Pharmacological agents targeting autophagy and their effects on lipolysis in human adipocytes

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**Abstract**

Adipose tissue of metabolically compromised humans with obesity is often characterized by impaired regulation of autophagy pathway. However, data on the role of autophagy in human adipocyte lipid catabolism is scarce. Therefore, we investigated the effect of pharmacological agents (including 3-methyladenine (3MA), bafilomycin A1 (BAF), chloroquine (CQ) and lalistat-2 (L-stat), that target different stages of the autophagy pathway on lipid hydrolysis in differentiated human multipotent adipose-derived stem cells (hMADs). Glycerol and fatty acid release were measured as marker of lipid hydrolysis following starvation and \( \beta \)-adrenergic stimulation. Microtubule-associated protein light chain 3 ratio (LC3II/LC3I) and HSL phosphorylation (pHSL) were analyzed by Western blot. Our data indicate that pharmacological inhibition of the autophagy pathway reduced lipid hydrolysis in human adipocytes, although to a limited extent (10-15%). However, further research is needed to reveal the exact mechanism of action of these pharmacological agents and their interplay with cytosolic lipid breakdown in human adipocytes.

### 1. Introduction

Human obesity is associated with intrinsic impairments of adipocyte cytosolic lipolysis, which might affect adipose tissue (AT) lipid buffering capacity and promote lipid overflow and ectopic lipid accumulation (Arner and Langin, 2014). Recent studies indicate that autophagy, an intracellular self-digestion system, which is essential for cell homeostasis and intracellular recycling, may be involved in selective lysosomal lipid hydrolysis, at least in murine adipocytes and hepatocytes, a process called “lipophagy” (Zhang et al., 2018). For instance, rodent studies revealed that autophagy is largely involved in adipocyte differentiation since adipogenesis and lipid accumulation was hampered following genetic and pharmacological inhibition in mice (Singh et al., 2009; Baerga et al., 2009; Heckmann et al., 2013; Singh and Cuervo, 2012; Zhang et al., 2009). Moreover, autophagic clearance in the white adipose tissue (WAT) is likely to be impaired in high fat diet-induced obese mice and genetically obese mice (Mizuno et al., 2017; Nunez et al., 2013; Jansen et al., 2012). In addition, in murine 3T3-L1 cells, autophagy was found to take part in lipid hydrolysis via a key regulator in endo-lysosomal trafficking, Ras-related protein Rab-7a, aside from cytosolic lipase (Lizaso et al., 2013). In recent years, autophagy has been found to be involved in human AT metabolism (Jansen et al., 2012; Clemente-Postigo et al., 2020). However, the exact mechanism of how autophagy involves in human AT is still unclear.

Dysregulated AT autophagy has been associated with human metabolic disorders like obesity and type 2 diabetes (Stienstra et al., 2014; Kosacka et al., 2015; Soussi et al., 2015; Haim et al., 2015; Xu et al., 2018; Kovsan et al., 2011). Cross-sectional data showed that mRNA and protein levels of autophagy genes (ATGs) are increased in WAT of overweight and obese subjects, which was accompanied by increased AT apoptosis and inflammation (Jansen et al., 2012; Kosacka et al., 2015; Kovsan et al., 2011; Rodríguez et al., 2012). On the other hand, isolated mature adipocytes from obese subjects have been shown to display a severely reduced autophagic flux (Soussi et al., 2015), indicating that the observed increase in AT autophagy gene expression is mainly related to non-adipocyte cells. Furthermore, we recently showed that mRNA expression of ATG7, essential in autophagosome formation, correlated negatively with the classic cytosolic lipases HSL and ATGL in WAT of lean and obese subjects (Xu et al., 2018). In addition, we showed that in differentiated human adipocytes the autophagic flux was increased...
2. Methods

