Plasma Kinetics of a Chylomicron-Like Emulsion in Normolipidemic Obese Women After a Short-Period Weight Loss by Energy-Restricted Diet

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Chylomicrons carry dietary fats in the bloodstream for storage in body tissues, and thus play an important role in obesity. The 2-step chylomicron metabolism consists of lipolysis by lipoprotein lipase (LPL) on vessel walls and hepatic uptake of triglyceride-depleted remnants. A triglyceride-rich emulsion that mimics chylomicrons, labeled with [9,10-^{3}H]glycerol-trioleate (TG) and [1-^{14}C] cholesteryl-oleate (CE) was intravenously injected into 14 obese women with body mass index between 30 and 40 kg/m^{2} (age, 30 to 40 years), before and after a 2-month energy-restricted diet and into non-obese controls for determination of radioactive lipid plasma kinetics. TG kinetics evaluates lipolysis, whereas CE kinetics evaluates remnant removal. The emulsion TG fractional clearance rate (FCR, in min^{-1}) was similar in obese women and their controls (0.126 ± 0.065; controls, 0.111 ± 0.031), but the CE-FCR was pronouncedly reduced in the obese subjects (0.028 ± 0.014; controls, 0.070 ± 0.009 min^{-1}; $P < .0001$). After the energy-restricted diet, TG-FCR was reduced in the obese women (0.075 ± 0.044 min^{-1}; $P < .05$), but CE-FCR was unchanged (0.032 ± 0.025 min^{-1}). Therefore, the lipolysis of the chylomicron-like emulsion is normal in obese women, but remnant removal from the plasma is diminished. After active weight loss by an energy-restricted diet, the remnant removal was unchanged but lipolysis was diminished, possibly due to adaptative changes in LPL activity.

OBESITY IS FREQUENTLY accompanied by alterations in plasma lipids, mainly by fasting hypertriglyceridemia.[1,2] As measured after a 12-hour fasting period, concentration in the plasma of triacylglycerols mostly reflects the concentration of very-low-density lipoprotein (VLDL), the triacylglycerol-rich lipoprotein synthesized by the liver. VLDL accumulation in obese subjects may be due to saturation of the lipolytic process by excessive production of the lipoprotein. Increased VLDL synthesis by the liver is consequent to the enhanced supply of free fatty acids drained from the abdominal fat stores to the liver. This process is mediated by endocrine factors and high plasma concentration of insulin and cortisol are often found in subjects with the central form of obesity.[3-5] Low plasma HDL-cholesterol may also be found in obesity consequent to high triacylglycerols. Because of the action of transfer proteins, net mass transfer of cholesterol from high-density lipoprotein (HDL) to VLDL occurs when VLDL concentration increases. Low-density lipoprotein (LDL)-cholesterol may be increased, but in some studies this was not found.[6,8]

Chylomicrons are the triacylglycerol-rich lipoproteins synthesized in the enterocyte from the fats and other lipids ingested in the diet and absorbed by the intestine. They share a common catabolic pathway with liver-produced VLDL. Similarly to VLDL, chylomicrons undergo the action of lipoprotein lipase (LPL) on the capillary wall, triggered by apolipoprotein CII (apo CII), one of the apolipoproteins present on the surface of the lipoprotein particles. Apo CII possesses a domain that binds to the enzyme and another that stimulates the enzymatic action. LPL can break chylomicron triacylglycerols down to glycerol and fatty acids, which are thus absorbed in several body tissues, especially muscle and adipose tissue, where the compounds are re-esterified and stored.[9,10] This mechanism is important for the energetic economy of the organism, since fat constitutes its greatest storage energy source. By the action of hormone-sensitive lipase, fats stored in the body tissues can again be broken down and released into the circulation for disposal by the liver when energy expenditure is required.[9,10] After lipolysis by LPL, the resulting smaller particles, called chylomicron remnants, unbind from the enzyme molecules and return to circulation. They are then sequestered into the space of Disse and taken up by the liver cells by various receptor mechanisms, mainly the LDL receptors and LDL receptor-related protein (LRP).[9-11] Apo E is the main ligand of chylomicron remnants to the hepatic receptors.[12] Among the lipoprotein classes, chylomicrons possess the most rapid mechanism for plasma clearance, their half-life being about 15 to 20 minutes.[9,10] As described above, the metabolism of chylomicrons is directly involved in the excess of fat stores that defines the obese organism.

