Biochimica et Biophysica Acta 1841 (2014) 588–594

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbalip

Adipose triglyceride lipase activity is inhibited by long-chain acyl-coenzyme A

Harald M. Nagy [1], Margret Paar [1], Christoph Heier, Tarek Moustafa, Peter Hofer, Guenter Haemmerle, Achim Lass, Rudolf Zechner, Monika Oberer, Robert Zimmermann *

Institute of Molecular Biosciences, University of Graz, Austria

ARTICLE INFO

Article history:
Received 7 November 2013
Received in revised form 20 December 2013
Accepted 6 January 2014
Available online 16 January 2014

Keywords:
Adipose triglyceride lipase
Hormone-sensitive lipase
Lipolysis
Regulation
acyl-CoA

ABSTRACT

Adipose triglyceride lipase (ATGL) is required for efficient mobilization of triglyceride (TG) stores in adipose tissue and non-adipose tissues. Therefore, ATGL strongly determines the availability of fatty acids for metabolic reactions. ATGL activity is regulated by a complex network of lipolytic and anti-lipolytic hormones. These signals control enzyme expression and the interaction of ATGL with the regulatory proteins CGI-58 and G0S2. Up to date, it was unknown whether ATGL activity is also controlled by lipid intermediates generated during lipolysis. Here we show that ATGL activity is inhibited by long-chain acyl-CoAs in a non-competitive manner, similar as previously shown for hormone-sensitive lipase (HSL), the rate-limiting enzyme for diglyceride breakdown in adipose tissue. ATGL activity is only marginally inhibited by medium-chain acyl-CoAs, diglycerides, monoglycerides, and free fatty acids. Immunoprecipitation assays revealed that acyl-CoAs do not disrupt the protein–protein interaction of ATGL and its co-activator CGI-58. Furthermore, inhibition of ATGL is independent of the presence of CGI-58 and occurs directly at the N-terminal patatin-like phospholipase domain of the enzyme. In conclusion, our results suggest that inhibition of the major lipolytic enzymes ATGL and HSL by long-chain acyl-CoAs could represent an effective feedback mechanism controlling lipolysis and protecting cells from lipotoxic concentrations of fatty acids and fatty acid-derived lipid metabolites.

1. Introduction

Adipose triglyceride lipase (ATGL, also referred to as patatin-like phospholipase domain containing 2 [PNPLA2] or desnutrin [1]) performs the first step in triglyceride (TG) hydrolysis generating diglyceride (DG) and free fatty acids (FFAs) [2]. Consequently, the enzyme controls the availability of FFAs, which may serve as energy substrates, precursors for other lipids, and lipid signaling molecules. This central function has a major impact on overall energy metabolism and becomes evident in fasted ATGL-deficient mice (ATGL-ko). In this mouse model, the lack of sufficient FFAs for energy conversion promotes the usage of glucose for energy conversion [3]. As a consequence, short fasting periods or moderate exercise leads to rapid consumption of glycogen stores. Fasting for more than 6 h results in hypoglycemia, hypometabolism, and hypothermia [3,4].

ATGL activity is regulated by a complex network of hormones which control enzyme expression and the interaction of the enzyme with regulatory proteins. ATGL is stimulated by the presence of an activator protein as observed for other TG lipases, such as pancreatic lipase or lipoprotein lipase. The activator of ATGL is termed comparative gene identification-58 (CGI-58) [or alpha/beta-hydrolase domain containing 5 (ABHD5)] [5]. Currently, the molecular mechanism on how CGI-58 stimulates ATGL activity is unknown [6]. However, loss of either ATGL or CGI-58 function causes systemic TG accumulation in humans and mice. This inherited disorder is known as Neutral Lipid Storage Disease (NLSD) [7]. A second regulatory protein of ATGL is G0/G1 switch gene-2 (G0S2). This protein was originally described to be required to commit cells to enter the G1 phase of the cell cycle [8]. Recent evidence suggests that G0S2 specifically inhibits ATGL activity in rodents and humans [9,10]. Both G0S2 and CGI-58 have been shown to interact with ATGL. Furthermore, they are present on lipid droplets and regulated by metabolic hormones. G0S2 appears to be regulated primarily on the expression level. The antilipolytic hormone insulin increases G0S2 expression in 3T3-L1 adipocytes, whereas activation of lipolysis by fasting, β-adrenergic agonists, and tumor necrosis factor-α has the opposite effect [9,11]. In contrast to G0S2, fasting and β-adrenergic stimulation have minor effects on CGI-58 protein expression in adipose tissue.

* Corresponding author at: Institute of Molecular Biosciences, University of Graz, Heinrichstrasse 31A, 8010 Graz, Austria. Tel.: +43 316 380 1900; fax: +43 316 380 9016.
E-mail address: robert.zimmermann@uni-graz.at (R. Zimmermann).
[1] Authors contributed equally.

http://dx.doi.org/10.1016/j.bbalip.2014.01.005
1388-1981/

This co-activator protein is regulated primarily by its reversible interaction with the lipid droplet coating protein perilipin 1 [12]. In non-activated adipocytes, CGI-58 is bound to perilipin 1 and lipolysis is low. Upon lipolytic stimulation by β-adrenergic agonists, perilipin 1 gets phosphorylated by protein kinase A leading to the release of CGI-58 which is now available for ATGL activation. In addition, ATGL activity is influenced by other members of lipid droplet coat proteins of the perilipin (PAT) family. Perilipin 2 has been shown to reduce the lipid droplet association of ATGL [13]. Recent data also suggest that perilipin 5 interacts with ATGL and inhibits its activity [14–17].

Up to date, it was unknown whether ATGL activity or its interaction with regulatory proteins is controlled by lipid metabolites arising during lipolysis. Here we show that ATGL is directly inhibited by long-chain acyl-CoA via a non-competitive mechanism.

2. Materials and methods

2.1. Materials

Acyl-CoA with various fatty acid chain lengths and triolein were obtained from Sigma-Aldrich (Taufkirchen, Germany). Radiolabeled [9,10(N)-^{3}H]triolein was obtained from PerkinElmer Life Sciences and hexadecyl-CoA was obtained from Avanti Polar Lipids.