2.1. Cell culture and experiments

Human AT-derived mesenchymal stem cells (hMADs) (Jocken et al., 2015) were cultured to 80% confluence and differentiated for 7 days using differentiation medium (Dulbecco’s Modified Eagle Medium DMEM/F-12 (Life Technologies, 17.5 mM glucose) supplemented with D-Pantothenate 17 μM, Insulin 0.1 μM, Dexamethasone 1 μM, IBMX 250 μM, Rosiglitazone 5 μM and PBS 5%). Thereafter, we replaced the differentiation medium for the control medium (D-Pantothenate 17 μM, Insulin 0.1 μM) and refreshed it every 2 days until day 14 (Jocken et al., 2015). Next, fully differentiated adipocytes were serum-starved and treated for 24 h with or without 3MA (Invivogen, 5 mM), BAFTT (Invivogen, 100 nM), CQ (sigma, 1 μM), or L-stat (10 μM, kind gift from Prof. Paul Helquist, Department of Chemistry & Biochemistry, University of Notre Dame, Notre Dame, Indiana U.S.A.). After 24 h treatment, the medium was collected and cells were incubated for 4 h with or without Isoprenaline (ISO, 1 μM; Stichting Apotheek der Haarlemse Ziekenhuizen, The Netherlands) to trigger β-adrenoceptor mediated lipolysis. For time and dose experiments (Figs. S2–S5), cells were treated with various doses of each blocker in serum deprived medium with or without ISO. For time experiments, cells were treated with specific doses of each blocker in serum deprived medium and incubated for up to 24 h.

2.2. Lipolysis measurement

As a measure for complete lipid hydrolysis (lipolysis), the amount of glycerol (μg/ml) was determined in the collected medium samples using the Glycerol Assay Kit (Sigma; #MAK117) according to the manufacturer’s instructions. In addition, free fatty acid (μg/ml) concentrations were determined in the collected medium samples using the Fatty Acid Assay Kit (Abnova; #KA1667) according to the manufacturer’s instructions.

2.3. Cell lysis and Western blotting

After specified treatments, cells were washed twice with ice-cold PBS (pH 7.5) and then lysed in 1 x RIPA Lysis buffer supplemented with protease/phosphatase inhibitor (Cell Signaling; #5872S). Lysis buffer was added on cells and collapsing by pipette tips was followed. Solution was collected in small tubes for every sample. BCA protein assay was used to measure the protein concentration of every sample. For Western blotting, 15 μg protein was subjected to SDS-PAGE and proteins were transferred to a nitrocellulose membrane (CriterionTM Gel system, BioRad). The membrane for detection of microtubule-associated protein 1A/1B-light chain 3 (LC3) was blocked with 5% non-fat milk for 1 h at room temperature, followed by incubation overnight with primary antibody against LC3 (1:1000 in 5% non-fat milk PBS 0.1%; Cell Signaling; #12741). The membrane for detecting phosphorylated HSL (pHSL) and total HSL was blocked with 5% BSA for 1 h at room temperature, followed by overnight incubation with primary antibody against pHSL (Cell Signaling; #4126), or total HSL (Cell Signaling; #4107). After overnight incubation, the blots were washed and incubated with secondary horseradish peroxidase-conjugated swine anti-rabbit antibody (Dako-2600 Glostrup, Denmark) at a dilution of 1:5000 for 1 h at room temperature. As a loading control, membranes were stained with Ponceau S and total protein was quantified.

2.4. Cell fractionation and immunoblotting

To investigate intracellular location and translocation of pHSL and PLIN1, cells were lysed in PBS containing protease/phosphatase inhibitor and the lysate was collected and vortexed five times for 30 s and kept on ice. The lysate was then centrifuged at 14000 rpm for 10 min. The supernatant and fat cake (top layer) were collected and after protein isolation as described above 15 μg protein was subjected to SDS-PAGE and proteins were transferred to a nitrocellulose membrane (Criterion Gel system, BioRad). The membrane for PLIN1 was blocked with 5% BSA in PBST for 1 h at room temperature, followed by incubation overnight with primary antibody against PLIN1 (Cell Signaling; #9349) 1:1000 in 5% BSA. After overnight incubation, the blot was incubated with secondary horseradish peroxidase-conjugated swine anti-rabbit antibody (Dako-2600 Glostrup, Denmark) at a dilution of 1:10000 for 1 h at room temperature and subsequently developed with chemiluminescence solution (ECL famto) according to the manufacturer’s instructions (Thermo Scientific; 34095S). Analysis of pHSL was performed as described above. As a control for fractionation, blots were incubated overnight with an antibody against GAPDH (Cell Signaling; #2118), 1:1000 in 5% non-fat milk, followed by incubation with secondary horseradish peroxidase-conjugated swine anti-rabbit antibody (Dako-2600 Glostrup, Denmark) at a dilution of 1:10000 for 1 h at room temperature. All membranes were subsequently developed with enhanced chemiluminescence solution (ECL famto) (Thermo Scientific 34095S) according to the manufacturer’s instructions.
2.5. Immunoprecipitation

