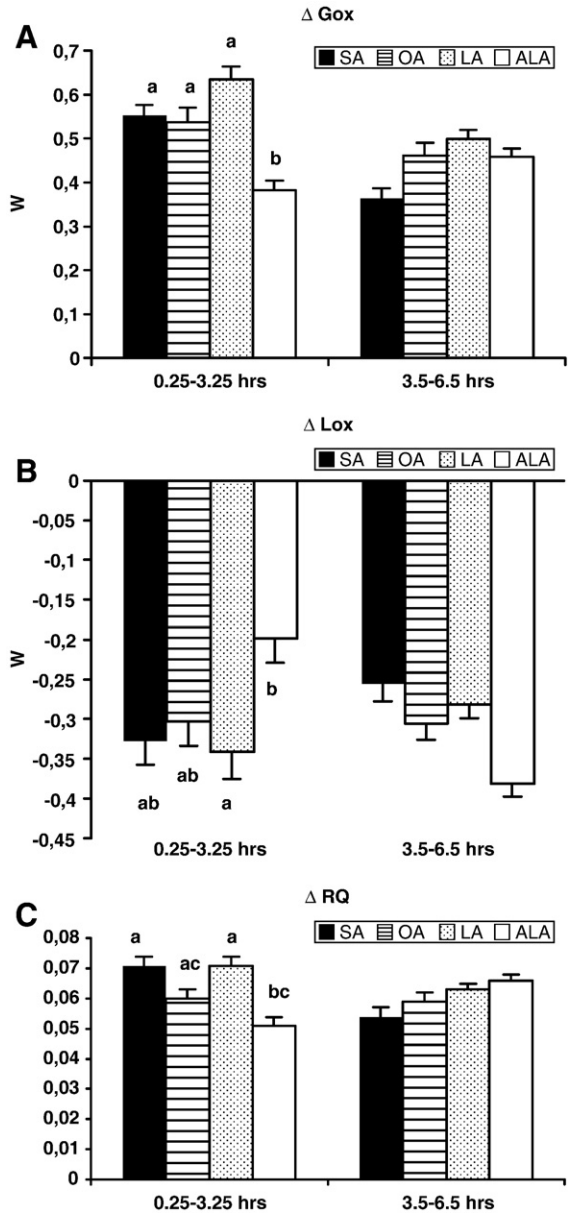

Fig. 3. Effects of meal-test type on substrate oxidation and RQ (relative to fasting state) within the 2 successive postprandial periods (0-3.25 and 3.50-6.50 hours). Postprandial changes in rGox (A), rLox (B), and RQ (means ± SEM) (C) were measured relative to the average 2-hour premeal fasting rate and therefore named *ΔGox*, *ΔLox*, and *ΔRQ*. Values not sharing the same superscript differed significantly at $P < .05$.

postprandial carbohydrate oxidation (see below). This is in line with most previous human studies showing that, in contrast with long-term studies [4], postprandial increases in plasma glucose and insulin do not vary with the nature of dietary fatty acids in response to a single fat bolus [13,15,27,28,37].

Regarding TG concentration, the effects of the fatty acid composition of the test meals are difficult to compare with the rather inconsistent literature data. In healthy humans, the postprandial TG rise in response to an SFA-rich meal has been reported to be higher [12-15], similar [27,28], or even lower [16,17] than that in response to MUFA- or PUFA-rich meals. In our hands, fatty acids with the same 18-carbon chain length but various levels of unsaturation induced similar postprandial hypertriglyceridemia, a result in agreement with a previous report in the rat [18]. However, total TG plasma concentration, which reflects the balance between intestinal absorption, de novo hepatic synthesis, and vascular catabolism of lipids, is less informative than plasma concentration of CM, which represents the metabolically active form of dietary lipids and is therefore more readily responsive to dietary conditions. Unexpectedly, CM concentration was the highest after the test meals rich in 18:2 n-6 (LA diet) and also in 18:3 n-3 (ALA diet), and the lowest after the one rich in 18:0 (SA diet). This differs from previous studies in which SFA induced the highest CM concentration. However, in these studies, SFA were provided as fats richer in palmitic acid (16:0) than in 18:0, whereas n-3 PUFA were provided as fish oil rich in 20:5 n-3 and 22:6 n-3 instead of 18:3 n-3 [13,14]. This confirms our hypothesis that not only the unsaturation degree, but also the carbon chain length, must be taken into account when addressing the metabolic role of fatty acid classes.

