Objective
To assess the effect of captopril, an angiotensin-converting enzyme inhibitor, on the metabolism of chylomicrons and their remnants and the possible alterations in the concentrations of plasma lipids caused by the drug in hypertensive hypercholesterolemic individuals.

Methods
The metabolism of chylomicrons was tested with the method of artificial lipid emulsion of chylomicrons labeled with $^{3}$H-cholesteryl oleate. The emulsion was injected intravenously in 10 patients with mild-moderate arterial hypertension before and 45 days after treatment with captopril (50 mg/day). After injection, blood samples were collected during 60 minutes at pre-established time intervals for determining the decay curve, the fractional catabolic rate (FCR in min$^{-1}$), and the plasma residence time of the artificial lipid emulsion by analyzing different compartments. The plasma concentrations of the lipids were also assessed before and after treatment.

Results
The fractional catabolic rate (min$^{-1}$) of the lipid emulsion before and after treatment with captopril (0.012±0.003 and 0.011±0.003, respectively; p=0.85, n.s.) did not change, but the total cholesterol and LDL-C levels decreased by 7% and 10%, respectively (p=0.02). The concentration of HDL-C, triglycerides, Lp(a), and apolipoproteins AI and B did not change.

Conclusion
Treatment with captopril, evaluated with the artificial lipid emulsion method, does not cause deleterious changes in the metabolism of chylomicrons and their remnants.

Keywords
captopril, chylomicrons, lipoproteins, metabolism

Original Article
Lack of Effect of Captopril on the Metabolism of an Artificial Lipid Emulsion Similar to Chylomicrons in Hypertensive Hypercholesterolemic Patients

Renato J. Alves, Jayme Diament, Rosângela F. Amâncio, Neusa Forti, Raul C. Maranhão
São Paulo, SP - Brazil

Treatment with some antihypertensive agents may cause unwanted changes in the lipid profile, attenuating their beneficial antiatherogenic effects of blood pressure reduction. The effects of antihypertensive drugs on lipid profile vary with both the pharmacological class and the drug specifically. Thiazide diuretics, such as hydrochlorothiazide and chlortalidone, and nondiuretics, such as betablockers without sympathomimetic activity, as propranolol, are major deleterious agents to the lipid profile 1-2. The increase in triglyceride levels and a reduction in HDL-C are 2 alterations caused by those agents. On the other hand, angiotensin-converting enzyme (ACE) inhibitors, such as captopril, seem to have a neutral effect on lipid profile, or even, to improve it in hypertensive hypercholesterolemic individuals due to a reduction in the LDL-C and total cholesterol levels 3.

Chylomicrons are the plasma lipoproteins responsible for the transportation of dietary lipids absorbed through the intestine. They have the same metabolic pathway of the VLDL produced by the liver. Similarly to VLDL, chylomicrons undergo lipoprotein lipase activity in capillary walls. That enzyme is stimulated by the apolipoprotein (apo) CII, one of the apop present on the surface of chylomicrons. The triglycerides of the chylomicron particles undergo hydrolysis by lipoprotein lipase, transforming into fatty acids and glycerol, which are absorbed by muscle and fatty tissues, where they are stored after re-esterification.

After lipolysis, the chylomicrons become smaller particles, the remnants of chylomicrons. These particles are sequestered in the Disse space and absorbed by liver cells, through specific receptors, among which are the LDL receptors and the LDL-receptor-related protein (LRP) 4. ApoE functions as the major ligand of the chylomicron remnants to liver receptors 5.

The chylomicron remnants are considered atherogenic lipoproteins and their slower removal is directly related to coronary artery disease 6. Assessment of that metabolism is important for the complete understanding of the lipid metabolism in hypertensive individuals using a certain pharmacological agent. In addition, the effects of antihypertensive drugs on chylomicron metabolism have been only rarely investigated due to methodological difficulty.

