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Differential uptake of subfractions of triglyceride-rich lipoproteins by THP-1 macrophages

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Abstract

It is well known that raised plasma triglycerides (TG) are positively linked to the development of coronary heart disease. However, triglycerides circulate in a range of distinct lipoprotein subfractions and the relative atherogenicity of these subfractions is not clear. In this study, three fractions of triglyceride rich lipoprotein (TRL) were isolated from normolipidaemic males according to their differing Svedberg flotation (S_f) rates: chylomicron (CM, $S_f > 400$), very low-density lipoprotein (VLDL)-1 (S_f 60–400) and VLDL-2 (S_f 20–60). These fractions were incubated with THP-1 monocyte-derived macrophages for determination of cholesterol and TG accumulation, in the presence and absence of the lipoprotein lipase (LPL) inhibitor orlistat. Expression of LDL receptor related protein (LRP) and apolipoprotein B48 receptor (apoB48R) was also examined in both differentiating monocytes, and monocyte-derived macrophages, incubated with TRL.

VLDL-1 caused a significantly greater accumulation of TG within macrophages compared to VLDL-2. Binding studies also tended to show a greater preference for VLDL-1. No change in expression of LRP or apoB48R was observed in fully differentiated macrophages incubated with VLDL-1, VLDL-2 or CM, although a greater expression of LRP mRNA was observed in differentiating monocytes exposed to VLDL-1, compared to those incubated with CM or VLDL-2. TG loading in response to all three TRL fractions was blocked by orlistat, suggesting that it is likely that the major pathway for uptake of TG was hydrolysis by LPL. Calculations suggested that direct uptake of particles accounts for between 12 and 25% of total TAG uptake. In conclusion, THP monocyte-derived macrophages demonstrate a preference for VLDL-1, both through the LPL pathway and by direct uptake of whole particles.

Keywords: Chylomicron; Lipoprotein lipase; Macrophage; Triglyceride-rich lipoproteins; Very low-density lipoprotein

1. Introduction

There is now abundant evidence showing that triglyceride-rich lipoproteins (TRL) have atherogenic properties and that postprandial lipid levels are an independent predictor of coronary heart disease [1–3]. Circulating TRL include chylomicrons (CM), very low-density lipoproteins (VLDL) and their remnants, and constitute a highly heterogeneous family of apoB-containing lipoproteins.

It has been shown that unmodified TRL interact with lipoprotein receptors on macrophages, leading to lipid accumulation [4]. This is in contrast to LDL, which requires chemical modification or oxidation in order to induce macrophage lipid loading and foam cell formation [5]. It has been assumed that larger lipoproteins are unable to penetrate the arterial intima, and become available for macrophage uptake in the sub-endothelial space, until they have become smaller through hydrolysis [6]. However, in addition to the reported presence of VLDL, IDL and LDL within atherosclerotic tissue [7], there is also persuasive evidence that CM are able to infiltrate the arterial intima [8], accumulate within me-

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dial smooth muscle cells [9] and penetrate endothelial cells, monocytes and macrophages [4].

A previous study reported differential uptake of type IIB dyslipidaemic subject derived VLDL-1 and VLDL-2 by human macrophages and attributed the majority of uptake to LPL-mediated hydrolysis [10]. However, while VLDL from normolipidaemic subjects has also been shown to result in TG accumulation in various monocyte/macrophage cell types [11–13], it is not clear whether normal VLDL-1 particles are also taken up in preference to VLDL-2. It is also unclear whether LPL plays a similar role in the uptake of TG from normolipidaemic TRL as it does from type IIB dyslipidaemic TRL. Milosavljevic et al. [10] note that the relative influence of VLDL from normolipidaemic versus dyslipidaemic subjects on macrophage LPL expression and activity remains controversial. In their studies, cells were incubated with TRL on the basis of 50 μg/ml total protein. This means that cells would have been exposed to approximately equivalent numbers of TRL particles and hence the greater uptake of TG from VLDL-1 was attributed to the higher TG/protein ratio in VLDL-1 as compared to VLDL-2. However, if most of the uptake of VLDL by monocyte-macrophages is indeed due to LPL-mediated hydrolysis, and if VLDL-1 is a better substrate for LPL—as has been suggested [14]—then the uptake of lipid from VLDL-1 should be greater than that from VLDL-2, even if particles are matched for TG content rather than protein content. The aim of the current study was (i) to compare the uptake of CM, VLDL-1 and VLDL-2 from normolipidaemic subjects, standardising particles for either apoB (particle number) or TG, (ii) to evaluate the contribution of the LPL pathway to uptake of TRL from normolipidaemic subjects by monocyte-macrophages and (iii) to examine the influence of TRL subfractions from normolipidaemic subjects on the expression of two proposed receptors for TRL macrophage uptake, the LDL-receptor related protein (LRP) [15–18] and the apolipoprotein B48 receptor (apoB48R) [19–21].

