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Insulin stimulates hepatic low density lipoprotein receptor-related protein 1 (LRP1) to increase postprandial lipoprotein clearance

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ABSTRACT

Background: While the role of insulin in glucose uptake and its aberration in diabetes are well established, the effect of insulin on lipoprotein clearance in the postprandial phase is not yet fully understood. The dietary lipids are carried in chylomicron remnants (CR) which are taken up into the liver mainly via LDLR-related protein 1 (LRP1). In this study, the effect of insulin on LRP1-mediated hepatic CR uptake was investigated.
Methods: The study was based on determining the subcellular localisation of LRP1 by subcellular fractionation and immunofluorescence microscopy and correlating those findings with the hepatic uptake of fluorescently or radioactively labelled LRP1-specific ligands and CR in hepatoma cells, primary hepatocytes and mouse models.
Results and conclusion: In vitro and *in vivo*, insulin stimulated the translocation of hepatic LRP1 from intracellular vesicles to the plasma membrane, which correlates with an increased uptake of LRP1-specific ligands. In wild-type mice, a glucose-induced insulin response increased the hepatic uptake of LRP1 ligands while in leptin-deficient obese mice (*ob*/*ob*), which are characterised by hepatic insulin resistance, insulin-inducible LRP1 ligand uptake was abolished. Finally, upon hepatic LRP1 knockdown, insulin no longer significantly enhanced CR uptake into the liver. The insulin-induced LRP1-mediated CR uptake, as demonstrated here, suggests that impaired hepatic LRP1 translocation can contribute to the postprandial lipaemia in insulin resistance.

1. Introduction

Hepatic lipoprotein uptake is a complex but well regulated process involving multiple molecular players. Although not all steps are completely understood, recent advances from *in vitro* studies and mouse model systems have led to a general concept for the internalisation and processing of intestinally derived chylomicron remnants (CR) in the liver (reviewed in [1,2]). From the sinusoid, CR enter the space of Disse through the fenestrated endothelium. Some particles then directly bind to the low density lipoprotein receptor (LDLR) and LDLR-related protein 1 (LRP1) [1,3], whereas the vast majority of the particles first attaches to cell surface structures such as heparan sulfate proteoglycans (HSPG), which may also directly facilitate internalisation [4]. Similarly, scavenger receptor class B, type I (SRBI) was described to mediate binding of CR [5]. Subsequently, CR are passed on to the LDLR and LRP1 for internalisation. Although the LDLR was at first considered to be central for the uptake of CR, accumulated evidence pointed to an important role for LRP1 in the hepatic clearance of postprandial lipoproteins [6,7]. LRP1 interacts with CR via apolipoprotein E (apoE) [8,9] and particle-bound lipoprotein lipase (LpL) *in vitro* and *in vivo* [10–12]. LRP1 is composed of a large extracellular (515 kDa) and a small transmembrane (85 kDa) subunit, which are derived from proteolytic processing of a precursor by the protease furin [13]. Maturation of LRP1 requires the receptor-associated protein (RAP), a chaperon preventing premature binding of LRP1 ligands some of which are synthesised alongside LRP1 [14]. The efficient binding

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to LRP1 makes RAP a suitable tool for investigating LRP1 binding. Similarly, activated α_2-macroglobulin (α_2M^*) is another ligand specifically binding to and cleared by LRP1.

Prolonged postprandial lipaemia, as it occurs in type 2 diabetes (T2D) has been recognised as a risk factor for atherosclerosis [15]. Several mechanisms leading to postprandial hyperlipidaemia in insulin resistant patients can be explained by reduced insulin sensitivity, in particular the overproduction of liver-derived VLDL1 [16,17], the decrease of LpL-mediated lipolysis of chylomicrons and the reduced binding of CR to HSPG [18–20]. In spite of these findings, it is yet unclear whether insulin also directly affects the hepatic clearance of postprandial lipoprotein remnant particles.

Clinical data linking LRP1 to lipid metabolism in humans is rare. Although some LRP1 polymorphisms were repeatedly confirmed in genetic studies to be associated with coronary artery disease [21], they failed to show an effect on plasma lipid levels [22]. The lack of functional phenotypes in *LRP1* polymorphisms in humans may be explained by the importance of this multiligand-receptor in various processes central to organism development and embryonic survival (reviewed in [23]). This view is supported by the murine LRP1 knockout, which leads to pre-natal death [24].

Since LRP1 has been described to translocate to the plasma membrane (PM) of rodent adipocytes after insulin stimulation [25,26], this work investigates the potential role of insulin in the regulation of the LRP1-dependent CR clearance in hepatocytes.

2. Materials and methods

2.1. Ligand preparation

RAP [27], α_2M^* [28] and human CR [29] were prepared as described. Cy3 Mono-Reactive Dye (Amersham) was used for fluorescence labelling of RAP, α_2M^* and recombinant apoE isoforms (kindly provided by K.A. Rye, Sydney). CR were associated with apoE [29] and re-isolated by ultracentrifugation. Radioactive labelling using non-degradable ^{125}I-tyramine-cellobiose was performed as described [30,31].

