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Fasting and post-prandial adipose tissue lipoprotein lipase and hormone-sensitive lipase in obesity and Type 2 diabetes

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ABSTRACT. ***Background:*** **Fasting and post-prandial abnormalities of adipose tissue (AT) lipoprotein lipase (LPL) and hormone-sensitive lipase (HSL) activities may have pathophysiological relevance in insulin-resistant conditions.** ***Aim:*** **The aim of this study was to evaluate activity and gene expression of AT LPL and HSL at fasting and 6 h after meal in two insulin-resistant groups – obese with Type 2 diabetes and obese without diabetes – and in non-diabetic normal-weight controls.** ***Material/subjects and methods:*** **Nine obese subjects with diabetes, 10 with obesity alone, and 9 controls underwent measurements of plasma levels of glucose, insulin, and triglycerides before and after a standard fat-rich meal. Fasting and post-prandial (6 h) LPL and HSL activities and gene expressions were determined in abdominal subcutaneous AT needle biopsies.** ***Results:*** **The diabetic obese subjects had significantly lower fasting and post-prandial AT heparin-releasable LPL activity than only obese and control subjects ($p<0.05$) as well as lower mRNA LPL levels. HSL activity was significantly reduced in the 2 groups of obese subjects compared to controls in both fasting condition and 6 h after the meal ($p<0.05$), while HSL mRNA levels were not different. There were no significant changes between fasting and 6 h after meal measurements in either LPL or HSL activities and gene expressions.** ***Conclusions:*** **Lipolytic activities in AT are differently altered in obesity and Type 2 diabetes being HSL alteration associated with both insulin-resistant conditions and LPL with diabetes *per se*. These abnormalities are similarly observed in the fasting condition and after a fat-rich meal.**
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INTRODUCTION

Adipose tissue (AT) activities of the main lipolytic enzymes, lipoprotein lipase (LPL) and hormone-sensitive lipase (HSL), play a fundamental role in the regulation of lipogenesis and/or lipolysis and, therefore, may be very important also in fat repartition during the post-prandial period (1). To this respect, insulin resistance seems to be one of the main determinants of AT LPL activity (2, 3), although Type 2 diabetes *per se*, independently of insulin resistance, may also play a role, as recently shown by our group (4). The role of insulin resistance on AT HSL activity is less clear, the few data available being very contradictory, some showing an increased or normal HSL activity and some just the opposite (5-7).

Even fewer and more contrasting are the data on the activities of these 2 enzymes during the post-prandial period (8-10), that is the condition in which Western societies spend most of their time. Reasons for these inconsistencies may be: 1) the type of meal tested, i.e., whether rich in carbohydrates or fat and, therefore, with different effects on the secretion of insulin, the key hormone in the regulation of expression and activity of both LPL and HSL and, more in general, in the regulation of lipogenesis/lipolysis; 2) the time points after the test meal when lipolytic activities have been evaluated; 3) the type of subjects studied: normal weight or obese, normo- or hypertriglyceridemic, with or without diabetes. In particular, in relation to Type 2 patients, characterized by a high prevalence of post-prandial plasma lipoprotein alterations, which may contribute to their increased cardiovascular risk (11), it is not known whether the AT lipolytic abnormalities present in the fasting state (12) are still observed in the post-prandial period, and if so whether to a more marked extent.

Moreover, it is not known whether lipolytic abnormalities are linked to insulin resistance, typical of patients with Type 2 diabetes as well as with obesity and other metabolic conditions, or to diabetes *per se*, independently of insulin resistance.

Therefore, the aim of our study was to evaluate activities and gene expressions of LPL and HSL in AT in the fasting conditions and after a fat-rich test meal in 2 groups of obese insulin-resistant subjects, one with and the other without Type 2 diabetes, and in a group of non-diabetic normal-weight subjects.

