Fatty acid composition of an oral load affects chylomicron size in human subjects

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HDL-phospholipids are determinants in reverse cholesterol transport. They are mostly derived from triacylglycerol (TG)-rich lipoproteins. Chylomicron size is important, therefore, because it is related to the ratio surface phospholipids : core TG and, thus, determines the availability of postprandial phospholipids for transfer to HDL. Eleven healthy young women each ingested four different fat loads supplemented with retinyl palmitate and containing 60 g sunflower oil (SO), oleic–sunflower oil (OSO), mixed oil (MO; (g/kg) linoleic acid 480, oleic acid 380, linolenic acid 13) or beef tallow (BT). At the peak of TG absorption for all loads (4 h) chylomicron diameters, determined by agarose-gel filtration, were larger after SO compared with OSO ($P < 0.05$) and BT ($P = 0.06$) and after MO compared with BT ($P < 0.05$). At 6 h chylomicron size was larger after the vegetable oils compared with BT ($P < 0.05$ in each case). After each fat load chylomicron size decreased at 6 and 8 h compared with that at 4 h ($P < 0.05$) except for OSO. Retinyl ester and TG concentrations were lower in chylomicrons after BT than after the other fats but not in the chylomicron-free serum (containing chylomicron remnants), suggesting absorption in the form of very small particles. Compared with the fasting value, the concentration of the Svedberg unit of flotation 20–400 fraction, which contains VLDL and chylomicron remnants, was lower 8 h after MO, the only fat to contain significant amounts of linolenic acid. We conclude that chylomicron size is dependent on the fatty acid composition of ingested fats and the time-course of digestion, being larger for polyunsaturated fatty acid-rich fats and in the early phase of digestion. On the basis of retinyl ester concentration there were no differences between fats in chylomicron-remnant clearance.

Fat saturation: Post-prandial lipaemia: Retinyl esters

There is ample evidence that the nature of chronically ingested fat has a major impact on serum lipid and lipoprotein profiles and, further, on the development of atherosclerosis (Arntzenius *et al.* 1985; Keys *et al.* 1986; Mensink & Katan, 1989; Grundy & Denke, 1990; Lichtenstein *et al.* 1994). Long-term effects can be evaluated in the fasted state by means of the balance between LDL and HDL, which are in an opposing relationship with respect to obstructive pathology (Castelli *et al.* 1986). But the immediate postprandial state is potentially atherogenic (Zilversmit, 1979; Patsch *et al.* 1992; Karpe *et al.* 1994) and greatly dependent on the nature of the fats in the meal (Groot *et al.* 1988; Harris *et al.* 1988; Weintraub *et al.* 1988; Van Heek & Zilversmit, 1990; De Bruin *et al.* 1993; Zampelas *et al.* 1994; Muesing *et al.* 1995). Studies conducted in animals or human subjects have revealed differences at many levels: digestibility (Carey *et al.* 1983; Bergstedt *et al.* 1990; Bracco,

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1994), absorption (Ockner *et al.* 1972; Jones *et al.* 1985; Bergstedt *et al.* 1990), chylomicron metabolism (Coiffier *et al.* 1987; Groot *et al.* 1988; Weintraub *et al.* 1988; Levy *et al.* 1991) and impact on other lipoprotein classes (Demacker *et al.* 1991; De Bruin *et al.* 1993; Muesing *et al.* 1995).

The size of triacylglycerol (TG)-rich lipoproteins (TRL) is largely determined by the rapidity of TG overall absorption, as apolipoprotein (apo) B synthesis appears to be essentially constant (Hayashi *et al.* 1990); large TRL are produced when amounts of TG increase (Boquillon *et al.* 1977; Bennett-Clark & Norum, 1978; Hayashi *et al.* 1990). Also, the quality of ingested or infused TG affects TRL size; large chylomicrons are produced after ingestion of polyunsaturated fatty acids (PUFA), compared with VLDL-size particles secreted after ingestion of saturated fatty acids (SFA) (Boquillon *et al.* 1977; Feldman *et al.* 1983; Levy *et al.* 1991; Kalogeris & Story, 1992*a*) which are characterized by an elevated phospholipid (PL) : TG ratio (Kalogeris & Story, 1992*a*). On the other hand, large size and/ or PUFA-rich chylomicrons are rapidly cleared from serum compared with small and/or SFA-rich chylomicrons (Groot *et al.* 1988; Weintraub *et al.* 1988; Levy *et al.* 1991) due to differences in susceptibility to lipolytic enzymes (Coiffier *et al.* 1987; Weintraub *et al.* 1988). The quality of ingested fat also affects the metabolism of chylomicron remnants, which are thought to be atherogenic (Zilversmit, 1979); their serum concentrations are increased after SFA ingestion compared with PUFA ingestion (Weintraub *et al.* 1988; Demacker *et al.* 1991) or by long-chain SFA in the *sn*-2 position on the TG structure (Redgrave *et al.* 1988).

To date, few studies have compared the effects of PUFA or monounsaturated fatty acids (MUFA) on chylomicron and chylomicron-remnant metabolism (De Bruin *et al.* 1993) and on chylomicron size (Kalogeris & Story, 1992*b*). Chylomicrons are the main source of HDL-PL, following intravascular lipolysis (Eisenberg, 1984), and chylomicron size is affected by the quality of ingested fat. It is, therefore, of interest to compare the impact of single meals differing in fatty acid composition on such postprandial variables as chylomicron size, chylomicron-remnant clearance and accretion of PL which are likely to affect the HDL-PL pool quantitatively and qualitatively and, thus, influence its capacity to promote reverse cholesterol transport (Johnson *et al.* 1986; Davidson *et al.* 1995). The fats tested were: sunflower and oleic–sunflower oils as sources of linoleic and oleic acids respectively, a mixed oil containing approximately equal proportions of both linoleic and oleic acids plus 13 g linolenic acid/kg and 1 kg beef tallow as a source of long-chain SFA.

SUBJECTS AND METHODS

Subjects

The subjects were eleven healthy young women aged 25·5 (SD 2·9) years, with a BMI of 21·1 (SD 1·7) kg/m^2 and normal fasting lipid and LP concentrations (total cholesterol (TC) 4·51 (SD 0·72) mmol/l, TG 0·68 (SD 0·28) mmol/l, HDL-cholesterol 1·34 (SD 0·30) mmol/l). They were non-drinkers, non-smokers and were not on medication known to affect plasma lipids. No attempt was made to synchronize the tests with a given period of the menstrual cycle.

