Adipocyte triglyceride lipase expression in human obesity

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Steinberg GR, Kemp BE, Watt MJ. Adipocyte triglyceride lipase expression in human obesity. Am J Physiol Endocrinol Metab 293: E958–E964, 2007. First published July 3, 2007; doi:10.1152/ajpendo.00235.2007.—We have investigated the gene and protein expression of adipocyte triglyceride lipase (ATGL) and triglyceride (TG) lipase activity from subcutaneous and visceral adipose tissue of lean and obese subjects. Visceral and subcutaneous adipose tissue was obtained from 16 age-matched lean and obese subjects during abdominal surgery. Tissues were analyzed for mRNA expression of lipolytic enzymes by real-time quantitative PCR. ATGL protein content was assessed by Western blot and TG lipase activity by radiometric assessment. Subcutaneous and visceral adipose tissue of obese subjects had elevated mRNA expression of PNPLA2 (ATGL) and other lipases including PNPLA3, PNPLA4, CES1, and LPLAL1 (P < 0.05). Surprisingly, ATGL protein expression and TG lipase activity were reduced in subcutaneous adipose tissue of obese subjects. Immunoprecipitation of ATGL reduced total TG lipase activity in adipose lysates by 70% in obese and 83% in lean subjects. No significant differences in the ATGL activator CGI-58 mRNA levels (ABHD5) were associated with obesity. These data demonstrate that ATGL is important for efficient TG lipase activity in humans. They also demonstrate reduced ATGL protein expression and TG lipase activity despite increased mRNA expression of ATGL and other novel lipolytic enzymes in obesity. The lack of correlation between ATGL protein content and in vitro TG lipase activity indicates that small decrements in ATGL protein expression are not responsible for the reduction in TG lipase activity observed here in obesity, and that post-translational modifications may be important.

triglyceride metabolism; adipose triglyceride lipase; triglyceride hydrolase activity

The ability to efficiently regulate the storage and release of fatty acids contained in adipose triglyceride (TG) requires tight coupling between hydrolysis and esterification. Fat stored in subcutaneous and visceral depots is mobilized by activation of lipolytic enzymes, which degrade TGs and release free fatty acids (FFAs) into the circulation for use as an energy substrate. Obesity is characterized by excessive adipose tissue deposition and increased FFA release that exceeds metabolic demands (17). Elevated postprandial FFAs are a hallmark of obesity (33) and contribute to metabolic disturbances including insulin resistance in skeletal muscle and liver (4, 44), pancreatic β-cell dysfunction (51), and increased VLDL triglyceride production (27).

Until recently, hormone-sensitive lipase (HSL) was considered the rate-limiting enzyme for lipolysis, but recent evidence suggests that adipose triglyceride lipase (ATGL) may also be important. HSL−/− mice have residual TG lipase activity (11, 31, 32) indicative of the presence of other TG lipases and maintain partial basal and catecholamine-stimulated lipolysis (41). Recent studies have identified the molecular lipolytic (and esterolytic) proteome in mouse adipose tissue (3) and 23 proteins that are incompletely characterized. Many of these proteins are predicted to contain an α/β-hydrolase fold that accommodates catalytic residues typical of many lipases (36). Of these recently identified lipases (3), ATGL (also known as desnutrin and calcium-independent phospholipase, iPLA2γ) has been demonstrated to be critical for both basal and catecholamine-stimulated TG hydrolysis, energy metabolism in cultured adipocytes, and weight maintenance in mice (10, 12, 15, 21, 40, 52). Homologues with similar functions have also been reported in Drosophila and Saccharomyces cerevisiae (9, 20, 38).