MATERIALS AND METHODS

Subjects

Nine patients with obesity and Type 2 diabetes mellitus, 10 with obesity alone, and 9 normal-weight control subjects were studied. All participants were males and their baseline characteristics are shown in Table 1. Both groups of obese patients had similarly high body mass idex (BMI) and waist circumference compared to controls and also similar levels of insulin resistance. All subjects had normal fasting plasma concentrations of both triglyceride and cholesterol.

Key-words: Adipose tissue, hormone-sensitive lipase, insulin resistance, lipoprotein lipase, obesity.

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Table 1 - *Characteristics of the subjects participating in the study.*

	Diabetic obese	Non-diabetic obese	Controls
Males (no.)	9	10	9
Age (yr)	48±8	47±8	37±8
Body mass index (kg/m^2)*	33±2	34±2	24±1
Waist circumference (cm)*	109±6	110±6	83±4
Plasma cholesterol (mg/dl)	170±22	188±36	162±26
Plasma triglycerides (mg/dl)	104±26	100±37	75±28
HDL cholesterol (mg/dl)*	33±4	40±7	47±9
Insulin sensitivity (M/I)*	2.1±1.2	1.7±0.8	7.6±3.4

mean±SD; *$p<0.05$ analysis of variance.

The subjects had no history or symptoms of any known disease, apart from diabetes, nor were they vegetarians or engaged in intensive physical activity. They were not taking any hypolipidemic drug. Diabetic patients were in stable glycemic control (glycated hemoglobin =6.5±1.5%) on diet alone. The study protocol was approved by the Federico II University Ethics Committee and informed consent by participants was obtained.

Experimental procedures

In the morning, after at least a 12-h fast, anthropometric measurements were taken before subjects were administered a standard meal. Before the meal and over the following 6 h blood samples were taken for determination of plasma levels of glucose, insulin, and triglycerides. Six hours after the meal a sample of abdominal subcutaneous AT was taken by needle biopsy for the determination of LPL and HSL activities and their gene expressions. On a different day, 1-2 weeks apart, a similar biopsy on the opposite side of the lower abdomen was taken in the fasting condition, and a hyperinsulinemic euglycemic clamp was performed.

Standard test meal

The standard meal consisted of a potato gateau (a pie made of mashed potato, whole milk, egg, cheese, ham, and butter), which was consumed in 15 min. The meal, which provided 944 kcal, was composed by 31% carbohydrates, 57% fat (34% saturated fat), and 12% protein.

Hyperinsulinemic euglycemic clamp

Regular human insulin was administered iv at a constant rate of 1.5 mU · kg body weight^{-1} · min^{-1} for 2 h. Blood glucose concentrations were maintained around 90 mg/dl by adjusting glucose infusion rate according to blood glucose measurements (Accuchek analyser, Roche, Switzerland). Whole body insulin sensitivity was calculated as mean glucose infusion rate during the last 30 min of the clamp (M value) divided by the corresponding plasma insulin concentrations (M/I).

Laboratory procedures

AT LPL activity

LPL heparin-releasable activity was determined as previously described (13). Briefly, 5-10 mg frozen AT were incubated in a buffer containing beef lung heparin. Thereafter, 100 µl eluate were incubated with 100 µl ^{3}H-trioleoylglycerol substrate emulsion. The ^{3}H-labeled oleic acid released was extracted and counted in a Wallac 1410 Liquid Scintillation Counter. Enzymatic activity is expressed as nmol of fatty acids released per gram of AT per hour at 37 C. The coefficient of variation (CV) for triplicates of a single AT aliquot was 4.9% and of two AT aliquots of the same biopsy was 4.7%.

LPL total activity was determined as modified from Taskinen et al. (14). Less than 10 mg frozen tissue samples were homogenized in a buffer pH 8.5 containing sucrose, bovine albumin, heparin, and detergent. After centrifugation, 100 µl of 12-fold diluted infranatant were incubated with 100 µl ^{3}H-trioleoylglycerol substrate emulsion and oleic acid was extracted and measured as described for heparin-releasable activity. The CV for triplicates of a single AT aliquot was 3.4% and of two AT aliquots of the same biopsy was 3.5%.

