A role of lipin in human obesity and insulin resistance: relation to adipocyte glucose transport and GLUT4 expression

Vanessa van Harmelen, Mikael Rydén, Eva Sjölin, and Johan Hoffstedt[1]

Department of Medicine, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden

Abstract The mouse lipin gene, *Lpin1*, is important for adipose tissue development and is a candidate gene for insulin resistance. Here, we investigate the adipose tissue expression levels of the human *LPIN1* gene in relation to various clinical variables as well as adipocyte function. *LPIN1* gene expression was induced at an early step in human preadipocyte differentiation in parallel with peroxisome proliferator-activated receptor γ. Lipin mRNA levels were higher in fat cells than in adipose tissue segments but showed no difference between subcutaneous and omental depots. Moreover, *LPIN1* expression levels were reduced in obesity, improved following weight reduction in obese subjects, and were downregulated in women with the metabolic syndrome. With respect to adipocyte function, adipose *LPIN1* gene expression was strongly associated with both basal and insulin-mediated subcutaneous adipocyte glucose transport as well as mRNA levels of glucose transporter 4 (*GLUT4*). We show that body fat accumulation is a major regulator of human adipose *LPIN1* expression and suggest a role of *LPIN1* in human preadipocyte as well as mature adipocyte function.— van Harmelen, V., M. Rydén, E. Sjölin, and J. Hoffstedt. **A role of lipin in human obesity and insulin resistance: relation to adipocyte glucose transport and GLUT4 expression.** *J. Lipid Res.* 2007. **48:** 201–206.

Supplementary key words adipogenesis • adipose tissue • fat cell • metabolic syndrome • mRNA

Obesity is a major risk factor for insulin resistance and associated metabolic aberrations. Although visceral fat accumulation is of pathophysiologic importance (1), associations with insulin resistance have been found also for increased total and subcutaneous fat mass (2). Thus, enlargement of subcutaneous adipocytes showing both resistance to insulin-mediated glucose uptake (3) and increased rate of lipolysis (4) may also play an important role in insulin resistance development. It has been suggested that the reduced triglyceride storage capacity of enlarged fat cells is followed by ectopic fat storage in liver, skeletal muscle, and pancreas, resulting in insulin resistance and other features of the metabolic syndrome (2, 5).

This overflow hypothesis is supported by findings from studies in patients lacking subcutaneous fat, lipodystrophy (6), a condition characterized by often severe insulin resistance due to excess accumulation of nonadipose tissue fat. In mice, the gene responsible for this syndrome, *Lpin1*, was recently isolated through positional cloning and found to encode an 891 amino acid protein, lipin, with a putative nuclear localization but hitherto unknown function (7). Lipin mRNA expression was induced during 3T3-L1 preadipocyte differentiation and was found predominately in adipose tissue, skeletal muscle, and testis. Moreover, two mutant alleles of the *Lpin1* gene, fld1 and fld2, were associated with a markedly reduced adipose tissue mass, consistent with an important role for lipin in adipose tissue development and function (7). Altogether, these findings suggest that *Lpin1* is a candidate gene in the pathogenesis of various adipose-related conditions, including obesity, insulin resistance, and type 2 diabetes mellitus.

In screening for mutations in the human gene ortholog, *LPIN1* (gene ID: 23175), no sequence variants exclusive for lipodystrophy were found (8). However, in a recent study, findings on both gene variation and adipose mRNA levels implicate *LPIN1* in human glucose metabolism (9). Here, we further investigate the adipose tissue expression of the human *LPIN1* gene in relation to clinical variables and fat cell function.

METHODS

Subjects

Four separate cohorts including obese and lean women were used. All were healthy and free of medication and ate a standard Swedish diet. They were all living in the Stockholm area and were at

---

This article is available online at http://www.jlr.org

Abbreviations: BMI, body mass index; GPDH, glycerol-3-phosphate dehydrogenase; HOMA, homeostasis model assessment algorithm; PPARγ2, peroxisome proliferator-activated receptor γ transcript variant 2.

[1] To whom correspondence should be addressed.
   e-mail: johan.hoffstedt@ki.se

least second generation Scandinavian. None were completely sedentary or involved in athletic performances and none had undergone a slimming effort or experienced a change in body weight >1 kg within 6 months of the study, according to self-report. The study was explained in detail to each subject and her informed consent was obtained. The study was approved by the hospital's committee on ethics.