Despite its crucial importance, only a few studies have addressed the issue of chylomicron metabolism in obese subjects. To shed more light on this issue, we studied a group of obese women before and after weight reduction was achieved by a 2-month energy-restricted diet period. In weight reduction programs, greater weight loss is usually achieved during this initial short period. Chylomicron metabolism was assessed using a triacylglycerol-rich emulsion model that mimics the intravascular behavior of chylomicrons.[13] The emulsion is double-labeled with radioactive cholesteryl esters and triacylglycerols. After intravenous injection into the subjects, determination of the plasma decaying curves of the labeled lipids allows the 2-step metabolism of chylomicrons to be followed. The

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chylomicron-like emulsion model is a useful tool that facilitates human studies of this metabolic pathway. The triglyceride-rich emulsion approach has been validated in human subject studies.[14,15] It has revealed chylomicron metabolism defects in malignant hypertension,[16] systemic lupus erythematosus,[17] and heart transplant recipients,[18] and has been used to investigate effects of lipid-lowering drugs on chylomicron metabolism.[19,20] In this study, we also determined the plasma kinetics of the chylomicron-like emulsion in a control group of normal-weight women.

SUBJECTS AND METHODS

Subjects

Fourteen obese female volunteers with body mass index (BMI) between 30 and 40 kg/m^2 and aged 30 to 40 years participated in the study. Exclusion criteria were apparent or reported diseases, amenorrhea, pregnancy or breast feeding, alcohol abuse, use of antihyperlipidemia or anti-obesity medications, as well as dietary regimen for weight loss for the last 6 months. All participants were sedentary, were not smokers, and none had arterial hypertension. A control group consisting of 14 volunteer women aged 30 to 40 years with BMI less than 25 kg/m^2 was also selected according to the criteria described above.

The design and the objective of the study were explained to each participant before the study, and informed written consent was obtained from all. The study was approved by the Scientific and Ethics Committee of the Heart Institute of the Medical School Hospital of the University of São Paulo.

Weight Loss Treatment

The group of obese women was submitted to treatment for weight loss over a period of 2 months with the assistance of a multiprofessional team composed of a nutritionist, endocrinologist, and psychologist. Treatment was based on nutritional education and stimulation of changes in eating behavior. The participants were seen individually by the nutritionist once a week for dietary education and planning and were instructed to reduce energy and fat ingestion and to increase the ingestion of vegetables rich in fiber. The changes in eating habits were induced while respecting the sociocultural context and the limits of each individual. The control of food ingestion was performed using a daily eating record beginning the week that preceded the experiment.

Emulsion Preparation

The emulsion was prepared as previously described,[21] with addition to the lipid mixtures of [1-^{14}C]cholesteryl-oleate (CE; specific activity, 2.07 Gbq/mmol) and [9,10-^{3}H]glycerol-trioleate (TG; specific activity, 518 GBq/mmol), supplied by Amersham International (Cardiff, UK). The emulsion was purified by 2-step ultracentrifugation, as described previously,[22] sterilized by passage through a 0.2-μm filter, and evaluated for sterility and pyrogenicity prior to injection into the patients.

Kinetics of the Emulsion

The determination of the plasma kinetics of the chylomicron-like emulsion was performed before the dietary intervention in both controls and obese subjects. In the 2 obese groups, the plasma kinetics test was repeated after the 2-month energy-restricted diet. The emulsion fraction injected in each subject had a lipid mass of approximately 3.0 mg in 500 μL vol. [^{14}C] and [^{3}H] radioactivities of the labeled lipids were 74 kBq (2 μCi) and 148 kBq (4.0 μCi), respectively. The emulsion was injected by intravenous bolus after a 12-hour fast. Blood samples were collected from another peripheral vein at pre-established intervals over 45 minutes. Blood was centrifuged and the radioactivity contained in 1.0 mL of plasma was measured by liquid scintillation counting (Packard 1.660 TR, Meridien, CT). The safety of the radioactive dose injected into the subjects was warranted according to radioprotection regulations[23] as described elsewhere.[15]

