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Clinica Chimica Acta 366 (2006) 309 – 315

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Postprandial lipemic response and lipoprotein composition in subjects with low or high cholesterol absorption efficiency

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Received 31 August 2005; received in revised form 9 November 2005; accepted 9 November 2005
Available online 20 December 2005

Abstract

Backgroud: The purpose of this study was to investigate the effect of differences in cholesterol absorption efficiency on the postprandial lipemia and lipoprotein composition.
Methods: Fifteen healthy subjects were divided into low and high cholesterol absorbers on the basis of serum cholestanol to cholesterol ratio. A high-performance liquid chromatographic method with evaporative light scattering detection was developed for quantitation of free and esterified cholesterol, triglycerides and major phospholipids from the same lipid extract in two runs utilizing the same internal standard.
Results: The free cholesterol to phosphatidylcholine ratio of chylomicrons was higher in the high cholesterol absorption group. The total increase of cholesterol in combined chylomicron and very low density lipoprotein (VLDL) fraction was also higher in this group. Chylomicron free cholesterol and cholesterol ester responses correlated with fasting low density lipoprotein (LDL) cholesterol. VLDL and VLDL1 triglyceride responses correlated inversely with fasting insulin and homeostasis model assessment of insulin resistance.
Conclusions: High cholesterol absorption efficiency was seen in chylomicrons as higher cholesterol to phosphatidylcholine ratio during the postprandial peak. Chylomicron cholesterol response was linked to fasting LDL cholesterol and low VLDL triglyceride response to fasting insulin.
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Keywords: Cholesterol absorption; Chylomicron; Insulin; Phospholipid; Postprandial lipemia; Very low density lipoprotein

1. Introduction

Compared to other dietary lipids the mechanism and regulation of cholesterol absorption is much more complex [1,2]. The cholesterol absorption efficiency has been reported to vary from 30% to 80% [3]. In addition, the ingested cholesterol is secreted from intestine during several subsequent postprandial periods [2]. There is evidence that cholesterol absorption efficiency and cholesterol synthesis are both genetically determined [4] and regulated by insulin [5–7], and they are inversely related to each other [8]. It is not clear, however, what is the hierarchy of absorption and synthesis in the regulation of cholesterol metabolism in different conditions.

The possible effect of the differences in the amount of absorbed cholesterol on the composition of chylomicrons is not known. In addition, the low cholesterol absorption efficiency has been associated with high fasting insulin and triglyceride levels [9]. These both have been related also to postprandial lipoprotein metabolism [10–12]. Therefore, we wanted to investigate the effect of cholesterol absorption efficiency on the composition of postprandial lipoprotein particles as well as on the magnitude of postprandial response. Most postprandial studies have concentrated on triglycerides and cholesterol and phospholipid levels have

Abbreviations: HDL, high density lipoprotein; HPLC, high-performance liquid chromatography; HOMA-IR, The homeostasis model assessment index of insulin resistance; LDL, low density lipoprotein; VLDL, very low density lipoprotein.

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0009-8981/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.cca.2005.11.006

been less frequently measured [13]. Furthermore, commonly used enzymatic methods may not be very accurate when concentrations of lipids are small in chylomicrons or very low density lipoprotein (VLDL). In the present study the postprandial responses of free and esterified cholesterol and triglycerides as well as major phospholipids were determined in subjects with low or high cholesterol absorption efficiency utilizing high-performance liquid chromatography (HPLC) and evaporative light scattering detection.

2. Subjects and methods

2.1. Subjects

Altogether 15 normo- or hypercholesterolemic men aged 29–71 years were recruited to the oral fat load test from the former study carried out at the Department of Clinical Nutrition, University of Kuopio [9]. None of the subjects had liver, kidney, thyroid, coronary artery disease, diabetes mellitus or gastrointestinal diseases. One subject used calcium channel blockers, two used beta-blocking agents, and two used ACE inhibitors for hypertension. None of the subjects had lipid-lowering therapy. Two were smokers. The subjects were requested to maintain their medication, weight, diet, possible alcohol consumption, smoking habits and physical activity constant before the study. One subject was excluded from the statistical analysis due to exceptionally high postprandial triglyceride response (area under the response curve differed over 4 standard deviations from the mean of other subjects).