Aside from HSL, the role of other TG lipases in the pathogenesis of human obesity is unclear. HSL expression is reduced in obesity (23, 29, 42), which is inconsistent with the elevated basal lipolysis observed in these individuals, implying that other TG lipases may be upregulated. Two recent reports (22, 29) suggest that ATGL mRNA is not regulated in human obesity, whereas the same group reports that ATGL mRNA and protein expression are negatively associated with insulin resistance and not obesity per se (13). Suppression of ATGL with short interfering RNA in human adipocytes derived from human mesenchymal stem cells indicates that ATGL is important for basal lipolysis but does not contribute to catecholamine-induced lipolysis (34). Despite the uncertainty regarding ATGL in obesity, single nucleotide polymorphisms within the ATGL gene are associated with free fatty acid levels and increased risk of type 2 diabetes mellitus (35). Similarly, mutations in the lipase family member comparative gene identification-58 (CGI-58), an essential co-regulator of ATGL (24), result in TG accumulation in several tissues, resulting in serious pathologies including cardiomyopathy and liver steatosis (26). Another TG lipase, adiponutrin, has been reported to be either increased (14) or unchanged (28) with obesity. Taken together, these data suggest that the upregulation of basal adipose tissue lipolysis in obesity may be associated with the increased expression of alternative TG lipases.

The first aim of this study was to characterize the mRNA and protein expression of ATGL in relation to obesity and compare these levels with in vitro TG lipase activity. The second aim of the study was to assess the contribution of ATGL to in vitro TG lipase activity. To gain further insight into the molecular control of adipocyte lipolysis, the third aim was to assay the mRNA content of selected enzymes that were previously identified to possess murine TG lipase activity and contain an α/β-hydrolase fold (3). Finally, we have extended previous studies (13) by examining both subcutaneous and visceral adipose tissue, because there is marked heterogeneity with regard to lipolysis regulation and fatty acid flux from these sites (17).

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METHODS

Subjects and Study Design

Lean [n = 8, body mass index (BMI) range = 21.0–27.9, mass = 59–75 kg; 4 male, 4 female] and obese (n = 8, BMI range = 30.0–37.1, mass = 86–113 kg; 2 male, 6 female) subjects participated in this study. None was taking medication or involved in an exercise program, and their body weight was essentially stable for 12 mo before all experiments. All female subjects were postmenopausal.

Participants were admitted to McMaster Health Sciences Center for a variety of abdominal surgical interventions after an overnight fast (~16 h) as previously described (5). Adipose tissue samples (from visceral and subcutaneous depots) were excised under general anesthesia and snap frozen in liquid N2 while a venous blood sample was obtained from the antecubital vein and stored on ice until processing (<5 min). All experimental procedures were performed according to the Declaration of Helsinki. Ethical clearance was granted by the McMaster University Ethics Committee, and informed consent was obtained after procedures were explained both verbally and in writing. All applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research.

Analytical Methods

Adipose tissue mRNA analysis. Total RNA was extracted from adipose tissue in 750 μl of Qiazol extraction reagent followed by isolation using an RNasy Lipid Tissue kit (Qiagen, Doncaster, Victoria, Australia) according to the manufacturer’s instructions. RNA quantity was determined at 260 nm (NanoDrop p2000 Spectrometer). RNA was reverse transcribed (Invitrogen, Mt. Waverley, Victoria, Australia), and gene products were determined by real-time quantitative RT-PCR (Stratagene Mx3000P, La Jolla, CA) using TaqMan Assays-on-Demand (Applied Biosystems, Scoresby, Victoria, Australia) as previously described (43). The final reaction mix consisted of 10 μl of TaqMan Universal PCR master mix, 0.5 ng of cDNA in 9 μl of RNase-free water, and 1 μl of supplied primers. PCR conditions were as follows: 2 min at 50°C and 10 min at 95°C, followed by 40 cycles of PCR reaction at 95°C for 15 s and 60°C for 45 s. 18S was used as a reference gene and did not vary between groups or adipose tissue depots [cycle threshold (CT) lean = 19.2 ± 0.1 vs. obese = 19.3 ± 0.2]. ATGL (PNPLA2) could not be identified using the TaqMan Assay-on-Demand probe. We therefore used 2× SYBR green PCR master mix, 2 ng of cDNA, and 10 μM primers (sense, 5′-GGTTCAGACGGCGAGAATG-3′; antisense, 5′-TGAGGAATGGAGGAGGATG-3′) as described previously (29). The PCR conditions were as described above, and the primer pairs yielded a single amplicon based on dissociation curves. The mRNA levels were determined by a comparative CT method. For each sample, a ΔCT value was obtained by subtracting 18S CT values from those of the gene of interest. Similar values were obtained when subtracting RPL-32. The average ΔCT value of the lean group was then subtracted from the sample to derive a Δ – ΔCT value. The expression of each gene was then evaluated by 2–ΔΔCT.

