The common *rs9939609* gene variant of the fat mass- and obesity-associated gene FTO is related to fat cell lipolysis

Kerstin Wåhlén, Eva Sjölin, and Johan Hoffstedt[1]

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

Abstract We investigated the *rs9939609* single nucleotide polymorphism of the FTO gene in relation to fat cell function and adipose tissue gene expression in 306 healthy women with a wide range in body mass index (18–53 kg/m^2). Subcutaneous adipose tissue biopsies were taken for fat cell metabolism studies and in a subgroup (n = 90) for gene expression analyses. In homozygous carriers of the T-allele, the in vitro basal (spontaneous) adipocyte glycerol release was increased by 22% (*P* = 0.007) and the in vivo plasma glycerol level was increased by ~30% (*P* = 0.037) compared with carriers of the A allele. In contrast, there were no genotype effects on catecholamine-stimulated lipolysis or basal or insulin-induced lipogenesis. We found no difference between genotypes for adipose tissue mRNA levels of FTO, hormone-sensitive lipase, adipose triglyceride lipase, perilipin, or CGI-58. Finally, the adipose tissue level of FTO mRNA was increased in obesity (*P* = 0.002), was similar in subcutaneous and omental adipose tissue, was higher in fat cells than in fat tissue (*P* = 0.0007), and was induced at an early stage in the differentiation process (*P* = 0.004). These data suggest a role of the FTO gene in fat cell lipolysis, which may be important in explaining why the gene is implicated in body weight regulation.—Wåhlén, K., E. Sjölin, and J. Hoffstedt. **The common *rs9939609* gene variant of the fat mass- and obesity-associated gene FTO is related to fat cell lipolysis. *J. Lipid Res.* 2008.** 49: **607–611.**

Supplementary key words adipocyte • lipid and glucose metabolism • messenger RNA • single nucleotide polymorphism

Obesity is becoming a serious health problem worldwide as a result of its association with various disorders, including type 2 diabetes mellitus and cardiovascular disease. In searching for gene variation implicated in obesity development, the recent discoveries of strong associations with obesity of common single nucleotide polymorphisms (SNPs) in the fat mass- and obesity-associated gene FTO in large populations are of particular interest. In a genome-wide search, Frayling et al. (1) found that several SNPs of the FTO gene, including *rs9939609*, were associated with increased body mass index (BMI), and after additional studies in almost 39,000 subjects, it was concluded that homozygous carriers of the risk allele weighed 3 kg more than those devoid of the allele, with an increased odds ratio for obesity of 1.67 and a population-attributable risk of 20%. Interestingly, these findings have been replicated in a case-control study by Dina et al. (2) identifying an at-risk haplotype of the FTO gene, which showed a population-attributable risk of 22% for common obesity, and in a genome-wide association scan associating the FTO gene with obesity-related traits (3).

All of the at-risk FTO gene variants are located within a 47 kb region encompassing the second exon as well as parts of the two first introns of the FTO gene (1, 2). Hitherto, there have been no clues to help define any of these SNPs as functional. The human FTO gene is widely expressed in both fetal and adult tissues, including adipose tissue, with the highest relative levels found in the brain (1). The findings of higher waist circumference as well as higher subcutaneous fat mass in individuals carrying the *rs9939609* risk allele (1) suggest that the FTO gene's effects on adipocyte function may be of physiological importance. Therefore, this study was designed to investigate the association of the *rs9939609* SNP of the FTO gene with the metabolic function of fat cells and to explore the gene expression levels in various adipose materials.

SUBJECTS AND METHODS

Subjects

This study comprised 306 women. They were healthy and free of medication and were recruited to study the influence of genetic variance on adipocyte metabolism. BMI ranged between 18 and 53 kg/m^2, and age ranged between 20 and 72 years. All subjects were living in the Stockholm area and were at least second generation Scandinavian. None was completely sedentary or involved in athletic performances. All ate a standard

Manuscript received 9 October 2007 and in revised form 6 November 2007 and in re-revised form 26 November 2007.

Published, JLR Papers in Press, November 29, 2007.
DOI 10.1194/jlr.M700448-JLR200

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

Copyright © 2008 by the American Society for Biochemistry and Molecular Biology, Inc.

