Amounts and Types of Fatty Acids in Meals Affect the Pattern of Retinoids Secreted in Human Chylomicrons After a High-Dose Preformed Vitamin A Intake

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High doses of preformed vitamin A are commonly used to correct vitamin A deficiency. Newly absorbed vitamin A is secreted mainly as retinyl esters in chylomicrons. The effect of changing types and amounts of fatty acids on fatty acid composition of chylomicron retinoid esters when a high dose of vitamin A is ingested have not been studied previously. In the present study, 10 healthy young men ingested, in a random order, mixed meals containing 15,000 retinol equivalents (RE) of vitamin A (as retinyl palmitate) and either no fat or 40 g of fat provided as butter, olive oil, or sunflower oil. Fasting and postprandial blood samples were obtained for 7 hours after meals. Free retinol and the main retinyl esters (retinyl palmitate/oleate, stearate, and linoleate) were measured in chylomicrons by high-performance liquid chromatography (HPLC). Chylomicron retinyl palmitate/oleate and retinyl stearate concentrations significantly increased after intake of the 4 test meals. Conversely, chylomicron retinyl linoleate and chylomicron free retinol significantly increased only after the sunflower and the fat-free meals, respectively. The main retinoid secreted in chylomicrons after the intake of the fat-rich meals was retinyl palmitate/oleate, accounting for 63% to 79% of total RE, but it was free retinol after the fat-free meal (51% of total RE). Thus, the retinoid pattern secreted in chylomicrons after the intake of a high dose of preformed vitamin A depends on type and amounts of fatty acids ingested. To explain this result we suggest that the esterification process of retinol in the enterocyte by lecithin:retinol acyltransferase can be overwhelmed by a high load of vitamin A. Consequently, a significant proportion of the retinol is esterified by acyl coenzyme A:retinol acyltransferase (ARAT) with ingested fatty acids, explaining the appearance of retinyl linoleate in chylomicrons after the sunflower oil meal. If a high dose of preformed vitamin A is ingested with a fat-free meal, a significant proportion of retinol is not esterified, owing to the lack of fatty acids for ARAT, which explains the appearance of free retinol in chylomicrons.

IN HUMANS, vitamin A is provided either by preformed vitamin A from animal sources, mainly as retinyl esters, or by provitamin A carotenoids from plant sources, mainly as β-carotene.[1] The dietary proportions of these 2 forms of vitamin A depend on dietary habits, and range from 100% vitamin A as provitamin A carotenoids in vegetarians to most vitamin A as preformed vitamin A in people eating very little fruit and vegetables. In developing countries vitamin A deficiency is still a major health problem causing night-blindness, blindness, and ultimately death. Among the proposed strategies to correct this deficiency, the distribution of capsules containing a high dose of preformed vitamin A is widely used.

Dietary retinyl esters are hydrolyzed in the duodenum lumen[2-4] and unesterified retinol is taken up by enterocytes, involving both diffusion and protein-mediated facilitated transport. At physiological concentrations retinol is absorbed by facilitated diffusion, whereas at pharmacological concentrations it can be absorbed by passive diffusion. Once in enterocytes, retinol complexed with CRBP(II) is re-esterified by lecithin:retinol acyltransferase (LRAT),[5] while uncomplexed retinol in membranes may be esterified by acyl coenzyme A:retinol acyltransferase (ARAT).[6-10] Under a physiological vitamin A load, LRAT is the primary enzyme responsible for the esterification of retinol. ARAT esterifies excess retinol when pharmacological doses of vitamin A are absorbed and CRBP(II) becomes saturated. Palmitate, stearate, oleate, and linoleate account for the great majority of the fatty acids that esterify retinol. It has been shown that after fat-containing meals, retinyl esters are secreted in the approximate relative proportions of 55% palmitate, 26% stearate, 12.5% oleate, and 6.5% linoleate.[9,11] It has been assumed, from early results,[11,12] that changing dietary fat has very little effect on the pattern of retinoids secreted in chylomicrons. Chylomicrons reach the general circulation by way of the intestinal lymph, and represent an important mechanism for delivering vitamin A to tissues.[13-16] They carry retinyl esters to the liver where they are either stored in hepatic stellate cells, after hydrolysis and re-esterification, or bound to retinol binding protein (RBP) and transthyretin (TTR), to be distributed to peripheral tissues.