Adipose tissue protein analysis. Adipose tissue was homogenized and processed as described previously (45). A rabbit ATGL polyclonal antibody was raised against the peptide CTNVAPFPDDLARMAPA based on the amino acid sequence of human ATGL (449–465) and was purified as described previously (7). The ATGL antibody showed one band at ~54 kDa (see Fig. 3A) and in some instances a fainter band at ~48 kDa. The ATGL expressed from a mammalian expression vector was also detected with this antibody at a similar molecular mass (see Fig. 3A). After a 45-min washing, membranes were incubated with protein G-horseradish peroxidase-conjugated secondary antibody (1:2,000), and the immunoreactive proteins were detected with enhanced chemiluminescence and quantified by densitometry (Scion Image, Scion). In additional immunoprecipitation (IP) experiments, 1 mg of adipose tissue lysate was incubated for 2 h at 4°C in the presence of ATGL antibody conjugated to protein A-Sepharose beads. The post-IP lysate was recovered for TG lipase activity assays. For control experiments, some samples were immunoprecipitated with an antibody to insulin receptor substrate-2 (IRS2; Upstate, Lake Placid, NY).

Adipose tissue TG lipase activity assay. Adipose tissue TG lipase activity was determined using an emulsified substrate consisting of 5 mmol/l triolein, 6.7 × 105 counts/min [9,10-3H]tri olein, 0.6 mg of phospholipid (phosphatidy lcholine-phosphatidylinositol, 3:1, wt/wt), 0.1 M potassium phosphate, and 20% BSA. Procedures for this assay have been described previously (42).

Plasma metabolite determination. One portion of heparinized whole blood was deproteinized 1:5 with 0.6% (wt/vol) per chloric acid and centrifuged. The extract was analyzed for blood glucose (2). A second portion of whole blood was centrifuged, and the plasma was removed for the determination of FFA by an enzymatic colorimetric method (Wako NEFA C test kit, Wako Chemicals) and insulin by radioimmunoassay (Coat-a-Count insulin test kit, Diagnostics Products).

Calculations and statistics. Insulin sensitivity of subjects was evaluated using the homeostasis model assessment of insulin resistance [HOMA-IR; (fasting glucose × fasting insulin)/22.5] (where glucose is expressed as mmol/l and insulin as μU/l). Comparisons were made between lean and obese subjects by unpaired t-tests. Values are reported as means ± SE. Statistical significance was set a priori at P < 0.05.

RESULTS

Gene Expression of Lipolytic Enzymes in Subcutaneous and Visceral Adipose Tissue of Lean and Obese Subjects

The clinical characteristics of lean and obese subjects after an overnight fast are listed in Table 1. Obese subjects had a greater body mass, BMI, and HOMA index relative to lean controls. Although plasma glucose and insulin were not different between groups, obese subjects displayed whole body insulin resistance as demonstrated by an increased HOMA index.

