Published in final edited form as:
Biochim Biophys Acta. 2007 February ; 1771(2): 177–184.

Effects of Rosiglitazone and High Fat Diet on Lipase/Esterase Expression in Adipose Tissue

Wen-Jun Shen, **Shailja Patel**, **Zaixin Yu**, **Dyron Jue**, and **Fredric B. Kraemer**
Division of Endocrinology, Stanford University and VA Palo Alto Health Care System, Palo Alto, CA 94304

Abstract

A number of intracellular lipase/esterase have been reported in adipose tissue either by functional assays of activity or through proteomic analysis. In the current work, we have studied the relative expression level of 12 members of the lipase/esterase family that are found in white adipose tissue. We found that the relative mRNA levels of ATGL and HSL are the most abundant, being 2–3 fold greater than TGH or ADPN; whereas other intracellular neutral lipase/esterases were expressed at substantially lower levels. High fat feeding did not alter the mRNA expression levels of most lipase/esterases, but did reduce CGI-58 and WBSCR21. Likewise, rosiglitazone treatment did not alter the mRNA expression levels of most lipase/esterases, but did increase ATGL, TGH, CGI-58 and WBSCR21, while reducing ADPN. WAT from HSL−/− mice showed no compensatory increase in any lipase/esterases, rather mRNA levels of most lipase/esterases were reduced. In contrast, BAT from HSL−/− mice showed an increase in ATGL and CGI-58 expression, as well as a decrease in ES-1, APEH and WBSCR21. Analysis of the immunoreactive protein levels of some of the lipases confirmed the results seen with mRNA. In conclusion, these data highlight the complexity of the regulation of the expression of intracellular neutral lipase/esterases involved in lipolysis.

Keywords

Lipase; esterase; rosiglitazone; high fat diet

Introduction

Two of the main characteristics that differentiate adipose tissue from other cell types are its storage of neutral lipids, mainly triacylglycerol (TAG) and sterol esters, in lipid droplets, and lipolysis, the process through which stored lipids are hydrolyzed and FFA released. Decreased lipase expression (1) and decreased response of lipolysis to hormone stimulation have been demonstrated in obesity and may be a cause of excess accumulation of body fat (2). The control of lipolysis represents a complex process involving multiple regulatory events (3,4). These broadly include lipolytic (ACTH, β-adrenergic agonists, etc.) and anti-lipolytic (insulin, adenosine, etc.) hormones, their cognate receptors and signaling pathways, lipid droplet-associated proteins, such as perilipins, as well as lipases. Hormone-sensitive lipase (HSL) is most highly expressed in adipose tissue, although it is also expressed in skeletal and cardiac muscle, and macrophages (5). Despite broad substrate specificity, the primary action of HSL

Corresponding author: Wen-Jun Shen, Ph.D., Division of Endocrinology, S025, Department of Medicine, Stanford University, Stanford, CA 94305-5103, Tel: (650)493 5000 X 60351, Fax:(650)849 0215 E-mail: Wen-Jun.Shen@stanford.edu

is to hydrolyze stored TAG in adipose tissue in response to hormone stimulation, i.e., lipolysis, and HSL was considered the key enzyme for TAG hydrolysis until the examination of the non-obese HSL−/− mouse, which indicated the existence and importance of other lipase(s) (6). Recently, three groups have independently cloned a lipase (7) (8) (9), adipose triglyceride lipase (ATGL), which seems to be predominantly responsible for the first step of TAG hydrolysis (10). Therefore, it has now been proposed that ATGL and HSL work together with monoglycerol lipase to mediate the complete hydrolysis of cellular TAG.

In addition to HSL and ATGL, a number of intracellular lipase/esterases have been reported in adipose tissue using either functional assays of activity (5) (11) (12) or through proteomic analysis (13). Most of these lipase/esterases contain α/β-hydrolase folds, and are predicted to have lipase or esterase activity. These lipase/esterases presumably can hydrolyze neutral lipids, releasing FA and glycerol. Results from studies to date demonstrate that the mobilization of fat storage in adipose tissue is mediated through the action of HSL and ATGL; however, the relative importance of these two enzymes is not clear nor is the contributions of other lipase/esterases.

Intake of high-fat food is well known to contribute to the development of obesity and its associated disorders such as glucose intolerance, diabetes, hyperlipidemia, hypertension, and atherosclerosis. One of the earliest abnormalities observed as the consequence of high-fat feeding is a decrease in insulin sensitivity and the development of insulin resistance in various tissues. Thiazolidinediones (TZDs) are effective insulin sensitizing drugs that are able to enhance clearance of whole body FFA and TG (14). One hypothesis for the insulin-sensitizing action of TZDs in muscle and liver is that TZD treatment increases the partitioning of circulating lipids away from muscle and liver and towards the storage of lipids in adipose tissue. Since TZDs are high-affinity ligands for PPAR-γ (15), it is believed that these effects are mediated predominantly through the activation of PPAR-γ, which is one of the key transcription factors required for adipogenesis and which is known to regulate the expression of a number of genes during adipocyte differentiation. Recently, the expression of lipases, such as LPL, HSL, and ATGL (16–18), and lipid-droplet associated proteins S3-12 and perilipin (19), which are instrumental for lipid accumulation and mobilization in adipose tissue, were shown to be regulated by PPAR-γ.

In the current work, we have studied the relative expression level of 12 members of the lipase/esterase family that are neutral lipase/esterases found in white adipose tissue (WAT) of wild type and HSL−/− mice. Furthermore, we have examined the changes of expression of these lipase/esterases in adipose tissue of mice under high fat feeding and with rosiglitazone, a PPAR-γ agonist.

Materials and Methods

Chemicals and Reagents

Reagents were obtained from the following sources: Bovine serum albumin (fraction V), TRIzol reagent (Invitrogen, Carlsbad, CA), RNeasy kit (QIAGEN, Valencia, CA). SyBr green Taqman PCR kit (Applied Biosystems, CA) SuperScript II (Invitrogen, CA), Insulin RIA kit from Linco Research, St. Charles, MO.

Animals

HSL−/− mice were generated by homologous recombination as previously described (6), and backcrossed five times with C57/BL/6J mice. Mice were maintained in the animal facility at the VA Palo Alto on a 12/12 hr light/dark cycle. For breeding experiments, mice heterozygous for the deleted HSL allele were used to generate homozygous HSL−/− mice and HSL+/+ wild

type littermates. Genotyping was performed by a single-step PCR using three primers as described previously (6). High-fat (35.9% w/w lard fat) diet (HF) and control normal chow (4.8% w/w fat) diet (NC) were obtained from Research Diets, Inc. (New Brunswick, NJ), product numbers D12309 and D12310, respectively. Rosiglitazone containing diets were custom formulated by adding 200 mg/kg rosiglitazone (20) to the high fat and normal chow diets to form the high fat with rosiglitazone (HF+R) and normal chow with rosiglitazone diets (NC+R), respectively. Twelve week-old female HSL+/+ and HSL−/− littermate mice were randomized to either high-fat, normal chow, high-fat with rosiglitazone, and normal chow with rosiglitazone diets ad libitum for 15 weeks. At the end of the diet treatment, animals were sacrificed and adipose tissue collected.

