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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

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ABSTRACT

Our aims were to compare the systemic effects of insulin on lipoprotein lipase (LPL) in tissues from subjects with different degrees of insulin sensitivity. The effects of insulin on LPL during a 4-hour hyperinsulinemic, euglycemic clamp were studied in skeletal muscle, adipose tissue, and postheparin plasma from young healthy subjects (YS), older subjects with type 2 diabetes mellitus (DS), and older control subjects (CS). In addition, we studied the effects of insulin on the expression of 2 recently recognized candidate genes for control of LPL activity: angiopoietin-like protein 4 (ANGPTL4) and glycosylphosphatidylinositol-anchored high-density lipoprotein binding protein 1. As an effect of insulin, LPL activity decreased by 20% to 25% in postheparin plasma and increased by 20% to 30% in adipose tissue in all groups. In YS, the levels of ANGPTL4 messenger RNA in adipose tissue decreased 3-fold during the clamp. In contrast, there was no significant change in DS or CS. Regression analysis showed that the ability of insulin to reduce the expression of ANGPTL4 was positively correlated with M-values and inversely correlated with factors linked to the metabolic syndrome. Expression of glycosylphosphatidylinositol-anchored high-density lipoprotein binding protein 1 tended to be higher in YS than in DS or CS, but the expression was not affected by insulin in any of the groups. Our data imply that the insulin-mediated regulation of LPL is not directly linked to the control of glucose turnover by insulin or to ANGPTL4 expression in

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adipose tissue or plasma. Interestingly, the response of ANGPTL4 expression in adipose tissue to insulin was severely blunted in both DS and CS.

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1. Introduction

Lipoprotein lipase (LPL) plays a central role for the clearance of triglyceride (TG)-rich plasma lipoproteins. The enzyme is primarily expressed in parenchymal cells of the adipose tissue, skeletal muscle, and heart and is secreted to the endothelium of local blood vessels [1]. The amount of LPL in this "endothelial pool" can be approximately estimated in plasma after intravenous injection of heparin that releases LPL into the blood.

Insulin is a key regulator of LPL [2-4]. In healthy humans, acute insulin infusion increases LPL messenger RNA (mRNA) and activity in adipose tissue [5-7] and decreases LPL mRNA and activity in skeletal muscle [5,8]. Kovár et al [9] found that insulin infusion reduces the LPL activity in postheparin plasma, but no effect has also been reported [10].

Type 2 diabetes mellitus and insulin resistance are associated with hypertriglyceridemia, low high-density lipoprotein (HDL) cholesterol, and high free fatty acid (FFA) levels [11]. Both increased very low-density lipoprotein secretion from the liver and decreased clearance of TG-rich plasma lipoproteins contribute to postprandial hypertriglyceridemia[12,13]. A reduction in LPL activity in postheparin plasma and in adipose tissue has been reported [14-17], whereas data are inconsistent with respect to LPL activity in skeletal muscle [18-21].

Angiopoietin-like protein 4 (ANGPTL4) is a peroxisome proliferator-activated receptor–regulated member of the angiopoietin-like protein family and has been shown to influence plasma TG metabolism by decreasing LPL activity [22-24]. The role of ANGPTL4 for regulation of LPL in humans is not yet known. One study reports decreased concentrations of circulating ANGPTL4 in subjects with type 2 diabetes mellitus [25]. ANGPTL4 levels in plasma were reported to be related to overall body adiposity and to be positively correlated to plasma FFA in the fasting state [26-29], and variations in the ANGPTL4 gene correlate to plasma TG levels [30-32].

Glycosylphosphatidylinositol-anchored high-density lipoprotein binding protein 1 (GPIHBP1) is a novel endothelial protein that mediates transendothelial transport of LPL and appears to be a main binding site for LPL on the luminal side of capillaries in the skeletal muscle, heart, and adipose tissue [33,34]. In experiments on mice, it was found that GPIHBP1 is regulated by feeding/fasting and by ligands for peroxisome proliferator-activated receptor–γ [35]. The importance of insulin for regulation of GPIHBP1 is unknown, and there are no previous studies on regulation of GPIHBP1 in vivo in humans.

Although studied for several decades, the regulation of LPL by insulin is still poorly understood in humans. This may partly be due to lack of integrative studies of LPL in well-characterized individuals under controlled conditions. In the present study, we have investigated subjects with and without type 2 diabetes mellitus with different degrees of insulin sensitivity to address the acute effects of insulin on LPL in adipose tissue, muscle, and postheparin plasma as well as on the recently recognized LPL-regulating factors, ANGPTL4 and GPIHBP1.