Methods

Test loads. Each subject received four consecutive fat loads in the same order at weekly intervals while maintaining her habitual diet. The tests were all performed at the same time of the day, on the same day of the week, in order to minimize behavioural variations. The

test load was given as an emulsion containing 35 g saccharose, 25 g skimmed-milk powder as protein source (Protifar Plus, Nutricia, France), 60 ml water and 60 g fat (67 % total energy) in the form of sunflower oil (SO), oleic–sunflower oil (OSO), mixed oil (MO; (g/kg) 420 SO, 380 OSO, 150 soyabean, 50 grapeseed oil) or beef tallow (BT), all provided by Lesieur Alimentaire (Boulogne-Billancourt, France). The fatty acid composition of each fat source is given in Table 1. The composition of MO was designed to provide balanced proportions of the fatty acids 18 : 2n-6 and 18 : 1n-9 with the addition of the essential fatty acid 18 : 3n-3 in a proportion sufficient to meet daily requirements. The BT load contained 1·1 mg cholesterol/g fat, while SO, OSO or MO contained only 4 mg phytosterols/g fat of which negligible amounts are absorbed (Lütjohann et al. 1995). Retinyl palmitate was added to the meal in a liquid form (30 000 μg; Arovit; Hoffman-La Roche).

On the evening before the test the subjects were requested to eat, no later than 20.30 hours, a standard meal containing 3·3 MJ. In the morning a small indwelling catheter was placed in the antecubital vein. The test load was ingested at 09.00 hours within a 15 min period. Water, but no food, was allowed during the following 8 h of the study. The four test meals were well tolerated by all subjects and no unpleasant side effects were reported.

Blood collection. Peripheral blood samples were collected in dry tubes before the fat load and at 2, 4, 6 and 8 h after the load. Serum was separated by centrifugation at 2500 rev./min for 15 min at 4°, protected from light and kept on ice.

Lipoprotein separation. Chylomicrons were removed from 6 ml serum layered with 4 ml NaCl (density 1·006 g/ml) by ultracentrifugation (25 000 rev./min, 30 min, 15°) in a SW41Ti rotor (Beckman, Gagny, France). Further subfractionation was achieved by a two-step ultracentrifugation procedure (Swaney et al. 1987) Chylomicron-free serum was first adjusted to a density of 1·21 g/ml and then centrifuged at 45 000 rev./min for 44 h at 15° in a 50TFT rotor (Kontron, Saint Quentin-en-Yvelines, France). Total lipoproteins were collected and separated on a discontinuous KBr gradient (density 1·006−1·21 g/ml) at 36 000 rev./min for 24 h at 15° in a SW41Ti rotor. The top 1 ml, containing the Svedberg

Table 1. *Fatty acid composition (g/100 g total fatty acids) of the oral fat loads* *

Fat source...	Sunflower	Oleic–sunflower	Mixed oil†	Beef tallow‡
Fatty acid				
14 : 0	—	—	—	3·3
16 : 0	6·2	4·0	6·2	26·3
16 : 1n-7	—	—	—	3·0
17 : 0	—	—	—	1·2
18 : 0	4·6	3·4	4·0	17·8
18 : 1n-9	19·1	71·7	37·7	36·8
18 : 1n-7	0·7	0·8	0·8	4·2
18 : 2n-6	67·2	18·0	48·4	4·4
18 : 3n-3	—	—	1·3	—
22 : 0	1·0	0·7	—	—
SFA	12·4	8·8	11·3	49·7
MUFA	20·3	73·1	39·0	45·1
PUFA	67·3	18·3	49·7	5·2

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; —, < 0·5 g/100 g total fatty acids.
*Values determined by GLC.
†Contained (g/kg): sunflower 420, oleic–sunflower 380, soyabean 150, grapeseed oil 50 (Lesieur Alimentaire, Boulogne-Billancourt, France).
‡Trans-fatty acids were included in 18 : 1n-9 and 18 : 1n-7.

unit of flotation (Sf) 20–400 fraction (VLDL and, postprandially, chylomicron remnants), was withdrawn with a syringe and the remainder of the gradient was collected through an ISCO density-gradient fractionator (ISCO, Lincoln, NE, USA) into 0·375 ml fractions, with simultaneous recording of optical density at 280 nm.

Biochemical determinations. TC, unesterified cholesterol (UC), TG, PL and non-esterified fatty acids (NEFA) were assayed by enzymic colorimetric procedures (for TC and UC, Boehringer Mannheim, Mannheim, Germany; for TG, PL and NEFA, Wako, Unipath, Dardilly, France). Proteins were quantified (Peterson, 1977) using bovine serum albumin as a standard. Chylomicron TG were obtained by extraction of total chylomicron lipids (Folch *et al.* 1957) followed by TLC (hexane–diethyl ether–formic acid; 80:19:1, by vol.) in the presence of butyryl hydroxytoluene (0·2 g/l). Fatty acids from the area containing TG were methylated (Morrison & Smith, 1964) and separated using a Carlo Erba gas chromatograph (model 4180; Fisons, Massy, France) equipped with an on-column injector and a capillary column (CPWax 52CB; length 50 m, diameter 0·3 mm; Chrompack, Les Ulis, France). Fatty acids were characterized by their retention time compared with that of standards. The peaks were integrated using Nelson software (Stang, Pavillon-sous-Bois, France). Retinyl esters (RE) in chylomicrons and chylomicron-free serum were assayed by reversed-phase HPLC (De Ruyter & De Leenheer, 1978).

Chylomicron diameter determination. Chylomicron diameters were estimated by filtration on 4 % agarose columns (A-15m; Biorad, France) in a 50 mM-Tris, 0·15 M-NaCl, pH 7·4 buffer. Diameters were calibrated using a standard suspension of IVELIP 20 % (Clintec, Velizy-Villacoublay, France) containing known proportions of particles with diameters accurately determined by centrifuge photosedimentation (CAPA 700 HORIBA). Of the particle population, 54 % had diameters between 100 and 300 nm, comprising the median of the distribution (200 nm). A linear relationship was obtained between the logarithm of particle diameters and their elution volumes (Fig. 1). Chylomicron populations were characterized by the mean diameter of the elution peak comprising over 55 % of the chylomicron population relative to diameters of IVELIP particles with the same elution characteristics. The method was sufficiently specific to show, in the same subject, a load effect (Fig. 2(A)) and a time effect (Fig. 2(B)).
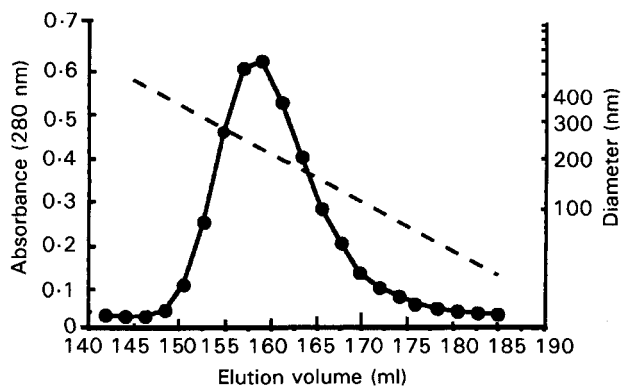

