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Original article

# Serum plant sterols as surrogate markers of dietary compliance in familial dyslipidemias

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SUMMARY

*Background & aims:* A well-balanced diet is the first-line treatment in hyperlipidemia. The objective was to study the association between serum phytosterols and dietary patterns to use them as surrogate markers of dietary compliance in primary dyslipidemias.
*Methods:* 288 patients with primary hyperlipidemias (192 autosomal dominant hypercholesterolemia (ADH) and 96 familial combined hyperlipidemia (FCHL)) were included. Principal factor analysis identified 2 major dietary patterns using a 137-item food frequency questionnaire. "Vegetable & Fruits pattern" was characterized by higher intake of fruits, green beans, nuts, tomatoes, roasted or boiled potatoes, lettuce and chard and lower of processed baked goods, pizza and beer. "Western pattern" was positively characterized by hamburgers, pasta, sunflower oil, rice, chickpeas, whole milk, veal, red beans and negatively with white fish. Serum non-cholesterol sterols were determined by HPLC-MS/MS.
*Results:* Plant sterols to-total cholesterol (TC) levels were lower with a higher adherence to a "Vegetable & Fruits pattern" ($P = 0.009$), mainly in ADH subjects ($R^2 = 0.019$). Their concentration was greater with higher compliance to "Western pattern" especially in FCHL ($P = 0.014$). Higher levels of synthesis markers-to-TC with a greater adherence to "Vegetable & Fruits pattern" were found ($P = 0.001$) ($R^2 = 0.033$ and $R^2 = 0.109$ in ADH and FCHL respectively).
*Conclusion:* In subjects with primary dislipidemia, dietary patterns associate with serum absorption and synthesis markers, but no with lipid concentrations. The influence of diet on non-cholesterol sterols levels is not powerful enough to use them as subrogate markers.


## 1. Introduction

The strong relationship between plasma cholesterol and cardiovascular disease (CVD), the leading cause of mortality in the world, is well accepted [1,2]. Lifestyle behavioral changes, including dietary modifications, increased physical activity and weight loss in overweight or obese patients, are the first-line option treatment in hypercholesterolemias. This is also reflected in the National Cholesterol Education Program (NCEP) dietary guidelines for the prevention of CVD recommending a healthy dietary pattern rich in monounsaturated fatty acids provided by plant sources, fruits, vegetables, whole grains, and low-fat dairy products and low in saturated fatty acids and cholesterol [3].

Dietary assessment is important in the following-up of the patients so as to identify "unhealthy" dietary habits to promote adherence to healthier dietary patterns. Dietary questionnaires such as food frequency questionnaires, 24 h recalls or food records are commonly used to assess dietary intake, however, such methods come with limitations as reliance on memory, misreporting and should be considered when evaluating nutrition behaviors [4,5]. Subrogate parameters which could objectively

*Abbreviations:* CVD, cardiovascular disease; ADH, autosomal dominant hypercholesterolemia; FCHL, familial combined hyperlipidemia; TC, total cholesterol; BMI, body mass index; CRP, C-reactive protein; FFQ, food frequency questionnaire; PCA, principal component analysis; GGT, γ-glutamyl transpeptidase.

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evaluate dietary compliance would be very useful both in epidemiological studies and in clinical practice.

Serum levels of phytosterols, commonly known as plant sterols, and cholestanol are positively correlated with cholesterol absorption and their ratios to cholesterol (relative concentrations) are considered to be reliable markers of intestinal sterols absorption efficiency [6,7]. Serum phytosterols are only partially dependent to their amount in the diet although this association has not been studied in subjects with primary dyslipidemias who show an abnormal cholesterol homeostasis [8,9].

Dietary pattern analysis has emerged as an alternative approach to examine the relationship between diet and the risk of chronic diseases; conceptually, dietary patterns provide a broader picture of food and nutrient consumption, and may thus be more predictive of disease risk than individual foods or nutrients [10,11]. The aim of the study was to examine the association between serum phytosterols levels and dietary patterns in primary dyslipidemias by proposing them as surrogate markers of dietary compliance in patients with primary dyslipidemias.