AT HSL activity

HSL activity was measured as previously described (13). Briefly, an aliquot of AT (15-20 mg) was homogenized in a buffer ph 7.4 containing sucrose, EDTA-Na_2, dithioerythritol and protease inhibitors and centrifuged. Thereafter, 30 µl of homogenate infranatant was incubated with ^{3}H-trioleoylglycerol substrate emulsion. After 45 min, the ^{3}H-fatty acids were extracted and counted in a liquid scintillator. Enzymatic activity is expressed as nmol of fatty acids released per gram of AT per hour at 37 C. The CV for triplicates of a single AT aliquot was 4.5% and of 2 AT aliquots of the same biopsy was 4.5%.

AT LPL and HSL gene expression

mRNA expression of LPL and HSL was evaluated by RT-PCR, as previously described (13). Primers used for LPL (Acc. M15856) were: sense 5′-CCT GGA GAT GTG GAC CAG C-3′, and antisense 5′-GTC CCA TAC AGA GAA ATC TC-3′ (338bp product). Primers used for HSL (Acc.NM-005357) were: sense 5′-TGG AGG AGT GCT TCT TCG CCT AC-3′; antisense 5′-GTC TCA GCT GAC AGC GAC ATC T-3′ generating two products, and, corresponding to hHSL-L (616bp) and hHSL-S (388bp). All PCR products were electrophoresed on 1.2% agarose gel and bands visualized by ethidium bromide staining. Semiquantitative analysis was performed by densitometric gel scanning and the results are expressed as the ratio between the gene of interest and glyceraldehyde 3-phosphate dehydrogenase in each sample analyzed.

Other measurements

Plasma glucose and triglyceride concentrations were assayed by enzymatic colorimetric methods (Roche Diagnostics, Milan, Italy) on a Cobas Mira autoanalyzer (ABX Diagnostics, Montpellier, France). Plasma insulin concentrations were measured by enzyme-linked immunosorbent assay (Technogenetics, Milan, Italy).

Statistical analysis

Data are expressed as mean±SD, unless otherwise stated. Differences between the 3 groups (diabetic obese, obese only, and control) were evaluated by analysis of variance and by *post-hoc* test between groups (LSD). Differences in enzymatic activities and gene expressions were evaluated after adjustment for age (covariance analysis). Differences between pre- and post-test meal values were evaluated by t-test for paired data. Two-tailed tests were used and a $p<0.05$ was considered statistically significant. Statistical analyses were performed using the Statistical Package for Social Sciences software (SPSS/PC, SPSS, Inc., Chicago, IL, USA).

Table 2 - *Plasma glucose, insulin, and triglycerides concentrations at fasting and 6 h after a standard test meal rich in fat.*

	Diabetic obese		Non-diabetic obese		Controls	
	Fasting	Post prandial	Fasting	Post prandial	Fasting	Post prandial
Plasma glucose (mg/dl)	131±37[a,b]	104±22[a,b,c]	90±14	80±4[c]	87.0±9.4	84.0±5.6
Plasma insulin (mU/l)	16.7±8.4[a]	17.3±10.5[a]	16.3±7.4[a]	17.2±6.4[a]	6.3±3.6	5.7±3.7
Plasma triglycerides (mg/dl)	104±22	170±69[a,c]	100±37	132±55[c]	75±27	98±32[c]

Data are expressed as mean±SD. [a]$p<0.05$ *vs* controls, [b]$p<0.05$ *vs* non-diabetic obese, [c]$p<0.05$ *vs* fasting.

RESULTS

As expected, blood glucose levels were higher in the diabetic subjects both at fasting and after the meal, compared to obese and controls (Table 2).

Plasma insulin levels were significantly higher in both groups of obese subjects than in controls (Table 2), although patients with diabetes showed a tendency to a blunted early insulin response (2 h after meal: 61.6±15.5 in only obese, 47.0±8.7 in obese with diabetes, 22.3±5.5 mU/l in controls).