Compartmental Analysis

The removal of the emulsion from the plasma was evaluated by compartmental analysis according to a modification of the model proposed by Redgrave et al.[14] Briefly, 4 compartments were employed to estimate the kinetic parameters for both ^{14}C-CE and ^{3}H-TG tracers. The plasma hydrolysis and removal of native chylomicrons, as well as chylomicron-like emulsions, displayed a rapid initial decay followed by a slow removal phase.[10,15,24] The $k_{x,y}$ constants represent the transfer or fractional catabolic rates (FCRs) from compartment x to compartment y. The kinetics of ^{14}C-CE and ^{3}H-TG metabolism are represented by compartments 1 to 4 and 5 to 8, respectively. The rapid and slow decay phases evaluated by the ^{14}C-CE and ^{3}H-TG tracers are represented by $k_{1,3}$ and $k_{2,3}$ and by $k_{5,7}$ and $k_{6,7}$, respectively. The model also takes into account the recirculation of the radioactive tracers in plasma in the form of newly synthesized VLDL (expressed by $k_{3,4}$ and $k_{7,8}$ for the ^{14}C and ^{3}H, respectively). The percentage of TG removed by the action of LPL was calculated from the differences between the areas under the curve for the removal of ^{14}C-CE and of ^{3}H-TG. All calculations were performed using a computer software.[25] The details of the compartmental analysis calculations were published previously.[20]

Biochemical Analysis

Serum triacylglycerols, total, VLDL-, LDL-, and HDL-cholesterol, apo A1 and apo B, and glucose were determined from blood samples taken after a 12-hour fast using a automatic instrument (Cobas Mira Plus, Roche, Basel, Switzerland). Total cholesterol and triacylglycerols were determined with the aid of enzymatic test kits (CHO-PAD, Boehringer [Penzberg, Germany] and Abbott [Lake County, IL], respectively). HDL-cholesterol was determined with the same method, after precipitation of LDL and VLDL with $MgCl_2$ and phosphotungstic acid. VLDL- and LDL-cholesterol were calculated using the Friedewald equation.[26] The obese subjects were submitted to a glucose tolerance test before dietary intervention.

Statistical Analysis

All recorded variables were tabulated as means $\pm$ SD or SEM. The differences in the obtained data were evaluated by the Student's *t* test, paired or unpaired when appropriate, with the level of significance set at $P < .05$ for all comparisons.

RESULTS

Plasma, Lipids, Apolipoproteins, and Glucose

Table 1 shows the individual physical characteristics and the plasma lipid and apoliprotein profiles of the obese women and their controls. Obese women had plasma lipid cholesterol and apo B and A1 values similar to the controls. Fast triacylglycerols showed a trend to be greater in the obese, but this was not statistically significant.

Fasting glucose plasma concentration in obese women was within the normal range (4.1 $\pm$ 1.2 mmol). Postprandial glycemia determined 2 hours after the ingestion of the glucose load was below the 7.77-mmol/L cut-off value for glucose intolerance[27] in all but 1 obese woman.

Table 1. Individual Physical Characteristics and Plasma Lipid and Apoliprotein Profiles (mean ± SD) in Controls and Obese Women Before and 2 Months After an Energy-Restricted Diet

		Obese Women	
	Controls	Baseline	After Diet
Age (y)	37 ± 4	37 ± 5	
BMI (kg/m^2)	23.3 ± 0.98	33.5 ± 3.4*	30.9 ± 4.2§
Weight (kg)	58.0 ± 4.70	85.9 ± 15.2*	79.7 ± 14.1§
Waist/hip ratio	0.75 ± 0.05	0.84 ± 0.06†	0.82 ± 0.07
Fasting glycemia (mmol/L)	3.88 ± 0.83	4.1 ± 1.2	4.1 ± 1.1
Postprandial glycemia (mmol/L)	4.33 ± 1.50	4.5 ± 1.6	
Triacylglycerols (mmol/L)	1.22 ± 0.62	1.49 ± 0.88	1.17 ± 0.76‡
Cholesterol			
Total (mmol/L)	4.68 ± 0.62	4.62 ± 0.47	4.55 ± 0.46
VLDL (mmol/L)	0.56 ± 0.29	0.67 ± 0.41	0.54 ± 0.34‡
LDL (mmol/L)	2.81 ± 0.60	2.82 ± 0.49	2.87 ± 0.52
HDL (mmol/L)	1.32 ± 0.33	1.14 ± 0.21	1.14 ± 0.21
Apo A1 (g/L)	1.72 ± 0.22	1.68 ± 0.43	1.59 ± 0.32
Apo B (g/L)	1.12 ± 0.18	0.95 ± 0.30	0.90 ± 0.29

*$P < .001$, †$P < .005$ by unpaired Student's t test for controls in relation to the obese women at baseline.