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

Downloaded from www.jlr.org by guest, on June 14, 2012

ASBMB JOURNAL OF LIPID RESEARCH

Swedish diet. None had undergone a slimming effort or experienced a change in body weight (>1 kg) within 6 months before the study, according to self-report. At ~7:30 AM after an overnight fast, a venous blood sample was obtained for DNA extraction and for analyses of plasma levels of glucose, insulin, triglycerides, cholesterol, HDL cholesterol, and glycerol, which were performed by the hospital's accredited chemistry laboratory. Insulin sensitivity was indirectly assessed (homeostasis model assessment) by a formula based on glucose and insulin values (4). Systolic and diastolic blood pressures were measured in the supine position after 15 min of rest. Measurement of total body fat was obtained by a formula based on age, BMI, and sex (5). This formula is accurate compared with more direct estimates of body fat content (6). Plasma glycerol divided by body fat was used as an indirect measure of lipolysis in vivo. An adipose sample (1–2 g) was obtained by needle biopsy from the abdominal subcutaneous area under local anesthesia. The study was approved by the ethics committee at Karolinska University Hospital, and informed consent was obtained from all participants.

Fat cell studies

The adipose tissue was treated with collagenase, and isolated fat cells were collected and subjected to lipolysis and lipogenesis experiments as described (7). In lipolysis studies, adipocytes were incubated in the absence (basal) or presence of increasing concentrations of noradrenaline. After 2 h of incubation, the medium was removed the for measurement of glycerol, which is a quantitative marker for lipolysis. Glycerol release was related to the number of incubated adipocytes. Noradrenaline caused a concentration-dependent stimulation of glycerol release that reached a plateau at the highest agonist concentration. Consequently, it was always possible to determine the concentration of agonist producing a half-maximum effect (sensitivity) as well as the maximal effect. In lipogenesis studies, adipocytes were incubated with radioactive glucose without (basal) or with increased concentrations of insulin. Incorporation into lipid was related to adipocyte number (nmol/2 h/10^7 cells), and the EC50 corresponding to insulin receptor sensitivity and maximum effect were calculated.

Genotyping

Genotype determination of the *rs9939609* T>A polymorphism of the FTO gene (http://www.ncbi.nlm.nih.gov/SNP) was accomplished using a TaqMan-based method (Applied Biosystems, Foster City, CA; C_30090620_10). Genotyping failed in seven subjects, who were excluded from further studies.

mRNA analyses

In 90 subjects, 76 obese (BMI > 30) and 14 nonobese (BMI < 26), there was subcutaneous fat tissue available also for gene expression studies of mRNA. In 18 obese women, we also obtained omental adipose tissue in association with abdominal gastric banding surgery. These mRNA analyses were performed as described (8) using a SYBR Green-based real-time PCR (Bio-Rad Laboratories, Inc., Hercules, CA). The primers used for 18S (reference gene), hormone-sensitive lipase (HSL), adipose triglyceride lipase (ATGL), perilipin, and peroxisome proliferator-activated receptor γ transcript variant 2 have been described elsewhere (8–10). The primers used for FTO were 5′-GACTGCCGAGGAACGAGAG-3′ (sense) and 5′-GGGTCAGATAAGGGAGCCAAG-3′ (antisense), and those used for the lipase-regulating gene CGI-58 were 5′-AGACCCAGGTTTGACAGTGATG-3′ (sense) and 5′-AGTAAGCAGCAGCCAAGAATCC-3′ (antisense). In 14 subjects, there was subcutaneous tissue available also for the comparison of adipose cell versus tissue mRNA expression. From 10 subjects, some of the subcutaneous adipose tissue was used to study preadipocytes. These cells were isolated and differentiated into adipocytes, as described previously (9). The cells reached full differentiation after 12–14 days. At days 4, 8, and 12, cells were lysed for the isolation of total RNA.

Statistical analysis

The Hardy-Weinberg equilibrium test was applied to ensure independent segregation of alleles. Parameter distributions were normalized when necessary by ^{10}logarithm transformation. Testing for differences among parameters between genotype groups was performed using ANOVA, Student's unpaired *t*-test, or analysis of covariance with BMI as covariate. The analyses were performed using StatView version 6.0 (Stata Corp., College Station, TX). Values are expressed as means ± SEM.

RESULTS

The genotype distribution of the FTO *rs9939609* T>A polymorphism was in Hardy-Weinberg equilibrium, and the frequency of the minor allele A was 43%, which is in agreement with the allele frequency of Centre d'Etude du Polymorphisme Humain Europeans (45%) reported by the International HapMap project (www.hapmap.org).

