

The effects of gemfibrozil upon the metabolism of chylomicron-like emulsions in patients with endogenous hypertriglyceridemia

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Received 13 March 2000; accepted 3 October 2000

Abstract

Objective: To evaluate the effects of gemfibrozil upon the intravascular metabolism of chylomicron-like emulsions in endogenous hypertriglyceridemia. **Methods:** We evaluated the plasma kinetics of a chylomicron-like emulsion in 39 subjects: 27 hypertriglyceridemics, total cholesterol (TC) expressed as median (%25; %75) 7.47 (6.1; 8.19) mmol/l and plasma triglycerides (TG) 4.28 (3.6; 18.5) mmol/l and in 12 normolipidemics, TC 4.7 (3.85; 5.37) mmol/l and TG 0.91 (0.64; 1.75) mmol/l. Hypertriglyceridemics were evaluated at baseline and after a 30-day 1200-mg/day gemfibrozil ($n=8$) or placebo treatment ($n=7$). The emulsion labelled with ¹⁴C-cholesteryl oleate (¹⁴C-CO) and ³H-triolein (³H-TO) was injected intravenously after a 12-h fast. The plasma kinetics of ³H-TO and ¹⁴C-CO were determined to assess, respectively, lipolysis and clearance of chylomicron and remnants by compartmental analysis. **Results:** The residence times (in minutes) of ³H-TO and ¹⁴C-CO of hypertriglyceridemics were roughly twice the values of normolipidemics, i.e. 8.0 (5.5; 12.0) versus 15.0 (11.0; 24.0) and 21.5 (14.0; 33.0) versus 44.0 (32.0; 72.0), $P=0.001$. Gemfibrozil treatment of hypertriglyceridemic patients reduced the residence times of ³H-TO and ¹⁴C-CO, respectively, by 46% ($P=0.003$) and 53% ($P=0.008$). Effects were noted on the slow phase of emulsion plasma removal, which was reduced in hypertriglyceridemics. After treatment, the emulsion residence times determined in hypertriglyceridemics attained the values of the normolipidemic group. **Conclusions:** Gemfibrozil treatment normalised the defects in chylomicron-like emulsion catabolism observed in endogenous hypertriglyceridemia patients. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Atherosclerosis; Cholesterol; Computer modelling; Lipid metabolism; Lipoproteins

1. Introduction

Both triglyceride-rich lipoproteins, chylomicrons originated in the intestine, and liver-synthesised VLDL, share common intravascular catabolic pathways [1–4]. Both undergo lipolysis by lipoprotein lipase stimulated by apolipoprotein (Apo) CII on the endothelium surface of capillary vessels. Efficient degradation of VLDL and chylomicrons is necessary for their optimal clearance from plasma. It has been suggested that the LDL receptor

removes not only LDL and VLDL remnants but is mainly responsible for the clearance from the plasma of chylomicron remnants [4,5].

VLDL is routinely evaluated by measuring the concentration of fasting plasma triglycerides. On the other hand, due to technical difficulties, the status of chylomicron metabolism in man is much less studied.

It has been shown that entry of chylomicrons into the bloodstream elicits accumulation in the plasma of VLDL [2,6]. This occurs because the presence in the circulation of a large concentration of chylomicrons saturates the lipolytic mechanism and perhaps the removal mechanisms that also promote VLDL catabolism and clearance. Con-

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versely, it is worthwhile to verify whether VLDL accumulation in the plasma also results in decreased removal of chylomicrons. Since fasting hypertriglyceridemia may involve defects in VLDL synthesis and catabolism the question is whether those defects would also lead to slow chylomicron catabolism and remnant removal. In fact, this issue has been addressed in studies that used fat tolerance tests with ingestion of vitamin A as chylomicron marker [7–9]. After the ingestion of the standard fatty meal, subjects with fasting hypertriglyceridemia have diminished vitamin A elimination from the plasma and higher postprandial triglyceridemia [7,9].

Chylomicron-like lipid emulsions are a practical and direct approach to evaluate chylomicron metabolism [10–15]. After injection into the plasma, these emulsions that are devoid of proteins, adsorb Apo CII and Apo E and other apolipoproteins that mediate this metabolism [10,11]. Thus, similarly to the processes lymph chylomicrons undergo when entering blood stream, emulsion triglycerides are hydrolysed by lipoprotein lipase and the resulting remnants bind to receptors that remove chylomicron remnants [11]. Emulsions can be labelled with radioisotopes such as ^{14}C -cholesteryl oleate (^{14}C -CO) and ^3H -triolein (^3H -TO). The plasma kinetics of ^{14}C -CO allows evaluation of the removal of the emulsion particles, since the transfer of this label to the plasma lipoproteins is negligible [10,12]. On the other hand, the kinetics of the ^3H -TO allows the evaluation of emulsion lipolysis [12]. This model introduced by Redgrave and Maranhão [10] has been previously validated in animals [10–12] and in human subjects [13–15].

Fibrates have been used to treat fasting hypertriglyceridemia by reducing VLDL plasma levels [16]. However, the effects of fibrates on chylomicron lipolysis and remnant removal have not yet been completely established in patients with endogenous hypertriglyceridemia [9,17,18]. In the current study, the intravascular metabolism of a chylomicron-like emulsion in hypertriglyceridemics was compared with that of normolipidemic subjects and the effects of gemfibrozil were evaluated. Our results show that hypertriglyceridemic patients have altered plasma kinetics of the chylomicron-like emulsion but after gemfibrozil treatment they approach the values of normolipidemic subjects.

