# Effects of Atorvastatin and T-786C Polymorphism of eNOS Gene on Plasma Metabolic Lipid Parameters

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## Abstract

**Background: Endothelial nitric oxide synthase (eNOS) activity may be modulated by high-density lipoprotein cholesterol (HDL-C), statins or polymorphisms, such as the T-786C of eNOS.**

**Objective: This study aimed at evaluating if the T-786C polymorphism is associated with changes of atorvastatin effects on the lipid profile, on the concentrations of metabolites of nitric oxide (NO) and of high sensitivity C-reactive protein (hsCRP).**

**Methods: Thirty male volunteers, asymptomatic, aged between 18 and 56 years were genotyped and classified according to absence (TT, n = 15) or presence (CC, n = 15) of the polymorphism. They were randomly selected for the use of placebo or atorvastatin (10 mg/day/14 days). After each treatment lipids, lipoproteins, $HDL_2$ and $HDL_3$ composition, cholesteryl ester transfer protein (CETP) activity, metabolites of NO and hsCRP were evaluated.**

**Results: The comparisons between genotypes after placebo showed an increase in CETP activity in a polymorphism-dependent way (TT, 12±7; CC, 22±12; $p \leq 0.05$). The interaction analyses between treatments indicated that atorvastatin has an effect on cholesterol, LDL, nitrite and lipid-protein ratios ($HDL_2$ and $HDL_3$) ($p \leq 0.001$) in both genotypes. Interestingly, we observed genotype/drug interactions on CETP ($p \leq 0.07$) and lipoprotein (a) (Lp(a)) ($p \leq 0.056$), leading to a borderline decrease in CETP, but with no effect on Lp(a). HsCRP showed no alteration.**

**Conclusion: These results suggest that statin treatment may be relevant for primary prevention of atherosclerosis in patients with the T-786C polymorphism of eNOS, considering the effects on lipid metabolism. (Arq Bras Cardiol. 2013;100(1):14-20)**

**Keywords: Lipid Metabolism; Polymorphism, Genetic; Lipid Regulating Agents; Nitric Oxide; Protein C.**

## Introduction

The vascular endothelium is a complex system, integrated by several chemical mediators. Among them, nitric oxide stands out by being a potent vasodilator, amongst other beneficial effects[1,2]. Its synthesis depends on the activity of endothelial nitric oxide synthase (eNOS)[3], which, in the plasma membrane and caveolae, is modulated mainly by laminar shear stress and the presence of agonists on the cell surface[4].

However, transcriptional and post-transcriptional mechanisms are also involved[5,6]. For example, high density lipoprotein (HDL) can mediate the activation of eNOS through interaction with apolipoprotein AI and scavenger receptor class B type I (SR-BI)[7], characterizing in part the vasodilation activity of this lipoprotein[8].

Moreover, the presence of polymorphisms in the eNOS gene may explain different levels of enzyme activity, which may be associated with increased cardiovascular risk[9]. The promoter of polymorphism T-786C is the most extensively studied case, where the presence of the C allele reduces transcriptional activity by approximately 50%[10], probably the result of the binding of the repressor protein RPA1 to the promoter region when this allele is present[11]. By reducing the enzymatic activity, the C allele has been associated with an impaired NO production[9,10], increased endothelial dysfunction and cardiovascular diseases[12,13].

Statins have known pleiotropic effects, which include the regulation of endothelial function reducing the oxidative stress and thrombogenicity[14]. Moreover, they are responsible for the inhibition of Rho protein and increased eNOS expression, plus the post-transcriptional activation of PI3K/AKT pathway[15].

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Manuscript received May 22, 2012; revised manuscript September 17, 2012; acepted September 26, 2012.

Given the importance of both expression and activity of eNOS, as well the presence of HDL particles for endothelial function, and the pleiotropic effects of statins on these two variables, this study aims to determine whether the presence of T-786C polymorphism and the use atorvastatin results in interactions with metabolic alterations in lipids, metabolites of NO and hsCRP.

