Molecular and Cellular Endocrinology 343 (2011) 71–78

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

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

Postprandial triglyceride-rich lipoproteins induce hepatic insulin resistance in HepG2 cells independently of their receptor-mediated cellular uptake

Tobias Tatarczyk, Christian Ciardi, Andreas Niederwanger, Michael Kranebitter, Josef R. Patsch, Michael T. Pedrini *

Department of Internal Medicine I, Medical University of Innsbruck, Anichstrasse 35, A-6020 Innsbruck, Austria

ARTICLE INFO

Article history:
Received 4 March 2011
Received in revised form 8 June 2011
Accepted 9 June 2011
Available online 15 June 2011

Keywords:
Glucose metabolism
Hepatic insulin resistance
Insulin signaling
Liver steatosis
Postprandial lipemia

ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is associated with hepatic insulin resistance with the molecular basis of this association being not well understood. Here we studied the effect of hepatic triglyceride accumulation induced by postprandial triglyceride-rich lipoproteins (TGRL) on hepatic insulin sensitivity in HepG2 cells. Incubation of HepG2 cells with purified TGRL particles induced hepatocellular triglyceride accumulation paralleled by diminished insulin-stimulated glycogen content and glycogen synthase activity. Accordingly, insulin-induced inhibition of glycogen synthase phosphorylation as well as insulin-induced GSK-3 and AKT phosphorylation were reduced by TGRL. The effects of TGRL were dependent on the presence of apolipoproteins and more pronounced for denser TGRL. Moreover, TGRL effects required the presence of heparan sulfate-proteoglycans on the cell membrane and lipase activity but were independent of the cellular uptake of TGRL particles by receptors of the LDL receptor family. We suggest postprandial lipemia to be an important factor in the pathogenesis of NAFLD.

1. Introduction

Type 2 diabetes is frequently associated with non-alcoholic fatty liver disease (NAFLD) which is characterized by hepatic fat accumulation and insulin resistance. Insulin resistance has been shown to promote hepatic fat accumulation through various mechanisms (Chen et al., 1987; Ginsberg, 2006). Whether hepatic fat accumulation, in turn, may be a direct cause of insulin resistance, in particular of hepatic insulin resistance, is still a matter of debate (Boden, 1997; Marchesini et al., 2005). One possible mechanism by which hepatic fat may induce hepatic insulin resistance is the development of oxidative stress (Videla, 2009).

The sources of fatty acids stored in liver as triglycerides are diverse and the quantitative contribution of various fatty acid sources to NAFLD in humans is not well defined. In the postabsorptive state, fatty acids mainly derive from adipose tissue. In the postprandial state, de novo lipogenesis, spillover of non-esterified fatty acids (NEFA) after lipoprotein lipase (LPL)-mediated lipolysis of intestinally derived chylomicrons and hepatic uptake of the resulting triglyceride-depleted remnant particles (Barrows and Parks, 2006) are the major sources of hepatic triglycerides. Using magnetic resonance spectroscopy, it could be demonstrated that postprandial triglycerides are rapidly incorporated into the hepatic triglyceride pool in both normal and diabetic subjects (Ravikumar et al., 2005). The liver has a central role in chylomicron remnant catabolism (Cooper, 1997) which is underlined by the observation that defenestration of the sinusoidal endothelium and, consequently, an abolishment of the livers capability to eliminate chylomicron remnants from the circulation is closely correlated with massive hypertriglyceridemia in animal models (Cogger et al., 2006). Studies have shown that a large portion of chylomicron remnants are bound to heparan sulfate proteoglycans (HSPG) on the endothelial wall of peripheral capillary beds where their triglyceride portion undergoes initial LPL-mediated lipolysis resulting in an increase in chylomicron remnant density (Karpe et al., 2007). With an increase in density, the affinity of chylomicron remnants to adhere to the endothelial wall decreases (Karpe et al., 1997). As a consequence, chylomicron remnants re-enter plasma and eventually reach the space of Disse after having reached a size corresponding to a Svedberg flotation rate (S_f) of up to 400 allowing them to pass the 100–200 nm sized pores in the protecting endothelium (Cogger et al., 2004). During the