2.2. Cell assays, microscopy and fluorescence quantification

Hepatocytes from wild-type and LDLR$^{-/-}$ mice were prepared [32] and incubated for 16 h in DMEM supplemented with 10% lipoprotein- and insulin-deficient serum. Cell lines were starved overnight in serum-free medium containing 2% BSA. Stimulation was performed with 0 to 100 nM human recombinant insulin (Sigma–Aldrich) prior to the incubation with the respective ligands. All concentration values given for insulin or LRP1 ligands refer to the final concentration in the media. Indirect immunofluorescence microscopy was performed as described previously [29] using an LSM510 META (Zeiss) confocal laser scanning microscope and employing mouse monoclonal antibodies 8G1, 5A6 (both Progen) and the rabbit polyclonal 377-4 (kindly provided by J. Herz, Dallas, TX) to detect human and rat LRP1, respectively. After uptake, cells were washed with cooled heparin/PBS (10 U per ml) to remove surface-bound material. Biotinylated cell surface proteins were prepared and purified using the Pinpoint Cell Surface Protein Isolation Kit from Pierce according to the manufacturer's recommendation. Western blot analyses were performed using a polyclonal sheep antibody against the cytoplasmic tail of LRP1 [33]. Fluorescence-based in-gel quantification was performed after SDS-PAGE using a Typhoon 9410 (Amersham) with ImageQuant 5.2 and FluorSep 2.2 software as described [34].

2.3. Animal experiments

Animal experiments were in accordance with the guidelines of the Federation of American Societies for Experimental Biology (FASEB) and approved by the Department of Veterinary Affairs of the State of Hamburg. Due to the natural feeding behaviour of the nocturnal mice, nighttime fasting would have meant 24 h food deprivation leading to a ketotic condition. Therefore, male C57BL/6 mice and insulin-resistant, leptin-deficient, obese *ob*/*ob*-mice (Taconic) were instead fasted for 6 h at daytime and received an *i.p.* insulin (1 U per kg), glucose (2 mg per g body weight) or NaCl injection as described [35]. After 40 min, radio-labelled ligand (20 μg RAP or 20 μg α_2M^*) was injected into the tail vein and organ uptake was measured 20 min later after perfusion with heparin/PBS (10 U per ml) to wash out not internalised material as described [31]. The mice were anaesthetised by *i.p.* injection of a rompun/ketanest mixture prior to organ explantation. Insulin levels were determined by ELISA (Crystalchem) and purified PM were prepared as described [36]. LRP1*flox* mice ([6]; kindly provided by J. Herz, Dallas, TX) were injected with 2.5×10^9 IFU AdCre or AdEGFP virus, respectively, as described to predominantly target liver cells [37]. Similar to the experimental procedure described above, 5 days after adenoviral infection 10 μg of ^{125}I-TC-radio-labelled CR were tail-vein injected and organ uptake was measured accordingly. Membrane preparations of infected livers were subjected to Western blotting against LRP1 (sheep polyclonal, [33]), LDLR (Progen), SR-BI (Novus Biologicals), apoE (Dako), β-actin (Sigma) and GAPDH (Novus Biologicals).

3. Results

3.1. Hepatic LRP1 translocates to the PM after insulin stimulation

Both LRP1 and the LDLR are involved in the hepatic uptake of CR [6]. Nevertheless, and in contrast to the LDLR, only small amounts of LRP1 were detectable in PM purified from mouse livers (Supplemental Fig. S1A), but LRP1 was abundant in total membrane preparations. Since it has been shown that insulin can stimulate LRP1 translocation to the PM in rodent adipocytes [25,26], we analysed the effect of insulin on LRP1 localisation in liver cells. Human adipocyte precursor cells (hMSC-TERT) [38] served as controls and showed a translocation of LRP1 to the PM under these conditions (Fig. 1A). Similarly, the incubation of hepatic human HuH7 cells and rat FAO cells with insulin led to a translocation of LRP1 to the PM as judged by immunofluorescence and confocal microscopy (Fig. 1A, arrows). Comparable results were obtained from primary hepatocytes of wild-type and LDLR-deficient (LDLR$^{-/-}$) mice (Fig. 1B). Consistently, cell surface biotinylation revealed an approximately 80% increase of LRP1 in the PM of FAO cells after 20 nM insulin treatment (Fig. 1C). *In vivo*, a significant 70% increase of LRP1 was found in preparations of purified PM isolated from murine livers after insulin injection while the abundance of the LDLR appeared slightly decreased (Fig. 1D).

3.2. Insulin stimulates LRP1-specific uptake

In order to analyse whether insulin-stimulated LRP1 translocation results in increased LRP1-specific ligand uptake, internalisation studies with fluorescence- and radio-labelled RAP and α_2M^* were performed *in vitro* and *in vivo* [39,40]. After insulin stimulation, fluorescence-labelled RAP was incubated with FAO cells. RAP was internalised much more efficiently according to immunofluorescence (Fig. 2A) and in-gel fluorescence quantifica-