2.2. Expression of recombinant proteins

For expression of murine ATGL and CGI-58 in *Escherichia coli*, sequences containing the complete open reading frame of murine ATGL and murine CGI-58 were amplified from cDNA by PCR using Phusion™ High Fidelity DNA Polymerase (Finnzymes, Espoo, Finland). Respective primers were designed to create 5′ and 3′ restriction endonuclease cleavage sites (underlined) for subsequent cloning strategies:

mATGL_fw: 5′-TCGGTACC CATGTTCCCGAGGGAGACCAA-3′

mATGL_rv: 5′-ACCTCGAG TCAGCAAGGCGGGAGGC-3′

mCGI-58_fw: 5′-GGGGATCC CAAAGCGATGGCGGCGG-3′

mCGI-58_rv: 5′-CTGATATC TCAGTCTACTGTGTGGCAGATCTCC-3′.

PCR products were inserted into the target vector pASK-IBA5plus (IBA, Goettingen, Germany) and transformed into *E. coli* (strain XL-1 and BL-21 for ATGL and CGI-58, respectively). Protein expression was induced by adding 200 ng/ml anhydro-tetracycline. Cells were harvested 3 h after induction. Expression of strep-tagged proteins was detected by Western blot analysis using mouse anti-Strep-tag II antibody (1:5000 dilution; IBA, Goettingen, Germany) as primary antibody and HRP-linked sheep-anti mouse antibody, (1:10,000; GE Healthcare Amersham, Buckinghamshire, UK) as secondary antibody.

Transient transfection of Monkey embryonic kidney cells (COS-7, ATCC CRL-1651) with pcDNA4/HisMax coding for His-tagged ATGL, HSL, or ß-galactosidase (LacZ) was performed with Metafectene™ (Biontex GmbH) as described [2]. Expression of His-tagged proteins was detected using anti-His monoclonal antibody (6xHis, BD Biosciences) and a horseradish peroxidase-conjugated anti-mouse antibody (GE Healthcare) as secondary antibody.

2.3. Preparation of cell and tissue extracts

E. coli and COS-7 cells were disrupted by sonication resuspended in lysis buffer (0.25 M sucrose, 1 mM dithiothreitol, 1 mM EDTA, 20 μg/ml leupeptine, 2 μg/ml antipain, 1 μg/ml pepstatin, pH 7.0). Lysates of E. *coli* were centrifuged at 15,000 ×*g* at 4 °C for 20 min. For the preparation of COS-7 cell extracts, nuclei and unbroken cells were removed by centrifugation at 1000 ×*g* at 4 °C for 5 min. Supernatants were collected and used for activity assays. The specific activity of these lysates ranged from 100 to 400 nmol/h·mg depending on the expression levels of recombinant proteins.

Mouse gonadal WAT was homogenized in lysis buffer (~1 ml/fat pad) using an Ultra Turrax Homogenizer (Fisher Scientific, Waltham, MA). The homogenate was centrifuged at 20,000 ×*g* at 4 °C for 1 h. The interphase was collected and used for activity assays.

2.4. Assay for TG hydrolase activity

The substrate for the measurement of TG hydrolase activity was prepared as described previously with minor modifications [2]. Briefly, triolein and [9,10-^{3}H]triolein (10 μCi/ml) were emulsified in the presence of phosphatidylcholine/phosphatidylinositol using a sonicator (Virsonic 475, Virtis, Gardiner, NJ) and adjusted to 2.5% BSA (FFA free). The final substrate concentration was 1.67 μmol/ml triolein and 0.15 mg/ml PC/PI (3:1). For kinetic investigations, the TG substrate was diluted to the indicated concentrations after sonication. Activity assays were performed using 0.1 ml of cell lysates and 0.1 ml substrate in a water bath at 37 °C for 20 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.

2.5. Protein interaction of ATGL and CGI-58

Cos-7 cells were co-transfected with Flag-tagged CGI-58 and His-tagged ATGL. After 3 h of binding, FLAG-beads were washed and incubated for 20 min with indicated concentrations of acyl-CoAs at 37 °C. Subsequently, beads were washed 3-times with lysis buffer, proteins were eluted by boiling in SDS-containing sample puffer, and probes were subjected to Western blot analysis using FLAG- and His-tag specific antibodies (Monoclonal mouse ANTI-FLAG® M2-Peroxidase (HRP) antibody, Sigma, A8592; Monoclonal mouse ANTI-HIS antibody, GE Healthcare, 27-4710-01) and sheep anti-mouse IgG (HRP-linked, GE Healthcare; NA931) as secondary antibody.

2.6. Protein determinations

Protein concentrations of cell lysates were determined by Bio-Rad protein assay kit according to manufacturer's instructions (Bio-Rad, Hercules, CA) using BSA as standard.

2.7. Statistical analysis

Data are presented as mean ± S.D. Statistical significance was determined by the Student's unpaired *t*-test (two-tailed). Group differences were considered significant for $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***).

3. Results

3.1. ATGL is inhibited by oleoyl-CoA

Inhibition of ATGL activity by lipid intermediates was first investigated in lysates of COS-7 cells expressing His-tagged ATGL and CGI-58. Lysates containing approximately equimolar concentrations of ATGL and CGI-58 (Fig. 1A) were incubated with a radiolabeled triolein substrate in the absence (control) or presence of various lipid metabolites. At a concentration of 50 μM, ATGL activity was almost completely inhibited in the presence of oleoyl-CoA. In comparison, oleic acid (OA) had little effect, whereas free CoA, monoolein (MO), and diolein (DO) did not affect enzyme activity (Fig. 1B). ATGL was also inhibited by a thioether analog of palmitoyl-CoA (hexadecyl-CoA, Fig. 1B) suggesting that protein acylation is not required for inactivation.

Addition of oleoyl-CoA led to inactivation of ATGL activity with an IC_{50} value of 33 μM (Fig. 1C).

