Adipose Triglyceride Lipase and Hormone-sensitive Lipase Are the Major Enzymes in Adipose Tissue Triacylglycerol Catabolism*

Received for publication, August 22, 2006, and in revised form, October 10, 2006 Published, JBC Papers in Press, October 30, 2006, DOI 10.1074/jbc.M608048200

Martina Schweiger1†, Renate Schreiber1‡, Guenter Haemmerle1§, Achim Lass1, Christian Fledelius2, Poul Jacobsen3, Hans Tornqvist3, Rudolf Zechner2, and Robert Zimmermann1‡

From the 1Institute of Molecular Biosciences, University of Graz, A-8010 Graz, Austria, the 2Diabetes Research Unit, Novo Nordisk A/S, Novo Nordisk Park, DK-2706 Måløv, Denmark, and the 3Department of Clinical Sciences, Diabetes, and Endocrinology, Malmö University Hospital, Lund University, S-22100 Lund, Sweden

The mobilization of free fatty acids from adipose triacylglycerol (TG) stores requires the activities of triacylglycerol lipases. In this study, we demonstrate that adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) are the major enzymes contributing to TG breakdown in in vitro assays and in organ cultures of murine white adipose tissue (WAT). To differentiate between ATGL- and HSL-specific activities in cytosolic preparations of WAT and to determine the relative contribution of these TG hydrolases to the lipolytic catabolism of fat, mutant mouse models lacking ATGL or HSL and a mono-specific, small molecule inhibitor for HSL (76-0079) were used. We show that 76-0079 had no effect on TG catabolism in HSL-deficient WAT but, in contrast, essentially abolished free fatty acid mobilization in ATGL-deficient fat. CGI-58, a recently identified coactivator of ATGL, stimulates TG hydrolase activity in wild-type and HSL-deficient WAT but not in ATGL-deficient WAT, suggesting that ATGL is the sole target for CGI-58-mediated activation of adipose lipolysis. Together, ATGL and HSL are responsible for more than 95% of the TG hydrolase activity present in murine WAT. Additional known or unknown lipases appear to play only a quantitatively minor role in fat cell lipolysis.

Fatty acids deposited as triacylglycerol (TG) in white adipose tissue (WAT) represent the primary energy store in animals. In periods of increased energy demand, TG is hydrolyzed, and free fatty acids (FFA) are released into the circulation. The hydrolysis of TG is catalyzed by adipose tissue lipases in sequential steps leading to the formation of FFA and glycerol. The first step within the hydrolysis cascade generating FFA and diacylglycerol (DG) is rate-limiting for subsequent reactions. Hormone-sensitive lipase (HSL) has long been considered as catalyzing this initial step. However, in recent years it became evident that at least one additional lipase must exist capable of hydrolyzing TG. Observations that HSL-deficient (HSL-ko) mice are not obese (1–3) but accumulate DG in adipose tissue and muscle (4) suggest that HSL may be rate-limiting for DG hydrolysis rather than for the first and rate-limiting step within the TG lipolysis cascade.

In addition to HSL, three other lipases have been reported to be involved in adipose TG hydrolysis. Triacylglycerol hydrolysis (TGH) is highly expressed in liver and adipose tissue and has been suggested to represent a major adipocyte lipase (5). The recently identified TGH-2 exhibits high homology to TGH and a similar tissue distribution pattern (6). Both TGH and TGH-2 are capable of hydrolyzing TG. However, they are much more efficient in hydrolyzing substrates esterified with short-chain fatty acids compared with TG esterified with long-chain fatty acids (6, 7), and in vivo evidence for a role of these enzymes in lipolysis is lacking. Adipose triglyceride lipase (ATGL; official name, PNPLA 2 (patatin-like phospholipase domain containing protein-2); alternative names are desnutrin (8), phospholipase A2ζ (9), and transport-secretion protein) is highly expressed in adipose tissue and specifically removes the first fatty acid from the TG molecule generating FFA and DG (10). The drastic impairment of adipocyte lipolysis in ATGL inhibition studies in vitro indicates that the enzyme is responsible for most of the HSL-independent lipolytic activity (10, 11). An essential role of ATGL in lipolysis became evident from studies in ATGL-deficient (ATGL-ko) mice (12). ATGL-ko mice exhibited enlarged fat deposits and TG accumulation in multiple tissues. FFA release in response to isoproterenol is drastically reduced in ATGL-ko WAT, suggesting a key role of the enzyme in hormone-stimulated lipolysis.