Cohort 1 was composed of 80 obese (body mass index [BMI] 31–53 kg/m^2) and 16 lean (BMI 20–24 kg/m^2) women. At ~07.30 AM, after an overnight fast, a venous blood sample was obtained for analyses of plasma levels of glucose, insulin, triglycerides, cholesterol, and HDL cholesterol, which were performed by the hospital's accredited chemistry laboratory. Insulin sensitivity was calculated from the homeostasis model assessment algorithm (HOMA) based on plasma glucose and plasma insulin (p-insulin × p-glucose/22.5). Systolic and diastolic blood pressures were measured in the supine position after 15 min of rest. Thereafter, an adipose sample (1–4 g) was obtained by needle biopsy from the abdominal subcutaneous area under local anesthesia, as previously described (10). This tissue was frozen in liquid nitrogen, kept frozen at −70°C, and used for subsequent mRNA analysis (see below). In 70 of the obese subjects, there was adipose tissue available also for fat cell glucose transport studies (see below).

Cohort 2 consisted of 18 obese (BMI 38–53 kg/m^2) women undergoing weight reduction surgery at the Department of Surgery, Karolinska University Hospital, Huddinge. From these women, perioperative fat biopsies from subcutaneous and omental adipose tissue (1–2 g) were taken for subsequent mRNA analysis using a laparoscopic technique. mRNA analyses were performed as described below. In 13 of these women, there was a second subcutaneous fat biopsy taken 2–4 years after surgery (cohort 3). All subjects were weight stable after the weight reduction, and their BMI had decreased by an average of approximately 15 kg/m^2. Cohort 4 consisted of 11 subjects from which adipose tissue biopsies were taken for preadipocyte differentiation, see below.

Fat cell isolation

Isolated fat cells were prepared and isolated as described previously (11). In brief, adipocytes were separated from stroma cells by treatment in a shaking bath at 37°C for 60 min with 0.5 mg/l collagenase in 5 ml Krebs-Ringer phosphate (KRP) buffer (pH 7.4) with 40 g/l of purified BSA. Adipocyte suspensions were then rinsed three times in collagenase-free buffer using nylon filters. Fat cell sizes were measured by direct microscopy, and the mean adipocyte diameter was calculated from measures of 100 cells. The total lipid weight of the incubated fat cells was determined after organic extraction. The number of fat cells incubated was determined by dividing total lipid weight by the fat cell weight.

Adipocyte glucose transport

We used an indirect method, which is described in detail elsewhere (12). In brief, isolated fat cells were incubated at a concentration of 2% (v/v) in KRP buffer (pH 7.4) containing albumin (20 mg/ml), (3-^{3}H]glucose (5 × 10^6 dpm/ml), unlabeled glucose (1 μmol/l), and human insulin in different concentrations (10^{-15}–10^{-6} M). The incubations were conducted for 2 h at 37°C with air as the gas phase. Incubations were stopped by rapidly chilling the incubation vials to 4°C and adding H$_2$SO$_4$. Thereafter, the incorporation of radiolabeled glucose into adipocyte lipids was determined. This incorporation into lipids (i.e., lipogenesis) reflects glucose transport, because at micromolar glucose concentrations, glucose transport is the rate-limiting step for lipogenesis in human fat cells, as discussed previously (12). Glucose transport, basal and maximal insulin-stimulated minus basal, was expressed as the amount of glucose incorporated per fat cell number (nmol/2 h/10^7 cells), as described previously (12). The half-maximum effective concentration (EC$_{50}$) of insulin was calculated by log-logit transformation of concentration-response curves and converted to the negative logarithm of mol/l (pD$_2$), which corresponds to insulin receptor sensitivity (12).

Studying gene expression during the preadipocyte differentiation process

From 11 subjects, 2 males and 9 females (cohort 4), adipose tissue (9 abdominal subcutaneous, 2 hip) biopsies were taken, from which preadipocytes were isolated and differentiated into adipocytes as described previously (13). The mean BMI and age in this subject group were 28.4 ± 6.1 kg/m^2 and 42 ± 11 years, respectively. The differentiation process was enhanced by adding rosiglitazone (10 μM) during the first 6 days of the differentiation process. The cells reached full differentiation after 12–14 days. At days 4, 8, and 12, cells were lysed for the isolation of total RNA or for the measurement of glycerol-3-phosphate dehydrogenase (GPDH) activity for assessment of differentiation capacity, which was performed as described previously (14).

