Atherosclerosis 233 (2014) 319–325

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Favorable effects of ezetimibe alone or in association with simvastatin on the removal from plasma of chylomicrons in coronary heart disease subjects

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ARTICLE INFO

Article history:
Received 13 May 2013
Received in revised form
10 December 2013
Accepted 11 December 2013
Available online 17 January 2014

Keywords:
Simvastatin
Ezetimibe
Chylomicrons
Triglyceride-rich lipoproteins
Emulsions
ApoB-48

ABSTRACT

Objective: Reductions on the clearance from plasma of chylomicrons are associated with atherosclerosis. Statins improve the removal from plasma of chylomicrons in a dose dependent manner. There is controversy whether ezetimibe modifies the plasma clearance of chylomicrons. Effects of ezetimibe alone or in combination with simvastatin were compared with low and high dose of the latter, upon the kinetics of a chylomicron-like emulsion in coronary heart disease (CHD) patients.

Methods: 25 CHD patients were randomized for treatment with ezetimibe 10 mg (group 1) or simvastatin 20 mg (group 2) with progression to ezetimibe + simvastatin 10/20 mg or simvastatin 80 mg, respectively. Kinetic studies were performed at baseline and after each treatment period of 6 weeks. The fractional catabolic rates (FCR) of the emulsion labeled with ^{14}C-CE and ^{3}H-TG, that represent respectively chylomicron remnant and triglyceride removal, were calculated. Comparisons were made by ANOVA.

Results: The ^{14}CE-FCR in group 1 were 0.005 ± 0.004, 0.011 ± 0.008 and 0.018 ± 0.005 min^{-1} and in group 2 were 0.004 ± 0.003, 0.011 ± 0.008 and 0.019 ± 0.007 min^{-1} respectively at baseline, after 6 and 12 weeks ($p < 0.05$ vs. baseline, and 6 vs. 12 weeks). The ^{3}H-TG-FCR in group 1 were 0.017 ± 0.011, 0.024 ± 0.011 and 0.042 ± 0.013 min^{-1} and in group 2 were 0.016 ± 0.009, 0.022 ± 0.009 and 0.037 ± 0.012 min^{-1} at baseline, after 6 and 12 weeks ($p < 0.05$ vs. baseline, and 6 vs. 12 weeks). There were no differences between groups in time.

Conclusion: Both treatments increased similarly the removal from plasma of chylomicron and remnants in CHD patients.

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1. Introduction

The impaired plasma removal of chylomicrons and remnants has been associated with the incidence and progression of coronary atherosclerosis even when fasting plasma lipids are within normal values [1–5]. These particles rapidly penetrate and accumulate in the subendothelial space of the arterial wall, leading to macrophage uptake and foam cell formation [6]. Changes in chylomicron metabolism are also implicated with high-density lipoprotein cholesterol (HDL-C) reduction and impaired reverse cholesterol transport [3]. In addition to being removed from plasma by their specific receptor [7] LRP, by heparan-sulphate proteoglycans (HSPG) [8] and by very low density lipoprotein (VLDL) receptors [9], chylomicron remnants are also removed by the LDL receptor (LDLR) [7].

Statins reduce plasma low-density lipoprotein cholesterol (LDL-C) levels by inhibiting hydroxy-methyl-glutaryl coenzyme A reductase (HMGCo-A reductase), which leads to decreased intracellular hepatic cholesterol pool and consequently up-regulation of the LDLR expression of in liver [10]. In addition to increasing the plasma clearance of LDL particles, statins also enhance the removal

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http://dx.doi.org/10.1016/j.atherosclerosis.2013.12.008

of chylomicrons and its remnants from the blood, an effect proportional to the potency of the statin [11,12].

Ezetimibe inhibits intestinal cholesterol absorption acting on the Niemman-Pick C1 Like 1 receptor located at the enterocyte brush border [13]. When used alone in subjects, ezetimibe reduces LDL-C by only 12–14% [13]. However, in combination with low dose statins there is a clear-cut synergic effect, and LDL-C lowering reaches 50–60% reduction, which is the range attained only by the most potent statins at the maximally approved dosage [14,15]. This effect has been ascribed to increased expression of LDLR consequent to the incremental reduction in intrahepatic cholesterol pool, which leads to greater removal from plasma of the apoB-100 containing lipoproteins [13,16].

Triglyceride-rich emulsions similar to small-sized chylomicrons have been extensively used to study chylomicron and remnant metabolism in different clinical sets. Reductions in the clearance from plasma of emulsion and remnants have been clearly shown in subjects with atherogenic dyslipidemia [17], familial hypercholesterolemia [18] and in subjects with stable coronary heart disease (CHD) under or not statin treatment [5,11,19,20]. In CHD patients reduction in emulsion clearance and lipolysis was associated with presence and progression of the atherosclerotic plaque, as well as with clinical cardiovascular events [20,21]. Procedures that change the expression of the LDLR, like cholesterol feeding [22] or the use of statins [11,12], have respectively reduced and increased the removal from plasma of the emulsion and remnants.

There is limited evidence from the literature that ezetimibe decreases the concentration of chylomicrons and remnants [23,24]. This is clearer when ezetimibe is added to statins in normolipidemic subjects without previous manifestation of CHD [24]. Furthermore, the mechanisms behind these findings are not fully understood.