The most important activators of lipolysis are catecholamines, which increase cellular AMP levels followed by the activation of protein kinase A (PKA) (13). PKA phosphorylates cytosolic HSL and the lipid droplet-associated protein, perilipin A. This process leads to the translocation...
of HSL to the lipid droplet where the enzyme gains access to TG stores (14). Phosphorylation of both proteins is necessary for the initiation of HSL-mediated lipolysis (15). The regulation of ATGL activity appears to be quite different compared with that described for HSL. ATGL is not a target for PKA-mediated phosphorylation and is localized on the lipid droplet in the basal and hormone-stimulated state of the cell (10). These observations suggest that ATGL is not activated by translocation to the lipid substrate as demonstrated for HSL. Instead, ATGL activity is regulated by an activator protein annotated as α/β-fold domain-containing protein 5 (ABHD5) (16), also known as CGI-58 (comparative gene identification 58). Mutant forms of CGI-58 carrying point mutations or protein truncations have been identified as causative for Chanarin-Dorfman syndrome (CDS) (17). Affected individuals accumulate TG in multiple tissues leading to the alternative designation, “neutral lipid storage disease” (18, 19). Mutant forms of CGI-58 associated with CDS lose their ability to activate ATGL (16) and cause misrouting of CGI-58 from the lipid droplet to the cytosol (20). These data indicate that TG accumulation in CDS is caused by defective ATGL activation (16). Furthermore, the multi-systemic TG accumulation observed in ATGL-ko mice (12) and in CDS suggests a similar function of CGI-58 and ATGL in humans and mice.

To further define the contribution of ATGL and HSL to murine adipose lipolysis, we determined whether additional lipases are involved in the hydrolysis of TG and whether CGI-58 is capable of activating lipolysis in the absence of ATGL. Our results demonstrate that TG hydrolysis is almost abolished when both ATGL and HSL are inactivated. CGI-58 failed to activate lipolysis in ATGL-deficient WAT, suggesting that ATGL represents the sole lipase activated by CGI-58.

EXPERIMENTAL PROCEDURES

Animals—Mice were maintained on a regular light-dark cycle (14 h light, 10 h dark) and kept on a standard laboratory chow diet (4.5% w/w fat). HSL-deficient and ATGL-deficient mice were generated by targeted homologous recombination as described (4, 12). Animals used for experiments were 12 to 16 weeks of age. Mice kept ad libitum were killed by cervical dislocation between 8 and 10 a.m.

Expression of Recombinant Proteins—GST-tagged murine CGI-58 (GST-CGI) was expressed in Saccharomyces cerevisiae and purified as described previously (16).

Preparation of Tissue Cytosolic Extracts—Gonadal WAT of wild-type (WT), ATGL-ko, and HSL-ko mice was surgically removed and washed in phosphate-buffered saline containing 0.3M EDTA. Homogenization was performed on ice in lysis buffer A (0.25 M sucrose, 1 mM EDTA, 1 mM dithiothreitol, 20 μg/ml leupeptin, 2 μg/ml antipain, 1 μg/ml pepstatin, pH 7.0) using an Ultra Turrax (IKA, Staufen, Germany). The WAT lysate was centrifuged at 1 h at 100,000 × g at 4 °C. The lipid-free infranatant (cytosolic fraction) was collected and used for TG hydrolase assays.

In Vitro Lipolysis of Isolated Gonadal WAT—Gonadal fat pads were surgically removed and washed several times with phosphate-buffered saline. Tissue pieces (~20 mg) were incubated in Dulbecco’s modified Eagle’s medium (Invitrogen) containing 2% fatty acid-free bovine serum albumin (Sigma) either in the presence or absence of 10 μM forskolin and/or the HSL inhibitor 76-0079 (NNC 0076-0000-0079, Novo Nordisk, Denmark) at 37 °C for 1 h. After this preincubation period, the fat pads were transferred into identical, fresh medium and incubated for a further 60 min at 37 °C. Thereafter, aliquots of the medium were removed and analyzed for FFA and glycerol content using commercial kits (Wako Chemicals, Neuss, Germany; Sigma). For protein determinations, fat pads were washed extensively with phosphate-buffered saline and lysed in 0.3 M NaOH, 0.1% SDS. Protein measurements were performed using the BCA reagent (Pierce).