2. Methods

2.1. Study subjects

This study evaluated subjects with endogenous hypertriglyceridemia with plasma triglycerides (TG) ≥ 2.26 mmol/l (200 mg/dl) and age between 18 and 70 years. The exclusion criteria were: type I or type V hyperlipidemia, use of lipid lowering drugs in the last 3 months before

enrolment in the study, uncontrolled diabetes mellitus, e.g. fasting blood glucose ≥ 7.5 mmol/l (135 mg/dl) or glycated hemoglobin $> 7.5\%$ [19], use of ethanol, presence of heart failure, hepatic, renal or thyroid disease, women within reproductive age without the use of a mechanical contraceptive method, use of hormones, uncontrolled clinical diseases other than hyperlipidemia, acute coronary or cerebrovascular event within the last 6 months, and any collateral effect or previous intolerance to gemfibrozil. Patients were compared to non-diabetic normolipidemic subjects, TG < 2.26 mmol/l (200 mg/dl), TC < 6.2 mmol/l (240 mg/dl), and LDL-C < 4.1 mmol/l (160 mg/dl) presenting none of the exclusion criteria previously cited.

2.2. Study design

This project was conducted in two parts. Firstly, the emulsion kinetics of 27 hypertriglyceridemic patients were compared with those of the 12 normolipidemic controls. Secondly, the emulsion kinetics were evaluated in 15 consecutive hyperlipidemics who were randomised to a 30-day 1200-mg/day gemfibrozil (Parke Davis Laboratories, Sao Paulo, Brazil) ($n=8$) or equivalent placebo treatment ($n=7$). This was a single blinded study, and neither patients nor those responsible for the calculations were aware of the treatment status of the patients. Attending physicians were aware of which medication was being used, however they strictly followed the rules of the randomisation sheet. The effects of gemfibrozil on emulsion kinetics in those eight patients were also compared to the values of the 12 normolipidemic subjects.

This study was performed in agreement with the rules of the Helsinki Conference and was approved by the Scientific Committee of The Heart Institute of the Hospital das Clínicas of the São Paulo University Medical School. Informed consent was obtained from all participants.

2.3. Laboratory evaluation

Plasma samples were collected after a 12-h fast on 2 consecutive days and the results were averaged. Plasma total cholesterol and triglycerides were determined using enzymatic methods (Roche Laboratories, Basel, Switzerland). HDL-cholesterol (HDL-C) was determined by the same method as total cholesterol, after chemical precipitation of Apo B-100 containing lipoproteins with MgCl and phosphotungstic acid. LDL-cholesterol (LDL-C) was calculated by the subtraction of HDL-C and VLDL-cholesterol (VLDL-C) from total cholesterol. VLDL-C was calculated by dividing triglycerides by 5 for triglyceride levels up to 4.5 mmol/l (400 mg/dl). Apo B and Apo AI were also determined by immunoturbidimetry (Roche Laboratories, Basel, Switzerland). All lipid, and apolipoprotein determinations were performed automatically in a COBAS-MIRA analyser (Roche Laboratories, Basel, Switzerland).

Lipoprotein electrophoresis was performed in cellulose acetate, with a barbital buffer, pH 7.2, and coloured by Sudam black. Quantification was made by optical densitometry.

Apo E genotyping was performed by the technique described by Hixson and Vermier [20].

2.4. Emulsion preparation

Chylomicron-like emulsions were prepared as described previously [11]. In short, dried lipid mixtures composed of 2% cholesterol, 23% lecithin, 6% cholesteryl oleate and 69% triolein (Sigma, St. Louis, USA) were prepared with ^{14}C -cholesteryl oleate (^{14}C -CO) and tri[9,10(n)- ^3H]oleate (triolein- ^3H -TO) (Amersham, Surrey, UK). The mixtures were emulsified by sonication in 6 ml of NaCl (density 1.101 g/ml, pH 7.0) with a Branson Cell Disrupter (Branson Ultrasonic Power, model B 450, Dranbury, USA) at 55°C for 30 min using a 1-cm probe with continuous output of 70–80 W. Purification of the crude emulsions was by ultracentrifugation using discontinuous NaCl gradients with densities of 1.065, 1.020, and 1.006 g/ml and a TH 641 rotor using a Sorvall ultracentrifuge (model OTD Comb, Wilmington, USA) at 12,000 rpm at 22°C for 15 min. The coarse lipid layer that initially floated to the top (first run) was discarded and replaced with a corresponding volume at 1.006-g/ml solution. The emulsions were recovered from the upper 3 ml of the top of the gradient after a second centrifugation at 36,000 rpm during 25 min. Emulsions were immediately sterilised through passage in a 0.2- μm filter (Millipore, São Paulo, Brazil) and stored in sterile vials. All glass hardware used in this study was made pyrogen-free by exposure to dried steam at 180°C during 2 h and sterilised by wet steam at 120°C during 30 min. All plastic materials were sterilised by ultra-violet light exposition. All mixing procedures were performed under laminar flux. The lipid composition of the emulsion as determined by standard laboratory methods was 1.9% cholesterol, 10.4% phospholipids, 11.2% cholesteryl ester and 76.5% triacylglycerols (% weight).