This study was aimed to investigate the effects of ezetimibe alone or in combination with low-dose simvastatin as compared to intermediate and maximum simvastatin doses upon the removal from plasma of a chylomicron-like emulsion in CHD patients. The results show that ezetimibe alone or in association with simvastatin improved the chylomicron metabolism pathways.

2. Methods

2.1. Study patients

Twenty-five stable CHD patients from the outpatient clinic of the Heart Institute (InCor) of the University of Sao Paulo Medical School Hospital were studied. None had an acute coronary or cerebrovascular event or revascularization in the last 6 months. The mean age was 60 years-old; 20 (80%) were of the male gender. Inclusion criteria were LDL-C >100 mg/dl and plasma triglycerides <500 mg/dl after a lipid lowering drug wash-out of 6 weeks, a safe period for wash-out in CHD patients [25]. All studied women were post-menopausal and were not in use of hormone replacement therapy. Exclusion criteria included heart, kidney and hepatic failure as well as type 2 diabetes and thyroid disease. This was a randomized, non-blinded study, with two arms (Groups 1 and 2):

Group 1: 13 subjects randomized to ezetimibe 10 mg/day for 6 weeks, followed by ezetimibe 10 mg plus simvastatin 20 mg/day for additional 6 weeks.
Group 2: 12 subjects randomized to simvastatin 20 mg/day for 6 weeks and after that simvastatin was up-titrated to 80 mg/day for additional 6 weeks.

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in *a priori* approval by the Ethics Committee of the Hospital das Clinicas of the University of São Paulo Medical School (CAPEPesq, protocol number 1068/06), and a written informed consent was obtained from all patients. This trial was registered at Clinicaltrials.gov with the number NCT00481351. Both simvastatin (Zocor®) and ezetimibe (Ezetrol®) were donated by MSD (São Paulo, Brazil). The design, development and analysis of this study were totally done by the investigators.

2.2. Plasma biochemical and apolipoprotein analysis

Fasting blood collection was performed at baseline, 6 weeks (second evaluation) and 12 weeks (third evaluation) after the start of the experiment. Total cholesterol (TC), HDL-C, and triglycerides (TG) were determined by enzymatic methods (commercial kits–Roche, Somerville, NJ, USA) and LDL-C was calculated by Friedewald formula (LDL = T C-HDL-TG/5) for values up to 400 mg/dL. When TG values were between 400 and 500 mg/dl, direct determination of LDL-C was carried out using and enzymatic homogeneous LDL-C kit (Roche, Somerville, NJ, USA). The quantification of apolipoproteins (apo) A-I and apoB-100 were done using commercial kits (Roche, Mannhein, Germany). Serum apoB-48 was quantified at fasting states, from serum samples frozen at −80 °C using an ELISA kit manufactured by Shibayagi Co (Gunma, Japan) at Boston Heart Diagnostics (Framingham, USA). The assay uses a monoclonal antibody that only recognizes apoB-48 and not apoB-100. Glucose, creatine kinase (CK) and alanine aminotransferase (ALT) levels were determined by standardized automated laboratory methods (Roche, Mannhein, Germany).

2.3. Chylomicron-like emulsion kinetic study

The chylomicron-like emulsions were prepared as previously described [5] by ultrasonic irradiation of lipid mixtures containing 2% cholesterol, 23% lecithin, 6% cholesteryl oleate (^{14}C-CE) and 69% triolein (^{3}H-TG) with 20 μCi of ^{14}C-CE and 40 μCi of ^{3}H-TG. Emulsions were purified by ultracentrifugation in density gradients as described previously [5] and sterilized by passage through a 0.2 μm filter. All kinetic studies were performed after a 12-h fast. One vein from each arm was cannulated and maintained with a saline flush. The chylomicron-like emulsion was injected in a *bolus* (volume of 200–300 μl), containing 148 kBq (4 μCi) of ^{3}H-TG and 74 (2 μCi) of ^{14}C-CE, followed by a 5 ml saline flush. Blood samples were collected from the contralateral arm vein at pre-established intervals during 60 min (2, 4, 6, 10, 15, 20, 30, 45 and 60 min after emulsion injection). Blood was collected into tubes containing 50 μl of sodium heparin and centrifuged at 2700 rpm for 10 min. An aliquot of 1 ml of plasma was transferred to counting vials and 5 ml of scintillation solution PPO: DM-POPOP: triton-100/toluene (5 g: 0.5 g: 333 ml:/667 ml) added to the vials. Radioactivity in the samples was determined using a Packard 1660 TR spectrometer (Packard Meridien). The calculated inter-assay coefficient of variation for those kinetic analyses was <3%. As previously described [5], the radiation dose injected in each experiment was much bellow the 50 mSV limit for radioactive intake, as determined by the International Commission on Radiological Protection [26]. For ^{14}C-CE, the dose was 0.04 mSV and for ^{3}H-TG, 0.0025 mSV. Patients were submitted to kinetic studies at baseline, 6 weeks and at 12 weeks of follow-up.