Since retinyl esters are by far the main retinoids recovered in chylomicrons after the intake of vitamin A with fat-containing meals,[11,12] the presence of free retinol in chylomicrons has not been systematically studied. Yet low amounts of this retinoid are secreted by caco-2 cells[17] and are found in rat chylomicrons.[12,18,19] Moreover, a mass transfer of free retinol from chylomicrons to plasma protein, low-density lipoprotein (LDL), and high-density lipoprotein (HDL), but not of retinyl esters, has been described,[18] suggesting that the fate of chylomicron free retinol is different from that of chylomicron retinyl esters. A better understanding of the factors that affect the proportion of free retinol in chylomicrons is needed.

The aim of this study was to assess the effect of varying the type and amount of fatty acids in meals on the pattern of

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retinoids secreted in chylomicrons after intake of a high dose of preformed vitamin A. For this purpose we measured, in 10 healthy young men, the chylomicron retinoid responses elicited by the intake of meals that provided a similar high load of preformed vitamin A together with varying amounts and types of fatty acids, given as dietary fatty acids.

MATERIALS AND METHODS

Subjects

Ten adult male volunteers (aged 20 to 29 years) participated in the study after giving their written informed consent to a protocol approved by the local Medical Ethics Committee (Comité Consultatif pour la Protection des Personnes se prêtant à des Recherches Biomédicales, Marseille). No volunteer suffered from any digestive or metabolic disease as checked by medical history and fasting blood parameters. No subject was obese (body mass index [BMI]: 22.1 ± 0.80 kg/m^2) and body weights did not vary significantly during the experiment. The subjects had not taken any medication known to interfere with lipid or vitamin A metabolism for months. They were instructed not to deviate from regular habits during the study period. The usual basal diet of each subject was monitored by a 3-day food record during the first week of the experiment and calculations were performed with a GENI software package (Micro 6, Nancy, France).

Subject Characteristics

The subjects ate a typical Western diet, with a moderate energy consumption (mean, 11,616.3 ± 491.9 kJ/d), and protein, carbohydrate, and fat accounting for 13.9% ± 0.7%, 40.0% ± 2.3%, and 45.5% ± 2.7% total energy intake, respectively. Daily intakes of saturated, monounsaturated, and polyunsaturated lipids were 66.4 ± 5.7, 58.0 ± 5.2, and 16.8 ± 1.5 g, respectively. Alcohol intake was negligible. Daily intake of retinol was 440 ± 84 μg.

The mean fasting lipid serum parameters were very close and not different ($P > .05$) at the time of the 4 tested meals, and lay in the normal range (triacylglycerols, 0.72 to 0.88 mmol/L; total cholesterol, 4.69 to 4.73 mmol/L).

Test Meals and Sampling

The 4 experimental test meals were presented in a random order. The interval between successive test meals was 5 to 7 days. As previously,[20] to prevent a possible effect of the previous meal, the subjects were asked to eat a light meal before 8 PM the evening before the experiment.

Three mixed meals containing the same amount of dietary fat (40 g) but from different fatty acid sources, ie, butter, olive oil, or sunflower oil, and a meal without any fat (fat-free) were tested. Butter mainly provided saturated fat (66.4 g/100 g) as C4:0-C12:0 (14.1 g/100 g), C14:0 (11.0 g/100 g), C16:0 (30.0 g/100 g), C18:0 (11.3 g/100 g), and C18:1 (25.0 g/100 g). Olive oil mainly provided C16:0 (11.0 g/100 g), C18:1 (75.5 g/100 g), and C18:2 (8.5 g/100 g). Sunflower oil mainly provided C18:2 (67.0 g/100 g) and C18:1 (21.2 g/100 g). The meals consisted of commercially available food and comprised 2 slices of French bread, 150 g of cooked pasta, 50 g of tomato sauce, one no-fat yogurt (125 g), and the tested fat (40 g) or no fat in the case of the fat-free meal. The vegetable oils were incorporated into the tomato sauce and butter spread on bread. The so-called no-fat test meal provided a negligible amount of fat (about 1.3 g). Retinyl palmitate (Theraplix, Montrouge, France) was added at 15,000 retinol equivalents (RE) per meal. During the 7-hour postprandial period, the participants were allowed to drink 200 mL of water and a decaffeinated coffee (100 mL).