2.5. Emulsion kinetic studies

All hyperlipidemic patients were following a low fat, low in simple carbohydrates diet for 30 days. During the study, patients were asked to keep to their diet and to abstain from ethanol. All kinetic studies were performed after a 12-h fast. Two veins from different arms were cannulated and kept with a saline flush. On average 100 μl of the emulsion containing 148 kBq (4 μCi) of ^3H -TO and 74 kBq (2 μCi) of ^{14}C -CO were injected in a bolus followed by a 5-ml saline flush. Blood samples were collected on the contra-lateral arm vein at 2, 4, 6, 10, 15, 30, 45 and 60 min after injection. Blood was dispensed into tubes containing 50 μl of sodium heparin (Liquemine,

Roche Laboratories, Sao Paulo, Brazil) and was immediately centrifuged at 2700 rpm during 10 min (Sorvall centrifuge, model RT7, Wilmington, USA). An aliquot of 1 ml of plasma was transferred to counting vials and 7 ml of scintillation solution PPO: DM-POPOP: Triton X-100/toluene (5 g: 0.5 g: 333 ml/667 ml) were added to the vials. Radioactivity in the samples was determined using a Packard 1660 TR spectrometer (Packard, Meridien, USA). The plasma decay of the radioisotopes was used to calculate the kinetics of the emulsion.

As previously described [15], the radiation dose injected in each experiment was well below the Annual Limit for Intake of Radionuclide, 50 mSv, determined by the International Commission on Radiological Protection. For ^{14}C -CO the dose was 0.0411 mSv and for ^3H -TO it was 0.0025 mSv.

2.6. Compartmental analysis

The compartmental analysis was performed according to a modification of the model proposed by Redgrave and Zech [12]. The full description of the model employed and the calculations are shown in Appendix A. In short, four compartments were employed to estimate the kinetic parameters for both ^{14}C -CO and ^3H -TO tracers (Fig. 1). The removal from plasma of chylomicrons as well as from chylomicron-like emulsions show a rapid decay followed by a slow removal phase [4,12,15]. The $k_{x,y}$ constants represent the transfer or fractional catabolic rates from compartment x to compartment y . The clearance of ^{14}C -CO reflects tissue uptake from plasma of remnant particles in various delipidation degrees [14] and is represented by compartments 1–4. The clearance from plasma of ^3H -TO represents the lipolytic mechanism and is represented by compartments 5–8. The kinetics of chylomicron-like emulsion is represented by the plasma residence times expressed in minutes of both radioisotopes and was calcu-

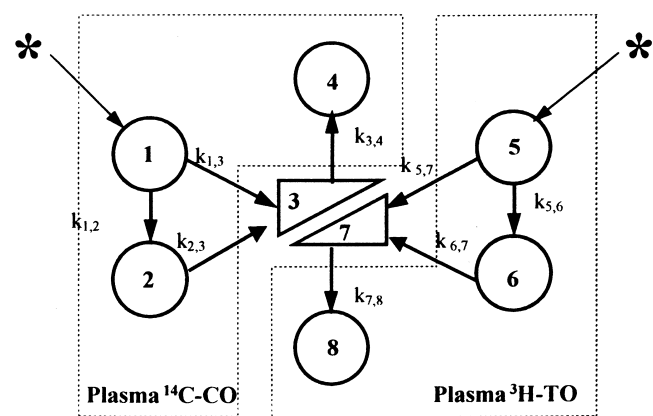


Fig. 1. Kinetic model adopted for the chylomicron-like emulsion in plasma.

lated according to the occupancy principle [12,14]. The rapid and slow decay phases evaluated by the ^{14}C -CO and ^3H -TO tracers are represented, respectively, by $k_{1,3}$ and $k_{2,3}$ and by $k_{5,7}$ and $k_{6,7}$. All calculations were performed using computerised software [21].

2.7. Statistical analysis

Data normal distribution was tested by the Kolmogorov–Smirnov procedure. Since most parameters were not normally distributed, lipid profile and kinetic data were expressed as median (%25; %75) unless otherwise stated. Categorical data were compared by Fisher's exact test. Spearman's rank correlation was used to evaluate any association between ^3H -TO and ^{14}C -CO residence times with age, body mass index, total plasma cholesterol, plasma triglycerides, HDL-C, Apo AI and Apo B levels. If a correlation was detected, non-parametric data were normalised using logarithmic transformation and linear regression was used. The multiple linear regression procedure was used to determine if the correlation persisted after adjustment for other variables. Non-paired data were compared by Student's *t*-test or Mann–Whitney's test when applicable. Paired data were compared by paired *t*-test or Wilcoxon's signed rank test when necessary. Hypertriglyceridemic patients that received gemfibrozil treatment were compared with the normolipidemic subjects by Student's *t*-test. Statistical significance was accepted at the level of 5%.

3. Results

3.1. Comparison of hypertriglyceridemics with normolipidemics

Clinical parameters, plasma lipids and apolipoproteins of the hypertriglyceridemic patients and normolipidemic volunteers at baseline are shown in Table 1. There were no differences between the groups regarding age, gender, body mass index, number of smokers and subjects with arterial hypertension. There were two diabetics on treatment in the hypertriglyceridemic group and none in the normolipidemic group. The number of patients using beta-blockers or thiazidic diuretics was also similar in the two groups (Table 1). However, in the hypertriglyceridemic group nine (33%) of the enrolled patients had a history of coronary artery disease and none among normolipidemics ($P=0.03$). A total of 18 patients presented a type IV pattern on lipid electrophoresis, eight presented type IIB and one showed broad β pattern (type III) with concomitant Apo E2/E2 genotype. There were no differences in the distribution of apolipoprotein E genotypes between the normolipidemic and hypertriglyceridemic subjects (Table 1).

