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Nutritional regulation of adipose tissue lipoprotein lipase is blunted in insulin resistant rats

Research Article

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Abstract: Background: Hypertriglyceridemia is a common lipid abnormality accompanying insulin resistance. This study was designed to assess the contribution of dysregulation of adipose tissue lipoprotein lipase (LPL) activity to the hypertriglyceridemia in a rat model of insulin resistance. Methodology: Hereditary hypertriglyceridemic (HHTg) rats were challenged for two weeks on a high sucrose diet and LPL activity, angptl-4 expression and FFA utilisation *in vitro* were determined in adipose tissue. Results: Compared to control rats (Wistar), HHTg rats exhibited hyperinsulinemia, impaired fatty acid storage in adipose tissue and elevated LPL activity both in fasting and after refeeding. The expression of angiopoietin-like protein 4 (angptl4), a fasting-induced control protein for LPL activity, was not increased in adipose tissue of fasted HHTg rats as it was in the control rats. Conclusion: We conclude that LPL remains in its active form to a higher extent in HHTg rat adipose tissue due to the low expression of angptl4 on fasting. This is a possible consequence of the hyperinsulinemia. In combination with the impaired storage of fatty acids as triglycerides in adipose tissue in HHTg rats, the inflexibility of angptl4 expression may contribute to the establishment of hypertriglyceridemia in the insulin-resistant animals.

Keywords: *Adipose tissue • Hypertriglyceridemia • Glucose metabolism • Fatty acids • Angiopoietin-like protein 4 • High-sucrose diet • HHTg rats*

1. Introduction

Hypertriglyceridemia, together with hyperinsulinemia, is a common lipid abnormality accompanying insulin resistance, glucose intolerance and peripheral insulin resistance.

Plasma levels of triglycerides and their partitioning among tissues are to a large extent determined by the activity of lipoprotein lipase ([LPL] EC 3.1.1.34) [1]. LPL hydrolyzes triglycerides (Tg) found in circulating Tg-rich lipoproteins into monoglycerides and non-esterified fatty acids (FFA). FFA are then either taken up by the fat cells or escape in the albumin-bound form into circulation [2,3]. The ability of adipose tissue to accommodate the released FFA may be an important factor contributing to the final serum Tg content [3,4].

LPL is regulated at multiple levels. Insulin is considered to be a major hormone regulating the changes in LPL activity by translational and posttranslational mechanisms [5]. Originally, the increase of the synthesis of the enzyme was supposed to be the predominant, if not the only effect of insulin [6]. Under normal conditions, insulin has been shown to correlate positively with the enzyme activity in white adipose tissue (WAT) [7]. The question still remains open to how insulin is involved in LPL regulation in the state of insulin resistance, which is accompanied by long-lasting hyperinsulinemia. In hyperinsulinemic states, the LPL synthesis may be increased due to the stimulating effect of insulin on proteosynthesis *via* mTOR/S6K1 signalling pathway. This pathway represents the link between insulin and nutrient signalling and cellular growth. It is

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overstimulated in the situation of nutrient excess [8] or in chronic hyperinsulinemia [9]. mTOR activation stimulates protein synthesis by phosphorylating eIF4E-binding protein–1 and the 70 kDa ribosomal protein S6 kinase (p70-S6K) [10]. Recent reports show that the effect of chronic hyperinsulinemia is tissue-specific. p70S6-kinase was highly activated in mesangial cells in diabetic obese db/db mice [11] and in the renal cortex of Zucker diabetic rats [12] and it is supposed to contribute to the renal hyperplasia. On the other hand, chronic hyperinsulinemia imposed on normal rats (5 days hyperinsulinemic-euglycemic clamp) had no effect on mTOR/p70-S6K pathway in white adipose tissue [9].

It is well known that the activity of LPL is rapidly modulated by changes in the nutritional state [1]. Nevertheless, these acute changes are usually not attributed to changes in the levels of LPL mRNA or LPL immunoreactive mass [13]. Bergö *et al*. showed that during short-term fasting LPL is regulated post-transcriptionally which allows for quick upregulation after refeeding. This regulation involves a dynamic balance between inactive (monomeric) and active (dimeric) forms of LPL [14]. Later studies show that in fasting rats a gene, different from LPL, is switched on in adipose tissue and its short-lived gene product makes the tissue produce an inactive form of LPL [15]. Refeeding inhibits expression of this putative factor, allowing newly synthesised LPL to remain active. Recently, it became evident, that activity of LPL is regulated by members of the angiopoietin-like protein family. In animal models, a specific role has been attributed to angptl4. Studies with mice lacking or overexpressing angptl4 have clearly demonstrated the impact of angptl4 on plasma Tg metabolism. Mice deficient in angptl4 exhibit low plasma Tg coinciding with elevated post-heparin plasma LPL activity, while whole-body angptl4 transgenic mice show high Tg levels together with low plasma LPL activity [16]. Subsequent studies employing transgenic mice overexpressing angptl4 specifically in white adipose tissue have shown that this protein significantly decreased post-heparin plasma LPL activity [17]. Numerous studies have provided firm evidence that angptl4 irreversibly inhibits LPL activity which is at least partly accounted for by promoting the conversions of active LPL dimers into inactive monomers [18]. In rodents, angptl4 is most highly expressed in adipose tissue followed by the liver, intestine, heart, kidney and ovary [19]. In these tissues, angptl4 expression is regulated by many signals. Angptl4 was originally identified as PPAR-α and –γ target gene in the liver and adipose tissue, respectively [20,21]. Beside that, a large body of evidence strongly implicates insulin as the major hormone in the regulation of angptl4 expression. Yamada *et al*. reported that angptl4 mRNA expression in 3T3 L1 adipocytes is down-regulated by insulin [22]. Ruge *et al*. [23] showed that acute infusion of insulin caused a highly significant decrease in levels of angptl4 mRNA in adipose tissue from young humans. Collectively, these results indicate that the effects of insulin on LPL could, at least partly, be mediated *via* regulation of angptl4 expression [24,25].