2. Research design and methods

2.1. Subjects

Characteristics of the subjects are shown in Table 1. Three groups matched for sex (male/female, 4/6) were recruited; young, healthy subjects (YS; n = 10; mean age, 26 years; body mass index [BMI], 22 kg/m^2; range, 20-26 kg/m^2); patients with type 2 diabetes mellitus (DS; n = 10; mean age, 61 years; BMI, 27 kg/m^2; range, 22-31 kg/m^2); and control subjects matched for age and BMI to the diabetic group (CS; n = 10; mean age, 60 years; BMI, 27 kg/m^2; range, 22-30 kg/m^2). The groups were previously described in a study on insulin-mediated regulation of inflammatory markers and adipokines [36].

2.2. Protocol

Subjects attended 3 different study days within a period of 2 months. On one study day, the subjects underwent a 4-hour hyperinsulinemic (56 mU/[m^2 min]), euglycemic clamp as previously described [36]; and biopsies from adipose tissue and skeletal muscle were taken before and at the end of the clamp. On another day, the subjects underwent a similar clamp; but this time, heparin (100 U/kg body weight) was

Table 1 – Characteristics of the study participants

	YS (n = 10)	CS (n = 10)	DS (n = 10)
Sex (M/F)	4/6	4/6	4/6
Age (y)	26 ± 2	60 ± 2 †	61 ± 3 ‖
BMI (kg/m^2)	22.5 ± 1	27.4 ± 1.0 †	27.5 ± 1.1 ‖
Body fat (%)	20 ± 2	36 ± 3 †	36 ± 2 ‖
WHR	0.82 ± 0.02	0.88 ± 0.02 *	0.92 ± 0.02 ‖
Serum glucose (mmol/L)	4.1 ± 0.1	4.5 ± 0.2 †	8.0 ± 0.4 ‡,‖
Serum insulin (mU/L)	5.0 ± 0.6	7.0 ± 0.7 †	10.0 ± 0.1 ‡,‖
Serum C-peptide (pmol/L)	580 ± 60	570 ± 60	940 ± 120 ‡,‖

Blood samples were collected from subjects fasted overnight. Data are expressed as means ± SEM. Student unpaired t test was used for analyses of the P values.
* $P < .05$, CS compared with YS.
† $P < .01$, CS compared with YS.
‡ $P < .01$, DS compared with CS.
‖ $P < .01$, DS compared with YS.

injected 10 minutes before the end of the clamp, and a postheparin plasma sample was collected in heparinized tubes 10 minutes later for analysis of LPL. The order of the 2 clamps was randomly assigned. On yet another day, the subjects arrived in the morning after a 10-hour fast; and basal blood samples were collected. Afterward, heparin was injected; and a blood sample was collected 10 minutes later to obtain postheparin plasma also in the basal state.

Angiopoietin-like protein 4 in basal and postheparin plasma was measured by the enzyme-linked immunosorbent assay kit from R&D systems (Abingdon, UK catalog no. DY3485) with slight modifications of the protocol to improve the sensitivity of the assay, including addition of 0.1% Tween 20 to the samples. Free fatty acids in plasma were measured with an enzymatic kit (Wako Chemicals, Neuss, Germany). Other blood chemistry was performed as previously described [36].

2.3. Biopsies

Following local dermal anesthesia (Xylocaine; AstraZeneca, Södertälje, Sweden), biopsies were taken from abdominal subcutaneous adipose tissue and skeletal muscle (musculus tibialis anterior). Biopsies were prepared as previously described and frozen at −70°C for subsequent analyses of LPL activity and mass [37]. Separate pieces were frozen for preparation of RNA. Adipocyte cell size and basal as well as insulin-stimulated glucose uptake were analyzed as previously reported [38]. The average fat cell diameter was significantly larger in DS and CS compared with that in YS: 105 ± 4 μm for CS, 107 ± 2 μm for DS, and 89 ± 2 μm for YS ($P < .05$).

2.4. Analyses of LPL, ANGPTL4, and GPIHBP1

Lipoprotein lipase activity and mass were measured as described previously [39]. Lipoprotein lipase mRNA was analyzed in both muscle and adipose tissue, whereas ANGPTL4 and GPIHBP1 mRNA was only analyzed in adipose tissue. Total RNA was extracted from adipose tissue using TRIzol reagent (Invitrogen, Stockholm, Sweden) and was treated with DNA-free kit (Ambion, Stockholm, Sweden). Complementary DNA was prepared, and the levels of LPL mRNA were determined by real-time polymerase chain reaction in comparison to β-actin mRNA as previously described [40]. The levels of ANGPTL4 mRNA were quantified similarly. The sequences for probe and primers used were as follows: 5′-Fam-TTCTTGTCCCTGCGGAGGTCGTGAT-TAMRA (ANGPTL4 probe), 5′-GGCCTCTCCGTACCCTTCTC (ANGPTL4 forward), 5′-TGGCCGTTGAGGTTGGAAT (ANGPTL4 reverse). For GPIHBP1 mRNA levels, the sequences for primers and probe were as follows: 5′-FAM CCCCTGCACCAGCTTTGGAGAATG-TAMRA (GPIHBP1 probe), 5′-GCCCTGCCAGCACTCTGT (forward), 5′-TCGCCCAAGACACTCCAAAT (reverse).