Fig 1. Calibration of an agarose column using a stable suspension (IVELIP 20%; Clintec, Velizy-Villacoublay, France) in which the distribution of diameters was accurately determined by centrifuge photosedimentation. By applying to the elution profile the percentage of each diameter class a linear regression was determined between elution volume and mean diameter in each class (- - - -). Calibration was repeated before each series of filtrations corresponding to a meal. For details of procedures, see p. 22.
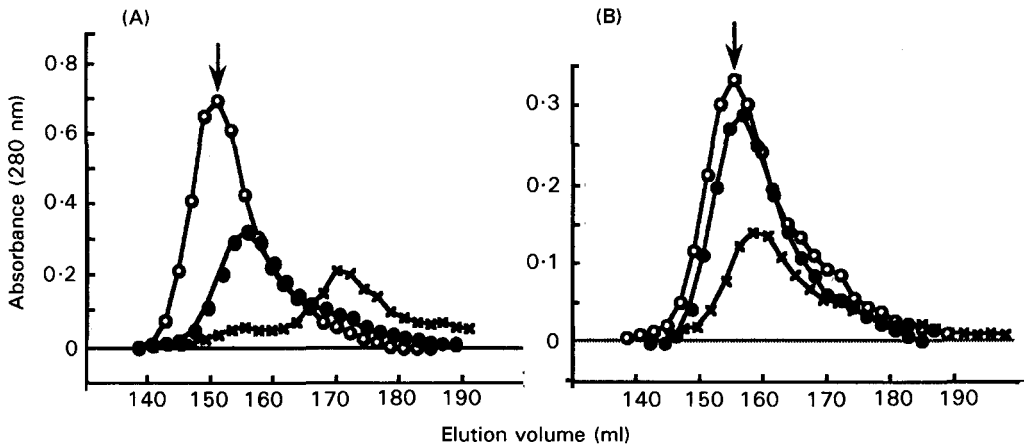
Fig. 2. Plots showing diameters derived from elution volumes in Fig. 1, for one subject (A) after different fat loads at peak elution (4 h), after sunflower oil (O; 280 nm), oleic–sunflower oil (●; 170 nm) or beef tallow (× ; 85 nm) and (B) at different times after OSO at 4 h (O; 170 nm), 6 h (●; 150 nm) or 8 h (× ; 120 nm). For details of procedures, see p. 22.

Statistical analysis. The magnitude of postprandial changes was quantified as the incremental area under the time curve (iAUC), calculated by the trapezoidal rule (Matthews *et al.* 1991). Results are reported as means and standard deviations and, in Figs, as means with their standard errors. All statistical tests were performed using the MGLH module of the Systat program (Wilkinson, 1990). Global ANOVA for repeated measurements including fat and time as trial factors was used. When F was significant, time effect for each fat effect at each time point were tested by ANOVA for two repeated measurements (paired *t* test). Wilcoxon's test was used in the comparison of chylomicron diameters. Associations between quantitative variables were tested by Pearson's correlation coefficient. A value of $P < 0.05$ was considered statistically different.

Ethical considerations. The study was approved by the Ethics Committee of Faculté de Médecine X, Bichat, Paris. The informed consent of each subject was obtained in the written form. Participation was remunerated.

RESULTS

Triacylglycerols

Chylomicron. TG response was affected by the fatty acid composition of ingested fat. While no difference was observed between vegetable oils, iAUC for chylomicron TG was two-thirds lower after BT than after SO, OSO or MO (Fig. 3(A)). Chylomicronaemia was lower after BT than after the other fats at each time point except 8 h postprandially. Chylomicron TG peaked at 2 and 4 h after the vegetable oils ($P < 0.001$ in each case except 4 h after OSO ($P < 0.01$) compared with fasting values) and later after the BT load. At 6 h after the fat load, chylomicron TG concentrations, although reduced by half compared with peak values after SO, OSO and MO, remained significantly elevated with respect to fasting values ($P < 0.001$ after SO and MO, $P < 0.01$ after BT or $P < 0.05$ after OSO) and even at 8 h for SO, MO and BT ($P < 0.01$, $P < 0.001$ and $P < 0.05$ respectively).
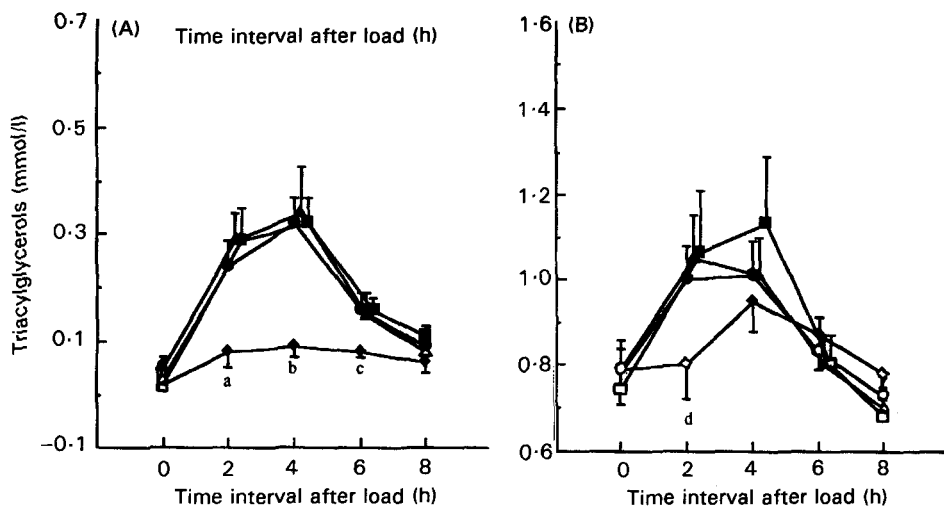
Fig. 3. Triacylglycerol concentrations (mmol/l) in (A) chylomicron and (B) chylomicron-free serum fraction after an oral load of sunflower oil (SO; ●, ○), oleic–sunflower oil (OSO; ▲, △), mixed oil (MO; ■, □;) or beef tallow (BT; ◆, ◇). Values are means with their standard errors represented by vertical bars for eleven subjects. For details of test loads and procedures, see Table 1 and pp. 20–23. Incremental area under the curve calculated by the trapezoidal rule was: (A) SO 1·36 (SE 0·18), OSO 1·32 (SE 0·36), MO 1·47 (SE 0·20), BT 0·41 (SE 0·09); (B) SO 0·91 (SE 0·23), OSO 0·85 (SE 0·28), MO 1·48 (SE 0·44), BT 0·48 (SE 0·15). ●, ▲, ■, ◆, Mean values were significantly different from those for fasting state. [a] Mean value was significantly different from those for SO ($P < 0.05$), OSO and MO ($P < 0.01$); [b] mean values were significantly different from those for SO ($P < 0.001$), OSO ($P < 0.05$) and MO ($P < 0.001$); [c] mean value was significantly different from SO ($P < 0.05$) and MO ($P < 0.001$); [d] mean value was significantly different from SO ($P < 0.01$), OSO ($P < 0.05$) and MO ($P < 0.01$).

