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Relationship between postprandial lipemia and atherogenic factors in healthy subjects by considering gender differences

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ABSTRACT

Background: Postprandial triglyceride concentrations are clinically significant and independent predictor of cardiovascular disease risk. The purpose of this study was to determine postprandial TG ranges in healthy subjects by considering gender differences. Secondly, assess the relationship between postprandial lipemia and atherogenic indicators. Finally, investigate the use of the postprandial 4h TG test instead of the area under the curve (AUC).

Methods: Postprandial lipemia was investigated using the standardized oral fat tolerance test (OFTT) in 96 healthy subjects (45 female/51 male). Study group was categorized into tertiles based on AUC calculated using TG concentrations at fasting and 2, 4 and 6h after OFTT. Lipid, lipoproteins, apolipoproteins, LDL subfractions and oxidized LDL (oxLDL) were evaluated in tertiles in both sex groups.

Results: The cut-off concentrations for postprandial 4-hour TG concentrations in female and male were 3.20 mmol/L and 4.59 mmol/L, respectively. We observed higher concentrations for atherogenic indicates like small dense-low density lipoprotein (sdLDL), oxLDL values in top tertiles for both groups ($P < 0.05$). Cohen's kappa coefficients for the agreement of AUC and 4h postprandial TG tests were 0.935, 0.970, 0.469 ($P = 0.0001$) in female, male and total study group, respectively.

Conclusion: Due to predominant effects of gender differences on postprandial lipemia, postprandial TG cut-off values for female and male subjects should be determined separately. Postprandial lipemia may be associated with atherogenic tendency by changing lipids, lipoproteins, sdLDL and oxLDL concentrations, especially in males. Four-hour postprandial TG concentrations emerged as a useful and reliable marker for evaluation of postprandial lipemia.

1. Introduction

Epidemiological and case-control studies in the last decade have demonstrated that non-fasting and postprandial (TG) concentrations are clinically significant and independent predictors of the cardiovascular disease (CVD) risk [1]. Since humans spend most of their time in a postprandial state, rather than fasting, it has been suggested that postprandial plasma lipid and lipoprotein concentrations are more meaningful and important than fasting values in evaluating coronary risk [2]. Several aspects related to non-fasting and postprandial TG and their role as risk factors for CVD were discussed by scientists and clinicians in February 2010 [3]. Among several possible factors, the increased highly atherogenic triglyceride (TG)-rich remnant lipoprotein particles associated with low high-density lipoprotein cholesterol (HDL-C) concentrations in plasma have been proposed as the most important attribute for the atherogenicity of postprandial lipemia [4]. Moreover, it has been suggested that the co-presence of increased more atherogenic small dense low-density lipoprotein (sdLDL) and decreased HDL-C is associated with an augmented atherogenic tendency in lipid and lipoprotein profiles [5].

Various lipoprotein ratios or atherogenic indices including total cholesterol (TC)/HDL-C, LDL-C/HDL-C and apo B/apo AI have also been described. It has been suggested that these risk indicators have a greater predictive value than isolated parameters used independently, particularly LDL-C [6]. Moreover, the TG/HDL-C ratio has been proposed as a good discriminator for CVD risk prediction [7]. This ratio has

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been shown to reflect atherogenic sdLDL particles and to be associated with insulin resistance and metabolic syndrome [8,9]. Our present knowledge of the postprandial changes in lipoproteins and their subclasses is relatively poor. Few previous studies have investigated postprandial changes in the above-mentioned atherogenic parameters [10].

The methodology for evaluating postprandial TGs using the oral fat tolerance test (OFTT) and a desirable TG concentration (≤ 2.5 mmol/L) for any time during the postprandial period was defined by an expert panel [3]. Only a few studies have investigated postprandial TG ranges in healthy subjects in the light of gender differences because of the lack of OFTT standardization [11].

The purpose of this study was i) to define postprandial TG ranges in healthy subjects by considering gender differences, ii) to evaluate the relationship between postprandial lipemia and atherogenic indices including lipids, lipoproteins, sdLDL and ox-LDL concentrations, and iii) to assess the use of the 4 h postprandial TG test, as suggested by the expert panel group, instead of the area under the curve (AUC) calculated using TG concentrations at fasting, and 2, 4 and 6 h after the OFTT.

2. Materials and methods

2.1. Subjects

Ninety-six healthy volunteers (51 male and 45 female) aged between 19 and 54 years and with different socio-economic levels were randomly enrolled in this study. All participants were recruited through e-mail and personal contact among students and staff and their acquaintances at Karadeniz Technical University (KTU), Turkey. Subjects' health status was evaluated by means of detailed medical history, physical examinations and laboratory tests of blood samples (complete blood cell count (CBC), lipids, lipoproteins and thyroid function tests including TSH, free-T4, liver and kidney function tests) at the KTU Medical Faculty. Exclusion criteria included smoking status, alcohol or drug abuse, presence of acute and chronic inflammatory diseases, chronic kidney disease, obesity and endocrine disorders related to lipid and lipoprotein metabolism, such as diabetes mellitus, menopause, estrogen replacement therapy and thyroid hormone disorders. Subjects performing heavy exercise and those on herbal medicines were also excluded. Body weights (kg), body fat percentages, body mass index (BMI) were obtained using impedance scales (Tanita Body Composition Analyzer, TBF-300, Illinois, USA). BMI was calculated using the formula weight/height^2 (kg/m^2). The waist to hip ratio (WHR) was calculated by measuring the circumferences at the waist (midway between the rib cage and iliac crest) and hip (maximal circumference between the iliac crest and thigh region). Since the use of the waist to-height ratio (WHtR) has been shown to be a better discriminator of coronary heart disease and cardiovascular risk factors than waist circumference and BMI [12], the WHtR was also evaluated in the present study. All subjects gave written consent to participate in the study, which was approved by the KTU Medical Ethics Committee.