For analysis of ANGPTL4 protein in plasma by Western blots, samples of 1 μL taken before and after 4 hours of insulin clamp were separated on 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis under reducing conditions. After transfer to Hybond C membrane (GE Healthcare, Umeå, Sweden), blotting was performed with rabbit polyclonal antibodies against the epitope CQGTEGSTDLPLAPE of human ANGPTL4 (a kind gift from Dr S Kersten, Wageningen University, the Netherlands) diluted 1:4000, followed by incubation with a horseradish peroxidase–conjugated mouse anti-rabbit IgG antibody (1:25.000, Sigma, Stockholm, Sweden). Peroxidase activity was detected using the Advance ECL detection system (GE Healthcare, Umeå, Sweden) on a ChemiDoc XRS (Bio-Rad, Sundbyberg, Sweden), and analyses was performed using the Quantity One software (Bio-Rad).

2.5. Statistics

Data are expressed as mean ± SEM. All data were tested for normal distribution, and the Student paired and unpaired t tests were used for comparisons. GPIHBP1 data were found to be nonnormally distributed; and therefore, Mann-Whitney U test was used. When appropriate, 2-way analyses of variance and simple and multiple regression analyses were used as indicated. The SPSS (Chicago, IL) software was used for all statistical calculations. Significance was taken as $P < .05$.

3. Results

3.1. Basal measurements of glycemia and blood lipids

Basal data for glucose and lipid metabolism are shown in Tables 1 and 2. As expected, DS showed signs of diabetic dyslipidemia and perturbations in glucose control. YS had significantly lower levels of glucose and insulin compared with YS and CS.

3.2. Effects of insulin on glucose metabolism

As previously reported [36], we observed no significant difference between YS and CS in the insulin-stimulated glucose utilization; and there were no differences in insulin levels during the clamp or in insulin sensitivity index (Fig. 1 and Table 3). DS had significantly lower M-values compared with CS and YS, and their insulin sensitivity index was significantly lower (M-value adjusted for insulin level; Table 3). No significant differences in basal glucose uptake in isolated adipocytes were seen between the 3 groups: 11.6 ± 4.2 fL per cell per second for DS, 8.8 ± 2.0 fL per cell per second for YS, and 16.2 ± 3.5 fL per cell per second for CS. Glucose uptake in isolated adipocytes from DS was increased by 75% ± 10% of basal uptake in the presence of insulin. This was lower than in adipocytes from YS (by 253% ± 61% of basal uptake, $P = .012$) and CS (by 112% ± 14% of basal uptake, $P = .045$; CS vs YS, $P = .055$).

3.3. Effects of insulin on LPL

3.3.1. Adipose tissue

The LPL activity in biopsies from adipose tissue increased by 20% to 30% in all 3 groups during the clamp (Fig. 2A). This reached statistical significance in YS (from 22 to 31 mU/g, $P < .05$), but not in CS (from 27 to 32 mU/g) or DS (from 19 to 24 mU/g). Basal LPL activity levels tended to be lower in DS compared with YS ($P = .10$) and CS ($P = .30$). The levels of LPL mass increased by about 30% in all 3 groups (Table 4). The differences reached statistical significance in DS and YS ($P < .05$), but not in CS ($P = .15$). The levels of LPL mRNA (Table 4)

Table 2 – Effects of insulin on blood lipids during a 240-minute hyperinsulinemic, euglycemic clamp

	YS			CS			DS		
	Basal	120 min	240 min	Basal	120 min	240 min	Basal	120 min	240 min
Serum TG (mmol/L)	1.0 ± 0.1	0.7 ± 0.1	0.6 ± 0.1 ‖	1.2 ± 0.2	0.9 ± 0.1	0.8 ± 0.1	2.7 ± 0.5 †,§	2.1 ± 0.5 *,‡	2.0 ± 0.4 *,‡
Serum cholesterol (mmol/L)	4.4 ± 0.2 ‖	3.8 ± 0.3 ‖	3.8 ± 0.2 ‖	5.7 ± 0.4	5.1 ± 0.4	5.1 ± 0.4	5.4 ± 0.2 †	4.9 ± 0.2 †	4.8 ± 0.2 †
Serum HDL (mmol/L)	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.5 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.1 ± 0.1 *,‡	1.0 ± 0.1 §	1.0 ± 0.1 ‡
Serum LDL (mmol/L)	2.6 ± 0.2 ‖	2.3 ± 0.2 ‖	2.3 ± 0.2 ‖	3.7 ± 0.4	3.3 ± 0.3	3.3 ± 0.4	3.2 ± 0.2 †	3.0 ± 0.2	2.9 ± 0.2
Plasma FFA (μmol/L)	367 ± 57	11 ± 3	1 ± 1	422 ± 56	13 ± 4	8 ± 3	552 ± 61	60 ± 12 †,§	45 ± 7 †,§

The effects of insulin on blood lipids are shown at steady state and at the end of the clamp and compared with basal values analyzed before the clamp. Data are expressed as means ± SEM for each group. Student unpaired *t* test was used for comparisons. n = 10 for each group. LDL indicates low-density lipoprotein.