‡$P < .005$, §$P < .01$ by paired Student's t test for baseline in relation to the obese women after diet.

Food Ingestion Before and During the Energy-Restricted Dietary Period

Table 2 lists data on the estimated dietary composition before and during the energy-restricted dietary period. During the week preceding the period of dietary intervention, there was a wide variation in the amount of ingested daily energy by the obese women, as calculated from their foodstuff intake records. Daily energy consumption was 8.36 ± 2.51 MJ/d (minimum, 4.48; maximum, 13.00 MJ/d). During the 2-month period of energy-restricted diet, the daily food intake was reduced to 4.60 ± 1.26 MJ/d, which corresponds to a 45% reduction in energy consumption.

Effect of the Energy-Restricted Diet on Anthropometric Measurements

After the 2-month dietary period, the obese women lost 7% of their initial weight. Figure 1 shows the week-to-week weight loss, expressed in grams. It is clear that there was a trend for diminished weight loss as the dietary period advanced. While the weight loss was on average 1,500 g/wk at the first week of the dietary period, at the last week it was only 300 g/wk. The obese women showed reductions in all monitored anthropometric measurements (including triceps, biceps, subscapular, and suprailiac skinfolds; data not shown), except for the waist/hip ratio, which was unchanged (Table 1).

Table 2. Dietary Composition (mean ± SD) in Obese Women Before baseline and During the Energy-Restricted Diet Period

	Baseline	Energy-Restricted Diet
Energy (MJ/d)	8.36 ± 2.51	4.60 ± 1.26†
Fat (g/d)	73 ± 35	31 ± 12†
Protein (g/d)	93 ± 28	55 ± 19†
Carbohydrate (g/d)	240 ± 114	148 ± 46*

*$P < .05$, †$P < .005$ by paired Student's t test for baseline in relation to the same group after diet.

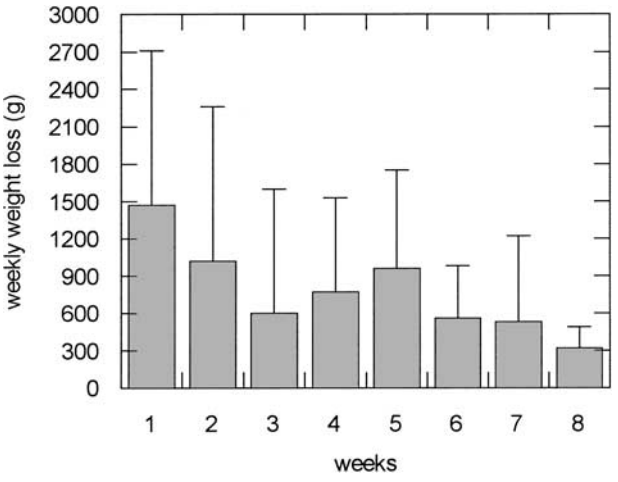

Fig 1. Weekly amount of weight loss during the 2-month dietary energy restriction period.

Effects of Energy-Restricted Diet on Plasma Lipids and Glucose

Table 1 shows the effects of the 2-month energy-restricted diet period on plasma lipids and apolipoproteins. Triacylglycerols and VLDL-cholesterol values were reduced. LDL- and HDL-cholesterol, as well as apo B, apo A1, and fasting plasma glucose, were unchanged.

Emulsion Plasma Kinetics in the Obese

Figure 2 shows the plasma decay curves of the emulsion radioactive lipids obtained in obese women and their controls studied before the 2-month energy-restricted diet period. As expected, the decay curve of the emulsion triacylglycerol moiety was faster than that of the emulsion cholesteryl ester in all subjects. It is apparent that the triglyceride decay curves of the obese subjects and the controls were similar, but the cholesteryl ester curve of the obese subjects was slower than that of the controls.

