

Effects of etofibrate upon the metabolism of chylomicron-like emulsions in patients with coronary artery disease

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Abstract

Slow chylomicron intravascular catabolism has been associated with coronary artery disease and screening for drugs that can speed-up this process can be important. In this study, the effects of etofibrate upon chylomicron metabolism was tested by determination of the plasma kinetics of a chylomicron-like emulsion model in 12 patients with coronary artery disease, aged 59 ± 11 years, (total cholesterol: 240 ± 41 mg/dl; triglycerides: 188 ± 42 mg/dl) submitted to a randomized, crossover, double-blind, placebo-controlled study with administration of 1 g per day etofibrate or placebo for 1-month. A 1-month washout period was inserted between the treatment periods. Patients were intravenously injected a chylomicron-like emulsion doubly labeled with ^{14}C -cholesteryl oleate and ^3H -triolein at baseline and after treatments. After etofibrate treatment, there was decrease of total cholesterol and triglyceride plasma levels and a trend to increase high-density lipoprotein cholesterol plasma levels. Etofibrate elicited 62% enhancement of post-heparin lipolytic activity and 100% increase of ^3H -triglyceride fractional clearance rate compared with placebo treatment. ^{14}C -cholesterol ester fractional clearance rate was 260% greater after etofibrate than after placebo. Therefore, a potent effect of etofibrate on both chylomicron lipolysis and remnant removal was achieved, indicating that this drug can be used to improve this metabolism in future prospective studies. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Chylomicrons are quasispherical particles constituted mostly of a triglyceride core (roughly 90% by weight) with small amounts of cholesterol esters and vitamins surrounded by a phospholipid monolayer [1]. Apolipoproteins are adhered to the surface of chylomicrons and usually do not exceed 2% of the total lipoprotein weight. After entering the blood stream, chylomicrons undergo the action of lipoprotein lipase on the capillary wall. Lipase action is triggered by apolipoprotein (apo) CII bound to the surface of the particle [2] and can break chylomicron triglycerides down to glycerol and

fatty acids that are absorbed and stored in several body tissues, mainly adipose tissue and muscle [3]. This mechanism is extremely important for the energetic disposal of the organism. The smaller and triglyceride-depleted chylomicron remnants resulting from lipolysis are sequestered into the space of Disse and taken-up by the liver cells by low-density lipoprotein (LDL) receptors and the LDL receptor related protein [4,5]. Apo E is the main ligand of chylomicron remnants to those receptors. Chylomicrons share common catabolic pathways with liver produced very low-density lipoprotein (VLDL) regarding lipolysis by lipoprotein lipase and at least in part the mechanisms of cellular uptake.

Among the lipoprotein classes, chylomicrons is the one with the most rapid removal from the plasma, their half-life being about 15 min in man. In this respect, the speed of chylomicron removal has been related with

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incidence of coronary artery disease (CAD): smaller chylomicron plasma clearance rates occur in CAD patients [6–8]. Therefore, it is tempting to achieve therapeutic measures to accelerate chylomicron catabolism to subsequently verify whether this would, indeed, diminish the incidence of the disease.

Among the lipid lowering drugs, fibrates have been successfully used to reduce fast triglyceride plasma concentration in patients with hypertriglyceridemia. Niacin has a lesser but nonetheless important hypotriglyceridemic effect and the concomitant use of the two drugs conveys synergistic effects. The concomitant use of clofibrate and niacin elicited a more intense reduction of triglycerides, LDL cholesterol and a greater increase of high-density lipoprotein (HDL) cholesterol along with an important reduction of CAD events [9–12]. The advantages of clofibrate and niacin association led to the development of etofibrate in which the two compounds are covalently linked aiming to achieve that synergistic effect in a single therapeutic agent. In contact with plasma hydrolases, both constituents are gradually released with a pharmacokinetic behavior similar to that of sustained release forms [13].