* $P < .05$, DS compared with YS.
† $P < .01$, DS compared with YS.
‡ $P < .05$, DS compared with CS.
§ $P < .01$, DS compared with CS.
‖ $P < .05$, YS compared with CS.

increased by around 40% in YS ($P < .05$) and 30% in DS (not significant), but did not change in CS. Similar to LPL activity, basal LPL mRNA levels tended to be lower in DS compared with YS ($P < .05$) and CS ($P = .20$).

3.3.2. *Skeletal muscle*

In contrast to adipose tissue, LPL activity in skeletal muscle did not change during the hyperinsulinemic clamp (Fig. 2B). The LPL mass tended to increase in all subjects: from 85 ± 16 to 110 ± 22 ng/g in YS (not significant), from 115 ± 14 to 129 ± 21 in CS (not significant), and from 119 ± 11 to 158 ± 14 ng/g in DS ($P < .05$). There were no significant differences between the groups in absolute values of LPL activity and mass before or after the clamp.

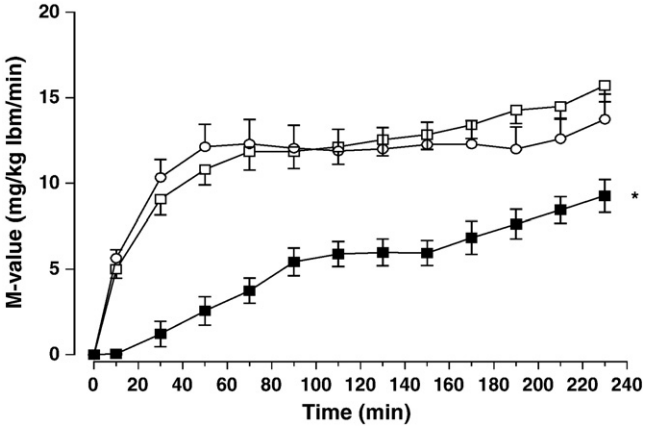

Fig. 1 – Whole-body insulin sensitivity during the hyperinsulinemic, euglycemic clamp. Glucose infusion rate is expressed as milligrams of glucose per lean body mass during the 20-minute intervals between 2 consecutive time points. Open circles = YS, open boxes = CS, and filled boxes = DS. Data are expressed as means ± SEM. *P < .01, DS compared with CS or YS. Two-way analysis of variance was used for comparisons. n = 10 in each group.

3.3.3. *Postheparin plasma*

The activity of LPL in postheparin plasma decreased by 20% to 25% during the hyperinsulinemic clamp (Fig. 2C), and this was statistically significant in all 3 groups ($P < .05$). There were no statistically significant differences in absolute values of basal or insulin-stimulated LPL activity in postheparin plasma between the groups.

3.4. *Effects of insulin on ANGPTL4*

Basal levels of ANGPTL4 mRNA in adipose tissue tended to be highest in YS and lowest in CS, but the differences in the basal levels were not statistically significant between the groups (Fig. 3A). The hyperinsulinemic clamp caused about a 70% reduction in ANGPTL4 mRNA in YS. No such effect was seen in CS or DS. In contrast, a tendency ($P = .14$) for an increase of ANGPTL4 mRNA was observed in DS, whereas no effect of insulin was seen in CS.

Plasma levels of ANGPTL4 protein was first studied by Western blots, but no visible differences could be detected between the groups. Quantification by enzyme-linked immunosorbent assay demonstrated that ANGPTL4 tended to be

Table 3 – M-values, insulin concentrations, and insulin sensitivity index during clamp

	YS (n = 10)	CS (n = 10)	DS (n = 10)
M-value (mg/kg lbm/min)	12.0 ± 0.6	12.3 ± 0.5	5.8 ± 0.4 †,§
Serum insulin (mU/L)	97 ± 3	101 ± 4	121 ± 6 *,‡
ISI	13.0 ± 1.6	12.7 ± 0.9	5.3 ± 0.9 †,§

Insulin was analyzed repeatedly during the clamp ("Research design and methods"). The insulin sensitivity index (ISI) was calculated for each group: 100 × the M-value at steady state (100-160 min)/the average insulin concentration (from 120 and 180 min). Data are expressed as means ± SEM for each group.

* $P < .05$, DS compared with CS.
† $P < .01$, DS compared with CS.
‡ $P < .05$, DS compared with YS.
§ $P < .01$, DS compared with YS.