Assay for Tissue TG Hydrolase Activities and Analysis of Lipolytic Reaction Products—The substrate for the measurement of TG hydrolysis activity containing triolein and [9,10-3H]tri-olein (PerkinElmer Life Sciences) as radioactive tracer was emulsified with phosphatidylcholine/phosphatidylinositol using a sonicator (Virsonic 475, Virtis, Gardiner, NJ). The final substrate concentration was 167 nmol of triolein/assay (8000 cpm of [9,10-3H]tri-olein/nmol). The cytosolic fractions (0.1 ml) supplemented with different concentrations of purified GST-CGI-58 and/or with a specific inhibitor of HSL were incubated with 0.1 ml of the substrate in a water bath at 37 °C for 60 min. The reaction was terminated by adding 3.25 ml of methanol/chloroform/heptane (10:9:7) and 1 ml of 0.1 M potassium carbonate, 0.1 M boric acid, pH 10.5. After centrifugation at 800 × g for 20 min, the radioactivity in 1 ml of the upper phase was determined by liquid scintillation counting.

For the analysis of lipolytic reaction products, the reaction was terminated by adding 1 ml of CHCl3/methanol (2:1) containing 1% acetic acid, 10 μg/ml oleic acid, and 10 μg/ml standards for tri-, di- (sn-1,2 and sn-1,3), and monoolein (Sigma). The mixture was vortexed and centrifuged (4000 × g, 10 min). The lower phase was quantitatively collected and evaporated. The lipid pellet was dissolved in CHCl3 and applied to thin layer chromatography (Merck, Darmstadt, Germany) with chloroform/acetone/acetic acid (96:4:1) as solvent. Lipids were visualized with iodine vapor, and the bands corresponding to mono-, di-, triolein and triglycerides and FFA were cut out. The co-migrating radioactivity was determined by liquid scintillation counting (4).

Statistical analysis was determined by Student’s unpaired t test (two-tailed). Group differences were considered significant for p < 0.05, 0.01, and 0.001.

RESULTS

ATGL and HSL Are the Major Lipases in Murine WAT Cytosol—To test the specificity of the HSL inhibitor 76-0079, we determined TG hydrolase activities in cytosolic preparations of WAT of ATGL-ko and HSL-ko mice in the absence and presence of 76-0079. As shown in Fig. 1A, 76-0079 reduced the TG hydrolase activity in WAT of ATGL-ko mice in a dose-dependent manner. Maximum inhibition was achieved at a concentration of ~2.5 μM. In contrast, 76-0079 had no effect on activity in HSL-ko cytosol, demonstrating that the inhibitor does not affect ATGL or other lipases. HSL is thought to represent the rate-limiting DG hydrolase in WAT (4). To deter-
mine whether 76-0079 causes DG accumulation in WT WAT, the lipolytic products were applied to TLC analysis, and the radioactivity present in DG, MG, and FFA bands was determined. As shown in Fig. 1B, 76-0079-mediated inhibition of FFA release was associated with increased DG levels. The DG/FFA ratio increased from 1:48 to 1:2.4 in the absence and presence of 76-0079, respectively, indicating severely reduced DG catabolism. MG did not accumulate, presumably because of reduced DG hydrolysis and of the high MG lipase activity present in WAT.

Fig. 1C shows the specific TG hydrolase activities obtained in cytosolic preparations of WT, ATGL-ko, and HSL-ko WAT. Compared with WT, HSL-ko and ATGL-ko WAT exhibited
Role of ATGL and HSL in Adipose Lipolysis