## Methods

This study was developed in partnership between the Department of Pharmacology (Faculdade de Medicina de Ribeirao Preto – USP) and the Department of Clinical Pathology (Faculdade de Ciências Médicas– UNICAMP), and was approved by the Ethics Committee of those institutions. All volunteers were informed of the research terms and signed the written free and informed consent form.

The number of subjects enrolled in this study was estimated to allow a statistical power of 80% ($\beta$ <0.20; $\alpha$ <0.05) to detect differences of 30% or more between the two groups of genotypes (TT and CC). According to the estimated frequency of TT (49%) and CC (9%) genotypes[10,16-18], 200 male volunteers were selected from the local population (Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, São Paulo, Brazil), aged between 18 and 56 years; they also were asymptomatic, Caucasian, nonsmokers, and did not use any medication. Female volunteers and smokers were excluded.

These volunteers were genotyped for presence of T-786C polymorphism of the eNOS gene, and for the present study, 15 individuals with the TT genotype and 15 with the CC genotype, were selected and matched by age and body mass index. They underwent thorough clinical examination and laboratory tests, and provided a complete health history.

The study had a placebo-controlled, single-blind design. Volunteers with CC or TT genotype received a placebo for 14 days, followed by further treatment for 14 days with atorvastatin (Lipitor® Pfizer, Brazil) 10 mg/day orally. Blood samples were drawn after 12-hour fasting and collected in tubes containing EDTA after both treatments and the samples were stored at -70 °C until analysis.

### Genotyping for the T-786C polymorphism

Genomic DNA was extracted from 1 mL of whole blood by salting-out method and stored at -20 °C until analysis. The genetic variants of the T-786C polymorphism located in the 5 'region of the eNOS gene were determined by amplification by polymerase chain reaction, as previously described[16].

The amplified products were digested with MspI at 37 °C for four hours, which produced fragments of 140 and 40 bp for the wild allele (T) or 90, 50 and 40 bp in the case of the polymorphic allele (C). The fragments were separated by electrophoresis (12% polyacrylamide gel) and visualized by silver staining.

### Biochemical measurements

Cholesterol, triglycerides, free fatty acids, phospholipids and free cholesterol from plasma and HDL subfractions were quantified using enzymatic-colorimetric methods in the automated Boehringer Mannheim-Roche Hitachi 917 system, using commercial reagents from Roche (Mannheim, Germany) and Waco (Waco Chemicals, USA). The proteins were determined by bicinchoninic acid assay (BCA) method.

Cholesterol esters were calculated by the formula: (total cholesterol – free cholesterol) x 1.67, and LDL-C by Friedewald formula (LDL cholesterol = TC − (HDL-C) − TG/2.2), whereas HDL-C was calculated by homogeneous direct methods[19].

Apolipoproteins AI, B-100 and lipoprotein (a) were determined by nephelometry through immunochemical reactions in BNII/ Marburg, using Dade-Behring reagents (Mannheim, Germany).

$HDL_2$ and $HDL_3$ were isolated using the micro-ultracentrifugation method[20].

LDL-C/apoB-100 and triglycerides/HDL-C were used to determine the estimated size of LDL[21], while the Castelli indexes I and II were used to estimate cardiovascular risk[22].

To determine the relative % of each component (lipids and proteins) of $HDL_2$ and $HDL_3$ related to the total HDL, the formula:

component*100/component $HDL_2$+$HDL_3$ was used.

The relative % of each component, in each subfraction, was determined by the formula:

HDL component/total mass HDL*100.

CETP activity (%) was determined using the exogenous radiometric method[23].

NO metabolites (nitrite/nitrate) were detected by the commercial assay Nitric Oxide ($NO^{2-}$/$NO^{3-}$) Assay Kit (StressGen, Assay Designs, Inc.). The variable $NO_x$ was calculated as the sum of the concentrations ($\mu$mole/L) of nitrite and nitrate, and the nitrite/nitrate ratio by the relationship between the two analytes. The reference values for NOx range between 11.5 and 76.4 $\mu$mole/L[24].