mRNA analysis

Total RNA was extracted from 300 mg of adipose tissue or adipocytes using the RNeasy mini kit (Qiagen; Hilden, Germany). The RNA concentration and purity were assessed spectrophotometrically. One microgram of total RNA from each sample was reverse transcribed to cDNA using the Omniscript RT kit (Qiagen) and random hexamer primers (Invitrogen; Tåstrup, Denmark). To minimize methodological errors resulting from variation in cDNA synthesis, cDNA synthesis was performed simultaneously for all subjects included in the first, second, third, and fourth cohorts, respectively, using exactly the same mix of primers and the same RT kit. The Agilent 2100 Bioanalyzer (Agilent Technologies; Kista, Sweden) was used to confirm the integrity of the RNA. In a final volume of 25 μl, 5 ng of cDNA was mixed with 2× SYBR green PCR master mix (Bio-Rad Laboratories, Inc.; Hercules, CA) and primers (Invitrogen). The primer pairs were selected to yield a single amplicon based on dissociation curves and analysis by agarose gel electrophoresis. The primers used were 5′-GAAAGGACAGGGCAGAAGAAC-3′ (sense) and 5′-TGC-TCAATGGGCTGGACTC-3′ (antisense) for lipin (NM_145693); 5′-ACCCAGAAAGCGATTCCTTCAC-3′ (sense) and 5′-CCACGG-AGCTGATCCCAAAG-3′ (antisense) for peroxisome proliferator-activated receptor γ transcript variant 2 (PPARγ2) (NM_015869); 5′-CATTCCTTGGTTCATCGTG-3′ (sense) and 5′-ATAGCCTCC-GCAACATAC-3′ (antisense) for GLUT4 (NM_001042); 5′-GGTC-TCGAACTCCTGGCCTA-3′ (sense) and 5′-TGAGATATCGACT-GGGCATGGT-3′ (antisense) for adiponectin (NM_004797); and 5′-TGACTCAACACGGGAAACC-3′ (sense) and 5′-TCGCTC-CACCAACTAAGAAC-3′ (antisense) for 18S rRNA. Quantitative real-time PCR was performed in an iCycler IQ™ (Bio-Rad Laboratories, Inc.). The mRNA levels were determined by a comparative C_t method (ABI Prism 7700, Applied Biosystems; Foster City, CA). The subject with the highest C_t value was used as a reference; all other C_t values for the target gene and reference gene, respectively, were subtracted from this C_t value. The C_t values were then normalized to rRNA for 18S. All PCR reactions were run in duplicate.

Statistical analysis

Parameter distributions were normalized when necessary by ^{10}logarithm transformation before statistical comparison. Values are mean ± SD. The Student's paired or unpaired t-test and single and multiple regression analyses were used for statistical evaluation.

A *P* value of 0.05 or less was considered statistically significant. The analyses were performed using StatView version 6.0 (Stata Corporation; College Station, Texas).

RESULTS

Lipin mRNA gene expression during preadipocyte differentiation

Lipin mRNA levels were measured during preadipocyte differentiation and compared with PPARγ2 mRNA levels. Lipin expression and PPARγ2 mRNA expression displayed similar patterns. Both mRNAs were detectable at day 4 and increased until day 8. Lipin mRNA remained constant after day 8, whereas PPARγ2 showed a slight decrease at day 12 in comparison with day 8. GLUT4 mRNA levels, on the other hand, were not detectable at day 4, but increased from day 8 to day 12. The pattern of GLUT4 mRNA levels during differentiation was very similar to the pattern of GPDH activity, which was used as an index of the differentiation capacity of the cells (**Fig. 1**).

Adipose specificity and regional distribution of LPIN1 gene expression

Lipin mRNA adipose expression in subcutaneous adipocytes versus adipose tissue was measured using preparations from 14 out of 18 women of cohort 2. As shown in **Fig. 2A**, the lipin as well as adiponectin mRNA/18S rRNA ratio was approximately twice as high in the fat cells as in the tissue, (100 ± 38% vs. 53 ± 29%, $P < 0.0001$; and 100 ± 25% vs. 49 ± 18%, $P < 0.0001$, respectively). The relative expression level of lipin was further compared between subcutaneous and omental adipose tissue from 18 obese women (cohort 2). No regional adipose tissue difference in lipin mRNA levels was found (subcutaneous 100 ± 29%, omental 100 ± 21%, $P = 0.84$), whereas the omental adiponectin mRNA levels (78 ± 23%) were lower than in the subcutaneous depot (100 ± 41%), $P = 0.04$ (Fig. 2B).

LPIN1 gene expression in obesity

The effect of obesity on *LPIN1* gene expression was analyzed in subcutaneous adipose tissue from 80 obese and 16 lean women (cohort 1). As seen in Fig. 2C, the lipin mRNA-to-18S rRNA ratio was markedly downregulated in obese subjects, (obese, 58 ± 29% vs. nonobese, 100 ± 50%, $P < 0.0001$). The corresponding values for adiponectin mRNA/18S rRNA were 78 ± 30% (obese) and 100 ± 24% (nonobese), $P = 0.008$. A similar expression pattern was found in 13 obese women (cohort 3) undergoing weight-reducing therapy by bariatric surgery. After weight reduction, an increased level of lipin, 76 ± 38% (before) versus 100 ± 38% (after), $P = 0.03$, as well as adiponectin mRNA/18S ratio, 78 ± 30% (before) versus 100 ± 24% (after), $P = 0.002$, was found (Fig. 2D).

