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Original Article

Cholesterol absorption and synthesis markers in Portuguese hypercholesterolemic adults: A cross-sectional study

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ABSTRACT

Objective: The dynamics of cholesterol homeostasis and the development of cardiovascular disease (CVD) are complex and multifactorial, to which adds individual variability in the proportion of cholesterol from exogenous versus endogenous sources. The aim of this study was to undertake the first characterization of cholesterol absorption and synthesis profiles in Portuguese hypercholesterolemic adults through the quantification of surrogate markers, and the analysis of the predictive value of age and sex on the cholesterol homeostasis biomarkers.
Methods: Serum samples for the measurement of lipid profiles and cholesterol homeostasis markers were obtained for 100 men and 112 women, aged 30–65, with TC ≥5.2 mmol/L (~200 mg/dL) and/or LDL-C ≥2.6 mmol/L (~100 mg/dL), none of whom were on any lipid-lowering therapy.
Results: Overall, sex-specific significant differences were observed in the cholesterol homeostasis markers and lipid profiles; women had lower cholesterol synthesis marker concentrations ($P < 0.01$ for lathosterol) and lipid parameters (except for HDL-C concentrations). Age-related significant differences were also found, including higher concentrations of cholesterol absorption markers in association with increasing age.
Conclusion: In our study, the predictors of higher levels of cholesterol absorption markers were higher age and female gender.

1. Introduction

Atherosclerosis, which leads to cardiovascular diseases (CVD), is usually caused by a combination of interacting risk factors. Some of these factors are related to lifestyle, while other risk factors, also modifiable, are elevated blood pressure, type 2 diabetes, and dyslipidemias, or non-modifiable, such as age and male sex.

The role of lifestyle patterns in the prevention/treatment of CVD has been extensively reviewed with most evidence showing that dietary factors, levels and types of physical activity, and tobacco cessation influence atherogenesis directly or through their effects on traditional risk factors and can also be used in the management of hypercholesterolemia. Total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels have been the primary targets of dyslipidemia therapy, precisely because they can be modified by lifestyle changes and drug therapies [1].

A novel strategy to complement the classical lipid profile determination (TC; LDL-C; high-density lipoprotein cholesterol (HDL-C); triglyceride (TG)) may be the analysis of the whole-body cholesterol homeostasis through the use of noncholesterol sterols (NCS) as surrogate markers of cholesterol metabolism. The circulating levels of NCS have been shown to correlate with the absolute cholesterol synthesis and absorption levels measured by the standard gold radio and stable isotopic methods [2–4]. The ease with which NCS can be measured compared to the complicated nature of more invasive isotopic methods has led to its wide use for the measurement and characterization of cholesterol metabolism across studies [5–7].

Among the cholesterol precursors, squalene, desmosterol, and lathosterol have been used as surrogate markers of cholesterol synthesis [3,4,8], and serum plant sterols (PS), mainly campesterol and sitosterol, and cholestanol, a 5α-saturated cholesterol derivative, as surrogate markers of cholesterol absorption [9].

Elevated cholesterol synthesis and reduced absorption have been reported in individuals with type 2 diabetes or hyperglycemia and have been associated with insulin resistance in cross-sectional studies [10–14]. Furthermore, several studies of NCS have described its

Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BMI, body mass index; CAD, coronary artery disease; CVD, cardiovascular disease; GC/MS-SIM, gas-chromatography/mass spectrometry-selective ion monitoring; HDL-C, high-density lipoprotein cholesterol; HMGR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; LDL-C, low-density lipoprotein cholesterol; NCS, noncholesterol sterols; PS, plant sterols; TC, total cholesterol; TG, triglycerides.

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association to established risk factors of CVD [15,16]. Matthan and colleagues have documented in patients with CAD that LDL-C concentrations were positively associated with cholesterol synthesis markers and negatively associated with cholesterol absorption markers [16]. Available data of the prognostic value of the cholesterol homeostasis markers on CVD events are still inconsistent [17].

More recently, Hyanek et al. evaluated the clinical practical use of NCS, showing that extending the laboratory lipid spectrum scan through routine determination of NCS improves the differential diagnosis of heterozygous familial hypercholesterolemia (in children and adolescents) and the titration of therapeutic statin doses and makes monitoring and/or treatment more precise [18].

The overall aim of this study was to undertake the (first) characterization of the cholesterol metabolism profiles in the adult (untreated) hypercholesterolemic Portuguese population through the quantification of serum cholesterol absorption and synthesis surrogate markers, resulting in better understanding of one of the main risk factors for CVD.

2. Material and methods

2.1. Study population and experimental design

The study was approved by the Ethics Committee of the Faculty of Medicine of the University of Coimbra and its institutional scientific review board, as well as by the Ethics Committee for Health of the Regional Governmental Office for Primary Health Care of the Central Region of Portugal. All procedures adhered to the guidelines of the Declaration of Helsinki. Informed consent was obtained from all individuals before they were enrolled into the study. By design, all participants were Caucasian, Portuguese, living/working in the Central Region of Portugal, included in the active working-age population. Individuals included in the study were selected at primary health care units in the Central Region of Portugal, on the basis of the last check-up registered in the medical database of patients coded for lipid metabolism alterations. The recruitment was conducted individually by phone invitation to participate in the study. A fasting serum sample was collected from each individual who agreed to participate. The fasting serum lipid criteria for enrollment were TC ≥5.2 mmol/L (~200 mg/dL) and/or LDL-C ≥2.6 mmol/L (~100 mg/dL)]. Exclusion criteria were as follows: diagnosis of sitosterolemia, history of liver or kidney chronic disease, mental illness, pregnancy and the current use of lipid-lowering medications, dietary supplements or functional foods active on plasma lipid levels for at least 3 months before the study.