^3H -TO and ^{14}C -CO residence times determined in the hypertriglyceridemic patients were roughly twice the values of normolipidemics ($P=0.001$), data shown in Table 2. In hypertriglyceridemics, $k_{1,2}$ ($k_{5,6}$) was 1.6 times the value of normolipidemics ($P=0.004$), while $k_{2,3}$ and $k_{6,7}$ were, respectively, 0.75 ($P=0.04$) and 0.5 ($P=0.014$) times the

Table 1
Clinical characteristics, baseline plasma lipids (mmol/l) and apolipoproteins (g/l) of the normolipidemic (NL) and hypertriglyceridemic (HTG) subjects studied

	NL	HTG	P
<i>n</i>	12	27	–
Age (years)	43.0±13.0	48.0±14.0	NS
Male, <i>n</i> (%)	7 (58)	18 (67)	NS
Female, <i>n</i> (%)	5 (42)	33 (9)	
Body mass index (kg/m ²)	26.0±6.0	27.0±3.0	NS
Hypertension, <i>n</i> (%)	5 (42)	12 (44)	NS
Diabetics, <i>n</i> (%)	0 (0)	2 (7)	NS
Coronary artery disease, <i>n</i> (%)	0 (0)	9 (33)	0.03
Smoking, <i>n</i> (%)	3 (17)	2 (7)	NS
Beta-blockers, <i>n</i> (%)	1 (8)	4 (15)	NS
Thiazidic diuretics, <i>n</i> (%)	1 (8)	6 (22)	NS
Total cholesterol ^a	4.7 (3.85; 5.37)	7.47 (6.1; 8.19)	<0.0001
Triglycerides ^b	0.91 (0.64;1.75)	4.28 (3.6; 18.5)	<0.0001
HDL-C ^a	1.26 (1.05; 1.29)	0.80 (0.69; 0.88)	<0.0001
Apolipoprotein B	1.1(1.0; 1.4)	1.6 (1.4; 1.7)	0.005
Apolipoprotein A-I	1.7 (1.5; 2.2)	1.5 (1.3; 1.8)	NS
Apo E3/E3 genotype, <i>n</i> (%)	11 (92)	22 (81)	NS
Apo E4/E3 genotype, <i>n</i> (%)	–	3 (11)	NS
Apo E2/E3 genotype, <i>n</i> (%)	1 (8)	1 (4)	NS
Apo E2/E2 genotype, <i>n</i> (%)	–	1 (4)	NS

^a Multiply by 38.7 to convert to mg/dl.

^b Multiply by 88.5 to convert to mg/dl.

Table 2

^{14}C -cholesteryl oleate (^{14}C -CO) and ^3H -triolein (^3H -TO) residence times (min) and transference $k_{x,y}$ constants (min^{-1}) of normolipidemics and hypertriglyceridemics

	Normolipidemics	Hypertriglyceridemics	P
n	12	27	–
^3H -TO residence time	8.0 (5.5; 12.0)	15.0 (11.0; 24.0)	0.001
^{14}C -CO residence time	21.5 (14.0; 33.0)	44.0 (32.0; 72.0)	0.001
$k_{1,2}=k_{5,6}$	0.104 (0.045; 0.262)	0.193 (0.170; 0.350)	0.004
$k_{1,3}$	0.203 (0.113; 0.38)	0.128 (0.05; 0.30)	NS
$k_{2,3}$	0.019 (0.0135; 0.023)	0.015 (0.007; 0.017)	0.04
$k_{3,4}$	0.0001 (0.0002; 0.003)	0.002 (0.004; 0.006)	NS
$k_{5,7}$	0.19 (0.151; 0.443)	0.30 (0.14; 0.60)	NS
$k_{6,7}$	0.063 (0.041; 0.073)	0.035 (0.03; 0.045)	0.014
$k_{7,8}$	0.0008 (0.0005; 0.002)	0.002 (0.0009; 0.003)	NS

values. The plasma decaying curves of the emulsion radiolabeled lipids in normolipidemic subjects and hypertriglyceridemics are shown in Fig. 2.

The residence time of ^{14}C -CO correlated positively with the values of total plasma cholesterol ($r=0.38$, $P=0.02$) and plasma triglycerides ($r=0.6$, $P<0.001$) and negatively with HDL-C ($r=-0.49$, $P=0.0025$). ^3H -TO residence time was correlated positively with total plasma cholesterol ($r=0.47$, $P=0.003$) and plasma triglycerides ($r=0.53$, $P=$

0.007). There was no correlation between the residence time of ^3H -TO and HDL-C. The residence times of both radioisotopes did not correlate with age, body mass index, Apo B or Apo AI levels. The univariate analysis confirmed the results of the Spearman's test (data not shown). Multivariate analysis showed that, after correcting for the other variables, only the logarithm of plasma triglycerides persisted in influencing ^{14}C -CO residence time (r^2 for the regression equation=0.4, $P=0.004$). On the other hand, the residence time of ^3H -TO was independently influenced by the logarithms of total plasma cholesterol and plasma triglycerides (r^2 for the multiple regression equation=0.2, $P=0.02$).

3.2. Comparison of the patients randomized to gemfibrozil or placebo

The clinical and laboratory characteristics of the hypertriglyceridemic patients that received gemfibrozil or placebo are shown in Table 3. There were no differences regarding clinical parameters and the use of thiazidic diuretics or beta-blockers. Also, total cholesterol and HDL-C levels were similar. Despite randomisation plasma triglyceride levels were higher in the group allocated to gemfibrozil ($P=0.001$). However, there were no differences regarding the residence times of the labelled emulsion lipids at baseline (Table 3).