Adipose tissue lipin mRNA levels and the metabolic syndrome

The obese women of cohort 1 were classified according to the presence or absence of the metabolic syndrome, as
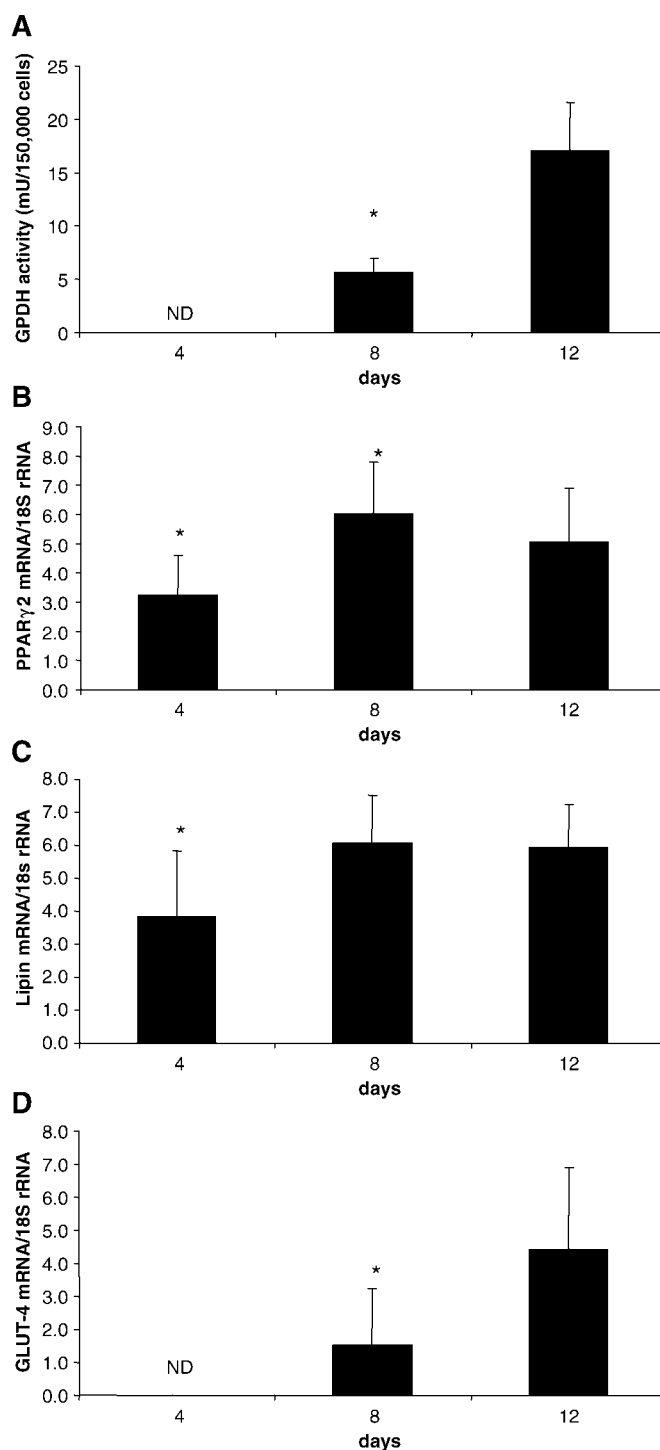

Fig. 1. Glycerol-3-phosphate dehydrogenase activity (A) and gene expression of *PPARγ2* (B), *LPIN1* (C), and *GLUT4* (D) during the differentiation of human preadipocytes to adipocytes. Number of experiments = 11. ND, nondetectable. Levels at day 4 or 8 were compared with levels at day 12 using a paired *t*-test. *$P < 0.05$. Values are mean ± SD.