C-reactive protein was determined by immunoturbidimetry in a high sensitivity method (hsCRP), using the Tina-quant CRP (Latex) HS-Roche.

### Statistical analyses

The Mann-Whitney test was used to compare the variables between the two genotype groups and the use of atorvastatin and placebo in the same subjects. ANOVA for repeated measures followed by Tukey's post-hoc, profile test by contrasts or the BOX-COX test was used for multiple comparisons and to detect interactions. The level of significance for statistical tests was 5% or $p \leq 0.05$, and for borderline values 5-9% ($p > 0.05$ and $\leq 0.09$).

This study was partially supported by the Foundation for Research Support of the State of São Paulo (FAPESP), SP, Brazil and by the National Council for Scientific and Technological Development, Brazil. No additional external funding was received for this study.

## Results

Baseline biochemical and anthropometric data (average ± SD) obtained during the selection period, in the TT and CC genotypes were, respectively: age (years) 27.6±1.8/31.1±2.1, systolic blood pressure (mmHg) 123±3/130±2, diastolic blood pressure (mmHg) 80±3/81±2, body mass index

(kg/m2) 23.9±0.7/25.1±0.7, total cholesterol (mg/dL) 161±10/160±10, triglycerides (mg/dL) 103±13/103±2, HDL-C (mg/dL) 36±2/35±2, LDL-C (mg/dL) 104±10/103±9 and VLDL-C (mg/dL) 21±3/21±2".

The biochemical parameters were evaluated in groups TT and CC after administration of placebo and atorvastatin and the differences are shown in Table 1.

The beneficial effects of atorvastatin on lipid metabolism parameters were independent from the genotype; this same pattern was observed regarding NO metabolites. Free fatty acids were lower in the CC group after atorvastatin, when compared with TT (Table 1).

CETP activity was significantly higher in CC group after placebo, but after atorvastatin this activity showed a borderline increase in TT and a decrease, albeit not significant, in CC.

The chemical compositions of $HDL_2$ after the treatments in both genotypes were also determined. No significant differences were seen regarding $HDL_2$ composition between TT and CC. However, significant differences were observed after atorvastatin in both genotypes, such as the increase in absolute and relative content of proteins, total lipoprotein mass, absolute content of TAG; and lowest relative CE and lipids/proteins ratio (Table 2). The same parameters were evaluated in $HDL_3$, as shown by Table 3.

**Table 1 – Biochemical parameters by genotype and treatment**

| Parameters | Placebo | | Atorvastatin | |
|---|---|---|---|---|
| | TT (n = 15) | CC (n = 15) | TT (n = 15) | CC (n = 15) |
| Cholesterol (mg/dL) | 140 ± 29 | 140 ± 26 | *102 ± 24‡ | *100 ± 12‡ |
| LDL-C (mg/dL) | 80 ± 35 | 85 ± 21 | *48 ± 24‡ | *49 ± 11‡ |
| TAG/HDL-C | 2.46 ± 2 | 2.91 ± 2 | *2.00 ± 1§ | *2.59 ± 2§ |
| Cholesterol/HDL-C | 3.92 ± 2 | 3.94 ± 1 | *2.72 ± 1§ | *3.04 ± 1§ |
| Apo B-100 (mg/dL) | 68 ± 25 | 69 ± 21 | *43 ± 15‡ | *41 ± 10‡ |
| Lp(a) (mg/dL) | 11 ± 12 | 10 ± 14 | 10 ± 9 | 14 ± 21 |
| FFA (mEq/L) | 0.37 ± 0.2 | 0.45 ± 0.4 | 0.38 ± 0.1 | †0.27 ± 0.1§ |
| CETP (%) | 12 ± 7 | †22 ± 12§ | *16 ± 8// | 16 ± 9 |
| Nitrite (µmole/L) | 62 ± 55 | 56 ± 55 | *24 ± 22‡ | *21 ± 21‡ |
| $NO_x$(µmole/L) | 50 ± 19 | 46 ± 21 | *21 ± 4‡ | *20 ± 3‡ |
| Nitrite/Nitrate | 15 ± 11 | 14 ± 11 | *6 ± 4‡ | *8 ± 5‡ |

