Consumption of Yerba Mate (*Ilex paraguariensis*) Improves Serum Lipid Parameters in Healthy Dyslipidemic Subjects and Provides an Additional LDL-Cholesterol Reduction in Individuals on Statin Therapy

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The objective of this study was to verify the effect of yerba mate (*Ilex paraguariensis*) consumption on lipid and lipoprotein levels in humans. One hundred and two individuals participated of this single-blind controlled trial. Normolipidemic ($n = 15$), dyslipidemic ($n = 57$), and hypercholesterolemic subjects on long-term statin therapy ($n = 30$) ingested 330 mL, 3 times/day, of green or roasted yerba mate infusions for 40 days. In normolipidemic subjects, yerba mate consumption reduced LDL-cholesterol by 8.7% ($p < 0.05$). Compared with the baseline period, yerba mate intake by dyslipidemic individuals for 20 and 40 days lowered LDL-cholesterol by 8.1 and 8.6% ($p < 0.001$) and non-HDL cholesterol by 5.4 and 6.5% ($p < 0.01$). After 20 days of yerba mate intake, apolipoprotein B was reduced by 6.0% ($p < 0.05$) and HDL-cholesterol was increased by 4.4% ($p < 0.01$). In all participants triglyceride levels remained unchanged. The consumption of yerba mate by hypercholesterolemic individuals on statin therapy promoted additional 10.0 and 13.1% reductions in LDL-C after 20 and 40 days, respectively ($p < 0.001$) and increased HDL-cholesterol by 6.2% after 40 days ($p < 0.05$). It was thus concluded that intake of yerba mate infusion improved the lipid parameters in normolipidemic and dyslipidemic subjects and provided an additional LDL-cholesterol reduction in hypercholesterolemic subjects on statin treatment, which may reduce the risk for cardiovascular diseases.

KEYWORDS: *Ilex paraguariensis*; yerba mate; hypercholesterolemia; statin; saponins; phenolic compounds; humans.

INTRODUCTION

Cardiovascular diseases (CVD) are the main cause of morbidity and mortality throughout the world. There are many factors involved in the etiology and progression of CVD, such as hypercholesterolemia—due to high levels of low-density lipoprotein-cholesterol (LDL-C)—and reduction of high-density lipoprotein-cholesterol (HDL-C), which are major risk factors for primary and established CVD (*1*). Moreover, it is well-known that the pathological processes related to CVD can be minimized by reducing plasma LDL-C and/or increasing HDL-C (*2, 3*).

In this context, the most powerful treatment for hypercholesterolemia is based on the use of statins, drugs that inhibit 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, the cholesterol biosynthesis rate-limiting enzyme (*4*). The development of statins was a major advancement for clinical practice, promoting a potent reduction in LDL-C and prevention of the atherosclerosis process. Statin therapy may decrease serum LDL-C by 30−40%, resulting in a proportional reduction of around 30%

in the relative risk of developing CVD (*5*). However, according to the National Cholesterol Education Program (*6*), prior to starting drug therapy in subjects with moderate hypercholesterolemia, the use of nonpharmacological treatments to reduce the plasma LDL-C is recommended, particularly steroids and viscous fibers from plants. There is therefore a growing interest in alternative treatments to reduce and/or prevent hypercholesterolemia, particularly through lifestyle changes and increasing the consumption of plant food with cholesterol-lowering products.

Yerba mate (*Ilex paraguariensis* St. Hill. Aquifoliaceae) is a phenolic- and saponin-rich plant species with potential lipid-lowering properties. In South American countries, including Brazil, Uruguay, Argentina, and Paraguay, the infusion or decoction of the aerial parts of *I. paraguariensis* is widely used in the preparation of a beverage appreciated for its peculiar bitter taste and stimulant properties, known as *chimarrão* and *tererê* (in Portuguese) or *mate* (in Spanish). For centuries, yerba mate was used as a stimulant beverage by the South American native peoples, especially the indigenous Guarani, and this practice was incorporated by colonial societies and persists until today.

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An estimated 1 million people in South American countries consume around 1−2 Lper day of mate infusion, which is the main alternative to coffee or black tea. Besides *chimarrão* and *tererê*—which are prepared with dried leaves of *I. paraguariensis* and hot and cold water, respectively—another tea-like beverage can be prepared using roasted (or burned) dried and minced leaves of *I. paraguariensis* and hot water. The roasted mate, or *mate tea*, as it is known in Brazil, is a beverage of soft and pleasant aroma, which is consumed in southern and southeastern Brazil and Argentina as a typical hot tea or in the form of refreshing ready-to-drink iced mate tea.

Since the pioneering study of Gugliucci and Stahl (*7*) reporting that the aqueous extract of *I. paraguariensis* inhibited LDL oxidation in vitro, several other studies have been published confirming the high antioxidant activity of the plant extracts, due to their various phenolic compound constituents—mainly caffeoylquinic acid derivatives—and suggesting that yerba mate might help to prevent atherosclerosis, diabetes, and inflammatory diseases through various mechanisms (see reviews in refs *8* and *9*).

Studies from our laboratory showed, for the first time, that the aqueous extract of *I. paraguariensis* inhibited the progression of atherosclerosis in cholesterol-fed rabbits (*10*) and improved the vascular contraction and relaxation in LDL receptor-knockout mice with atherosclerosis (*11*). In humans, we reported that acute ingestion of yerba mate infusion increased the antioxidant protection of plasma and LDL particles against ex vivo copper-mediated lipid peroxidation (*12*).

In folk medicine, yerba mate infusion has been used for the treatment of a number of diseases, including hepatic and digestive disorders, arthritis, rheumatism, and other inflammatory diseases, obesity, hypertension, and hypercholesterolemia. The potential cholesterol-lowering property suggested for mate may be due to the presence of saponins (*13*), which have the ability to form complexes with bile acids and/or cholesterol. Additionally, phenolic compounds such as chlorogenic, gallic, and caffeic acids, flavonoids, and caffeine, which are also constituents of *I. paraguariensis* (*8*,*9*), may play a role in the hypocholesterolemic effect of mate (*14*−*18*). Recently, it was reported that the aqueous extract of *I. paraguariensis* reduced the serum levels of the VLDL-LDL fraction, total cholesterol, and triglycerides in rats (*19*, *20*).