CGI-58 fails to Activate lipolysis in the Absence of ATGL—ATGL activity is stimulated by the presence of CGI-58 (16). As shown in Fig. 2A, the addition of CGI-58 to WT cytosol increased TG hydrolyase activity in a dose-dependent manner up to ~100%, indicating that endogenously produced CGI-58 in the cytosolic fraction is not sufficient to obtain maximum ATGL activity. To determine the effect of CGI-58 on TG hydrolyase activity in WT, HSL-ko, and ATGL-ko mice, cytosolic fractions were incubated with the TG substrate in the absence and presence of purified GST-tagged CGI-58. CGI-58 increased TG hydrolyase activity in WT and HSL-ko preparations by 1.7- and 2.1-fold (Fig. 2B), respectively, but was not capable of stimulating the activity in ATGL-ko WAT. Similar data were obtained in the presence of 76-0079. CGI-58 stimulated TG hydrolyase activity in cytosol of WT and HSL-ko WAT by 1.9- and 2.2-fold, respectively, and had no effect in ATGL-ko WAT. These data demonstrate that ATGL is the sole target for CGI-58-mediated activation of adipose lipolysis. In contrast, incubation of ATGL-deficient samples with CGI-58 inhibited TG hydrolyase activity by 25%, suggesting a moderate inhibitory effect of CGI-58 on HSL activity in in vitro assays.

FFA Mobilization in WAT Is Ablated in the Absence of ATGL and HSL Activity—The measurement of lipolytic activity in tissue preparations in vitro does not necessarily reflect the situation in living cells. Therefore, we tested the implication of ATGL and HSL in lipolysis in organ cultures of WAT. To determine the effect of 76-0079 on lipolysis, fat pads from WT mice were incubated with various amounts of the inhibitor in the presence or absence of forskolin. As shown in Fig. 3A, 76-0079 reduced the forskolin-stimulated glycerol release in WAT of WT mice to basal levels even at the lowest inhibitor concentration used (1 μM). Maximum inhibition of FFA release was achieved at ~25 μM 76-0079 (~65%). Fig. 3B compares the FFA release obtained with fat pads of WT, HSL-ko, and ATGL-ko mice. The treatment of fat pads with forskolin increased basal FFA release severalfold in all genotypes. Compared with WT, forskolin-stimulated FFA release in HSL-ko and ATGL-ko WAT was reduced by 38 and 70%, respectively. The addition of 76-0079 had no effect on FFA release in HSL-ko WAT, again demonstrating that the inhibitor is specific for HSL. In fat pads of WT and ATGL-ko mice, 76-0079 inhibited forskolin-stimulated FFA release by 68 and 94%, respectively. The remaining FFA mobilization from ATGL-ko fat was ~2% of the release obtained in WAT expressing functional enzymes.

DISCUSSION

WAT stores and releases FFA according to the requirements of the body. An imbalance of TG synthesis and hydrolysis may result in metabolic disorders such as lipodystrophy, obesity, type 2 diabetes, and metabolic syndrome. The availability of FFA is determined predominantly by the lipase-dependent hydrolysis of TG stores in WAT. In obesity, plasma FFA levels are increased, and a clear relationship exists between sustained decreased TG hydrolase activities (~65 and ~72%, respectively). In the presence of 76-0079, the activities in WT and ATGL-ko WAT were reduced by 70 and 84%, respectively. The activity in HSL-ko WAT was not affected. In comparison with WT conditions, where both ATGL and HSL are active, inhibition of both enzymes resulted in more than 95% reduction of TG hydrolyase activity. Thus, TG hydrolyase activities in cytosolic preparations of murine adipose tissue are mainly composed of ATGL and HSL activity.

FIGURE 3. Implication of ATGL and HSL in lipolysis in WAT organ cultures. Basal and forskolin-stimulated release of FFA and glycerol from gonadal white adipose tissue isolated from non-fasted mice. WAT pieces (~20 mg) were preincubated in Dulbecco’s modified Eagle’s medium, 2% bovine serum albumin (FFA-free) in the absence (basal) or presence of 10 μM forskolin and/or 76-0079 at various concentrations for 1 h at 37 °C. Thereafter, WAT pieces were transferred into an identical, fresh medium, and FFA or glycerol release was determined after incubation for another hour under the same conditions. A, dose-dependent effect of 76-0079 on FFA and glycerol release in WAT pieces from WT mice. Measurements for each data point were obtained from three single fat pads. B, effect of 76-0079 on FFA release from WAT of WT, ATGL-ko, and HSL-ko mice. Data were obtained from WAT pieces of five single mice of each genotype and are presented as mean ± S.D. (**, p < 0.01; ***, p < 0.001).
Role of ATGL and HSL in Adipose Lipolysis

elevated plasma FFA levels and the development of insulin resistance (21). Recent studies in mice with targeted ablation of ATGL suggest that a reduction of adipose lipolysis may result in improved glucose tolerance and insulin sensitivity (12). Thus, lipolysis is linked to the pathogenesis of diabetes in obesity and related disorders, and adipose tissue lipases might represent molecular targets for the development of novel anti-diabetic drugs.