To our knowledge, this is the first study that examined the effects on energy metabolism of the 4 major 18-carbon fatty acids by using indirect calorimetry. Postprandial energy metabolism differed with the unsaturation degree of dietary fatty acids. Interpretation of data may be discussed in relation to (*a*) the possible adverse effects of SFA and (*b*) the relative influence of the 3 unsaturated fatty acids.

Previous studies dedicated to SFA used test meals containing TG rich in medium-chain (8-16 carbons) fatty acids, and these lead to inconsistent results. In the present study, SFA were provided as 18:0 and compared with the three 18-carbon unsaturated fatty acids. In our hands, postprandial thermogenesis of the test meal rich in 18:0 (SA group) tended to be lower than that of the test meal rich in 18:1 n-9 (OA group) (Table 4 and Fig. 1). This is consistent with some studies comparing medium-chain fatty acids and 18:1 n-9 [27,38], but differs from other studies in which postprandial thermogenesis in response to medium-chain fatty acids was identical [28] or even higher [26] than that in response to 18:1 n-9. Besides, postprandial thermogenesis of the test meal rich in 18:0 (SA group) was significantly lower than that of the test meal rich in 18: n-6 (LA group), but did not differ from that of the 18:3 n-3–rich test meal (ALA group). To our knowledge, the present study is the first one assessing the postprandial thermogenesis in response to a test meal rich in 18:0. Further studies are therefore needed to confirm the thermogenic value of 18:0 when compared with PUFA with 18 carbons and also to clarify the importance of the fatty acid chain length when assessing the role of SFA in postprandial thermogenesis, in relation to the risk of obesity, by comparing test meals rich in either medium-chain fatty acids or 18:0. As concerns fatty acid oxidation, in the present study, the decrease in Lox during the early postprandial period (0.25-3.25 hours after

the test meal) did not differ in response to 18:0, 18:1 n-9, and 18:2 n-6 and was greater than that after the test meal rich in 18:3 n-3 (Fig. 3B).This suggests that the switch from energy provided by the oxidation of fatty acids released from the adipose tissue in the fasting state to energy provided by the oxidation of dietary carbohydrates was less pronounced in response to 18:3 n-3. This was not true in the latter postprandial period (between 3.5 and 6.5 hours after the test meal), during which Lox increased progressively up to the fasting value, without significant effect of the dietary fatty acid pattern. This apparently differs from previous data using tracers, in which 18:0 was less oxidized than unsaturated 18 carbon fatty acids, in rats [18,23] as well as in humans [21]. This may be surprising because, in our experimental conditions, test meals were high in lipids and each of the 18-carbon fatty acids was prominent in one of the test meals. It was therefore expected that the main fatty acid in each test meal would be a major substrate of oxidative process, with Lox after the 18:0-rich test meal differing from the other ones. The reason for this discrepancy is probably that, in tracer studies, (1) only the metabolic fate of tracers could be assessed and (2) the various labeled fatty acids were administered together with diets that did not differ in their fatty acid composition. In contrast, the present study allowed assessing the metabolic fate of lipids as a whole, including dietary fatty acids and those resulting from de novo lipogenesis. Under these conditions, even if 18:0 is the poorer substrate for oxidative processes and if it is the main fatty acid in a given experimental diet, its specific influence is limited by the very complex pathways of the postprandial lipid metabolism. Besides, as regards the comparison of dietary lipids rich in either 18:0 or medium-chain SFA, the similar postprandial Lox after test meal rich in 18:0, 18:1 n-9, or 18:2-6 differs from other studies showing that oxidation of medium-chain fatty acids provided as dairy fat was generally found to be the lowest when compared with 18-carbon unsaturated fatty acids [27,28,38]. As in the case of thermogenesis, 18:0 obviously modulates lipid oxidation differently from shorter-chain SFA.