However, despite the great number of people who ingest *I. paraguariensis* infusions, there are no clinical or epidemiological studies associating its consumption to lowering plasma lipid levels or to decreasing cardiovascular diseases. Therefore, the purpose of this study was to evaluate the hypocholesterolemic potential of green and roasted *I. paraguariensis* infusions in healthy subjects with normo- or dyslipidemia. In addition, we investigated whether the yerba mate infusion could provide an additional cholesterol reduction in individuals ingesting a stable statin dose.

MATERIALS AND METHODS

Plant Material and Chemicals. Yerba mate infusions were prepared from commercially available green or roasted loose leaves of *I. paraguariensis*, purchased from Leao Junior SA (Curitiba-PR, Brazil). The yerba mate was collected in Irati-PR, Brazil, and a specimen of the plant was identified as authentic *I. paraguariensis* at the Botanical Department of the Federal University of Santa Catarina. A sample of *I. paraguariensis* was deposited in the Herbarium of the Botanical Department (Florianopolis-SC) under the number FLOR 37066.

Reagent kits for the measurement of total cholesterol, HDL-C, triglycerides, glucose, uric acid, urea, creatinine, and the enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and γ-glutamyl transferase (GGT) were obtained from Wiener Laboratorios SAIC (Rosario, Argentina). Reagents for the quantification of apolipoproteins A-I and B-100 were purchased from Dade Behring (Marburg, Germany). Ursolic acid, vanillin, chlorogenic

acid, 4,5-dicaffeoylquinic acid, caffeic acid, gallic acid, *p*-coumaric acid, protocatechuic acid, ferulic acid, epicatechin, gallocatechin, caffeine, theobromine, theophyline, and perchloric acid were purchased from Sigma (St. Louis, MO).

Preparation of Yerba Mate Infusions. Infusions of yerba mate were prepared by mixing boiling water and dried and minced leaves of commercial green or roasted yerba mate in the proportion of 50 or 20 mg/mL, respectively, representing the usual amount consumed by the population. After 10 min of extraction, the mixture was filtered and consumed immediately by the volunteer subjects.

Determination of Total Saponins Content in Yerba Mate Infusions. The content of total saponins in green and roasted mate infusions was measured by a spectrophotometric method after acid hydrolysis of the yerba mate saponins and extraction of sapogenins as described by Gnoatto et al. (*21*) and reaction with vanillin and perchloric acid according to Fan and He (*22*). Ursolic acid, the major triterpenic nucleus of saponins present in *I. paraguariensis* (*21*), was used as the standard, and the results were expressed as milligram equivalents of ursolic acid per milliliter. The interassay coefficient of variation of the total saponins contents of the different preparations was < 10%. The equation obtained for the ursolic acid standard curve was $y = 0.0003972x + 0.00216$, $r^2 = 0.988$.

Total Polyphenol Content in Yerba Mate Infusions. The total polyphenol content of the green and roasted yerba mate infusions was measured according to the modified Folin−Ciocalteu method as described by Singleton et al. (*23*). The interassay coefficient of variation was < 10%, and the equation for the chlorogenic acid standard curve was $y = 0.0005846x + 0.00179$, $r^2 = 0.997$.

Chromatographic Analysis of Phenolic Compounds and Xanthines. Phenolic compounds and methylxanthines in the green and roasted yerba mate infusions were measured by reversed-phase HPLC (RF-HPLC) (Shimadzu LC-10, Kyoto, Japan), using a Shim-pack C18 column (4.6 mm ID × 250 mm length) with a UV−visible detector (Shimadzu SPD 10A, 280 nm), as previously described (*24*). For the determination of phenolic compounds, the yerba mate infusions were centrifuged (2300*g*, 10 min) and filtered (0.22 μm), and aliquots were injected into an HPLC column thermostated at 40 °C, with UV detection at 280 nm. The isocratic mobile phase was constituted of water/acetic acid/*n*-butanol (350:1:10, v/v/v), and a flow rate of 0.8 mL/min was used. Quantitative determination was based on the external standard method by comparison with the standard retention time of several pure phenolics. Standard calibration curves were obtained by plotting the peak areas against different concentrations of gallic acid ($y = 9781x$, $r^2 = 0.99$) and gallocatechin ($y = 795.09x$, $r^2 = 0.99$).

Methylxanthinic alkaloids were isolated from the green and roasted yerba mate infusions by incubation with 60 mL of dichloromethane for 1 h. The solvent extract was recovered and concentrated to 2 mL under reduced pressure, and aliquots were injected into a Shim-pack C18 column thermostated at 30 °C, with detection at 272 nm. An isocratic mobile phase of acetonitrile/0.1% formic acid (15:85, v/v) was used with a flow rate set at 1.0 mL/min. Quantitative analysis was performed using standard calibration curves of caffeine ($y = 14502.02x$, $r^2 = 0.99$) and theobromine ($y = 34931.57x$, $r^2 = 0.99$). For all samples the final concentration of compounds was determined by averaging the results for three consecutive injections.

Subjects and Experimental Design. One hundred and eighteen potential volunteers (44 males and 74 females) were recruited through an announcement at the Federal University of Santa Catarina requesting subjects to take part in the study. The general health condition and the dyslipidemia status of potential volunteers were verified by the application of a standard questionnaire and the measurement of biochemical and hematological parameters, such as lipid profile, glucose, urea, creatinine, and the enzymes AST, ALT, ALP, and GGT, and hemograms (counts of red blood cells, white blood cells, and platelets, and measurement of hemoglobin level).

Exclusion criteria were obesity [body mass index (BMI) > 30 kg/m^2], presence of hepatic, renal, or gastrointestinal disorders, cancer, hypothyroidism, hyperthyroidism, diabetes, or alcoholism, and athletes practicing intense physical activity. After the beginning of the study, participants who had ingested medication affecting lipid metabolism or changed the drug doses (e.g., statins or antihypertensive), who had intolerance to yerba mate

infusions, or who discontinued the mate infusion intake for three or more consecutive days were excluded from the study. Throughout the study period the participants were instructed to maintain their regular lifestyle, such as the practice of moderate physical activities and their usual diet. All participants gave written, informed consent, and the Federal University of Santa Catarina Human Studies Committee approved the study (no. 080/2006).