For a confidence level of 95%, a margin of error of 5%, and a 55% prevalence of hypercholesterolemia, a statistical sample of N = 201 individuals was calculated, based on the population of the Central Region of Portugal (2 381 068 individuals on the basis of the last 2011 census register). The women/men ratio in the study reflects the proportion in the whole population (proportional allocation).

In total, fasting serum samples for measurement of lipid profile and cholesterol homeostasis markers were obtained for 100 men and 112 women, aged 30–65 years old. These participants were also evaluated with a questionnaire (designed, produced and pre-tested by the authors for the purposes of this study) to obtain socio-demographic, lifestyle (regular physical activity, use of tobacco, dietary pattern), and clinical (body mass index (BMI), use of concurrent medication that might influence cholesterol levels) characterizations.

Current smokers were defined as those who reported smoking ≥1 cigarette per day. Regular physical activity and/or aerobic exercise was defined as 150 minutes of brisk walking per week (moderate intensity), 75 minutes of running (vigorous intensity), or an equivalent combination [19].

Dietary pattern recommendations were defined to obtain the lowest level of chronic disease risk, including consumption of white meat instead of red meat (preferable); low consumption of red meats and meat products (≤2 servings/week); moderate to high fish consumption (≥3 servings/week) (especially oily fish ≥2 servings/week); moderate alcohol intake, usually in the form of red wine with meals (1 glass/day for women – 10 g of alcohol or 2 glasses/day for men – 20 g of alcohol) [20]. Daily reference values for water intake (in the form of beverages) for the Portuguese population are ≥1.9 L for men and ≥1.5 L for women [21].

2.2. Anthropometric and biochemical analyses

BMI was calculated (kg/m^2) using height and weight, as measured by a trained nurse in each health care unit following standardized and appropriate technique. A properly calibrated (by professional service) electronic digital scale (200 × 0.1 kg) with stadiometer (110–200 cm) (Prodoc PD 300, Detecto, Webb City, MO, USA) was utilized for accurate weighing and height measuring of the study participants.

Fasting serum TC, HDL-C and TG concentrations were measured using standardized enzymatic techniques. LDL-C concentrations were calculated by Friedewald's equation [22].

The cholesterol homeostasis markers analyzed included campesterol, sitosterol, and cholestanol as absorption markers, and desmosterol and lathosterol as synthesis markers. The serum values were expressed in terms of 10^2 mmol/mol of cholesterol by dividing NCS concentrations with the cholesterol value in order to eliminate the changing concentrations of sterol transporters. The ratios to cholesterol of serum precursors reflect whole-body cholesterol synthesis and those of PS and cholestanol, cholesterol absorption. We also calculated synthesis/absorption ratios (lathosterol/campesterol, lathosterol/ sitosterol, and lathosterol/cholestanol) to depict cholesterol metabolism with one variable. Serum concentrations of NCS were quantified using gas-chromatography/mass spectrometry-selective ion monitoring (GC/MS-SIM), as previously described [23]. The validation of the analytical method has been already reported by our group of researchers [24].

2.3. Statistical analyses

All statistical analyses were performed using IBM SPSS statistics version 22.0 Inc. (IBM Corporation, New York, USA). *P* values <0.05 were considered statistically significant. Independent-samples *t*-test was used to compare the lipid, lipoprotein, and NCS values between women and men and also to explain differences on the basis of sex that were not explained by differences in BMI. Differences among groups were determined using one-way ANOVA with Tukey's post hoc comparison test for localizing statistically significant effects. To investigate whether sex and age explained the variability of cholesterol metabolism,

Table 1
Study population: Clinical characterization (N = 212).

Variable	30–39 y (N = 56)	40–49 y (N = 59)	50–59 y (N = 52)	60–65 y (N = 45)
BMI, kg/m²				
Women	24.0 ± 3.7	25.3 ± 3.9	25.4 ± 2.7	25.0 ± 5.1
Men	26.9 ± 3.2	27.2 ± 3.9	27.6 ± 2.6	27.4 ± 3.9
Overall	25.5 ± 3.8	26.2 ± 4.0	26.5 ± 2.8	26.6 ± 4.6
*Concomitant medication**, N (%)				
Women	0 (0)	2 (6.3)	7 (25.9)	8 (32.0)
Men	2 (7.1)	3 (11.1)	8 (32.0)	7 (35.0)
Overall	2 (3.6)	5 (8.5)	15 (28.8)	15 (33.3)

Values are expressed as mean ± SD or N (%).
BMI, body mass index.
* Concomitant medication that may affect cholesterol level (ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; β-blocker; calcium channel blocker; diuretics).