In studies using the oral fat-load test, fibrates were shown to decrease post-prandial triglyceridemia. Remnant removal from the plasma evaluated by the inclusion of vitamin A in the test diet was shown increased by fibrate treatment in some studies and unchanged in others [14–20]. The effects of niacin and etofibrate upon postprandial lipidemia or chylomicron metabolism were poorly explored. Aiming to clarify this issue, in the current study the effects of etofibrate treatment upon the metabolism of a triglyceride-rich emulsion that mimics chylomicron intravascular metabolic behavior when injected into the plasma compartment was evaluated in patients with CAD in a randomized, crossover, double-blinded and placebo-controlled protocol. Our data show that etofibrate treatment accelerates chylomicron metabolism.

2. Materials and methods

2.1. Patients

We studied 12 patients (ten males), mean age 59 ± 11 years, from the outpatient clinics of the Heart Institute of the São Paulo University Medical School Hospital. They had stable CAD without ventricular dysfunction, as confirmed by cardiac catheterization. Inclusion criteria were plasma concentration of triglycerides < 400 mg/dl (range 99–290) and LDL cholesterol > 130 mg/dl (range 147–284). Exclusion criteria were alcoholism, liver, renal, metabolic, inflammatory and neoplastic disease. Subjects with diabetes were not included and all women were post-menopausal. All patients were

oriented to follow the Step-One Diet of National Cholesterol Education Program starting 3 months before the treatment periods and none of the patients received hormonal replacement or lipid-lowering therapies in the last 6 months before the study. The enrolled patients with CAD were submitted to a randomized, double-blind, placebo-controlled and cross-over program with either 1000 mg etofibrate (Tricerol, Searle, São Paulo, Brazil) or placebo administered twice a day during 1 month. A 1-month washout time was inserted between the treatment periods. Assays of plasma lipids, lipase activity and determination of the chylomicron-like emulsion kinetics were made after the dietary and treatment periods. To confirm the trend for delayed chylomicron removal documented in our previous study, the plasma kinetics of the patients as determined at baseline were compared with the data of 15 subjects with no evident CAD from the laboratory database, paired by age and sex, body mass index (BMI) and with equivalent ranges of plasma lipids. These subjects had no evident coronary lesions at the cinecoronariographic exam that had been performed to investigate thoracic pain. The laboratorial evaluation of this control group of subjects was proceeded in less than 2 months after cinecoronariography was made. The experimental protocol was approved by the Ethical and Scientific Committee of the Heart Institute and a written informed consent was given by all participants.

2.2. Lipids and lipoprotein analysis

Blood samples were obtained after a 12-h overnight fast, at baseline and after the 1-month treatment periods. Plasma cholesterol and triglyceride determinations were made by commercial enzymatic methods (Merck and Abbott, respectively). HDL cholesterol determination was made after LDL and VLDL chemical precipitation. VLDL and LDL cholesterol were estimated by the Friedewald formula [21].

2.3. Post-heparin lipolytic activity

After a 12-h fast, blood samples were collected from the patients 10 min after heparin injection (100 U/kg-body weight). Assays for lipolytic activity were performed by using the emulsion labeled with glycerol $\text{tri}[^3\text{H}]\text{oleate}$ (Amersham, UK) as the substrate. The chylomicron-like emulsion (final composition: triolein $76.5 \pm 4.1\%$; free cholesterol $1.9 \pm 0.3\%$; cholesterol oleate $11.2 \pm 3.0\%$ and phosphatidylcholine $10.4 \pm 1.3\%$) was made from lipid mixtures emulsified by ultrasonic irradiation and purified by ultracentrifugation in density gradients as described previously [6]. Plasma samples and the emulsion were incubated at 37°C for 5, 10, 15, 30, 45, 60, 120 or 180 min. Lipids were then extracted and separated by thin layer chro-

matography (TLC). The amount of radioactivity present in the TLC band corresponding to the triglyceride and free fatty acid (FFA) fraction was measured in a scintillation solution (PPO: DM-POPOP: Triton X-100: Toluene; 5 g: 333: 667 ml), using a Packard 1660 TR Beta-Spectrometer (Meridien, CT). The incubation time elapsed to yield 50% hydrolysis of glycerol tri [^3H]oleate was used to calculate the clearance of FFA expressed as $\mu\text{mol/ml}$ per min. The calculated interassay coefficient of variation for this procedure was 2.2%.