Taqman real time PCR analysis

Tissues were homogenized in TRIzol reagent and total RNA was extracted and purified using the RNeasy kit, and treated with RNase-free DNase I. Total RNA was reverse-transcribed in a 20µ1 reaction containing random primers and Superscript II enzyme. Real-time PCR was performed with an ABI Prism 7900 System using SYBR green master mix reagent and specific primer pairs selected with Primer Express software as described previously (21). The primer pairs used are listed in Table 1. The relative mass of specific RNA was calculated by the comparative cycle of threshold detection method according to the manufacturer's instruction. Three independent sets of Taqman real time PCR were performed using different RNA preparations from adipose tissue; each run of Taqman real-time PCR was conducted in triplicate.

Immunoblot Analysis

Adipose tissue was homogenized in ice-cold fractionation buffer (50 mM Tris-HCl, pH 7.4, 8% sucrose, 1 mM EDTA, 0.1 mM sodium orthovanadate [Na_3VO_4], and 50 mM sodium fluoride [NaF]). Fat cake and cytosolic fractions were prepared following published procedures with modification (22,23). The cell lysate was incubated on ice for 15 min and then centrifuged at 20,000×*g* for 30 min at 4 °C. The fat cake representing intracellular lipid droplets floated on top of the tube. The cytosolic fraction was localized below the layer of the fat cake. The fat cake and cytosolic fractions were collected and extracted with 1% SDS in the same buffer for 20 min at room temperature. Fractions were then centrifuged at 20,000 × g for 20 min at 4 °C, and aliquoted for protein quantification using Pierce reagent. 25 µg of total protein were then mixed with concentrated sample buffer (50 mM Tris-HCl, pH 6.8, 5% SDS, 1% β-mercaptoethanol, 0.1 mM Na_3VO_4, 50 mM NaF, and 15% glycerol). The samples were heated to 95 °C for 5 min and cleared at 12,000×*g* for 10 min, prior to loading on SDS-PAGE. Immunoblot analyses were performed as previously described (24). Following SDS-PAGE the samples were transferred to a nitrocellulose membrane. The membrane was incubated with rabbit anti-ATGL antibody (Cayman Chemical Co., Ann Arbor, MI) and anti-β-actin antibody (Santa Cruz Biotechnology, Santa Cruz, CA) and then with horseradish peroxidase-linked anti-rabbit IgG (Amersham). The membranes were visualized with chemiluminescence reagent ECL (Amersham), exposed to Kodak XAR film, and then analyzed by a Fluor-S multi-image analyzer (Bio-Rad, Hercules, CA).

Statistical Analysis

Results are given as the mean ± SE and statistical significance was tested using ANOVA with Bonferroni as post test or unpaired two-tailed Student's t test, except where otherwise stated, using StatView (version 4.5, Abacus Concepts, Berkley, CA) and InStat (version 2.03, GraphPad Software, San Diego, CA) software for Macintosh.

Results

Relative expression level of lipase/esterases in adipose tissue and changes in expression with high fat feeding

The presence of lipase/esterases in adipose tissue has been reported using either functional assays of activity or through proteomic analysis. To determine the relative level of expression of neutral lipase/esterases, gonadal WAT was collected from 6 months old female wild-type mice. Total RNA was isolated and reverse transcribed as described in the methods. Quantitative RT-PCR analysis was performed on 12 of the reported lipase/esterases: ATGL (8), adiponutrin (ADPN) (25), triacylglycerol hydrolase (TGH), also known as carboxylesterase-3, (26) (27), α/β hydrolase domain containing mRNA 5, also known as CGI-58, and α/β hydrolase domain containing mRNA 11, also known as Beuren-Williams Syndrome Critical Region 21 (WBSCR21), (28), acylpeptide hydrolase (APEH), esterase 1 (EST-1) (29), esterase D (ESD-1) (30), esterase 1 homolog (ES-1) (13), carboxylesterase ML1 (ML-1) (13), membrane-associated calcium-independent phospholipase A2γ (MiPLA-2) (31), as well as HSL. The level of expression of the various lipase/esterases is presented relative to the control house keeping gene acidic ribosomal phosphoprotein 36B4. As shown in Figure 1, the relative mRNA levels of ATGL and HSL appear to be most abundant, and, although ATGL expression tended to be higher than HSL, their levels are not significantly different from each other; however, both ATGL and HSL are expressed at significantly higher levels compared with other lipase/esterases ($P< 0.05$). TGH and ADPN mRNA are expressed at approximately 50% and 35% of the level of HSL ($P< 0.05$), whereas miPLA-2, ESD-1, CGI-58 and ML-1 are all expressed at relatively similar mRNA levels and are approximately 22%, 19%, 17%, and 15% of the level of HSL, respectively. Expression levels of ES-1, APEH, WBSCR21 and EST-1 mRNA were extremely low and averaged only 6%, 4%, 1% and 0.6% of HSL, respectively.

High fat feeding has been shown to reduce insulin sensitivity and to promote fat accumulation. In order to examine the effects of high fat-feeding on the expression levels of lipase/esterases in adipose tissue, 12 weeks old, wild-type female mice were randomized to either high fat or normal chow diets. After 15 weeks of diet, high fat fed mice were 35% heavier compared to mice fed normal chow (NC 29.46 ± 5.50 g vs. HF 40.21 ± 11.46 g $P<0.05$) that was reflected in white adipose tissue (WAT) showing a significant increase with high fat diet feeding (NC 3.25 ± 0.64 g, HF 9.59 ± 3.83 g, $p<0.001$). For analysis of expression of the lipase/esterases, WAT was collected and total RNA isolated as described earlier, and quantitative RT-PCR analysis was performed on the selected lipase/esterases. The level of expression of the various lipase/esterases is presented relative to the control house keeping gene acid ribosomal phosphoprotein 36B4 in Figure 1. High fat feeding did not alter the mRNA expression of most of the lipase/esterases examined; however, high fat feeding did result in a 70% reduction in the expression of CGI-58 and a 50% reduction in WBSCR21 ($P<0.05$).

Changes of expression pattern of lipase/esterases with rosiglitazone

Treating mice with rosiglitazone for 15 weeks resulted in greater weight gain whether on a normal chow or high fat diet (NC + R body weight gain 7.55 ± 2.41 g, and NC 5.14 ± 1.19 g $p< 0.05$; HF+R 16.75 ± 5.0 g, HF 11.34 ± 3.75 g, $p<0.05$), consistent with previous reports showing that rosiglitazone increases body weight gain in rats and mice (32) (33) (34). The weight of adipose tissue showed a trend to increase with rosiglitazone treatment, but did not reach statistical significance. In order to examine whether rosiglitazone alters the expression of lipase/esterases, quantitative RT-PCR analysis was performed on WAT for the selected lipase/esterases. Figure 2A shows the levels of expression of the selected lipase/esterases of rosiglitazone-treated animals relative to the expression level of each respective gene in normal chow fed mice. ATGL (1.7-fold), TGH (1.7-fold), CGI-58 (2.3-fold), and WBSCR21 (2.5-fold) were significantly increased by rosiglitazone ($P<0.05$); only ADPN expression was

significantly reduced (~30%). The expression of none of the other lipase/esterases was significantly affected by rosiglitazone with normal chow feeding. In the setting of a high fat diet (Figure 2B), rosiglitazone also increased the expression of CGI-58 (2.7-fold, $P<0.05$) and WBSCR21 (1.6-fold, $P<0.05$); however, ATGL and TGH were no longer significantly affected by rosiglitazone, although there were positive trends. ADPN expression was reduced 3-fold by rosiglitazone in the setting of a high fat diet ($P<0.05$).