Gemfibrozil treatment reduced the residence times of ^3H -TO and ^{14}C -CO by, respectively, 46% ($P=0.003$) and 53% ($P=0.008$). There was a 92% increase in $k_{2,3}$ ($P=0.008$) and a 51% increase in $k_{6,7}$ ($P=0.008$). There were no alterations in the rapid clearance component represented by $k_{1,3}$ and $k_{5,7}$. Data are shown in Table 4. The radioisotopic decaying curves before and after gemfibrozil treatment are shown in Fig. 3. The residence times of the tracers were not affected by placebo administration, respectively, for ^{14}C -CO from 47.0 (30.0; 75.5) to 55.0 (37.0; 87.0) min and for ^3H -TO from 17.5 (13.0; 24.0) to 13.0 (9.0; 25.0) min, for both parameters, $P=NS$ (Table 4).

After treatment, the residence times of ^3H -TO and

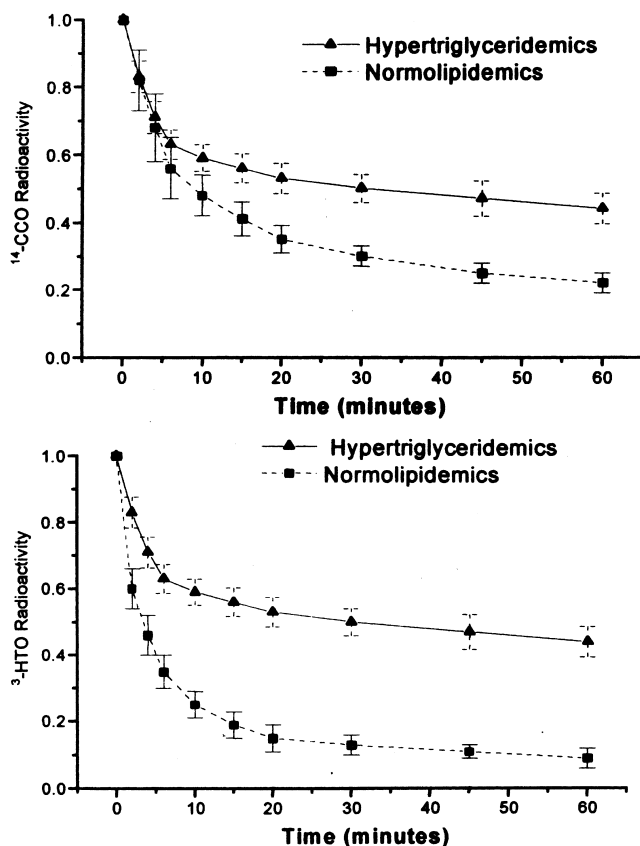


Fig. 2. Plasma decaying curves of ^{14}C -cholesteryl oleate (^{14}C -CO) and ^3H -triolein (^3H -TO) in normolipidemic ($n=27$) and hypertriglyceridemic ($n=12$) patients. Data expressed as mean \pm S.E.M.

Table 3

Clinical characteristics, baseline plasma lipids (mmol/l) and ^{14}C -cholesteryl oleate (^{14}C -CO) and ^3H -triolein (^3H -TO) residence times (min) of the hypertriglyceridemic subjects who were randomized to gemfibrozil or placebo

	Gemfibrozil	Placebo	<i>P</i>
<i>n</i>	8	7	–
Age (years)	48.0±11.0	52.0±20.0	NS
Male, <i>n</i> (%)	6 (75.0)	5 (61.5)	NS
Female, <i>n</i> (%)	2 (25.0)	2 (38.5)	
Body mass index (kg/m ²)	28.0±4.0	27.0±3.0	NS
Arterial hypertension, <i>n</i> (%)	5 (62.5)	3 (42.9)	NS
Diabetes mellitus, <i>n</i> (%)	1 (12.5)	1 (14.3)	NS
Coronary artery disease, <i>n</i> (%)	2 (25.0)	2 (28.6)	NS
Smoking, <i>n</i> (%)	0 (0)	0 (0)	NS
Beta-blockers, <i>n</i> (%)	1 (12.5)	1 (14.3)	NS
Thiazidic diuretics, <i>n</i> (%)	0 (0)	1 (14.3)	NS
Total cholesterol ^a	6.85 (6.15; 7.49)	8.21 (6.64; 10.82)	NS
Triglycerides ^b	5.71(2.89; 8.11)	2.91 (2.78; 2.99)	0.001
HDL-C ^a	0.75 (0.69; 0.87)	0.72 (0.67; 0.85)	NS
^{14}C -CO residence time	43.0 (34.0; 114.0)	47.0 (30.0; 75.5)	NS
^3H -TO residence time	13.0 (11.0; 20.0)	17.5 (13.0; 24.0)	NS

^a Multiply by 38.7 to convert to mg/dl.

^b Multiply by 88.5 to convert to mg/dl.

^{14}C -CO in patients that received gemfibrozil became similar to those of normolipidemics (Tables 2 and 4). Compared to baseline, gemfibrozil treatment improved plasma lipids by reducing total plasma cholesterol by 23%

($P=0.03$), plasma triglycerides by 59% ($P=0.002$) and increasing HDL-C by 25% ($P=0.008$). These values attained after treatment with the drug were no longer different of those of normolipidemics (Tables 2 and 4).