2.4. Emulsion kinetic studies

The chylomicron-like emulsion was prepared as described above. A total of 3–5 mg of the emulsion total lipid (200–300 μl) containing 74 kBq (2 μCi) of the ^{14}C and 148 kBq (4 μCi) of the ^3H label were injected in a bolus into the forearm vein. Plasma samples were collected from a vein of the other forearm at intervals of 2, 4, 6, 10, 15, 20, 30, 45 and 60 min. Radioactivity in aliquots (1 ml) of plasma was measured in the same scintillation solution used for lipase activity analysis. The decay curve calculations of the plasma decay of ^{14}C -cholesteryl oleate and ^3H -triolein (Amersham, UK) radioactivity were made by the non-linear least squares method, as described previously, and were expressed as the fractional clearance rate (FCR) for both labels [6]. The radiological dose injected as emulsion label was evaluated according to the guidelines of the International Commission on Radiological Protection, ICRP [22] and its safety was assured as discussed previously

[6]. The calculated interassay coefficient of variation for both emulsion label kinetic studies was $< 3.0\%$.

2.5. Statistical analysis

Data were expressed as mean \pm standard deviation. The Kolmogorov-Smirnov test was applied for testing the normality of the data along the three evaluations altogether (baseline, placebo and etofibrate). Repeated measure analysis of variance was used to study: the effect of interaction between factors group (etofibrate-placebo or placebo-etofibrate) and drug (placebo or etofibrate); the difference between groups etofibrate-placebo and placebo-etofibrate and, finally, the difference among placebo and etofibrate treatments.

The residual effect from the first to the second phase was evaluated through items 1 and 2 above. There was no residual effect of etofibrate treatment period on placebo period. Values of P less than 0.05 were considered statistically significant. SPSS for Windows was used to perform statistical calculations.

3. Results

Table 1 shows baseline clinical and laboratorial characteristics of the participant subjects with and without CAD. There was no difference in age, sex, BMI and lipid profile between the enrolled patients with CAD and subjects without CAD.

Fig. 1 shows the plasma disappearance curves of the labeled emulsion cholesterol esters and triglycerides of the CAD patients and those of subjects without CAD from our laboratory database. It is apparent that both curves were slower in the CAD patients compared with the subjects without CAD. In fact, the FCR of ^3H -triglycerides were 38% smaller and ^{14}C -cholesterol ester was 57% smaller in the CAD group (Table 1).

Table 2 shows that when our CAD patients were submitted to the 30-day treatment with etofibrate their total cholesterol and triglyceride plasma levels decreased significantly compared to the 30-day placebo treatment period. From Table 2, it can be seen that compared to the placebo treatment, LDL cholesterol was not altered and HDL cholesterol tended to increase after etofibrate treatment.

Fig. 2 shows the patient plasma radioactivity decay curves of ^3H -triglycerides and ^{14}C -cholesterol ester after etofibrate and placebo treatments. It appears that etofibrate treatment accelerated the decay curve of ^3H -triglycerides compared with the curve obtained after placebo treatment (Fig. 2A). Likewise, ^{14}C -cholesterol ester curve was also accelerated by etofibrate (Fig. 2B). In fact, from the data displayed in Table 2 it can be estimated that FCR ^3H -triglycerides was 100% and

Table 1
Baseline characteristics of subjects with and without CAD^a

	Without CAD	With CAD	<i>P</i>
<i>N</i>	15	12	
Male	12	10	0.9
Age (years)	57 \pm 9	59 \pm 11	0.6
BMI	26.7 \pm 2.54	26.6 \pm 2.67	0.9
Hypertension	10	9	0.9
Smoking	5	4	0.9
Physical inactivity	9	7	0.9
Total cholesterol (mg/dl)	239 \pm 25	240 \pm 41	0.5
Triglycerides (mg/dl)	180 \pm 38	188 \pm 42	0.48
HDL cholesterol (mg/dl)	48 \pm 15	39 \pm 7	0.08
VLDL cholesterol (mg/dl)	36 \pm 8	39 \pm 11	0.47
LDL cholesterol (mg/dl)	154 \pm 22	162 \pm 38	0.13
FCR ^{14}C -CE	0.028 \pm 0.017	0.012 \pm 0.006	0.006
FCR ^3H -TG	0.047 \pm 0.015	0.029 \pm 0.019	0.003

^a HDL C, HDL cholesterol; VLDL C, VLDL cholesterol; LDL C, LDL cholesterol. FCR, fractional clearance rate. Lipid values are in mg/dl and FCR in min^{-1} .