Chylomicron-free serum. TG concentrations in the chylomicron-free serum also peaked at 2 and 4 h after vegetable oils ($P < 0.001$ after SO, $P < 0.01$ after MO and $P < 0.001$ and $P < 0.01$ at 2 and 4 h after OSO, compared with respective fasting values; Fig. 3(B)). After BT the maximum occurred later (4 h, $P < 0.001$, 6 h, $P < 0.01$, compared with fasting value) as in chylomicrons and at 2 h, TG concentrations were lower than those after the other fats. The differences in TG concentrations were smaller than those in the chylomicrons and the iAUC were not significantly different between fats.

Retinyl esters

Fasting serum RE concentrations of all subjects were below the detection limit. Postprandial concentrations were determined only at 4 and 8 h, i.e. at the peak of TG concentrations for all loads and after return to baseline respectively. Differences in RE paralleled those in TG concentrations; 4 h after the load the increase in chylomicron RE concentrations was lower for BT than those for SO, OSO and MO (Fig. 4(A)). In chylomicron-free serum, however, RE concentrations were not significantly lower after BT than after the other fats (Fig. 4(B)).

No significant differences between fat loads were observed in the RE : TG molar ratio in either the chylomicron or the chylomicron-free serum fraction (2·00 (SD 1·39), 1·85 (SD 1·00), 1·26 (SD 1·33), 1·09 (SD 1·20) mmol/mol in chylomicron fraction and 0·69 (SD 0·62), 0·53 (SD 0·22), 0·48 (SD 0·35), 0·42 (SD 0·32) mmol/mol in chylomicron-free serum 4 h after SO, OSO, MO and BT loads respectively). There were also no differences between ratios calculated at 4 and 8 h (data not shown).
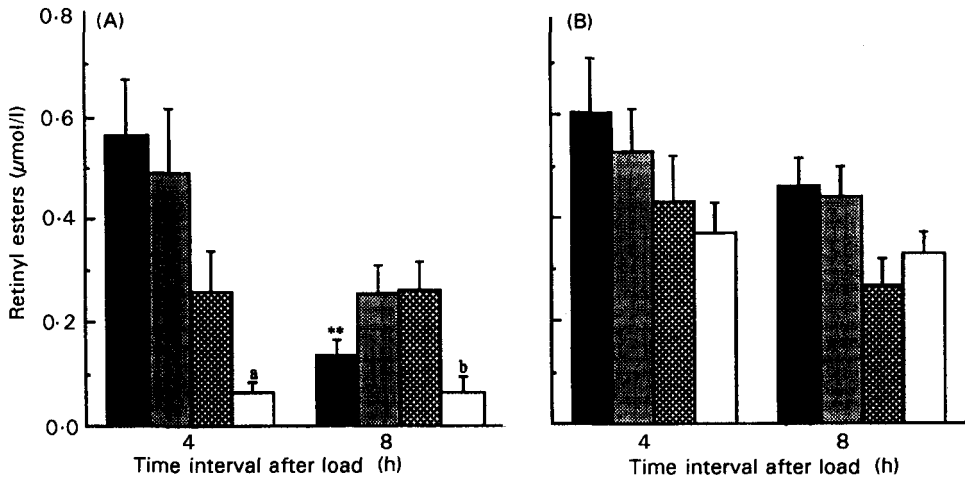
Fig. 4. Retinyl ester concentrations (μmol/l) in (A) chylomicron and (B) chylomicron-free serum fraction after an oral load of sunflower oil (SO; ■); oleic–sunflower oil (OSO; ■); mixed oil (MO; ▨); beef tallow (BT, □). Values are means with their standard errors represented by vertical bars for eleven subjects. For details of test loads and procedures, see Table 1 and pp. 20–22. [a]Mean value was significantly different from those for SO, OSO ($P < 0.01$), OSO and MO ($P < 0.05$); [b]mean value was significantly different from that for OSO ($P < 0.01$). Mean value was significantly different from fasting value: **$P < 0.01$.

Non-esterified fatty acids in whole serum

NEFA accumulated in serum after each fat load and their profile reflected that of the meal (Fig. 5). As expected, PUFA iAUC, calculated by the trapezoidal rule, was higher after SO than after OSO and BT ($P < 0.001$ and $P < 0.05$, respectively) and also after MO compared with OSO and BT ($P < 0.01$); MUFA accumulated in their free form after OSO, MO and BT ($P < 0.001$, $P < 0.05$ and NS respectively compared with SO).

Chylomicron triacylglycerol fatty acid composition

TG-fatty acids in the chylomicron fraction were determined at peak TG concentration after each fat load in three subjects. As shown in Table 2, fatty acids in chylomicron TG closely reflected those of the corresponding meal (Table 1).