ATGL-ko and HSL-ko mice exhibit a decreased capacity to mobilize FFA from adipose stores, demonstrating that both enzymes contribute to the hydrolysis of TG (1, 4, 12, 22). However, it remained obscure whether additional lipases existed that were capable of hydrolyzing TG. The present study demonstrates that ATGL and HSL are the rate-limiting enzymes in FFA mobilization. The drastically reduced DG hydrolysis activity in vitro and the ablated FFA release from organ cultures of WAT in the absence of both ATGL and HSL activity demonstrate that other enzymes cannot compensate for these lipases. According to their specific activities, ATGL and HSL act coordinately in hydrolyzing TG stores. ATGL predominantly performs the initial hydrolysis and provides DG for subsequent reactions. HSL efficiently degrades DG into MG and FFA (10). As demonstrated in this study, forskolin-activated FFA mobilization is present in organ cultures of ATGL-ko and HSL-ko WAT, indicating that both enzymes are capable of mobilizing TG and that both enzymes are sensitive to hormonal stimulation. FFA mobilization in ATGL-ko WAT is severely reduced, suggesting that HSL is less efficient in initiating TG hydrolysis compared with ATGL. Conversely, ATGL or other enzymes are not capable of compensating for HSL-mediated DG hydrolysis. Thus, efficient lipolysis in murine WAT is dependent on the provision of DG by ATGL and subsequent hydrolysis of DG by HSL.

The inhibition of lipolysis in WAT of WT mice using an HSL-specific inhibitor resulted in severely reduced DG catabolism and DG accumulation similar to that observed in HSL-ko mice. FFA release from WAT cultures was reduced by approximately two-thirds. This value would be expected when DG hydrolysis is blocked, because two fatty acids remain trapped within the DG molecule. Similar results were obtained in a previous study using a specific HSL inhibitor in isolated WT mouse adipocytes (23). In comparison, the FFA release from HSL-ko WAT was less affected. This discrepancy is consistent with a previously observed reduction in TG synthesis in HSL-ko WAT. This compensatory mechanism results in less efficient re-esterification of mobilized FFA and might therefore be responsible for the unexpectedly moderate decrease in FFA release in HSL-ko adipose tissue (24).

CGI-58 and ATGL are essential components of the lipolytic system, yet the molecular mechanism leading to ATGL activation remains to be elucidated. Our data demonstrate that CGI-58 is not capable of increasing TG hydrolyase activity in ATGL-deficient WAT, suggesting that ATGL is the major target for CGI-58-mediated activation of lipolysis. Lipolysis in adipocytes is primarily stimulated by signals that activate PKA (13). In contrast to HSL, ATGL is not a target for PKA-mediated phosphorylation, and its presence on lipid droplets in the basal and the activated state excludes an activation mechanism comparable with the PKA-driven translocation of HSL (10). However, the localization of CGI-58 is influenced by PKA. In the basal state, CGI-58 is present on adipose lipid stores and reversibly interacts with perilipin A (25). Perilipin A is abundantly present on lipid droplets and protects TG stores from hydrolysis. Although it has been suggested that this is mostly protection from HSL-mediated lipolysis, several studies indicate that perilipin A has a protective effect independently of the presence of HSL (26–28). In response to PKA stimulation, perilipin A is phosphorylated and CGI-58 is released from the lipid droplet (25). This reversible binding of CGI-58 to perilipin A could potentially represent an indirect, PKA-dependent mechanism controlling ATGL activity. It is conceivable that, in the basal state, CGI-58 is bound to perilipin A, resulting in incomplete or absent activation of ATGL. Upon lipolytic stimulation, CGI-58 is released from perilipin A and becomes available for ATGL resulting in increased TG hydrolysis. However, additional experiments are required to unfold the molecular mechanisms regulating ATGL activity and the role of perilipin in this process. Notably, CGI-58 is capable of activating TG hydrolyase activity not exclusively in WAT but also in brown adipose tissue, skeletal muscle, cardiac muscle, testes, and liver (16). CGI-58 appears to be expressed ubiquitously, and the tissue distribution pattern of CGI-58 mRNA differs from that of ATGL (16, 25). These observations raise the possibility that CGI-58 possesses an additional function in lipid metabolism or activates other lipases than ATGL in specific tissues.

