Atherosclerosis 222 (2012) 222–228

Contents lists available at SciVerse ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Postprandial accumulation of chylomicrons and chylomicron remnants is determined by the clearance capacity

Martin Adiels[a,b,*,1], Niina Matikainen[c,1], Jukka Westerbacka[c], Sanni Söderlund[c], Thomas Larsson[a], Sven-Olof Olofsson[a], Jan Borén[a], Marja-Riitta Taskinen[c]

[a] Sahlgrenska Center for Cardiovascular and Metabolic Research/Wallenberg Laboratory, Department of Molecular and Clinical Medicine, University of Gothenburg, Sweden
[b] Department of Mathematical Sciences, University of Gothenburg, Sweden
[c] Department of Medicine, University of Helsinki, Finland

ARTICLE INFO

Article history:
Received 3 July 2011
Received in revised form 31 January 2012
Accepted 1 February 2012
Available online 7 February 2012

Keywords:
Insulin resistance
Kinetics
Postprandial lipemia
Stable isotopes
Triglyceride-rich lipoproteins
VLDL

ABSTRACT

Objective: To better understand the postprandial clearance of triglyceride-rich lipoproteins (TRLs) and its relation to the fasting kinetics of TRLs.
Methods: Two studies were performed on 30 male subjects: a fasting kinetic study to determine the fasting secretion and clearance rates of apolipoprotein B (apoB) 100 and triglycerides in the very low-density lipoprotein 1 and 2 ($VLDL_1$ and $VLDL_2$) fractions; and a postprandial study to determine the postprandial accumulation of apoB48, apoB100 and triglycerides in the chylomicron, $VLDL_1$ and $VLDL_2$ fractions. Results from these two studies were combined to characterize the postprandial clearance of TRLs in a physiologically relevant setting.
Results: Our results show that postprandial accumulation of the apoB48-carrying chylomicrons can be predicted from the clearance capacity of the lipolytic pathway, determined in the fasting state. Furthermore, we show that chylomicrons and $VLDL_1$ particles are not cleared equally by the lipoprotein lipase pathway, and that chylomicrons seem to be the preferred substrate. Subjects with a rapid fasting lipid metabolism accumulate lower levels of postprandial triglycerides with less accumulation of apoB100 in the $VLDL_1$ fraction and a faster transfer of apoB100 into the $VLDL_2$ fraction. In contrast, fasting $VLDL_1$ secretion does not predict postprandial triglyceride accumulation.
Conclusions: Non-fasting triglyceride levels have recently been identified as a major predictor of future cardiovascular events. Here we show that the capacity of the lipolytic pathway is a common determinant of both the fasting and non-fasting triglyceride levels and may thus play an important role in the development of dyslipemia and atherosclerosis.

1. Introduction

Fasting hypertriglyceridemia has been identified as an independent risk factor for cardiovascular disease [1,2] but recent evidence suggests that postprandial lipemia increases the risk of atherogenesis [3]. The Atherosclerosis Risk in Communities study found that although LDL cholesterol is the most important lipid factor in early stages of atherogenesis, the metabolism of postprandial triglyceride rich lipoproteins (TRLs) and its effects on LDL and HDL may be more relevant to later atherothrombotic processes [4]. Furthermore, several studies have shown that non-fasting triglycerides are associated with increased risk of cardiovascular events [3,5], and that non-fasting triglyceride levels are a better predictor of cardiovascular disease than the fasting levels [5]. These data showing the clinical significance of postprandial lipemia for the development of atherosclerosis emphasize the importance of increasing our understanding of the mechanisms and dynamics of postprandial lipid metabolism.

In the fasting state, the majority of triglycerides are contained in very low-density lipoproteins (VLDL), which are secreted from the liver and contain one copy of the apolipoprotein (apo) B100. The concentration of triglycerides is determined by the level of triglyceride secretion (i.e. particle count and triglyceride content), the rate at which triglycerides are removed from the particles by lipases (forming denser, less triglyceride-rich particles) and the rate at which particles are completely removed from the circulation by uptake mechanisms.

Lipoprotein lipase is a key enzyme in the metabolism of VLDL [6]. However, lipoprotein lipase activity is not directly related to

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein.

* Corresponding author at: Wallenberg Laboratory, Sahlgrenska University Hospital, SE-413 45 Gothenburg, Sweden. Tel.: +46 31 3422210; fax: +46 31 823762.
E-mail address: martin.adiels@wlab.gu.se (M. Adiels).

[1] These authors contributed equally to this work.

0021-9150/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.atherosclerosis.2012.02.001

the storage of VLDL triglycerides in the fasting state [7], suggesting that lipoprotein lipase is in excess and that other factors, such as apoC-III [8], determine the rate of lipolysis. Indeed, we have recently shown that although apoC-III correlates to turnover of VLDL particles, lipoprotein mass does not [9].

In the non-fasting state, the intestine secretes large triglyceride-rich chylomicrons, uniquely identified by having one copy of the apoB48 protein. Chylomicron metabolism may interfere with VLDL metabolism by competition for lipoprotein lipase [10–12], thus prolonging the circulation time of VLDL particles and resulting in elevated postprandial VLDL levels [10,13]. In addition, insulin acutely suppresses VLDL secretion [14–16], suggesting that VLDL secretion may be suppressed in the postprandial state when insulin is present. Thus, the metabolism of lipoproteins of hepatic and intestinal origin is strongly related.