Expression levels of lipase/esterases in adipose tissue of HSL−/− mice

HSL−/− mice display decreased lipolysis, but retain ~50–60% of normal TAG hydrolytic activity in WAT (6,21). In order to examine whether there are any compensatory changes in the expression levels of other lipase/esterases, WAT was collected from female wild-type and HSL−/− mice, RNA isolated and the expression of selected lipase/esterases was analyzed by quantitative RT-PCR. The results are shown in Figure 3A and are presented relative to the expression of the respective gene in wild type mice. None of the lipase/esterases assessed was increased in HSL−/− mice. Thus, there was no evidence for compensatory changes. However, the expression levels of several lipase/esterases were reduced quite substantially in WAT of HSL−/− mice. ATGL and TGH ($P<0.05$) were 70% lower ($P<0.005$), ADPN 95% lower ($P<0.05$), miPLA-2 60% lower ($P<0.05$), CGI-58 80% lower ($P<0.05$), ES-1 more than 95% lower ($P<0.01$) and WBSCR21 85% lower ($P<0.005$). The other lipase/esterases measured, APEH, EST-1, ESD-1 and ML-1 all showed trends for lower expression levels, although the decreases failed to reach statistical significance.

The TAG hydrolytic activity in brown adipose tissue (BAT) of HSL−/− mice has been reported either not to be significantly reduced (6) or to be decreased 74% (8). In order to examine whether there are any compensatory changes in the expression levels of other lipase/esterases, BAT was collected from female wild-type and HSL−/− mice, RNA isolated and the expression levels of selected lipase/esterases were analyzed by quantitative RT-PCR. The results are shown in Figure 3B and are presented relative to the expression of the respective gene in wild type mice. As opposed to the findings in WAT, there was evidence for compensatory changes in BAT, where both ATGL and CGI-58 mRNA expression levels were significantly increased in HSL−/− mice ($P<0.05$). Meanwhile the expression levels of several lipase/esterases were reduced substantially in BAT of HSL−/− mice. ES-1 was 80% lower ($P<0.01$), APEH 20% lower ($P<0.01$) and WBSCR21 40% lower ($P<0.01$). The other lipase/esterases measured, TGH, ADPN, MiPLA-2, ESD, and EST-1 were not changed significantly. There was no detectable mRNA of ML-1 in the BAT of either WT and HSL−/− mice.

Analysis of immunoreactive ATGL and HSL in WAT of mice

To confirm the changes observed in the expression of HSL and ATGL in adipose tissue and to further examine their subcellular localization, we prepared cytosolic and fat cake fractions; proteins were extracted from both fractions and analyzed for the presence of immunoreactive HSL and ATGL. As shown in Fig. 4, with high fat feeding, the amount of both ATGL and HSL protein in the cytosolic fraction was decreased, yet the amount of both proteins in fat cake fraction did not seem to change. Rosiglitazone feeding increased the amount of ATGL and HSL in the fat cake fraction, but appeared to decrease the amount of the proteins in the cytosolic fraction.

Analysis of immunoreactive ATGL in adipose tissue of HSL−/− mice

To confirm the changes in the expression of ATGL in adipose tissue in HSL−/− mice, we analyzed immunoreactive ATGL in WAT and BAT of HSL−/− mice by Western blot. For this set of experiments, we also separated the cytosolic and fat cake fractions and extracted proteins from each fraction separately. As shown in Figure 5, the amount of immunoreactive ATGL in the cytosolic fraction of WAT did not show a statistical difference; however, the amount of

ATGL in the fat cake fraction of WAT of HSL−/− decreased to only 22% of that seen in wild type mice ($P<0.05$). When the fat cake and cytosolic fractions were combined, the total immunoreactive ATGL was decreased in WAT ($P<0.05$). In BAT, the amount of ATGL in the cytosolic fraction decreased to about 20% ($P<0.05$) of wild type, but there was no difference in the amount of ATGL in the fat cake fraction in HSL−/− and wild type mice. When the fat cake and cytosolic fractions were combined, the amount of ATGL in BAT of HSL−/− mice showed no significant difference from wild type mice.

Discussion

Intracellular lipases are instrumental in lipolysis, the process through which stored TAG is hydrolyzed to release FFA. It has long been thought that HSL was the rate-limiting enzyme for lipolysis; however, the examination of the non-obese HSL−/− mouse indicated the existence and importance of other lipase(s) (6). Recently, three groups have independently cloned a lipase (7–9), ATGL, which seems to be predominantly responsible for the first step of TAG hydrolysis (10), and which possesses an α/β hydrolase fold, typical of many lipases (5) (13). Nonetheless, there are many proteins that have an α/β hydrolase fold and that are predicted to have lipase or esterase activities. However, the relative expression in adipose tissue and the involvement of these lipases in response to different physiological conditions are not clear.

Using quantitative real-time PCR analysis, we have confirmed that under normal chow fed conditions, ATGL and HSL are the predominant neutral lipases expressed in WAT, but with significant amounts of TGH and ADPN and modest amounts of MiPLA-2, ESD-1, CGI-58 and ML-1 present; whereas only extremely low levels of ES-1, APEH, WBSCR21 and EST-1 are expressed. Although the current studies establish the relative mRNA expression levels of these neutral lipase/esterases, it is important to note that due to the availability of antibodies, we were only able to confirm the changes of the protein levels of ATGL and HSL. The protein expression and the amount of cellular lipase/esterase activity attributable to each of the respective lipase/esterases were not determined in this study. Thus, the relative physiological importance of each of the lipase/esterases in adipose TAG metabolism awaits further study.

In addition to establishing the relative mRNA expression levels of neutral lipase/esterases in WAT, we explored the physiological regulation of these lipase/esterases by high fat feeding and by rosiglitazone. Interestingly, most of the lipase/esterases examined were unaffected by high fat feeding; however, the expression of CGI-58, ML-1, WBSCR21 and ES-1 were significantly reduced. The physiological significance of these changes is currently unclear, but the changes in CGI-58 may be noteworthy. CGI-58 is an a/β hydrolase containing protein whose catalytic serine within the reactive triad is replaced by asparagine and, thus, lacks hydrolytic activity against neutral lipids (28). However, naturally occurring mutations in CGI-58 have been identified in Chanarin-Dorfman Syndrome (28), a neutral lipid storage disorder characterized by ichthyosis, hepatic steatosis and hepatomegaly, developmental defects, and the accumulation of TAG-containing lipid droplets in leukocytes, basal keratinocytes, hepatocytes, myocytes, and other cells (28,35,36). Recent studies have reported that CGI-58 interacts with perilipin (37), and that this interaction is regulated by lipolytic and anti-lipolytic conditions (38). Moreover, CGI-58 has recently been reported to activate ATGL mediated lipolysis (39). These observations lead to the speculation that the reduction in CGI-58 expression with high fat diets might contribute to some of the alterations in lipolysis observed under these conditions.