To investigate PLIN1 phosphorylation cells were lysed in RIPA buffer. Subsequently, anti-phosphoserine antibody (Invitrogen; 61-8300) was added and rotated overnight at 4 °C. Next, protein G coated Sepharose beads (GE healthcare) were washed 3 times with PBS and then 50 μl was added to each tube and left to rotate for 4 h at 4 °C. After incubation, beads were washed with RIPA buffer to remove the non-specific binding, and 5 μl of Laemmli Sample Buffer (Bio-Rad; #1610747) was added to each tube, and boiled for 5min. After centrifugation, the supernatant was collected and subjected to Western blot analysis as described before.

2.6. Data analysis

Results are presented as mean ± SEM for a minimum of 3 independent experiments. Statistical significance was defined as p < 0.05 and analysis were using GraphPad Prism 7.0 software for Windows (GraphPad Prism, California, USA). Graphs were prepared using GraphPad Prism 7.0 software (GraphPad Prism, California, USA).

3. Results

3.1. Effect of pharmacological targets for autophagy on starvation-induced and β-adrenoceptor mediated lipolysis

To investigate whether pharmacological agents that target the autophagic machinery can affect starvation-induced lipolysis in human adipocytes, we incubated differentiated hMADs for 24 h with 4 different agents (3MA, BAF, CQ or L-stat) in serum-free medium. After 24 h serum starvation, differentiated hMADs treated with acute (4 h) basal and β-adrenoceptor mediated lipolysis using the non-selective β-adrenoceptor agonist ISO (1 μM). 3MA (5 mM) increased basal (p = 0.04) and β-adrenoceptor mediated glycerol (p = 0.01) and fatty acid release by 2-3 fold compared to control-treated cells. CQ (1 μM) had no effect on basal or stimulated lipolysis (Fig. 1A). Furthermore, BAF (100 nM) decreased basal (p = 0.01) and β-adrenoceptor mediated glycerol release (p = 0.04) by almost 15% (Fig. 1A). But no effect was observed on fatty acid release (Fig. S1). L-stat (10 μM) decreased basal and β-adrenoceptor mediated glycerol (p = 0.002, Fig. 1A) and fatty acid (p < 0.05, Fig. S1) release by almost 50% following 4 h incubation.

3.2. Effect of 3MA on cytosolic lipolysis and autophagic flux

To investigate whether the observed effects following treatment with the pharmacological agents that target the autophagic machinery were independent of changes in cytosolic lipolysis, we checked the degree of HSL phosphorylation by Western blotting. As shown in Fig. 1B, an elevated pHSL660 was found in adipocytes treated with 3MA, and in adipocytes treated with 3MA + ISO compared to ISO-treated control cells, indicating that the 3MA-induced glycerol release is partly explained by the increased HSL phosphorylation and thereby increased cytosolic lipolysis. To ensure that the pronounced lipolytic effect of 3MA was solely due to activation of cytosolic lipolysis and not to changes in the autophagic flux, we evaluated the LC3II/LC3I. As shown in Figure 1B, 3MA did not change the LC3II/LC3I, indicating that the autophagic flux was not affected by 3MA treatment. Together these results indicate that 3MA is a potent stimulator of cytosolic lipolysis, via induction of HSL phosphorylation. Interestingly, in the 3MA dose and time experiment, all tested concentrations of 3MA (1 mM, 5 mM and 20 μM) showed a significant increase in basal glycerol release compared to control-treated cells. Fig. 1. (A). Summary of 7 independent experiment. Relative glycerol release in medium of serum starved adipocytes pre-treated with different autophagy inhibitors for 24 h and following incubation with and without ISO for 4 h. Glycerol release is expressed relative to baseline control treated cells and data are presented as mean ± SEM. * indicate the significant difference with Baseline control, *p < 0.05, **p < 0.01 and # indicate the significant difference with Isoprenaline control, *p < 0.05, **p < 0.01, paired t-test. (B). Representative Western blots for pHSL660, total HSL, LC3 in differentiated human adipocytes treated with or without autophagy inhibitors and Ponceau S as loading control.
mM) significantly increased glycerol release at 4 h incubation (Fig. S2A). And with 5 mM, 3MA consistently increased glycerol release from 1 h up to 24 h incubation (Fig. S2B), indicating that 3MA’s effect on increasing cytosolic lipolysis is independent of time and dose.