Abbreviations: BMI, body mass index; DAPI, 4′,6-diamidino-2-phenylindole; DMEM, dulbeccos minimal essential media; FCS, fetal calf serum; GS, glycogen synthase; GSK-3, glycogen synthase kinase 3; HL, hepatic lipase; HOMA-IR, homeostasis model assessment of insulin resistance; HSPG, heparan sulfate proteoglycans; LPL, lipoprotein lipase; LRP, LDL-receptor-related protein; NAFLD, non-alcoholic fatty liver disease; PBS, phosphate buffered saline; RAP, receptor-associated protein; ROS, reactive oxygen species; S_f, Svedberg flotation rate; TGRL, triglyceride-rich lipoproteins; THL, tetrahydrolipstatin.

* Corresponding author. Tel.: +43 512 504 23326; fax: +43 512 504 28539.
E-mail address: michael.pedrini@i-med.ac.at (M.T. Pedrini).

0303-7207/$ - see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.mce.2011.06.008

process of lipolysis, chylomicron remnants become enriched with Apo E (Batal et al., 2000) which has been shown to bind to LPL (Huff et al., 1997), hepatic lipase (HL) (Krapp et al., 1996) and LDL-receptor-related protein (LRP), a receptor mediating hepatic chylomicron remnant clearance (Beisiegel et al., 1989; Herz et al., 1991). LPL and HL also possess LRP-binding properties of their own (Krapp et al., 1996; Olivecrona and Lookene, 1997), further enhancing the affinity of chylomicron remnants towards the LRP.

In the present work, we sought to analyze the effects of hepatic triglyceride accumulation induced by postprandial triglyceride-rich lipoproteins (TGRL) with a $S_f < 400$, i.e. chylomicron remnants and VLDL/VLDL remnants, on several parameters of hepatic insulin sensitivity using the human hepatoblastoma-derived cell line HepG2. Our decision to study postprandial lipoproteins was based on the following observations suggesting that the postprandial state plays an important role in the development of NAFLD, hepatic insulin resistance and skeletal muscle insulin resistance. Firstly, we have previously shown that insulin sensitivity is impaired during the state of postprandial lipemia in healthy men (Pedrini et al., 2006) and, moreover, that postprandial TGRL particles cause insulin resistance in cultured skeletal muscle cells (Pedrini et al., 2005). Secondly, postprandial lipids represent a substantial source of hepatic triglycerides in vivo in humans (Barrows and Parks, 2006; Ravikumar et al., 2005), yet have been incompletely studied as a potential factor in the pathogenesis of NAFLD and hepatic insulin resistance. Thirdly, in Westernized societies, a large part of the day or even of life is spent in the state of postprandial lipemia.

2. Materials and methods

2.1. Materials

The HepG2 cell line was obtained from ATCC (Manassas, VA, USA); DMEM and amyloglucosidase were purchased from Sigma (St. Louis, MO, USA), FCS from PromoCell (Heidelberg, Germany) and fatty acid-/insulin-free BSA from Valeant Pharmaceuticals (Bryan, OH, USA). Sepharose 2B, [14C]-UDPG and the ECL kit were purchased from Amersham Biosciences (Buckinghamshire, UK), and the 4–15% linear-gradient mini gels from Biorad (Hercules, CA, USA). The anti-GSK-3 (anti-glycogen synthase kinase 3) antibody was obtained from Upstate (Charlottesville, VA, USA) and the LPL antibody from Abcam (Cambridge, UK); anti-phospho-GSK-3/β-ser21/9, anti-phospho-Akt-ser473 and anti-Akt, anti-GS (anti glycogen synthase) and anti-phospho-GS antibodies were from Cell Signaling Technology (Beverly, MA, USA). The receptor-associated protein (RAP) fusion protein was obtained from Progen (Heidelberg, Germany), Tetrahydroplipstatin (Orlistat®) from Roche (Hertfordshire, UK) and purified LPL from Sigma.