Prolonged supplementation of rosiglitazone to a normal chow diet resulted in increased expression of ATGL, TGH, CGI-58 and WBSCR212. These findings are consistent with recent reports showing that the levels of expression of ATGL and TGH are increased during adipocyte differentiation following treatment with PPARγ agonists (7–9,40), and extend these

observations to include CGI-58 and WBSCR21 as PPARγ up-regulated genes. In contradistinction, ADPN is also markedly increased during adipocyte differentiation (41), but its expression was decreased with prolonged supplementation of rosiglitazone to a normal chow diet, which is consistent with a report that troglitazone decreased ADPN expression in 3T3-L1 adipocytes (42). There is clearly an interaction between diet and the actions of PPARγ agonists, since rosiglitazone increased only CGI-58 and WBSCR21, while decreasing ADPN, in the setting of a high fat diet. Fast-acting hormones stimulate lipolysis through phosphorylation of both HSL and perilipin and result in the translocation of HSL to the surface of lipid droplets (43–45). With the cloning of ATGL and the study of ATGL-/- mice, it has become apparent that ATGL plays an important role in cellular TAG hydrolysis (10,46). However, HSL is still the only lipase whose activity has been shown to be regulated by fast-acting hormones. In HSL−/− mice, hormone-stimulated lipolysis is significantly reduced (6). Although there are differences among the various HSL−/− colonies, the ablation of HSL results in a decrease in lipolysis with the retention of a significant amount of basal TAG hydrolytic activity in both WAT and BAT (6) (8). Several groups have attempted to identify the lipases responsible for the residual TAG hydrolytic activity in HSL−/− mice, reporting that ATGL (8) and TGH (26) are responsible for residual TAG hydrolytic activity in this setting. In the current studies we surveyed a number of lipase/esterases for their expression in adipose tissue of HSL−/− mice to determine whether there is any increased expression to compensate for the loss of HSL. Interestingly, we observed that none of the lipase/esterases that we assessed displayed any compensatory increase in WAT. Rather, the expression of most lipase/esterases was decreased in WAT of HSL−/−. Thus, the ablation of HSL in vivo appears to affect the expression of multiple lipase/esterases in WAT, raising the possibility that the reduction in lipolysis observed in HSL−/− mice is due not only to a loss of HSL, but also to a lower expression of other lipase/esterases. In contrast to WAT, we did observe a compensatory increase in ATGL and CGI-58 expression in BAT, providing a possible explanation for the normal TAG hydrolytic activity previously observed in BAT in HSL−/− mice.

In conclusion, these studies document the complex regulation of intracellular neutral lipase/esterases in adipose tissue under important physiological conditions.

Acknowledgements

This work was supported in part by research grants from American Diabetes Association (W.-J. S), the Research Service of the Department of Veterans Affairs and by grant DK 46942 from the National Institutes of Health (F.B. K.).

References

1. Large V, Reynisdottir S, Langin D, Fredby K, Klannemark M, Holm C, Arner P. Decreased expression and function of adipocyte hormone-sensitive lipase in subcutaneous fat cells of obese subjects. JOURNAL OF LIPID RESEARCH 1999;40:2059–2066. [PubMed: 10553009]
2. Langin D, Dicker A, Tavernier G, Hoffstedt J, Mairal A, Ryden M, Arner E, Sicard A, Jenkins C, Viguerie N, van Harmelen V, Gross R, Holm C, Arner P. Adipocyte lipases and defect of lipolysis in human obesity. Diabetes 2005;54:3190–3197. [PubMed: 16249444]
3. Londos C, Brasaemle DL, Schultz CJ, Adler-Wailes DC, Levin DM, Kimmel AR, Rondinone CM. On the control of lipolysis in adipocytes. Ann. N. Y. Acad. Sci 1999;892:155–168. [PubMed: 10842661]
4. Holm C, Osterlund T, Laurell H, Contreras J. Molecular Mechanisms Regulating Hormone-Sensitive Lipase And Lipolysis. Annu. Rev. Nutr 2000;20:365–393. [PubMed: 10940339]
5. Kraemer F, Shen W-J. Hormone-sensitive lipase: control of intracellular tri-(di-) acylglycerol and cholesteryl ester hydrolysis. J. Lipid Res 2002;43:1585–1594. [PubMed: 12364542]
6. Osuga J, Ishibashi S, Oka T, Yagyu H, Tozawa R, Fujimoto A, F S, Yahagi N, Kraemer F, Tsutsumi O, Yamada N. Targeted disruption of hormone-sensitive lipase results in male sterility and adipocyte hypertrophy, but not in obesity. Proc Natl Acad Sci U S A 2000;97:787–792. [PubMed: 10639158]