The subjects were divided into low and high cholesterol absorption efficiency groups according to baseline serum cholestanol to cholesterol ratio [14]. Those with cholestanol level higher than median (131 $10^2 \times$ mmol/mol of cholesterol) formed the high absorber group and the rest the low absorber group.

The subjects gave their informed consent for the study, and the study protocol was approved by the Ethics Committee of the University of Kuopio.

2.2. Oral fat-loading test

The oral fat-loading test started at 7.30–8.00 am after a 12 h fast. Subjects were advised not to drink alcohol and to avoid strenuous exercise for 3 days before the test. After collecting fasting blood samples, subjects consumed a drink containing a mixture of milk and vegetable fat-based creams, fish oil (9.3 g/m^2 body surface), yolk (150 mg/m^2 body surface), and squalene (250 mg/m^2 body surface). The drink contained a small amount (<0.2 g) of deuterium labeled stearic acid in triacylglycerol form. The cream mixture contained in ratio of 1:1 cream (38% fat) (Valio, Finland) and vegetable fat-based cream (20% fat) (Unilever Finland Oy). The amount of total fat load was 35 g/m^2 body surface (mean 68 g) and that of cholesterol 234 mg/m^2 body surface (mean 456 mg). Postprandial blood samples were collected at 15, 30, 45 and 60 min, and at 2, 3, 4, 6 and 8 h after the test meal.

2.3. Laboratory measurements

To separate chylomicrons plasma (1.8 ml) was overlaid with 1.6 ml of NaCl solution (d=1.006 g/ml) and ultracentrifuged with 50.4 Ti rotor (Beckman Instruments, Palo Alto, CA) at 18,000 rpm for 30 min [15]. The top milliliter was aspirated to remove chylomicrons. The infranate was overlaid again with 0.9 ml of NaCl solution (d=1.006 g/ml) and samples were ultracentrifuged to separate VLDL and combined low density lipoprotein (LDL) and high density lipoprotein (HDL) fractions (37,000 rpm, 15 h). A part of this combined fraction was taken to analyze HDL cholesterol concentrations after precipitation of LDL with dextran sulfate and $MgCl_2$. For the separation of S_f 60–400 fraction (VLDL1) plasma (1.0 ml) was overlaid with 2.4 ml of NaCl solution (d=1.006 g/ml) and ultracentrifuged at 18,000 rpm for 30 min. The top milliliter was aspirated to remove chylomicrons. The infranate was overlaid again with 1.0 ml of the same NaCl solution as earlier. The samples were ultracentrifuged at 35,000 rpm for 2 h 20 min and top 0.7 ml was aspirated to collect VLDL1 fraction. Isolated fractions were stored at −70 °C until analyzed.

Cholesterol and triglyceride concentrations in plasma or serum and separated lipoprotein fractions were determined with enzymatic photometric methods using commercial kits (Monotest® Cholesterol and Triglyceride GPO-PAP, Boehringer Mannheim GmbH Diagnostica, Mannheim, Germany). Plasma glucose was measured with the dehydrogenase method (Granutest 500, Merck, Darmstadt, Germany), and serum insulin with the RIA method (Phadeseph Insulin RIA, Pharmacia, Uppsala, Sweden). Serum cholestanol was quantitated by gas–liquid chromatography as previously described [16]. The homeostasis model assessment index of insulin resistance (HOMA-IR) was calculated as insulin (μU/ml) × fasting glucose (mmol/l) / 22.5 [17].

HPLC was used to separate lipid classes. Lipids of lipoprotein fractions were extracted with chloroform–methanol (2:1) [18]. Monocaprylin (30–80 μg) was added to samples to serve as internal standard. Two separate runs were made from each extract to separate neutral lipid and phospholipid classes. For the separation of neutral lipids normal-phase silica column was used (Luna, 250 × 4.6 mm i.d., 5 μm particle size, 100 Å pore size; Phenomenex, Torrance, CA). A guard column was used to prevent the accumulation of phospholipids to the column. The multistep gradient system is shown in Table 1. The complete separation of triglycerides, cholesterol esters, cholesterol, diglycerides and monoglycerides was obtained. In addition, monocaprylin was separated from monoglycerides with long-chain fatty acid. Normal-phase silica column was used also for the separation of phospholipids (Phenosphere,

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