Our findings in mouse adipose tissue may not reflect the situation in human adipose tissue. Next to ATGL, another member of the adiponutrin family, GS-2 (PNPLA4), has been reported to possess TG and retinyl ester hydrolyase activity as well as transacylase activity (29). The mouse ortholog of GS2 is unknown (30) raising the possibility that additional lipases contribute to human adipose lipolysis. Langin et al. (23) interpreted their findings in human adipocytes using a specific HSL inhibitor to mean that HSL is the major lipase for hormone-stimulated lipolysis, whereas ATGL might participate in basal lipolysis. However, human ATGL (hATGL) is highly expressed in human adipocytes (10, 23) and hATGL as well as human CGI-58 (hCGI-58) exhibit the same biological activity as demonstrated for their mouse orthologs. hATGL hydrolyzes TG and is stimulated by hCGI-58 (16). Mutants of hCGI-58 present in subjects suffering from CDS lose their ability to activate ATGL, and a systemic TG accumulation is observed in CDS as well as in ATGL-ko mice (12, 16). Together, these observations suggest that CGI-58 and ATGL exert similar functions in lipid metabolism in humans and mice. Earlier studies suggested that TG accumulation in CDS occurs because of abnormalities in phospholipid (PL) metabolism. Igal and Coleman (31, 32) reported defective acylglycerol recycling from TG stores to PL as well as increased de novo PL synthesis in skin fibroblasts obtained from a CDS patient. These alterations in PL metabolism may be explained by the specific activities of ATGL and HSL. ATGL generates DG from TG, which can be utilized for PL synthesis. In contrast, HSL-mediated lipolysis of TG does not result in DG accumulation because of the high specific activity of HSL against DG (10). Thus, defective ATGL activation as observed in CDS fibroblasts (16) could reduce the availability of DG for
Role of ATGL and HSL in Adipose Lipolysis

PL synthesis, resulting in decreased acylglycerol recycling and, as a compensatory process, in increased de novo PL synthesis.

In conclusion, our data demonstrate that ATGL and HSL are quantitatively the most important lipases in murine adipose tissue. Additional known or unknown lipases appear to play only a minor role in TG hydrolysis. The absence of CGI-58-mediated stimulation of TG hydrolysis in ATGL-deficient tissue. Additional known or unknown lipases appear to play

PL suggests that ATGL represents the only adipose lipase mediated stimulation of TG hydrolysis in ATGL-deficient tissue. Additional known or unknown lipases appear to play

Acknowledgment—We thank Dr. E. Zechner for reviewing the manuscript.

REFERENCES

1. Osuga, J., Ishibashi, S., Oka, T., Yagyu, H., Tozawa, R., Fujimoto, A., Shionoiri, F., Yahagi, N., Kraemer, F. B., Tsutsumi, O., and Yamada, N. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 787–792.

2. Wang, S. P., Laurin, N., Himms-Hagen, J., Rudnicki, M. A., Levy, E., Robert, M. F., Pan, L., Oligny, L., and Mitchell, G. A. (2004) J. Biol. Chem. 279, 4806–4815.

3. Okazaki, H., Igarashi, M., Nishi, M., Tajima, M., Sekiya, M., Okazaki, S., Yahagi, N., Ohashi, K., Tsukamoto, K., Amemiya-Kudo, M., Matsuzaka, T., Shimano, H., Yamada, N., Aoki, J., Morikawa, R., Takanezawa, Y., Arai, H., Nagai, R., Kadowaki, T., Osuga, J., and Ishibashi, S. (2006) Diabetes 55, 2091–2097.

4. Jenkins, C. M., Mancuso, D. J., Yan, W., Sims, H. F., Gibson, B., and Gross, R. W. (2004) J. Biol. Chem. 279, 48968–48975.

5. Subramanian, V., Rotenberg, A., Gomez, C., Cohen, A. W., Garcia, A., Bhattacharyya, S., Shapiro, L., Dolios, G., Wang, R., Lisanti, M. P., and Arner, E. (2004) J. Biol. Chem. 279, 42062–42071.