Insulin sensitivity was similarly reduced in the 2 groups of obese patients compared to controls (Table 1). Plasma triglyceride concentrations 6 h after the test meal were higher in diabetic patients than in the other 2 groups (Table 2).

The results on lipases activity and gene expression in AT are shown in Figures 1 and 2. Heparin-releasable LPL activity was significantly lower in diabetic patients compared to obese ($p<0.05$) and control subjects ($p<0.05$) both at fasting and in the post-prandial phase (Fig.1) as previously reported (4). Total LPL activity was reduced in both groups of obese subjects compared to controls, reaching statistical significance in the post-prandial condition ($p<0.05$). There were no significant changes be-

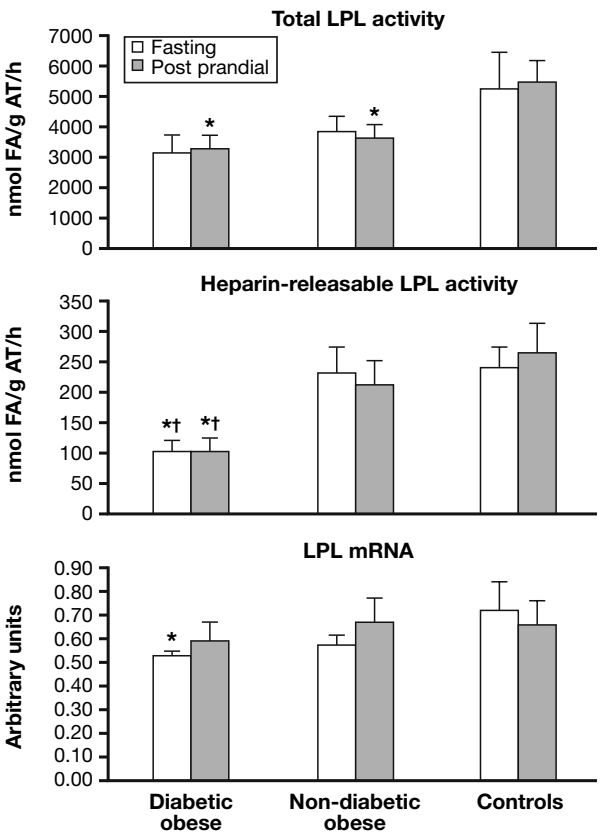

Fig. 1 - *Total and heparin-releasable lipoprotein lipase (LPL) activity and LPL mRNA levels in subcutaneous adipose tissue (AT) at fasting and 6 h after a standard test meal rich in fat in diabetic obese, non-diabetic obese and normal-weight control subjects. FA: fatty acids.* *p<0.05 vs *controls;* †p<0.05 vs *non-diabetic obese. Adjusted for age.*