This study was designed to investigate the association of the *rs9939609* polymorphism on various aspects of fat cell metabolism. As shown in **Table 1**, no association of the FTO genotypes and various clinical data were found. With respect to fat cell function, there were no associations between the FTO genotypes and maximal noradrenaline-induced lipolysis, basal or maximal insulin-stimulated lipogenesis, or EC50 for noradrenaline or insulin (**Table 2**). However, there was a significant difference in basal adipocyte lipolysis between genotypes. Therefore, we pooled the AA homozygotes (n = 49) with the heterozygotes (AT; n = 158) and made comparisons with the TT homozygous women (n = 92). As seen in **Fig. 1**, a 22% greater level of in vitro basal unstimulated adipocyte glycerol release (μmol glycerol/10^7 cells) was found in TT subjects (10.1 ± 0.8) than in TA/AA subjects (8.3 ± 0.5; P = 0.007). This was accompanied by a 28% greater in vivo plasma glycerol level of TT homozygotes (3.2 ± 0.4 vs. 2.5 ± 0.1 glycerol/kg body fat; P = 0.037) (Fig. 1).

Table 3 shows the association of the FTO polymorphism on mRNA expression levels of FTO and various genes implicated in lipolysis regulation, including HSL, ATGL, perilipin, and CGI-58. However, no difference in mRNA expression levels between TT, AT, and AA subjects was found for any of the genes analyzed.

Finally, we investigated the relative expression levels of FTO mRNA in various adipose materials. As seen in **Fig. 2A**, whereas obese (BMI > 30) compared with nonobese (BMI < 26) subjects showed an increased level of subcutaneous adipose tissue FTO mRNA, no regional difference in FTO gene expression between omental and subcutaneous adipose tissue was found (Fig. 2B). By comparing the relative expression levels in adipocytes and adipose tissue, we found that there is an enrichment of FTO mRNA in fat cells (Fig. 2C). In studying preadipo-

ASBMB JOURNAL OF LIPID RESEARCH

Downloaded from www.jlr.org by guest, on June 14, 2012

TABLE 1. Clinical data in subjects genotyped for the *rs9939609* SNP of the FTO gene

Characteristic	Genotype TT	AT	AA	P
Number	92	158	49	
Age (years)	38 ± 1	39 ± 1	40 ± 1	0.72
BMI (kg/m^2)	33 ± 1	34 ± 1	35 ± 1	0.44
Waist (cm)	103 ± 2	103 ± 2	107 ± 3	0.43
Body fat (%)	43 ± 1	44 ± 1	46 ± 2	0.40
P-Glucose (mmol/l)	5.4 ± 0.1	5.3 ± 1	5.3 ± 1	0.69
P-Insulin (mU/l)	12 ± 1	12 ± 1	12 ± 1	0.82
Homeostasis model assessment (index)	3.1 ± 0.3	2.9 ± 0.2	3.0 ± 0.3	0.84
P-Triglycerides (mmol/l)	1.4 ± 0.1	1.4 ± 0.1	1.2 ± 0.1	0.57
P-Cholesterol (mmol/l)	4.8 ± 0.1	5.0 ± 0.1	5.1 ± 0.1	0.11
P-HDL cholesterol	1.3 ± 0.04	1.3 ± 0.03	1.4 ± 0.06	0.60
P-Glycerol/kg body fat	3.2 ± 0.4	2.5 ± 0.2	2.5 ± 0.2	0.11
Systolic blood pressure (mmHg)	125 ± 2	124 ± 1	123 ± 2	0.66
Diastolic blood pressure (mmHg)	76 ± 1	76 ± 1	77 ± 1	0.91

BMI, body mass index; SNP, single nucleotide polymorphism. Values are means ± SEM and were compared using ANOVA.

cytes, we observed that FTO mRNA expression was induced at an early stage in the differentiation process (Fig. 2D). No correlation between FTO mRNA and lipolysis was observed except for a borderline relationship with plasma glycerol ($P = 0.057$).

DISCUSSION

The mechanism underlying the effect of FTO on body fat regulation is unknown (11). This study sheds some light, for the first time, on the possible mechanisms by which this gene may regulate fat mass. We demonstrate that healthy women, who are homozygous for the more common obesity-protective FTO allele, have ~30% increased in vivo lipolytic activity (measured as circulating glycerol corrected for total body fat) compared with other genotypes, independent of BMI. In addition, the spontaneous (basal) lipolysis in fat cells is increased by ~20% in homozygous women. The latter finding most likely explains the increased in vivo lipolytic activity, because the effect of noradrenaline, a major lipolytic hormone in humans, on adipocyte lipolysis was not influenced by the FTO polymorphism.