Several reports have shown that fasting triglyceride levels predict the postprandial triglyceride levels and, in particular, that subjects with elevated fasting triglyceride levels have higher postprandial levels [17,18]. This is intriguing since it suggests that the number of apoB48- and/or apoB100-containing particles that accumulate in the postprandial phase is related to the number of apoB100-containing particles circulating in the fasting state. There is no obvious reason why fasting triglyceride or apoB100 levels per se should predict postprandial apoB48 levels. We thus hypothesized that the capacity of the clearance pathway is an important predictor of postprandial clearance and a link between fasting and non-fasting triglyceride concentrations.

To test the hypothesis that the relation between fasting and non-fasting triglyceride levels (in particular, triglycerides carried by apoB48) is due to a common determinant, namely the rate of clearance, we combined results from a fasting kinetic study using stable isotopes and an oral fat tolerance test, both performed in the same 30 subjects.

2. Subjects and methods

2.1. Study subjects

Thirty male subjects were recruited for the study. The subjects were recruited by advertisements in local newspapers. Inclusion criteria included no known diagnoses other than type 2 diabetes or nonalcoholic fatty liver disease (NAFLD). Each subject underwent a physical examination and laboratory tests to exclude hepatic diseases (other than NAFLD), renal, thyroid and hematological abnormalities. Study subjects with coronary heart disease, retinopathy, microalbuminuria, total cholesterol > 6.2 mmol/L, triglycerides > 5.0 mmol/L, body mass index > 40 kg/m^2, or regular daily alcohol consumption over 2 doses (i.e. 5 g pure alcohol) were excluded from the study. Subjects without known diabetes underwent an oral glucose tolerance test. The diagnosis of type 2 diabetes was based on the use of oral hypoglycemic agents or an oral glucose tolerance test. Ten subjects fulfilled the criteria for type 2 diabetes. All subjects with diabetes were treated with diet, sulfonylurea, metformin or a combination of these. None of the subjects were taking lipid-lowering treatment or insulin. The participants abstained from alcohol and physical exercise at leisure time for two days before each examination. The Helsinki University Central Hospital ethics committee approved the study design and each subject gave written informed consent before participation in the study. All the samples were collected in accordance with the Helsinki Declaration. Kinetics and postprandial data from some subjects have been published before in separate papers [19,20].

2.2. Oral fat tolerance test

After a 12 h fast, the subjects ate a standardized fat-rich mixed test meal (oral fat tolerance test) consisting of bread, butter, cheese, sliced sausage, a boiled egg, fresh pepper, soured whole milk, orange juice and coffee. The meal contained 72 g fat (P/S ratio of 0.08; 490 mg cholesterol), 50 g carbohydrates and 35 g protein. The total energy content was 1000 kcal. Blood samples were drawn before the meal and at 3, 4, 6, and 8 h after the meal.

2.3. Nomenclature of density fractions and lipoproteins

Throughout this article, the $S_f > 400$ density fraction is termed the 'chylomicron fraction' and the term 'chylomicrons' refers to the apoB48-carrying particles in this fraction. The S_f 60–400 density fraction is termed the 'VLDL$_1$ fraction' and the terms 'VLDL$_1$ particles' and 'large chylomicron remnants' refer to the apoB100- and apoB48-carrying particles in this fraction, respectively. The S_f 20–60 density fraction is termed the 'VLDL$_2$ fraction' and the terms 'VLDL$_2$ particles' and 'small chylomicron remnants' refer to the apoB100- and apoB48-carrying particles in this fraction, respectively.

2.4. Lipoprotein and biochemical analyses for oral fat tolerance test

Particles in the chylomicron, VLDL$_1$ and VLDL$_2$ fractions were separated by density gradient ultracentrifugation as previously described [21,22]. Aliquots of the isolated fractions were frozen immediately. Concentrations of apoB48 and apoB100 were measured using SDS-PAGE as described previously [21,22]. The Coomassie-blue stained gels were photographed (Electrophoresis Documentation and Analysis System 120, Kodak, NY), and the bands representing apoB48 and apoB100 were analyzed with Image-Master 1-D software (Amersham Pharmacia Biotech, UK). The detection limit for apoB48 and apoB100 ranged from 0.01 to 0.02 mg/L. Triglyceride concentrations were analyzed in total plasma and lipoprotein fractions by automated enzymatic methods using the Cobas Mira S analyzer (Hoffman-La Roche, Basel, Switzerland). In addition, concentrations of glucose, serum free fatty acids, C-peptide and insulin were measured at each time point as previously described [22].

2.5. Fasting kinetic study and triglyceride and apoB100 turnover protocol

The fasting kinetic study was performed on a separate occasion from the oral fat tolerance test. After a 12 h fast, leucine (5,5,5-D3), 7 mg/kg body weight, and glycerol (1,1,2,3,3-D5), 500 mg (Isotec, Miamisburg, OH), were injected as a bolus. Blood sampling, sample analysis, mathematical modeling and calculation of kinetic parameters were performed as previously described [23].

HDL cholesterol concentrations and LDL peak sizes were measured at the fasting kinetic study visit.

2.6. Determination of liver fat

Liver fat was determined using proton magnetic resonance spectroscopy as described [24].

2.7. Statistical analysis and additional calculations

Changes of concentrations were expressed as incremental area under the curve (iAUC), which accurately describes the triglyceride response to an oral fat load in both healthy and type 2 diabetic subjects [25]. The iAUC was calculated using the trapezoid method.