7. Jenkins C, Mancuso D, Yan W, Sims H, Gibson B, Gross R. Identification, cloning, expression, and purification of three novel human calcium-independent phospholipase A2 family members possessing triacylglycerol lipase and acylglycerol transacylase activities. J Biol Chem 2004;279:48968–48975. [PubMed: 15364929]
8. Zimmermann R, Strauss J, Haemmerle G, Schoiswohl G, Birner-Gruenberger R, Riederer M, Lass A, Neuberger G, Eisenhaber F, Hermetter A, Zechner R. Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. Science 2004;306:1383–1386. [PubMed: 15550674]
9. Villena J, Roy S, Sarkadi-Nagy E, Kim K, Sul H. Desnutrin, an adipocyte gene encoding a novel patatin domain-containing protein, is induced by fasting and glucocorticoids: ectopic expression of desnutrin increases triglyceride hydrolysis. J Biol Chem 2004;279:47066–47075. [PubMed: 15337759]
10. Zechner R, Strauss J, Haemmerle G, Lass A, Zimmermann R. Lipolysis: pathway under construction. Curr Opin Lipidol 2005;16:333–340. [PubMed: 15891395]
11. Dolinksky V, Sipione S, Lehner R, Vance D. The cloning and expression of a murine tracylglycerol hydrolase cDNA and the structure of its corresponding gene. Biochem. Biophys. Acta 2001;1532:162–172. [PubMed: 11470237]
12. Karlsson M, Contreras J, Hellman U, Tornqvist H, Holm C. cDNA cloning, tissue distribution, and identification of the catalytic triad of monoglyceride lipase. Evolutionary relationship to esterases, lysophospholipases, and haloperoxidases. J Biol Chem 1997;272:27218–27223. [PubMed: 9341166]
13. Birner-Gruenberger R, Susani-Etzerodt H, Waldhuber M, Riesenhuber G, Schmidinger H, Rechberger G, Kollroser M, Strauss J, Lass A, Zimmermann R, Haemmerle G, Zechner R, Hermetter A. The Lipolytic Proteome of Mouse Adipose Tissue. Mol Cell Proteomics 2005;4:1710–1717. [PubMed: 16048907]
14. Dumasia R, Eagle K, Kline-Rogers E, May N CL, Mukherjee D. Role of PPAR-g agonist thiazolidinediones in treatment of pre-diabetic and diabetic individuals: a cardiovascular perspective. Curr. Drug Tragets Cardiovasc Haematol. Disord 2005;5:377–386.
15. Wilson TM, B P, Sternbach DD, Henke BR. The PPARs: from orphan receptors to drug discovery. J. Med. Chem 2000;43:527–550. [PubMed: 10691680]
16. Kageyama H, Hirano T, Okada K, Ebara T, Kageyama A, Murakami T, Shioda S, Adachi M. Lipoprotein lipase mRNA in white adipose tissue but not in skeletal muscle is increased by pioglitazone through PPAR-gamma. Biochem Biophys Res Commun 2003;305:22–27. [PubMed: 12732191]
17. Deng T, S S, Li PP, Shen ZF, Lu XP, Cheng J, Ning ZQ. Peroxisome proliferator-activated receptor-gamma transcriptionally up-regulates hormone-sensitive lipase via the involvement of specificity protein-1. Endocrinology 2006;147:875–884. [PubMed: 16269451]Epub 2005 Nov 2003
18. Kim J, Tillison K, Lee J, Rearick D, Smas C. The adipose tissue triglyceride lipase ATGL/PNPLA2 is downregulated by insulin and TNF-alpha in 3T3-L1 adipocytes and is a target for transactivation by PPARgamma. Am J Physiol Endocrinol Metab 2006;291:E115–E127. [PubMed: 16705060]Epub 2006 May 2016
19. Dalen K, Schoonjans K, Ulven S, Weedon-Fekjaer M, Bentzen T, Koutnikova H, Auwerx J, Nebb H. Adipose tissue expression of the lipid droplet-associating proteins S3-12 and perilipin is controlled by peroxisome proliferator-activated receptor-gamma. Diabetes 2004;53:1243–1252. [PubMed: 15111493]
20. Watkins SM, Reifsnyder PR, Pan HJ, German JB, L EH. Lipid metabolome-wide effects of the PPARgamma agonist rosiglitazone. J. Lipid Res 2002;43:1809–1817. [PubMed: 12401879]
21. Harada K, Shen W-J, Patel S, Natu V, Wang J, Osuga J, Ishibashi S, Kraemer F. Resistance to high fat diet-induced obesity associated with altered expression of adipose specific genes in hormone-sensitive lipase deficient mice. Am J Physiol Endocrinol Metab 2003;285:E1182. [PubMed: 12954598]
22. Clifford G, Londos C, Kraemer F, Vernon R, Yeaman S. Translocation of hormone-sensitive lipase and perilipin upon lipolytic stimulation of rat adipocytes. JOURNAL OF BIOLOGICAL CHEMISTRY 2000;275:5011–5015. [PubMed: 10671541]
23. He J, Jiang H, Tansey JT, Tang C, Pu S, G X. Calyculin and okadaic acid promote perilipin phosphorylation and increase lipolysis in primary rat adipocytes. Biochim Biophys Acta 2006;1761:247–255. [PubMed: 16545598]

24. Shen W-J, Patel S, Natu V, Kraemer F. Mutational analysis of structural features of rat hormone-sensitive lipase. BIOCHEMISTRY 1998;37:8973–8979. [PubMed: 9636039]
25. Lake A, Sun Y, Li J, Kim J, Johnson J, Li D, Revett T, Shih H, Liu W, Paulsen J, Gimeno R. Expression, regulation, and triglyceride hydrolase activity of Adiponutrin family members. J Lipid Res 2005;46:2477–2487. [PubMed: 16150821]
26. Dolinsky V, Gilham D, Alam M, Vance D, Lehner R. Triacylglycerol hydrolase: role in intracellular lipid metabolism. Cell Mol Life Sci 2004;61:1633–1651. [PubMed: 15224187]
27. Soni K, Lehner R, Metalnikov P, O'Donnell P, Semache M, Gao W, Ashman K, Pshezhetsky A, Mitchell G. Carboxylesterase 3 (EC 3.1.1.1) is a major adipocyte lipase. J Biol Chem 2004;279:40683–40689. [PubMed: 15220344]
28. Lefevre C, Jobard F, Caux F, Bouadjar B, Karaduman A, Heilig R, Lakhdar H, Wollenberg A, Verret JL, Weissenbach J, Ozguc M, Lathrop M, Prud'homme JF, Fischer J. Mutations in CGI-58, the gene encoding a new protein of the esterase/lipase/thioesterase subfamily, in Chanarin-Dorfman syndrome. Am. J. Hum. Genet 2001;69:1002–1012. [PubMed: 11590543]
29. Kadner S, Katz J, Finlay T. Esterase-1: developmental expression in the mouse and distribution of related proteins in other species. Arch Biochem Biophys 1992;296:435–441. [PubMed: 1632635]
30. Peters J, Nash H. Esterases of Mus musculus: substrate and inhibition characteristics, new isozymes, and homologies with man. Biochem Genet 1978;16:553–569. [PubMed: 216346]
31. Su X, Mancuso D, Bickel P, Jenkins C, Gross R. Small interfering RNA knockdown of calcium-independent phospholipases A2 beta or gamma inhibits the hormone-induced differentiation of 3T3-L1 preadipocytes. J Biol Chem 2004;279:21740–21748. [PubMed: 15024020]
32. Larsen T, Toubro S, Astrup A. PPARgamma agonists in the treatment of type II diabetes: is increased fatness commensurate with long-term efficacy; Int J Obes Relat Metab Disord 2003;27:147–161. [PubMed: 12586994]
33. Yamauchi T, Kamon J, Waki H, Murakami K, Motojima K, Komeda K, Ide T, Kubota N, Terauchi Y, Tobe K, Miki H, Tsuchida A, Akanuma Y, Nagai R, Kimura S, Kadowaki T. The mechanisms by which both heterozygous peroxisome proliferator-activated receptor gamma (PPARgamma) deficiency and PPARgamma agonist improve insulin resistance. J Biol Chem 2001;276:41245–41254. [PubMed: 11533050]Epub 42001 Aug 41231
34. de Souza C, Eckhardt M, Gagen K, Dong M, Chen W, Laurent D, Burkey B. Effects of pioglitazone on adipose tissue remodeling within the setting of obesity and insulin resistance. Diabetes 2001;50:1863–1871. [PubMed: 11473050]
35. Srebrnik A, Brenner S, Ilie B, Messer G. Dorfman-Chanarin syndrome: morphologic studies and presentation of new cases. Am. J. Dermatopathol 1998;20:79–85. [PubMed: 9504676]
36. Igal RA, Rhoads JM, Coleman RA. Neutral lipid storage disease with fatty liver and cholestasis. J. Pediatr. Gastroenterol. Nutr 1997;25:541–547. [PubMed: 9360211]
37. Yamaguchi T, Omatsu N, Matsushita S, Osumi T. CGI-58 interacts with perilipin and is localized to lipid droplets. Possible involvement of CGI-58 mislocalization in Chanarin-Dorfman syndrome. J Biol Chem 2004;279:30490–30497. [PubMed: 15136565]
38. Subramanian V, Rothenberg A, Gomez C, Cohen A, Garcia A, Bhattacharyya S, Shapiro L, Dolios G, Wang R, Lisanti M, Brasaemle D. Perilipin A mediates the reversible binding of CGI-58 to lipid droplets in 3T3-L1 adipocytes. J Biol Chem 2004;279:42062–42071. [PubMed: 15292255]
39. Lass A, Zimmermann R, Haemmerle G, Riederer M, Schoiswohl G, Schweiger M, Kienesberger P, Strauss J, orkiewicz G, Zechner R. Adipose triglyceride lipase-mediated lipolysis of cellular fat stores is activated by CGI-58 and defective in Chanarin-Dorfman Syndrome. Cell Metab 2006;3:309–319. [PubMed: 16679289]
40. Dolinsky V, Gilham D, Hatch G, Agellon L, Lehner R, Vance D. Regulation of triacylglycerol hydrolase expression by dietary fatty acids and peroxisomal proliferator-activated receptors. Biochim Biophys Acta 2003;1635:20–28. [PubMed: 14642773]
41. Baulande S, Lasnier F, Lucas M, Pairault J. Adiponutrin, a transmembrane protein corresponding to a novel dietary- and obesity-linked mRNA specifically expressed in the adipose lineage. J Biol Chem 2001;276:33336–33344. [PubMed: 11431482]
42. Polson D, Thompson M. Adiponutrin gene expression in 3T3-L1 adipocytes is downregulated by troglitazone. Horm Metab Res 2003;35:508–510. [PubMed: 12953171]