TABLE 2. Fat cell function in subjects genotyped for the *rs9939609* SNP of the FTO gene

Variable	Genotype TT	AT	AA	P
Number	92	158	49	
Lipolysis (μmol glycerol/10^7 cells)				
Basal	10.1 ± 0.8	8.2 ± 0.5	8.8 ± 0.9	0.03
Maximal (−basal)	12.9 ± 0.6	12.9 ± 0.6	12.9 ± 0.9	0.98
EC_{50}	−8.3 ± 0.1	−8.2 ± 0.1	−8.1 ± 0.2	0.85
Lipogenesis (nmol glucose/2 h/10^7 cells)				
Basal	2.4 ± 0.3	2.5 ± 0.2	2.4 ± 0.4	0.89
Maximal (−basal)	3.4 ± 0.4	3.4 ± 0.2	3.1 ± 0.4	0.95
EC_{50}	−12.9 ± 0.2	−12.8 ± 0.1	−13.2 ± 0.2	0.10

Values are means ± SEM and were compared using analysis of covariance with BMI as covariate.

Our findings were obtained with a rather small number of subjects (~300). However, it is by far the largest existing cohort for genetic lipolysis studies, and the FTO polymorphism is very common (minor allele frequency ~ 40%). We studied women and subcutaneous fat only, and there are variations in lipolysis between the sexes and adipose tissue regions (12, 13). Therefore, we cannot

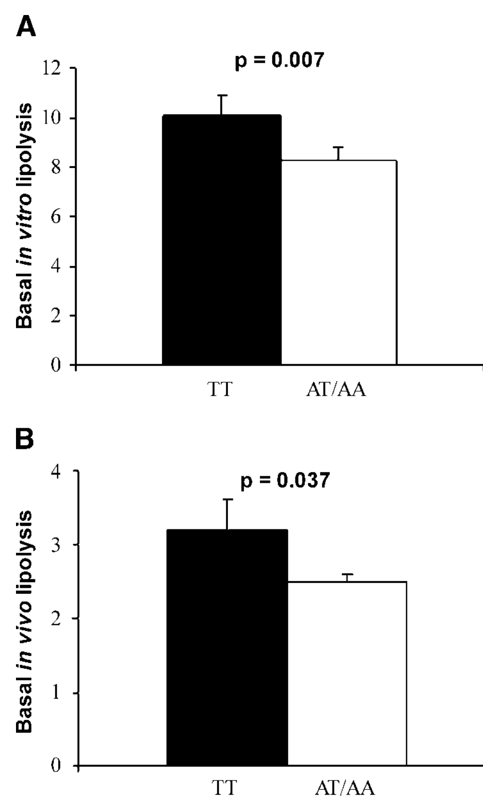

Fig. 1. Basal in vitro lipolysis (glycerol release/2 h/10^7 fat cells) (A) and basal in vivo lipolysis (P-glycerol/l/kg body fat) (B) in subjects genotyped for the *rs9939609* single nucleotide polymorphism of the FTO gene. Values are means ± SEM and were compared using analysis of covariance with body mass index as covariate (in vitro lipolysis) (A) or ANOVA (in vivo lipolysis) (B).

ASBMB JOURNAL OF LIPID RESEARCH JLR

Downloaded from www.jlr.org by guest, on June 14, 2012

TABLE 3. FTO *rs9939609* SNP in relation to adipose tissue gene expression levels

mRNA	Genotype TT	AT	AA	P
Number	27	46	17	
FTO/18S (au)	1.8 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	0.21
Hormone-sensitive lipase/18S (au)	4.6 ± 0.4	4.8 ± 0.3	5.0 ± 0.5	0.52
Adipose triglyceride lipase/18S (au)	5.9 ± 0.4	5.8 ± 0.3	5.6 ± 0.5	0.72
Perilipin/18S (au)	3.7 ± 0.2	3.8 ± 0.2	3.7 ± 0.4	0.76
CGI-58/18S (au)	1.4 ± 0.1	1.1 ± 0.1	1.3 ± 0.2	0.55

au, arbitrary units. Values are means ± SEM and were compared using analysis of covariance with BMI as covariate.

say whether the findings are true also for men or for other fat depots, such as the visceral one. The subcutaneous adipose tissue, on the other hand, is by far the body's largest fat depot.