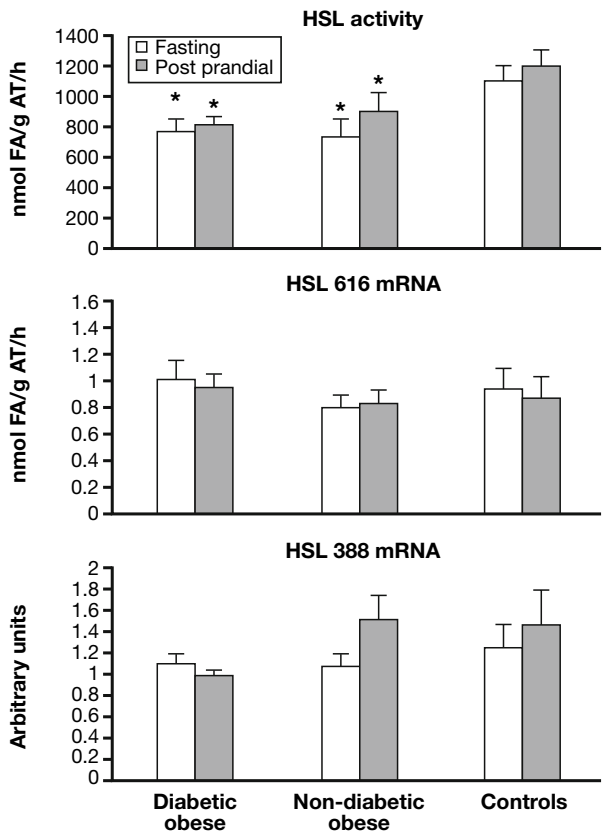

Fig. 2 - Hormone-sensitive lipase (HSL) activity and mRNA levels (HSL 616 and HSL 388 forms) in subcutaneous adipose tissue (AT) at fasting and 6 h after a standard test meal rich in fat in diabetic obese, non-diabetic obese, and normal-weight control subjects. FA: fatty acids. *p<0.05 vs *controls. Adjusted for age.*

tween fasting and 6-h post-meal values in any group (Fig. 1).
LPL mRNA fasting levels in AT were significantly reduced in diabetic patients compared to controls ($p<0.05$). LPL mRNA levels did not change significantly 6 h after the meal compared to fasting in any group (Fig. 1).
HSL activity was significantly lower in both groups of obese subjects compared to controls in either fasting condition and 6 h after the standard test meal. HSL activity did not significantly change after the meal in any group (Fig. 2).
HSL mRNA levels, whether HSL 616 or HSL 388 forms, were not significantly different between the 3 groups at fasting, and were not changed after the standard test meal (Fig. 2).

DISCUSSION

This study shows that enzymatic lipolytic activities in AT are differently regulated according to the presence of obesity and/or diabetes and these differences are observed both in the fasting and post-prandial conditions.
The first finding is that both groups of obese patients, with and without diabetes, were characterized by a decreased HSL activity compared with non-diabetic normal-weight controls. These data were somewhat unexpected since both groups of obese subjects were insulin resistant, and insulin resistance has been generally considered to associate with a higher lipolytic rate. On the other hand, these data are in line with those shown in diabetic patients with a lower degree of overweight (5).
There are some possible explanations for the reduced HSL activity in the presence of obesity. First of all, other lipases could be more relevant than HSL in the regulation of fat cell lipolysis in obese individuals. In this respect, however, adipose triglyceride lipase, which has a significant role in AT lipolysis in rodents (15, 16), seems to have a low *in vitro* triglyceride hydrolase activity in humans and is not regulated by obesity (17). Another possibility is that HSL activity may be reduced in subcutaneous AT, as measured in our study, while increased in visceral AT (15-17). A more likely explanation is an inhibitory effect on HSL by chronic hyperinsulinemia, that was present in both groups of obese patients, overcoming the effects of insulin resistance (5). The effect seems to be post-translational, since it regarded HSL activity and not its mRNA expression which did not differ between the 3 groups. The different behavior between gene expression and enzyme activity may also be the consequence of a possible feedback mechanism involving the lipolysis reaction end-products free fatty acids/glycerol, that could be mediators of the effect of insulin on HSL gene expression, taking it back to normal levels (18). The lack of differences with controls concerned both forms of HSL mRNA, which have been found to translate in functionally different proteins, indicating that the proportion of active *vs* inactive form of HSL was not responsible for the reduced activity in the obese individuals.