The aim of our study was to test the hypotheses that the expression of angptl4 is deranged in insulin resistance and that dysregulation of LPL in adipose tissue could contribute to development of hypertriglyceridemia. We have used a rat model of insulin resistance, the HHTg rat, challenged with a high sucrose diet for two weeks. We found that LPL activity in adipose tissue was significantly up-regulated in the fasting state and tended to be elevated in the refed state compared to normal Wistar rats on the same diet. The HHTg rats exhibited hyperinsulinemia and decreased expression of angptl4 in the fasting state compared to controls. To our knowledge this is the first study to illustrate an association between blunted down-regulation of adipose tissue LPL activity on fasting and dysregulation of angptl4 expression in an animal model of dyslipidemia. We further demonstrate an impaired ability of HHTg rat adipose tissue to store exogenous fatty acids as Tg. Based on these findings we propose that the dysregulation of angptl4 in adipose tissue is one of several possible mechanisms underlying the development of the dyslipidemia in insulin-resistant states.

2. Experimental Procedures

2.1 Animals and experimental protocol

Male hereditary hypertriglyceridemic rats (HHTg) and control (C) Wistar rats were kept in a temperature-controlled room with a 12:12-h light-dark cycle. Animals had free access to drinking water and diet if not stated otherwise. Wistar rats were obtained from the Institute of Physiology, Czech Academy of Sciences. The strain of HHTg rats was originally selected from Wistar strain rats in our laboratory [26]. All experiments were performed in agreement with the Animal Protection Law of the Czech Republic 311/1997 which is in compliance with European Community Council recommendations for the use of laboratory animals 86/609/ECC and were approved by the ethical committee of IKEM.

Starting at 3 months of age (b. wt. 300±20 g) all animals were fed a high sucrose diet (HSD with 70% of energy derived from sucrose) for 2 weeks. The animals designated as the “refed” group were trained to intake the whole daily food allowance during 3 h (6 a.m. till 9 a.m.), the group designated as “fasted” had free

access to food during the two-week feeding period but were deprived of food for 24 hours prior to decapitation.

2.2 Oral glucose tolerance test (OGTT)

In order to avoid disturbing LPL metabolism, a separate group of animals, subjected to the same feeding protocol as described above, was used for the determination of glucose tolerance. Before the test, the animals were subjected to overnight fasting (16 hours). T_0 blood samples were obtained from fasted animals at 9 a.m. and then the animals were administered a single dose of glucose (3 g/kg b.wt.) intragastrically. Samples of tail vein blood were collected at time intervals 30, 60, 120 and 180 min after glucose administration. The results are expressed as area under the curve (AUC).

2.3 Electrophoretic separation and immunodetection of proteins

Samples of adipose tissue (0.5 g) were harvested *in situ* and stored in liquid nitrogen until further utilization. The homogenate was prepared by Ultra-Turax homogenizer (IKA works, Sweden) in homogenization buffer (150 mM NaCl, 2 mM EDTA, 50 mM Tris, 20 mM glycerolphosphate, 1 mM Na_3VO_4, 2 mM sodium pyrophosphate, 1 mM PMSF, leupeptin 10 µg/ml, aprotinin 10 µg/ml) 1:1 (wt/vol). A crude membrane fraction was prepared by centrifugation of the homogenate at 100000x*g* and solubilisation was carried out in 1% Triton X-100, 0.1% SDS and 0.5% deoxycholate. The proteins were separated by electrophoresis and transferred onto polyvinyl difluoride membranes. The expression of angptl4 was determined using the rabbit polyclonal to FIAF [=angptl4] antibody from Abcam, Cambridge. Phosphorylated forms of mTOR (Ser2448), p70-S6K (Thre389) and PKB (Ser473) were immunodetected using specific rabbit polyclonal antibodies. The total expression of mTOR, p70-S6K and PKB proteins were determined on the same membrane after striping and reblotting using target specific antibodies (mTOR: Cell Signalling, Boston, MA; p70-S6K Abcam, Camebridge UK; PKB: Cell Signalling, Beverly, MA).The expression of β-actin served as a loading control (polyclonal rabbit antibody, Abcam, Camebridge, UK). The bands obtained from immunoblotting were quantified using a FUJI LAS-3000 imager and Quantity One software.