43. Sztalryd C, Xu G, Dorward H, Tansey J, Contreras J, Kimmel A, Londos C. Perilipin A is essential for the translocation of hormone-sensitive lipase during lipolytic activation. J. Cell Biol 2003;161:1093–1103. [PubMed: 12810697]

44. Zhang H, Souza S, Muliro K, Kraemer F, Obin M, Greenberg A. Lipase-selective functional domains of perilipin A differentially regulate constitutive and protein kinase A-stimulated lipolysis. J Biol Chem 2003;278:51535–51542. [PubMed: 14527948]

45. Miyoshi H, Souza S, Zhang H, Strissel K, Christoffolete M, Kovsan J, Rudich A, Kraemer F, Bianco A, Obin M, Greenberg A. Perilipin promotes HSL-mediated adipocyte lipolysis via phosphorylation-dependent and independent mechanisms. J Biol Chem. 2006*Apr 4; [Epub ahead of print]:* Apr 4; [Epub ahead of print]

46. Haemmerle G, Lass A, Zimmermann R, Gorkiewicz G, Meyer C, Rozman J, Heldmaier G, Maier R, Theussl C, Eder S, Kratky D, Wagner E, Klingenspor M, Hoefler G, Zechner R. Defective lipolysis and altered energy metabolism in mice lacking adipose triglyceride lipase. Science 2006;312:734–737. [PubMed: 16675698]

Abbreviations

ACTH, adrenocorticotropic hormone; ADPN, adiponutrin; APEH, acylpeptide hydrolase; ATGL, adipose triglyceride lipase; BAT, brown adipose tissue; CGI-58, α/β hydrolase domain containing mRNA 5; ES-1, esterase 1 homolog; ESD-1, esterase D; EST-1, esterase 1; FFA, free fatty acids; HSL, hormone-sensitive lipase; NCEH, neutral cholesteryl ester hydrolase; MiPLA-2, membrane-associated calcium-independent phospholipase A2γ-; ML-1, carboxylesterase; ML1, RT-PCR:reverse transcriptase-polymerase chain reaction; PPAR, peroxisomal proliferator activated receptor; TAG, triacylglycerol; TGH, triacylglycerol hydrolase; TZD, thiazolidinedione; WAT, white adipose tissue; WBSCR21, Williams- Beuren Syndrome Critical Region 21..

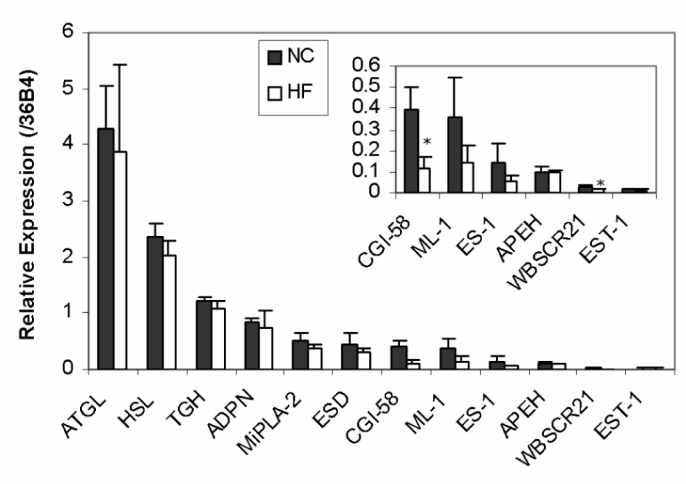

Figure 1.

Expression of lipase/esterases in WAT and changes with high fat feeding. 12 week old female C57Bl6 wild type mice were fed normal chow and a high fat diet for 15 weeks with five mice in each group. At the end of the diet treatment, gonadal WAT was collected. Total RNA was isolated, reverse transcribed and the expression of lipase/esterases was analyzed using Taqman quantitative PCR. The level of expression is presented relative to the control house keeping gene, 36B4. Taqman RT-PCR was performed in triplicate, and results presented are the summary of experiments with five mice in each group. The data are presented as means ±S.E. *, $P< 0.05$.