2.4. Kinetic analysis

Fig. 1 shows the kinetic model used in this study. After entering the plasmatic compartment emulsions adsorb apolipoproteins like apoE, apoC-II and apoC-III [27] and are quickly incorporated into the plasma lipoprotein pool. The plasma decay curves of the

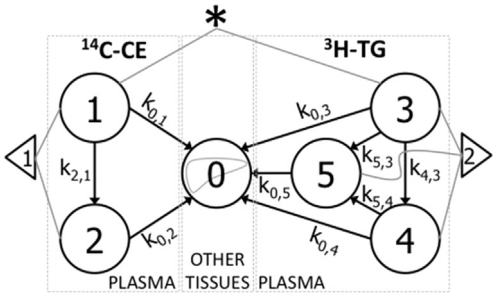

Fig. 1. Biokinetic model for [^{14}C-CE] and [^{3}H-TG] labeled chylomicron-like core. 1 – chylomicron-like core labeled with ^{14}C-CE; 2 – chylomicron-like core labeled with ^{14}C-CE assimilated by other plasmatic lipoproteins; 3 – chylomicron-like core labeled with ^{3}H-TG; 4 – chylomicron-like core labeled with ^{3}H-TG assimilated by other plasmatic lipoproteins; 5 – lipolysis components of ^{3}H-TG; 0 – extra-plasmatic tissues including the liver. The input of the radioactive tracer chylomicron-like core is represented by an asterisk. The triangles represent the radioactive measurement content in the sample.

emulsion ^{14}C-CE and ^{3}H-TG were evaluated according to a modification of the model proposed by Redgrave and Zech [28] and considering the model proposed by Schwartz et al. [29]. The kinetic model is based on the experimental curve which shows the following profile: both radioactivity decay curves (^{14}C-CE and ^{3}H-TG) show a rapid decay followed by a slow decay and finally the curve tends to a plateau (Fig. 2). Compartments (1) and (3) correspond to the chylomicron-like core injected into the plasma, and (1) corresponds to the fraction of ^{14}C-CE and (3) the fraction of ^{3}H-TG. Compartments (2) and (4) represent respectively the chylomicron-like core labeled with ^{14}C-CE and ^{3}H-TG assimilated by the plasmatic lipoprotein pool as suggested by Schwartz et al. [29]. Compartment (5) represents lipolysis components of ^{3}H-TG and finally compartment (0) represents extra-plasmatic tissues including the liver. The constants $k_{i,j}$ (min^{-1}) represent the fractional catabolic rate (FCR) or transfer from compartment j to compartment i over time. The model proposed by Redgrave and Zech [27] does not consider a direct output of the compartments (1) and (3), but the initial absence of a plateau in the decay curve, as shown previously [5], suggests that a fraction of the injected particles, called in our study as $k_{0,1}$ and $k_{0,3}$ is removed directly from plasma by the liver or other tissues.

Due to its trace concentrations the artificial chylomicron complex kinetic phenomenon is governed basically by the adsorbed apolipoproteins [27,28]. Since the concentration of natural lipoproteins found in plasma is considerably higher, the reactions between the emulsion and plasmatic lipoproteins can be assumed as a first order reaction. Therefore the constant $k_{0,1}$ is considered $= k_{0,3}$, $k_{2,1} = k_{4,3}$ and $k_{0,2} = k_{0,4}$ respectively. However, the removal from plasma of the artificial chylomicron labeled with ^{3}H-TG differs from the one seen by the ^{14}C-CE by the TG lipolysis and free fatty acid removal expressed by $k_{5,3}$ and $k_{5,4}$. The constants $k_{0,1}$ and $k_{0,3}$ represent the removal of chylomicron immediately after entering the plasma compartment by the liver and other tissues, and correspond the fast component of the radioisotope curve decay seen respectively by the ^{14}C-CE and ^{3}H-TG tracers. The constants $k_{2,1} = k_{4,3}$ correspond to the emulsion transference rates to a complex plasma lipoprotein pool as described by a model of Schwartz et al. [29]. As previously shown [18] the constants $k_{0,2}$ and

