Experimental and Molecular Pathology 95 (2013) 325–329

Contents lists available at ScienceDirect

Experimental and Molecular Pathology

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

LDL but not HDL increases adiponectin release of primary human adipocytes

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ARTICLE INFO

Article history:
Received 4 October 2013
Available online 22 October 2013

Keywords:
Lipoprotein
LDL apheresis
Familial hypobetalipoproteinemia

ABSTRACT

Adipocytes in obesity have inappropriately low cholesterol while adiponectin release is reduced. Cholesterol shortage may contribute to low adiponectin and 3T3-L1 cells treated with lovastatin have diminished adiponectin in cell supernatants. LDL and HDL deliver cholesterol to adipocytes. LDL but not HDL increases adiponectin in cell supernatants of primary human adipocytes. The effect of LDL is not blocked by receptor associated protein suggesting that members of the LDL-receptor family are not involved. To evaluate whether these in vitro observations translate into changes in systemic adiponectin, adiponectin was measured in serum of three patients before, immediately after and 3 d after LDL-apheresis. Whereas circulating lipoproteins are reduced immediately after apheresis adiponectin is not changed. Therefore, acute lowering of lipoproteins does not affect systemic adiponectin also excluding that plenty of adiponectin is bound to lipoprotein particles. Accordingly, levels of adiponectin in purified lipoproteins are quite low. Familial hypobetalipoproteinemia (FHBL) is a rare disorder associated with low plasma LDL. Serum adiponectin is, however, similar compared to healthy controls. Thus, neither LDL nor HDL directly contributes to circulating adiponectin concentrations.

Introduction

Adiponectin is an extensively studied adipokine whose adipose tissue and serum levels are reduced in obesity. Beneficial effects of this adipokine in glucose and lipid metabolism have been described in various studies (Buechler et al., 2011; Kadowaki et al., 2006; Ye and Scherer, 2013). Adiponectin is nearly exclusively produced in adipocytes and serum levels are defined by its synthesis in different fat pads and hepatic clearance (Halberg et al., 2009). In obesity adiponectin clearance is even reduced indicating markedly impaired synthesis in hypertrophic adipocytes (Halberg et al., 2009). Various studies have shown that inflammation and oxidative stress in obesity lower adiponectin synthesis (Shehzad et al., 2012). Adiponectin multimerization which affects its release and biological function is also impaired in obesity (Liu et al., 2008).

Triglyceride accumulation in adipocytes is accompanied by increased cholesterol which mostly exists as free cholesterol. Cellular cholesterol distribution is disturbed in obesity and low plasma membrane cholesterol levels affect adipocyte function (Le Lay et al., 2001, 2004; Yu et al., 2010). Statins inhibit 3-hydroxy 3-methyl glutaryl CoA reductase which is the rate-limiting enzyme in cholesterol biosynthesis (Danesh et al., 2003). In terminally differentiated murine adipocytes atorvastatin treatment reduces adiponectin mRNA levels while IL-6 is increased suggesting that cholesterol shortage may have a role in the pro-inflammatory phenotype of hypertrophic adipocytes (Mauser et al., 2007). Simvastatin selectively lowers the release of high-molecular weight adiponectin which is the most active biological form of this adipokine (Khan et al., 2009; Simpson and Whitehead, 2010).

Adipocyte endogenous cholesterol synthesis is limited and cells depend on the uptake of cholesterol from circulating lipoproteins. High-density lipoprotein (HDL) cholesterol uptake occurs through scavenger receptor type-BI dependent and independent pathways (Yu et al., 2010; Zhang et al., 2010). Low-density lipoprotein receptor is expressed on adipocytes and mediates endocytosis of LDL (Kraemer et al., 1994). Cholesterol exchange between LDL and adipocytes not mediated by members of the LDL receptor family has also been identified (Angel et al., 1981; Kovanen and Nikkila, 1976).

Negative correlations of adiponectin with LDL and positive correlations with HDL have been described (Izadi et al., 2013) and may be related to the effects of adiponectin on hepatic lipoprotein synthesis and peripheral catabolism. Adiponectin lowers apolipoprotein B-100 (apoB-100) in hepatocytes and this may contribute to reduced VLDL and LDL in the circulation (Neumeier et al., 2007). Adiponectin further enhances lipoprotein lipase activity and VLDL receptor expression in skeletal muscle and subsequently VLDL catabolism (Qiao et al., 2008). Further, ATP-binding cassette transporter A1

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0014-4800/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.yexmp.2013.10.002

protein and systemic apolipoprotein A-I are reduced in adiponectin deficient mice suggesting that this protein also regulates HDL assembly (Oku et al., 2007).

Cellular cholesterol homeostasis is essential for adipocyte function (Le Lay et al., 2001, 2004; Yu et al., 2010) and may also have a role in adiponectin production. To our knowledge only one study so far has shown that HDL directly increases adiponectin mRNA expression in immature adipocytes (Van Linthout et al., 2010). Here, effects of lovastatin, LDL and HDL on adiponectin levels in the supernatants of adipocytes have been studied. Systemic adiponectin has been determined in patients with transiently low LDL and HDL and patients with chronically low LDL.