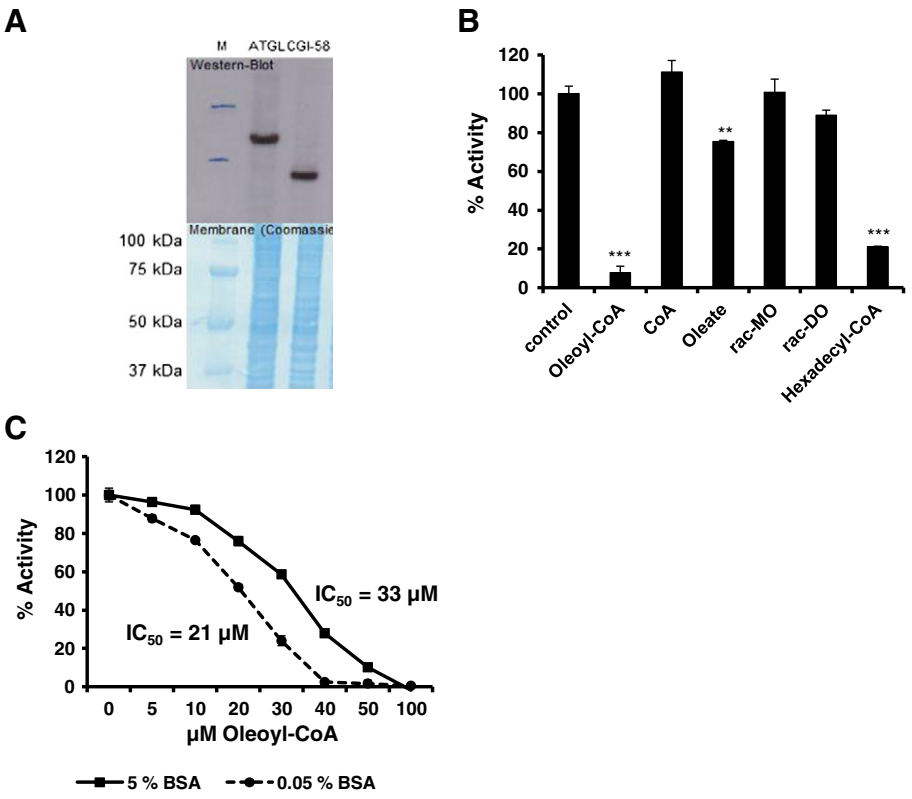

Fig. 1. ATGL is inhibited by oleoyl-CoA. (A) Western blot analysis of COS-7 cell lysates overexpressing ATGL and CGI-58. (B) TG hydrolase activity of ATGL in presence of 50 μM oleoyl-CoA, free CoA, free oleic acid, rac-MO, rac-DO and a thioether analog of palmitoyl-CoA (Hexdecyl-CoA). (C) Effect of BSA on oleoyl-CoA mediated inhibition of ATGL activity. The specific activity decreased from 3.2 μmol/h·mg to 1.7 μmol/h·mg when the BSA concentration was reduced from 5% (360 μM) to 0.05%. Data are presented as mean ± S.D. from triplicate determinations and representative for at least three independent experiments.

It is important to note that all assays have been performed in the presence of excess BSA (360 μM) which harbors high affinity sites for acyl-CoA [18]. When BSA was omitted from the reaction, we observed an almost complete inhibition of ATGL activity implicating that the enzyme requires an FFA acceptor for full activity. In the presence of low amounts of BSA (3.6 μM), the enzyme retained ~50% of its activity. Under these conditions, we determined an IC_{50} value of 21 μM suggesting that BSA moderately interferes with acyl-CoA-mediated enzyme inhibition (dashed line, Fig. 1C).

3.2. ATGL and HSL are inhibited by long-chain acyl-CoAs

HSL has previously been shown to be inhibited by long-chain acyl-CoAs (LCAs) [19]. To compare the effect of acyl-CoAs of different chain length on ATGL and HSL activity, we expressed these enzymes in COS-7 cells and determined TG hydrolase activity in the presence of various acyl-CoAs. The acyl-CoA-mediated inhibition of TG hydrolysis strongly depended on fatty acid length. Both, ATGL and HSL were inhibited by oleoyl-CoA and palmitoyl-CoA (Fig. 2A). Lauroyl-CoA inhibited HSL but had no effect on ATGL activity. Acyl-CoA with shorter chain length did not inhibit either enzyme. Next, we tested the effect of acyl-CoAs in lysates of mouse white adipose tissue (WAT) where ATGL and HSL are together responsible for more than 95% of the neutral TG hydrolase activity [20]. In WAT lysates of wild-type and ATGL-ko mice, TG hydrolase activity was inhibited by acyl-CoAs exhibiting a fatty acid chain length ≥12 carbon atoms (Fig. 2B, C). In WAT lysates of HSL-deficient (HSL-ko) mice, where ATGL represents the major TG lipase, TG hydrolase activity was inhibited by palmitoyl- and oleoyl-CoA (Fig. 2D) whereas lauroyl-CoA had no effect. Thus, both ATGL and HSL are inhibited by LCA and only HSL is sensitive to lauroyl-CoA allowing discrimination between ATGL and HSL activity in biological samples.

3.3. Acyl-CoAs interact with the N-terminal domain of ATGL and do not disrupt the protein interaction of ATGL and CGI-58

Next we investigated whether LCAs inhibit ATGL activity directly by binding to the enzyme or indirectly by binding to CGI-58 and interfering with ATGL/CGI-58 interaction. To test if the inhibition is dependent on CGI-58, we omitted CGI-58 from the reaction. As shown in Fig. 3A, ATGL was sensitive to LCA-mediated inhibition in the absence of CGI-58 indicating that LCAs directly interact with the enzyme. To restrict the binding site of LCAs to N- and C-terminal domains, we used a truncated version of ATGL lacking the C-terminal part of the enzyme (Q289ter). This truncated ATGL variant comprises the active patatin-like phospholipase domain and has previously been shown to exhibit increased lipase activity in comparison to the full-length enzyme [21]. As shown in Fig. 3C, Q289ter was inactivated by oleoyl-CoA which suggests that LCAs interact with the N-terminal domain of ATGL comprising the catalytic patatin-like region [22].

To investigate whether LCAs affect the interaction of ATGL and CGI-58, we performed immunoprecipitation assays using His-tagged CGI-58 and FLAG-tagged ATGL. As expected, we could clearly detect an interaction between these proteins when co-expressed in COS-7 cells (Fig. 3B). Addition of oleoyl-CoA or lauroyl-CoA had no effect suggesting that acyl-CoAs do not disrupt the interaction of ATGL and its activator protein.