It is noteworthy that the differences in AT HSL activity between obese and control individuals were also observed 6 h after a fat-rich standard meal, indicating that the factors operating at fasting were equally effective in the post-prandial condition. In this respect, concerning hyperinsulinemia, it must be considered that plasma insulin concentrations 6 h after meal were back to fasting levels but, anyhow, higher in the 2 obese groups. Previous data on post-prandial changes in AT HSL activity are very scant. A time curve after a mixed meal by *in vivo* measurement of arteriovenous substrate concentrations showed a suppression of HSL activity lasting about 5 h and, thereafter, a return to baseline values (19). This is therefore in line with the lack of changes in HSL activity we observed between fasting and 6-h samples in all groups, although different changes in the early post-prandial phase cannot be excluded.
A second finding of this study is that, at odds with the similar behavior of obese diabetic and non-diabetic subjects in respect to HSL, we observed a reduced activity of AT heparin-releasable LPL only in patients with diabetes, when compared either to obese or control individuals. This was observed both at fasting and 6 h after meal. The reduced activity in the diabetic subjects only concerned heparin-releasable activity, while total activity was lower in both groups of obese subjects than in controls. In addition, AT LPL mRNA expression was significantly reduced in diabetic patients compared to controls, but not to obese subjects. In our study, diabetic subjects, compared to the obese only, had a slightly lower LPL gene expression while having a more evident significant reduction of LPL activity in AT. The greater decrease in activity compared to gene expression indicates that the differences observed were mainly based on a post-translational process, likely concerning the transposition of LPL to the vessel wall, as they regarded the heparin-releasable and not the total LPL activity. A role in the altered LPL regulation could be played by recently shown mechanisms like di- monomerisation changes by angiopoietin-like proteins (20).
We observed no significant differences in LPL activity between fasting and post-prandial changes in all groups. A first explanation for this finding may be that 6 h after meal sampling time is too late to still observe a post-prandial change. In fact, AT LPL activity after an early post-meal increase (3-4 h) has been shown to return to baseline values after 6 h (21). The lack of post-prandial changes in LPL in our study may also be due to the type of meal, as we administered a meal very rich in fat, especially in saturated fat. In this regard, Nilsson-Ehle et al. (22) found in healthy controls an increase in AT LPL activity lasting 4 h after a glucose challenge, while LPL activity did not change after a corn oil load for as long as 6 h. In this study the LPL increase after the glucose challenge was related to the increase in insulin levels, observed after glucose but not after corn oil load. Since post-prandial insulin responses were not greatly increased in our study participants, it may be that our test meal was not able to stimulate insulin secretion to an extent able to increase AT LPL activity.
The lack of post-prandial changes in AT LPL activity implies that the lower LPL activity, which characterizes diabetic patients both at fasting and post-prandially, can become of major relevance in a condition of metabolic stress, such as the post-prandial state, which alters