defined by the National Cholesterol Education Program (15). According to this definition, 29 women fulfilled the criteria of the metabolic syndrome, whereas 51 did not. In **Fig. 3**, the effect of the metabolic syndrome on the subcutaneous adipose tissue mRNA levels of lipin and
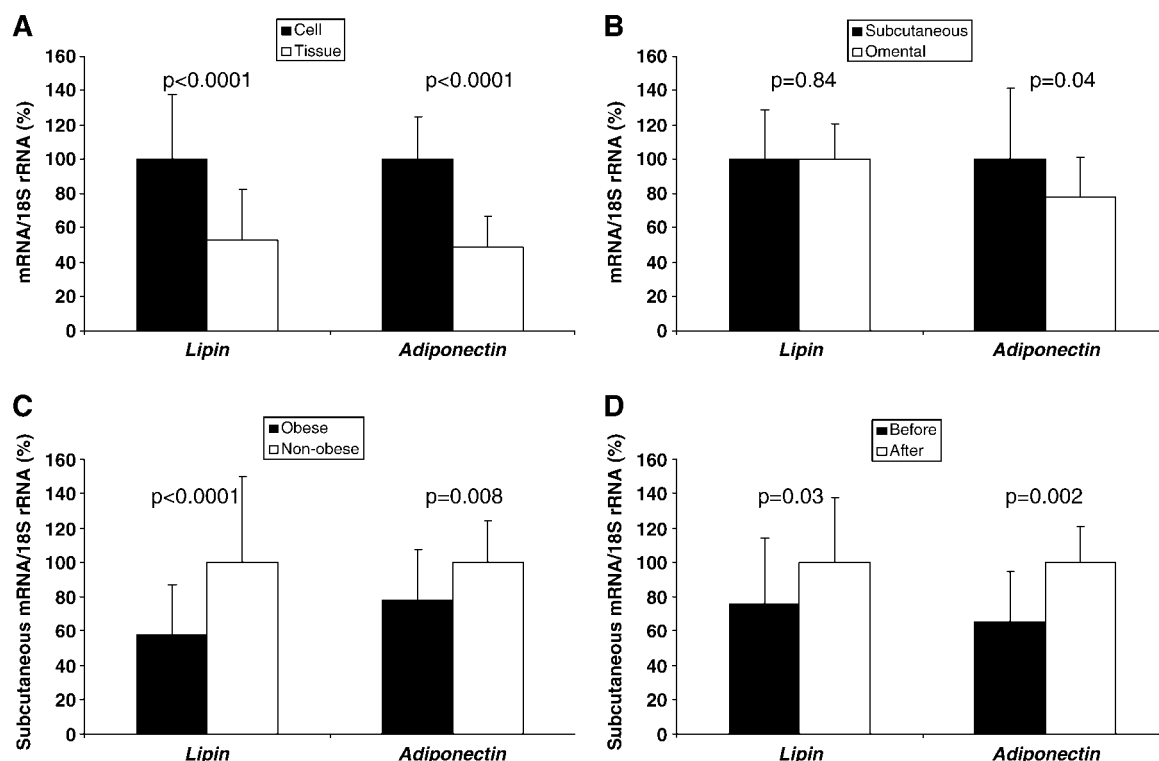
Fig. 2. Lipin and adiponectin mRNA levels in subcutaneous fat cells versus tissue (A), in omental versus subcutaneous adipose tissue (B), in subcutaneous adipose tissue from obese versus nonobese subjects (C), and in subcutaneous adipose tissue before and after weight reduction (D). Values are mean ± SD and were compared using Student's paired or unpaired *t*-tests.

adiponectin is shown. In women with the metabolic syndrome, the lipin (67 ± 25%) as well as the adiponectin (74 ± 35%) mRNA/18S rRNA were reduced as compared with women that did not meet the metabolic syndrome criteria (100 ± 47%, $P = 0.0007$, and 100 ± 32%, $P = 0.001$, respectively).

LPIN1 gene expression and subcutaneous adipocyte glucose transport

In 70 of the obese subjects of cohort 1, there was enough tissue available to investigate the association of lipin mRNA
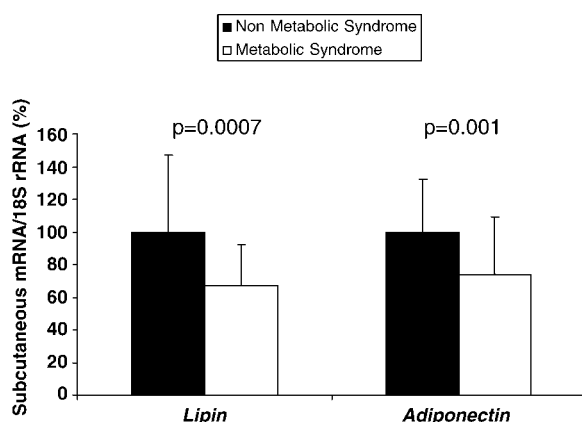

Fig. 3. Lipin and adiponectin mRNA levels in obese subjects with or without the metabolic syndrome. Values are mean ± SD and were compared using Student's unpaired *t*-test.

with human fat cell metabolism. Subcutaneous adipocyte lipogenesis, as assessed by basal and maximal minus basal insulin-stimulated glucose transport as well as pD$_2$ for insulin-mediated glucose transport, was related to adipose tissue lipin mRNA levels. As seen in **Fig. 4**, strong relationships between both basal and maximal insulin-stimulated minus basal glucose transport were found that were independent of age, BMI, and insulin sensitivity measured as HOMA (multiple regression analysis). A significant but weaker association was also demonstrated between lipin mRNA expression and pD$_2$ for insulin-stimulated glucose transport (insulin receptor sensitivity). However, this association did not remain significant when adjusted for HOMA. The subcutaneous adipose tissue adiponectin mRNA levels were also correlated with both basal ($r = 0.42$, $P = 0.0003$) and maximal minus basal glucose transport ($r = 0.39$, $P = 0.0008$) and pD$_2$ for insulin-mediated glucose transport ($r = 0.25$, $P = 0.04$), although the latter did not remain significant after adjustment for age, BMI or HOMA.