Chylomicron diameter

At 6 and 8 h after each load, chylomicron diameters were markedly smaller than at peak TG absorption time ($P < 0.05$ at 6 and 8 h compared with 4 h), except after the OSO load for which a significant decrease with time was observed only between 6 and 8 h ($P < 0.05$; Fig. 6). Chylomicron size was influenced by the type of fat ingested. At the time of peak triacylglycerolaemia, i.e. 4 h, the SO load produced chylomicrons with consistently higher diameters than OSO ($P < 0.05$) and BT ($P = 0.06$). Also at 4 h, chylomicrons after the MO load were larger than those after BT ($P < 0.05$) and had an intermediate size between SO and OSO. At 6 h postprandially, chylomicrons from BT were smaller than those from vegetable oils ($P < 0.05$ in each case). At 8 h the difference was not significant.
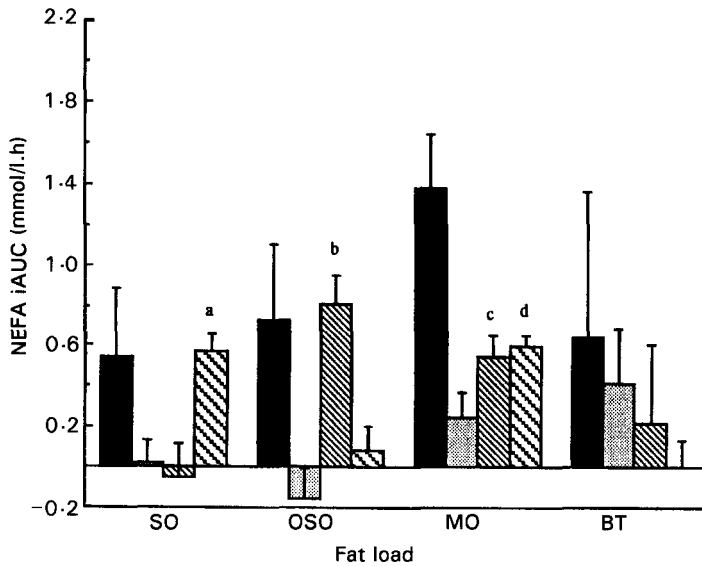
Fig. 5. Incremental area under the curve (iAUC) for non-esterified fatty acids (NEFA) in whole serum, expressed as total (■), saturated (▨), monounsaturated (▧) or polyunsaturated (▨) fatty acids, after an oral load of sunflower oil (SO), oleic–sunflower oil (OSO), mixed oil (MO) or beef tallow (BT). Values are means with their standard errors represented by vertical bars for eleven subjects. For details of test loads and procedures, see Table 1 and pp. 20–23. [a]Mean value was significantly different from those for OSO ($P < 0.001$) and BT ($P < 0.05$); [b,c]mean values were significantly different from those for SO: b $P < 0.001$, c $P < 0.05$; [d]mean value was significantly different from those for OSO and BT ($P < 0.01$).

Table 2. *Fatty acid composition (g/100 g fatty acids) of chylomicron triacylglycerols**

(Mean values and standard deviations for three subjects)

Fat source...	Sunflower		Oleic–sunflower		Mixed oil		Beef tallow	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fatty acid								
14:0	1·5	1·6	0·6	0·0	0·7	0·3	3·2	0·5
16:0	12·5	3·4	7·8	0·5	10·1	1·4	28·7	1·2
16:1n-9	—		—		—		1·0	0·5
16:1n-7	1·2	1·0	—		0·6	0·4	2·8	0·5
17:0	—		—		—		0·9	0·1
18:0	3·6	2·1	3·9	0·4	3·7	1·3	13·5	3·2
18:1n-9	21·5	1·8	68·7	1·3	40·2	2·1	39·1	1·6
18:1n-7	1·0	0·1	0·6	0·5	0·9	0·1	3·4	0·1
18:2n-6	55·9	2·1	15·2	0·1	41·5	6·8	5·8	0·6
18:3n-3	—		—		0·7	0·1	—	
SFA	18·4	2·5	13·4	1·0	15·2	3·7	47·2	1·9
MUFA	24·2	0·4	70·4	0·8	42·1	2·8	46·5	1·4
PUFA	57·3	1·9	16·2	0·2	42·6	6·6	6·4	0·5

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; —, < 0.5
    g/100 g total fatty acids.
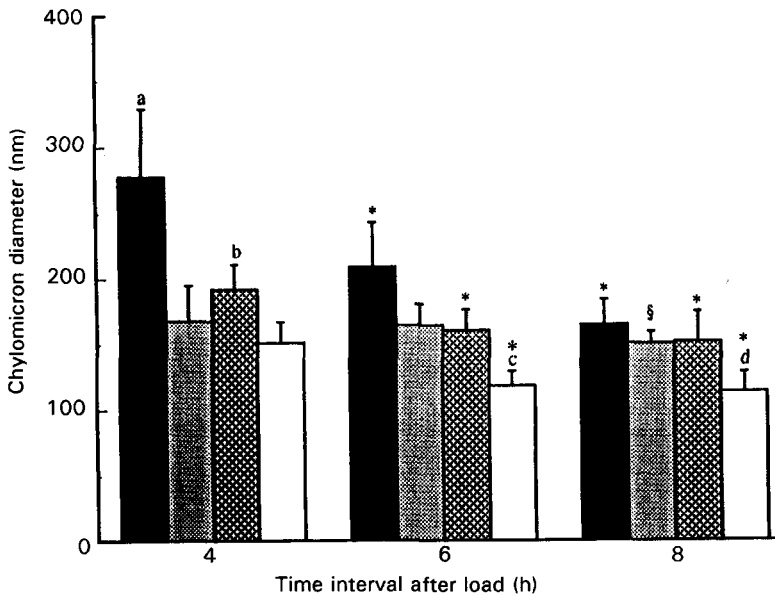*Values at the peak of chylomicron triacylglycerol concentration as determined by GLC.

Fig. 6. Chylomicron diameter, as determined by agarose-gel filtration (for details, see p. 22), 4, 6 or 8 h after an oral load of sunflower oil (SO; ■), oleic–sunflower oil (OSO; ▩), mixed oil (MO; ▨) or beef tallow (BT; □). Values are means with their standard errors represented by vertical bars for nine subjects. For details of test loads and procedures, see Table 1 and pp. 20–22. [a]Mean value was significantly different from those for OSO ($P < 0.05$) and BT ($P = 0.06$); [b]mean value was significantly different from that for BT ($P < 0.05$); [c]mean value was significantly different from those for SO, OSO and MO ($P < 0.05$); [d]mean value was significantly different from that for OSO ($P < 0.05$). Mean values were significantly different from those at *4 and §6 h ($P < 0.05$).

Table 3. *Total concentrations (mg/l)†of the Sf 20-400 fraction after the oral fat loads‡*

(Mean values and standard deviations for eleven subjects)

Fat source...	Sunflower		Oleic–sunflower		Mixed oil		Beef tallow	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Time (h)								
0	477	241	530	224	538	262	496	304
2	654	363*	813	317***	811	460**	565	271
4	773	439**	748	353*	856	380**	669	286**
6	519	200	625	289	527	206	589	308
8	413	199	430	195	358	98*	466	220

Mean values were significantly different from fasting value: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.
†Total concentration (mg/l) was calculated by addition of individual component concentrations.
‡For details of procedures and composition of test loads, see Table 1 and pp. 20–22.