The eligible volunteers ($n = 102$; 36 male and 66 female; mean age = 48.4 ± 1.35 years) were distributed into three groups according to the values of baseline serum lipids and lipoproteins, as described in the IV Brazilian Guideline for Dyslipidemia and Prevention of Atherosclerosis (*25*): (i) normolipidemic individuals ($n = 15$); and (ii) dyslipidemic individuals ($n = 57$; LDL-C $\geq$ 160 mg/dL; triglycerides $\geq$ 150 mg/dL; HDL-C $\leq$ 50 and 40 mg/dL, for women and men, respectively; or LDL-C/HDL-C ratio $\geq$ 2.5). The third group comprised hypercholesterolemic subjects on statin therapy ($n = 30$). The inclusion criteria for this last group were a stable statin dose for more than 3 months, LDL-C > 90 mg/dL, and triglycerides < 300 mg/dL.

The study was a single-blind, controlled trial. All participants remained for 30 days (baseline period) following their normal lifestyle (diet and physical activities). However, at least two weeks before the initial visit and throughout the baseline period, subjects had to discontinue consuming yerba mate-containing beverages and using lipid-lowering products or drugs, other than statins. To verify the effect of prolonged intake of yerba mate infusions on the serum lipid variables, the subjects were instructed to prepare the mate infusions daily, as described above, with no sugar or any sugar-like substances, and consume 330 mL of mate, 3 times/day, for 40 days, immediately before or during breakfast and before or during the additional two main meals, that is, lunch and supper. The participants had the option of drinking green (*chimarrão* type) or roasted (mate tea) yerba mate infusions, according to their preference. However, each participant ingested the same yerba mate infusion throughout the study. Blood samples were collected before (baseline period, at days -30, -15, and 0) and after 20 and 40 days of mate consumption, after $12-14$ h fasting, by vein puncture with a vacuum system (Vaccuntainer-BD, São Paulo-SP, Brazil) into tubes without or with EDTA. The tubes without anticoagulant were immediately centrifuged ($1000g$, 15 min) to obtain serum for the measurement of lipid parameters. Hematological variables were determined in the EDTA–blood sample. In addition, the potential toxicity of yerba mate consumption was determined through routine biochemical and hematological tests during the baseline period and after 20 and 40 days of mate infusion ingestion.

The participants served as their own control, as we compared all data obtained during the yerba mate consumption period with the average baseline values. During each visit, blood pressure, body height, and body weight were measured using standard procedures. BMI was calculated as body weight (kg) divided by height (m) squared.

Dietary Record. All of the subjects followed a free diet throughout the study. The nutrient composition of the usual dietary intake of each individual was calculated from 3-day dietary records (one day at the weekend and two days during the week). The food intakes during the baseline and study periods were calculated as the mean value of the 3-day dietary records evaluated in the first and fourth weeks of the baseline period and after 20 and 40 days of yerba mate infusion consumption. Food records were checked by a nutritionist and analyzed using the Avanutri software, version 3.15 (UNICAMP-SP, Brazil).

Laboratory Analyses. Serum levels of total cholesterol and triglycerides were determined by enzymatic methods, using the enzymes cholesterol oxidase and glycerol-phosphate oxidase, respectively, and HDL-C was determined by a homogeneous method using automated equipment (Wiener Analyzer, Wiener Laboratories SAIC, Rosario, Argentina). LDL-C was calculated by using the Friedewald equation [LDL = total cholesterol $-$ (HDL-C + triglycerides/5)] (*26*), and non-HDL-cholesterol (non-HDL-C) was obtained by the difference between total cholesterol and HDL-C. Serum concentrations of apolipoprotein A-I and B-100 were determined by nephelometry (Dade Behring, Marburg, Germany). Additional biochemical (urea, creatinine, glucose, uric acid, and the activity of the enzymes AST, ALT, ALP, and GGT) and hematological parameters (complete blood count) were evaluated by routine laboratory methods using automated equipment Wiener Analyzer and Sysmex XE-2100D (Kobe, Japan), according to the manufacturers' instructions.

Statistical Analyses. The descriptive statistics were presented as mean and standard error of the mean (SEM). Baseline categorical and continuous variables were analyzed by the chi-square test (x^2) and analysis of variance, respectively. Kolmogorov–Smirnov or Shapiro–Wilk tests were applied to verify the normality of continuous variables. When necessary, the logarithmic transformation of data was used. The repeated measures analysis of variance (RM-ANOVA) and the post hoc Tukey test were applied to verify the effects of green or roasted mate consumptions within each group (*27*). The Pearson correlation was used to determine the association between LDL-C changes and the baseline LDL-C level. Differences between the phenolic compounds, caffeine, and saponin content of green and roasted mate infusions were evaluated by Student's *t* test. A significance level of < 5% was considered to indicate that samples were statistically different. The software Statistical Package for Social Science (SPSS), version 12.0, was used for all of the analyses.

RESULTS

Total Saponin Content of Yerba Mate Infusions. The total saponin content of green mate infusion, prepared in the proportion of 50 mg of leaves/mL of water, was about 2.6-fold higher than that of the roasted mate infusion (20 mg of leaves/mL of water) on the basis of infusion volume (0.350 ± 0.011 vs 0.132 ± 0.007 mg/mL, $p < 0.05$). However, taking into account the dried leaves of yerba mate used for infusion preparations, the two types of yerba mate contain a similar amount of total saponins (7.01 ± 0.23 mg/g of green leaves vs 6.60 ± 0.35 mg/g of roasted leaves, $p = 0.348$; **Table 1**).

Total Polyphenol Content, Characterization of Phenolic Compounds and Methylxanthines of Yerba Mate Infusions. The total polyphenol content of the green yerba mate infusion, on a volume basis, was higher than that of the roasted infusion (5.51 ± 0.2 vs 1.74 ± 0.1 mg/mL, $p < 0.05$). On a dry weight basis, the green yerba mate also had a higher total phenol content than the roasted yerba mate (green, 110.3 ± 3.8 mg/g, vs roasted, 87.2 ± 1.3 mg/g, $p < 0.05$; **Table 1**). A similar pattern was observed for all polyphenol compounds and methylxanthines; that is, green yerba mate contains a slight higher but significant amount of bioactive constituents than the roasted mate (**Table 1**), as previously described by Bastos et al. (*8*).