Table 4

Plasma lipids (mmol/l), ^{14}C -cholesteryl oleate (^{14}C -CO) and ^3H -triolein (^3H -TO) residence times (min) and transference $k_{x,y}$ constants (min⁻¹) of hypertriglyceridemic subjects who were randomized to gemfibrozil ($n=8$) or placebo ($n=7$)

	Before gemfibrozil	After gemfibrozil	<i>P</i>	Before placebo	After placebo	<i>P</i>
Total cholesterol ^a	6.85 (6.15; 7.49)	5.27 (4.88; 6.17)	0.03	6.54 (5.78; 6.87)	6.66 (4.78; 715)	NS
Triglycerides ^b	5.71 (3.89; 8.11)	2.32 (1.62; 3.43)	0.003	2.91 (2.78; 2.99)	4.3 (2.5; 5.7)	NS
HDL-C ^a	0.75 (0.69; 0.87)	1.00 (0.82; 1.24)	0.008	0.7 (0.67; 0.85)	0.67 (0.70; 0.88)	NS
^{14}C -CO residence time	43.0 (34.0; 114.0)	20.0 (15.0; 26.0)	0.008	47.0 (30.0; 75.5)	55.0 (37.0; 87.0)	NS
^3H -TO residence time	13.0 (10.0; 20.0)	7.0 (5.0; 9.0)	0.015	17.5 (13.0; 24.0)	13.0 (9.0; 25.0)	NS
$k_{1,2}$	0.18 (0.13; 0.3)	0.22 (0.12; 0.39)	NS	0.193 (0.18; 0.30)	0.190 (0.07; 0.44)	NS
$k_{1,3}$	0.19 (0.11; 0.28)	0.33 (0.098; 0.40)	NS	0.095 (0.007; 0.15)	0.182 (0.08; 0.28)	NS
$k_{2,3}$	0.013 (0.004; 0.015)	0.025 (0.021; 0.033)	0.008	0.015 (0.014; 0.017)	0.010 (0.004; 0.01)	0.047
$k_{3,4}$	0.002 (0.0017; 0.004)	0.0026 (0.0017; 0.0044)	NS	0.002 (0.002; 0.01)	0.002 (0.0005; 0.004)	NS
$k_{5,7}$	0.38 (0.19; 0.63)	0.47 (0.28; 0.70)	NS	0.254 (0.160; 0.32)	0.50 (0.193; 0.90)	NS
$k_{6,7}$	0.033 (0.027; 0.039)	0.050 (0.042; 0.086)	0.008	0.04 (0.03; 0.042)	0.03 (0.02; 0.004)	NS
$k_{7,8}$	0.0015 (0.001; 0.0025)	0.0007 (0.0005; 0.001)	NS	0.002 (0.0004; 0.007)	0.0009 (0.0005; 0.0018)	NS

^a Multiply by 38.7 to convert to mg/dl.

^b Multiply by 88.5 to convert to mg/dl.

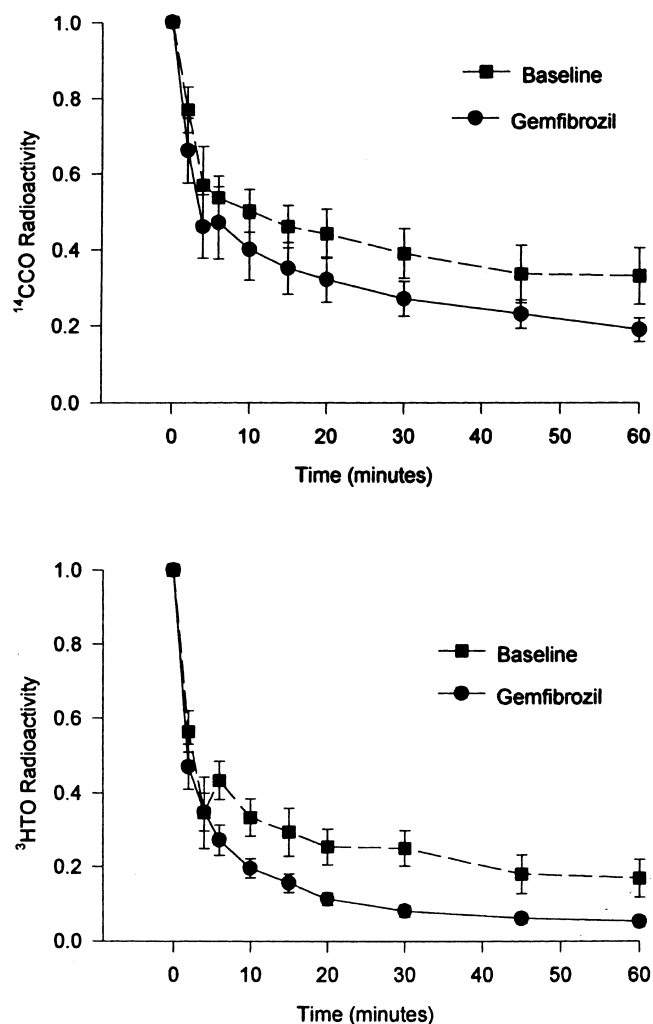


Fig. 3. Plasma decaying curves of ^{14}C -cholesteryl oleate (^{14}C -CO) and ^3H -triolein (^3H -TO) in hypertriglyceridemics at baseline and after gemfibrozil treatment ($n=8$). Data mean \pm S.E.M.