2.4 Adipose tissue incubation procedure

Distal parts of epididymal adipose tissue (approx. 150±25 mg) were rapidly dissected and the tissues were incubated for 2 hours in a Krebs-Ringer bicarbonate buffer with 5 mmol/l glucose, 1 mmol/l unlabeled palmitic acid, 18.5 kBq (U-^{14}C)-palmitate/µmol (UVVR, Czech Republic) and 4% bovine serum albumin, in a gas phase of 95% O_2 and 5% CO_2. All the incubations were carried out at 37°C in sealed vials in a shaking water bath.

2.5 Measurement of lipogenesis in adipose tissue *in vitro*

Estimation of ^{14}C-palmitate incorporation into neutral lipids was carried out after extraction in chloroform methanol as described previously [27]. After removal of the aquaeous phase, an aliquot of the chloroform extract was evaporated and reconstituted in scintillation liquid. The radioactivity was then measured by scintillation counting.

2.6 LPL assay

The tissues were stored in liquid nitrogen. Prior to assay, 0.5 g of adipose tissue was homogenized either in 1 ml of 0.05 M Tris–HCl buffer (pH=7.4) containing heparin 0.15 M NaCl and 7 U/ml (heparin extract) or in 0.15 M NaCl, 0.05 M Tris–HCl buffer (pH=7.4), 1% Triton X-100 and 0.5% deoxycholate (detergent extract). The homogenate was centrifuged for 20 min at 12000x*g*, after which the intermediate phase (between the floating fat and the pellet) was used to assay LPL activity and immunoreactivity. The substrate for LPL assay was prepared according to [4]. For this, 200 mg of cold triolein, 24 mg of lecithin and 26 mg of glycerol were dissolved in benzene and mixed together. ^{3}H-triolein in toluene was added (462 kBq) and all the organic solvents were evaporated under a stream of nitrogen. One ml of 6% FFA free albumin was added and the mixture was sonicated (Hielsler sonicator UP200S, amplitude 1) in an ice/water bath according to the following scheme: 3 min continuous sonication, 1 min break, 2 min continuous sonication. This substrate gave reproducible results on inner standard (LPL from bovine milk) and was stable for at least one week. The LPL activity was measured as described by Bengtsson-Olivecrona and Olivecrona [28]. 80 µl of the homogenate was incubated for 60 min at 26°C with the substrate in the presence of 10 µl of heat inactivated serum from fasted rats (as the source of apolipoprotein CII), 10 µl of radioactive substrate and 100 µl of reaction buffer containing 0.3 M Tris, 0.2 M NaCl, 0.02% heparin and 12% FFA free BSA. The total volume was 200 µl. After termination of the reaction, the released fatty acids were extracted according to [29] and counted for radioactivity.

2.7 Biochemical analysis

FFA, insulin, Tg and serum content of glucose were determined using commercially available kits (FFA: FFA half micro test, Roche Diagnostics GmbH Germany; triglycerides: Pliva-Lachema Czech Republic; glucose: Pliva-Lachema Czech Republic; insulin: Mercodia, Sweden).

2.8 Statistical analysis

Data are presented as mean ± SEM. Statistical analyses were performed using Kruskal-Wallis test with multiple comparisons. Differences were considered statistically significant at the level of $P<0.05$. The correlation between LPL activity and angptl4 protein expression in adipose tissue was evaluated using Spearman´s correlation coefficient.

3. Results and Discussion

3.1 Characterics of the experimental groups and effects of a high sucrose diet

HHTg rats kept on laboratory chow diet had increased levels of serum Tg and FFA, but they did not differ from control rats in their levels of glucose or insulin. The weight gain was comparable in both groups during the two week feeding period (control rats: 32±8; HHTg rats: 38±5 g, n=6 in each group). Changing to a high sucrose diet (HSD) led to a significantly higher increase in serum Tg in refed HHTg animals compared to refed controls (120 *vs* 50%) and to a higher level of serum FFA in fasted HHTg animals compared to fasted controls (30% *vs* no increase). In contrast to the control animals, the HHTg rats kept for two weeks on HSD developed glucose intolerance as determined by OGTT. Further disturbances of glucose metabolism manifested themselves in insulinemia both in the fasted and the refed HHTg animals and in glycemia in the refed HHTg animals (Table 1).

3.2 The ability of adipose tissue of HHTg rats to store exogenous fatty acids was impaired

As the HHTg rats display elevated serum FFA levels, we tested whether the ability of their adipose tissue to esterify and store fatty acids as Tg was impaired. For this, the incorporation of ^{14}C-palmitic acid into triacylglycerols during incubation of adipose tissue explants *in vitro* at two concentrations of albumin-bound palmitate (0.5 mM and 1 mM) was investigated. The results showed that the adipose tissue of HHTg rats exhibited lower ability than that of control animals to entrap FFA from the medium and store them as Tg (Figure 1). This was the case both at low (0.5 mM) and at high (1 mM) concentrations of exogenous palmitate. As calculated from these data, an increase of the FFA concentration in the medium from 0.5 to 1 mM resulted in a 100% increase in incorporation into Tg in control rats but only a 27% increase in the HHTg rats. Our previous results showed that insulin signalling with