lipoprotein profile also in subjects perfectly normotriglyceridemic at fasting as was the case of our diabetic subjects.

Our findings have been obtained in a group of Type 2 diabetic patients in good blood glucose control on diet alone. Therefore, their extrapolation to all Type 2 diabetic patients should be done with caution.

In conclusion, this study shows that: 1) a reduced activity of AT HSL is present in obese patients with and without diabetes, who are characterized by insulin resistance; 2) a reduced activity of AT LPL is present only in diabetic patients and, therefore, this abnormality seems to be linked to diabetes per se; 3) these lipolytic alterations are observed both in the fasting and post-prandial conditions.

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REFERENCES

1. Frayn KN. Adipose tissue as a buffer for daily lipid flux. Diabetologia 2002, 45: 1201-10.
2. Taskinen MR, Nikkilä EA. Lipoprotein lipase activity of adipose tissue and skeletal muscle in insulin-deficient human diabetes. Relation to high-density and very low-density lipoprotein and response to treatment. Diabetologia 1979, 17: 351-7.
3. Eckel RH, Yost TJ, Jensen DR. Alterations in lipoprotein lipase in insulin resistance. Int J Obesity Int J Obes Relat Metab Disord 1995, 19 (Suppl 1): S16-21.
4. Annuzzi G, Giacco R, Patti L, et al. Postprandial chylomicrons and adipose tissue lipoprotein lipase are altered in type 2 diabetes independently of obesity and whole-body insulin resistance. Nutr Metab Cardiovasc Dis 2008, 18: 531-8.
5. Watt MJ, Carey AL, Wolsk-Petersen E, Kraemer FB, Pedersen BK, Febbraio MA. Hormone-sensitive lipase is reduced in the adipose tissue of patients with type 2 diabetes mellitus: influence of IL-6 infusion. Diabetologia 2005, 48: 105-12.
6. Reynisdottir S, Angelin B, Langin D, et al. Adipose tissue lipoprotein lipase and hormone-sensitive lipase. Contrasting findings in familial combined hyperlipidemia and insulin resistance syndrome. Arterioscler Thromb Vasc Biol 1997, 17: 2287-92.
7. Arner P, Bolinder J, Engfeldt P, Ostman J. The antilipolytic effect of insulin in human adipose tissue in obesity, diabetes mellitus, hyperinsulinemia, and starvation. Metabolism 1981, 30: 753-60.
8. Goldberg IJ, Kako Y, Lutz EP. Responses to eating: lipoproteins, lipolytic products and atherosclerosis. Curr Opin Lipidol 2000, 11: 235-41.
9. Panarotto D, Remillard P, Bouffard L, Maheux P. Insulin resistance affects the regulation of lipoprotein lipase in the postprandial period and in an adipose tissue-specific manner. Eur J Clin Invest 2002, 32: 84-92.
10. Lithell H, Boberg J, Hellsing K, Lundqvist G, Vessby B. Lipoprotein-lipase activity in human skeletal muscle and adipose tissue in the fasting and the fed states. Atherosclerosis 1978, 30: 89-94.
11. Karpe F. Postprandial lipoprotein metabolism and atherosclerosis. J Int Med 1999, 246: 341-55.
12. Taskinen MR, Nikkila EA, Kuusi T, Harmo K. Lipoprotein lipase activity and serum lipoproteins in type 2 (insulin-independent) diabetes associated with obesity. Diabetologia 1982, 22: 46-50.
13. Rivellese AA, Giacco R, Annuzzi G, et al. Effects of monounsaturated vs. saturated fat on postprandial lipemia and adipose tissue lipases in type 2 diabetes. Clin Nutr 2008, 27: 133-41.
14. Taskinen MR, Nikkilä EA, Huttunen JK, Hilden H. A micromethod for assay of lipoprotein lipase activity in needle biopsy samples of human adipose tissue and skeletal muscle. Clin Chim Acta 1980, 104: 107-17.
15. Okazaki H, Igarashi M, Nishi M, et al. Identification of a novel member of the carboxylesterase family that hydrolyzes triacylglycerol: a potential role in adipocyte lipolysis. Diabetes 2006, 55: 2091-7.
16. Kershaw EE, Hamm JK, Verhagen AW, Peroni O, Katic M, Flier JS. Adipose triglyceride lipase: function, regulation by insulin, and comparison with adiponutrin. Diabetes 2006, 55: 148-57.
17. Mairal A, Langin D, Arner P, Hoffstedt J. Human adipose triglyceride lipase (PNPLA2) is not regulated by obesity and exhibits low in vitro triglyceride hydrolase activity. Diabetologia 2006, 49: 1629-36.
18. Plée-Gautier E, Grober J, Duplus E, Langin D, Forest C. Inhibition of hormone-sensitive lipase gene expression by cAMP and phorbol esters in 3T3-F442A and BFC-1 adipocytes. Biochem J 1996, 318: 1057-63.
19. Frayn KN, Coppak SW, Fielding BA, Humphreys SM. Coordinated regulation of hormone-sensitive lipase and lipoprotein lipase in human adipose tissue in vivo: implications for the control of fat storage and fat mobilization. Adv Enzyme Regul 1995, 35: 163-78.
20. 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: 17450-5.
21. Eriksson JW, Buren J, Svensson M, Olivecrona T, Olivecrona G. Postprandial regulation of blood lipids and adipose tissue lipoprotein lipase in type 2 diabetes patients and healthy control subjects. Atherosclerosis 2003, 166: 359-67.
22. Nilsson-Ehle P, Carlström S, Belfrage P. Rapid effect on lipoprotein lipase activity in adipose tissue after carbohydrate and lipid intake. Time course and relation to plasma glycerol, triglyceride, and insulin levels. Scand J Clin Lab Invest 1975, 35: 373-8.