2.2. Oral fat tolerance test

Participants consumed routine daily meals prior to the OFTT. They were instructed to avoid alcohol intake 24 h before the test. After 12 h overnight fasting, blood samples were collected at 8:00 am. Each participant was then given an OFTT meal containing a total 75 g fat in line with the expert panel suggestion [3]. The test meal consisted of toast bread, cheese and butter served as a toasted sandwich. In order to increase the digestibility and tolerability of the meal, 200 mL liquid 'ayran', a traditional homogenous drink made from yoghurt and water, was also provided. All test foods and drink were provided in the form of commercial products with specific ingredients approved by the Ministry of Food Agriculture and Livestock of the Republic of Turkey. The OFTT meal closely matched the expert panel recommendations of 62.5% fat, 24.1% carbohydrate and 13.4% protein. The test meal was consumed within 20 min, after which all subjects were instructed not to consume anything orally for the following 6 h, except for water. The test meal was well tolerated by all subjects. The OFTT was conducted at the KTU Medical Faculty Medical Biochemistry Department, where participants spent the entire test and were not allowed bed-rest or heavy exercise.

2.3. Biochemical analyses

Blood samples were drawn into the tubes without any anticoagulant for serum acquisition, as well as EDTA-anticoagulant tubes for plasma, by venipuncture before the test and every 2 h there after over a 6 h period. After centrifugation at 1800*g* for 10 min (Eppendorf 5804, Hamburg, Germany), serum and plasma samples were obtained and stored at −80 °C for 2 months until analysis. In order to reduce inter-assay measurement errors, samples were processed using the same test kit batch.

Concentrations of glucose, TC, TG, HDL-C and LDL-C were measured in serum samples using enzymatic methods. These assays were performed on an AU 5800 autoanalyzer (Beckman Coulter, Shizuoka, Japan) and using its original reagents. Quantitative determination of insulin was performed using an IMMULITE 2000 XPi analyzer with its original reagents (Siemens, Munich, Germany). Apolipoprotein AI and B were assessed on the immunonephelometry BN II system (Siemens, Munich, Germany). These parameters were run after daily quality control procedures at the Clinical Biochemistry Laboratory as approved by the Ministry of Health at the KTU Medical Faculty. Insulin resistance was determined using the homeostasis model assessment method-insulin resistance (HOMA-IR) method and calculated with the appropriate formula [13]. AUC values were calculated from TG concentrations obtained at the baseline fasting period and at 2, 4, and 6 h after the OFTT using the trapezoidal rule [14].

LDL subfractions were measured in serum samples using a Lipoprint System (Quantimetrix Inc., Redondo Beach, CA, USA). This system separates lipoproteins in a non-denaturing gel gradient of polyacrylamide on the basis of net surface charge and size. The dye binds proportionally to the relative amount of cholesterol in each lipoprotein. After electrophoresis, densitometric analyses and proportional concentrations of lipoprotein classes and subclasses were calculated on Lipoware software. Very low-density lipoprotein (VLDL) and intermediate density lipoprotein (IDL) fractions were also determined in this system. In addition to providing seven LDL subfractions (LDL-1-7), we divided LDL particles into "large" and "small" species as previously described by Vega et al. [15]. Reagents supplied by the manufacturer were used for each analysis. Oxidized LDL (ox-LDL) was measured in plasma samples using the ELISA method (Mercodia, Uppsala, Sweden).

Cut-off values were calculated for postprandial 4 h TG concentrations for females and males. The mean values plus 1.96 standard deviation (SD) were used to obtain cut-off value for the sexes.

2.4. Statistical analysis

Data were expressed as mean ± SD for normally distributed and as median (interquartile range) values for non-normally distributed variables. The distribution of variables was assessed using Kolmogorov-Smirnov test. Comparison of two groups was performed using Student's *t*-test or Mann–Whitney *U* test. Each sex group was categorized into three equal subgroups based on tertiles of AUC values. Participants with low AUC values were enrolled in Group 1, those with intermediate values in Group 2 and those with high values in Group 3. One-way analysis of variance (ANOVA) and Kruskal-Wallis test were used to compare parameters among tertiles. Tukey's and Tamhane's T2 tests were performed for post-hoc comparisons. Pearson or Spearman correlation analysis was used to assess the relationships between postprandial lipemia and atherogenic indices including lipids, lipoproteins, sdLDL and oxLDL in the light of the skewness of data distribution.