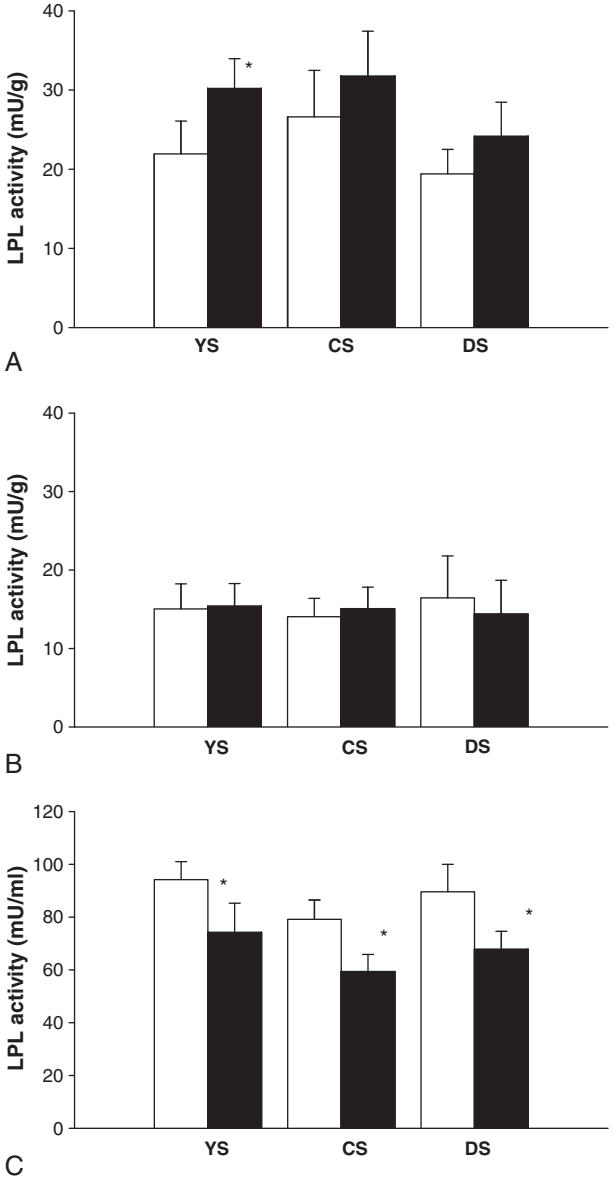

Fig. 2 – Effects of insulin on LPL activity in adipose tissue, skeletal muscle, and postheparin plasma. The LPL activity in homogenates of adipose tissue (A), in homogenates of skeletal muscle (B), and in postheparin plasma (C) was analyzed before (open bars) and after (filled bars) 4 hours of insulin administration. Data are expressed as means ± SEM. *P < .05, when data before and after insulin administration were compared. Student paired t test was used for comparison. n = 10 in each group.

higher in plasma of YS both before and after the clamp when compared with CS and YS (Fig. 3B, borderline significance). As an effect of insulin, plasma ANGPTL4 decreased in YS ($P < .05$) and DS ($P = .07$), but not in CS.

Plasma ANGPTL4 increased significantly in all 3 groups after heparin infusion (Fig. 3C compared with Fig. 3B, $P < .01$). Similarly, insulin caused a significant decrease in plasma ANGPTL4 levels after heparin in YS ($P < .05$) and DS ($P < .05$), but not in CS.

3.5. *Effect of insulin on GPIHBP1*

Hyperinsulinemia during 4 hours had no effect on the level of GPIHBP1 mRNA in adipose tissue from any of the groups (Fig. 4). Expression of GPIHBP1 in DS and CS tended to be lower compared with YS both before and after insulin infusion (P around .20 for these comparisons).

3.6. *Regression analyses on LPL, ANGPTL4, GPIHBP1, and metabolic variables*

Regression analyses were performed in the combined study cohorts. M-values were not significantly associated with insulin-induced changes in LPL activity in adipose tissue, skeletal muscle, or postheparin plasma LPL activity. No association between sex and any of the insulin-induced changes in LPL activity in adipose tissue, skeletal muscle, or postheparin plasma LPL activity was observed. Neither was there any correlation between insulin action on adipocyte glucose uptake and any of the LPL variables. In contrast, the insulin-induced suppression in ANGPTL4 mRNA in adipose tissue was inversely correlated with BMI ($r = –0.59$, $P = .003$), body fat percentage ($r = –0.56$, $P = .006$), waist to hip ratio (WHR) ($r = –0.45$, $P = .033$), age ($r = –0.62$, $P = .001$) , fasting glucose ($r = –0.48$, $P = .019$), and TG levels ($r = –0.51$, $P = .012$); and it was positively associated with M-value ($r = 0.51$, $P = .014$) and HDL cholesterol ($r = 0.42$, $P = .049$). The insulin-induced change in adipose tissue ANGPTL4 mRNA was significantly associated with the concomitant change in plasma TG levels ($r = –0.51$, $P = .016$). In multiple regression analyses adjusting for sex and age, BMI and M-value remained significantly and independently associated with the insulin-induced reduction in ANGPTL4 mRNA (standard β coefficient = –0.36 and 0.36, $P = .024$ and .020, respectively). In addition age, but not sex, contributed significantly to the model (standard β coefficient = – 0.46, $P = .005$). This model explained about 60% of the variability in ANGPTL4 mRNA change during insulin infusion (adjusted $r^2 = 0.61$, $P < .001$). Replacing BMI with body fat percentage or WHR attenuated the model. Expression of GPIHBP1 after insulin was inversely correlated to age ($r = –0.42$, $P < .05$). In contrast to the insulin-induced change, basal levels of GPIHBP1 and ANGPTL4 mRNA did not display any association with anthropometric or metabolic variables.