In this study, the effects of captopril on the removal of chylomicron remnants from the circulation were evaluated in a group of individuals with mild-moderate arterial hypertension and concomitant hypercholesterolemia. That is a very common pathological condition, in which not only blood pressure control is important to reduce the atherosclerotic risk, but also hypercholesterolemia control and appearance of other pro-atherogenic conditions,
Lack of Effect of Captopril on the Metabolism of an Artificial Lipid Emulsion Similar to Chylomicrons in Hypertensive Hypercholesterolemic Patients

such as alterations in the metabolism of triglyceride-rich lipoproteins and retention of chylomicron remnants. The intravascular metabolism of chylomicrons was assessed through the plasma removal of an artificial triglyceride-rich lipid emulsion, which simulates the intravascular behavior of chylomicrons. The emulsion, labeled with radioactive cholesteryl oleate (\(^{3}\)H-CO) and intravenously injected after a 12-hour fast, reflects the plasma kinetics of the chylomicron remnants. The method facilitates the metabolic study of those lipoproteins in human beings, overcoming the gastrointestinal component and not being affected by the individual variability of intestinal absorption. That artificial lipid emulsion has been used to evidence disorders in the metabolism of chylomicrons in several diseases and to investigate the effects of lipid-lowering drugs on that metabolism.

Methods

This study comprised 10 individuals (8 females) with mild-moderate arterial hypertension, aged 60.7 ± 2.2 years, cholesterolemia > 240 mg/dL, triglycerides < 200 mg/dL, and fasting glycemia < 110 mg/dL. Table I shows the characteristics of the patients. The antihypertensive treatment was interrupted 30 days before the study, and the diagnosis of arterial hypertension was based on the VI report of the Joint National Committee.

The exclusion criteria were as follows: coronary heart disease; diabetes mellitus; premenopausal women; alcoholism; renal, hepatic or thyroid dysfunction; acute inflammatory disease; and neoplasm. No patient received hormone replacement therapy or lipid-lowering therapy in the 6 months preceding the study.

The patients underwent treatment with captopril for 45 days, at the dosage of 25 mg, twice a day (50 mg/day). The plasma kinetics of the lipid emulsion and the determination of the plasma levels of lipids and apolipoproteins were performed before and after therapy with captopril.

The study protocol was approved by the scientific committee on ethics of the Instituto do Coração of the Hospital das Clínicas of the medical school of the University of São Paulo, and all patients provided written informed consent.

Total cholesterol and triglyceride levels were determined through the enzyme colorimetric method (CHOD-PAP), Boeringher and Abbott, respectively. Apolipoproteins A1 and B were measured by radial immunodiffusion (Boeringher), and Lp(a) by immunoturbidimetry.

The metabolism of chylomicrons was assessed by using the method of artificial lipid emulsion labeled with \(^{3}\)H-cholesteryl oleate. The clearance of cholesteryl oleate reflects the plasma removal of the emulsion particles labeled with radionuclide, while the triolein clearance reflects the lipolytic process.

The lipid emulsion was prepared with ultrasound radiation of the lipid mixture composed by triolein, 69%; cholesteryl oleate, 6%; phospholipids, 23%; and free cholesterol, 2%, in an aqueous medium with the addition of \(^{3}\)H-cholesteryl oleate, and followed by ultracentrifugation in a saline-density gradient, as previously described. Then, the emulsion was sterilized by passage through a 0.2-µm filter. Approximately 3 to 5 mg of the lipid emulsion in an approximate volume of 100 to 200 µL, containing 4µci of tritium-labeled cholesteryl oleate (\(^{3}\)H-CO), was intravenously injected in bolus. Blood samples were collected at pre-established time intervals.

Aliquots of 1.0 mL of plasma were pipeted in scintillation tubes. Then, 7.0 mL of the PPO/POPOP/tritonX-100/toluene scintillation solution (0.5g: 0.5g: 333 mL:/ 667 mL) were added to determine the radioactivity present in the samples by using a Beta counter (Packard, model 1660 TR, USA).

Blood samples were collected for 60 minutes at pre-established time intervals of 2, 4, 6, 10, 15, 20, 30, 45, and 60 minutes. The plasma decay curve of residual radioactivity of the emulsion was determined before and after treatment with captopril as a function of time. The fractional catabolic rate of the cholesteryl ester (FCR-CE), in min\(^{-1}\), representing the fraction of the particle that is extracted from the plasma compartment, was calculated through analysis of the compartments with the aid of a computer program (AnaComp 4.1) developed for analyzing the kinetics of artificial emulsions.