Subcutaneous adipose tissue. Subcutaneous fat located in the upper body is thought to contribute ~65% of systemic FFA flux and is likely the dominant source of the excess systemic fatty acids released in obesity (17). The mRNA expression of lipolytic proteins in subcutaneous adipose tissue of lean and obese subjects is shown in Fig. 1. We first examined members of the “patatin-like phospholipase domain-containing proteins” (PNPLA) family. These proteins contain a patatin-like domain that shows acyl-hydrolase activity and are highly conserved across all life forms. PNPLA proteins are expressed in mouse adipose tissue (18) and demonstrate marked TG lipase activity in vitro (21, 52). PNPLA2 (ATGL) mRNA expression levels

Table 1. Clinical characteristics of lean and obese subjects

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th>Obese</th>
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<tr>
<td>Age, yr</td>
<td>59.5±6.6</td>
<td>57.5±5.4</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>66.9±4.6</td>
<td>93.6±6.7*</td>
</tr>
<tr>
<td>BMI, kg/m2</td>
<td>24.6±1.25</td>
<td>32.6±1.5*</td>
</tr>
<tr>
<td>Plasma glucose, mM</td>
<td>6.61±0.22</td>
<td>6.08±0.41</td>
</tr>
<tr>
<td>Plasma insulin, μU/ml</td>
<td>7.29±0.97</td>
<td>11.3±1.6</td>
</tr>
<tr>
<td>Plasma FFA, mM</td>
<td>1.19±0.04</td>
<td>1.16±0.11</td>
</tr>
<tr>
<td>HOMA</td>
<td>21.3±2.3</td>
<td>34.4±2.2*</td>
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Values are means ± SE. BMI, body mass index; FFA, free fatty acid; HOMA, homeostasis model assessment. *P < 0.05 vs. lean.
tended to be higher in obese (Fig. 1A, P = 0.08) than in lean subjects. PNPLA2 and PNPLA4 mRNA contents were increased 2.7- and 1.8-fold, respectively, in obese vs. lean subjects (Fig. 1B and C). PNPLA8 mRNA expression was unaltered between groups (Fig. 1D). We did not probe for PNPLA1 because its physiological function is unknown, and we were unable to detect PNPLA5 mRNA with the assay conditions listed, consistent with a previous report using Northern blot analysis (21). LIPE (HSL) mRNA expression was not altered by obesity (Fig. 1E). The human carboxylesterase-1, or triacylglycerol hydrolase, has been shown to play a role in hepatic lipid metabolism (1). The expression of its gene, CES1, was increased 2.2-fold in obesity (Fig. 1F). There were no changes in ABHD5 mRNA (Fig. 1G), whereas LYPLAL1 mRNA was increased in obesity (Fig. 1H).

The abundance of ATGL and HSL transcripts was highly correlated ($R^2 = 0.74$, $P = 0.004$; Fig. 1I). There were no significant relationships between the other transcripts assessed. Further correlative analysis with BMI as the independent variable indicated that PNPLA2 (ATGL) and LIPE (HSL) are not obesity dependent (BMI vs. ATGL: $R^2 = 0.08$, $P = 0.30$). Previous studies have demonstrated that peroxisome proliferator-activated receptor (PPAR)γ increases ATGL transcription (15, 16, 19). PPAR γ protein was decreased ($P < 0.01$) by 48 ± 6% in subcutaneous adipose tissue of obese subjects (data not shown). No significant correlation was reported between ATGL mRNA and PPAR γ protein ($R^2 = 0.11$, $P = 0.56$).

Visceral adipose tissue. Visceral fat is positively associated with whole body insulin resistance (25) and contributes ~15% to systemic FFA flux (17). The gene expression profile of the PNPLA family was similar to our observations in subcutaneous adipose tissue. PNPLA2 was increased 2.2-fold (Fig. 2A) and PNPLA3 2.8-fold (Fig. 2B), and PNPLA4 tended to increase ($P = 0.11$, Fig. 2C). PNPLA8 was not different between groups (Fig. 2D). LIPE was elevated 2.3-fold in the visceral adipose tissue of obese individuals (Fig. 2E), whereas CES1 (Fig. 2F), ABHD5 (Fig. 2G), and LYPLAL1 (Fig. 2H) were not different between groups. Consistent with our observations in subcutaneous adipose tissue, PNPLA2 and LIPE were positively correlated (Fig. 2I).