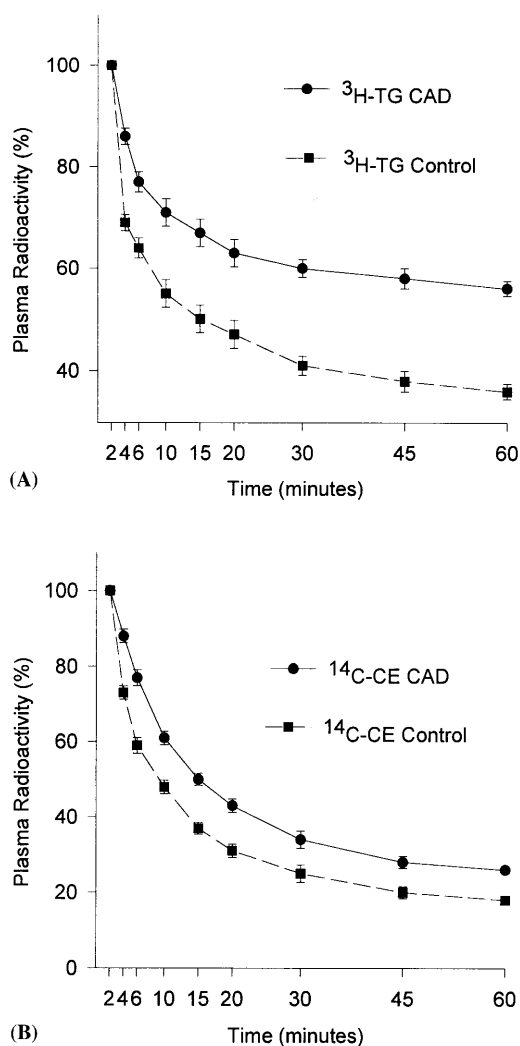


Fig. 1. Plasma decaying curves of emulsion ^3H -triglycerides (A); and ^{14}C -cholesteryl ester (B) in patients with coronary artery disease (continuous line) and control subjects (dashed line).

FCR ^{14}C -cholesterol ester 260% greater after etofibrate treatment in comparison with the data obtained after the placebo treatment period. Table 2 also shows that etofibrate treatment resulted in enhancement of lipolytic activity compared with placebo.

4. Discussion

A strong case has been made for the involvement of the post prandial lipoprotein status in atherogenesis. A trend for greater accumulation of triglyceride-rich lipoproteins after a fatty meal has been documented in patients with CAD [14–20]. However, it is difficult to establish whether this is as a result of chylomicrons or liver-originated VLDL and VLDL remnants because the separation of both lipoprotein classes is difficult to accomplish. Chylomicron metabolism has long been

evaluated in subjects by the so-called fat load test whereby triglyceride plasma levels are determined for some hours after the ingestion of a standard fatty meal. Considering that the bulk of basal fasting triglyceride levels are contained in the VLDL fraction, increments in triglyceride concentration after the ingestion of the meal would be ascribed to lymph chylomicrons entering the intravascular compartment. However, lipid recirculation also takes place because during the several-hour period of fat intestinal absorption, chylomicron lipids taken-up by the liver are secreted back into the plasma as part of newly formed VLDL. On the other hand, as chylomicrons compete with VLDL for lipolysis, the latter accumulates in the plasma during the post-prandial period. Therefore, post-prandial increment in plasma triglyceride is in fact not only as a result of chylomicron input but also to lipid recirculation and VLDL accumulation [23–25]. Relative to the use of retinyl palmitate mixed with the test meal as a marker of chylomicrons in the plasma, it may partially shift to other lipoprotein classes while in the circulation [25]. A specific although technically laborious strategy for determination of the chylomicron appearance-disappearance curve consists in the measurement after the fatty meal of the plasma concentration of apo B48 that is the apo B form synthesized in the intestine and present in chylomicrons and of liver produced apo B100 present in VLDL and its catabolic products [26,27].