Table 3 lists the plasma kinetic data calculated from the curves. TG-FCR was similar compared to the controls. CE-FCR, however, was smaller in the obese groups compared to the controls. Thus, the rate of triacylglycerols removed by lipolysis was estimated to be approximately 4-fold that of the controls. This difference in CE-FCR was largely due to the $k_{1\text{-}3}$ constant, which was 3-fold smaller in the obese subjects. According to the compartmental model used to analyze the data, the $k_{1\text{-}3}$ constant is related to the first exponential of the decay curve.

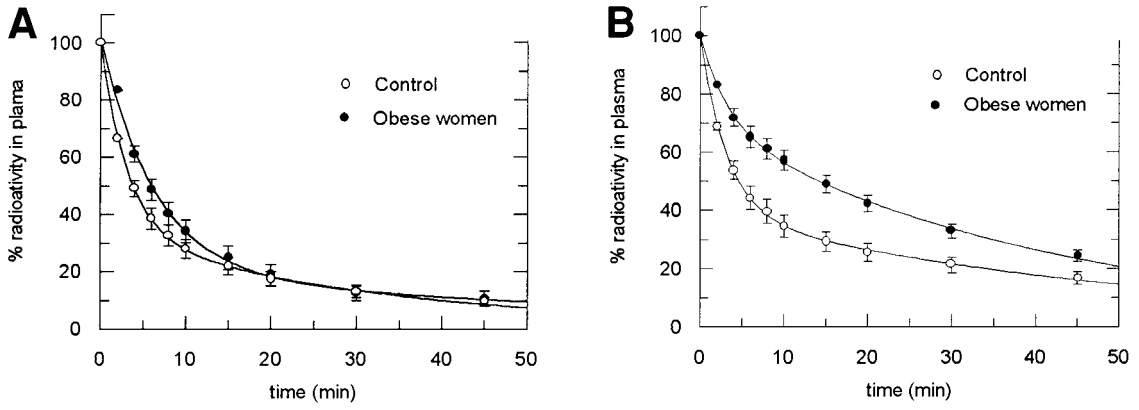

Fig 2. Removal from the plasma of the emulsion (A) [9,10-^{3}H]glycerol-trioleate and (B) [1-^{14}C]cholesteryl-oleate in the obese women and in the control normal-weight group. The emulsion labeled with the radioactive lipids was injected intravenously after a 12-hour fast. Plasma samples were taken at regular intervals over 45 minutes to determine the radioactivity remaining in the plasma in a scintillation solution.

Effects of Energy-Restricted Diet on Emulsion Kinetics

As shown Table 3 and Fig 3, TG-FCR was reduced by approximately 40% in obese women after the 2-month dietary period. Regarding triglyceride kinetics, the transfer constant $k_{6,7}$ was reduced, while $k_{7,8}$ was increased by the energy restriction, resulting in a net reduction of TG-FCR.

CE-FCR was apparently unchanged. Because TG-FCR was reduced by the diet, the rate of emulsion triacylglycerols removed by lipolysis was reduced. A 62% reduction was estimated.

DISCUSSION

Although not confirmed statistically, the obese women showed the trend for increase in fasting plasma triacylglycerols that in other studies has been associated with obesity[1,6] and ascribed to VLDL overproduction by the liver.[28]

The 7% weight loss obtained at the end of the 2-month dietary period confirms that the adherence of the participants to the program was good, similar to other studies that followed equivalent restriction dietary protocols. Weight reductions in the 5% to 10% range lead to changes in plasma lipids,[29,30] mainly in fast triacylglycerols.[31,32] In fact, after the dietary period, the obese women showed diminution of triacylglycerols. On the other hand, the weight loss had no effects on total, LDL-, or HDL-cholesterol, nor on the plasma concentrations of apo B and apo A1. This outcome is consistent with results of other studies in female subjects, where the alterations in LDL- and HDL-cholesterol after weight reduction were small or absent.[33,34]