After an overnight fast, an antecubital vein was catheterized with an intravenous cannula equipped with disposable obturators (Jelco-Critikon, Chatenay-Malabry, France). A baseline (0 hours) fasting blood sample was collected. The subjects then ingested the test meal in 20 minutes. Blood samples (15 to 20 mL) were obtained every hour for 7 hours as previously.[20,21]

From blood samples collected in tubes containing EDTA, plasma was separated from whole blood by centrifugation (910 × g, 4°C, 10 minutes).

Chylomicron Separation

Chylomicrons (Sf > 1,000) were isolated as described previously[20] from 1 mL plasma layered under 2 mL NaCl 0.9% by ultracentrifugation at 10°C for 20 minutes at 25,000 × g in a Beckman TL100 ultracentrifuge and 100.3 rotor (Palo Alto, CA). Chylomicrons were stored at −80°C under nitrogen until analyzed.

Chromatographic Analysis of Retinoids

Chylomicron retinoid extraction was performed at room temperature under red light. Proteins were precipitated by adding 1 vol of ethanol containing retinyl laurate as internal standard and 50 mg/mL butylated hydroxytoluene as antioxidant. Two volumes of hexane (containing 50 mg/mL butylated hydroxytoluene) were added. The mixture was then vortexed and centrifuged (5 minutes, 500 × g, 4°C). Samples were hexane-extracted twice, and the upper hexane phases were pooled and evaporated to dryness under nitrogen. The residue was then dissolved in a dichloromethane/methanol (65/35 vol/vol) mixture, and subjected to reverse-phase high-performance liquid chromatography (HPLC; Kontron apparatus with MT2 software [Kontron, Lyon, France]) to measure retinol and retinyl esters (retinyl palmitate + retinyl oleate, which were not separated under these chromatographic conditions, retinyl stearate and retinyl linoleate). Compounds were separated on a monomeric C_{18} column (Nucleosil, 5μm, 250 × 4.6 mm, Interchim, Montluçon, France). The mobile phase was 100% methanol (2 mL/min). Compounds were detected at 325 nm using a UV detector (Detector 430, Kontron) and identified by retention times of pure (>95%) retinoid standards. All-*trans* retinol was a gift from Hoffmann La Roche (Basel, Switzerland). Retinyl palmitate, retinyl stearate, and retinyl linoleate were prepared as previously described.[22] Retinyl laurate[22] was used as internal standard.

Calculations and Statistical Analysis

In this randomized study, each subject experimented the four test meals and served as his own control. Results are expressed as means ± SEM. Incremental meal response values (mean of 10 determinations) are expressed as variations of concentration over baseline (fasting baseline values being zero). The areas under the curves (AUCs) of the postprandial chylomicron retinoid responses (delta from fasting values) were calculated by the trapezoidal rule. Changes in postprandial chylomicron retinoid concentration were analyzed by 1-way analysis of variance (ANOVA) with time as factor. AUCs were compared with ANOVA. When significant ($P < .05$) differences were detected, means were compared between each other using the post-hoc Tukey/Kramer test. The statistical comparisons were performed using StatView software version 5.0 (SAS Institute, Cary, NC).