Biodemographic Characteristics of Participants. One hundred and eighteen subjects started the study but 16 of them were excluded after consumption of mate infusions (four individuals had adverse effects, such as irritation of the oral or stomach mucosa, insomnia, or nausea; nine changed their diet habits and/or physical activities, and three altered medicine doses or had intake of another drug). Of the remaining participants ($n = 102$), 16 drank green mate (4 normolipidemic and 12 dyslipidemic subjects) and 86 individuals consumed roasted mate infusion (11 and 45 normolipidemic and dyslipidemic individuals, respectively). All hypercholesterolemic subjects on statin therapy drank roasted yerba mate. After $20-30$ days of mate ingestion, 12 additional individuals interrupted their participation in the study due to personal reasons.

The ingestion of both green and roasted yerba mate infusions affected similarly the serum lipid profile of participants within each group. Therefore, data on the green and roasted mate infusions were pooled. The baseline characteristics are presented in **Table 2**.

Food Records. Table 3 shows the 3-day dietary food records of normolipidemic, dyslipidemic, and hypercholesterolemic individuals on statin therapy during the baseline and yerba mate ingestion periods. In general, analysis of the food records showed agreement with health guidelines, mainly for total fat. However, it should be noted that saturated fatty acid consumption was higher than that recommended (< 7% total energy) (*25*). There were no

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Table 1. Total Saponin and Total Polyphenol Contents and Characterization of Phenolic Compounds and Methylxanthines in the Green and Roasted Yerba Mate Infusions[a]

.	green yerba mate		roasted yerba mate	
	mg/mL	mg/g	mg/mL	mg/g
total saponins[b]	0.350 ± 0.011 a	7.01 ± 0.23 a	0.132 ± 0.007 b	6.60 ± 0.35 a
total phenols[b]	5.51 ± 0.06 a	110.3 ± 3.8 a	1.74 ± 0.01 b	87.2 ± 1.3 b

	green yerba mate		roasted yerba mate	
	μg/mL	mg/g	μg/mL	mg/g
chlorogenic acid	804.1 ± 11.7 a	16.08 ± 0.23 a	170.0 ± 4.5 b	8.50 ± 0.22 b
4,5-dicaffeoylquinic acid	216.5 ± 4.6 a	4.33 ± 0.09 a	51.79 ± 3.09 b	2.59 ± 0.15 b
caffeic acid	10.39 ± 0.07 a	0.21 ± 0.001 a	4.83 ± 0.07 b	0.24 ± 0.003 a
gallic acid	201.3 ± 4.5 a	4.03 ± 0.09 a	59.1 ± 2.0 b	2.95 ± 0.10 b
p-coumaric acid	6.30 ± 0.05 a	0.13 ± 0.001 a	1.18 ± 0.04 b	0.06 ± 0.002 b
protocatechuic acid	40.31 ± 1.99 a	0.81 ± 0.04 a	9.34 ± 0.07 b	0.47 ± 0.003 b
ferulic acid	tr	tr	tr	tr
epicatechin	101.1 ± 2.9 a	2.02 ± 0.06 a	34.07 ± 1.52 b	1.70 ± 0.08 a
gallocatechin	458.9 ± 8.1 a	9.18 ± 0.16 a	47.4 ± 2.1 b	2.37 ± 0.10 b
caffeine	157.4 ± 1.5 a	3.15 ± 0.03 a	109.9 ± 3.8 b	5.49 ± 0.19 b
theobromine	48.12 ± 1.38 a	0.96 ± 0.03 a	26.98 ± 0.77 b	1.35 ± 0.04 b
theophylline	nd		nd	

[a] Data are mean ± SEM. Mean values on the same line and unit expressions followed by different letters are significantly different ($p < 0.05$). tr, traces; nd, not detected. [b] Colorimetric assays. The assays were carried out in triplicate of 8−10 different extractions for each green or roasted yerba mate infusion.

Table 2. Baseline Characteristics of Subjects at the Beginning of the Study[a]

characteristic	normolipidemic	dyslipidemic	hypercholesterolemic on statin
no. of subjects (M/F)	1/14 a	23/34 b	12/18 c
age (y)	42.0 ± 3.2 a	45.8 ± 1.6 a	55.3 ± 2.5 b
weight (kg)	59.6 ± 2.4 a	70.3 ± 2.1 b	75.6 ± 2.1 b
BMI (kg/m^2)	23.0 ± 0.7 a	25.4 ± 0.6 a	28.0 ± 0.6 b
SBP (mmHg)	109.7 ± 3.4 a	117.0 ± 1.9 a	121.4 ± 2.6 b
DBP (mmHg)	71.3 ± 2.1 a	77.5 ± 1.4 a	78.7 ± 1.7 a
cigarette smoking (%)	13.3 a	6.2 a	3.3 a
family history of CVD (%)	66.7 a	70.8 a	73.3 a
physical inactivity (%)	33.3 a	37.5 a	26.7 a
total cholesterol (mg/dL)	195.9 ± 7.4 a	233.1 ± 5.0 b	212.7 ± 8.9 a
LDL-C (mg/dL)	114.3 ± 4.7 a	158.8 ± 4.7 b	136.5 ± 14.1 a
HDL-C (mg/dL)	67.0 ± 3.7 a	48.4 ± 1.5 b	44.8 ± 2.6 b
triglycerides (mg/dL)	84.4 ± 6.7 a	152.6 ± 10.9 b	169.9 ± 20.1 b

[a] Data are mean ± SEM. Mean values in the same line followed by different letters are significantly different ($p < 0.05$). Abbreviations: M/F, male/female; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; CVD, cardiovascular disease; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