The chylomicron-like emulsion technique used in the current study is a practical and straightforward tool to evaluate this metabolism, mainly because it bypasses the gastrointestinal component. When injected in the bloodstream, the double-labeled emulsion promptly incorporates apolipoproteins as apo CII that stimulates LPL action, and apo E that binds the lipolysed emulsion to the liver receptors [28,29]. The emulsion does not acquire apo B48, but this is irrelevant because this apolipoprotein apparently plays no important role in

Table 2

Etofibrate and Placebo effects on lipid profile, post-heparin lipase activity and fractional clearance rate of chylomicron-like emulsion kinetics^a

	Etofibrate	Placebo	P
Total cholesterol	230 ± 44	243 ± 50	0.018
Triglycerides	160 ± 38	237 ± 89	0.002
HDL C	42 ± 8	39 ± 6	0.06
VLDL C	32 ± 8	47 ± 18	0.006
LDL C	155 ± 40	157 ± 44	0.9
FCR ^{14}C -CE	0.018 ± 0.003	0.009 ± 0.002	0.03
FCR ^3H -TG	0.049 ± 0.009	0.019 ± 0.002	0.0005
Lipase activity	67.200 ± 24.571	36.000 ± 14.727	0.008

^a HDL C, HDL cholesterol; VLDL C, VLDL cholesterol; LDL C, LDL cholesterol. Lipase activity is expressed as FFA $\mu\text{mol}/\text{ml}$ per min; FCR, fractional clearance rate. Lipid values are in mg/dl and FCR in min^{-1} .

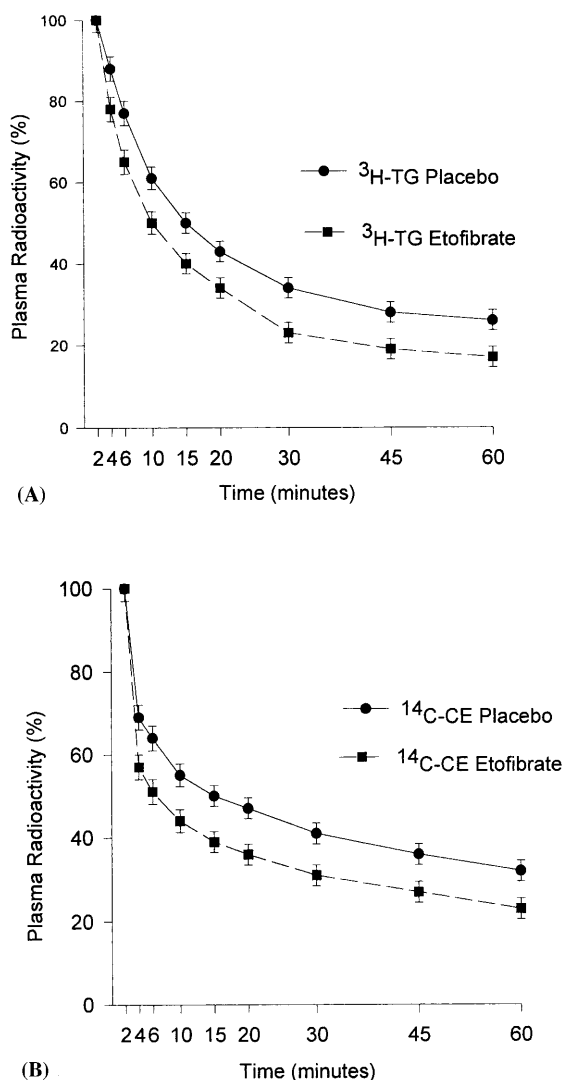


Fig. 2. Plasma decaying curves of emulsion ^3H -triglycerides (A); and ^{14}C -cholesteryl ester (B) in patients with coronary artery disease after placebo (continuous line) and etofibrate (dashed line) treatment periods.