To exclude that other cofactors are required for LCA-mediated inhibition of ATGL, we switched to a heterologous expression system and expressed Strep-tagged ATGL and CGI-58 in *E. coli*. As shown in Fig. 3C, ATGL was active in *E. coli* lysates and inactivated by the addition

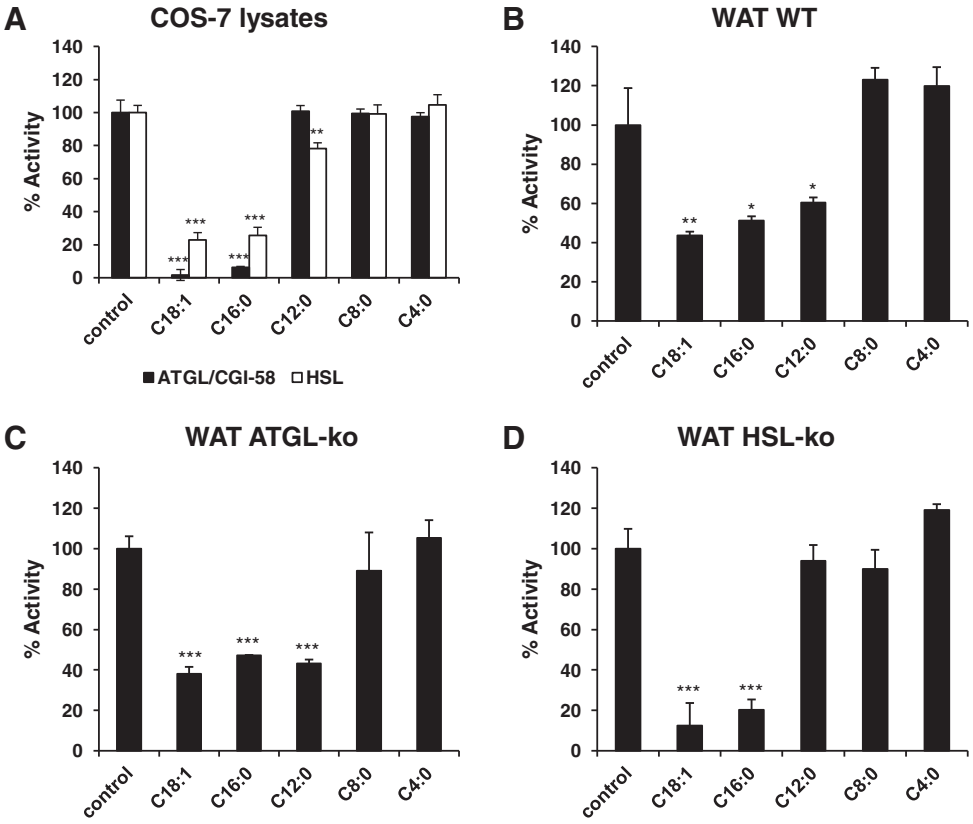

Fig. 2. ATGL and HSL are specifically inhibited by long-chain acyl-CoAs. (A) TG hydrolase activity of COS-7 cell lysates overexpressing ATGL/CGI-58 or HSL in presence of acyl-CoAs with different acyl-chain lengths. (B), (C), (D) Acyl-CoA-mediated inhibiton of TG hydrolase activity in WAT lysates of wild-type, ATGL-ko, and HSL-ko mice, respectively. The specific activity of wild-type lysate was 412 ± 77 nmol/h·mg. TG hydrolase activities in ATGL-ko and HSL-ko samples are decreased by 65% and 72%, respectively, as described earlier [20]. Data are presented mean ± S.D. from triplicate determinations and representative for two independent experiments.

of oleoyl-CoA with an IC_{50} value of 17 μM. Together, these observations suggest that LCA directly inhibit ATGL.

3.4. Long-chain acyl-CoAs inhibit ATGL in a non-competitive manner

To get insight into the mechanism of inhibition, we performed inhibitor kinetic studies. For this purpose, we used *E. coli* lysates containing ATGL and CGI-58. First, we tested whether we can apply Michaelis–Menten kinetics. As shown in Fig. 4A, saturation kinetics revealed an almost linear increase in enzyme activity up to 800 μM substrate. Furthermore, time course experiments demonstrated that the reaction was linear for at least 30 min in the absence and presence of oleoyl-CoA suggesting that steady-state conditions are achieved (Fig. 4B). Inhibitor kinetics were performed using different substrate and inhibitor concentrations in a concentration range of 200–800 μM triolein and 0, 10, 20, and 40 μM oleoyl-CoA, respectively. As shown in Fig. 4C, inhibition of ATGL by oleoyl-CoA was almost independent of the substrate concentration. Accordingly, Lineweaver–Burk analysis revealed that oleoyl-CoA reduced V_{max} but did not affect K_m demonstrating that acyl-CoA-mediated inhibition occurs in a non-competitive manner (Fig. 4D). Using nonlinear regression analysis (GraphPad Prism 5, GraphPad Inc.) and a model for mixed inhibition kinetics we calculated a Ki of 19 ± 5 μM.

4. Discussion

Generally, FFAs have to be activated to acyl-CoAs for further metabolization such as β-oxidation, synthesis of complex lipids, or protein acylation. It is well known that acyl-CoAs are not only short-lived metabolites, but directly regulate central enzymes in energy and lipid metabolism including mitochondrial adenine nucleotide translocase, acetyl-CoA carboxylase, pyruvate dehydrogenase, and phosphofructokinase [23]. Furthermore, LCAs regulate the activity of different protein kinase C subtypes and are directly or indirectly involved in the control of gene expression, ion fluxes, and membrane trafficking [23].

Previous studies demonstrated that LCAs inhibit tissue TG lipase activity [24,25] and HSL by non-competitive inhibition [19,26]. Here we show that LCA also target ATGL and inhibit the enzyme in a non-competitive manner. Our data indicate that differences exist with respect to the efficacy of acyl-CoA species, since HSL but not ATGL is inhibited by lauroyl-CoA. We assume that this observation does not have physiological relevance, since lauric acid is a minor component of cellular lipids (~0.3% in WAT; [27]). Conversely, both enzymes are inhibited by acyl-CoA esterified with the highly abundant fatty acid species palmitic acid and oleic acid. Since inactivation of ATGL and HSL almost completely abolishes WAT fatty acid release [20], our data suggest that LCA control the activity of the major lipolytic enzymes. It is important to note that FFA metabolism is causally linked to metabolic disease. Increased circulating FFAs, as observed in obesity, can cause FFA overload of non-adipose tissues resulting in ectopic TG accumulation which is associated with impaired metabolic functions of these tissues, insulin resistance, and inflammation. These changes are not caused by the increase of the inert TG storage pool. It is believed that elevated cellular FFA levels promote the synthesis of lipotoxic metabolites such as ceramides, acyl-CoAs, and diacylglycerol [28–30]. FFA overload may result from increased lipolysis and impaired β-oxidation [31] and both processes can elevate cellular acyl-CoA concentrations. Notably, acyl-CoA concentrations are increased in tissues of insulin resistant subjects and the correlation between muscle acyl-CoA content and insulin resistance is stronger than that between muscle TG stores