How can FTO regulate lipolysis? Our findings suggest that the gene is markedly expressed in adipocytes and that the mRNA for FTO is enriched in these cells compared with total adipose tissue. FTO expression is regulated in human adipose tissue. It decreases during adipocyte differentiation and is increased in obesity, but it is not influenced by adipose region. However, there seems to be no straightforward link between FTO gene expression and lipolysis rate in adipose tissue. We found no relationship between FTO mRNA levels and lipolysis, although the in vivo lipolytic activity tended to correlate ($P = 0.057$). The polymorphism is not associated with the level of mRNA expression of FTO. We also investigated the association of the FTO genotype with expression levels of genes that have been shown to be important in regulating basal adipocyte lipolysis, including the two lipases HSL and ATGL and the two lipid droplet-associated proteins perilipin and CGI-58 (14), but no positive relations were found. However, a number of additional putative lipolysis-regulating genes may be associated with the FTO genotype. It is also possible that FTO has some indirect effects on lipolysis, which are not known at present. Because there were no differences in BMI or other metabolic parameters between genotypes, these putative lipolysis-regulating effects most likely are not secondary to body fat accumulation per se.

It appears that high lipolytic activity in vivo is an important protective factor for excess body fat, at least at the level of gene variance. We recently observed that an orphan G-protein receptor termed GPR74 is involved in the regulation of human fat cell lipolysis and that a common haplotype, ATAG, in the GPR74 gene was associated with protection from obesity, high lipolytic activity in vivo, and increased catecholamine-induced lipolysis in fat cells (6). Thus, high spontaneous (this study) as well as enhanced stimulated (6) rates of lipolysis in fat cells may be protective against the development of obesity. The present data are partly in agreement with genetic animal models of obesity, including perilipin-null mice, which are characterized by increased basal, but attenuated stimulated, lipolysis, resulting in resistance to diet-induced obesity (15, 16).

In summary, the more common obesity-protective allele in the FTO gene is in its homozygous form associated with increased adipocyte lipolytic activity both in vivo and in vitro, suggesting that FTO may, at least in part, regulate body fat mass through lipolysis, although the precise mechanisms of action need to be defined.

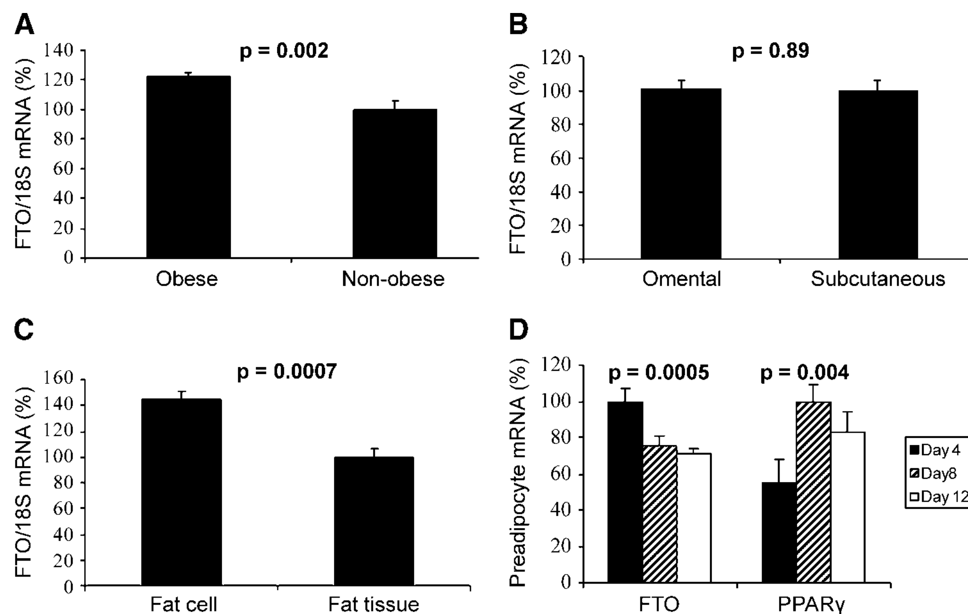

Fig. 2. FTO mRNA adipose expression levels in subcutaneous adipose tissue from obese and nonobese subjects (A), in omental and subcutaneous adipose tissue (B), in fat cells and fat tissue (C), and in preadipocyte differentiation (D). Values are means ± SEM and were compared using Student's unpaired *t*-test. PPARγ, peroxisome proliferator-activated receptor γ.

Downloaded from www.jlr.org by guest, on June 14, 2012

This study was supported by the Swedish Research Council, AFA Life Insurance, and the Swedish Medical Association.