The kinetics was expressed in FCR and in plasma residence times, calculated as 1/FCR. In the model used, shown in figure 1, the plasma kinetics of the radioactive lipid components was assessed. From that model and based on the method of the nonlinear minimum square, the FCR(K) of the labeled lipid was calculated between the compartments. In figure 1, compartment number 1 represents the emulsion labeled with \(^{3}\)H-CO introduced into the intravascular space; K1.0 represents the fraction of the emulsion that is extracted from the plasma compartment through a nonspecific pathway; K1.2 represents the fraction of the emulsion that undergoes lipoprotein lipase activity, transforming into the chylomicron remnant, which is represented by compartment 2; K2.0 represents the fraction of the chylomicron remnant that is extracted from the plasma compartment, mainly through hepatic uptake.

The experimental decay curve of plasma radioactivity of cholesteryl oleate (\(^{3}\)H-CO) was obtained by plotting the raw and percentage values of the residual plasma radioactivity in a graph as a function of time, at the above pre-established time intervals. The result showed the double exponential aspect of the curve that is characterized by a rapid drop in residual radioactivity, representing the fast removal of the particles. After this fast drop, slower plasma decay follows. Finally, a trend towards a plateau occurs, or even mild elevation, indicating that the radioactive components recycle becoming incorporated into the VLDL recently synthesized in the liver.

The Wilcoxon test was used to compare FCR-CE, blood pressure and plasma lipid levels before and after therapy with captopril. Values P<0.05 were considered significant, adopting the 95% confidence interval.

Results

Table I shows the treatment with captopril reduced the total cholesterol and LDL-C levels by 7% and 10%, respectively (P=0.02). However, this therapy did not change the levels of HDL-C, triglycerides, Lp(a), and apolipoproteins A1 and B. Figure 2 depicts the decay curves of plasma radioactivity of the lipid emulsion obtained before and after treatment with captopril. No difference was observed between the 2 curves.

The plasma residence times and FCR-CE shown in table I did not change after treatment (P=0.85).

Discussion

Arterial hypertension is one of the major risk factors for atherosclerosis and coronary artery disease, and its treatment has proved to be beneficial for preventing those pathologies. Because...
Lack of Effect of Captopril on the Metabolism of an Artificial Lipid Emulsion Similar to Chylomicrons in Hypertensive Hypercholesterolemic Patients

Dyslipidemia has been frequently associated with arterial hypertension, being also a strong risk predictor of coronary artery disease, one could assume that antihypertensive drugs should not have unwanted effects on lipid profile.

This study, using the artificial lipid emulsion test, showed that treatment with an ACE inhibitor (captopril) in hypertensive hypercholesterolemic patients, and, therefore, with a greater predisposition to atherosclerosis, does not cause retention of chylomicron remnants (atherogenic particles) into the bloodstream. Similarly to the chylomicron secretion in the lymph by the intestine, the emulsion metabolism consists of 2 stages: 1) lipolysis by lipoprotein lipase; 2) removal of chylomicron remnants by the liver. The esterified cholesterol of the emulsion, which reproduces the plasma kinetics of the emulsion particles, remains inside the particle in the plasma compartment 15, while the triglycerides are continuously removed from the emulsion particles by the lipoprotein lipase activity, reflecting the lipolytic process 16. In this study, because the radioactive labeling was performed only on the cholesterol ester of the emulsion, but not on the triglycerides, the lipolytic process was not studied. However, double labeling would not be necessary. When lipolysis is reduced, the emulsion particles, similarly to chylomicrons in the lymph, are more slowly removed. An efficient process of lipolysis is required for an effective removal from the plasma 10,15,16. As no difference was observed in the plasma kinetics of the cholesterol ester in the emulsion, one may assume that lipolysis did not also change with treatment.

It is worth stressing that in normolipidemic patients with coronary artery disease, chylomicron removal from the plasma is attenuated 6,17,18. The role played by chylomicron remnants in atherogenesis has been investigated 19 and postprandial lipemia has been associated with coronary artery disease in humans through the oral fat overload test. Simpson et al 20, Groot et al 17, and Patsch et al 21 reported an increase in the postprandial concentration of triglycerides or vitamin A, or both, in patients with coronary artery disease. Simons et al 18 reported an elevation in apoB48 in patients with coronary artery disease. Retinyl ester and apoB48 (apoB form found in chylomicrons) are used as markers of chylomicrons in the bloodstream. Maranhão et al 6, using the same lipid emulsion used in this study for assessing the metabolism of chylomicrons, found a decreased removal of cholesteryl ester and triglycerides from the bloodstream, indicating that both lipolysis and removal of the remnants are decreased in patients who develop coronary artery disease.