Table 3. Dietary Composition of the Subjects during the Baseline and Yerba Mate Ingestion Periods[a]

	normolipidemic group			dyslipidemic group			hypercholesterolemic on statin		
	baseline	20 days	40 days	baseline	20 days	40 days	baseline	20 days	40 days
energy (kcal)	2103.6 ± 412.5	2157.8 ± 151.3	2263.1 ± 443.1	1801.6 ± 232.8	2099.4 ± 614.2	1779.1 ± 311.6	1807.3 ± 261.4	2047.9 ± 267.7	2122.7 ± 365.5
carb (%)	52.8 ± 1.0	53.5 ± 2.2	52.1 ± 2.1	50.1 ± 5.4	46.8 ± 8.9	50.0 ± 7.9	50.0 ± 2.4	52.8 ± 5.4	51.9 ± 0.9
proteins (%)	17.7 ± 2.2	15.5 ± 2.8	16.5 ± 2.5	17.2 ± 3.6	18.4 ± 6.0	17.2 ± 0.7	16.9 ± 1.9	18.4 ± 6.1	15.6 ± 3.2
total fat (%)	29.5 ± 1.4	30.9 ± 3.6	31.4 ± 3.3	32.6 ± 1.7	34.9 ± 5.0	32.9 ± 7.8	33.2 ± 3.4	28.8 ± 1.2	32.5 ± 3.8
SFA (%)	10.8 ± 0.7	11.6 ± 0.5	9.9 ± 0.7	9.5 ± 1.9	11.0 ± 2.6	8.6 ± 0.7	11.5 ± 1.2	7.4 ± 2.7	9.4 ± 4.0
PUFA (%)	21.1 ± 1.7	16.6 ± 0.5	20.5 ± 0.7	10.9 ± 5.1	14.2 ± 7.5	13.4 ± 5.3	20.4 ± 2.9	19.9 ± 2.0	17.6 ± 1.9
chol (g/day)	0.16 ± 0.02	0.18 ± 0.03	0.18 ± 0.02	0.17 ± 0.04	0.19 ± 0.06	0.15 ± 0.05	0.19 ± 0.01	0.18 ± 0.06	0.17 ± 0.03

[a] Data are mean ± SEM. Macronutrients are presented as percentage of total energy intake. There were no statistically significant differences between the paired variables in the same group (baseline vs 20 days vs 40 days) (RM-ANOVA). Abbreviations: carb, carbohydrates; SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids; chol, cholesterol.

statistically significant differences regarding the daily intake of nutrients before and 20 or 40 days after the yerba mate consumption period.

Effect of Yerba Mate Intake on Serum Lipid Parameters. Table 4 and **Figure 1** present the results for the serum lipid parameters of normolipidemic and dyslipidemic subjects not taking statin, before and after consumption of mate infusions. In normolipidemic subjects, the intake of yerba mate infusions caused 8.7 and

7.3% (or 9.9 and 8.3 mg/dL) reductions in LDL-C and 16 and 10% reductions in the LDL-C/HDL-C ratio, after 20 and 40 days, respectively ($p < 0.05$), as compared to mean baseline values. Yerba mate consumption did not change the levels of total cholesterol, HDL-C, non-HDL-C, triglycerides, apo B-100, or apo B/apo A-I ratio ($p > 0.05$, **Table 4** and **Figure 1A**).

In the dyslipidemic individuals, the intake of yerba mate infusions for 20 and 40 days reduced the total cholesterol by

Table 4. Effect of Green and Roasted Yerba Mate (*Ilex paraguariensis*) Infusion Consumption (Pooled Data) for 20 and 40 Days on Serum Lipid Parameters of Normolipidemic and Dyslipidemic Subjects[a]

	cholesterol (mg/dL)				triglycerides (mg/dL)	LDL-C/ HDL-C	apo B-100 (mg/dL)	apo B/apo A-I
	total	LDL	HDL	non-HDL				
normolipidemic								
baseline (*n* = 15)	195.9 ± 7.4	114.3 ± 4.7 a	67.0 ± 3.7	128.9 ± 4.7	84.4 ± 6.8	1.77 ± 0.10 a	86.2 ± 5.4	0.47 ± 0.03
after 20 days (*n* = 15)	192.6 ± 9.3	104.4 ± 7.4 b	69.6 ± 4.0	123.0 ± 7.5	95.5 ± 8.0	1.49 ± 0.12 b	78.9 ± 5.2	0.45 ± 0.04
after 40 days (*n* = 14)	191.0 ± 9.6	106.0 ± 7.6 b	68.1 ± 3.7	122.9 ± 7.3	84.0 ± 7.3	1.6 ± 0.11 b	77.6 ± 5.7	0.44 ± 0.04
dyslipidemic								
baseline (*n* = 57)	233.4 ± 4.4 a	159.1 ± 4.2 a	48.1 ± 1.4 a	185.4 ± 4.2 a	151.7 ± 10.3	3.31 ± 0.1 a	119.4 ± 4.8 a	0.78 ± 0.04 a
after 20 days (*n* = 57)	225.3 ± 4.3 b	146.2 ± 4.0 b	50.2 ± 1.4 b	175.3 ± 4.2 b	146.9 ± 9.5	2.91 ± 0.1 b	112.2 ± 4.1 b	0.73 ± 0.03 b
after 40 days (*n* = 46)	222.7 ± 4.6 b	145.4 ± 4.2 b	49.4 ± 1.6 a	173.4 ± 4.4 b	147.3 ± 9.4	2.94 ± 0.1 b	115.6 ± 4.6 a	0.77 ± 0.04 a

[a] The results are expressed as mean ± SEM. Mean values in the same column of a respective group with different letters are significantly different ($p < 0.01$). The baseline values are the mean of three determinations with 15 day intervals.
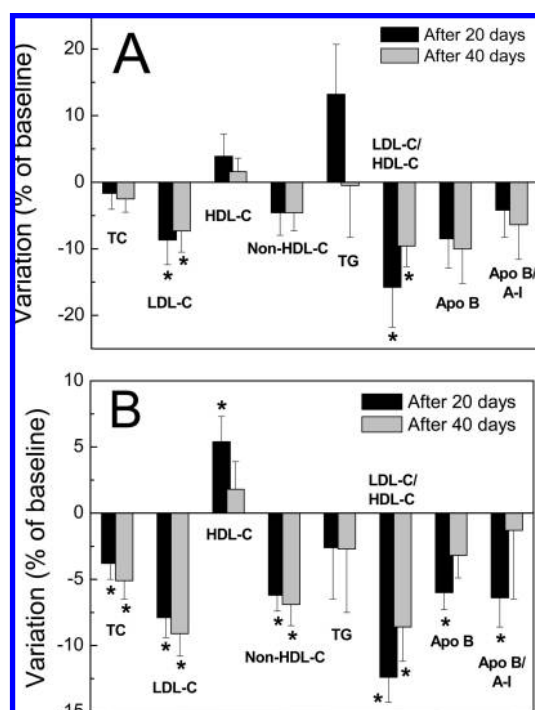

Figure 1. Percentage change in lipid parameters of normolipidemic (**A**) or dyslipidemic (**B**) subjects after 20 and 40 days of consumption of green or roasted mate (pooled data) in relation to their baseline values. The results are expressed as mean ± SEM. TC, total cholesterol; TG, triglycerides. ∗, $p < 0.05$ (RM-ANOVA and Tukey's test).