Sf 20-400 fraction

The total concentration of the Sf 20-400 fraction (VLDL only in the fasting state, VLDL plus chylomicron remnants postprandially) increased significantly 2 and 4 h after SO, OSO and MO, but only at 4 h after BT (Table 3). At the end of the experimental period, concentrations were, in all cases, lower than those in the fasting state, and were significantly lower after the MO load. The chemical composition of the Sf 20-400 fraction

was influenced by the nature of ingested fat. The Sf 20-400 fraction was significantly enriched with PL at 6 and 8 h after the OSO load (18·2 (SD 1·8) and 18·5 (SD 2·2) *v.* 17·2 (SD 1·3) g/100 g, ($P < 0.01$ and $P < 0.05$ respectively) compared with fasting value) as well as at 8 h after BT (18·7 (SD 1·8) *v.* 17·5 (SD 0·7) g/100 g ($P < 0.05$) compared with fasting value) but not after SO and MO (data not shown).

DISCUSSION

Chylomicrons are the source of a major proportion of HDL surface material, in particular PL (Eisenberg, 1984). In this respect their size is of importance since the smaller they are the greater the PL : TG ratio (Fraser, 1970; Kalogeris & Story, 1992*a*), i.e. the greater the amount of PL available for transfer to HDL after TG lipolysis. During fat infusion, intestinal output of apoB does not change in the rat, and it is the size of the particles which increases, not their number (Bennett-Clark & Norum, 1978; Hayashi *et al.* 1990), depending on the availability of fatty acids to the enterocytes for esterification. This, in turn, is dependent on luminal concentration, lipolysis (Carey *et al.* 1983; Bergstedt *et al.* 1990) and intracellular esterification (Ockner *et al.* 1972). Unsaturated fatty acids, which have a lower melting point and a greater affinity for the fatty acid-binding protein (Ockner & Manning, 1976), are transported more rapidly and should thus give rise to larger chylomicrons than SFA. Experiments conducted in the rat have yielded contrasting results, some claiming no impact of fat saturation (Fraser *et al.* 1968; Renner *et al.* 1986), others finding larger particles after unsaturated fatty acids (Boquillon *et al.* 1977; Feldman *et al.* 1983; Levy *et al.* 1991; Kalogeris & Story, 1992*a,b*). Few data were available for human subjects. Our results show that chylomicron size is larger with SO at the peak of TG absorption and decreases with time after ingestion, especially in the case of SO, as TG transport slows down. The limited time effect with OSO, BT and MO is linked to the absence of very large chylomicrons at 4 h.

Chylomicron size was evaluated by a novel procedure involving the use, as a standard, of stabilized liposomes intended for intravenous infusion. The diameters of the standard preparations, provided by the manufacturer, were calculated with great accuracy on the basis of their combined sedimentation characteristics and density. These liposomes, comprising mainly TG coated with PL, are very close in structure to chylomicrons. It should be pointed out, however, that chylomicrons were isolated from the serum before agarose filtration. The possibility that they underwent some structural modifications in the process cannot be excluded, although cold storage, known to alter specific volume of SFA-rich chylomicrons (Bennett-Clark *et al.* 1982), was avoided. The purpose was not, however, as much to measure absolute volumes as to provide comparative values with respect to a common standard.

In the present experiment the fats were given in non-random order, in order to prevent storage of fats over extended periods. The order of ingestion was, however, unlikely to have any effect since a single meal is not likely to induce long-term changes. Also, they were given 1 week apart, a delay sufficient for complete renewal of the intestinal mucosa (Green & Glickman, 1981). No attempt was made to synchronize the tests with a given period of the menstrual cycle because the events studied were mostly of a digestive nature. Moreover, while lipoprotein metabolism is markedly affected by sex hormones when pre- and postmenopausal women are compared (Miller, 1994), their impact is limited within the cycle. Synchronization would have meant spreading the experiments over 4 months with the possibility of behavioural or seasonal changes of much greater importance.

The BT load produced the lowest chylomicronaemia and smallest chylomicrons,

although sizing was carried out at 20° in order to avoid, as far as possible, the artefacts linked to low temperatures (Bennett-Clark *et al.* 1982). On the one hand, the presence of long-chain SFA-rich TG is likely to result in poor digestibility (Carey *et al.* 1983; Berg-stedt *et al.* 1990) and, therefore, poor absorption. Moreover, the presence of long-chain SFA in the *sn*-1 and *sn*-3 positions is associated with poor absorption (Small, 1991), especially for stearic acid (Bracco, 1994). Based on its TG structure, BT should have been 60–70 % absorbed (Bracco, 1994). On the other hand, SFA-rich chylomicrons are less rapidly catabolized than PUFA-rich chylomicrons (Groot *et al.* 1988; Levy *et al.* 1991). Thus, lower chylomicronaemia after BT should result more from poor digestibility than accelerated catabolism. The TG and RE concentrations were very significantly lower in chylomicrons, but not in chylomicron-free serum containing chylomicron remnants. Moreover, the increase in NEFA concentration, reflecting chylomicron-TG lipolysis, was the same after BT as that after the other fats. It does appear, therefore, that BT was absorbed to a greater extent than that reflected by chylomicronaemia. Experiments conducted *in vitro* have shown that Caco-2 cells exposed to the fatty acid 18:2 secrete TG in the form of large chylomicrons with little PL (Van Greevenbroek *et al.* 1995). When the fatty acid is 16:0, more is incorporated into PL, the particles secreted are smaller and display a density in the IDL-LDL range (Van Greevenbroek *et al.* 1995). The high chylomicron-free serum RE:chylomicron RE ratio after BT, as well as the significant enrichment of the Sf 20-400 fraction with PL at 8 h, are compatible with the presence of this type of very small PL-rich particles in a fraction with a density higher than that of chylomicrons which usually contains mostly chylomicron remnants. This could also be the case for OSO which produced smaller chylomicrons than SO at 4 h and a PL enrichment of the VLDL and chylomicron-remnant-containing fraction.

Thus, it appears that in human subjects, as in the rat, the nature of ingested fatty acids qualitatively influences chylomicron production. The repective importances of rapidity of transport and of PL synthesis in the determination of size remain to be evaluated. An impact of the fatty acid composition of the diet on the supply of PL to HDL may contribute to the reduction in the HDL pool found with linoleic acid-enriched diets (Grundy & Denke, 1990).