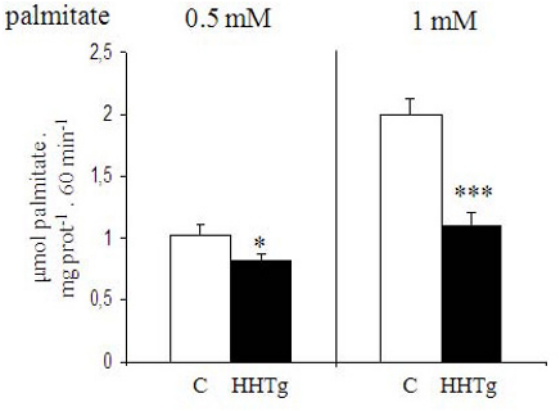

Figure 1. Palmitate incorporation into the adipose tissue in control and insulin resistant HHTg rats *in vitro*. Parts of epididymal adipose tissue were incubated in the presence of 0.5 mM (A) or 1 mM (B) palmitic acid. The utilization of palmitate was determined according to the incorporation into neutral Tg. Data are given as mean ± S.E.M., n=6. open bars = controls, closed bars = HHTg. * $P<0.05$ HHTg *vs* C; *** $P<0.001$ HHTg *vs* C

		control		HHTg	
HSD administration (days)		0	14	0	14
s-Tg (mmol/l)	fasted	0.9±0.04	1.3±0.12	2±0.5^{x}	1.7±0.2
	refed	0.9±0.07	1.4±0.18	2.7±0.4^{x}	5.9±0.4*
s-FFA (mmol/l)	fasted	0.8±0.03	0.8±0.05	1±0.08^{x}	1.3±0.07*
	refed	0.5±0.05	0.57±0.04	0.6±0.04	0.6±0.2
s-glucose (mmol/l)	fasted	4.8±0.3	4.9±0.4	4.2±0.4	4.5±0.2
	refed	7.3±0.1	7.2±0.1	7.7±0.2	8.6±0.2*
s-insulin (pmol/l)	fasted	48±5	61±9	55±8	103±13*
	refed	122±10	195±15	165±12	400±35*
AUC (mmol/180 min)		973±35	1068±48	1039±25	1325±33*

Table 1. Effects of high sucrose diet on the characteristics of control and HHTg animals. Data are given as means ± SEM, n=6. ^{x} $P<0.05$ HHT/0 *vs* C/0; * $P<0.05$ HHTg/14 *vs* C/14

respect to glucose metabolism is impaired in adipose tissue of the HHTg rats [30]. In that study we found no differences between glucose incorporation into Tg in the basal state, but the effect of insulin was 70% higher in the control rats compared with HHTg rats (3.4±0.2 *vs* 2±0.16 µmol glucose g^{-1}, P<0.01). The diminished uptake of exogenous fatty acids by adipose tissue in HHTg rats may thus be explained by the impaired transport of glucose into adipocytes and thereby its lower availability for fatty acid esterification.

3.3 Adipose tissue heparin-releasable LPL activity is higher in HHTg rats

Both total (detergent extractable) and heparin-releasable LPL activity extracted from adipose tissue of the HHTg animals was significantly higher than in controls (Figure 2A,B). One explanation for this difference might be stimulation of LPL synthesis as a consequence of the general stimulatory effect of insulin on protein synthesis *via* mTOR/p70-S6K signalling pathway that represents the link between insulin and nutrient signalling and cellular growth. As it was previously demonstrated, in some tissues, *i.e.* mesangial cells or in renal cortex [11,12] the sensitivity of mTOR/p70-S6K pathway is preserved in insulin resistance and this pathway is being continuously over-stimulated due to the persisting hyperinsulinemia. Nevertheless, insulin signalling was significantly attenuated in white adipose tissue of HHTg rats (Figure 3). The effect of refeeding on mTOR/p70-S6K pathway was significantly lower in the HHTg group compared with controls (Figure 3A,B) as well as the phosphorylation of PKB (Figure 3C). Based on these results we do not suppose that the increased LPL synthesis due to the hyperinsulinemia may explain the elevated LPL activity in fasted HHTg rats.

3.4 The increased activity of adipose tissue LPL in HHTg rats is associated with low expression of angptl4

The previously held idea was that the default state of LPL in adipose tissue is a low, basal activity state that can be temporarily up-regulated after meals. The observation of Bergo *et al*. [15], and the discovery of the role of angiopoietin-like proteins as nutrient-dependent regulators of lipid metabolism [24,25], advocated another perspective on the regulation of LPL. According to this view, the LPL default state is characterised by high synthesis of LPL protein from a stable mRNA, where the newly synthesized LPL is processed mainly into the catalytically active form. During periods of caloric restriction, the LPL activity may be suppressed by transcriptional up-regulation of the fasting-induced protein angptl4 [24]. The observation that insulin rapidly inhibits angptl4 mRNA expression [31] indicates that this protein could modulate the short-time changes in adipose tissue LPL activity that depends on nutrition. Furthermore, Sukonina *et al*. demonstrated an inverse relation between LPL activity and angptl4 mRNA levels [24] in adipose tissue of normal Sprague Dawley rats.