*Values are the mean ± SD; p : Mann-Whitney test; LDL-C : low-density lipoprotein cholesterol; TAG : triglycerides; HDL-C : high-density lipoprotein cholesterol; TAG/HDL-C : estimate the size of LDL; Apo B-100 : apolipoprotein B-100; Lp(a) : lipoprotein (a); FFA : free fatty acids; CETP : cholesteryl ester transfer protein; $NO_x$ : nitrite + nitrate; *differences between groups (placebo/atorvastatin); †differences between genotypes (TT/CC); ‡ p≤ 0.001; §p≤0.05; //p=0.065.*

**Table 2 - Significant differences of absolute (mg/dL) and relative (% total HDL) values of chemical components of $HDL_2$ after placebo and atorvastatin**

| Parameters | Placebo | | Atorvastatin | |
|---|---|---|---|---|
| | TT (n=15) | CC (n=15) | TT (n=15) | CC (n=15) |
| CE | 4.66 ± 1.51 | 4.64 ± 1.96 | 5.69 ± 2.84 | 5.46 ± 1.22 |
| Relative % CE | (58.31 ± 3.69) | (59.08 ± 5.32) | *(55.71 ± 3.00)‡ | *(55.42 ± 1.46) ‡ |
| TAG | 1.16 ± 0.36 | 1.08 ± 0.96 | *2.25 ± 0.88§ | *1.88 ± 0.98§ |
| Relative % TAG | (35.40 ± 11.73) | (28.20 ± 12.41) | (39.04 ± 17.21) | (33.36 ± 16.33) |
| Protein | 15.54 ± 9.84 | 12.96 ± 12.75 | *101.58 ± 16.33‡ | *132.17 ± 129.06‡ |
| Relative % Protein | (27.37 ± 16.68) | (21.17 ± 16.69) | *(44.49 ± 2.29) § | *(43.87 ± 5.96) ‡ |
| Total mass $HDL_2$ | 32.44 ±9.90 | 29.26 ± 13.84 | *121.09 ± 17.96‡ | *150.66 ± 129.17§ |
| Mass of lipids | 16.89 ± 2.26 | 16.30 ± 4.58 | *19.51 ± 3.69‡ | 18.50 ± 3.15 |
| Lipids/protein ratio | 3.60 ± 5.14 | 3.71 ± 5.20 | *0.19 ± 0.05§ | *0.18 ± 0.05§ |

*Values are the mean ± SD; p : Mann-Whitney test; Relative % : HDL component/total mass HDL*100; CE : cholesteryl ester; TAG : triglycerides; protein : total protein from $HDL_2$; total mass $HDL_2$ : sum of cholesterol, free cholesterol, CE, TAG, phospholipids and protein; mass of lipids : sum of cholesterol, free cholesterol, CE, TAG and phospholipids; *differences between groups (placebo/atorvastatin); ‡p ≤ 0.001; §p ≤ 0.05.*

**Table 3 - Significant differences of absolute (mg/dL) and relative (% total HDL) values of chemical components of HDL3 after placebo and atorvastatin**

| Parameters | Placebo | | Atorvastatin | |
|---|---|---|---|---|
| | TT (n=15) | CC (n=15) | TT (n=15) | CC (n=15) |
| CE | 3.37 ± 1.24 | 3.30 ± 1.52 | 4.55 ± 2.32 | 4.41 ± 1.09 |
| Relative % CE | (41.69 ± 3.69) | (40.92 ± 5.32) | *(44.29 ± 3.00) § | *(44.58 ± 1.46) § |
| Protein | 40.95 ± 19.97 | 42.34 ± 19.98 | *111.98 ± 6.55‡ | *120.00 ± 18.10‡ |
| Relative % Protein | (72.63 ± 16.68) | (78.83 ± 16.69) | *(55.51 ± 2.29) § | *(56.13 ± 5.96) ‡ |
| Total mass $HDL_3$ | 77.69 ± 24.12 | 79.18 ± 23.75 | 97.52 ± 50.41 | 101.29 ± 62.04 |
| Mass of lipids | 36.74 ± 6.20 | 36.84 ± 9.84 | *56.78 ± 15.23§ | *54.58 ± 5.11‡ |
| Lipids/protein ratio | 0.76 ± 0.41 | 0.86 ± 0.50 | *0.40 ± 0.21‡ | *0.35 ± 0.15‡ |