## 2. Materials and methods

### 2.1. Study population

Patients attending to the Lipid Unit of the Hospital Universitario Miguel Servet (Zaragoza, Spain) from January 2011 to September 2012 were recruited. 288 subjects with familial hypercholesterolemias were recruited as part of a genetic and metabolic wider study whose study details has been already published elsewhere [12]. Inclusion criteria were being over 18 years of age and the presence of familial hyperlipidemia by including autosomal dominant hypercholesterolemia (ADH) and familial combined hyperlipidemia (FCHL). ADH was diagnosed in subjects with off-treatment LDL cholesterol levels above the age- and sex-specific 95th percentile of a Spanish reference population, triglyceride below 200 mg/dL and familial vertical transmission with at least one first-degree relative with LDL cholesterol above age- and sex-specific 95th percentiles. The diagnosis of FCHL was based on the presence of primary combined hyperlipidemia in untreated patients whose serum cholesterol and triglyceride concentrations were above the sex- and age-specific 90th percentiles for the Spanish population, serum total apolipoprotein B levels $\geq$120 mg/dL and there was at least one first-degree relative with hyperlipidemia (total cholesterol (TC) and/or triglycerides >90th percentile) [13]. Secondary causes of hyperlipidemia (e.g. body mass index (BMI) $\geq$30 kg/m$^2$, alcohol intake over 30 g and 20 g in men and women respectively) and subjects with plant sterols supplements intake were excluded. Written informed consent was obtained by all study participants. The study protocol was approved by the Ethical Committee of our Institution (Comité Ético de Investigación Clínica de Aragón).

### 2.2. Clinical and laboratory determination

Clinical parameters obtained included anthropometric measures (weight, height and waist circumference) and blood pressure. BMI was calculated (weight in kg. divided by the square of height in meters) and all subjects were assessed for personal and/or family history of early-onset coronary heart disease, clinical history, tobacco consumption and demographic characteristics by a personal interview.

Fasting blood was drawn following at least 4 weeks without lipid-lowering drugs treatment. Cholesterol, triglycerides, HDL cholesterol and $\gamma$-glutamyl transpeptidase (GGT) were measured by spectrophotometry with standard enzymatic methods. LDL cholesterol was estimated with the Friedewald formula when serum triglycerides were <400 mg/dL. Non-HDL cholesterol was calculated as TC minus HDL cholesterol. Apolipoprotein B, lipoprotein(a), and C-reactive protein (CRP) were determined by nephelometry using IMMAGE-Immunochemistry System (Beckman Coulter).

### 2.3. Dietary assessment

Dietary intakes were determined using an interviewer-administered 137-item food frequency questionnaire (FFQ). One registered dietician (RM-G) performed the interviews. More details of the FFQ validity, which has been previously used to study other diet-disease association including plant sterols, could be found elsewhere [14–17]. A full version of the FFQ can be downloaded (http://www.unav.es/departamento/preventiva/predi_educationals). Food and nutrient intakes were calculated as frequency $\times$ nutrient composition of specified portion sizes, where frequencies were measured in 9 categories (never, 1–3 times a month, 1 time a week, 2–4 times a week, 5–6 times a week, 1 time a day, 2–3 times a day, 4–6 times a day and >6 times a day) for each food item. The total energy and nutrients intakes were calculated based on previously validated Spanish food composition tables [18]. When possible and applicable, the 137 foods were grouped into categories based on similar nutritional values. Food items ($N = 18$) with low prevalence of consumption (less than 15%) were not considered in the final analysis ($N = 119$) to avoid possible bias in the dietary patterns calculation.

### 2.4. Serum non-cholesterol sterols determination

Serum non-cholesterol sterol concentrations were analyzed by high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) [19]. Briefly, ([$^2H_6$] cholesterol-26,26,26,27,27,27 D6) (4 μg/g) was added to serum (0.1 ml) as the internal standard. After alkaline hydrolysis, extraction and solid phase extraction, the sterols were separated using reverse-phase C18 HPLC. A 40 μl aliquot of the extract (100% 2-propanol) was loaded onto an RP-HPLC column (Zorbax Eclipse Plus $C_{18}$ 2.1 $\times$ 150 mm, 3.5 μm particle; Agilent, Spain) equipped with a guard column ($C_{18}$, 4 $\times$ 2.5 mm). The HPLC (Agilent 1200RRLC) was coupled to a 4000 QTrap triple quadrupole ion trap mass spectrometer (Applied Biosystems, Foster City, CA) through an APCI by Heated Nebulizer (Turbo VTM Source). In each run, cholestanol, campesterol, sitosterol, sitostanol and stigmasterol were quantified.

### 2.5. Statistical analysis

Statistical analysis was performed using SPSS software version 15.0 (Chicago, Illinois, USA) using a significance level of $P < 0.05$.

Data are expressed as means $\pm$ standard deviation (SD) for continuous variables with normal distribution and medians (percentile 25–percentile 75) for variables with a skewed distribution. Student-*t* or Mann–Whitney tests were used accordingly. Categorical variables were compared using a chi-square test. ANOVA and Kruskal–Wallis tests were performed to multiple independent variables comparison. Non-cholesterol sterols levels were adjusted by those variables which have been shown more influential in its concentration: age, gender, BMI and APOE genotype [20]. PCA with varimax rotation was used to derive dietary patterns based on the 61 foods or food groups [10,21,22]. The factors were rotated by an orthogonal transformation (resulting in uncorrelated factors) to achieve a simpler structure with greater interpretability. In determining the number of factors to retain, we considered components with an eigenvalue >1, the Scree test and the interpretability of the factors. The factor