In our experiments, the LPL activity in adipose tissue of refed HHTg rats was marginally, but significantly, increased compared to normal Wistar rats. The difference was, however, larger in the fasted animals. The elevation of LPL activity in fasted HHTg rats indicated a blunted ability of the HHTg rats to down-regulate LPL activity upon fasting, just like what was previously described for old, obese and presumably insulin resistant normal rats [32]. Analyses of the angptl4 protein by Western blots demonstrated slightly lower levels of angptl4 in refed HHTg rats compared to controls. The HHTg rats showed little or no change in

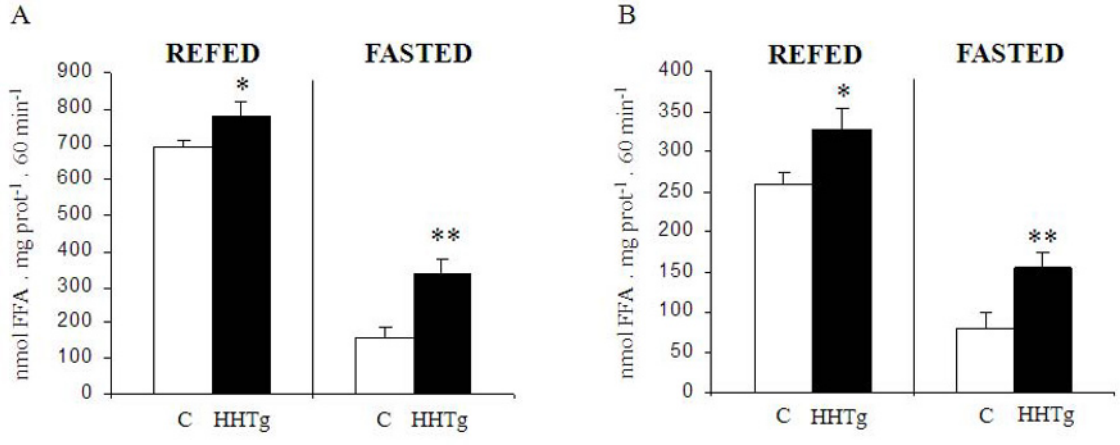

Figure 2. LPL activity in the adipose tissue in control and insulin resistant HHTg rats. A: LPL activity in detergent extract; B: LPL activity in heparin extract. Data are given as mean ± S.E.M., n=6. open bars = controls, closed bars = HHTg. * P<0.05 HHTg *vs* C; ** P<0.01 HHTg *vs* C