RESULTS

Postprandial Chylomicron Retinyl Palmitate/Oleate and Stearate Responses to the Test Meals

Figure 1 shows that whatever the test meal there was a significant increase in chylomicron retinyl palmitate/oleate and stearate concentrations ($P < .05$, ANOVA with time as factor). In the case of the fat-free meal, the retinyl palmitate/oleate and retinyl stearate peaks were observed 3 hours after meal intake. In the case of the butter meal, they appeared after 2 to 3 hours,

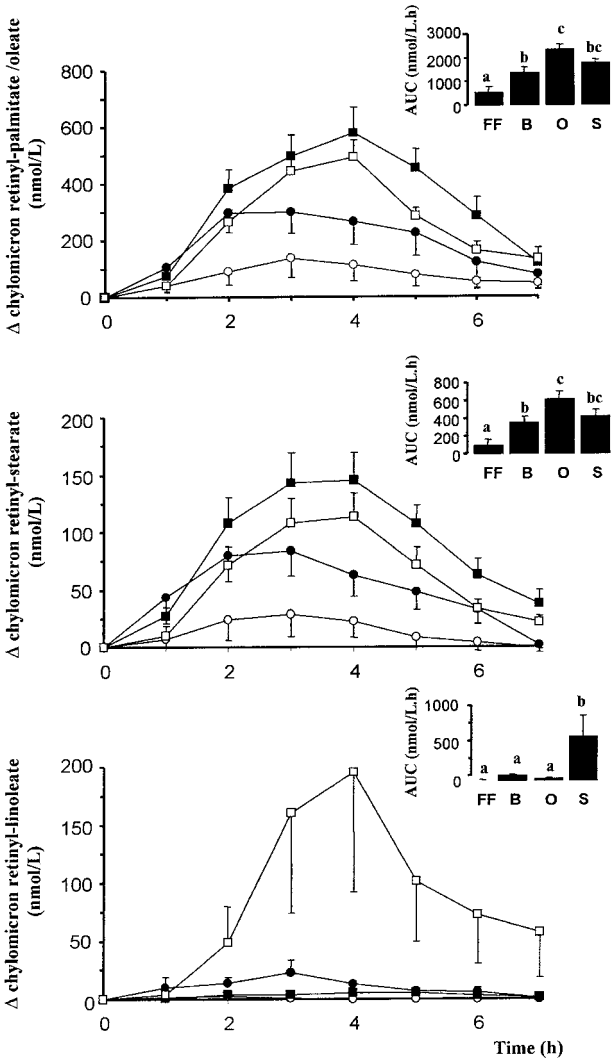

Fig 1. Postprandial changes in chylomicron retinyl palmitate (A) and retinyl stearate (B) and retinyl linoleate (C) concentrations elicited by intake of meals that contained no fat (○) or 40 g fat as butter (●), olive oil (■), or sunflower oil (□). Inserts show the calculated AUCs obtained after each test meal: FF (fat-free meal), B (butter meal), O (olive oil meal), S (sunflower oil meal). Results are expressed as mean ± SEM (n = 10). Different letters indicate a significant difference (*P* < .05) between AUCs obtained for each retinyl ester (ANOVA followed by post-hoc Tukey/Kramer's test).

but after 4 hours in the case of the olive and sunflower oil meals. Chylomicron retinyl palmitate/oleate AUCs, which represented the whole postprandial chylomicron retinyl palmitate/oleate responses, were significantly higher after the long-chain fatty acid–rich meals (2,330 ± 219 and 1,756 ± 168 nmol/L · h for the olive and sunflower oil meals, respectively) than after the fat-free meal (534 ± 261 nmol/L · h). Similar results were observed for retinyl stearate responses, ie, AUCs for the long-chain fatty acid–rich meals were significantly higher than those observed for the fat-free meal (611 ± 85; 419 ± 70 and 93 ± 70 nmol/L · h for the olive, sunflower, and fat-free meals, respectively).