*Values are the mean ± sd; p : Mann-Whitney test; Relative % : HDL component/total mass HDL*100; CE : cholesteryl ester; protein : total protein from $HDL_3$; total mass $HDL_3$ : sum of cholesterol, free cholesterol, CE, triglycerides, phospholipids and protein; mass of lipids : sum of cholesterol, free cholesterol, CE, triglycerides and phospholipids; *differences between groups (placebo/atorvastatin); ‡p ≤ 0.001; §p ≤ 0.05.*

$HDL_3$ subfraction showed no differences between TT and CC genotypes. After atorvastatin, both TT and CC showed a significant increase in relative CE, proteins (absolute) and lipids mass, while lipids/proteins ratio and proteins (relative) were lower.

In order to measure changes induced by atorvastatin in both genotypes and the $HDL_2$ and $HDL_3$ subfractions, we assessed the percentage by weight of each component after administration of placebo and atorvastatin (Table 4).

Regarding the composition of $HDL_2$, it was observed that there was a reduction in the percentage by weight of all lipid compounds of this subfraction, and a significant increase of proteins, regardless of genotype. $HDL_3$ showed a decrease in triglycerides and an increase in proteins, regardless of the genotype (Table 4).

The significant and borderline results obtained between the TT and CC genotypes and between treatments are shown in Table 5, which also shows the analysis of interaction between the polymorphism and atorvastatin.

The T-786C polymorphism was not responsible for any changes in the studied parameters (Table 5). Regarding the use of placebo and atorvastatin, a clear effect of statins in reducing serum lipids, apolipoproteins and nitrite is shown. Still, there were modifications in $HDL_2$ and $HDL_3$, which resulted in significant reduction of the lipid/protein ratios of each subfraction, with different metabolic implications. Finally, we showed borderline interactions between the polymorphism and atorvastatin for CETP and Lp(a).

## Discussion

The T-786C polymorphism of eNOS gene is functionally characterized by a reduction of the gene promoter activity, with consequent influence on NO production[11,25]. Recent studies have demonstrated that individuals homozygous for this polymorphism have a reduced sensitivity to laminar shear stress[26] as well as an increase in serum inflammatory markers in patients with established CAD[27] and increased risk of mortality and development of renal dysfunction when undergoing emergency heart surgery[28].

Although the effect of statins on the CC genotype has been previously assessed, especially the anti-inflammatory effect of atorvastatin[25], there are still some unexplored points, such as the impact of the interaction between the polymorphism and the drug on lipids metabolism and HDL subfractions.

Our study demonstrated that atorvastatin had an effect that was primarily genotype-independent, given the reductions in lipids, ratios of lipid/protein ($HDL_2$ and $HDL_3$), Castelli I index, free fatty acids and a higher estimated LDL size; this lipid-lowering effect are in line with previous studies[29,30].

TT and CC genotypes were not different regarding the chemical composition of $HDL_2$ and $HDL_3$; all changes in the composition were determined by atorvastatin. In $HDL_2$ we observed a decrease in all lipids, with a significant increase in proteins. This finding suggests that $HDL_2$ became more delipidated, with no change in particle number. Furthermore, $HDL_3$ had an enrichment of lipids and proteins - with a consequent decrease in the lipid/protein ratio, which points to an increase in the number of $HDL_3$ particles.

