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HDL-sphingomyelin reduction after weight loss by an energy-restricted diet is associated with the improvement of lipid profile, blood pressure, and decrease of insulin resistance in overweight/obese patients

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ABSTRACT

Background: Sphingomyelin (SM) diminishes the fluidity of the surface monolayer of high-density lipoproteins (HDL), affecting their intravascular metabolism and antiatherogenic properties. Since overweight is associated with an altered HDL structure, weight loss may result in changes in HDL subclasses, particularly in their SM content. Therefore, we determined the plasma SM concentrations associated to both total HDL and HDL subclasses after weight loss in obese patients.
Methods: Fifty overweight patients, 40 women and 10 men, aged 38.6 ± 6.4 y, were given an energy-restricted diet according to their sex, age, and height. No physical activity was prescribed. Plasma SM concentrations of HDL subclasses were determined by a gel surface method developed for this study. Cholesterol of HDL subclasses was also determined by enzymatic methods performed on a gel surface.
Results: Mean weight lost was 3.5 ± 0.4 kg after 6 weeks of dietary intervention. As expected, insulin resistance and blood pressure decreased whereas lipid profile improved, except for HDL-cholesterol. SM in plasma and in all HDL subclasses significantly decreased after intervention. The magnitude of HDL-SM reduction was statistically associated with the amelioration of the components of the metabolic syndrome; the reduction of BMI explained the decrement of HDL-SM in a multivariate analysis.
Conclusion: HDL-SM decreased after weight loss by an energy-restricted diet. Further, the association of this decrement with the improvement of blood pressure, lipid profile and the decrease of insulin resistance, was statistically significant; all HDL subclasses were similarly affected. Whether a reduction in HDL-SM contributes to the cardiovascular benefits of weight loss remains to be elucidated.

1. Introduction

The concurrence of excess of abdominal fat, hypertension, hyperglycemia, insulin resistance, hypertriglyceridemia, and low high-density lipoproteins (HDL)-cholesterol plasma levels drastically increases the risk for coronary artery disease (CAD) [1]. HDL includes a heterogeneous group of lipoproteins that can be classified by their size in HDL2b, HDL2a, HDL3a, HDL3b, and HDL3c [2]. These HDL subclasses differ in their physicochemical properties and probably in their antiatherogenic characteristics [2,3]. It is likely that some of the functional properties of HDL are related to their role to deliver lipids into endothelial cells, including SM [4–6].

Sphingomyelin (SM) is an important component of cellular plasma membranes that contributes to the structure of the lipid rafts [7,8], and it is a precursor of molecules involved in cell signaling, inflammatory processes, and apoptosis [9–11]. SM is a structural phospholipid of HDL that diminishes the fluidity of the surface monolayer, affecting the intravascular metabolism of these lipoproteins [12,13]. SM present in HDL stimulates ABCA1-mediated cholesterol efflux [14], and induces a decrease of cholesterol influx via SR-BI in COS-7 cells [15]. Additionally, HDL-SM modulates several enzymes and transport proteins involved in the intravascular metabolism of HDL such as LCAT and PLTP [12,16–18]. Taken together, these evidences strongly suggest that SM content of HDL determines the plasma size distribution of these lipoproteins and probably their functionality; yet, little is known in this field.

Since HDL subclasses are altered in metabolic syndrome [19–21], and an energy-restricted diet ameliorates some components of the syndrome, we hypothesize that HDL-SM content is modified during such

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intervention. To test this hypothesis, the need for a practical method to quantify the SM levels of HDL subclasses became apparent. Therefore, we have first developed an enzymatic method to determine the proportion of HDL subclasses based on their SM content on a native electrophoresis gel surface. We further characterized the plasma concentrations of SM in HDL subclasses in a group of patients, before and after energy-restricted diet consumption.

2. Methods

2.1. Reagents

Sphingomyelinase (SMase), alkaline phosphatase, choline oxidase, peroxidase, sodium cholate, triton 100×, thiazolyl blue tetrazolium bromide (MTT), phenazine methosulphate (PMS), carboxymethylcellulose, 4-aminoantipyrine and DAOS (N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline, sodium salt) and SM were from Sigma-Aldrich.

2.2. Study subjects and intervention

Fifty overweight or obese individuals were included in the study (age ≥ 18 y). Actually, they had participated in a previous trial conducted at the Instituto Nacional de Cardiología Ignacio Chávez (México City) [22]. Subjects were excluded if they had a personal history of diabetes, hypertension, chronic kidney disease, liver disease, anemia, pregnancy, malignancy, or if they were taking any medications. Patients were given a total dietary plan of 1500, 1800, or 2000 kcal according to their age, sex, and height as reported in a previous study [22]. No physical activity was prescribed. Measurements of blood pressure, body weight, abdominal and hip circumference were obtained with standardized techniques. The study was approved by the Ethics Committee of the National Institute of Cardiology in Mexico. All eligible participants were informed of the study objectives, and those who agreed to participate signed a letter of informed consent.

2.3. Laboratory assessment

All patients were instructed to avoid strenuous exercise and to eat a light dinner the day before blood sampling was performed. Serum and blood-EDTA plasma samples were obtained after 12-h overnight fasting. Plasma and serum samples were separated and analyzed, or frozen at −80 °C until analysis.