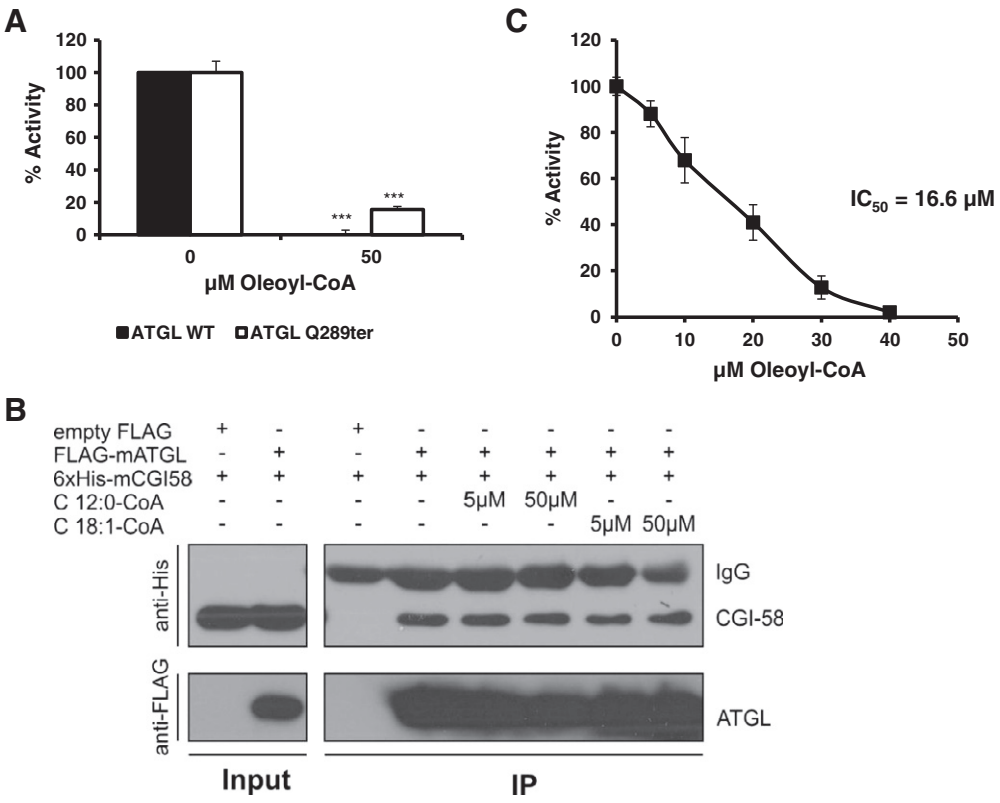

Fig. 3. Oleoyl-CoA directly interacts with the N-terminal domain of ATGL and does not affect the protein interaction of ATGL and CGI-58. (A) Oleoyl-CoA-mediated inhibiton of wild-type ATGL and the truncated Q289ter mutant without addition of CGI-58. (B) Effect of C12-CoA and C18:1-CoA on the protein–protein interaction of ATGL and CGI-58. Cos-7 cells were co-transfected with His-tagged CGI-58 and FLAG-tagged ATGL. After incubation with the lysates, FLAG-beads were incubated for 20 min with indicated concentrations of acyl-CoAs at 37 °C. After extensive washing, proteins were eluted by boiling in SDS-containing sample puffer and subjected to Western blot analysis. (C) Oleoyl-CoA-mediated inhibiton of Strep-tagged ATGL and CGI-58 expressed in *E. coli*. The specific activity of these lysates ranged from 100 to 400 nmol/h·mg depending on the expression levels of recombinant proteins. Activity data are presented as mean ± S.D. from triplicate determinations and representative for three independent experiments.

and insulin resistance [32]. Under such conditions, acyl-CoA-mediated inhibition of lipolysis could represent a principle feedback mechanism reducing FFA concentrations and promoting the storage of inert TG.

It is interesting to note that the increase in circulating FFA levels in obese patients is modest in comparison to the enormous expansion of WAT [33]. This suggests that WAT maintains its ability to control lipolysis despite hypertrophy and hyperplasia. Actually, FFA release per kilogram fat mass is reduced in obesity [33] and elevated plasma FFA levels may result from increased adipose mass. Acyl-CoA-mediated inhibition of lipolysis may be one mechanism preventing TG degradation in obese subjects and this could be specifically important in the insulin resistant state, since insulin is considered as the major suppressor of lipolysis [34].

Cellular LCA concentrations have been reported to be in the range of 5–160 µM and strongly depend on the metabolic state and tissue-type [35]. Accordingly, the IC_{50} values determined for ATGL inhibition are clearly within the physiological range. However, it has to be considered that LCA are primarily bound to acyl-CoA binding protein (ACBP) acting as acyl-CoA transporter [36]. Additionally, liver fatty acid binding protein can bind LCA with high affinity [37]. Because of the high cellular concentrations of LCA binding proteins, it is assumed that LCAs are present in their free form in very small amounts [36]. Currently, it is unclear how ACBP-bound LCA affect ATGL activity. Yet, it has been shown that ACBP-bound LCA are available for metabolic or regulatory processes such as beta-oxidation, synthesis of lipids, and signal transduction [35]. Furthermore, addition of ACBP promotes the inhibitory effect of palmitoyl-CoA on partially purified HSL [25]. It was also shown that LCA stimulate non-HSL lipase activity in pancreatic islets and this stimulatory effect was blocked by the addition of ACBP [25]. Thus, we assume that free and ACBP-bound LCAs can affect ATGL activity. Obviously, extensive studies are required to determine the role of ACBPs in lipolysis. In this respect, it is interesting to note that ACBP-deficient mice show a complex metabolic phenotype. These mice go through a crisis with overall weakness at weaning [38] indicating that ACBP is important for metabolic adaption which might also include regulation of lipolysis.