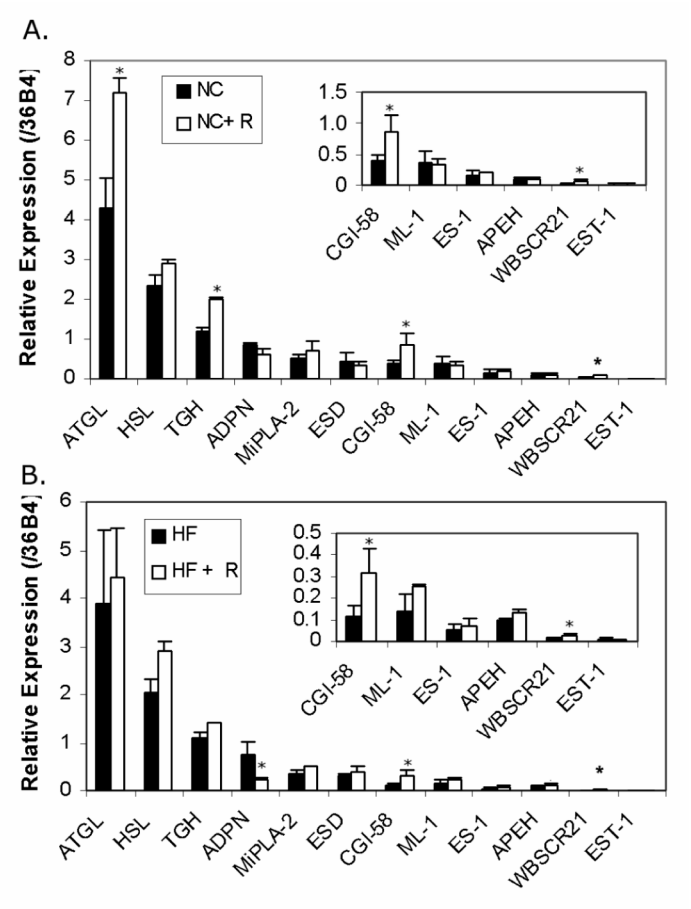

Figure 2.

Effects of rosiglitazone on the expression of lipase/esterases in WAT. Panel A: 12 week old female wild-type C57Bl6 mice were fed normal chow supplemented with 200 mg/kg rosiglitazone for 15 weeks with five mice in each group. Panel B: 12 week old female wild-type C57Bl6 mice were fed a high fat diet supplemented with 200 mg/kg rosiglitazone for 15 weeks with five mice in each group. At the end of the diet treatment, gonadal WAT was collected. Total RNA was isolated, reverse transcribed and the expression of lipase/esterases was analyzed using Taqman quantitative PCR. The level of expression is presented relative to the control house keeping gene, 36B4. Taqman RT-PCR was performed in triplicate, and results presented are the summary of experiments with five mice in each group. *, $P<0.05$.

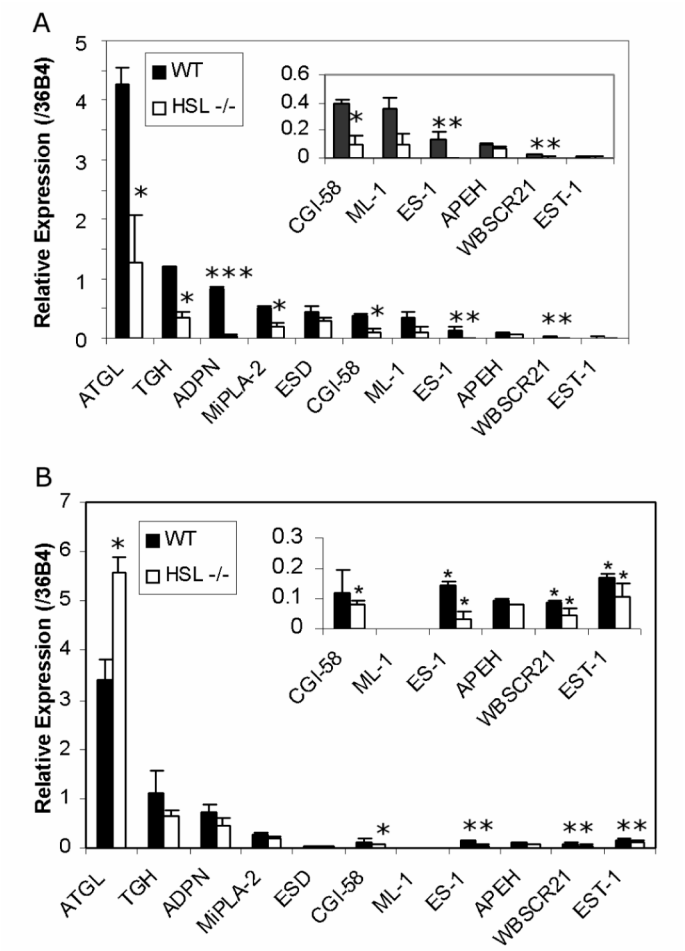

Figure 3.

Expression of lipase/esterases in adipose tissue of HSL−/− mice. Gonadal WAT (3A) and brown adipose tissue (BAT, 3B) were collected from 6 month old female wild-type and HSL −/− mice. Total RNA was isolated, reverse transcribed and the expression of lipase/esterases was analyzed using Taqman quantitative PCR. The level of expression is presented relative to the control house keeping gene, 36B4. Taqman RT-PCR was performed in triplicate, and results presented are the summary of three experiments with four mice in each group. The data are presented as means ± S.E. *, $P< 0.05$; **, $P<0.01$; ***, $P<0.005$.

ND: not detectable.

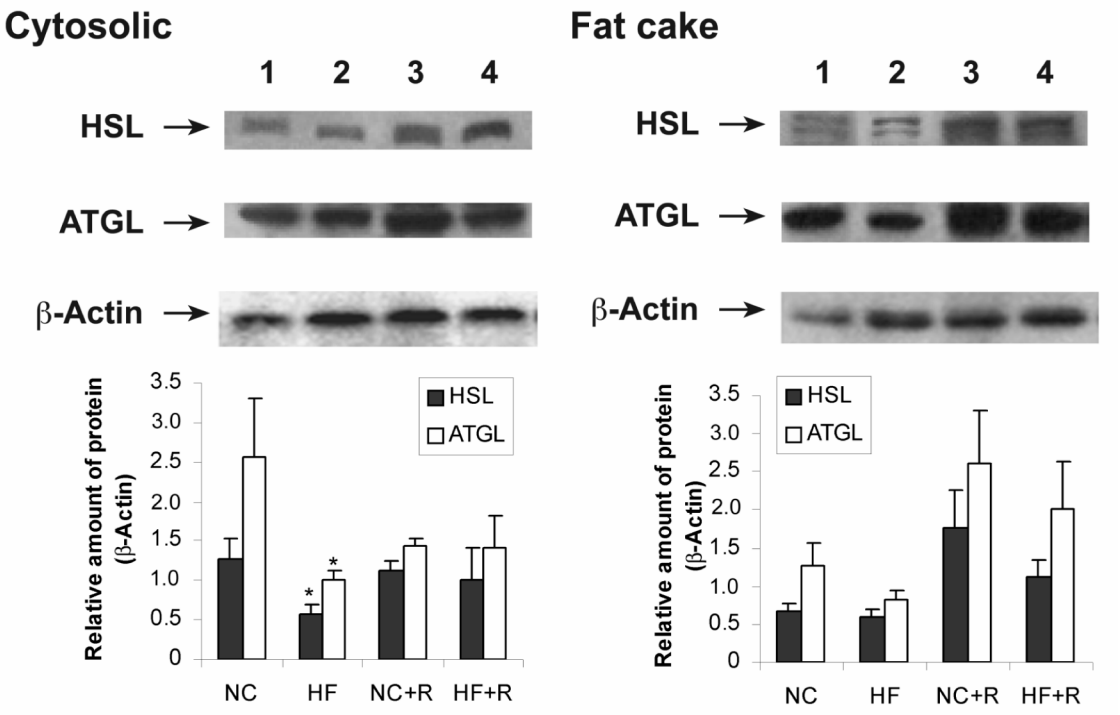

Figure 4.