3.5 and 4.6% (or 8.1 and 10.7 mg/dL), respectively ($p < 0.01$); LDL-C by 8.1 and 8.6% (or 12.9 and 13.7 mg/dL), respectively ($p < 0.001$); non-HDL-C by 5.4 and 6.5% (or 10.1 and 12.0 mg/dL), respectively ($p < 0.05$); and the LDL-C/HDL-C ratio by 12.1 and 11.2%, respectively ($p < 0.01$), compared with the baseline period (**Table 4** and **Figure 1B**). After 20 days of mate consumption, HDL-C had increased by 4.4% (2.1 mg/dL) ($p < 0.01$), apo B-100 was reduced by 6.0% ($p < 0.05$), and the apo B/apo A-I ratio was lowered by 6.4% ($p < 0.05$). On the other hand, the intake of yerba mate infusions did not change the values of triglycerides ($p > 0.05$, **Table 4**, **Figure 1B**). There was no significant relationship between the baseline LDL-C level and the extent of LDL-C variation after yerba mate intake ($r = -0.198$, $p = 0.191$; **Figure 3A**).

Effect of Yerba Mate Consumption on Serum Lipid Parameters of Hypercholesterolemic Individuals on Statin Therapy. The results of the effect of yerba mate ingestion, concomitantly with statin treatment, on the serum levels of LDL-C and HDL-C in
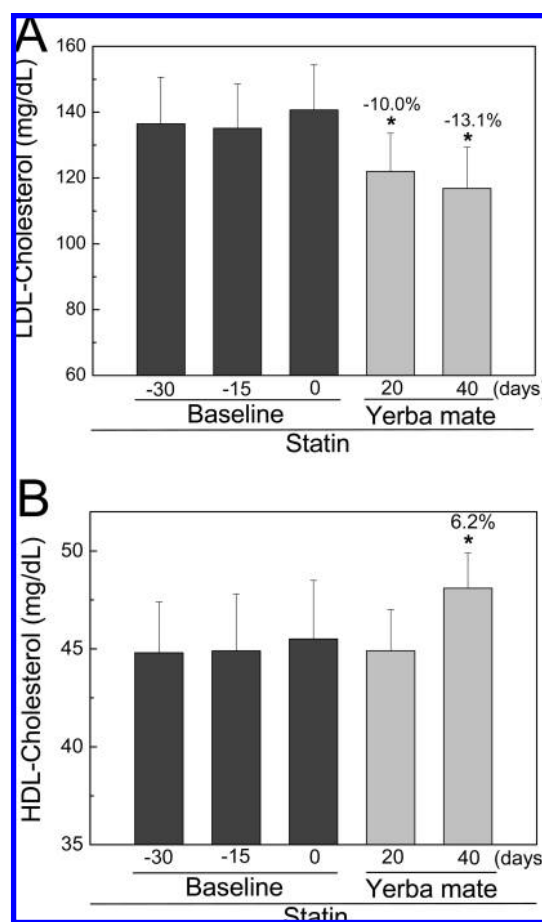

Figure 2. LDL-cholesterol (**A**) and HDL-cholesterol (**B**) levels during the baseline and roasted yerba mate ingestion periods by hypercholesterolemic subjects on statin therapy. The results are expressed as mean ± SEM ($n = 30$). ∗, $p < 0.05$ compared to baseline values (RM-ANOVA and Tukey's test).

hypercholesterolemic subjects are shown in **Figure 2**. The statin and daily doses were simvastatin (10 mg), atorvastatin (20 mg), or lovastatin (40 mg). It can be observed that in the baseline period of 30 days the serum levels of LDL-C and HDL-C remained almost stable due to the statin therapy ($p > 0.05$). The ingestion of roasted yerba mate infusion, in addition to the usual statins therapy, reduced the serum level of LDL-C by 10.0% (or 13.5 mg/dL), from 135.4 ± 15.1 to 121.9 ± 13.0 mg/dL ($p < 0.01$) after 20 days and by 13.1% (17.7 mg/dL) after 40 days ($p < 0.05$; **Figure 2A**). Although the small number of subjects in each statin dose level made statistical analysis difficult, the individuals on

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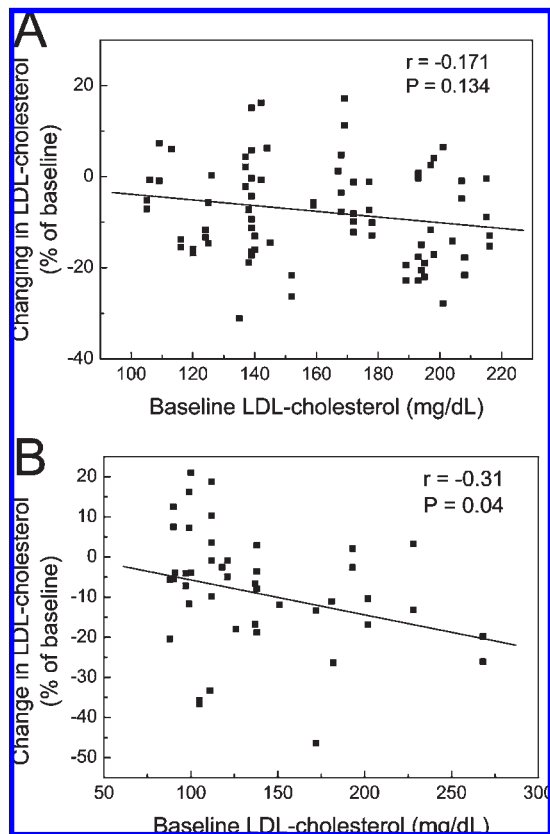*J. Agric. Food Chem.,* Vol. 57, No. 18, 2009 **8321**

Figure 3. Correlation between variation in LDL-cholesterol after 20 and 40 days of yerba mate ingestion versus baseline LDL-cholesterol levels in (**A**) dyslipidemic and (**B**) hypercholesterolemic subjects on statin therapy (Pearson correlation).

atorvastatin (20 mg) and lovastatin (40 mg) therapies tended to show a higher yerba mate hypocholesterolemic effect in comparison to simvastatin (10 mg) (data not shown).