4. Discussion

We have investigated the response of the LPL system to insulin in patients with type 2 diabetes mellitus compared with nondiabetic young individuals and to age- and BMI-matched elderly control subjects. For this, we studied LPL itself and 2 novel proteins that appear to play major roles for LPL action: ANGPTL4 and GPIHBP1. The main conclusions are that there were relatively small effects on LPL, but large effects on ANGPTL4 gene expression. Insulin tended to cause an increase in LPL mRNA and mass levels, as well as activity, in adipose tissue; and this was similar in all 3 groups of subjects. In contrast, there was a clear reduction in LPL activity in postheparin plasma from all 3 groups. These changes were not related to the insulin sensitivity of the subjects, as reflected

Table 4 – Effects of insulin on LPL mass and mRNA in adipose tissue

	YS		CS		DS	
	Basal	240 min	Basal	240 min	Basal	240 min
LPL mass (ng/g)	122 ± 19	158 ± 25 *	138 ± 20	170 ± 30	119 ± 11	158 ± 14 *
LPL mRNA (LPL/β-actin)	2.9 ± 0.7	5.4 ± 1.2 *	3.5 ± 0.6	3.6 ± 0.9	1.8 ± 0.4 †	2.5 ± 0.2 †

Data are expressed as means ± SEM for each group.
n = 10 for LPL mass measurements. For LPL mRNA measurements, YS: n = 6, CS: n = 7, and DS: n = 9.
* $P < .05$, when data before and at the end of the clamp were compared. Student paired *t* test was used for these analyses of the *P* values.
† $P < .05$, when values of LPL mRNA for DS were compared with YS.

by whole-body or adipocyte glucose uptake measured in vivo and in vitro, respectively. Our study indicates that a major source for LPL activity in postheparin plasma (the heparin-releasable "endothelial pool") after insulin clamp is not directly dependent on the expression of LPL in adipose tissue. The reduction of LPL activity in some other major tissue, most likely skeletal muscle and heart, is likely to be reflected in the decreased LPL activity in postheparin plasma because, in adipose tissue, the activity increased after insulin infusion. Finally, we show that acute insulin infusion caused decreased levels of ANGPTL4 mRNA in adipose tissue from young and healthy subjects; but that was blunted in elderly control subjects and in patients with type 2 diabetes mellitus.

In the present study, the direct effect of insulin on LPL in adipose tissue did not seem to be compromised by insulin resistance. This suggests that the increase of LPL following hyperinsulinemia occurs via mechanisms that are, at least in part, different from those involved in regulation of glucose metabolism. A previous study from our group suggests that skeletal muscle may be the major source of LPL activity in

