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Effect of obesity and metabolic syndrome on plasma oxysterols and fatty acids in human

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ABSTRACT

Background: Obesity and the related entity metabolic syndrome are characterized by altered lipid metabolism and associated with increased morbidity risk for cardiovascular disease and cancer. Oxysterols belong to a large family of cholesterol-derived molecules known to play crucial role in many signaling pathways underlying several diseases. Little is known on the potential effect of obesity and metabolic syndrome on oxysterols in human.
Objectives: In this work, we questioned whether circulating oxysterols might be significantly altered in obese patients and in patients with metabolic syndrome. We also tested the potential correlation between circulating oxysterols and fatty acids.
Methods: 60 obese patients and 75 patients with metabolic syndrome were enrolled in the study along with 210 age- and sex-matched healthy subjects, used as control group. Plasma oxysterols were analyzed by isotope dilution GC/MS, and plasma fatty acids profiling was assessed by gas chromatography coupled with flame ionization detection.
Results: We found considerable differences in oxysterols profiling in the two disease groups that were gender-related. Compared to controls, males showed significant differences only in 4α- and 4β-hydroxycholesterol levels in obese and metabolic syndrome patients. In contrast, females showed consistent differences in 7-oxocholesterol, 4α-hydroxycholesterol, 25-hydroxycholesterol and triol. Concerning fatty acids, we found minor differences in the levels of these variables in males of the three groups. Significant changes were observed in plasma fatty acid profile of female patients with obesity or metabolic syndrome. We found significant correlations between various oxysterols and fatty acids. In particular, 4β-hydroxycholesterol, which is reduced in obesity and metabolic syndrome, correlated with a number of saturated and mono-unsaturated fatty acids that are end-products of *de novo* lipogenesis.
Conclusions: Our data provide the first evidence that obesity and metabolic syndrome are associated with major, gender-specific, changes in circulating oxysterols and fatty acids. These findings suggest a metabolic link between oxysterols and fatty acids, and that oxysterols may contribute to the epidemic diseases associated with obesity and metabolic syndrome in female.

1. Introduction

Obesity is a medical condition characterized by excessive accumulation of fat in the body and is better defined as a body mass index of 30 and above. It is a major epidemic and a public health concern worldwide [1]. Some obese patients may develop important complications, including the metabolic syndrome, that favor premature death [2]. Metabolic syndrome is the most studied complication in obesity and is diagnosed by the co-occurrence of three out of five of the following conditions: abdominal (central) obesity, high blood pressure, high fasting plasma glucose, high serum triglycerides, and low high-density cholesterol (HDL) levels [3]. Additional complications such as liver disorders (non alcoholic fatty liver diseases) [4], inflammatory bowel diseases [5], reproductive disorders including infertility [6], autoimmune diseases [7] and many cancers [8] may also develop in obesity. Interestingly, preclinical and clinical studies have provided

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evidence for the part played by lipids in the etiology of diseases associated with obesity.

Oxysterols are a complex family of oxidized species of cholesterol [9]. They are powerful bioactive lipids able to influence an array of biological processes [10] and may be involved in the pathologies associated with obesity. There are several mechanisms by which oxysterols influence cell signaling and physiology. Oxysterols are potent regulators of cholesterogenic pathways [11] and they act as ligands for receptors such as the Liver X Receptors (LXRs) [12], which are nuclear receptors that act as oxysterol sensors and regulate gene expression [13]. They are highly expressed in the liver where they regulate the expression of genes involved in cholesterol and fatty acid metabolism [14]. More recently, it has also been shown that 27-hydroxycholesterol act as selective estrogen receptor modulators (SERM) [15] and influence the cardiovascular system, bone biology and cancer [16].

There are little data on the relationship between oxysterol and metabolic diseases in human. In this work we investigated whether obesity and metabolic syndrome might have a significant impact on serum oxysterol profile. Because cholesterol metabolism shows important gender specificity and oxysterols act as SERMs we questioned this both in males and in females. In addition, various independent works provided evidence that not only fatty acid metabolism is regulated by oxysterol receptors (LXRs) [14,17] but also that fatty acids influence LXR activity [18,19]. Therefore, we tested whether changes in oxysterol concentrations may be correlated to changes in circulating fatty acids.