chylomicron intravascular metabolism. By acquiring apo CII, the emulsion suffers extensive lipolysis that can be evaluated by the ^3H -triglyceride decay curve. As shift of cholesteryl ester to native lipoprotein classes by cholesterol ester transfer protein (CETP) is negligible as measured in subjects [6], the emulsion ^{14}C -cholesteryl ester is in fact the marker of the emulsion particles while it remains in the plasma. Due to lipolysis, the ^3H -triglyceride decay curve is faster than that of ^{14}C -cholesteryl ester. Similarly to lymph chylomicrons, the emulsion is rapidly cleared from the circulation and is almost completely taken-up by the liver while only trace amounts are trapped by the spleen and other tissues [30]. Interestingly, Hultin et al. [31] observed that the degree of intravascular lipolysis suffered by these emulsions are much greater than that of emulsions used for lipid parenteral nutrition such as In-

tralipid, while approaching the intensity of lipolysis suffered by lymph chylomicrons. Parameters of a kinetic model estimated for both emulsions and lymph chylomicrons injected into rats using the SAAM/CONSAM programs were compatible, as well as the plasma residence times of the emulsion obtained from human subjects [32]. In rats [30] and in subjects [6], the injected chylomicron-like emulsion competes with chylomicrons for the same lipolytic mechanisms and hepatic receptor sites in a dose-dependent way. The plasma decay curves of chylomicron-like emulsions were also largely as expected for lymph chylomicrons in patients with dyslipidemia [6,33]. All those observations confirm the adequacy of the emulsion used in the current study as a physical model of native chylomicrons [6,28].

Accumulation of chylomicrons and remnants in the bloodstream could lead to increased thrombogenesis, endothelial dysfunction and arterial lipid deposition that would favor the genesis of CAD [34–40]. Simons et al. [27] reported elevated post-prandial levels of apo B48 in CAD patients. In case-control studies, normolipidemic patients with CAD demonstrated by cinecoronariography had higher postprandial plasma levels of chylomicron remnants than subjects with no evident lesions [7,26]. Therefore, despite the limitations of cinecoronariography to exclude CAD, these findings at least suggest that chylomicron metabolism is delayed in subjects with obstructive CAD. Furthermore, the progression of the coronary lesion was significantly correlated with the plasma levels of chylomicron remnants in a 5-year angiographic follow-up [8]. Maranhão et al. [6] showed that both lipolysis and removal from the plasma of chylomicron-like emulsion are diminished in normolipidemic patients with CAD, a finding that is being confirmed by our data. In this perspective, it is important to screen for drugs that can speed up chylomicron metabolism.

In our study etofibrate treatment increased post-heparin lipolytic activity as evaluated by the acceleration of the removal from plasma of the emulsion triglycerides and also by the *in vitro* assay of emulsion triglyceride hydrolysis in post-heparin plasma. It is noteworthy to point out that etofibrate not only intensified lipolysis but also accelerated remnant removal, as indicated by the increased FCR of the emulsion cholesterol ester moiety. Enhancement of lipolysis without increasing the remnant removal could perhaps be atherogenic because it would imply in the generation and retention in the circulation of the remnant products that are conceivably more atherogenic than the precursor lipoprotein. In this regard, it was shown that treatment with some fibrates, as fenofibrate and bezafibrate, resulted in increased lipolysis without change in remnant removal [18–20]. On the other hand, after gemfibrozil and etophylline clofibrate treatments both processes were intensified [14–17]. Addition of niacin to a fibrate may

bring enhancement of both chylomicron lipolysis and remnant removal, because niacin has been shown to reduce VLDL synthesis [41]. Because chylomicrons and VLDL compete for common catabolic pathways, decrease in VLDL concentration in the plasma as a result of synthesis reduction or increased lipolysis presumably facilitates chylomicron catabolism. Therefore, decreased VLDL by etofibrate treatment resulting in less competition for the lipoprotein lipase sites is a possible contributing factor for the accelerated emulsion catabolism found in this study.

Etofibrate effect on the two-step chylomicron catabolic process may be the result of other mechanisms of action, including increase of lipoprotein lipase synthesis and inhibition of apo CIII synthesis that would lead to increase of remnant uptake. Those actions have been pointedly related to the interaction of fibrates, including clofibrate, with peroxisome proliferator-activated receptor [42,43].

In conclusion, this study shows that etofibrate is capable of promoting overall acceleration of chylomicron intravascular catabolism in patients with CAD wherein this process was previously slow. Therefore this drug may be chosen for studies aiming to clarify whether acceleration of chylomicron catabolism may be useful for atherosclerosis primary or secondary prevention.

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