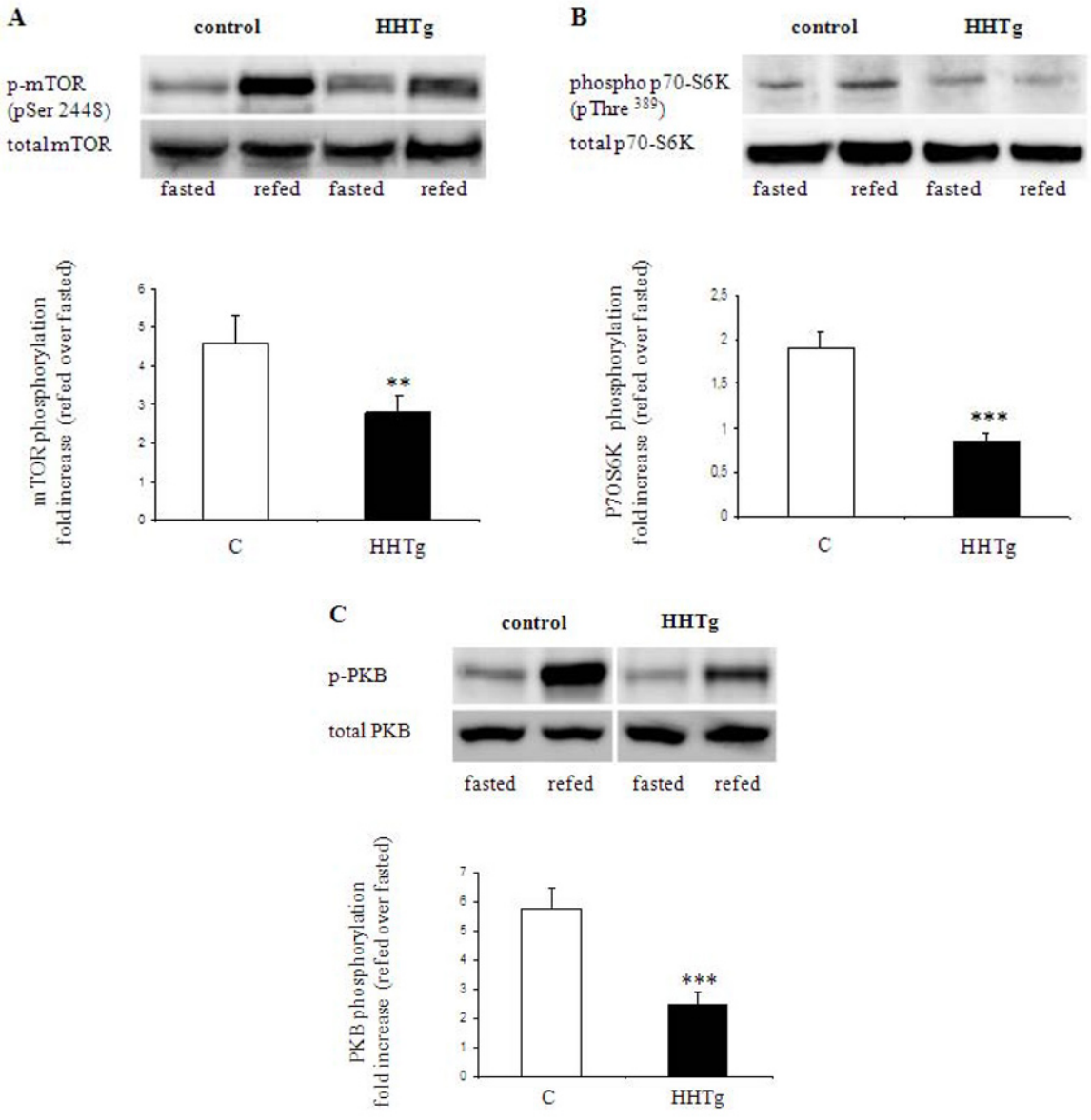

Figure 3. Insulin signaling in adipose tissue of HHTg and control rats. A: mTOR; B:p70-S6K; C: PKB phosphorylation. All results are expressed as a fold increase in the refed relative to the fasted animals. Representative Western blots are shown in the upper part of the figure. The total mTOR or total p70-S6K expression was determined after striping the membrane and reblotting with anti-mTOR, anti p70-S6K or anti-PKB antibodies. Values represent means ± S.E.M., n=6 animals in each group. ** $P<0.01$; *** $P<0.001$ HHTg *vs* control

the expression of angptl4 in going from the refed to the fasted state, while in the control rats the level of angptl4 protein increased about two-fold upon fasting (Figure 4). The angptl4 expression in adipose tissue exhibited a negative correlation with LPL activity (r^2=-0.86) in control rats. In the HHTg rats, no relationship between LPL activity and angptl4 appeared to exist (Figure 5). These findings indicate that in the HHTg rats, most of the newly synthesised LPL protein remains in the active form regardless of nutritional status. We propose that this is due to the hyperinsulinemia that continuously suppresses the expression of angptl4 and allows LPL to remain active also in fasted animals.

Hypertriglyceridemia is a common lipid abnormality in patients with diabetes. Several authors [33,34] have suggested that a diminished clearance of Tg-rich lipoproteins caused by reduced LPL activity is one of several possible underlying mechanisms. Based on our results, we propose that two alternative mechanisms operate in the HHTg rat model of insulin resistance. One

is that the ability of adipose tissue to esterify fatty acids and store them as Tg is seriously impaired, probably as a consequence of lower glucose availability due to the insulin resistance. The other is a lack of control of adipose tissue LPL activity in the fasted state due to insensitivity of the transcriptional regulation of angptl4, probably resulting from the continuous hyperinsulinemia in these animals. Failure to down-regulate LPL activity and the reduced ability of adipose tissue to store Tg could result in excessive generation of circulating FFA, in addition to the FFA generated from intracellular lipolysis. This could in turn lead to product inhibition of LPL at the sites of lipolysis at the endothelium [35] and therefore to inefficient hydrolysis of Tg-rich lipoproteins. Furthermore, the FFA are to a large extent taken up in the liver where they are transformed into VLDL and secreted as such into the circulation, thus contributing to the hypertriglyceridemia.

Acknowledgements

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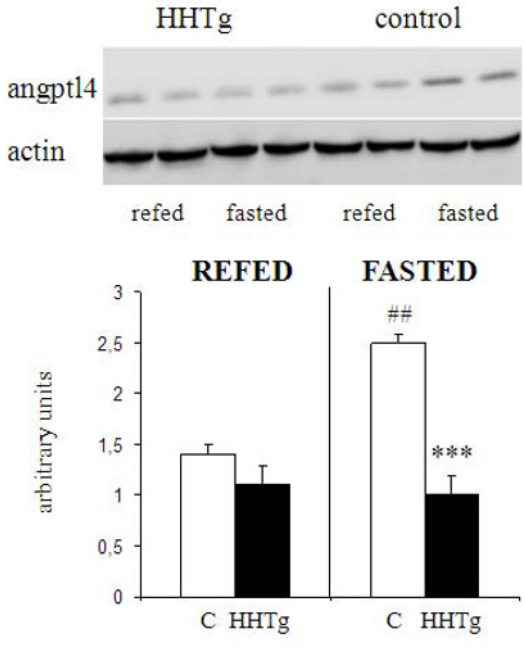

Figure 4. Angptl4 expression in adipose tissue in fasted and refed HHTg and control rats. Values represent means ± S.E.M., n=6 in each group. *** P<0.001 HHTg *vs* control; ## P<0.01 fasted *vs* refed.