The mechanism of LCA-mediated ATGL inhibition appears to be independent of CGI-58, since inactivation of the enzyme was also observed in the absence of its co-activator protein and acyl-CoA did not disrupt the protein interaction of ATGL and CGI-58. LCAs were able to inhibit full-length ATGL and the truncated variant Q289ter lacking the C-terminal domain indicating that they bind to the N-terminal patatin-like phospholipase domain (PNPLA). Furthermore, ATGL was sensitive to LCA inhibition in a heterologous expression system excluding an important contribution of other co-factors. Notably, several PNPLA proteins have been shown to possess acyl-CoA dependent acyltransferase activity such as adiponutrin [39], GS2 [40], and yeast lipases Tgl3p, Tgl4p, and Tgl5p [41,42]. To our knowledge ATGL does not exhibit this activity, but it is reasonable to assume that ATGL has a yet uncharacterized conserved LCA-binding motif with regulatory function.

5. Conclusion

LCAs can directly inhibit ATGL in a non-competitive manner. Considering that LCAs also inhibit HSL, LCA-mediated inhibition of lipolytic enzymes could represent an effective cellular mechanism controlling lipolysis. Inhibition of lipases could be important in adipose and non-adipose tissues, avoid FFA-mediated lipotoxicity, and promote TG

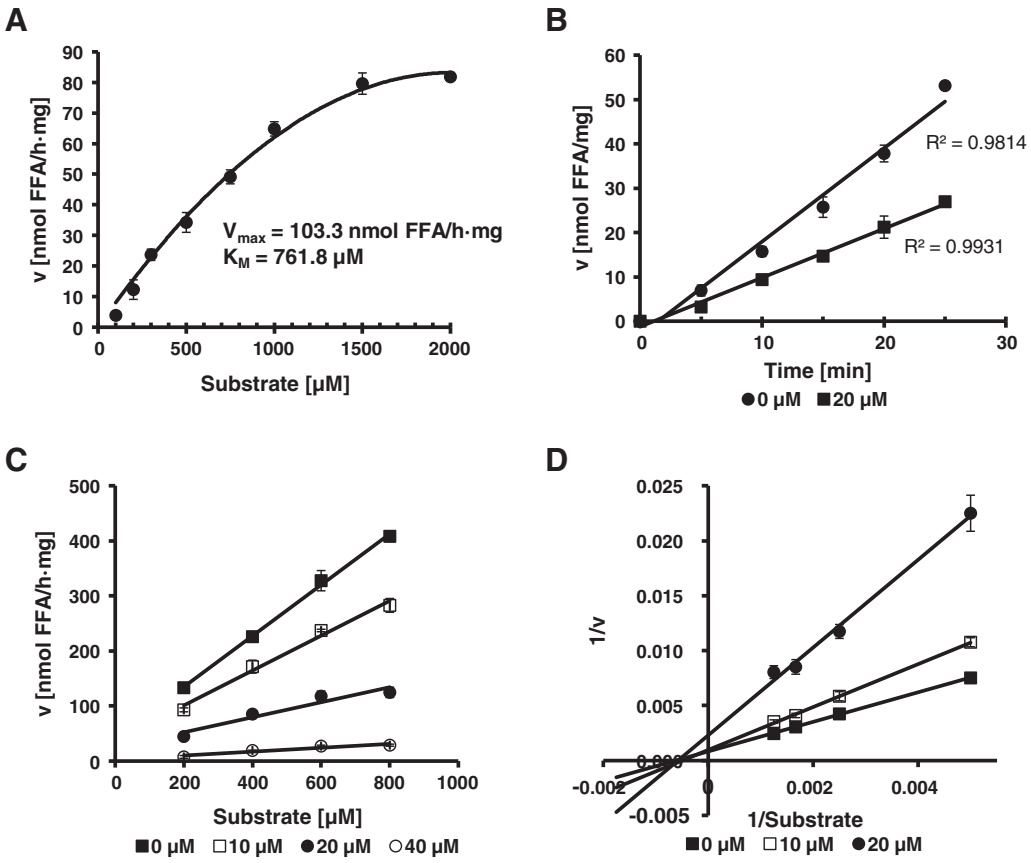

Fig. 4. Oleoyl-CoA inhibits ATGL in a non-competitive manner. TG hydrolase activity of ATGL was determined in lysates of *E. coli* overexpressing Strep-tagged ATGL and Strep-tagged CGI-58. (A) Substrate saturation. (B) Time-dependent release of fatty acids in the absence and presence of oleoyl-CoA. (C) Inhibition kinetics raw data. Inhibition kinetics assays where performed in a concentration range of 200 to 800 μM substrate and the indicated concentrations of oleoyl-CoA. (D) Lineweaver–Burk blot of the data shown in (C) indicating a non-competitive inhibition mechanism. Data are presented as mean ± S.D. from triplicate determinations and are representative for two independent experiments.

accumulation when tissues are chronically exposed to high FFA levels as frequently observed in obesity.

Abbreviations

ACBP	acyl-CoA binding protein
ATGL	adipose triglyceride lipase
ATGL-ko	ATGL-deficient
CGI-58	comparative gene identification-58
DG	diacylglycerol
DO	diolein
FFA	free fatty acid
HSL	hormone-sensitive lipase
HSL-ko	HSL-deficient
LCA	long-chain acyl-CoA
MGL	monoglyceride lipase
MO	monoolein
OA	oleic acid
PNPLA	patatin-like phospholipase domain containing protein
TG	triglyceride
WAT	white adipose tissue

Acknowledgments

This work was supported by the doctoral program Molecular Enzymology (Ro. Zi., M. O., Ru. Ze.), the SFB Lipotox (M. O., Ru. Ze.), and project P22170 (M. O.) funded by the Austrian Science Fund (FWF), and GOLD, Genomics of Lipid-Associated Disorders (M. O., Ro. Zi, Ru. Ze.), as part of the Austrian Genome Project GEN-AU funded by the Forschungsförderungsgesellschaft und Bundesministerium für Wissenschaft und Forschung.