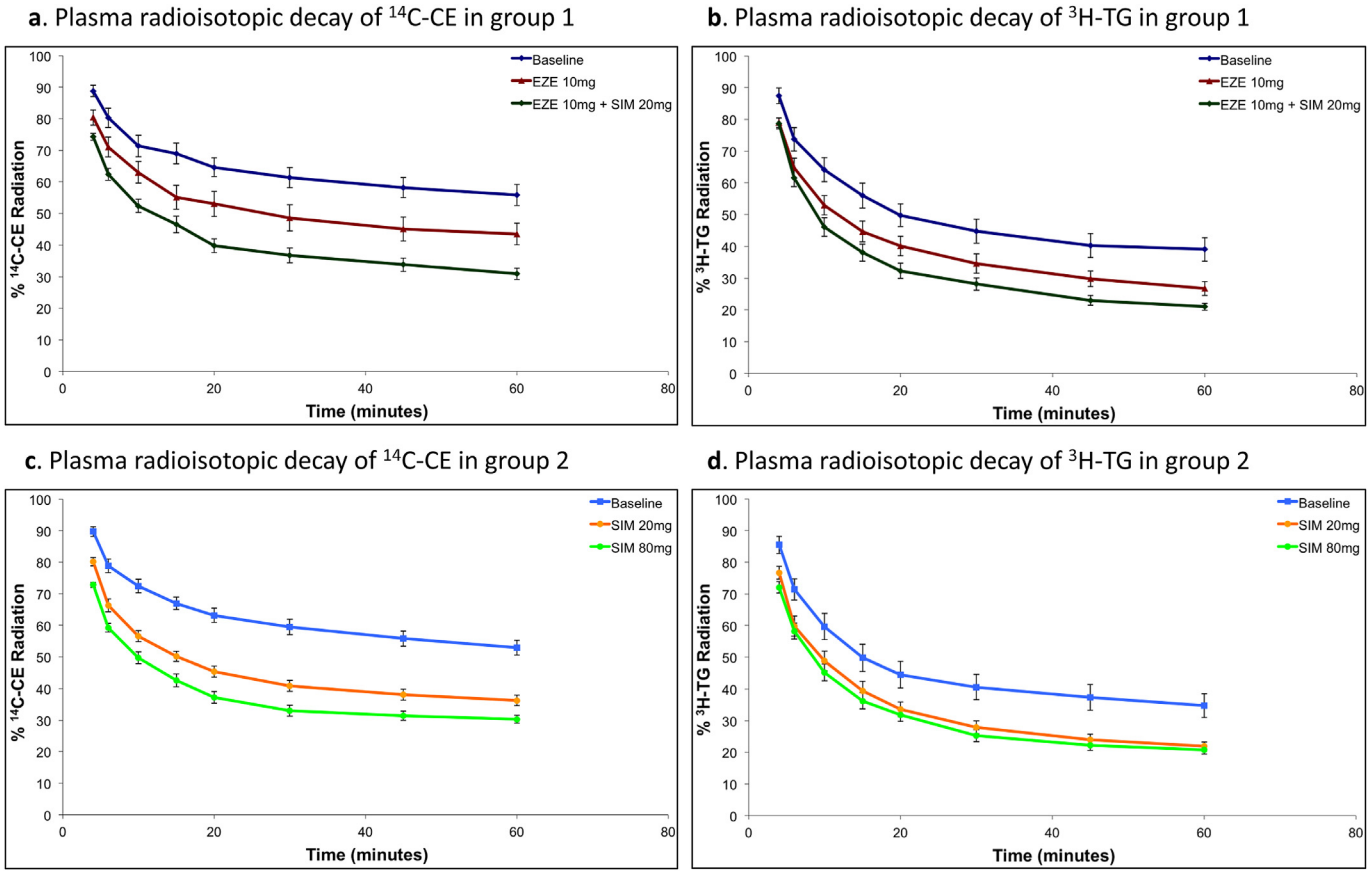

Fig. 2. Plasma radioisotopic decay of ^{14}C-CE and ^{3}H-TG in Groups 1 and 2 at baseline and after 6 weeks and 12 weeks of pharmacological treatment. Data expressed as mean± standard error of the mean.

$k_{0,4}$ are significantly smaller than $k_{0,1}$ and $k_{0,3}$ and represent the removal of the emulsion, mainly by the liver, from the plasma lipoprotein pool and correspond to the slow decay component of the radioisotope curve. Finally, $k_{0,5}$ represents the disappearance of free fatty acids from the intravascular compartment. The precision of parameters of the kinetic model (transference rates) presented an averaged coefficient of variation of 12.76 ± 10.93%.

Removal of chylomicron-like emulsions from the plasma was estimated by the fraction catabolic rates (FCR) of ^{14}C-CE and ^{3}H-TG (min^{-1}), calculated with the assistance of the ANACOMP code [18,30,31] as previously described. This software allows the determination of the transfer rates ($k_{i,j}$) among the compartments yields the parameters of the exponential time course of emulsion decay curves and calculates the FCR of both radioisotopes.

Emulsion ^{3}H-TG peeling or lipolysis is evaluated mainly by the delipidation index (DI) as proposed by Redgrave and Zech [28] by the following equation:

$$DI = 1 - [(1/^{3}\text{H-TG FCR})/(1/^{14}\text{C-CE FCR})]$$

2.5. Statistical analysis

Continuous data are shown as mean and standard deviations, except for apoB-48 and delipidation index shown as medians (ranges). Categorical variables are expressed as *N* (%). Data normality was evaluated using the Kolmogorov–Smirnov test. Comparison of clinical variables was done by Student's *t* test. Categorical variables were evaluated by Fisher's exact test. Laboratory and emulsion kinetic parameters at baseline and during treatment were compared by the use of two-factors repeated measures ANOVAs assuming an unstructured correlation matrix among evaluations. Differences among groups were detected by Tukey's test. A two-tailed *p*-value <0.05 was considered statistically significant. This is a non-inferiority study testing the effects of ezetimibe alone or in association with simvastatin upon the kinetics of chylomicron-like emulsion in comparison with 2 different dosages of simvastatin. Sample size was calculated based on a previous observation using equipotent doses of atorvastatin in relation to simvastatin vs. placebo [12]. Considering a study power of 95% and a *p* value of 5%, 12 and 9 patients would have to be evaluated respectively on each group at week 6 and 9 at week 12 to show differences vs. baseline.

Tests were performed at a significance level of 5%. Statistical analysis was performed using the SAS 8.0 (SAS Institute Inc. Cary, North Carolina) and SPSS 18.0 (SPSS Inc.,Chicago, Illinois) software.