Materials and methods

LDL-apheresis

Three patients (one man, 43 years old and two women, 52 and 56 years old) with high levels of serum lipoprotein (a) that were on weekly therapeutic LDL-apheresis (DALI system, Fresenius, Bad Homburg, Germany) were enrolled. Each patient provided written informed consent and the study was performed in accordance with the Declaration of Helsinki. Serum was obtained of the three patients right before, right after and 3 d after LDL apheresis.

Familial hypobetalipoproteinemia (FHBL)

Serum was also collected from members of a family with FHBL where the grandmother, the mother (index patient), one daughter and two sons were affected while one daughter was not affected. The affected members have a heterozygous nonsense mutation at Glu3545 (C to T, p.Glu3545Ter) and a heterozygous missense mutation at Arg3611 (G to A, p.Arg3611Gln) in the *APOB*-gene while the non-affected daughter does not have these sequence changes implicating that both sequence changes are on the same allele. Serum lipid levels of these patients are summarized in Table 1.

Measurement of standard serum parameters

Plasma total cholesterol, triglycerides, HDL, VLDL and LDL were measured by standard techniques at the Institute of Clinical Chemistry and Laboratory Medicine, Regensburg University Hospital, Germany.

Material

In vitro differentiated primary human subcutaneous adipocytes were ordered from BioCat GmbH (Heidelberg, Germany). Cells were obtained from 5 females, median age was 33 (24–58) years and median BMI 22 (22–24) kg m^{-2}. ELISAs for murine and human adiponectin and human leptin were from R&D Systems (Wiesbaden-Nordenstadt, Germany). Lovastatin was ordered from Sigma (Deisenhofen, Germany). Recombinant Receptor Associated Protein (RAP) was from Calbiochem (Darmstadt, Germany) and was used at a concentration of 1μM.

Table 1
Serum cholesterol (C), triglycerides (TG), HDL, LDL, VLDL, apolipoprotein B-100 (ApoB-100) and gender of the family members with familial hypobetalipoproteinemia.

	Sex	C	TG	HDL	LDL	VLDL	ApoB-100
		mg/dl	mg/dl	mg/dl	mg/dl	mg/dl	mg/dl
Proband 1	female	173	40	116	59	8	37.4
Proband 2	female	177	57	82	92	3	65.8
Proband 3	female	113	39	43	57	13	48.8
Proband 4	male	122	24	92	25	5	24.6
Proband 5	male	121	26	82	37	2	31.6
Reference values		<200	20 –200	35 – 55	<150	0 –40	60 –150

ELISA

ELISA was performed as recommended by the distributor. Adipocyte supernatants were diluted 1:250 and serum was diluted 1:5000 to determine adiponectin. To measure leptin serum was diluted 1:50 fold.

Adipocyte cell culture

3T3-L1 preadipocytes were purchased from the American Type Culture Collection (ATCC, Manassas, VA) and cultured as described (Bauer et al., 2011). Lovastatin was added to the cells 6 days after hormonal induction of adipogenesis until day 9 where cells have the typical appearance of mature adipocytes. LDL and HDL were added to differentiated cells for 24 h.

Purification of human lipoproteins

Human LDL and HDL were isolated from the plasma of healthy blood donors by sequential preparative ultracentrifugation in KBr gradients as described (Havel et al., 1955; Lindgren et al., 1975) followed by extensive dialysis and filter sterilization. Protein concentrations were determined by use of Lowry's method (Lowry et al., 1951).

Incubation of human adipocytes with lipoproteins

Differentiated human adipocytes were cultivated in DMEM medium (Biochrom, Berlin, Germany) without other additives and in the identical medium supplemented with 100 μg LDL or HDL for 24 h. RAP was added 10 min before the addition of the respective lipoproteins.

Statistical analysis

Data are presented as box plots indicating median, lower and upper quartiles and range of the values. Statistical differences were analyzed by two-tailed Mann–Whitney U Test or paired Student's *t*-test and a value of $p < 0.05$ was regarded as significant.

Results

Lovastatin reduces adiponectin release of 3T3-L1 cells

To evaluate whether cholesterol depletion by lovastatin treatment affects adiponectin release 3T3-L1 cells were differentiated for 6 d and then lovastatin (0.5 μM and 5 μM) was added until day 9. Cholesterol content was significantly reduced (data not shown), and adiponectin in the supernatants was similarly diminished by both concentrations of lovastatin (Fig. 1A). LDL delivers cholesterol to adipocytes but adiponectin was not altered by human LDL in 3T3-L1 cells (Fig. 1B). Further human HDL (100 μg/ml) had no effect on adiponectin in the supernatants (not shown).

LDL but not HDL enhances adiponectin in primary human adipocytes

Fully differentiated primary human adipocytes of 5 different donors were incubated with purified LDL (100 μg/ml) or HDL (100 μg/ml) for 24 h. LDL significantly increased adiponectin in the supernatants whereas HDL had no effect (Fig. 1C, D). Receptor associated protein prevents binding of ligands to LDL-receptor family members (Li et al., 2002) but did not block LDL-mediated upregulation of adiponectin. To exclude that HDL and LDL affect cell viability LDH was measured in the supernatants of these cells and was similar to control incubated cells (not shown).