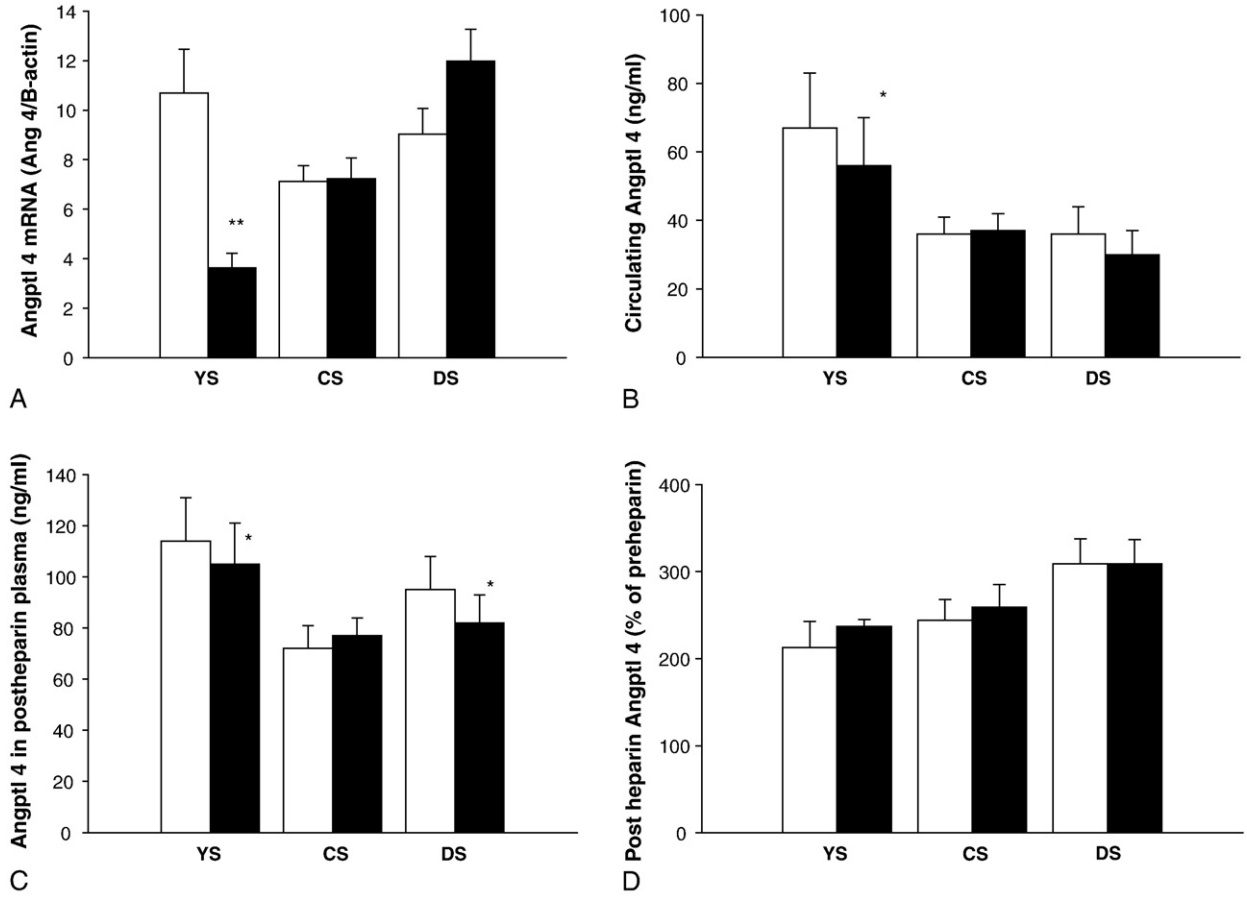

Fig. 3 – Effects of insulin on the levels of ANGPTL4 in adipose tissue and blood/plasma. ANGPTL4 mRNA in adipose tissue and protein mass in blood was analyzed before (open bars) and after 4 hours of insulin administration (filled bars). A, ANGPTL4 mRNA levels in adipose tissue. B and C, ANGPTL4 protein mass in blood plasma before and after heparin infusion, respectively. D, The postheparin concentration of ANGPTL4 expressed as percentage of preheparin (basal) concentration of ANGPTL4 in blood. Data are expressed as means ± SEM. *P < .05 and **P < .001, when data before and after insulin administration were compared. Student paired *t* test was used for comparison. n = 10 in each group.

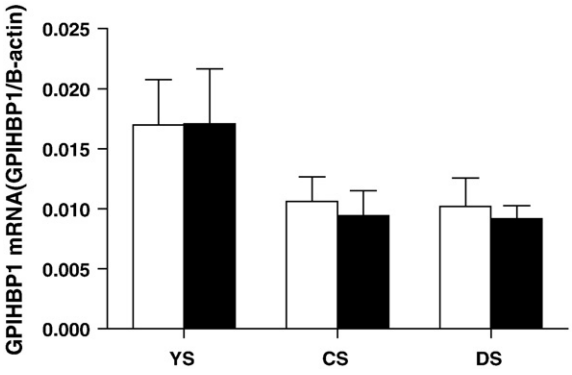

Fig. 4 – Effect of insulin on the levels of GPIHBP1 mRNA in adipose tissue. GPIHBP1 mRNA in adipose tissue was analyzed before (open bars) and after (filled bars) 4 hours of insulin administration. Data are expressed as means ± SEM; n = 6 for YS, n = 9 for CS, and n = 10 for DS.

postheparin plasma in humans [37]. The LPL activity in skeletal muscle, as measured in homogenates of the tissue, did not change during the insulin clamp and did not correlate to whole-body insulin sensitivity. Similarly, postheparin plasma LPL activity was not related to overall insulin sensitivity. Our results are in concert with those of Farese et al [5], and it appears that insulin is not a strong determinant of total LPL activity in skeletal muscle [21].