3.3. Effect of CQ on cytosolic lipolysis and autophagic flux

In line with our fatty acid and glycerol data, no effect was observed following CQ (1 μM) treatment on pHSL660 content (Fig. 1B). However, CQ elevated LC3II/LC3I compared to non-treated cells both at starvation- and ISO-stimulated conditions, indicating that the autophagic flux was blocked. Moreover, CQ dose (0–25 μM) and time experiment (1 μM, 0–24 h) showed no consistent effect on glycerol release (Figs. S3A and B).

3.4. Effect of BAF on cytosolic lipolysis and autophagic flux

Treatment with BAF (100 nM) had no effect on pHSL660 content (Fig. 1B) and induced a modest elevation of the LC3II/LC3I ratio compared to non-treated cells both at starvation- and ISO-stimulated conditions (Fig. 1B). Of note, a clear elevated LC3II/LC3I ratio was observed for adipocytes treated with BAF for up to 7 h (Fig. S4C).
together, these data indicate that the observed reduction in starvation- and ISO-induced lipolysis (10–15%) following BAF treatment (100 nM) is partly mediated by autophagy inhibition and independent of changes in cytosolic lipolysis measured by HSL-phosphorylation.

3.5. Effect of L-stat on cytosolic lipolysis and autophagic flux

As indicated above, L-stat treatment of differentiated hMADs induced a pronounced reduction (50%) of glycerol and fatty acid release both at basal and ISO-stimulated conditions (Fig. 1A). Dose or time experiments showed that L-stat significantly reduced basal and β-adrenoceptor mediated lipolysis only at 10 μM compared to non-treated cells, independent of time from 4 h (p = 0.001 and p = 0.016, Figs. S5A and B). To investigate whether this effect was dependent on changes in cytosolic lipolysis or autophagic flux, we performed Western blotting analysis. Surprisingly, and in contrast to the observed blunted glycerol release, adipocytes treated with L-stat showed an increased pHSL660 content both under basal and ISO-stimulated conditions, indicating increased activation of cytosolic lipolysis (Fig. 2A). No significant changes on LC3II/LC3I were observed following L-stat treatment, indicating that the autophagic flux was unaffected (Fig. 2A). Despite the increase of pHSL, L-stat significantly decreased glycerol release suggesting a blockade in the cytosolic lipolytic cascade. Since the interaction of pHSL with the lipid droplet requires the phosphorylation and translocation of PLIN1 from the droplet membrane to the cytosol, we separated the membrane from the cytosolic fraction to see if PLIN1 translocated from fat droplet to the cytosol following L-stat treatment. As shown in Fig. 2B, no measurable change in PLIN1 translocation was observed following L-stat treatment. Since translocation first requires PLIN1 to be phosphorylated, immunoprecipitation was performed after the L-stat treatment with a phosphoserine antibody. Subsequent Western blotting of PLIN1 showed that L-stat treatment did not induce PLIN1 phosphorylation (Fig. 2C). Together, these results indicate that L-stat increases pHSL but prevents phosphorylation and translocation of PLIN1, suggesting that the increased amount of pHSL is unable to access the lipid droplet for subsequent TAG hydrolysis, which partly explains the observed blunted glycerol and fatty acid release.

4. Discussion

Autophagy serves as a catabolic mechanism to recycle damaged or unwanted cellular components to produce substrates for energy production in times of extra energy need (e.g. starvation) (Kim and Lee, 2014). There is evidence for a role of autophagy in lipid catabolism (also called lipolysis) in murine hepatocytes and adipocytes, and there is an indication that the autophagy pathways are altered in metabolically compromised states, contributing to impaired lipolysis and AT dysfunction, but human data are scarce (Singh and Cuervo, 2012). Of interest, recent evidence indicates that alterations in the autophagy pathway in metabolically compromised individuals might contribute to impaired lipolysis and AT dysfunction, but human data are scarce (Singh and Cuervo, 2012). The present study investigated the effects of different pharmacological agents that target the autophagic machinery on human adipocyte lipid hydrolysis. 3MA, often II PI3K-stimulated cytosolic lipolysis but had no effect on the autophagic flux. On the other hand, the V-ATPase inhibitor BAF blocked the autophagic flux, which was accompanied by a decreased glycerol release (10–15%). On the other hand, CQ (0–25 μM) that blocks the fusion of autophagosomes with lysosome and lysosomal protein showed no effect on lipid hydrolysis in human adipocytes. Finally, the lysosomal lipase inhibitor L-stat (10 μM) decreased glycerol release, possibly by interfering with cytosolic lipolysis via impaired serine phosphorylation and translocation of HSL and PLIN1 to and from the lipid droplet membrane.