2. Materials and methods

2.1. Materials

Refined palm oil and deodorised cocoa butter contained in the test meal were provided by Anglia Oils Ltd. (Kingston-upon-Hull, UK) and ADM Cocoa (Hull, UK), respectively. Cell culture media and fetal calf sera were from Autogen-Bioclear (Wiltshire, UK) whilst delipidated and human sera were obtained from First Link (UK) Ltd. Primers for RT-PCR were obtained from MWG Biotech (Germany). Antibodies against LRP 85 kDa were obtained from Molecular Innovations (MI, USA) whilst secondary antibodies were from Serotech Ltd. (Oxford, UK). Tetrahydrolipstatin (orlistat) was kindly provided by Roche Pharmaceuticals (Basel, Switzerland). All other chemicals were obtained from Sigma-Aldrich (Poole, UK) unless otherwise stated in methodology.

Table 1
Fatty acid composition of test oil mixtures (g/50 g)

Fatty acid	Palm oil	Cocoa butter	Test meal
12:0	0.1		0.1
14:0	0.6	0.1	0.4
16:0	19.8	13	16.9
18:0	2.1	17.3	8.4
20:0	0.2	0.5	0.3
22:0			
24:0			
14:1			
16:1	0.2	0.2	0.2
18:1	20.7	17.3	19.3
20:1			
22:1			
24:1			
18:2	4.0	1.8	3.1
18:3	0.2		0.1

Test meal = 29 g refined palm oil + 21 g deodorised cocoa butter.

2.2. Subjects

The University of Reading Research and Ethical Committee approved the study protocol, and written informed consent was obtained from each participant. Healthy male volunteers ($n = 18$) were recruited in the age range 35–60 years (mean age 50 ± 6.6 years), with normal fasted plasma lipids (mean cholesterol 5.7 ± 1.0 mmol/l, mean TG 1.6 ± 0.6 mmol/l) and BMI in the range 21–33 (mean BMI 28.0 ± 3.4 kg/m^2). Volunteers were given a test meal, provided as a warm drink (skimmed milk 150 ml, milk powder 15 g, Nesquik 15 g, palm oil 29 g and cocoa butter 21 g) with white toast (70 g) and jam (20 g). The fatty acid composition of the meal is described in Table 1. Peripheral blood samples were obtained in lithium heparin before (fasted) and at 5 h after the oral fat load (postprandial).

2.3. Separation of lipoproteins

TRL were sub-fractionated by cumulative density gradient ultra-centrifugation, as previously described by Karpe et al. [22] and Mero et al. [23]. Briefly, plasma was adjusted to $d = 1.10$ g/ml with KBr and a discontinuous density gradient (NaCl 1.100–1.006 g/ml) constructed above it in Ultraclear tubes (14 × 95 mm; Beckman Instruments Inc., Palo Alto, CA). Three ultracentrifugation steps were performed (40,000 rpm, 15 °C in Beckman L-90K ultracentrifuge) to isolate lipoprotein subfractions with Svedberg's flotation index (S_f) > 400 (32 min; CM), S_f 60–400 (3 h 28 min; VLDL-1) and S_f 20–60 (16 h; VLDL-2). To prevent apolipoprotein cleavage, a cocktail of preservatives was used in all lipoprotein subfractions (5% v/v) [24]. Plasma lipoprotein fractions were protected from light and kept at 4 °C. Before addition to cell cultures lipoproteins were passed through PD-10 desalting columns (Amersham Biosciences, Bucks UK) and further concentrated in 'vivaspin' concentrators (Vivascience AG, Hanover, Germany). These were rinsed through with phos-