This set of changes suggest an increased activity of hepatic lipase, in contrast to studies which show that atorvastatin would be responsible for the decrease in its activity[31]. On the other hand, the increase in $HDL_3$ particles suggests that atorvastatin promoted an exchange between the neutral lipids and lipoproteins and HDL2 particles became triglyceride enriched, an ideal substrate for PLTP to form HDL3 particles[32,33].

In our study no interactions between hsCRP and atorvastatin were observed, despite a 40% of reduction after the treatment. Gensini et al[34] demonstrated that treatment with atorvastatin, particularly at a dose of 80 mg/day, effectively reduces the plasma levels of hsCRP, despite the presence of metabolic syndrome or diabetes mellitus.

We observed that NO metabolites decreased significantly after atorvastatin treatment; the same results were seen in patients with peripheral arterial disease[35]. However, most studies show an increase or maintenance of these levels[36].

Borderline interactions between atorvastatin and CC

**Table 4 - Chemical composition expressed as % by weight of HDL2 and HDL3 of TT and CC subjects after placebo and atorvastatin**

| Parameters | $HDL_2$ | | | |
|---|---|---|---|---|
| | Placebo | | Atorvastatin | |
| | TT(n= 15) | CC(n= 15) | TT(n= 15) | CC(n= 15) |
| Cholesterol | 4.28 ± 2.13 | 5.23 ± 2.60 | *1.01 ± 0.73‡ | *0.98 ± 0.55‡ |
| FC | 4.20 ± 5.11 | 3.99 ± 1.93 | *0.86 ± 0.32‡ | *0.82 ± 0.35‡ |
| CE | 15.69 ± 7.79 | 17.93 ± 8.88 | *4.62 ± 1.96‡ | *4.33 ± 1.21‡ |
| TAG | 3.83 ± 1.31 | 3.61 ± 2.56 | *1.94 ± 0.83‡ | *1.61 ± 1.06§ |
| PL | 32.99 ± 10.38 | 37.66 ± 15.65 | *8.80 ± 1.52‡ | *8.28 ± 2.62‡ |
| Protein | 43.29 ± 18.04 | 36.81 ± 23.86 | *83.79 ± 2.55‡ | *84.96 ± 4.46‡ |
| **Parameters** | $HDL_3$ | | | |
| | Placebo | | Atorvastatin | |
| | TT (n= 15) | CC(n= 15) | TT (n= 15) | CC (n= 15) |
| TAG | 3.22 ± 1.68 | 3.23 ± 1.74 | *0.72 ± 0.26‡ | *0.79 ± 0.26‡ |
| Protein | 50.39 ± 10.21 | 51.35 ± 14.26 | *78.68 ± 2.06‡ | *77.08 ± 2.41‡ |

*Values are the mean ± sd; p - Mann-Whitney; FC - free cholesterol; CE - cholesteryl ester; TAG – triglycerides; PL – phospholipids; *differences between groups (placebo/atorvastatin); ‡ p≤ 0.001; §p≤0.05.*

**Table 5 - Comparisons of variables and interactions between genotypes and treatments**

| Variables | †Polymorphisms | *Treatments | ¶Interactions |
|---|---|---|---|
| Cholesterol (mg/dL) | - | p ≤ 0.001# | - |
| LDL-C (mg/dL) | - | p ≤ 0.001# | - |
| VLDL-C (mg/dL) | - | p ≤ 0.032** | - |
| TAG (mg/dL) | - | p ≤ 0.043** | - |
| Apo B-100 (mg/dL) | - | p ≤ 0.001# | - |
| Lp(a) (mg/dL) | - | p ≤ 0.067# | p ≤ 0.056 |
| FFA (mEq/L) | - | - | - |
| CETP (%) | - | - | p ≤ 0.070 |
| Nitrite (µmole/L) | - | p ≤ 0.001‡‡ | - |
| $HDL_2$ CE (mg/dL) | - | p ≤ 0.033** | - |
| $HDL_3$ CE (mg/dL) | - | p ≤ 0.002** | - |
| $HDL_2$ TAG (mg/dL) | - | p ≤ 0.001# | - |
| $HDL_3$ TAG (mg/dL) | - | p ≤ 0.048# | - |
| $HDL_2$ PROT (mg/dL) | - | p ≤ 0.001# | - |
| $HDL_3$ PROT (mg/dL) | - | p ≤ 0.001# | - |
| Total mass $HDL_2$ (mg/dL) | - | p ≤ 0.001# | - |
| Lipid mass $HDL_2$ (mg/dL) | - | p ≤ 0.012†† | - |
| Lipid mass $HDL_3$ (mg/dL) | - | p ≤ 0.001# | - |
| Total $HDL_2$ to proteins ratio | - | p ≤ 0.001# | - |
| Total $HDL_3$ to proteins ratio | - | p ≤ 0.001# | - |