3. Results

As shown in Tables 1–3, Groups 1 and 2 were not different regarding age, gender, waist circumference, plasma lipids, apolipoproteins, safety parameters (hepatic enzymes, glucose and CK) and the FCRs of the emulsion radioactive labels. The body mass index (BMI), however, was higher in Group 2 ($p = 0.02$).

3.1. Effects of the treatments upon plasma lipids and apolipoproteins

In Group 1 (Table 2), treatment with 10 mg ezetimibe alone resulted in a 20% reduction of LDL-C ($p < 0.001$) concentrations. When 20 mg simvastatin was added, LDL-C was further reduced by 48% from baseline ($p < 0.001$). In contrast, HDL-C was changed neither by 10 mg ezetimibe alone nor by the association of 20 mg simvastatin to the latter. TG and apoB-100 were not significantly changed by ezetimibe alone but changes attained formal significance level by the association of the two drugs by, respectively, −18% ($p = 0.007$) and −39% ($p < 0.001$). ApoA-I was increased by the association of the two drugs only, with a 9% increase ($p = 0.02$), and did not respond to 10 mg ezetimibe alone.

Table 1
Physical and clinical characteristics of the participant subjects allocated to Group 1, treated with 10 mg ezetimibe during 6 weeks followed by 10 mg ezetimibe +20 mg simvastatin in the ensuing 6 weeks; and Group 2, treated with 20 mg simvastatin during 6 wks followed by 80 mg simvastatin in the ensuing 6 weeks.

	Group 1	Group 2	*p*
	N = 13	*N* = 12	
Age (years)	61 ± 6	60 ± 3	0.14
Male gender *n* (%)	10 (77%)	10 (83%)	1.0
BMI (kg/m^2)	27 ± 3	28 ± 3	0.02
Waist circumference (cm)	94 ± 9	97 ± 8	0.53
Smoking *n* (%)	4 (30%)	2 (16%)	0.64
Hypertension *n* (%)	8 (61%)	10 (83%)	0.38

In Group 2, (Table 2) treatment with 20 mg simvastatin resulted in 34% reduction of LDL-C ($p < 0.001$). Escalation of simvastatin dose to 80 mg resulted in further reduction of LDL-C to 49% from baseline ($p < 0.001$). HDL-C was changed neither by the lower nor the higher dose. TG remained unchanged with 20 mg simvastatin, but responded to 80 mg simvastatin with a 30% reduction ($p = 0.007$). ApoB-100 was reduced by both 20 mg and 80 mg simvastatin, by 28% and 38% ($p < 0.001$) from baseline, but apoA-I was increased only by the high dose, in 9% ($p = 0.02$).

Comparing data from Group 1 with those from Group 2, LDL-C was more strongly reduced by 20 mg simvastatin than by 10 mg ezetimibe ($p = 0.048$). On the other hand, the association 10 mg ezetimibe +20 mg simvastatin had equal effect on LDL-C as 80 mg simvastatin ($p = 0.99$). ApoB-100 reduction was not greater with 20 mg simvastatin than with the 10 mg ezetimibe treatment ($p = 0.14$) and the 10 mg ezetimibe +20 mg simvastatin treatment and the 80 mg simvastatin resulted in equal reductions of apoB ($p = 0.99$). There were non-significant changes in apoB-48 concentrations in time with both treatments.

Regarding the safety parameters, namely plasma glucose, ALT and CK, they were altered by none of the treatments.

3.2. Effects of treatments upon emulsion kinetics

Fig. 2a and b show the plasma decaying curves of both radioisotopes in Group 1. Table 3 depicts the emulsion kinetic parameters of studied subjects. Treatment with 10 mg ezetimibe increased the emulsion ^{14}C-CE FCR and ^{3}H-TG FCR by 80% ($p = 0.001$) and 23% ($p = 0.036$), respectively, as compared to baseline. Association of 20 mg simvastatin further increased ^{14}C-CE FCR by 260% ($p = 0.002$) and ^{3}H-TG FCR by 152% ($p < 0.001$) compared to baseline. Regarding the other descriptive compartmental parameters, $k_{0,2} = k_{0,4}$ was increased by 10 mg ezetimibe in 300% ($p < 0.001$), and further by 10 mg ezetimibe + simvastatin 20 mg in 600% ($p < 0.001$) from baseline. Differently, $k_{0,1} = k_{0,3}$ was only increased by 10 mg ezetimibe + simvastatin 20 mg, with 171% increase ($p = 0.001$), but not by 10 mg ezetimibe alone. In relation to the other transference rates, $k_{0,5}$, $k_{2,1} = k_{4,3}$ and $k_{5,3}$ were reduced with the drug association, with, respectively, 54% ($p = 0.008$), 57% ($p = 0.023$) and 30% ($p = 0.023$) reduction, but not with ezetimibe alone.

Fig. 2c and d show respectively the plasma decaying curves of ^{14}C-CE and ^{3}H-TG in Group 2. The use of 20 mg simvastatin increased the emulsion ^{14}C-CE FCR and ^{3}H-TG FCR by 100% ($p < 0.001$) and 31% ($p = 0.036$), respectively, as compared to baseline (Table 3). Escalation of simvastatin dose to 80 mg further increased ^{14}C-CE FCR by 375% ($p = 0.002$) and ^{3}H-TG FCR by 131%

Table 2
Effects of lipid-lowering therapy on lipids, apolipoproteins and safety parameters in groups 1 and 2.