For lipoprotein isolation and plasma lipid profile, samples were processed within 2 h after collection. Commercially available kits from Randox Laboratories (Antrim, UK) were used to determine total cholesterol, triglycerides and glucose. The phosphotungstic acid-Mg^{2+} method was used to precipitate apoB-containing lipoproteins before quantifying plasma concentrations of HDL cholesterol. Low-density lipoprotein cholesterol was estimated in samples with triglycerides <400 mg/dl. Serum samples were used to quantify SM as previously described [23] with slight modifications. Briefly, plasma was incubated with bacterial SMase, alkaline phosphatase, choline oxidase, peroxidase, DAOS and 4-aminoantipyrine for 45 min. A blue dye with an optimal absorption at 595 nm was generated. HDL-SM was determined by analytical ultracentrifugation at a density of 1.063 g/ml. The infranatant was quantitatively recovered and analyzed by the enzymatic method reported above.

2.4. Isolation of HDLs

HDLs were separated by ultracentrifugation in a Beckman optima TLX table centrifuge at 100,000 rpm in 1 ml polycarbonate tubes as described previously [24]. HDLs were dialyzed against 0.09 mol/l Tris/0.08 mol/l boric acid (TB) buffer, pH 8.4.

2.5. Enzymatic staining of SM and cholesterol on polyacrylamide gel

HDLs were separated by their hydrodynamic diameter in a 8 × 10 × 0.15 cm non-denaturing 3–30% gradient polyacrylamide gel electrophoresis, using Tris 0.09 mol/l, Boric Acid 0.08 mol/l, $CaCl_2$ 3 mmol/l (TB-$CaCl_2$) buffer, pH 8.4, during 20 h at 180 V.

Twenty-five micrograms of HDL protein sample were deposited per well. Gels were stained for SM using an enzymatic mixture of SMase, alkaline phosphatase, choline oxidase and peroxidase at a final concentration of 0.0625 U/ml, 5 U/ml, 0.25 U/ml and 3.12 U/ml, respectively. A buffer of 50 mmol/l Tris, 3 mM $CaCl_2$, pH 8 was used too. The reaction mixture also included 3 mmol/l sodium cholate, 0.1% Triton 100×, 0.4 mmol/l MTT, and 0.6 mmol/l PMS, as well as carboxymethylcellulose as the viscosifying agent.

Electrophoresis gels were kept in contact with the reaction mixture during 1 h at 37 °C in the dark. At the end of the incubation time, the reaction mixture was removed and the gels were gently washed in PBS to eliminate any remaining residue of carboxymethylcellulose. Electrophoresis gels were then scanned in a GS-670 BioRad densitometer, distained and further restained and scanned for proteins as previously described [25]. The relative proportions of SM and protein in each HDL subclass were estimated by optical densitometry as previously documented [19,20,25,26], and their content was expressed as the percentage of the total HDL area under the curve, integrated from 7.94 to 13.59 nm.

Considering that the area under the curve in the densitogram represents 100% of the SM in the HDL, the SM plasma concentration of each HDL subclass was estimated as follows: HDLn-SM = (% HDLn determined by SM × HDL-SM) / 100 where n represents the HDL subclass, and HDL-SM is the HDL-SM plasma concentration. As for HDL subclassification, we considered the following size intervals: HDL 3c, 7.94–8.45 nm; HDL 3b, 8.45–8.98 nm; HDL 3a, 8.98–9.94 nm; HDL 2a, 9.94–10.58 nm, and HDL 2b, 10.58–13.59 nm [25]. The method was linear from 5 to 30 μg of HDL protein. The intra- and interassay variability coefficients were 6.1 and 5.9%, respectively, determined by 10 replicates of the same sample.

Cholesterol HDL subclasses were determined by a similar procedure used for SM as previously described [25].

2.6. Statistical analysis

Central tendency and dispersion measurements were estimated by conventional methods. Normal distribution of the variables was evaluated by the Kolmogorov–Smirnoff test. The significance of the differences among parameters between men and women was tested by the Student's t-test for normally distributed variables. Comparisons of non-normally distributed variables were either performed by Wilcoxon test for paired variables, or logarithmically transformed for parametric statistical analysis. Partial correlations, adjusted by age and gender, were performed and statistical significance was set a $P < 0.05$. Unless otherwise indicated, values are expressed as mean ± SD for variables with normal distribution and as median and interquartile interval for non-normally distributed variables. Statistical analysis was performed using SPSS V11 software.

3. Results

Forty females and 10 males were enrolled in the study. Their anthropometric and biochemical data are presented in Table 1. As expected, weight loss was associated with the amelioration of the MS components; namely, waist circumference, blood pressure, triglycerides, glucose, as well as HOMA-IR diminished. Either in women and men, HDL-cholesterol remained unchanged after weight loss (39.2 ± 10.1 and 33.9 ± 10.9 vs. 39.5 ± 10.7 vs. 36.9 ± 7.4, respectively, $P > 0.05$). In contrast, both total plasma SM and HDL-SM concentrations significantly decreased about 17 and 29%, respectively.