Among the methods to evaluate chylomicron metabolism, the use of double-labeled chylomicron-like emulsions injected into the bloodstream provides a useful study tool. Although devoid of apolipoproteins, the emulsions in contact with plasma acquire exchangeable apolipoproteins such as apo CII and apo E. Thus, the emulsion triacylglycerols undergo hydrolysis by LPL stimulated by apo CII and the resulting remnant particles are taken up by receptors in the liver that recognize apo E, similarly to chylomicron metabolic behavior. As documented previously,[15] the cholesteryl ester component is not substantially removed from the emulsion to other lipoprotein density classes.[35] Therefore, the radioactive cholesteryl ester is in fact the marker of the emulsion particles while in the intravascular compartment, and the finding of diminished CE-FCR indicates that the formed emulsion remnants were slowly removed from the circulation. This can be ascribed to the inhibitory effect of great fat ingestion on the receptor mechanisms that remove lipoproteins such as the LDL receptor, which also plays a major role in remnant removal.[11]

Because the TG-FCR was equal to that of the controls, it is presumed that the emulsion suffered greater lipolysis in the obese subjects, since lipolysis is estimated from the CE-FCR minus TG-FCR integration. Increased lipolysis rates in the

Table 3. Plasma Kinetics Parameters of the Emulsion (mean ± SEM) in Obese Women Before and After 2 Months on an Energy-Restricted Diet

Parameters	Controls	Obese Women	
		Baseline	After diet
TG-FCR (min^{-1})	0.111 ± 0.031	0.126 ± 0.065	0.075 ± 0.044§
$k_{5,6}$	0.092 ± 0.031	0.138 ± 0.189	0.202 ± 0.023
$k_{5,7}$	0.305 ± 0.129	0.261 ± 0.308	0.231 ± 0.270
$k_{6,7}$	0.042 ± 0.018	0.061 ± 0.024	0.032 ± 0.013§
$k_{7,8}$	0.001 ± 0.001	0.001 ± 0.001	0.003 ± 0.003§
Lipolysis (%)	26.6 ± 9.65	70.0 ± 17.5*	26.5 ± 35.0‡
CE-FCR (min^{-1})	0.070 ± 0.009	0.028 ± 0.014*	0.032 ± 0.025
$k_{1,2}$	0.092 ± 0.031	0.138 ± 0.171	0.202 ± 0.122
$k_{1,3}$	0.277 ± 0.114	0.076 ± 0.130†	0.157 ± 0.104
$k_{2,3}$	0.019 ± 0.002	0.021 ± 0.019	0.022 ± 0.024
$k_{3,4}$	0.001 ± 0.001	0.001 ± 0.001	0.000 ± 0.003

*$P < .0001$, †$P < .01$ by unpaired Student's *t* test for controls in relation to the obese women at baseline.

‡$P < .005$, §$P < .05$ by paired Student's *t* test for baseline in relation to the same group after diet.

Abbreviations: CE-FCR, [1-^{14}C] cholesteryl-oleate fractional clearance rate; TG-FCR, [9,10-H_3] glycerol-trioleate fractional clearance rate.

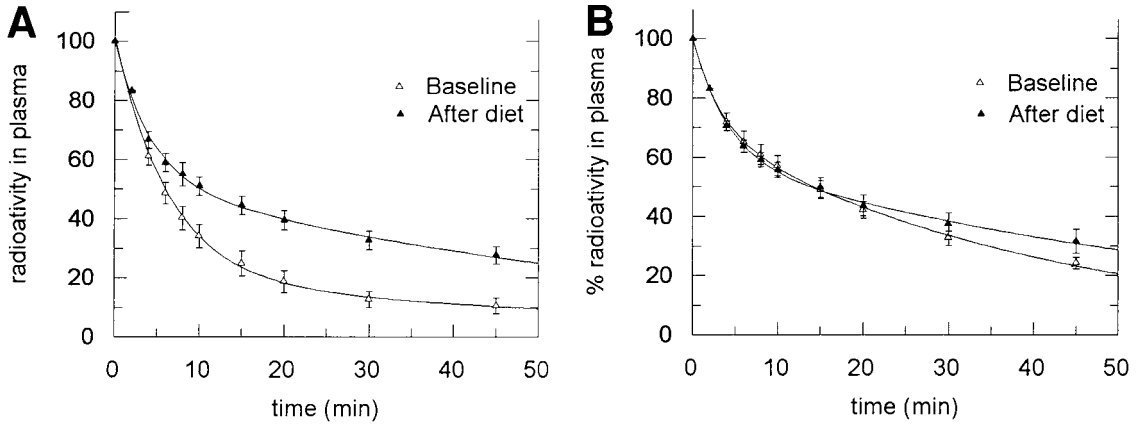

Fig 3. Removal from the plasma of obese women of the emulsion (A) [9,10-^{3}H]glycerol-trioleate and (B) [1-^{14}C]cholesteryl-oleate before and after the 2-month period of energy-restricted diet.