*Variables transformed in ranks due the absence of normal distribution; p : ANOVA for repeated measures, followed by Tukey post-hoc test and profile test by contrasts; LDLC : low-density lipoprotein cholesterol; VLDL-C : very low-density lipoprotein cholesterol; TAG : triglycerides; Apo B-100 : apolipoprotein B-100; Lp(a) : lipoprotein (a); FFA : free fatty acids; CETP : cholesteryl ester transfer protein; HDL- high-density lipoprotein; CE : cholesteryl ester; PROT : proteins; total mass HDL : sum of cholesterol, FC, CE, PL, TAG and proteins; lipid mass HDL : sum of cholesterol, FC, CE, TAG and PL; † polymorphisms: TT x CC; *treatments: placebo x atorvastatin; ¶Interactions: polymorphisms x treatment; #significant differences between treatments : Placebo ≠ Atorvastatin for both genotypes; ** significant differences between treatments : Placebo ≠ Atorvastatin only for CC; ††significant differences between treatments : Placebo ≠ Atorvastatin only for TT; ‡‡ BOX-COX between treatments.*

genotype were seen for CETP and Lp(a). Lp(a), although it was not different between groups, had a tendency to increase in CC after atorvastatin. Furthermore, previous studies showed that the presence of polymorphism T-786C in diabetic patients is an independent risk factor for the impairment of endothelium-dependent vasodilatation[37] .

Interestingly, CC volunteers show significant higher CETP activity, which decreased, although not significantly, after statin use. On the other hand, after statins, TT group show a borderline increase. The increased activity of CETP reduces HDL and increases its catabolism by hepatic lipase[38]. Thus, the use of atorvastatin "corrected" the initial condition of CETP in the CC group, as atorvastatin was able to reduce its activity[15,31]. A study conducted in the Chinese population showed that the presence of T-786C and Taq1B (a polymorphism of CETP gene) is responsible for an increased predisposition to non-valvular atrial fibrillation[39], contributing to the possibility that there are more complex relationships between these two variables in addition to the increased activity of CETP.

In the CC genotype a significant reduction of free fatty acids after the use of atorvastatin was shown, but there were no changes in the TT genotype. This suggests that the polymorphism may have a beneficial effect through lower mobilization of free fatty acids from adipose tissue to plasma and liver, facilitating the suppressive effect of statins on plasma free fatty acids. The involved mechanisms are not fully elucidated[40].

We would like to comment some points in this study that could be considered for further study, such as the fact that we have not assessed the effects of other doses of atorvastatin and/or other statins. Moreover, the study was conducted only in healthy Caucasian men, and in a relatively small number of subjects, a fact that may have limited the power to detect differences between the studied groups.

## Conclusion

These results together suggest that statin treatment may be relevant for primary prevention of atherosclerosis in patients with the eNOS gene T-786C polymorphism from the point of view of the lipid metabolism repercussions.

## Acknowledgments

We would like to thank FAPESP (Foundation for Research Support of the State of São Paulo) and CNPq (National Council for Scientific and Technological Development) for the financial support to this work.

### Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

### Sources of Funding

This study was funded by FAPESP and CNPq.

### Study Association

This article is part of the thesis of master submitted by Vanessa Helena de Souza Zago, from Faculdade de Ciências Médicas – Universidade Estadual de Campinas.

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