Postprandial Chylomicron Retinyl Linoleate Responses to the Test Meals

As shown in Fig 1C, despite the wide variability in chylomicron retinyl linoleate concentrations observed after the sunflower oil meal, the chylomicron retinyl linoleate concentrations significantly increased only after intake of this linoleic acid–rich meal ($P < .05$, ANOVA with time as factor). Consequently the chylomicron retinyl linoleate AUC was significantly higher after intake of the sunflower oil meal than after intake of the other meals (611 ± 80 nmol/L · h *v* 2 ± 4, 72 ± 18, and 23 ± 12 nmol/L · h for the fat-free, butter, and olive oil meals, respectively). Note that the maximum increase in chylomicron retinyl linoleate was observed at 4 hours after meal intake.

Postprandial Chylomicron Retinol Responses to the Test Meals

The chylomicron retinol concentrations exhibited a wide variability (Fig 2). No significant increase in chylomicron retinol concentrations was observed after the fat-rich meals (ANOVA with time as factor). Conversely, a significant variation in chylomicron retinol concentrations was observed after intake of the fat-free meal. The resulting AUC was 634 ± 189 nmol/L · h.

Total Amount of Retinoids and Pattern of Retinoids Secreted in Chylomicrons After the Different Meals

The postprandial chylomicron retinol and retinyl ester responses were converted into retinol equivalents (RE/L · h) and the whole chylomicron retinoid responses obtained after each test meal were calculated (Fig 3A). The intake of preformed vitamin A with the long-chain fatty acid–rich meals (olive oil and sunflower oil meals) resulted in significantly higher retinoid responses (10,283 ± 1,032 and 9,741 ± 994 RE/L · h, for the olive oil and the sunflower oil meal, respectively) than its

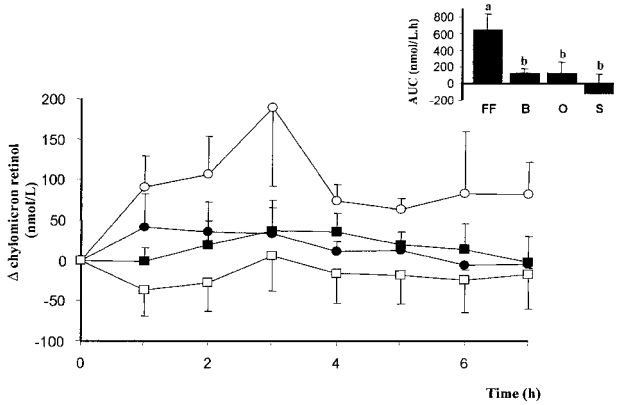

Fig 2. Postprandial changes in chylomicron retinol concentration elicited by intake of meals which contained no fat (FF, ○) or 40 g fat as butter (●), olive oil (■), or sunflower oil (□). Insert shows the calculated AUCs obtained after each test meal: FF (fat-free meal), B (butter meal), O (olive oil meal), S (sunflower oil meal). Results are expressed as mean ± SEM (n = 10). Different letters indicate a significant difference, (*P* < .05) between AUCs (ANOVA followed by post-hoc Tukey/Kramer's test).