Immunoblot analysis of ATGL and HSL in WAT of mice. 12 week old female wild-type C57Bl6 mice were randomly assigned to four groups and fed normal chow, high fat, or normal chow supplemented with 200 mg/kg rosiglitazone and high fat diet supplemented with 200 mg/kg rosiglitazone for 15 weeks (n=5 for each group). At the end of the diet treatment, gonadal WAT was collected. Total proteins were extracted in TES with 10 μg/ml leupeptin; 20 μg of protein from tissue homogenates were subjected to SDS-PAGE and transferred to a nitrocellulose membrane. The membrane was incubated with rabbit anti-ATGL antibody, rabbit anti-HSL and then with horseradish peroxidase-linked anti-rabbit IgG. The membranes were visualized with chemiluminescence reagent ECL, exposed to Kodak XAR film, and then analyzed by a Fluor-S multi-image analyzer. Quantitation of the density of the immunoreactive bands was performed with five mice from each group. *, $P<0.05$.

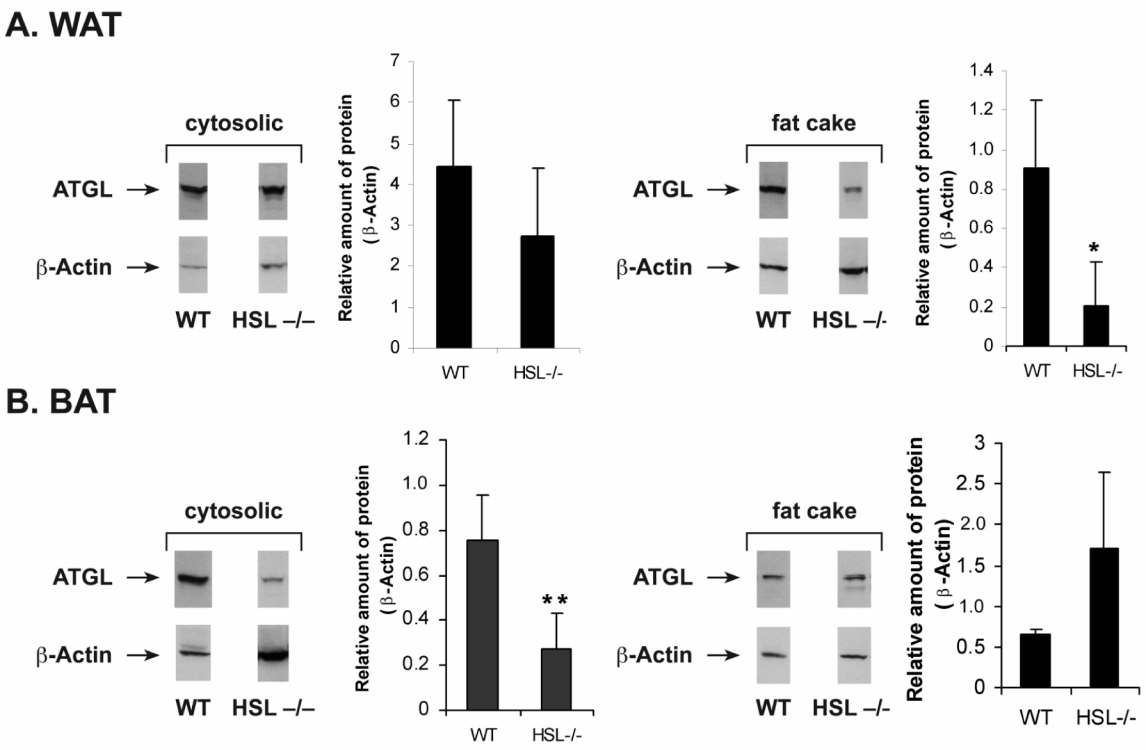

Figure 5.

Immunoblot analysis of ATGL in WAT and BAT of HSL−/− mice. WAT and BAT were isolated from 6 month old female wild-type and HSL−/− mice. Total proteins were extracted in TES with 10 μg/ml leupeptin; 20 μg of protein from tissue homogenates were subjected to SDS-PAGE and transferred to a nitrocellulose membrane. The membrane was incubated with rabbit anti-ATGL antibody and then with horseradish peroxidase-linked anti-rabbit IgG. The membranes were visualized with chemiluminescence reagent ECL, exposed to Kodak XAR film, and then analyzed by a Fluor-S multi-image analyzer. Quantitation of the density of the immunoreactive bands was performed with five mice from each group. *, $P<0.05$. **, $P<0.01$

Table 1

Primer pairs for Taqman RT-PCR.

Gene name	Accession number	primer name	seq
mouse acylpeptide hydrolase	NM_146226	APEH-f	GAG TCT CCT TCA GGC ACC AT
		APEH-r	CCC AGA CCT CCA AGA ACT GT
mouse ML1	BC013479	ML1-f	GGA ATG GGA ACC CTA ATG G
		ML1-r	TTC CTT CAG TCT CTG GGC TT
mouse esterase1	NM_007954	EST1-f	GTC TGG GCT TCT CTT GCT GT
		EST1-r	GAC TTT GCC TTG TGT GGT GT
mouse ES1	NM_138601	ES1-f	CCT ACC GCT CTC CAG ATA CC
		ES1-r	TCC GTC ATA GAC TCC ACA GC
mouse WBSCR21	NM_145215	WBSCR21-f	TCC TGC TTG GTG GAA ATT CT
		WBSCR21-r	TGA GGG AAG AGT CGC CTA AT
mouse esteraseD	NM_016903	ESD-f	GTG AAT GCC ACT GAA GAT CC
		ESD-r	TGG GAA ATT GGC ATT TAT GA
mouse CGI-58	NM_026179	CGI-58-f	TCT TGC TTG GAC ACA ACC TG
		CGI-58-r	GAG GTG ACT AAC CCT TGA TGG
Mouse TGH	NM_053200	TGH-f	GCCCTGGAGCTTCGTGAA
		TGH-r	CCTGCCCTCCAACAGCAT
mouse adiponutrin	NM_054088	ADPN-f	AGA ACG TGC TGG TGT CTG AG
		ADPN-r	GGA AGG AAG GAG GGA TTA GG
Mouse HSL	NM_010719	HSL-f	ACC GAG ACA GGC CTC AGT GTG
		HSL-r	GAA TCG GCC ACC GGT AAA GAG
mouse ATGL	AK031609	ATGL-f	AAC ACC AGC ATC CAG TTC AA
		ATGL-r	GGT TCA GTA GGC CAT TCC TC
MiPLA2	gi13385668	MiPLA2-f	TCATCAAGATGGAGGTTTGC
		MiPLA2-r	TGGCCAGATGCATTTACATT