4. Discussion

In fat load test, the area under the ascending-descending curve of post-prandial plasma triglycerides and vitamin A supposedly represents the input and output of chylomicron lipids into the circulation [9]. However, while the absorption process lasts several hours, chylomicron remnants are rapidly taken-up by the liver and their lipids start to recirculate back into the plasma compartment incorporated into newly synthesised VLDL [7,22]. Because chylomicrons compete with VLDL for lipolysis by lipoprotein lipase [1], accumulation of VLDL occurs in the post-prandial period, adding-up to the increase of post-prandial lipids [2,3]. Therefore, the area under the curve of post-prandial plasma triglycerides and vitamin A represents the summation of complex phenomena and is thus difficult to interpret. In contrast, the labelled chylomicron-like emulsions are injected intravenously in a bolus [11,15]. The gastrointestinal component is thus bypassed and the decay-

ing curves of the emulsion radiolabels assess, by analogy, chylomicron degradation and removal from the plasma. Injected after a 12-h fast in minimal amounts the emulsion does not disturb VLDL metabolism as occurs in fat-load tests [2,3]. The residence times of the two emulsion components injected into normolipidemic subjects calculated according to the technique described above were similar to those obtained for native chylomicrons labelled with vitamin A and reinjected into the subjects [7,22,23].

The type IV and IIB patients studied here showed defects in both emulsion lipolysis and removal from the plasma despite the fact that they had not displayed chylomicron accumulation after 12-h fast by lipoprotein electrophoresis. Chylomicrons and chylomicron-like emulsions are removed from plasma in a two-phase manner, reflected by the grossly bi-exponential decay curve [4,12,14,15]. Initially, there is a rapid removal accounted for by the redistribution of the injected chylomicron particles to sites where they may access such as the space of Disse and to lipolytic sites located on the endothelium, referred as chylomicron margination [24,25]. The second (slower) removal phase depends initially on chylomicron lipolysis and generation of remnants that subsequently enter the space of Disse [4]. There, remnants can bind directly to the LDL receptor or the low density lipoprotein related protein (LRP) receptor [4,5], a mechanism that is dependent on Apo E, and are thus taken-up by the liver cells. According to the adopted kinetic model, the differences between hypertriglyceridemic patients and their normolipidemic controls arise in the slow phase of the decaying curve expressed by $k_{2,3}$ and $k_{6,7}$. In contrast, there were no differences between the two groups regarding $k_{1,3}$ and $k_{5,7}$ that represent the transference rates of the rapid phase of the decaying curve. In the hypertriglyceridemic group, the reduction of $k_{2,3}$ and $k_{6,7}$ values could eventually be attributed to competition of the emulsion with accumulated VLDL for the common catabolic pathway [1], because it has been shown that excess VLDL reduces chylomicron clearance from plasma [9,14]. Similarly to the post-prandial triglyceridemia curves obtained from oral fat load tests [17,26], the plasma kinetics of both emulsion labels correlated with the fast triglyceride values. This finding is important for the understanding of the differences in emulsion kinetic data between hypertriglyceridemics and normolipidemics as well as the improvement of emulsion clearance after gemfibrozil treatment. However, even when multiple regression analysis was performed we were able to explain no more than 40% of the radioisotope's residence time values. The intravascular metabolism of the emulsion may be influenced in the same manner as chylomicrons by factors such as apolipoprotein E genotype [27], lipoprotein and hepatic lipase activities [10,26], and insulin plasma levels [28]. In this respect, in our study, differences in emulsion kinetic data between normolipidemics and hypertriglyceridemics cannot be ascribed to Apo E genotypes, because their frequency was similar in the two groups.

It is noteworthy that gemfibrozil improved not only the emulsion lipolysis, as would be expected since fibrates stimulate lipoprotein lipase activity [16], but also the removal of the $^{14}\text{C-CO}$ moiety that stands for the removal of the emulsion remnants. There is evidence that gemfibrozil reduces fasting triglyceride levels by two main mechanisms: increase in lipoprotein lipase activity and VLDL synthesis reduction [16,29]. The reduction of expression of Apo C-III, which is a lipoprotein lipase inhibitor, is accounted for by the increased activity of this enzyme [16]. However, decreased VLDL synthesis induced by this drug would lead to a reduced competition between emulsion and lipoproteins for lipolytic sites, as well as for Apo C-II and Apo E binding. The reduction of Apo C-III levels may also facilitate binding to receptors since this lipoprotein reduces the Apo E-dependent clearance [30]. We also cannot discard stimulatory effects of gemfibrozil on hepatic lipase [31] and on LDL receptor expression [32], both factors that increase remnant clearance [4,5]. Previously, by vitamin A fat load tests it had been shown in patients with endogenous hypertriglyceridemia that fibrates can reduce post-prandial lipemia [9,17,18,33]. It was found that gemfibrozil reduces post-prandial lipemia in type IV [9] as well as in diabetic patients with moderate hypertriglyceridemia [33]. However, studies do not show uniformly that both lipolysis and remnant removal were improved by treatment. The study of Simpson et al. with fenofibrate [17] showed improvement only in lipolysis, without enhancement of remnant removal. Attia et al. [18] showed that bezafibrate use led to slow remnant removal in diabetic patients with very high triglyceride levels. It remains undefined whether those findings are consequent to differences in the used drugs or in the studied patient groups or, else, to limitations of the vitamin A test.

Evaluated by either fat load tests [34–36] or by chylomicron-like emulsions [15], delayed chylomicron intravascular catabolism has consistently been documented in patients with coronary artery disease. Whether chylomicron remnant accumulation predisposes directly to atherogenesis by arterial deposition [37,38], or indirectly by hindrance of VLDL remnants catabolism [6,7], or by reducing HDL-C levels [38,39], remains to be settled. There is evidence that fibrates may reduce atherosclerosis progression and clinical events in selected groups of patients [40–44]. Therefore, improvement in chylomicron clearance by use of gemfibrozil, as in the case of our study evaluated by a chylomicron-like emulsion may have potential anti-atherogenic mechanisms.