Parameter	Group 1 ($N = 13$)			Group 2 ($N = 12$)			p intragroup ANOVA
	Baseline	10 EZT (6 wks)	10 EZT + 20 SIM (12 wks)	Baseline	20 SIM (6 wks)	80 SIM (12 wks)	
TC	222 ± 24	193 ± 21[a,c]	152 ± 21[a,b]	215 ± 33	168 ± 18[a,c]	144 ± 24[a,b]	<0.001
HDL-C	40 ± 11	50 ± 15	51 ± 16	45 ± 13	48 ± 15	44 ± 14	0.19
LDL-C	141 ± 21	112 ± 19[a,c]	74 ± 16[a,b]	139 ± 22	91 ± 15[a,c]	71 ± 15[a,b]	<0.001
TG	165 ± 80	164 ± 84	134 ± 54[a]	213 ± 112	136 ± 91	149 ± 81[a]	0.007
ApoB-100	123 ± 32	109 ± 33	74 ± 14[a,b]	115 ± 19	82 ± 20[a]	71 ± 18[a]	<0.001
ApoA-I	139 ± 29	152 ± 33	152 ± 26[a]	136 ± 30	143 ± 26	148 ± 21[a]	0.024
ApoB-48	0.85 (0.12; 11.30)	0.85 (0.21; 6.47)	0.64 (0.19; 1.50)	0.55 (0.14; 7.56)	0.56 (0.21; 4.32)	0.53 (0.24; 3.60)	0.214
Glucose	93 ± 9	97 ± 8	99 ± 7	102 ± 11	97 ± 10	95 ± 14	0.91
ALT	24 ± 11[c]	30 ± 19	30 ± 16	20±4[c]	20 ± 7	18 ± 8	0.40
CK	164 ± 86	149 ± 71	169 ± 95	119 ± 52	129 ± 83	131 ± 97	0.74

EZE = ezetimibe; SIM = simvastatin; wks = weeks; Data expressed as mean and standard deviation, except for ApoB-48 expressed as medians and ranges; a = $p < 0.05$ vs. baseline; b = $p < 0.05$ 6 weeks vs. 12 weeks; c = $p < 0.05$ in the same time points intergroup assessment (ANOVA and Tukey's test); Lipids, apolipoproteins and glucose in mg/dl; safety parameters in IU/ml.

($p < 0.001$) compared to baseline. Regarding the transference rates, $k_{0,2} = k_{0,4}$ were increased by 20 mg simvastatin in 200% ($p < 0.001$), and further by 80 mg simvastatin in 366% ($p < 0.001$) from baseline. In a different way, $k_{0,1} = k_{0,3}$ were only increased by simvastatin 80 mg, with 98% increase ($p = 0.001$), but not by the 20 mg dose. Regarding the other analysis parameters, similarly to Group 1, $k_{0,5}$, $k_{2,1} = k_{4,3}$ and $k_{3,5}$ were reduced with the high dose simvastatin, with, respectively, 65% ($p = 0.008$), 33% ($p = 0.023$) and 54% ($p = 0.023$) reduction, but not with simvastatin 20 mg.

Table 3 shows that there were non-significant changes in the delipidation index with both treatments in time, showing that emulsion lipolysis was not overall affected by the treatments.

4. Discussion

In this study 10 mg ezetimibe alone increased the removal from plasma of both emulsion ^{3}H-TG and ^{14}C-CE in stable CHD subjects. This effect was increased when 20 mg simvastatin was associated to 10 mg ezetimibe. The effect on chylomicron emulsion metabolism of the 10 mg ezetimibe +20 mg simvastatin combination equaled that of 80 mg simvastatin. Similar effects were seen on pro-atherogenic lipids and apoB-100 concentrations.

The catabolism of chylomicrons and remnants has been extensively studied in both animals [27] and in subjects [5,11,12,17,32] by the use of triglyceride-rich chylomicron-like emulsions. These emulsions similar in size and composition to small chylomicrons are devoid of apolipoproteins, however adsorb apolipoprotein E and C-II when injected into the blood circulation [27]. These apolipoproteins modulate both emulsion lipolysis and liver receptor binding and remnant removal. In the emulsion kinetic approach used here, the plasma decay curves of the radioactively labeled cholesteryl esters mark the removal from the plasma compartment of the emulsion remnant particles, while the decay curves of the labeled triglycerides evaluate both emulsion removal and the lipolytic process the emulsion undergo in the circulation, by catalysis mediated by lipoprotein lipase on the endothelial surface of the capillaries.

Defects in emulsion removal have been associated with the presence [5,20] and prospectively with progression of angiographic detected atherosclerosis [20] and with clinical events in stable CHD patients. Indeed in this study the FCR of both radioisotopes were reduced at baseline in groups 1 and 2 in comparison with subjects without CAD as shown previously [21].

Improvements on chylomicron-like emulsions plasma FCR have been shown in association with the use of fibrates [17], which increase TG lipolysis and decrease production of VLDL particles. Increased removal from plasma of emulsion and remnants has also been clearly shown in a dose dependent manner with the use of statins [11,12,32].