Lipin mRNA levels in relation to GLUT4 expression

A major regulator of glucose uptake in fat cells is glucose transporter 4, *GLUT4*. We therefore hypothesized that *LPIN1* gene expression might be related also to levels of GLUT4 mRNA in the 80 obese subjects of cohort 1. A strong association between lipin and GLUT4 mRNA levels was found that was independent of age, BMI, and HOMA (**Fig. 5**). A similar but weaker correlation with GLUT4 was found also for adiponectin mRNA ($r = 0.59$, $P < 0.0001$).
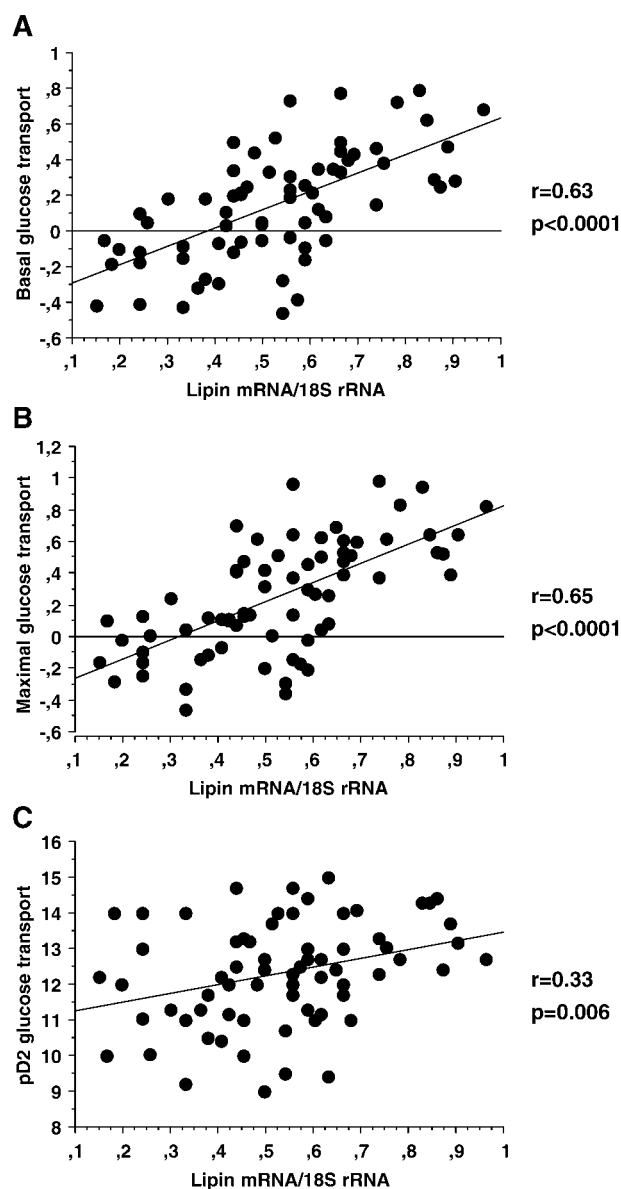
Fig. 4. Subcutaneous adipose tissue lipin mRNA levels in relation to subcutaneous adipocyte (A) basal glucose transport (log nmol/$2 h/10^7$ cells), (B) maximal insulin-stimulated minus basal glucose transport (log nmol/$2 h/10^7$ cells), and (C) pD_2 insulin-stimulated glucose transport from obese subjects. Values were analyzed using simple regression.

Fig. 5. Lipin mRNA levels in relation to GLUT4 mRNA levels in subcutaneous adipose tissue from obese subjects. Values were analyzed using simple regression.

DISCUSSION

Previous studies have shown that the *LPIN1* gene is an important regulator of fat cell function and a candidate gene for insulin resistance and type 2 diabetes mellitus. Here, we show that lipin mRNA was readily detected in both human subcutaneous and omental adipose tissue and showed a similar level of expression in both depots. We also found that lipin mRNA levels in human adipose tissue were downregulated in obesity and that weight reduction in obese subjects was linked to an upregulation of lipin mRNA levels. Moreover, lipin mRNA levels were reduced in women with the metabolic syndrome. These
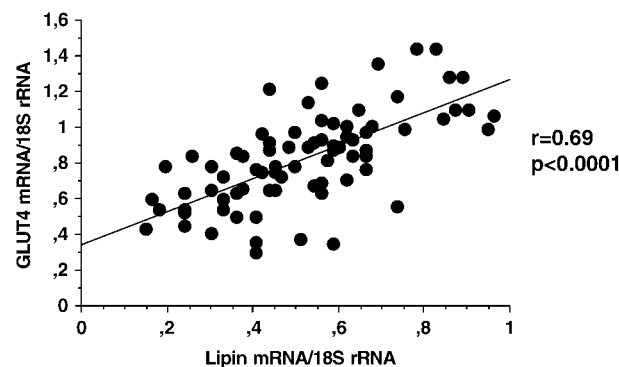
data are in agreement with findings from fld1/fld2 mice that either do not express lipin mRNA and protein or have a defective protein but develop glucose intolerance and insulin resistance (7), which, in turn, has been hypothesized to be a side effect of the diminished adipose tissue mass in these animals. The present results are also in accordance with two recent human studies in which reduced levels of human adipose tissue lipin mRNA were demonstrated in subjects with insulin resistance (9) and HIV-associated lipodystrophy (16). Unfortunately, no antibody specific for human lipin is available at the moment, so we do not know how mRNA levels relate to the protein expression of lipin. As a positive control, along with measures of lipin mRNA, adipose tissue levels of adiponectin mRNA were analyzed, and the results are in line with previous studies demonstrating that adiponectin levels are reduced in obesity and insulin resistance, are increased after weight reduction (17, 18), and are expressed at lower levels in omental as compared with subcutaneous adipose tissue (19).

