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Lipid metabolism after an oral fat test meal is affected by age-associated features of metabolic syndrome, but not by age

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ABSTRACT

Objective: Postprandial lipemia influences the development of atherosclerosis. Age has been defined as a regulating factor of the extent of postprandial lipemia, but its independence of other age-associated phenotypic features, such as metabolic syndrome, has not been fully elucidated.
Methods: To investigate if age is an independent factor influencing postprandial lipemia, we compared the lipemic response to a rich fatty meal (60% fat) of 88 healthy young men (<30 years old) and 97 older participants (77 metabolic syndrome patients aged > 40; and 20 healthy people > 65) (all ApoE3/E3), at fasting state and at 2nd and 4th postprandial hours.
Results: We didn't find differences between the healthy young men and the healthy elderly. The metabolic syndrome patients displayed a higher postprandial TG area below the curve than the other two cohorts $p < 0.001$. ANOVA for repeated measurements confirmed that these differences were significant at every time-point (fasting, 2 h and 4 h). Concomitant higher responses for Large and Small TRL-carried TG and Chol were found in these metabolic syndrome patients. Interestingly, the most significant differences were found for Small-TRL-carried particles, which suggest that this fact may be mainly due to impaired lipid clearance.
Conclusion: Metabolic syndrome may account for the differences in postprandial lipemia that have been attributed to age. In our study, there were no significant differences in postprandial lipemia between a young population (mean age 22.6 years) and a healthy people >65 years one (67.2 years) without metabolic syndrome.

Postprandial lipemia following a fat-rich meal is a situation characterized by the generation of an atherogenic environment in the bloodstream, derived by the conjunction of the direct atherogenic properties of some lipid particles, especially those carried in the triglyceride-rich lipoproteins, and by the activation of the inflammatory and hemostatic systems [1,2]. These last features may be influenced by the amount and type of fat in the meal [1,3–5], and are partly mediated by mononuclear cells, which respond directly to the increase in triglyceride remnants in the blood, to the apparition in the blood of proteins of bacterial origin (like LPS) and to stimuli secreted from intestinal endothelial cells (via TLR4 receptors) [6–8]. Furthermore, endothelial vasodilatory capacity is transiently impaired after a high-fat meal, a fact linked to the nitric oxide synthase pathway [9]. All these features turn the postprandial state into an atherogenic environment, and the extent of this period has been related to increased atherosclerosis [10].

Dietary background has been identified as the main extrinsic factor influencing postprandial lipemia [11,12], while genetics, sex,

Abbreviations: ApoB, apolipoprotein B; Chol, cholesterol; HDL-C, high density lipoproteins cholesterol; MetS, metabolic syndrome; SFA, saturated fatty acids; SNP, single nucleotide polymorphism; T2DM, type 2 diabetes mellitus; TG, triglycerides; TRL, triglyceride-rich lipoproteins.

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and age have been identified as potent intrinsic modifiers of postprandial lipemia [11,13,14]. For example, ApoE genotyping is a clearly established modifier of triglyceride levels, and the ApoE3/E3 genotype (the commonest) identifies those persons with an average response to diet and lipid overload [15–19]. However, although the influence of sex and genetics is easier to isolate from other interfering factors, age is often associated to related conditions, such as the appearance of metabolic syndrome. In developed countries, up to 35% of the general population suffers from metabolic syndrome [20]. The exact contribution of the presence of metabolic syndrome to age-associated enlarged postprandial lipemia has not been explored much.

1. Subjects and methods

1.1. Subjects

We evaluated one hundred and eighty-five persons who participated in three different studies performed in our unit: Eighty-eight healthy young men (<30 years old), and ninety-seven persons ranging from 40 to 70 years old, and who, in turn, came from two studies: 77 participants with metabolic syndrome from the LIPGENE study, and 20 healthy participants from the coenzyme Q and age study. Detailed information of these three studies has been published elsewhere [12,21–24]. The metabolic syndrome in the LIPGENE study was determined using a modified version of the NCEP criteria for MetS [25], where subjects were required to fulfill at least three of the following five criteria: waist circumference > 102 cm (men) or >88 cm (women); fasting glucose 5.5–7.0 mmol/L; TAG ≥ 1.5 mmol/L; HDL cholesterol < 1.0 mmol/L (men) or < 1.3 mmol/L (women); blood pressure ≥ 130/85 mmHg or treatment of previously diagnosed hypertension. Notably, all participants were previously genotyped for the ApoE genotype, and only ApoE3/E3 subjects were selected. None of the 88 healthy young males had any criteria of metabolic syndrome. All participants provided their written informed consent before enrolling for the study, according to the Declaration of Helsinki II. The study was approved by the local committee for scientific ethics.