REFERENCES

1. Frayling, T. M., N. J. Timpson, M. N. Weedon, E. Zeggini, R. M. Freathy, C. M. Lindgren, J. R. Perry, K. S. Elliott, H. Lango, N. W. Rayner, et al. 2007. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. *Science.* **316:** 889–894.
2. Dina, C., D. Meyre, S. Gallina, E. Durand, A. Korner, P. Jacobson, L. M. Carlsson, W. Kiess, V. Vatin, C. Lecoeur, et al. 2007. Variation in FTO contributes to childhood obesity and severe adult obesity. *Nat. Genet.* **39:** 724–726.
3. Scuteri, A., S. Sanna, W-M. Chen, M. Uda, G. Albai, J. Strait, S. Najjar, R. Nagaraja, M. Orrú, G. Usala, et al. 2007. Genomewide association scan shows genetic variants in the *FTO* gene are associated with obesity-related traits. *PLoS Genet.* **3:** e115.
4. Matthews, D. R., J. P. Hosker, A. S. Rudenski, B. A. Naylor, D. F. Treacher, and R. C. Turner. 1985. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia.* **28:** 412–419.
5. Gallagher, D., M. Visser, D. Sepulveda, R. N. Pierson, T. Harris, and S. B. Heymsfield. 1996. How useful is body mass index for comparison of body fatness across age, sex, and ethnic groups? *Am. J. Epidemiol.* **143:** 228–239.
6. Dahlman, I., A. Dicker, H. Jiao, J. Kere, L. Blomqvist, V. van Harmelen, J. Hoffstedt, K. Borch-Johnsen, T. Jorgensen, T. Hansen, et al. 2007. A common haplotype in the G-protein-coupled receptor gene GPR74 is associated with leanness and increased lipolysis. *Am. J. Hum. Genet.* **80:** 1115–1124.
7. Lofgren, P., J. Hoffstedt, E. Naslund, M. Wiren, and P. Arner. 2005. Prospective and controlled studies of the actions of insulin and catecholamine in fat cells of obese women following weight reduction. *Diabetologia.* **48:** 2334–2342.
8. Mairal, A., D. Langin, P. Arner, and J. Hoffstedt. 2006. Human adipose triglyceride lipase (PNPLA2) is not regulated by obesity and exhibits low in vitro triglyceride hydrolase activity. *Diabetologia.* **49:** 1629–1636.
9. Ryden, M., E. Arvidsson, L. Blomqvist, L. Perbeck, A. Dicker, and P. Arner. 2004. Targets for TNF-alpha-induced lipolysis in human adipocytes. *Biochem. Biophys. Res. Commun.* **318:** 168–175.
10. van Harmelen, V., M. Ryden, E. Sjolin, and J. Hoffstedt. 2007. A role of lipin in human obesity and insulin resistance: relation to adipocyte glucose transport and GLUT4 expression. *J. Lipid Res.* **48:** 201–206.
11. Groop, L. 2007. From fused toes in mice to human obesity. *Nat. Genet.* **39:** 706–707.
12. Blaak, E. 2001. Gender differences in fat metabolism. *Curr. Opin. Clin. Nutr. Metab. Care.* **4:** 499–502.
13. Lafontan, M., and M. Berlan. 2003. Do regional differences in adipocyte biology provide new pathophysiological insights? *Trends Pharmacol. Sci.* **24:** 276–283.
14. Arner, P., and D. Langin. 2007. The role of neutral lipases in human adipose tissue lipolysis. *Curr. Opin. Lipidol.* **18:** 246–250.
15. Martinez-Botas, J., J. B. Anderson, D. Tessier, A. Lapillonne, B. H-J. Chang, M. J. Quast, D. Gorenstein, K-H. Chen, and L. Chan. 2000. Absence of perilipin results in leanness and reverses obesity in *Leprdb/db* mice. *Nat. Genet.* **26:** 474–479.
16. Tansey, J. T., C. Sztalryd, J. Gruia-Gray, D. L. Roush, J. V. Zee, O. Gavrilova, M. L. Reitman, C-X. Deng, C. Li, A. R. Kimmel, et al. 2002. Perilipin ablation results in a lean mouse with aberrant adipocyte lipolysis, enhanced leptin production, and resistance to diet-induced obesity. *Proc. Natl. Acad. Sci. USA.* **98:** 6494–6499.

Downloaded from www.jlr.org by guest, on June 14, 2012