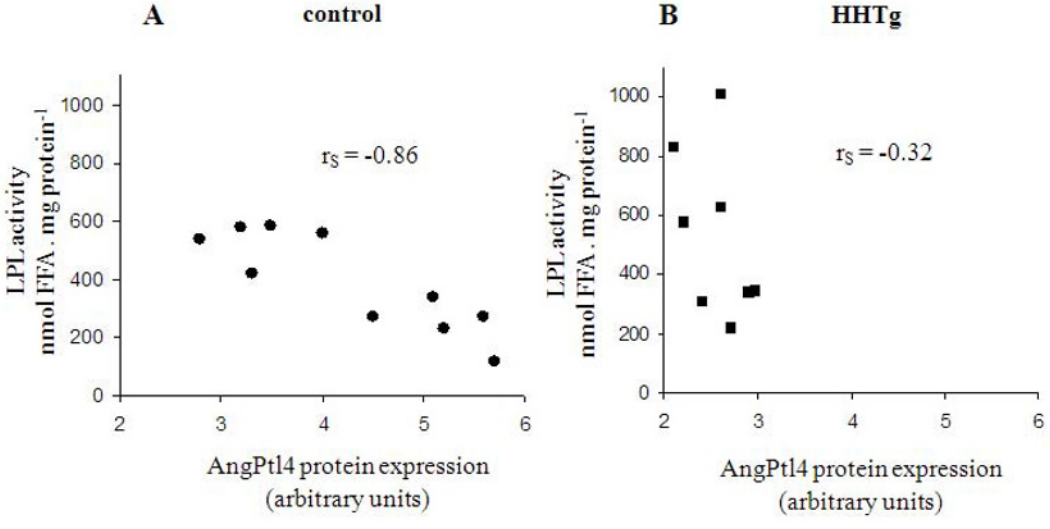

Figure 5. Correlation between LPL activity and angPtl4 protein expression in adipose tissue. A: control rats; B: HHTg rats. Heparin-releasable LPL activity was determined as the release of ^{3}H-labeled fatty acids from emulgated ^{3}H-triolein. AngPtl4 expression was estimated by immunoblotting. r_s represents Spearman´s correlation coeficient.

References

[1] Preiss-Landl K., Zimmermann R., Hammerle G., Zechner R., Lipoprotein lipase: the regulation of tissue specific expression and its role in lipid and energy metabolism, Curr. Opin. Lipidol., 2002, 13, 471-481

[2] Hultin M., Savonen R., Olivecrona T., Chylomicron metabolism in rats: lipolysis, recirculation of triglyceride-derived fatty acids in plasma FFA, and fate of core lipids as analyzed by compartmental modelling, J. Lipid. Res., 1996, 37, 1022-1036

[3] Bickerton A.S., Roberts R., Fielding B.A., Hodson L., Blaak E.E., Wagenmakers A.J., et al., Preferential uptake of dietary Fatty acids in adipose tissue and muscle in the postprandial period, Diabetes, 2007, 56, 168-176

[4] Sadur C.N., Eckel R.H., Insulin stimulation of adipose tissue lipoprotein lipase. Use of the euglycemic clamp technique, J. Clin. Invest., 1982, 69, 1119-1125

[5] Ashby P., Robinson D.S., Effects of insulin, glucocorticoids and adrenaline on the activity of rat adipose-tissue lipoprotein lipids, Biochem. J., 1980, 188, 185-192

[6] Eckel R.H., Lipoprotein lipase. A multifunctional enzyme relevant to common metabolic diseases, N. Engl. J. Med., 1989, 320, 1060-1068

[7] Boivin A., Montplaisir I., Deshaies Y., Postprandial modulation of lipoprotein lipase in rats with insulin resistance, Am. J. Physiol., 1994, 267, E620-E627

[8] Khamzina L., Veilleux A., Bergeron S., Marette A., Increased activation of the mammalian target of rapamycin pathway in liver and skeletal muscle of obese rats: possible involvement in obesity-linked insulin resistance, Endocrinogy, 2005, 146, 1473-1481

[9] Ueno M., Carvalheira J.B., Tambascia R.C., Bezerra R.M., Amaral M.E., Carneiro E.M., et al, Regulation of insulin signalling by hyperinsulinaemia: role of IRS-1/2 serine phosphorylation and the mTOR/p70 S6K pathway, Diabetologia, 2005, 48, 506-518

[10] Lynch C.J., Hutson S.M., Patson B.J., Vaval A., Vary T.C., Tissue-specific effects of chronic dietary leucine and norleucine supplementation on protein synthesis in rats, Am. J. Physiol. Endocrinol. Metab., 2002, 283, E824-E835

[11] Mori H., Inoki K., Masutani K., Wakabayashi Y., Komai K., Nakagawa R., et al, The mTOR pathway is highly activated in diabetic nephropathy and rapamycin has a strong therapeutic potential, Biochem. Biophys. Res. Commun., 2009, 384, 471-475

[12] Zdychova J., Kazdova L., Pelikanova T., Lindsley J.N., Anderson S., Komers R., Renal activity of Akt kinase in obese Zucker rats, Exp. Biol. Med., 2008, 233, 1231-1241

[13] Bergo M., Olivecrona G., Olivecrona T., Diurnal rhythms and effects of fasting and refeeding on rat adipose tissue lipoprotein lipase, Am. J. Physiol., 1996, 271, E1092-E1097

[14] Bergo M., Olivecrona G., Olivecrona T., Forms of lipoprotein lipase in rat tissues: in adipose tissue the proportion of inactive lipase increases on fasting, Biochem. J., 1996, 313, 893-898

[15] Bergo M., Wu G., Ruge T., Olivecrona T., Down-regulation of adipose tissue lipoprotein lipase during fasting requires that a gene, separate from the lipase gene, is switched on, J. Biol. Chem., 2002, 277, 11927-11932