2.2. Cell culture

HepG2 cells were stored in liquid nitrogen and cultured to confluence using low glucose DMEM containing 10% FCS.

2.3. Lipoprotein isolation

TGRL were isolated from healthy male individuals 3 h after ingestion of a standardized fatty meal (Patsch et al., 1983) by zonal ultracentrifugation as previously described (Pedrini et al., 2005). The mean age of our study subjects was 35.1 ± 3.5 years, the mean body mass index (BMI) was 21.4 ± 1.52 kg/m^2 and the mean homeostasis model assessment of insulin resistance (HOMA-IR) was 0.75 ± 0.10. Informed consent was obtained from all donors. Three fractions were obtained by pooling appropriate volume contents of the rotor: firstly, a lipoprotein fraction with a S_f of 20–400 corresponding mainly to VLDL/VLDL remnants and chylomicron remnants; secondly, a fraction with a S_f of 20–60 corresponding mainly to VLDL remnants and small dense chylomicron remnants and, thirdly, another fraction with a S_f of 60–400 corresponding mainly to VLDL and more buoyant chylomicron remnants. Prior to the experiments, lipoproteins were extensively dialyzed against phosphate buffered saline (PBS).

2.4. Analysis of lipoprotein fatty acid composition

The fatty acid composition of postprandial TGRL was analyzed by gas chromatography according to the method described by Kang and Wang, 2005.

Using known standards, we were able to identify 98% of the area under the gas chromatography curve of fatty acids extracted from lipoproteins. The postprandial lipoproteins used for our experiments were composed of 33% palmitic acid, 21% oleic acid, 11% stearic acid, 7% arachidoic acid and 4% linoleic acid.

2.5. Analysis of glycogen content

After incubation with TGRL, incubation media was replaced by serum-free DMEM containing 0.25% BSA. Cells were then incubated without or with 100 nmol/l insulin for 3 h at 37 °C. Subsequently, cells were analyzed for glycogen content as previously described (Pedrini et al., 2005). For the experiments using RAP, which blocks binding of ligands to receptors of the LDL family, this protein was added to the incubation media at a concentration of 1 μmol/l 5 min prior to the addition of TGRL. Tetrahydrolipstatin (THL), a potent lipase inhibitor, was prepared as described elsewhere (Krebs et al., 2000) and co-incubated at a concentration of 250 μg/l with lipoproteins.

2.6. NEFA determination

NEFA levels in the culture media were determined using a commercial kit from WAKO (Germany) with a Cobas MIRA analyzer.

2.7. Determination of hepatocellular triglyceride content

Cells were collected in trypsin, centrifuged at 3000*g* for 10 min and extensively washed with PBS containing 500 U/ml Heparin to remove any excess triglyceride on the cell surface. After another centrifugation step, the pellet was lysed using 0.1 mol/l NaOH and a brief sonication step. The supernatant was analyzed for triglyceride content using the TG kit from Roche.

2.8. Glycogen synthase activity

After incubation of cells without or with 100 nmol/l insulin for 30 min at 37 °C, glycogen synthase activity was determined by a method recently established in our laboratory (Niederwanger et al., 2005).

2.9. Western blot analysis

After incubation with TGRL, cells were incubated without or with 100 nmol/l insulin for 5 min. All subsequent steps for Western blot analysis were performed as previously described (Pedrini et al., 2005). The dilution of the glycogen synthase, the phospho-glycogen synthase and the LPL antibodies, respectively, was1:1000. The dilution of the secondary antibody (goat anti rabbit) was 1:20000.