References

[1] J.A. Villena, S. Roy, E. Sarkadi-Nagy, K.H. Kim, H.S. Sul, 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. 279 (2004) 47066–47075.
[2] R. Zimmermann, J.G. Strauss, G. Haemmerle, G. Schoiswohl, R. Birner-Gruenberger, M. Riederer, A. Lass, G. Neuberger, F. Eisenhaber, A. Hermetter, R. Zechner, Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase, Science 306 (2004) 1383–1386.
[3] G. Haemmerle, A. Lass, R. Zimmermann, G. Gorkiewicz, C. Meyer, J. Rozman, G. Heldmaier, R. Maier, C. Theussl, S. Eder, D. Kratky, E.F. Wagner, M. Klingenspor, G. Hoefler, R. Zechner, Defective lipolysis and altered energy metabolism in mice lacking adipose triglyceride lipase, Science 312 (2006) 734–737.
[4] G. Schoiswohl, M. Schweiger, R. Schreiber, G. Gorkiewicz, K. Preiss-Landl, U. Taschler, K.A. Zierler, F.P. Radner, T.O. Eichmann, P.C. Kienesberger, S. Eder, A. Lass, G. Haemmerle, T.J. Alsted, B. Kiens, G. Hoefler, R. Zechner, R. Zimmermann, Adipose triglyceride lipase plays a key role in the supply of the working muscle with fatty acids. J. Lipid Res. 51, 2010, 490–499.
[5] A. Lass, R. Zimmermann, G. Haemmerle, M. Riederer, G. Schoiswohl, M. Schweiger, P. Kienesberger, J.G. Strauss, G. Gorkiewicz, R. Zechner, Adipose triglyceride lipase-mediated lipolysis of cellular fat stores is activated by CGI-58 and defective in Chanarin–Dorfman Syndrome, Cell Metab. 3 (2006) 309–319.
[6] M. Oberer, A. Boeszoermenyi, H.M. Nagy, R. Zechner, Recent insights into the structure and function of comparative gene identification-58, Curr. Opin. Lipidol. 22 (2011) 149–158.
[7] J. Fischer, C. Lefevre, E. Morava, J.M. Mussini, P. Laforet, A. Negre-Salvayre, M. Lathrop, R. Salvayre, The gene encoding adipose triglyceride lipase (PNPLA2) is mutated in neutral lipid storage disease with myopathy, Nat. Genet. 39 (2007) 28–30.