The HDL-C level was increased by 6.2% (2.8 mg/dL) after 40 days ($p = 0.006$; **Figure 2B**). Moreover, the ingestion of roasted yerba mate lowered the LDL-C/HDL-C ratio by 9.4 ± 0.1 and $19.9 \pm 0.2\%$, after 20 and 40 days, respectively ($p < 0.05$). On the other hand, there were no significant changes in the serum levels of total cholesterol, triglycerides, and non-HDL-C (results not shown). **Figure 3B** shows a slight but significant inverse correlation between baseline LDL-C levels and the percentage of change due to yerba mate ingestion ($r = -0.310$, $p = 0.04$).

Effect of Yerba Mate Intake on Blood Pressure. The consumption of green or roasted mate infusions affected the systolic blood pressure of normolipidemic and dyslipidemic individuals, regardless of the statin intake, to the same extent. Therefore, the data on the mate infusions and the different groups were pooled. The systolic blood pressure was reduced by 2.3%, on average (117.5 ± 1.4 vs 114.8 ± 1.2 mmHg; $p < 0.05$), 40 days after intake of yerba mate infusions. However, yerba mate intake did not modify the diastolic pressure of participants (data not shown).

Effect of Yerba Mate Consumption on Body Weight. Neither green nor roasted yerba mate infusions were effective in reducing the body weight of participants, except the dyslipidemic individuals, who showed a slight body weight decrease of 0.5 kg (from 70.9 ± 1.6 to 70.4 ± 1.6 kg; $p = 0.02$), 40 days after mate ingestion.

Safety Monitoring. In the routine biochemical and/or hematological laboratory tests there were no significant or clinically relevant differences between baseline and 20 or 40 day values after consumption of mate infusions (data not shown).

DISCUSSION

In this study we demonstrated, for the first time, that consumption of green or roasted yerba mate by normolipidemic and dyslipidemic individuals significantly ameliorated serum lipid parameters. Moreover, our results clearly showed that yerba mate infusion provided further LDL-cholesterol reduction in subjects undergoing statin therapy.

After 40 days of consuming approximately 1 L of yerba mate infusion per day, the normolipidemic subjects showed a significant reduction in LDL-C of around 8.0% or 9.0 mg/dL (mean value of two measurements after 20 and 40 days), similar to results reported for other plant products (*28, 29*). It should be noted that approximately 67% of the subjects had a family history of CVD. Thus, by considering the already favorable lipid metabolism in normolipidemic individuals, our results suggest that daily ingestion of mate infusions might help to prevent the onset of future hypercholesterolemia. It should also be emphasized that a large variation in the response to yerba mate intake was observed, ranging from an absence of effects, the presence of negative effects, and up to a maximum LDL-cholesterol reduction of 40% in normolipidemic individuals (results not shown). A similar pattern was found for dyslipidemic (32% LDL-C reduction) and hypercholesterolemic subjects on statin therapy (45% LDL-C maximum reduction). Such variation might be due to the presence of males as well as females in the groups, to individual variability in metabolizing yerba mate constituents, and/or to the slight difference in PUFA ingestion, among other unknown reasons, which may be considered a limitation of our study.

In the dyslipidemic individuals not taking statins, the ingestion of mate infusion for 20 and 40 days also provided significant reductions in total cholesterol of 3.5 and 4.6%, corresponding to 8.1 and 10.7 mg/dL, respectively, and in LDL-C of 8.1 and 8.6% (12.1 and 13.7 mg/dL; $p < 0.001$), respectively. Similar levels of LDL-C reduction have been described for black and green teas in dyslipidemic individuals on a controlled diet (*30, 31*). Other important serum lipid parameters such as non-HDL-C and LDL-C/HDL-C ratio were also significantly reduced by approximately 6.0 and 12%, respectively, after intake of yerba mate infusions. Non-HDL-C represents very low-density lipoprotein (VLDL) plus LDL and intermediate-density lipoprotein (IDL), and it has been considered as a secondary target of therapy to consider the atherogenic potential associated with remnant lipoproteins in patients with hypertriglyceridemia (*5*). Additionally, apolipoprotein B-100 also represents the total number of atherogenic lipoproteins in plasma and the intake of green or roasted yerba mate by subjects with dyslipidemia decreased significantly apo B-100 (6%), as well as the apo B/apo A-I index (5.1%), which has emerged as an important additional parameter for the assessment of the risk of developing CVD (*32, 33*).

To our knowledge, this is the first clinical trial showing a cholesterol- and lipoprotein-lowering propriety of yerba mate infusion in humans. Previous results from our laboratory showed that administration of an aqueous extract of yerba mate to normolipidemic rabbits reduced the serum total cholesterol by around 30% (*10*). Paganini-Stein et al. (*19*) were the first to report a significant reduction in serum total cholesterol and triglycerides of cholesterol-fed rats after administration of *I. paraguariensis* aqueous extract. Recently, it was also shown that *I. paraguariensis* extract decreased the VLDL−LDL fraction in obese rats (*20*). However, contrary to these results in rats, here we did not find significant effects of yerba mate ingestion on the serum levels of triglycerides, as has also been observed after the consumption of different plant extracts or green tea (*29, 34*).

A probable mechanism for the LDL-C lowering ability of yerba mate is the blocking of cholesterol absorption in the small intestine and/or the inhibition of cholesterol synthesis in the liver, which can be attributed to the presence of saponins, phenolic compounds, flavonoids, and/or caffeine in the mate infusion. Indeed, Ferreira et al. (*35*) showed that the aqueous extract of *I. paraguariensis,* or its isolated saponins formed complexes with cholic acid in vitro, inhibiting its passage through a cellulose membrane. Catechins, a series of flavanol-type flavonoids, suppressed intestinal absorption of cholesterol by decreasing its micellar solubility in rats (*16*), and quercetin, a typical flavonoid, enhanced the excretion of cholesterol in feces in cholesterol-fed rats (*17*). Caffeine also inhibited the intestinal absorption of cholesterol in rats (*18*). Furthermore, the inhibitory effect of flavonoids and phenolic acids on the synthesis of cholesterol in the liver has also been described. The activity of hepatic HMG-CoA reductase in hypercholesterolemic rats was decreased by the intake of quercetin (*36*) and caffeic acid (*15*). Gebhardt (*37*) described that luteolin and, to a lesser extent, chlorogenic acid modulated indirectly the HMG-CoA reducatse activity in cultured rat hepatocyte, resulting in the inhibition of cholesterol biosynthesis. In addition, Yeh et al. (*15*) also reported that caffeic acid inhibited the acyl-Coa:cholesterol acyltransferase activity in the liver of cholesterol-fed rats. Overall, considering that yerba mate contains all of these compounds, we may hypothesize that a synergic effect of interaction between constituents may occur, as well as a dual mechanism leading to the final hypocholesterolemic result.