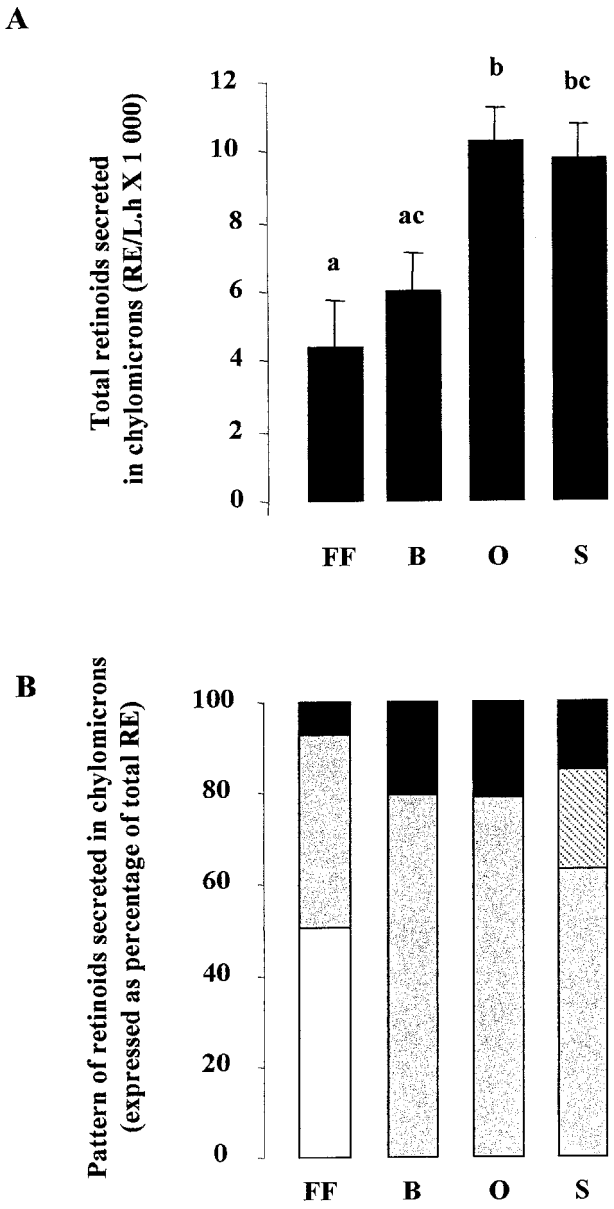

Fig 3. (A) Total retinoids (RE) secreted in chylomicrons after intake of meals that provided no fat (FF) or 40 g fat as butter (B), olive oil (O) or sunflower oil (S). Only retinoids that significantly increased in each meal were summed. Results are mean ± SEM (n = 10). Different letters indicate a significant difference ($P < .05$) between bars (ANOVA followed by post-hoc Tukey/Kramer's test). (B) Pattern of retinoids present in chylomicrons after intake of the different meals: (□) retinol, (▩) retinyl palmitate/oleate, (■) retinyl stearate, (▨): retinyl linoleate. Results are expressed as percent of total retinoids (% RE), mean (n = 10).

intake with the fat-free meal (4,444 ± 1,370 RE/L · h). Butter intake gave an intermediate response (5,957 ± 1,095 RE/L · h).

For each meal the distribution of the various retinoids recovered in the chylomicrons was expressed as whole retinoids recovered in the chylomicrons (Fig 3B). Note that only retinoids whose concentrations varied significantly in the postprandial period were taken into account for these calculations. The main retinoid secreted in the chylomicrons after the fat-rich meals was retinyl palmitate/oleate which accounts for 63% to 79% of total RE response. After the fat-rich meals retinyl stearate accounted for 15% to 21% of total RE response. After the sunflower oil meal, retinyl linoleate, whose concentration did not significantly vary following the other meals, represented 22 % of the total RE response. Finally, the main retinoid secreted in chylomicrons after consumption of the fat-free meal was retinol (51% of total RE response).

DISCUSSION

Three main effects of fatty acids in meals on postprandial vitamin A metabolism were observed in this study. They concern the amount of retinoids secreted in chylomicrons, the pattern of retinoids secreted in chylomicrons, and the presence of free retinol in these lipoproteins.

Figure 3A clearly shows that the intake of a high dose of preformed vitamin A without fat clearly impairs total retinoid secretion in chylomicrons. It could be wrongly concluded that preformed vitamin A bioavailability was diminished when the meal did not contain fat. However, retinoids were measured only in large chylomicron remnants and we missed retinoids present in smaller chylomicron remnants. Yet these remnants can represent a large proportion of blood retinoids in the postprandial period, particularly after a low-fat diet where chylomicron lipolysis by lipoprotein lipase is very efficient, owing to the smaller amount of chylomicrons. It has been shown that preformed vitamin A bioavailability was not significantly diminished with a fat-free meal when whole plasma retinyl esters were measured.[23]