2. Methods

2.1. Study population and design

To investigate the characteristics of plasma FA and oxysterols pattern in patients with obesity and metabolic syndrome we consecutively recruited 142 subjects at Sapienza University outpatient centers for Obesity and Vascular Medicine (obesity patients, $n = 67$; MS, $n = 75$) between January 2009 and July 2011. Inclusion criteria for obese patients were based on BMI > 30 in the absence of dyslipidemia, diabetes or hypertension. Diagnosis of metabolic syndrome was based on the ATPIII criteria [3]. Exclusion criteria included, previous angina, myocardial infarction, TIA or stroke, familial dyslipidemias, thyroid disease, kidney disease, systemic inflammatory diseases or cancer.

Healthy controls ($n = 210$), matched for age and sex, were recruited in the same geographical area, after carefully reviewing their medical history, among blood donors referring to the AVIS center of Latina. Healthy subject definition was based on the absence of any known clinical or anamnestic disease, and the absence of cardiovascular risk factors apart from smoking.

The study procedure was developed according to the guidelines of the Ethic Committee, which approved the protocol, and the Helsinki Declaration of 1975. All subjects signed written informed consent.

2.2. Plasma biochemistry

Serum glucose, bilirubin, uric acid, alanine transaminase, cholesterol, triglycerides, HDL-and cholesterol were determined by standard automated techniques as previously described [20]. Laboratory analyses were done in a blinded fashion. The clinical picture of the patients referred to in this study is described in Table 1.

2.3. Lipid biochemistry

To measure plasma FAs, peripheral venous blood was obtained after overnight fasting and was centrifuged at 4 °C. Plasma was then removed and stored at −80 °C until assay. Before analysis FAs were processed for direct transesterification with acetyl chloride according to previously published method [21]. Analyses were performed on an Agilent 7820A Plus Gas Chromatograph (Agilent Technologies; Palo Alto, CA) equipped with a G4513A automatic liquid sampler and a flame ionization detector. Separation was carried out on a 100 m capillary column (Supelco, SP-2560 100 m × 0.25 mm ID, 0.20 μm thickness) (Sigma Aldrich, Milan, Italy). Identification, precision, and accuracy were evaluated using mixtures of authentic methylated FA standards, and a control plasma pool as previously described [22,23]. The list of FAs species that were analysed is reported in Supplementary Table 1.

Oxysterols were determined by GC–MS using deuterium-labelled internal standards, as previously described [24]. Prior to analysis the isolated oxysterol fraction was submitted to base hydrolysis, hence the concentrations measured are for total oxysterols comprising the sum of esterified and non-esterified molecules. 5α-OH,6-oxocholesterol, d6-5α-OH,6-oxocholesterol, cholestane, triol and d6-triol were synthesized and kindly donated by Dr. Marc Poirot (Institut Claudius Régaud, Toulouse, France). 4α- and 4βOH-Cholesterol were kindly donated by Dr. G. Lizard (Faculté des Sciences Gabriel, Dijon, France). All other oxysterols and deuterated oxysterols were from Avanti Polar Lipids. The list of oxysterols measured here is reported in Supplementary Table 2.

2.4. Statistical analysis

Kruskal–Wallis test was used to compare oxysterol and fatty acid concentrations between the three health status groups (healthy, obesity, metabolic syndrome). This is a non-parametric univariate test that treats each variable independently. Multiple testing corrections (adjusted p-values), the false discovery rate, were applied to avoid false positive [25].

A heatmap was used to represent relationships between fatty acids and oxysterols. This is a graphical representation of Kendall correlation coefficients using darker colors to indicate high correlations, and brighter colors to indicate low correlations. Statistical analysis was performed using R software (http://www.r-project.org/).

3. Results

3.1. Clinical characteristics of the study population

Based on the ATPIII criteria, participants were classified as healthy controls, metabolic syndrome or obesity patients. We used these groups to investigate the respective influence of obesity and metabolic syndrome on plasma oxysterols and fatty acids. Demographic and clinical characteristics of the study population are shown in Table 1. Oxysterol profile showed significant differences in the three groups (Supplementary Table 3). However, analysis performed independently in patients from each gender revealed that these changes were markedly gender-dependent.

3.2. Influence of obesity and metabolic syndrome on plasma oxysterol concentration in males

Interestingly, we found that obesity alone has little impact on plasma oxysterols concentration when compared to healthy controls (Table 2). Only 4β-hydroxycholesterol and 4α-hydroxycholesterol were significantly different in males. Both 4β-hydroxycholesterol and 4α-hydroxycholesterol were lower in obese patients compared to healthy subjects. In patients with metabolic syndrome the level of 4β-hydroxycholesterol was lower and the level of and 4α-hydroxycholesterol was higher compared to healthy

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