When comparing the relative effects of the 3 unsaturated fatty acids, the increase in RMR during the 6.5 hours after the ALA test meal tended to be lower ($P = .0545$) than that after the OA test meal and was significantly lower than that after the LA test meal, indicating that meal-induced thermogenesis was reduced in response to a fat meal rich in 18:3 n-3 compared with 18:1 n-9 and 18:2 n-6 (Fig. 2 and Table 4). In humans, a lower postprandial thermogenesis was observed after a test meal rich in 18:3 n-3 compared with test meal rich in 18:1 n-9 or 18:2 n-6 when 18:3 n-3 was provided, as in our present study, in the form of linseed oil (50%-60% 18:3 n-3 as percentage of total fatty acids) [29]. In contrast, postprandial thermogenesis was strictly identical when a test meal rich in 18:1 n-9 was compared with a test meal based on walnut oil, rich in 18-carbon PUFAS, but containing only 15% 18:3 n-3 [27]. Furthermore, postprandial variations in thermogenesis did not differ markedly between the groups that received the test meals rich in 18:1 n-3 (OA) and 18:2 n-6 (LA), in accordance with previous human studies [21,29]. Taken together, the present results and previously published data suggest that only a high proportion of 18:3 n-3 in the test meal may affect meal-induced thermogenesis. In parallel, postprandial lipid oxidation did not statistically differ in the 3 groups fed the test meals rich in unsaturated fatty acids (Table 4). However, when considering the early postprandial period (0-3.5 hours), postprandial lipid oxidation after the ALA test meal rich in 18:3 n-3 was reduced to a lesser extent than that after the other test meals, especially when compared with the LA test meal (Fig. 3A). This suggests that, although dietary carbohydrate oxidation predominates during the early postprandial period, some of the dietary lipids are also oxidized. This stimulation of lipid oxidation by dietary 18:3 n-3, compared with 18:1 n-9, was suggested by previous human studies using indirect calorimetry; but the differences only approached significance [27,29]. This is consistent with previous studies showing that, among 18-carbon fatty acids, 18:3 n-3 was the most rapidly oxidized in rats [18] as well as in humans [21], in accordance with this fatty acid being the preferential substrate of β-oxidation in both mitochondria and peroxisomes [39,40]. Moreover, 18:3 n-3, as other n-3 PUFA, favors the oxidation of other dietary fatty acids by activating the transcription of the key enzymes of β-oxidation [41]. Besides, this higher oxidation of lipids originating from the ALA test meal apparently had a sparing effect on short-term dietary carbohydrate oxidation (thus resulting in the above-quoted reduced thermogenesis) without detrimental effect on postprandial hyperglycemia, which was not higher than that in the 3 other groups (Fig. 3A and Table 2).

5. Conclusion

Under our experimental conditions of a single high-fat test meal challenge, the degree of unsaturation of the 18-carbon fatty acids did not influence postprandial variations in plasma glucose, TG, or cytokines. In contrast, the metabolic fate of nutrients appeared to depend on the number of double bounds. Contrary to what has been described with other SFA, 18:0 intake did not induce a lower lipid oxidation and a higher CM concentration. Indeed, CM concentration was the lowest after the test meal rich in 18:0 (SA group). In this respect, 18:0 may elicit less postprandial adverse effects than other SFA. On the contrary, a test meal rich in 18:2 n-6 (LA group) resulted in a particularly low postprandial Lox during the early postprandial period (0.25-3.25 hours after the test meal), which may explain the high persistence of blood CM. Lasting exposure to blood CM, as TG-rich lipoproteins, is considered to drive most of the adverse effects of fatty acids, in connection with the risk of metabolic syndrome [42,43]. As concerns 18:3 n-3 (ALA group), the corresponding test meal failed to significantly reduce CM concentration.

However, rats fed the ALA test meal differed from the 3 other groups by a better preservation of postprandial lipid oxidation and a sparing of dietary carbohydrates during the early postprandial period (0.25-3.25 hours) as compared with other meals. This suggests that the postprandial fuel partitioning may be beneficially modified by 18:3 n-3. Taken together, the present study indicates also that the degree of unsaturation of dietary fatty acids has to be taken into consideration independently of their chain length when evaluating the magnitude and the metabolic consequences of postprandial events resulting from a high lipid consumption.

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