According to the kinetic model both ezetimibe alone and simvastatin 20 mg acted mainly by increasing the slow hepatic and other tissue removal component of the decaying curve represented by $k_{0,2}$ and $k_{0,4}$. However, the drug association and simvastatin 80 mg not only acted by increasing $k_{0,2}$ and $k_{0,4}$, but also increased $k_{0,1}$ and $k_{0,3}$ in relation to baseline. The increments in $k_{0,1}$ and $k_{0,3}$ represent a greater removal of the emulsion by the fast component. Consequently less emulsion particles enter the lipoprotein pool as suggested by Schwartz et al. [29] and therefore reducing $k_{2,1}$ and $k_{4,3}$. This phenomenon might also explain reduction in $k_{5,3}$ and $k_{0,5}$ values that characterize respectively generation and removal of ^{3}H-free fatty acids from the intravascular compartment. However, it is important to emphasize that overall emulsion lipolysis was not reduced by both treatments since there was no significant change in the delipidation index.

Table 3
Effects of lipid-lowering therapy on artificial chylomicrons biokinetic parameters in groups 1 and 2 at baseline and after treatments.

Parameter (min^{-1})	Group 1			Group 2			p intragroup ANOVA
	Baseline	10 EZT (6 wks)	10 EZT + 20 SIM (12 wks)	Baseline	20 SIM (6 wks)	80 SIM (12 wks)	
^{14}C-CE FCR	0.005 ± 0.004	0.011 ± 0.008[a]	0.018 ± 0.005[a,b]	0.004 ± 0.003	0.011 ± 0.008[a]	0.019 ± 0.007[a,b]	<0.001
^{3}H-TG FCR	0.017 ± 0.011	0.024 ± 0.011[a]	0.042 ± 0.013[a,b]	0.016 ± 0.009	0.022 ± 0.009[a]	0.037 ± 0.012[a,b]	<0.001
$k_{0,1} = k_{0,3}$	0.100 ± 0.098	0.143 ± 0.102	0.170 ± 0.065[a]	0.095 ± 0.053	0.140 ± 0.05	0.170 ± 0.05[a]	0.001
$k_{0,2} = k_{0,4}$	0.004 ± 0.004	0.010 ± 0.005[a]	0.018 ± 0.010[a,b]	0.003 ± 0.002	0.01 ± 0.004[a]	0.014 ± 0.005[a,b]	<0.001
$k_{0,5}$	0.460 ± 0.363	0.280 ± 0.07	0.210 ± 0.133[a]	0.432 ± 0.450	0.28 ± 0.250	0.153 ± 0.070[a]	0.003
$k_{2,1} = k_{4,3}$	0.140 ± 0.125	0.100 ± 0.06	0.067 ± 0.03[a]	0.093 ± 0.047	0.07 ± 0.034	0.06 ± 0.025[a]	0.003
$k_{5,3}$	0.099 ± 0.094	0.08 ± 0.063	0.076 ± 0.05[a]	0.115 ± 0.03	0.09 ± 0.038	0.051 ± 0.03[a]	0.017
$k_{5,4}$	0.020 ± 0.009	0.013 ± 0.020	0.012 ± 0.020	0.016 ± 0.007	0.015 ± 0.022	0.012 ± 0.023	0.43
Delipidation index (%)	70 (14; 90)	58 (−3.8; 88)	55 (−5; 74)	62 (23; 96)	58 (−4; 88)	48 (6; 80)	0.397

EZE = ezetimibe; SIM = simvastatin; wks = weeks; a = $p < 0.05$ vs. baseline; b = $p < 0.05$ at 6 weeks vs. 12 weeks; Data expressed as mean and standard deviation except for the delipidation index medians (ranges). Intergroup analysis: groups 1 and 2 did not significantly differ in all observations in time according to ANOVA and Tukey's test.

By acting upon the Niemman-Pick C1 like 1 receptor [33] ezetimibe reduces cholesterol absorption, and consequently the influx of cholesterol to the liver by chylomicrons and remnants [23]. Despite induction of cholesterol synthesis, the total hepatic pool of cholesterol is reduced by ezetimibe [34]. There is evidence that ezetimibe alone optimizes VLDL, IDL and LDL FCRs [35], which suggests that the expression and activity of LDL receptor is increased in the liver. Indeed, it has been demonstrated respectively a 70% and 240% increase of LDL receptor m-RNA concentrations in mini-pig hepatocytes by ezetimibe alone or when it is associated with simvastatin [34].

However, studies in subjects cast doubts about the effects of ezetimibe alone or in combination with statins upon the expression of the LDL receptor. Recently, Gouni-Bertoldi et al. [2] evaluated the effects of ezetimibe alone or associated with simvastatin on the genetic expression of the LDL receptor protein in human mononuclear cells. Although simvastatin increased the expression of LDL receptor m-RNA neither drug induced a greater concentration of the LDLR protein. Despite these results, it is important to emphasize that the model of mononuclear cells, although used to evaluate the behavior of genes and proteins that regulate the cholesterol metabolism, may not correspond precisely to what occurs in the liver. Indeed Tremblay et al. [35] showed in a human kinetic model, an increase in plasma FCR of apoB-100 containing lipoproteins that depend on LDLR expression, with ezetimibe. The present study results, made with the chylomicron-like emulsion that is in great part removed by the LDLR [18,22], corroborate with the postulated increment of ezetimibe upon the expression of this receptor. However, we cannot discard that ezetimibe alone or in association with simvastatin could have enhanced emulsion removal by other associated mechanisms. For instance both high dose simvastatin and the association of simvastatin 20 mg with ezetimibe reduced fasting plasma TG and apoB-100 levels in our study, this reflecting previous described reductions of apoC-III containing VLDL particles and therefore inducing less competition for removal pathways [36,37]. Also these medications especially statins could induce a greater expression of other receptors that clear TG-rich remnant lipoproteins like the VLDL receptor in non-hepatic tissues [9,38]. The possible more intense effects on these mechanisms by the high statin those and the lipid modifying medication association justify the increments in both fast ($k_{0,1} = k_{0,3}$) and slow emulsion removal components ($k_{0,2} = k_{0,4}$) and consequently the greater emulsion FCR at week 12 for both treatments.