Glycosylphosphatidylinositol-anchored high-density lipoprotein binding protein 1 is a novel endothelial binding partner for LPL [33]. It was previously shown that the number of binding sites for LPL in the heart is higher during fasting and lower after refeeding [41-44], which is in concert with the general statement that heparin-releasable LPL activity is low in the heart and muscles in the fed state. GPIHBP1 expression in mice is increased in skeletal muscle on fasting and returns to fed levels by 6 hours after refeeding [33,35]. It is therefore an exciting possibility that GPIHBP1 is involved in the rapid nutritional regulation of the "endothelial" pool of LPL in both skeletal muscle and heart. Our studies regarding GPIHBP1 expression were unfortunately restricted to adipose tissue due to lack of sufficient amounts of skeletal muscle tissue. In adipose tissue, we did not see any insulin-dependent changes in GPIHBP1 expression after the 4-hour insulin infusion. No significant correlations between the levels of GPIHBP1 mRNA and postheparin plasma LPL activity could be found. Interestingly, the expression level of GPIHBP1 in adipose tissue tended to be lower in the elderly groups of subjects (both CS and DS) than in young subjects.

The fasting-induced protein ANGPTL4 plays an important role for the regulation of LPL activity in experimental animal models [23,24]. This is explained by the ability of ANGPTL4 to interact with LPL and thereby inactivate the enzyme [45]. Whether ANGPTL4 has a similar role in humans is not known, but loss-of-function mutations of ANGPTL family members, including ANGPTL4, were reported to contribute to plasma TG levels [30-32]. There are studies on the levels of ANGPTL4 in human plasma, [25,28,46-50], but no previous studies of ANGPTL4 expression in human adipose tissue in relation to LPL activity have been reported. We found no significant correlations between the levels of ANGPTL4 mRNA and any of the LPL parameters in adipose tissue (mRNA, mass, or activity). This is in disagreement to the findings in rats, where an inverse correlation was found between ANGPTL4 mRNA and LPL activity [45]. This may indicate that ANGPTL4 does not have a similar modulating function on LPL in humans as in rodents. A similar conclusion was reached by Staiger et al [29] from studies of plasma levels of ANGPTL4 in relation to plasma TG. A possible explanation is that, in humans, the expression of ANGPTL4 in adipose tissue is much lower than in the liver, whereas, in rodents, the situation is the opposite [51]. We saw a strong downregulation of ANGPTL4 mRNA by insulin infusion in YS, resembling its response to food intake in animals [52,53]. However, in both CS and DS, this response was absent. Yamada et al [30,50] demonstrated that insulin failed to decrease the level of ANGPTL4 mRNA in an insulin-resistant 3T3-L1 adipocyte model induced by tumor necrosis factor–α. Our regression analyses suggest that an attenuated effect of insulin to suppress ANGPTL4 expression in adipose tissue is linked to insulin resistance and BMI and may contribute to the hypertriglyceridemia found in insulin-resistant conditions. Interestingly, a lack of response of ANGPTL4 to insulin was also seen in the CS group.

We also assessed blood levels of the ANGPTL4 protein. We found lower levels following insulin infusion, and this was most evident in the YS group. To quantify the endothelial amount of ANGPTL4, the concentration of ANGPTL4 in plasma after heparin infusion was subtracted with the value before heparin infusion. This pool of heparin-releasable ANGPTL4 seemed to be higher in DS when compared with CS ($P < .05$) and YS (borderline significant). We have visualized this in Fig. 3, showing the postheparin-releasable pool of ANGPTL 4 as a percentage of basal circulating ANGPTL4. No significant effect of insulin was observed. Linear regression analyses revealed an independent (tested for BMI and age) negative association of the heparin-releasable pool of ANGPTL4 with M-value ($r = 0.35$, $P = .07$) and positive association to fasting glucose ($r = 0.48$, $P < .05$) concentration as well as to hemoglobin A_{1c} ($r = 0.56$, $P < .05$). No association to any LPL parameters was found. Taken together, these data suggest that an increase in the heparin-releasable pool of ANGPTL4 in the vasculature may contribute to the impaired TG clearance found in type 2 diabetes mellitus. However, the hypothesized link to LPL activity remains unclear.

We acknowledge that the number of subjects in this study was low. However, subjects underwent a sophisticated protocol and were studied with cumbersome investigations. The subjects were well matched for sex in all 3 groups and also for age in DS and CS, which should support appropriate evaluation of the key questions related to insulin resistance and type 2 diabetes mellitus. We believe that the results presented here provide interesting observations that warrant further studies.

In conclusion, we have found that an acute hyperinsulinemia under euglycemic conditions increases LPL activity in subcutaneous adipose tissue, whereas the total pool of endothelial LPL released into plasma after heparin infusion is reduced. These effects did not differ significantly between our 3 study groups of young or older nondiabetic subjects and patients with type 2 diabetes mellitus. Our data imply

that the insulin-mediated regulation of LPL is not directly linked to the control of glucose turnover by insulin. In contrast, the insulin-mediated regulation of ANGPTL4 was blunted in subjects with insulin resistance and in elderly subjects. We did not find an association between ANGPTL4 expression and LPL activity in adipose tissue. Nonetheless, an elevation of the amount of endothelial ANGPTL4 can play a role in the dysregulation of plasma lipids found in type 2 diabetes mellitus.

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Conflict of Interest

The authors have nothing to disclose.

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