Acknowledgements

We would like to thank Carlos H. Mesquita, Ph.D., for the help with the compartmental analysis and the use of his ANACOMP[®] software. This work was supported by grant number 95/0591-9 from the Fundação de Amparo a

Pesquisa do Estado de São Paulo (FAPESP), São Paulo, Brazil. R.D. Santos was supported by a postgraduation scholarship from the Conselho Nacional de Pesquisa (CNPq), Brasília, Brazil. R.C. Maranhão holds a Research Award from CNPq.

Appendix A

As previously described [12], the kinetic model used in this study agrees with the fitting of experimental curve which shows the following profile: both radioactivity decay curves ($^{14}\text{C-CO}$ and $^3\text{H-TO}$) show a rapid decay followed by a slow decay and finally the curve tends to a plateau or to a smooth increase suggesting recycling of the radioactivity compounds incorporated into VLDL secreted by the liver. These features are similar to the biphasic plasma decay curve of natural chylomicrons [4]. Compartment 1 (Fig. 1) corresponds to the chylomicron-like emulsion $^{14}\text{C-CO}$ label injected into plasma. From the injected particles a fraction $k_{1,2}$ (min^{-1}) is transformed in another sub-population of particles, represented by compartment 2. A fraction $k_{2,3}$ (min^{-1}) from this sub-population is taken up by the hepatic cells (compartment 3). It was proposed that the constant $k_{2,3}$ depends on the action of hepatic receptors [12]. Clearance of $^{14}\text{C-CO}$ reflects tissue uptake from plasma of remnant particles in various delipidation degrees [13]. Specifically for $^{14}\text{C-CO}$ the model described by Redgrave and Zech [12] does not consider a direct output from compartment 1 but the absence of a plateau at the beginning of the decaying curve as previously shown [13–15] suggests that a fraction of the injected particles, denominated $k_{1,3}$ in our study is directly removed from the plasma by the liver or other tissues thus bypassing the delay time demanded by compartment 2. After removal from plasma, lipids from compartments 1 and 2 are supposed to be utilized in VLDL hepatic synthesis (compartment 3) and recycled to the plasma (compartment 4). The $^3\text{H-TO}$ compartments were denoted from number 5 to 8 and correspond to compartments 1–4 of the $^{14}\text{C-CO}$, respectively. The same model described by Redgrave and Zech [12] was used for $^3\text{H-TO}$ and compartment 7 represents the extra-vascular compartment (liver and peripheral tissues). Recirculation of $^3\text{H-TO}$ was also considered in the form of VLDL secretion (compartment 8). The kinetics of $^3\text{H-TO}$ allow evaluation of the kinetics of lipolytic mechanisms. The constant $k_{6,7}$ (min^{-1}) was assumed to represent the combination of the transference rate of the remnant particle ($k_{2,3}$) and removal from plasma of the $^3\text{H-free}$ fatty acid.

In order to achieve more accurate parameters, the curve fitting was done by pooling the data from $^{14}\text{C-CO}$ and $^3\text{H-TO}$. Moreover, the $k_{1,2}$ was assumed to be equal to $k_{5,6}$. The emulsion radioactivity was measured before its injection in the bloodstream allowing definition of the ratio:

$$R = \frac{{}^3\text{H-TO radioactivity}}{{}^{14}\text{C-CO radioactivity}} \quad (1)$$

Therefore, in the regression process the initial radioactivity of the $^3\text{H-TO}$ was assumed as:

$$\text{Radioactivity of } ^3\text{H-TO in plasma (dpm/ml)} \\ = (\text{initial radioactivity of } ^{14}\text{C-CO in plasma}) \cdot R \quad (2)$$

These procedures were able to increase 2 df in the regression process. The regression parameters were calculated using computerised software [21]. This software gives the parameters from the exponential time course of chylomicron decay curve. The functions defined for the compartments 1 and 5 were, respectively:

$$f1(t) = C1,1 \cdot \exp(-w1 \cdot t) \quad (3)$$

and

$$f5(t) = C5,5 \cdot \exp(-w5 \cdot t) \quad (4)$$

while for compartments 2 and 6 their functions are:

$$f2(t) = C2,1 \cdot \exp(-w1 \cdot t) + C2,2 \cdot \exp(-w2 \cdot t) \quad (5)$$

and

$$f6(t) = C6,4 \cdot \exp(-w4 \cdot t) + C6,5 \cdot \exp(-w5 \cdot t) \quad (6)$$

where $C_{i,j}$ are the linear parameters and w_j are the exponential coefficients estimated by the non-linear least square method. The mean transit time or residence time in minutes were calculated in terms of the occupancy principle, i.e.

$$\text{Residence time} = \frac{\int_0^{\infty} f_j(t) \cdot dt}{I_j} \quad (7)$$

where $f_j(t)$ is the time course curve of the concentration of the interest substance in j -compartment and I_j is the initial value at time zero (moment of the emulsion bolus injection). The residence time equations for $^{14}\text{C-CO}$ and $^3\text{H-TO}$ were, respectively:

$$\text{Residence time}_{^{14}\text{C-CO}} = \frac{\frac{C1,1 + C2,1}{w1} + \frac{C2,2}{w2}}{I1} \quad (8)$$

and

$$\text{Residence time}_{^3\text{H-TO}} = \frac{\frac{C5,4 + C6,4}{w4} + \frac{C6,5}{w5}}{I5} \quad (9)$$

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