After the MO load, concentrations of Sf 20-400 were lower by 35 % with respect to the fasting value and by 17 % with respect to the other fat loads at 8 h. This preparation contained nearly equal amounts of linoleic acid and oleic acid which, when given alone, yielded Sf 20-400 concentrations which were not different. The main difference in composition was the presence of 13 g linolenic acid /kg in MO. Whether the effect is one of stimulation of chylomicron-remnant uptake or of repression of hepatic TG synthesis, it is interesting to note that such a small proportion of linolenic acid is likely to have a significant effect, although it has not been as clearly identified as an inhibitor of VLDL synthesis as the longer-chain *n*-3 PUFA (Harris, 1989).

REFERENCES

Arntzenius, A. C., Kromhout, D., Barth, J. D., Reiber, J. H. C., Bruschke, A. V. J., Buis, B., van Gent, C. M., Kempen-Voogd, N., Strikwerda, S. & van der Velde, E. A. (1985). Diet, lipoproteins, and the progression of coronary atherosclerosis. The Leiden intervention trial. *New England Journal of Medicine* **312**, 805–811.

Bennett-Clark, S., Atkiuson, D., Hamilton, J. A., Forte, T., Russell, B., Feldman, E. B. & Small, D. M. (1982). Physical studies of d < 1·006 g/ml lymph lipoproteins from rats fed palmitate-rich diets. *Journal of Lipid Research* **23**, 28–41.

Bennett-Clark, S. & Norum, K. R. (1978). Intestinal lymph chylomicron cholesteryl ester during duodenal triolein infusion at increasing rate. *Scandinavian Journal of Gastroenterology* **13**, 983–990.

Bergstedt, E. E., Hayashi, H., Kritchevsky, D. & Tso, P. (1990). A comparison of absorption of glycerol tristearate and glycerol trioleate at rat small intestine. *American Journal of Physiology* **259**, G386–G393.

Boquillon, M., Paris, R. & Clement, J. (1977). The effect of various dietary factors on the size distribution of lymph fat particles in rat. *Lipids* **12**, 500–504.

Bracco, U. (1994). Effect of triglyceride structure on fat absorption. *American Journal of Clinical Nutrition* **60**, Suppl., 1002S–1009S.

Carey, M.C., Small, D. M. & Bliss, C. M. (1983). Lipid digestion and absorption. *Annual Review of Physiology* **45**, 651–677.

Castelli, W. P., Garrison, R. J., Wilson, P. W. F., Abbott, R. D., Kalousdian, S. & Kannel, W. B. (1986). Incidence of coronary heart disease and lipoprotein levels: The Framingham Study. *Journal of the American Medical Association* **256**, 2835–2838.

Coiffier, E., Paris, R. & Lecerf, J. (1987). Effects of dietary saturated and polyunsaturated fats on lipoprotein lipase and hepatic triglyceride lipase activity. *Comparative Biochemistry and Physiology* **88B**, 187–192.

Davidson, W. S., Gillotte, K. L., Lund-Katz, L., Johnson, W. J., Rothblat, G. H. & Phillips, M. C. (1995). The effect of high density lipoprotein phospholipid acyl chain composition on the efflux of cellular free cholesterol. *Journal of Biological Chemistry* **270**, 5882–5890.

De Bruin, T. W. A., Brouwer, C. B., van Linde-Sibenius, T. M., Jansen, H. & Erkelens, D. W. (1993). Different postprandial metabolism of olive oil and soybean oil: a possible mechanism of the high density lipoprotein conserving effect of olive oil. *American Journal of Clinical Nutrition* **58**, 477–483.

Demacker, P. N. M., Reijnen, I. G. M., Katan, M. B., Stuyt, P. M. J. & Stalenhoef, A. M. F. (1991). Increased removal of remnants of triglyceride-rich lipoproteins on a diet rich in polyunsaturated fatty acids. *European Journal of Clinical Investigation* **21**, 197–203.

De Ruyter, M. G. M. & De Leenheer, A. P. (1978). Simultaneous determination of retinol and retinyl esters in serum or plasma by reversed-phase high-performance liquid chromatography. *Clinical Chemistry* **24**, 1920–1923.

Eisenberg, S. (1984). High density lipoprotein metabolism. *Journal of Lipid Research* **25**, 1017–1058.

Feldman, E. B., Russel, B. S., Hawkins, C. B. & Forte, T. (1983). Intestinal lymph lipoproteins in rats fed diets enriched in specific fatty acids. *Journal of Nutrition* **113**, 2323–2334.

Folch, J., Lees, M. & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* **226**, 497–509.

Fraser, R. (1970). Size and lipid composition of chylomicrons of different Svedberg units of flotation. *Journal of Lipid Research* **11**, 60–65.

Fraser, R., Cliff, W. J. & Courtice, F. C. (1968). The effect of dietary fat load on the size and composition of chylomicrons in thoracic duct lymph. *Quarterly Journal of Experimental Physiology* **53**, 390–398.

Green, P. H. R. & Glickman, R. M. (1981). Intestinal lipoprotein metabolism. *Journal of Lipid Research* **22**, 1153–1173.

Groot, P. H. E., De Boer, B.C. J., Haddman, E., Houtsmuller, U. M. T. & Hülsmann, W. C. (1988). Effect of dietary fat composition on the metabolism of triacylglycerol-rich plasma lipoproteins in the postprandial phase in meal-fed rats. *Journal of Lipid Research* **29**, 541–551.

Grundy, S. M. & Denke, M. A. (1990). Dietary influences on serum lipids and lipoproteins. *Journal of Lipid Research* **31**, 1149–1172.

Harris, W. S. (1989). Fish oils and plasma lipid and lipoprotein metabolism in humans: a critical review. *Journal of Lipid Research* **30**, 785–807.

Harris, W. S., Connor, W. E., Alam, N. & Illingworth, D. R. (1988). Reduction of postprandial triglyceridemia in humans by dietary n-3 fatty acids. *Journal of Lipid Research* **29**, 1451–1460.

Hayashi, H., Fujimoto, K., Cardelli, J. A., Nutting, D. F., Bergstedt, S. & Tso, P. (1990). Fat feeding increases size, but not number, of chylomicrons produced by small intestine. *American Journal of Physiology* **259**, G709–G719.