1.2. Protocol

More detailed protocols have been published previously [12,21–24]. To sum up, participants fasted for 12 h, and then received a fatty meal, containing 1 g fat and 7 mg cholesterol per kg of body weight in the case of the healthy young men, and 0.7 g fat and 5 mg cholesterol per kg of body weight in the case of the other two cohorts. The meals contained 60–65% of energy as fat, 10–15% of energy as protein, and 25% of energy as carbohydrates, and were consumed in 20 min. We measured the lipid particles at the fasting state, and via blood drawn performed at different time-points in the postprandial state: with the young cohort, we assessed these fractions every hour until the sixth, and then every 2.5 h until 11th. With the metabolic syndrome patients, we measured them twice-hourly for 8 h, while with the cohort of persons above 65 years, for ethical reasons, we performed a short lipemia study (twice-hourly until the 4th hour). In the present study, only the common time-points (fasting, 2nd and 4th postprandial hour measurements) of all cohorts are considered.

1.3. Blood test

Extensive laboratory methodology has been published for the three populations elsewhere [22,24,26]. Blood drawn and TRL (Large and Small) isolation were performed by standard methodology, as published previously [24]. In short, plasma was separated from red cells by centrifugation at 1500× *g* for 15 min at 4 °C. The chylomicron fraction of TRL (Large-TRL) was isolated from 4 mL of plasma overlayered with 0.15 mol/L NaCl, 1 mmol/L EDTA (pH 7.4, $d < 1.006$ kg/L) by a single ultracentrifugal spin (36,200 *g*, 30 min, 4 °C) in a 50-type rotor (Beckman Instruments, Fullerton, CA). Large-TRL, contained in the top layer, was removed by aspiration after cutting the tubes, and the infranatant was centrifuged at a density of 1.019 kg/L for 24 h at 183,000 *g* in the same rotor. The non-chylomicron fraction of TRL (also referred to as Small-TRL) was removed from the top of the tube. All operations were done in subdued light. Large and Small TRL fractions were stored at −70 °C until biochemical determinations were performed. Total cholesterol (Chol) and triglycerides (TG) in plasma and lipoprotein fractions were assayed by enzymatic procedures. APOA1 and APOB were determined by turbidimetry [27]. HDL-C was measured by the dextran sulfate-Mg^{2+} method, as described in Ref. [28]. LDL-C levels were estimated using the Friedewald formula [29].

1.4. Statistics

In the baseline fasting comparisons, we used univariate ANOVA. We tested the size of the postprandial lipid fractions by univariate ANOVA for the area below the curve (AUC), defined as the area within the plasma concentration-versus-time curve, using the trapezoidal rule and treating the cohort as independent variable, with each of the lipid fractions as dependent variables. Then, to estimate if the influence was regular throughout the lipemia, we performed repeated ANOVA measurements, testing all the time-points (fasting, 2nd hour and 4th hour) in the Post-Hoc analysis, using Bonferroni's corrections. Additionally, we tested if the postprandial findings were influenced by fasting values by relativizing each tested variable to its fasting figures. For any given variable, fasting value was set to 1, and the relativized values for the 2nd and 4th hours were calculated as the quotient of the real value of that given variable in the studied time-point by that of the fasting value.

To infer if the short lipemia (4 h) was suitable to represent the whole postprandial lipemia, we used our existing data with the complete set of extracted blood for the young men's cohort (hourly after the meal for 6 h, then every 2.5 h until 11th) and the metabolic syndrome cohort (twice-hourly for 8 h), and we performed the correlation tests to obtain the R^2 index and the equation for the linear regression function.

A *p*-value of less than 0.05 was considered significant. All the data presented in the text and tables are expressed as mean ± SE unless otherwise specified. SPSS 19.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical comparisons.

2. Results

Baseline characteristics of the groups are described in Table 1. Young men differed from the metabolic syndrome and the healthy >65 years old cohorts in BMI, total cholesterol, and LDL cholesterol (all $p < 0.001$, Table 1). Metabolic syndrome patients exhibited higher baseline TG levels, while healthy >65 years patients showed higher HDL concentrations than the other two groups (Table 1).

The metabolic syndrome cohort showed a higher postprandial TG response than the other two groups ($p < 0.001$), both in AUC (Fig. 1) and in repeated ANOVA measurements, with differences found at the baseline and at every time-point blood drawn (Fig. 2).

When evaluating the different postprandial lipid fractions, we found differences for metabolic syndrome patients versus the other two groups for Large-TRL-TG ($p < 0.05$), Small-TRL-Chol ($p < 0.001$) and Small-TRL-TG ($p < 0.001$), both in AUC (Fig. 1) and in the repeated ANOVA measurements (Fig. 3). When evaluating the differences in the different time-points, we found higher Large-TRL-TG in metabolic syndrome patients than in the other two