6. Kershaw, E. E., Ham, J. K., Verhagen, L. A., Peroni, O., Katic, M., and Flier, J. S. (2006) Diabetes 55, 148–157.

7. Lehner, R., and Verger, R. (1997) Biochemistry 36, 1861–1868.

8. Villena, J. A., Roy, S., Sarkadi-Nagy, E., Kim, K. H., and Sul, H. S. (2004) J. Biol. Chem. 279, 47066–47075.

9. Jenkins, C. M., Mancuso, D. J., Yan, W., Sims, H. F., Gibson, B., and Gross, R. W. (2004) J. Biol. Chem. 279, 48968–48975.

10. Zimmermann, R., Strauss, J. G., Hamerle, G., Schoiswohl, G., Birner-Gruenberger, R., Riederer, M., Lass, A., Neuberger, G., Eisenhaber, F., Hermetter, A., and Zechner, R. (2004) Science 306, 1383–1386.

11. Hemmerle, G., Lass, A., Zimmermann, R., Gorkiewicz, G., Meyer, C., Rozman, J., Heldmaier, G., Maier, R., Theuissl, C., Eder, S., Kratky, D., Wagner, E. F., Klingenspor, M., Hoefler, G., and Zechner, R. (2006) Science 312, 734–737.

12. Holm, C. (2003) Biochem. Soc. Trans. 31, 1120–1124.

13. Clifford, G. M., Londos, C., Kraemer, F. B., Vernon, R. G., and Yeaman, S. J. (2000) J. Biol. Chem. 275, 5011–5015.

14. Chen, K., Chen, S., Wang, C., and Zechner, R. (2003) J. Biol. Chem. 278, 43615–43619.

15. Saavedra, J. M., and Zechner, R. (2006) Cell Metab. 3, 309–319.

16. Chanarin, I., Patel, A., Slavin, G., Wills, E. J., Andrews, T. M., and Stewart, G. (1975) Br. Med. J. 1, 553–555.

17. Dorfman, M. L., Hershko, C., Eisenberg, S., and Sagher, F. (1974) Arch. Dermatol. 110, 261–266.

18. Yamaguchi, T., Omatsu, N., Matsuhashi, S., and Osumi, T. (2004) J. Biol. Chem. 279, 30490–30497.

19. Boden, G., and Shulman, G. I. (2002) Eur J. Clin. Investig. 32, Suppl. 3, 14–23.

20. Haemmerle, G., Hamerle, G., Wagner, E. F., and Zechner, R. (2002) J. Biol. Chem. 277, 4806–4815.

21. Haemmerle, G., Zimmermann, R., Strauss, J. G., Kratky, D., Riederer, M., Knipping, G., and Zechner, R. (2002) J. Biol. Chem. 277, 12946–12952.

22. Langin, D., Dicker, A., Tavernier, G., Hofstedt, J., Mairal, A., Ryden, M., Arner, E., Sicard, A., Jenkins, C. M., Vigueir, N., van Harmelen, V., Gross, R. W., Holm, C., and Arner, P. (2005) Diabetes 54, 3190–3197.

23. Zimmermann, R., Haemmerle, G., Wagner, E. F., Strauss, J. G., Kratky, D., and Zechner, R. (2003) J. Lipid Res. 44, 2089–2099.

24. Subramanian, V., Rotenberg, A., Gomez, C., Cohen, A. W., Garcia, A., Bhattacharyya, S., Shapiro, L., Dolios, G., Wang, R., Lisanti, M. P., and Arner, E. (2004) J. Biol. Chem. 279, 42062–42071.

25. Schaeffer, J. E., Dallas, G. E., Wang, X., Kraemer, F. B., Obin, M., and Greenberg, A. S. (2002) J. Biol. Chem. 277, 8267–8272.

26. Tansey, J. T., Huml, A. M., Vogt, R., Davis, K. E., Jones, J. M., Fraser, K. A., Braesamle, K., Kimmel, A. R., and Londos, C. (2003) J. Biol. Chem. 278, 8401–8406.

27. Gao, J., and Simon, M. (2005) J. Invest. Dermatol. 124, 1259–1266.

28. Zechner, R., Strauss, J. G., Haemmerle, G., Lass, A., and Zimmermann, R. (2005) Eur J. Clin. Investig. 35, 333–340.

29. Igal, R. A., and Coleman, R. A. (1996) J. Biol. Chem. 271, 16644–16651.

30. Igal, R. A., and Coleman, R. A. (1998) J. Lipid Res. 39, 31–43.