Johnson, W. J., Bamberger, M. J., Latta, R. A., Rapp, P. E., Phillips, M. C. & Rothblat, G. H. (1986). The bidirectional flux of cholesterol between cells and lipoproteins: effects of phospholipid depletion of high density lipoprotein. *Journal of Biological Chemistry* **261**, 5766–5776.

Jones, P. J. H., Pencharz, P. B. & Clandinin, M. T. (1985). Absorption of ^{13}C-labeled stearic, oleic, and linoleic acids in humans: application to breath tests. *Journal of Laboratory and Clinical Medicine* **105**, 647–652.

Kalogeris, T. J. & Story, J. A. (1992a). Lymph chylomicron size is modified by fat saturation in rats. *Journal of Nutrition* **122**, 1634–1642.

Kalogeris, T. J. & Story, J. A. (1992b). Lymph chylomicron composition and size are modified by level of intestinally infused cholesterol and triglyceride source in rats. *Journal of Nutrition* **122**, 1045–1055.

Karpe, F., Steiner, G., Uffelman, K., Olivecrona, T. & Hamsten, A. (1994). Postprandial lipoproteins and progression of coronary atherosclerosis. *Atherosclerosis* **106**, 83–97.

Keys, A., Menotti, A., Karvonen, M. J., Aravanis, C., Blackburn, H., Buzina, R., Djordjevic, B. S., Dontas, A. S., Fidanza, F., Keys, M. H., Kromhout, D., Nedeljkovic, S., Punsar, S., Seccareccia, F. & Toshima, H. (1986). The diet and 15 year death rate in the seven countries study. *American Journal of Epidemiology* **124**, 903–915.

Levy, E., Roy, C. C., Goldstein, R., Bar-On, H. & Ziv, E. (1991). Metabolic fate of chylomicrons obtained from rats maintained on diets varying in fatty acid composition. *Journal of the American College of Nutrition* **10**, 69–78.

Lichtenstein, A. H., Ausman, L. M., Carrasco, W., Jenner, J. L., Ordovas, J. M. & Schaefer, E. J. (1994). Hypercholesterolemic effect of dietary cholesterol in diets enriched in polyunsaturated and saturated fat. Dietary cholesterol, fat saturation, and plasma lipids. *Arteriosclerosis and Thrombosis* **14**, 168–175.

Lütjohann, D., Björkhelm, I., Beil, U. F. & Von Bergmann, K. (1995). Sterol absorption and sterol balance in phythsterolemia evaluated by deuterium-labeled sterols: effect of sitostanol treatment. *Journal of Lipid Research* **36**, 1763–1773.

Matthews, J.N.S., Altman, D. G., Campbell, M.J. & Royston, P. (1991). Analysis of serial measurements in medical research. *British Medical Journal* **300**, 230–235.

Mensink, R. P. & Katan, M. B. (1989). Effect of a diet enriched with monounsaturated or polyunsaturated fatty acids on levels of low-density and high-density lipoprotein cholesterol in healthy women and men. *New England Journal of Medicine* **321**, 436–441.

Miller, V. T. (1994). Lipids, lipoproteins, women and cardiovascular disease. *Atherosclerosis* **108**, Suppl., S73–S82.

Morrison, W. & Smith, L. (1964). Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. *Journal of Lipid Research* **5**, 600–608.

Muesing, R. A., Griffin, P. & Mitchell, P. (1995). Corn oil and beef tallow elicit different postprandial responses in triglycerides and cholesterol, but similar changes in constituents of high-density lipoprotein. *Journal of the American College of Nutrition* **14**, 53–60.

Ockner, R. K. & Manning, J. A. (1976). Fatty acid binding protein. Role in esterification of absorbed long chain fatty acid in rat intestine. *Journal of Clinical Investigation* **58**, 632–641.

Ockner, R. K., Pittman, J. P. & Yager, J. L. (1972). Differences in the intestinal absorption of saturated and unsaturated long chain fatty acids. *Gastroenterology* **62**, 981–992.

Patsch, J. R., Miesenböck, G., Hopfervieser, T., Mühlberger, V., Knapp, E., Dunn, J. K., Gotto, A. M. Jr & Patsch, W. (1992). Relation of triglyceride metabolism and coronary artery disease. Studies in the postprandial state. *Arteriosclerosis and Thrombosis* **12**, 1336–1345.

Peterson, G. L. (1977). A simplification of the protein assay method of Lowry *et al.* which is more generally applicable. *Analytical Biochemistry* **83**, 346–356.

Redgrave, T. G., Kodali, D. R. & Small, D. M. (1988). The effect of triacyl-*sn*-glycerol structure on the metabolism of chylomicrons triacylglycerol-rich emulsions in the rat. *Journal of Biological Chemistry* **263**, 5118–5123.

Renner, F., Samuelson, A., Rogers, M. & Glickman, R. M. (1986). Effect of saturated and unsaturated lipid on the composition of mesenteric triglyceride-rich lipoproteins in the rat. *Journal of Lipid Research* **27**, 72–81.

Small, D. M. (1991). The effects of glyceride structure on absorption and metabolism. *Annual Review of Nutrition* **11**, 413–434.

Swaney, J. B., Orishimo, M.W. & Girard, A. (1987). Enzymatically induced alterations in the structure of rat serum lipoproteins. *Journal of Lipid Research* **28**, 982–992.

Van Greevenbroek, M. M. J., Voorhout, W. F., Willem Erkelens, D., Van Meer, G. & De Bruin, T. W. A. (1995). Palmitic acid and linoleic acid metabolism in Caco-2 cells: different triglyceride synthesis and lipoprotein secretion. *Journal of Lipid Research* **36**, 13–24.

Van Heek, M. & Zilversmit, D. B. (1990). Postprandial lipemia and lipoprotein lipase in the rabbit are modified by olive and coconut oil. *Arteriosclerosis* **10**, 421–429.

Weintraub, M.S., Zechner, R., Brown, A., Eisenberg, S. & Breslow, J. L. (1988). Dietary polyunsaturated fats of the n-6 and n-3 series reduce postprandial lipoprotein levels. *Journal of Clinical Investigation* **82**, 1884–1893.

Wilkinson, L. (1990). *Systat, The System for Statistics. Version* 5.03 Evanston, IL: Systat Inc.

Zampelas, A., Peel, A.S., Gould, B. J., Wright, J. & Williams, C. M. (1994). Polyunsaturated fatty acids of the n-6 and n-3 series: effects on postprandial lipid and apolipoprotein levels in healthy men. *European Journal of Clinical Nutrition* **48**, 842–848.

Zilversmit, D. B. (1979). Atherogenesis: a postprandial phenomenon. *Circulation* **60**, 473–485.

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