Previously, Iaina et al 22 reported that the area under the curve of postprandial plasma concentration of retinyl ester, used as a marker of chylomicron remnants, was reduced after 25 to 75 mg/day of captopril for 3 months. This finding suggests that the retention of chylomicron remnants in plasma decreases after treatment with captopril. This effect may be antiatherogenic, as suggested by the association between coronary artery disease and reduced removal of remnants in the literature 6,17,19.

### Table I - Clinical and metabolic characteristics of the patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men (n)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Women (n)</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Age (years)</td>
<td>60.7±2.20</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.48±0.63</td>
<td>26.48±0.63</td>
</tr>
<tr>
<td>White</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Black</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>155±3.07</td>
<td>139±3.14 †</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>99±1.80</td>
<td>88±3.33 †</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>265.5±21.7</td>
<td>248.4±21.1*</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>132.4±16.9</td>
<td>143.4±20.1</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>55.1±3.2</td>
<td>53.8±3.2</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>183.9±19.7</td>
<td>165.9±18.8*</td>
</tr>
<tr>
<td>Lipoprotein(a) (mg/dL)</td>
<td>45.24±9.76</td>
<td>43.91±10.97</td>
</tr>
<tr>
<td>Apolipoprotein AI (g/L)</td>
<td>1.55±0.05</td>
<td>1.52±0.04</td>
</tr>
<tr>
<td>Apolipoprotein B (g/L)</td>
<td>1.46±0.15</td>
<td>1.35±0.16</td>
</tr>
<tr>
<td>FCR-CE (min⁻¹)</td>
<td>0.012±0.003</td>
<td>0.011±0.003</td>
</tr>
<tr>
<td>Plasma residence time (min)</td>
<td>83.3±20.8</td>
<td>90.9±22.5</td>
</tr>
</tbody>
</table>

LDL = low-density lipoprotein; HDL = high-density lipoprotein; BMI = body mass index; FCR-CE = fractional catabolic rate of cholesteryl ester. Data expressed as mean ± standard error of the mean.

* p < 0.05.

![Compartment model used to demonstrate the plasma kinetics of artificial chylomicrons.](image1)

![Fig. 2 – Plasma decay curve of the radioactive emulsion obtained in patients before and after treatment with captopril.](image2)
Lack of Effect of Captopril on the Metabolism of an Artificial Lipid Emulsion Similar to Chylomicrons in Hypertensive Hypercholesterolemic Patients

use of captopril did not cause faster removal of chylomicron remnants in our study, the result that the drug does not change the kinetics of the remnants evidences the safety of the drug in regard to the chylomicron metabolism. In addition, and as in other studies, that therapy resulted in a reduction of the LDL-C levels, which is a very well-documented antiatherogenic effect.

The differences found between our results and those by Iaina et al. may be attributed to the fact that this study’s patients are hypercholesterolemic and non-diabetic. In the study by Iaina et al., the individuals might have insulin resistance, as suggested by the glucose plasma levels, which improved with treatment with captopril. In our study, the patients with fasting glycemia ≥ 110 mg/dL were excluded.

The mechanisms that may be implicated in these results reside primarily in the fact that the LDL receptors, which proved to be defective in the presence of hypercholesterolemia, are also responsible for the removal of chylomicron remnants from the circulation. Secondly, the action of lipoprotein lipase, resulting in the generation of remnants, also influences chylomicron removal, and its function depends on the insulin levels. In the abovementioned study, the decrease in insulin resistance achieved with captopril use, could have caused the improvement in the clearance of retinyl palmitate.

In conclusion, the treatment with captopril at the dosage of 50 mg/day administered to hypertensive hypercholesterolemic patients neither changed the metabolism of chylomicrons and their remnants nor caused deleterious changes in plasma lipids and apolipoproteins A1 and B.

References


515