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Effect of different fat-enriched meats on non-cholesterol sterols and oxysterols as markers of cholesterol metabolism: Results of a randomized and cross-over clinical trial

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Abstract *Background and aim:* Different kinds of fatty acids can affect the synthesis, absorption, and elimination of cholesterol. This study was carried out to assess the associations of cholesterol metabolism with the intake of two meats with different fatty acid composition in healthy volunteers.
Methods and results: The study group was composed of 20 subjects (12 males and eight females; age, 34.4 ± 11.6 years; body mass index (BMI), 23.5 ± 2.3 kg/m^2; low-density lipoprotein (LDL) cholesterol, 2.97 ± 0.55 mmol/l; high-density lipoprotein (HDL) cholesterol, 1.61 ± 0.31 mmol/l; triglycerides (TG), 1.06 ± 0.41 mmol/l) who completed a 30-day randomized and cross-over study to compare the cholesterol metabolism effect of 250 g of low-fat lamb versus 250 g of high-fat lamb per day in their usual diet. Cholesterol absorption, synthesis, and elimination were estimated from the serum non-cholesterol sterol and oxysterol concentrations analyzed by a high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS). No changes in weight, plasma lipids, or physical activity were observed across the study. Cholesterol intestinal absorption was decreased with both diets. Cholesterol synthesis and elimination decreased during the low-fat lamb dietary intervention ($\rho = 0.048$ and $\rho = 0.005$, respectively).
Conclusion: Acute changes in the diet fat content modify the synthesis, absorption, and biliary elimination of cholesterol. These changes were observed even in the absence of total and LDL cholesterol changes in plasma.
Registration number for clinical trials: ClinicalTrials.gov PRS, NCT02259153.

Introduction

The effects of dietary fats on the risk of coronary artery disease (CAD) have traditionally been mainly attributed to their effects on plasma levels of cholesterol and its distribution on lipoproteins [1,2]. Food products rich in saturated fatty acids (SFAs), especially on lauric (C:10), myristic (C14:0), and palmitic acids (C16:0), such as dairy fats, red meat, or tropical oils, are considered the most harmful fats mostly because they increase low-density lipoprotein (LDL) cholesterol. By contrast, diets low in saturated fat, although rich in monounsaturated fatty acids (MUFA), are considered healthy diets because of their beneficial effects on LDL cholesterol and high-density lipoprotein (HDL)

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cholesterol [3]. Three nutritional factors have been identified that raise serum LDL cholesterol levels: SFA, cholesterol itself, and excess caloric intake [4]. Although complex carbohydrates and MUFAs represent the preferred replacements for SFAs in the diet, modest increases in polyunsaturated fatty acids (PUFAs) and stearic acid (C18:0) probably are safe, and they may provide for a greater variety in the diet [5]. This is due to the fact that the effects of fatty acids (FAs) on lipid metabolism depend on the saturation of the carbohydrate chain, but also on the length, *cis*/*trans* disposition, and the amount of calories from different FAs [6]. Furthermore, there is an interindividual variability of the lipid response to different FAs, and, therefore the perfect diet, if available, remains to be determined.

The knowledge about the mechanism throughout FAs modifying cholesterol metabolism is derived, mostly, from "in vitro" studies [3]. Consistently, medium-size SFAs, with the exception of stearic acid, increase apolipoprotein B (apoB) and very low-density lipoprotein (VLDL) secretion in hepatic-derived cells. Human "in vivo" studies show an increase in hepatic cholesterol synthesis when diets are rich in myristic and palmitic acids [7]. The effects of different FAs on cholesterol intestinal absorption and bile acid synthesis, another two important mechanisms on cholesterol homeostasis, have been less studied and with conflicting results [8]. Changes produced in serum cholesterol precursors have been found to be a useful indicator of cholesterol metabolism during long-term dietary intervention with fat-modified diets [9].

The assessment of cholesterol absorption, synthesis, and elimination is very laborious in clinical studies. For this reason, serum non-cholesterol sterols and oxysterols have been examined as relative markers of whole-body cholesterol metabolism under steady-state conditions with subjects consuming a normal habitual diet [10]. Levels of serum cholesterol precursors (squalene, desmosterol, lanosterol, and lathosterol) reflect the activity of cholesterol synthesis; serum plant sterols (campesterol, sitosterol, and stigmasterol) and cholestanol reflect the absorption efficiency of cholesterol [11–13]. The oxysterols, 24S-hydroxycholesterol, 27-hydroxycholesterol, and 7α-hydroxy-4-cholesten-3one, are established biomarkers of bile acid synthesis [14]. We hypothesized that SFAs modify not only cholesterol synthesis but also cholesterol absorption and bile acid production. Those changes in cholesterol metabolism will be detected "in vivo" measuring serum non-cholesterol sterols by a high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) [15]. Hence, the objective of this study was to assess the effect of two diets with different FA composition on cholesterol metabolism. For this purpose, two synthesis markers (desmosterol and lanosterol), three plant sterols (sitosterol, campesterol, and stigmasterol), cholestanol, and three oxysterol (24S-hydroxycholesterol, 27-hydroxycholesterol, and 7α-hydroxy-4-cholesten-3one) concentrations in serum were analyzed during a high-fat and low-fat lamb dietary clinical trial.

Methods

Study subjects

Healthy volunteers (n = 20), aged >18 years, were recruited and provided written informed consent into a protocol previously approved by the ethics committee of our institution (Comité Etico de Investigación Clínica de Aragón, CEICA). Exclusion criteria included any disease that could interfere with the ability to comply with the study protocol, any chronic medication, except oral contraceptives, obesity (body mass index (BMI) of >30 kg/m^2), weight variation in the last 3 months (>3 kg), and pregnancy or intention of pregnancy.

Dietary intervention

Participants were randomized in a single-blind and crossover design with two-diet sequences for 10-day periods (ClinicalTrials.gov PRS, NCT02259153. The effect of different fat-enriched meats on the hepatic cholesterol synthesis). One of the diets included 250 g per day of leg and rib from the lamb of Rasa Aragonesa breed (a medium-wool breed, rustic type of sheep raised for its meat, with the protected origin label "Ternasco de Aragon" from Spain [16]), and the other diet included 250 g per day of ribs from lamb with high fat composition. The FA composition of both meats is presented in Table 1. A 10-day washout period was incorporated between the two meat diets. After the washout period, participants crossed over to the alternate diet sequence; thus, those who started with lean lamb switched to fat lamb and vice versa (Fig. 1). Study variables were evaluated at four-time visits (days 0, 10, 20, and 30 of the intervention period). Study period length was selected because previous intervention studies demonstrated the stabilization of non-cholesterol sterols in <1 week after intervention [17,18]. Subjects received the fat and lean lamb in daily portions during the study periods, but no monetary compensation.

Clinical parameters included the following: medical history, anthropometric measures (weight, height, and waist circumference), and blood pressure. Fasting blood samples for biochemical profiles were drawn at the four visits. Dietary assessment consisted of a validated food frequency questionnaire [19] that was performed at

Table 1 High-fat and low-fat lamb lipid composition per 100 g of meat.

	High-fat lamb	Low-fat lamb
Energy, kilocalories	310	218
Fat, g	27.5	17.2
Saturated fatty acids, g	11.1	7.2
Stearic acid, g	3.3	2.59
Palmitic acid, g	6.95	4.07
Myristic acid, g	0.77	0.5
Lauric, g	0.1	0.04
Monounsaturated fatty acids, g	13.8	7.5
Polyunsaturated fatty acids, g	2.12	2.0