ATGL Protein Content and Neutral Lipase Activity in Adipose Tissue of Lean and Obese Subjects

We first confirmed the specificity of our antibody. The ATGL polyclonal antibody revealed a single band at the expected molecular mass of ~54 kDa (Fig. 3A). In some samples, a faint band was also detected at ~48 kDa. The prevalence of this 48-kDa band was not prominent in any particular group. Further experiments revealed that ATGL expressed from a mammalian expression vector was detected with our antibody at the expected molecular mass (Fig. 3A). We next performed IP experiments and reprobed, using a commercially available ATGL antibody (Cell Signaling, Danvers, MA). In the

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**Fig. 1.** A–H: mRNA expression of triglyceride (TG) lipases in subcutaneous adipose tissue of lean and obese subjects. I: correlation between PNPLA2 and LIPE mRNA expression levels in subcutaneous adipose tissue. Values are means ± SE ($n = 8$ subjects).
post-IP fraction, no ATGL was observed, suggesting that all ATGL protein was immunoprecipitated (Fig. 3A). There was no reduction in HSL protein after ATGL IP (data not shown). To further evaluate the role of ATGL in adipose tissue lipolysis, we measured its protein expression in subcutaneous and visceral adipose tissue samples using immunoblotting. ATGL protein expression was lower (24%, \( P = 0.04 \)) in the subcutaneous adipose tissue of obese compared with lean subjects (Fig. 3B). ATGL protein expression in visceral adipose tissue was not different between groups (\( P = 0.09 \)).

TG lipase activity was assessed in adipose tissue lysates in vitro. TG lipase activity was decreased in the subcutaneous (\( P = 0.02 \)), but not visceral, adipose tissue of obese compared with lean subjects (Fig. 3C). ATGL protein expression in visceral adipose tissue was not different between groups (\( P = 0.09 \)).

TG lipase activity was assessed in adipose tissue lysates in vitro. TG lipase activity was decreased in the subcutaneous (\( P = 0.02 \)), but not visceral, adipose tissue of obese compared with lean subjects (Fig. 3C). Overall, TG lipase activity did not show any significant correlations with ATGL protein in either adipose tissue depot (\( R^2 = 0.06 \)). Even when subjects were divided into groups based on high (18.8 \( \pm \) 0.7 nmol·h\(^{-1}\)·mg protein\(^{-1} \), \( n = 8 \)) or low (7.5 \( \pm \) 1.0 nmol·h\(^{-1}\)·mg protein\(^{-1} \), \( n = 8 \)) TG lipase activity, ATGL protein expression was remarkably similar (high-TG lipase group, 1.79 \( \pm \) 0.15 arbitrary units; low-TG lipase group, 1.79 \( \pm \) 0.17 arbitrary units). We directly tested the importance of ATGL for total TG lipase activity in human adipose tissue in vitro. To do this, we examined total TG lipase activity in adipose lysates before and after IP of ATGL. We were unable to measure both visceral and subcutaneous depots because of a lack of tissue; hence, the data are pooled (\( n = 4 \) visceral and \( n = 4 \) subcutaneous for each group). IP removed the majority of ATGL protein from the adipose lysate as determined by Western blot analysis (Fig. 3A). TG lipase activity was reduced post-IP by 83 \( \pm \) 7% and 70 \( \pm \) 6% in lean and obese subjects, respectively (Fig. 3D). The percent reduction in TG lipase activity post-IP was not different between groups (\( P = 0.15 \)). TG lipase activity was unaffected in the post-IP lysates when an unrelated antibody (IRS2) was used in control experiments (data not shown).