[16] Mandard S., Zandbergen F., van Straten E., Wahli W., Kuipers F., Muller M., et al., The fasting-induced adipose factor/angiopoietin-like protein 4 is physically associated with lipoproteins and governs plasma lipid levels and adiposity, J. Biol. Chem., 2006, 281, 934-944

[17] Lichtenstein L., Berbee J.F., van Dijk S.J., van Dijk K.W., Bensadoun A., Kema I.P., et al., Angptl4 upregulates cholesterol synthesis in liver via inhibition of LPL- and HL-dependent hepatic cholesterol uptake, Arterioscler. Thromb. Vasc. Biol., 2007, 27, 2420-2427

[18] Yau M.H., Wang Y., Lam K.S., Zhang J., Wu D., Xu A., A highly conserved motif within the NH2-terminal coiled-coil domain of angiopoietin-like protein 4 confers its inhibitory effects on lipoprotein lipase by disrupting the enzyme dimerization, J. Biol. Chem., 2009, 284, 11942-11952

[19] Mattijssen F., Kersten S., Regulation of triglyceride metabolism by Angiopoietin-like proteins, Biochim. Biophys. Acta, 2011, DOI: 10.1016/j.bbalip.2011.10.010

[20] Kersten S., Mandard S., Tan N.S., Escher P., Metzger D., Chambon P., et al., Characterization of the fasting-induced adipose factor FIAF, a novel peroxisome proliferator-activated receptor target gene, J. Biol. Chem., 2000, 275, 28488-28493

[21] Yoon J.C., Chickering T.W., Rosen E.D., Dussault B., Qin Y., Soukas A., et al., Peroxisome proliferator-activated receptor gamma target gene encoding a novel angiopoietin-related protein associated with adipose differentiation, Mol. Cell. Biol., 2000, 20, 5343-5349

[22] Yamada T., Ozaki N., Kato Y., Miura Y., Oiso Y., Insulin downregulates angiopoietin-like protein 4 mRNA in 3T3-L1 adipocytes, Biochem. Biophys. Res. Commun., 2006, 347, 1138-1144

[23] Ruge T., Sukonina V., Kroupa O., Makoveichuk E., Lundgren M., Svensson M.K., et al., Effects of hyperinsulinemia on lipoprotein lipase, angiopoietin-like protein 4, and glycosylphosphatidylinositol-anchored high-density lipoprotein binding protein 1 in subjects with and without type 2 diabetes mellitus, Metabolism, 2011, DOI: 10.1016/j.metabol.2011.09.014

[24] Sukonina V., Lookene A., Olivecrona T., Olivecrona G., Angiopoietin-like protein 4 converts lipoprotein lipase to inactive monomers and modulates lipase activity in adipose tissue, Proc. Natl. Acad. Sci. USA, 2006, 103, 17450-17455

[25] Li C., Genetics and regulation of angiopoietin-like proteins 3 and 4, Curr. Opin. Lipidol., 2006, 17, 152-156

[26] Vrana A., Kazdova L., The hereditary hypertriglyceridemic nonobese rat: an experimental model of human hypertriglyceridemia, Transplant. Proc., 1990, 22, 2579

[27] Pravenec M., Kazdova L., Cahova M., Landa V., Zidek V., Mlejnek P., et al., Fat-specific transgenic expression of resistin in the spontaneously hypertensive rat impairs fatty acid re-esterification, Int. J. Obes., 2006, 30, 1157-1159

[28] Bengtsson-Olivecrona G., Olivecrona T., Assay of lipoprotein lipase and hepatic lipase, In: Skinner R., Converse C. (Eds.) Lipoprotein analysis. A practical approach, Oxford University Press, Oxford, 1992

[29] Belfrage P., Vaughan M., Simple liquid-liquid partition system for isolation of labeled oleic acid from mixtures with glycerides, J. Lipid. Res., 1969, 10, 341-344

[30] Cahova M., Vavrinkova H., Meschisvilli E., Markova I., Kazdova L., The impaired response of non-obese hereditary hypertriglyceridemic rats to glucose load is associated with low glucose storage in energy reserves, Exp. Clin. Endocrinol. Diabetes, 2004, 112, 549-555

[31] Koster A., Chao Y.B., Mosior M., Ford A., Gonzalez-DeWhitt P.A., Hale J.E., et al., Transgenic angiopoietin-like (angptl)4 overexpression and targeted disruption of angptl4 and angptl3: regulation of triglyceride metabolism, Endocrinology, 2005, 146, 4943-4950

[32] Bergo M., Olivecrona G., Olivecrona T., Regulation of adipose tissue lipoprotein lipase in young and old rats, Int. J. Obes. Relat. Metab. Disord., 1997, 21, 980-986

[33] Simsolo R.B., Ong J.M., Saffari B., Kern P.A., Effect of improved diabetes control on the expression of lipoprotein lipase in human adipose tissue, J. Lipid. Res., 1992, 33, 89-95

[34] Taylor K.G., Galton D.J., Holdsworth G., Insulin-independent diabetes: a defect in the activity of lipoprotein lipase in adipose tissue, Diabetologia, 1979, 16, 313-317

[35] Bengtsson G., Olivecrona T., Lipoprotein lipase. Mechanism of product inhibition., Eur. J. Biochem., 1980, 106, 557-562