Our participants consumed about 130 and 350 mg of saponins and 1.7 and 5.5 g of total phenols per day, from roasted and green yerba mate infusions, respectively. Despite the lower content of total saponins and phenolic compounds in the roasted mate, similar hypocholesterolemic effects for both yerba mate infusions were observed, suggesting that the amount of bioactive compounds in the roasted mate might be sufficient for cholesterol-lowering purposes. However, at present these results cannot be considered as conclusive due to the small number of individuals who drank the green yerba mate infusion. Therefore, further studies with higher numbers of subjects should be carried out to elucidate this topic.

Another risk factor for CVD is low levels of HDL-C (*38*). In this context, the ingestion of green or roasted mate for 20 days increased significantly HDL-C by 2.1 mg/dL in dyslipidemic individuals. In normolipidemic individuals, a similar improvement was observed, but it lacked statistical significance, probably because of the small number of participants. On the basis of the NCEP III recommendations, which state that for every 1 mg/dL increase in HDL-C the relative risk of developing CVD is reduced by approximately 3% (*39*), we may speculate a proportional reduction in this risk of around 6% in the dyslipidemic subjects. The mechanism by which yerba mate may increase HDL-C levels is still not clear. Here, we found no significant increase in apolipoprotein A-I level after mate ingestion (data not shown), indicating that HDL synthesis may not play a role in the observed HDL-C enhancement. Additional studies should be conducted to clarify this biochemical mechanism.

In this study, the hypothesis of a synergistic effect of mate with statin on the reduction of LDL-C was investigated. The results showed that the consumption of roasted yerba mate infusion in addition to statin ingestion promoted a significant and additional reduction in LDL-C of 10%, corresponding to 13.5 mg/dL, and of 13.1%, equivalent to 17.7 mg/dL, after 20 and 40 days, respectively. These results are comparable and slightly superior to those from clinical studies that used sterols in tablet form, or sterol-enriched margarines, to produce a further reduction in LDL-C of 7−10% in addition to that of statin alone (*40, 41*). It is interesting to note that in subjects on statin treatment who had already lowered their LDL-C by 27%, each doubling of the dose thereafter decreased the LDL-C only by an additional 7% (*42*). Therefore, yerba mate infusion showed a synergistic effect with statins and it could be considered as a potential adjunctive therapy for LDL-C reduction in patients on statin treatment. These results may also assume great clinical relevance in light of the possibility of lowering the doses of statin and, consequently, minimizings adverse effects. The synergistic effect of yerba mate with statin can be explained on the basis of a dual cholesterol inhibition, that is, blocking of intestinal absorption by *I. paraguariensis* and decreasing endogenous biosynthesis by the statins, as well as by yerba mate phenolic compounds. Furthermore, the subjects who ingested yerba mate in addition to statin experienced a significant increase in HDL-C of 2.8 mg/dL (6.2%) and a decrease in the LDL-C/HDL-C ratio of around 20%.

According to the NCEP Adult Treatment Strategies Panel III recommendations on the management of high serum LDL-C, it is suggested that for every 30 mg/dL reduction in LDL-C, the relative risk of developing CVD is lowered by around 30% (*5, 6*). Overall, in light of the results presented above, that is, a mean LDL-C reduction of 13.3 mg/dL in dyslipidemic individuals and a reduction of around 17.7 mg/dL LDL-C in hypercholesterolemic subjects on statin therapy (average of the two determinations carried out after 20 and 40 days of yerba mate consumption), we may speculate proportional decreases of 13.3 and 18%, respectively, in the risk of CVD development in these individuals.

It was demonstrated here that for the statin-treated hypercholesterolemic subjects, in contrast to dyslipidemic individuals not taking statin, the baseline LDL-C level was a partial, but significant, determinant of the response to yerba mate. A slight inverse correlation ($r = -0.31$; $p = 0.04$) was found between the percentage variation in LDL-C and the baseline level of LDL-cholesterol, indicating that some subjects who did not respond well to statin therapy might have high cholesterol absorption and, consequently, are more susceptible to the yerba mate cholesterol absorption inhibition. Our results are consistent with findings described by Goldberg et al. (*40*) using plant stanols in statin-treated hypercholesterolemic patients.

Finally, it should be noted that the slight body weight losses of 0.5 kg seen after mate consumption, although statistically significant, can be considered of low clinical relevance and might have had no impact on plasma lipid levels. In addition, this study demonstrated that prolonged ingestion of green or roasted yerba mate infusions may be considered a safe practice on the basis of the absence of abnormalities in the routine biochemical and/or hematological tests.

We conclude that consumption of green or roasted yerba mate infusions for 20 or 40 days by normolipidemic and healthy dyslipidemic individuals improved the serum lipid parameters, particularly reducing significantly the levels of LDL-C, non-HDL-C, and apo B-100 and the LDL-C/HDL-C ratio and increasing HDL-C. In addition, we have clearly demonstrated that yerba mate infusion produced additional LDL-C lowering in hypercholesterolemic subjects who were on stable statin therapy. On the basis of these results, it is suggested that epidemiological and/or long-term prospective studies should be carried out to investigate the antiatherosclerotic properties of *I. paraguariensis* in humans.

ABBREVIATIONS USED

LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Apo, apolipolipoprotein; CVC, cardiovascular diseases; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; BMI, body mass index; ALT, alanine aminotransferase;

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AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, γ-glutamyl transferase; SBP, systolic blood pressure; DBP, diastolic blood pressure; SEM, standard error of the mean.

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