**DISCUSSION**

In the present study, we investigated the mRNA expression profile of several novel TG lipases and ATGL protein expression and the relationship with in vitro TG lipase activity in subcutaneous and visceral adipose tissue from lean and obese subjects. We have assessed adipose tissue from male and female subjects. Although it is documented that lipolysis control varies according to sex, owing mainly to differences in sex hormones and adrenergic receptor sensitivity (46), this remains controversial (6). In fact, estrogen treatment in postmenopausal women does not affect the expression of proteins and genes involved in adipocyte signal transduction (49). Regardless, adipose tissue in this study was obtained from postmenopausal women, which most likely minimizes potential sex differences. The results of this study indicate the following. 1) The mRNA expression of ATGL (\( P = 0.08 \) in subcutaneous) and HSL is increased in adipose tissue of obese subjects, but this increase...
TG lipase activity is reduced in subcutaneous adipose tissue of obese subjects despite increases in lipase gene expression. And 5) ATGL is essential for efficient TG lipase activity, but ATGL protein expression is not related to TG lipase activity in subcutaneous or visceral adipose tissue from lean or obese subjects.

ATGL belongs to the patatin-like phospholipase family and shares a close evolutionary relationship with other lipid acyl-hydrolases (21). ATGL possesses several key structural features including an NH2-terminal patatin homology domain, which is a feature of proteins that possess acyl-hydrolase activity on lipid substrates (50), and an α/β-hydrolase fold and an active site GXSXG consensus sequence, both common features of many lipases (36). ATGL specifically hydrolyzes TG (12, 21, 52) and is predominantly expressed in adipose tissue. Inhibition of ATGL reduces TG breakdown in cell culture (15, 38) and mice in vivo (10). In the present investigation, we examined the influence of obesity on ATGL mRNA expression, as ATGL mRNA is induced during adipogenesis in murine adipocytes (15, 16, 40, 52) and is elevated in genetically obese mice (21, 40). We found that ATGL mRNA expression was increased in subcutaneous (P = 0.08) and visceral adipose tissue of obese compared with lean humans; however, ATGL mRNA expression was not related to BMI. The obese subjects in this study were mildly insulin resistant, and ATGL mRNA was positively correlated with HOMA (R² = 0.306, P < 0.05). So, rather than obesity per se, these findings in obese, mildly insulin-resistant humans are consistent with studies demonstrating transcriptional inhibition of ATGL by insulin (15, 16, 19) and increased ATGL mRNA expression in murine models of insulin deficiency (streptozotocin-induced diabetes) and insulin resistance (fat-specific insulin receptor knockout) (15). Also, preliminary data from our laboratory show a marked upregulation of ATGL mRNA (2.2-fold, n = 5) in the subcutaneous adipose tissue of nonobese type 2 diabetes patients. Collectively, these data support the concept that ATGL gene expression becomes resistant to the inhibitory action of insulin and is not regulated by obesity. In contrast to our results, it was reported that ATGL mRNA was reduced in insulin-resistant individuals, independent of fat mass in obese individuals (13). It is difficult to reconcile our results in obese individuals with those of Jocken et al. (13). It must be stressed that the increased ATGL mRNA in the obese subjects from the present study is entirely consistent with the premise that insulin’s demonstrated inhibitory effects were obviated in these insulin-resistant individuals, thus resulting in increased ATGL mRNA expression.