obese can be consequent to greater insulinemia associated with obesity because insulin stimulates the synthesis of LPL.[36]

There are reports in the literature on postprandial lipidemia in obesity evaluated by the fat-load test. In this test, after the ingestion of a standard fatty meal to which vitamin A is added as a chylomicron particle marker, plasma triacylglycerols and vitamin A are measured over several hours. Lewis et al[37] observed that obese subjects had smaller rates of triacylglycerols removed than normal-weight subjects, but the removal of chylomicron remnants evaluated by the appearance-disappearance curve of vitamin A in the plasma was not altered. Vansant et al[38] also showed that obese women with an average BMI of 38 kg/m^2 had increased plasma triacylglycerols after a standard fatty diet, but their vitamin A curves were normal. Recently, Couillard et al[39] and Taira et al[40] reported both decreased post-prandial triacylglycerols and decreased vitamin A removal in subjects with high visceral adipose tissue compared to those with low visceral adiposity.

It is known that cholesteryl ester transfer protein (CETP) activity is enhanced in the obese.[41,42] By transferring cholesteryl esters from the emulsion to the plasma lipoprotein fractions that have intrinsically much smaller plasma clearances, enhanced CETP would conceivably decrease the FCR of the emulsion cholesteryl esters. However, in previous experiments we have shown that the CETP effect on the emulsion lipids is negligible,[15] probably due to the short plasma half-life of the emulsion, so that this hypothesis is unlikely to explain our results.

In patients with coronary artery disease or other conditions associated with coronary artery disease development, diminished removal of chylomicron-like emulsions and higher concentration of postprandial lipids in fat-load tests were reported.[15,16,43-46] This suggests that deficiencies in chylomicron intravascular catabolism are involved in atherogenesis. Remnant retention can therefore be related to increased atherosclerosis risk in obesity.

The effects on the chylomicron metabolism of the administration of an energy-restricted diet to obese women have not yet been documented. As our subjects had a less than 50-g/d weight reduction in the week preceding the post-diet chylomicron-like emulsion test, they should be considered as being in a stable weight period.[47] The results of the emulsion test showed diminution of lipolysis after the dietary period as estimated by the diminished emulsion triacylglycerol FCR and mainly by the diminished lipolysis index. In contrast, the fast triacylglycerol concentration did decrease after weight loss, indicating a lower VLDL concentration. This is conceivably due to diminished post-dietary period synthesis of this lipoprotein by the liver.[48,49] A number of factors could account for this metabolic response to the energy restriction. First, a decrease of LPL synthesis may follow dietary restriction.[50,51] As described by Taskinen and Nikkilä,[52] during an energy-restricted diet period, LPL activity falls by 50% in adipose tissue and by 40% in skeletal muscle. This can be consequent to that diminished insulinemia that follows diminution of the caloric intake and the change of the chemical composition and physical form of carbohydrate in the dietary energy-restricted period,[51,53,54] considering that LPL is insulin-dependent.[36] The difference between the pre- and the post-diet period occurred in the second phase of the biexponential decay curve of the triglycerides, as seen in Fig 2A, involving $k_{6,7}$ and $k_{7,8}$. An increase in $k_{7,8}$ may also raise the possibility of post-diet increased recirculation of the emulsion lipids as newly synthesized VLDL.[14,55]

It is noteworthy that the removal of remnants, estimated by the emulsion cholesteryl ester, was not affected by the dietary restriction and weight loss.

In conclusion, obese women show increased emulsion lipolysis that diminishes after a short energy-restricted diet possibly due to the influence of diet-induced insulin level changes on LPL activity. Our results also showed a diminished ability to remove remnants, which did not improve after this short dietary period. Because delayed chylomicron remnant removal is associated with atherogenesis,[15,56-58] this finding can contribute to the increased incidence of coronary artery disease in obese subjects.

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