Previously, Yunoki et al. [23] have shown that ezetimibe alone reduces postprandial plasma triglycerides as well as remnant lipoproteins and apoB-48 concentrations. In disagreement with their findings, Tremblay et al. [35] did not find changes in the apoB-48 pool in 8 men with moderate hypercholesterolemia after ezetimibe treatment. In a subsequent study [24] the same authors tested the association of ezetimibe and simvastatin upon apoB-48 and apoB-100 kinetics. There was a non-significant trend of increase in apoB-48 FCRs, despite a clear increment in apoB-100 removal from plasma. However, reductions in apoB-48 production rates were induced by treatment. The authors recognized that their study might have been underpowered to show effects upon apoB-48 FCR, due to the variability in apoB-48 measurements and to detect a net effect of the drug association upon the removal from plasma of this lipoprotein. Similarly in our study there were non-significant reductions in fasting apoB-48 concentrations with both treatments (Table 2). Also changes in these apolipoproteins did not correlate with changes in emulsion's FCR (data not shown). To clarify these findings it's important to discuss the concept and possible limitations of the chylomicron-like emulsion in comparison with apoB-48 concentration determinations. The former must be seen as a good tool to study removal mechanisms and not a marker of apoB-48 pool [27]. Indeed, emulsions don't adsorb apoB-48 from natural lipoproteins and therefore do not reflect this apolipoprotein concentration. This approach also does not evaluate the postprandial state where chylomicrons and remnants are in grater concentrations. In addition we cannot discard the influence of high variability in apoB-48 concentrations seen in this and previous studies. Therefore our relatively small number of study subjects [39], and possible lack of study power, could be the reasons for the lack of study drugs effects upon apoB-48 concentrations.

Maximum dose statins can have their usefulness limited due to their potential toxicity, especially simvastatin [40]. In this regard, the FDA has recently issued a recommendation to limit high-dose simvastatin use. Synergic effect of drugs with different mechanisms of action may attain equivalent effects at low dose levels and ezetimibe has thus been proposed as an adjuvant to potentiate the statin effects [14,15]. As shown in this study, in addition to reducing LDL-C and other pro-atherogenic apoB-100 containing lipoproteins statins also improve the removal from plasma of pro-atherogenic chylomicrons and remnants. This study also confirms that the intravascular metabolism of chylomicrons and remnants also responds to the combined drugs in the same manner. It supports the assumption that the association of low-dose statin with ezetimibe is an alternative to maximal doses of statins to the improvement of the lipid plasma metabolism.

4.1. Study limitations

This study was conducted with coronary artery disease patients in clinical outpatient follow-up. Patients were followed for a total period of 12 weeks, after 6 weeks of lipid-lowering drug suspension. The long-term effect of therapeutic regimens on the kinetics of artificial chylomicrons was not tested.

Although there were several patients in the sample diagnosed with metabolic syndrome, patients with diabetes mellitus were excluded, not fitting therefore conclusions regarding this population.

Also worth pointing out that, although the distribution has been made randomly, neither patients nor the staff were blinded to which treatment was being administered. However, statistical analysis was carried out blindly.

Finally, this study is an assessment of atherosclerotic disease surrogate endpoint. Several studies demonstrated a difference between the artificial chylomicrons removal in subjects with CAD from normal population [5,20,21] and differences between individuals with different disease severity [21]. It has been demonstrated the effect of statin use on such removal [11,12]. However, despite the demonstrated benefits, it is not a study of clinical outcome.

5. Conclusion

In conclusion, 10 mg ezetimibe alone or in association with 20 mg simvastatin induced changes in the intravascular metabolism of chylomicron like emulsion that were similar to those induced respectively by 20 and 80 mg doses of simvastatin. These effects could have potential anti-atherogenic mechanisms.

Conflict of interest

Raul D. Santos: speakers bureau Pfizer, Astra Zeneca, Abbott, Merck, Novartis, Eli-Lilly. Consulting for Astra Zeneca, Bristol Myers Squibb, Merck, Abbott, Genzyme-ISIS, Amgen, Aegerion, Biolab, Pfizer, Eli-Lilly. All other authors nothing to declare.

Funding

This study was funded by the Fundação de Amparo a Pesquisa do Estado de São Paulo (Sao Paulo state foundation for research) project number 408048-3, named "The output of free cholesterol from lipoproteins and deposition of this cholesterol in arterial wall, a new mechanism of atherogenesis".

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