As an extension of previous human studies (22, 29), we examined ATGL protein expression in adipose tissue and compared this with TG lipase activity in fasted individuals. ATGL protein content was decreased in the subcutaneous adipose tissue of obese individuals, whereas no differences were observed in visceral fat. The reduced ATGL protein in subcutaneous fat is consistent with a previous report (13) and may reflect increased degradation, since ATGL mRNA expression was increased in obesity. The factors underpinning this apparent mismatch are presently unclear but may involve proteasome degradation via a ubiquitination pathway, as has been described previously for other proteins important for lipolytic regulation including perilipin and adipose differentiation-related protein (47, 48).
HSL content was previously shown to be associated with total neutral lipase activity (23, 42), and, given the central role of ATGL in murine TG lipolysis (10, 15, 52), we hypothesized that ATGL content would also be positively related to TG lipase activity in humans. Surprisingly, we found no correlation between ATGL content and in vitro TG lipase activity despite finding that total TG lipase activity was reduced by 70–85% after ATGL immunoprecipitation, indicating that ATGL is essential for efficient TG lipase activity. Our observations agree with a recent study in isolated human adipocytes that demonstrated a 50% reduction in basal lipolysis when ATGL expression was reduced by short interfering RNA administration in vitro (34). Subcutaneous ATGL protein and total TG lipase activity were concomitantly decreased in obesity; however, there were no significant correlations between ATGL protein content and TG lipase activity. The discordant regulation between ATGL protein expression and TG lipase activity may be due to a combination of factors. First, the interactions of ATGL with co-factors such as perilipin (8, 30) and CGI-58 (24, 39) are clearly important for lipolytic control in vitro and are likely to be relevant in vivo. Second, the regulation of ATGL activity, like HSL, may be more dependent on posttranslational modifications such as phosphorylation and translocation (8) than expression levels. Further studies examining the regulation of ATGL by these co-factors may be informative on the level/s of aberrant lipolytic control in human obesity.

We report increased gene expression of a number of novel TG lipases (PNPLA2, PNPLA3, PNPLA4, CES1, and LYPAL1) in subcutaneous adipose tissue of obese subjects, several of which were not previously described in humans. Surprisingly, total TG lipase activity was reduced, suggesting that increased lipase gene expression in obesity may be a homeostatic mechanism intended to enhance TG metabolism, perhaps to facilitate the disposal of the enlarged adipose mass. Several studies, including our own, demonstrate a close correlation between PNPLA2 and LIPE mRNA expression that indicates co-regulation of these two major lipolytic proteins in humans (22, 29); however, it is presently unclear whether a single transcription factor can mediate generic changes in both lipolytic genes. With respect to the other lipolytic genes assessed, the observed responses are unlikely to be due to insulin, glucose, or PPARγ, as these have opposite effects on PNPLA2 and PNPLA3 transcription (15). While this paper was in preparation, Schweiger et al. (37) reported that ATGL and HSL account for ~95% of total murine TG lipase activity, indicating that additional known or unknown lipases may play a quantitatively minor role in fat cell lipolysis. The present data do not conflict with this report; rather, they provide valuable information with respect to the expression of alternative lipolytic genes in humans in vivo. Indeed, we report in human adipose lysates that ATGL is important for TG lipase activity, and that non-ATGL lipases are unable to compensate for the loss of ATGL in human adipose tissue. In light of the observed dissociation between ATGL mRNA and protein content in this study, and the previous observation of proteosomal degradation of other proteins involved in lipolysis (47, 48), future studies examining the protein expression of these novel lipases combined with studies examining TG lipase activity in the presence of HSL and ATGL inhibitors will reveal whether these newly identified lipases are important in the regulation of human adipose tissue lipolysis.

In conclusion, we demonstrate that ATGL is an important enzyme for efficient TG lipase activity in human adipose tissue. ATGL mRNA content is increased in the adipose tissue of obese individuals, and this appears to be related to insulin resistance rather than adiposity. Surprisingly, ATGL protein content is decreased in subcutaneous but not visceral adipose tissue in obesity and was not associated with changes in TG lipase activity assessed in vitro. Collectively, these observations suggest that small reductions in ATGL protein expression are not important for human adipose tissue lipolysis in obesity; rather, posttranslational modifications may be more critical for the regulation of lipolysis.

REFERENCES

ATGL IN HUMAN OBESITY