A principal characteristic of insulin resistance is reduced insulin-mediated glucose uptake in muscle and adipose tissue, a process mediated by GLUT4 (20). In skeletal muscle, this is mainly due to impaired recruitment of GLUT4 to the plasma membrane despite normal GLUT4 expression (21). In contrast, insulin resistance in adipocytes is associated with reduced expression of GLUT4 (22, 23). Moreover, mice having an adipose-selective reduction of GLUT4 show a markedly impaired insulin-stimulated glucose uptake in adipocytes accompanied by insulin resistance in both muscle and liver, which is in accordance with the overflow hypothesis, and results in ectopic fat storage (24). Here, we found that lipin mRNA levels in adipose tissue were highly related to both basal and insulin-stimulated adipocyte glucose transport as well as GLUT4 mRNA levels in a large cohort of obese human subjects. The relation between lipin mRNA and insulin receptor sensitivity (pD_2) was, on the other hand, rather weak. Accordingly, a previous study has shown that insulin may regulate lipin protein activity by phosphorylation via a mammalian target of the rapamycin-mediated pathway (25). This pathway is located distal to IRS1/2 and PI3K in

the signaling cascade and has been shown to regulate adipocyte glucose transport (26). Whether lipin is a primary regulator of adipocyte glucose transport remains, however, to be studied.

In lipin-deficient and transgenic mouse models, it has been found that lipin is a critical factor in the adipogenic gene transcription program of adipocyte differentiation, being required prior to induction of key transcriptional regulators including PPARγ and C/EBPα (27). In human preadipocytes, we also show that lipin mRNA is expressed at an early stage of the differentiation process and that it is further induced more or less in parallel with PPARγ, indicating that lipin may also have a role in human adipogenesis.

In conclusion, *LPIN1* gene expression is induced at an early stage in human preadipocyte differentiation in parallel with *PPARγ*. Moreover, adipose tissue *LPIN1* expression levels are reduced in obesity and the metabolic syndrome and are associated with both basal and insulin-stimulated glucose transport in human fat cells as well as adipose *GLUT4* expression. Altogether, these data suggest a role for the *LPIN1* gene in human adipogenesis and fat cell function.⬛