[8] A.D. Cristillo, S.P. Heximer, L. Russell, D.R. Forsdyke, Cyclosporin A inhibits early mRNA expression of G0/G1 switch gene 2 (G0S2) in cultured human blood mononuclear cells, DNA Cell Biol. 16 (1997) 1449–1458.
[9] X. Yang, X. Lu, M. Lombes, G.B. Rha, Y.I. Chi, T.M. Guerin, E.J. Smart, J. Liu, The G(0)/G(1) switch gene 2 regulates adipose lipolysis through association with adipose triglyceride lipase, Cell Metab. 11 (2010) 194–205.
[10] M. Schweiger, M. Paar, C. Eder, J. Brandis, E. Moser, G. Gorkiewicz, S. Grond, F.P. Radner, I. Cerk, I. Cornaciu, M. Oberer, S. Kersten, R. Zechner, R. Zimmermann, A. Lass, G0/G1 switch gene-2 regulates human adipocyte lipolysis by affecting activity and localization of adipose triglyceride lipase, J. Lipid Res. 53 (2012) 2307–2317.
[11] X. Yang, X. Zhang, B.L. Heckmann, X. Lu, J. Liu, Relative contribution of adipose triglyceride lipase and hormone-sensitive lipase to tumor necrosis factor-alpha (TNF-alpha)-induced lipolysis in adipocytes, J. Biol. Chem. 286 (2011) 40477–40485.
[12] J.G. Granneman, H.P. Moore, R. Krishnamoorthy, M. Rathod, Perilipin controls lipolysis by regulating the interactions of AB-hydrolase containing 5 (Abhd5) and adipose triglyceride lipase (Atgl), J. Biol. Chem. 284 (2009) 34538–34544.
[13] L.L. Listenberger, A.G. Ostermeyer-Fay, E.B. Goldberg, W.J. Brown, D.A. Brown, Adipocyte differentiation-related protein reduces the lipid droplet association of adipose triglyceride lipase and slows triacylglycerol turnover, J. Lipid Res. 48 (2007) 2751–2761.
[14] H. Wang, M. Bell, U. Sreenivasan, H. Hu, J. Liu, K. Dalen, C. Londos, T. Yamaguchi, M.A. Rizzo, R. Coleman, D. Gong, D. Brasaemle, C. Sztalryd, Unique regulation of adipose triglyceride lipase (ATGL) by perilipin 5, a lipid droplet-associated protein, J. Biol. Chem. 286 (2011) 15707–15715.
[15] J.G. Granneman, H.P. Moore, E.P. Mottillo, Z. Zhu, L. Zhou, Interactions of perilipin-5 (Plin5) with adipose triglyceride lipase, J. Biol. Chem. 286 (2011) 5126–5135.
[16] N.M. Pollak, M. Schweiger, D. Jaeger, D. Kolb, M. Kumari, R. Schreiber, S. Kolleritsch, P. Markolin, G.F. Grabner, C. Heier, K.A. Zierler, T. Rulicke, R. Zimmermann, A. Lass, R. Zechner, G. Haemmerle, Cardiac-specific overexpression of perilipin 5 provokes severe cardiac steatosis via the formation of a lipolytic barrier, J. Lipid Res. 54 (2013) 1092–1102.
[17] H. Wang, U. Sreenivasan, D.W. Gong, K.A. O'Connell, E.R. Dabkowski, P.A. Hecker, N. Ionica, M. Konig, A. Mahurkar, Y. Sun, W.C. Stanley, C. Sztalryd, Cardiomyocyte-specific perilipin 5 overexpression leads to myocardial steatosis and modest cardiac dysfunction, J. Lipid Res. 54 (2013) 953–965.
[18] E.W. Richards, M.W. Hamm, J.E. Fletcher, D.A. Otto, The binding of palmitoyl-CoA to bovine serum albumin, Biochim. Biophys. Acta 1044 (1990) 361–367.
[19] D.L. Severson, B. Hurley, Inhibition of the hormone-sensitive lipase in adipose tissue by long-chain fatty acyl coenzyme A, Lipids 19 (1984) 134–138.
[20] M. Schweiger, R. Schreiber, G. Haemmerle, A. Lass, C. Fledelius, P. Jacobsen, H. Tornqvist, R. Zechner, R. Zimmermann, Adipose triglyceride lipase and hormone-sensitive lipase are the major enzymes in adipose tissue triacylglycerol catabolism, J. Biol. Chem. 281 (2006) 40236–40241.
[21] M. Schweiger, G. Schoiswohl, A. Lass, F.P. Radner, G. Haemmerle, R. Malli, W. Graier, I. Cornaciu, M. Oberer, R. Salvayre, J. Fischer, R. Zechner, R. Zimmermann, The C-terminal region of human adipose triglyceride lipase affects enzyme activity and lipid droplet binding, J. Biol. Chem. 283 (2008) 17211–17220.
[22] I. Cornaciu, A. Boeszoermenyi, H. Lindermuth, H.M. Nagy, I.K. Cerk, C. Ebner, B. Salzburger, A. Gruber, M. Schweiger, R. Zechner, A. Lass, R. Zimmermann, M. Oberer, The minimal domain of adipose triglyceride lipase (ATGL) ranges until leucine 254 and can be activated and inhibited by CGI-58 and G0S2, respectively, PLoS One 6 (2011) e26349.
[23] N.J. Faergeman, J. Knudsen, Role of long-chain fatty acyl-CoA esters in the regulation of metabolism and in cell signalling, Biochem. J. 323 (Pt 1) (1997) 1–12.
[24] K.H. McDonough, J.R. Neely, Inhibition of myocardial lipase by palmityl CoA, J. Mol. Cell. Cardiol. 20 (Suppl. 2) (1988) 31–39.
[25] L. Hu, J.T. Deeney, C.J. Nolan, M.L. Peyot, A. Ao, A.M. Richard, E. Luc, N.J. Faergeman, J. Knudsen, W. Guo, M. Sorhede-Winzell, M. Prentki, B.E. Corkey, Regulation of lipolytic activity by long-chain acyl-coenzyme A in islets and adipocytes, Am. J. Physiol. Endocrinol. Metab. 289 (2005) E1085–E1092.
[26] C.A. Jepson, S.J. Yeaman, Inhibition of hormone-sensitive lipase by intermediary lipid metabolites, FEBS Lett. 310 (1992) 197–200.
[27] M. Kunesova, P. Hlavaty, E. Tvrzicka, B. Stankova, P. Kalouskova, N. Viguerie, T.M. Larsen, M.A. van Baak, S.A. Jebb, J.A. Martinez, A.F. Pfeiffer, A. Kafatos, T. Handjieva-Darlenska, M. Hill, D. Langin, A. Zak, A. Astrup, W.H. Saris, Fatty acid composition of adipose tissue triglycerides after weight loss and weight maintenance: the DIOGENES study, Physiol. Res. 61 (2012) 597–607.
[28] J.E. Schaffer, Lipotoxicity: when tissues overeat, Curr. Opin. Lipidol. 14 (2003) 281–287.
[29] L. Liu, X. Shi, K.G. Bharadwaj, S. Ikeda, H. Yamashita, H. Yagyu, J.E. Schaffer, Y.H. Yu, I. J. Goldberg, DGAT1 expression increases heart triglyceride content but ameliorates lipotoxicity, J. Biol. Chem. 284 (2009) 36312–36323.
[30] L.O. Li, E.L. Klett, R.A. Coleman, Acyl-CoA synthesis, lipid metabolism and lipotoxicity, Biochim. Biophys. Acta 1801 (2010) 246–251.
[31] D.E. Kelley, Skeletal muscle fat oxidation: timing and flexibility are everything, J. Clin. Invest. 115 (2005) 1699–1702.
[32] B.A. Ellis, A. Poynten, A.J. Lowy, S.M. Furler, D.J. Chisholm, E.W. Kraegen, G.J. Cooney, Long-chain acyl-CoA esters as indicators of lipid metabolism and insulin sensitivity in rat and human muscle, Am. J. Physiol. Endocrinol. Metab. 279 (2000) E554–E560.
[33] F. Karpe, J.R. Dickmann, K.N. Frayn, Fatty acids, obesity, and insulin resistance: time for a reevaluation, Diabetes 60 (2011) 2441–2449.
[34] P. Chakrabarti, K.V. Kandror, Adipose triglyceride lipase: a new target in the regulation of lipolysis by insulin, Curr. Diabetes Rev. 7 (2011) 270–277.
[35] J. Knudsen, T.B. Neergaard, B. Gaigg, M.V. Jensen, J.K. Hansen, Role of acyl-CoA binding protein in acyl-CoA metabolism and acyl-CoA-mediated cell signaling, J. Nutr. 130 (2000) 294S–298S.
[36] B.B. Kragelund, J. Knudsen, F.M. Poulsen, Acyl-coenzyme A binding protein (ACBP), Biochim. Biophys. Acta 1441 (1999) 150–161.
[37] B. Rolf, E. Oudenampsen-Kruger, T. Borchers, N.J. Faergeman, J. Knudsen, A. Lezius, F. Spener, Analysis of the ligand binding properties of recombinant bovine liver-type fatty acid binding protein, Biochim. Biophys. Acta 1259 (1995) 245–253.
[38] D. Neess, M. Bloksgaard, S. Bek, A.B. Marcher, I.C. Elle, T. Helledie, M. Due, V. Pagmantidis, B. Finsen, J. Wilbertz, M. Kruhoffer, N. Faergeman, S. Mandrup, Disruption of the acyl-CoA-binding protein gene delays hepatic adaptation to metabolic changes at weaning, J. Biol. Chem. 286 (2011) 3460–3472.
[39] M. Kumari, G. Schoiswohl, C. Chitraju, M. Paar, I. Cornaciu, A.Y. Rangrez, N. Wongsiriroj, H.M. Nagy, P.T. Ivanova, S.A. Scott, O. Knittelfelder, G.N. Rechberger, R. Birner-Gruenberger, S. Eder, H.A. Brown, G. Haemmerle, M. Oberer, A. Lass, E.E. Kershaw, R. Zimmermann, R. Zechner, Adiponutrin functions as a nutritionally regulated lysophosphatidic acid acyltransferase, Cell Metab. 15 (2012) 691–702.
[40] J. Gao, M. Simon, Identification of a novel keratinocyte retinyl ester hydrolase as a transacylase and lipase, J. Invest. Dermatol. 124 (2005) 1259–1266.
[41] S. Rajakumari, G. Daum, Multiple functions as lipase, steryl ester hydrolase, phospholipase, and acyltransferase of Tgl4p from the yeast Saccharomyces cerevisiae, J. Biol. Chem. 285 (2010) 15769–15776.
[42] S. Rajakumari, G. Daum, Janus-faced enzymes yeast Tgl3p and Tgl5p catalyze lipase and acyltransferase reactions, Mol. Biol. Cell 21 (2010) 501–510.