The fatty acid composition of chylomicron retinyl esters obtained after the fat-containing meals was in close agreement with previous results.[9,11] Retinyl palmitate/oleate and retinyl stearate were the main retinoids recovered in chylomicrons representing about 70% and 25% total RE activity, respectively. Conversely, the fact that the retinyl ester pattern was affected by the type of fatty acids ingested (there was a significantly higher proportion of retinyl linoleate after the sunflower oil meal than after the olive oil and butter meals) apparently conflicts with the assumption that dietary fat does not markedly affect the fatty acid composition of chylomicron retinyl esters.[13,24] However, careful reading of the original study[11,12] reveals that the proportion of retinyl linoleate was higher after intake of a trilinolein meal than after intake of triolein and corn-olive oil meals, which agrees with our results. The wide variability of retinyl linoleate response after the sunflower oil meal (see Fig 1 SEM) was due to the fact that 5 subjects out of 10 did not exhibit a chylomicron retinyl linoleate increase after the sunflower oil meal. This suggests that the events that led to the appearance of significant amounts of retinyl esters in the chylomicrons depended on individual characteristics.

The fact that chylomicron free retinol significantly increased after the fat-free meal, and that free retinol was the main retinoid (in % of total RE activity) recovered in the chylomicrons after this meal, was noteworthy. Although it has been shown that free retinol is secreted by caco-2 cells[6,17] and that it is incorporated in rat chylomicrons,[18] this is the first time that such a huge proportion of free retinol is described in human

chylomicrons. Levin[6] hypothesized that free retinol secretion by caco-2 cells in his experiment was due to an insufficient total lipid load to stimulate chylomicron formation and/or secretion.

From the results obtained in this study, and from previous knowledge of retinol metabolism in the enterocyte,[25] we suggest the following mechanisms to explain the results obtained. When a pharmacological dose of preformed vitamin A is ingested (about 20 times the vitamin A recommended daily allowance in this study), LRAT capacities are overwhelmed, the esterification of retinol by ARAT becomes significant, and retinyl esters are produced using ingested fatty acids. This can explain the appearance of retinyl linoleate after the sunflower meal. When a high dose of preformed vitamin A is ingested together with a small amount of fatty acids, LRAT capacities are overwhelmed, and ARAT cannot esterify the excess of free retinol because of shortage of newly absorbed fatty acids. In these conditions a large fraction of retinol is incorporated in its free form in chylomicrons. This mechanism can explain why amounts and species of triacylglycerols affect the pattern of retinoids secreted in chylomicrons only when a pharmacological dose of preformed vitamin A is ingested.

In conclusion, this study establishes that the retinoid pattern secreted in chylomicrons can be affected by the type and amount of dietary fatty acids when high-dose preformed vitamin A is ingested. Two new findings on postprandial vitamin A metabolism have been obtained: first, a significant proportion of retinoids can be found as free retinol in chylomicrons when a meal provides a pharmacological dose of preformed vitamin A and trace amounts of fatty acids. Second, the proportion of chylomicron retinyl linoleate can become significant when a pharmacological dose of preformed vitamin A is ingested with a meal rich in linoleic acid.

It is not known whether different retinoids have different metabolisms in terms of tissue distribution of vitamin A, but since it has been shown that free retinol can transfer from chylomicrons while esterified retinol cannot,[18] it is possible that chylomicron retinol will reach peripheral tissues more easily than retinyl esters. This might have practical consequences when high-dose preformed vitamin A is given to vitamin A–deficient people. If, as is usual, the vitamin A–deficient people also have a poor protein status, the normal route of vitamin A delivery, ie, the RBP-TTR-retinol complex, is impaired. Then it would better to give vitamin A supplement without triacylglycerols to produce chylomicron retinol, and thus facilitate vitamin A distribution to peripheral tissues. On the contrary, the fact that significant amount of free retinol can be produced when pharmacological amounts of vitamin A are ingested with trace amounts of fatty acids raise the question of the potential toxicity of this supplementation. Indeed, there is the possibility that such a supplementation lead to the production of potentially toxic amount of retinoic acid from free retinol. Further research is required to verify these assumptions, but our findings should be kept in mind when high-dose preformed vitamin A is given to vitamin A–deficient persons.

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