REFERENCES

1. Björntorp, P. 1990. "Portal" adipose tissue as a generator of risk factors for cardiovascular disease and diabetes. *Arteriosclerosis.* **10:** 493–496.
2. Heilbronn, L., S. R. Smith, and E. Ravussin. 2004. Failure of fat cell proliferation, mitochondrial function and fat oxidation results in ectopic fat storage, insulin resistance and type II diabetes mellitus. *Int. J. Obes. Relat. Metab. Disord.* **28 (Suppl.):** 12–21.
3. Czech, M. P. 1976. Cellular basis of insulin insensitivity in large rat adipocytes. *J. Clin. Invest.* **57:** 1523–1532.
4. Large, V., and P. Arner. 1998. Regulation of lipolysis in humans. Pathophysiological modulation in obesity, diabetes, and hyperlipidaemia. *Diabetes Metab.* **24:** 409–418.
5. Bays, H., L. Mandarino, and R. A. DeFronzo. 2004. Role of the adipocyte, free fatty acids and ectopic fat in the pathogenesis of type 2 diabetes mellitus: peroxisome proliferator-activated receptor agonists provide a rational therapeutic approach. *J. Clin. Endocrinol. Metab.* **89:** 463–478.
6. Hegele, R. A. 2004. Phenomics, lipodystrophy and the metabolic syndrome. *Trends Cardiovasc. Med.* **14:** 133–137.
7. Peterfy, M., J. Phan, and K. Reue. 2001. Lipodystrophy in the fld mouse results from a mutation in a new gene encoding a nuclear protein, lipin. *Nat. Genet.* **27:** 121–124.
8. Cao, H., and R. A. Hegele. 2002. Identification of single-nucleotide polymorphisms in the human LPIN1 gene. *J. Hum. Genet.* **47:** 370–372.
9. Suviolahti, E., K. Reue, R. M. Cantor, J. Phan, M. Gentil, J. Naukkarinen, A. Soro-Paavonen, L. Oksanen, J. Kaprio, A. Rissanen, et al. 2006. Cross-species analyses implicate Lipin 1 involvement in human glucose metabolism. *Hum. Mol. Genet.* **15:** 377–386.
10. Kolaczynski, J. W., L. M. Morales, J. H. Moore, Jr. R. V. Considine, Z. Pietrzkowski, P. F. Noto, J. Colberg, and J. F. Caro. 1994. A new technique for biopsy of human abdominal fat under local anaesthesia with Lidocaine. *Int. J. Obes. Relat. Metab. Disord.* **18:** 161–166.
11. Rodbell, M. 1964. Metabolism of isolated fat cells. *J. Biol. Chem.* **239:** 375–380.
12. Arner, P., and P. Engfeldt. 1987. Fasting-mediated alteration studies in insulin action on lipolysis and lipogenesis in obese women. *Am. J. Physiol.* **253:** 193–201.
13. van Harmelen, V., A. Dicker, M. Ryden, H. Hauner, F. Lonnqvist, E. Naslund, and P. Arner. 2002. Increased lipolysis and decreased leptin production by human omental as compared with subcutaneous preadipocytes. *Diabetes.* **51:** 2029–2036.
14. van Harmelen, V., T. Skurk, K. Rohrig, Y. M. Lee, M. Halbleib, I. Aprath-Husmann, and H. Hauner. 2003. Effect of BMI and age on adipose tissue cellularity and differentiation capacity in women. *Int. J. Obes. Relat. Metab. Disord.* **27:** 889–895.
15. Expert Panel. 2001. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *J. Am. Med. Assoc.* **285:** 2486–2497.
16. Lindegaard, B., L. F. Larsen, A-B. E. Hansen, J. Gerstoft, B. K. Pedersen, and K. Reue. Adipose tissue lipin expression levels distinguish HIV patients with and without lipodystrophy. *Int. J. Obes.* Epub ahead of print. July 18, 2006; doi: 10.1038/sj.ijo.0803434 .
17. Kadowaki, T., T. Yamauchi, N. Kubota, K. Hara, K. Ueki, and K. Tobe. 2006. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. *J. Clin. Invest.* **116:** 1784–1792.
18. Serra, A., M. L. Granada, R. Romero, B. Bayés, A. Cantón, J. Bonet, M. Rull, A. Alastrue, and X. Formiguera. 2006. The effect of bariatric surgery on adipocytokines, renal parameters and other cardiovascular risk factors in severe and very severe obesity: 1-year follow-up. *Clin. Nutr.* **25:** 400–408.
19. Lihn, A. S., J. M. Bruun, G. He, S. B. Pedersen, P. F. Jensen, and B. Richelsen. 2004. Lower expression of adiponectin mRNA in visceral adipose tissue in lean and obese subjects. *Mol. Cell. Endocrinol.* **219:** 9–15.
20. Dugani, C. B., and A. Klip. 2005. Glucose transporter 4: cycling, compartments and controversies. *EMBO Rep.* **6:** 1137–1142.
21. Björnholm, M., and J. R. Zierath. 2005. Insulin signal transduction in human skeletal muscle: identifying the defects in Type II diabetes. *Biochem. Soc. Trans.* **33:** 354–357.
22. Defronzo, R. A. 1997. Pathogenesis of type 2 diabetes: metabolic and molecular implications for identifying diabetes genes. *Diabetes Rev.* **5:** 177–269.
23. Shepherd, P. R., and B. B. Kahn. 1999. Glucose transporters and insulin action—implications for insulin resistance and diabetes mellitus. *N. Engl. J. Med.* **341:** 248–257.
24. Dale Abel, E., O. Peroni, J. K. Kim, K. Young-Bum, O. Boss, E. Hadro, T. Minnemann, G. I. Shulman, and B. B. Kahn. 2001. Adipose-selective targeting of the *GLUT4* gene impairs insulin action in muscle and liver. *Nature.* **409:** 729–733.
25. Huffman, T. A., I. Mothe-Satney, and J. C. Lawrence, Jr. 2002. Insulin-stimulated phosphorylation of lipin mediated by the mammalian target of rapamycin. *Proc. Natl. Acad. Sci. USA.* **99:** 1047–1052.
26. Tremblay, F., A. Gagnon, A. Veilleux, A. Sorisky, and A. Marette. 2005. Activation of the mammalian target of rapamycin pathway acutely inhibits insulin signaling to Akt and glucose transport in 3T3-L1 and human adipocytes. *Endocrinology.* **146:** 1328–1337.
27. Phan, J., M. Peterfy, and K. Reue. 2004. Lipin expression preceding peroxisome proliferator-activated receptor-gamma is critical for adipogenesis in vivo and in vitro. *J. Biol. Chem.* **279:** 29558–29564.