3MA is an inhibitor of class III phosphatidinositol 3-kinase and is frequently used to inhibit the formation of autophagosome precursors at the early stage of autophagy (Wu et al., 2010; Galluzzi et al., 2009). In the present study 3MA (5 mM) increased glycerol and fatty acid release, and HSL-phosphorylation in human adipocytes, while not affecting the autophagic flux (i.e., LC3II/LC3I) following starvation- and ISO-stimulated conditions. In contrast, a similar study in murine 3T3-L1 cells showed that glycerol release was significantly decreased following 3MA treatment (at concentration 10 μM for 4 h), which was accompanied by a reduction of cAMP and no effect on pHSL (Lizaso et al., 2013). On the other hand, our observations are in line with another study in 3T3-L1 adipocytes, in which 3MA stimulated glycerol release (at which concentration) despite ATG5 knockdown (Heckmann et al., 2013), a protein specifically required in the elongation phase of autophagy. Of interest, the lipolytic effect of 3MA was disrupted only following ATGL knockdown (Heckmann et al., 2013), indicating an effect of 3MA on cytosolic lipolysis rather than on the autophagic cascade in human and murine adipocytes. Furthermore, the authors concluded that the lipolytic effect of 3MA was partly explained by PKA-mediated increase in HSL phosphorylation and an increased cAMP concentration. Although we did not investigate PKA activity here, it is tempting to assume that in human adipocytes the same mechanism is involved. In line with these data, we have also not observed any effect of 3MA treatment on autophagic flux based LC3II protein accumulation, which supports the possibility that 3MA activates HSL-dependent cytosolic lipolysis rather than attenuating autophagic flux in human adipocytes. With this, is 3MA a possible target for increasing lipolysis and a target for combating obesity.

BAF is a widely used inhibitor of V-ATPase in cells, and as such it inhibits the acidification of lysosomes and endosomes (Yamamoto et al., 1998). As early as 1998, Yamamoto et al. reported that BAF could prevent the maturation of autophagic vacuoles by inhibiting the fusion between autophagosomes and lysosomes in rat hepatoma cell line H-4-II-E (Yamamoto et al., 1998). A recent study using cells from the Drosophila fat body showed that the inhibition of autphagosome-lysosome fusion is not due to changes in acidification but might be rather mediated by the effect of BAF on the calcium pump SERCA (Mauvezin and Neufeld, 2015; Mauvezin et al., 2015). Results from our study showed that glycerol release decreased both in starvation- and β-adrenoceptor mediated adipocytes following BAF treatment, which is in line with observations in murine 3T3-L1 adipocytes (Lizaso et al., 2013). Although the fatty acid release did not show a clear effect, which might be due to the limited sample size or fatty acid re-uptake and subsequent intracellular re-esterification or metabolism. Therefore, future research should include measurements of both intra- and extra-cellular metabolite concentrations combined with markers of cell viability/cells death to get more insight on the effect of these agents on metabolite turnover rates. It has been reported that if cells are subjected to long-term starvation, both LC3I and LC3II disappear (Mizushima and Yoshimori, 2007). It was demonstrated that the effect of BAF on LC3II degradation differs according to the time of treatment in different cell types (Kliksny et al., 2008). This may explain why we observed an increased LC3II, and LC3II/LC3I ratio, together indicating a blockage of the autophagic flux, in cells treated for 7 h (short-term) with BAF but not following 24 h (long-term). In addition, no changes in HSL phosphorylation were observed following BAF treatment. As such, our findings indicate that inhibition of the autophagic flux following BAF treatment is accompanied by a blunted glycerol release, suggesting that autophagy in human adipocytes might contribute to lipid hydrolysis, although to a limited extent (10–15%), which is the maximum that we could expect. Of note, BAF is able to influence cellular Ca2+–flux (Mauvezin and Neufeld, 2015). Increasing intracellular calcium exerts an antilipolytic effect mainly by activation of phosphodiesterase (PDE), leading to a decrease in cAMP and HSL-phosphorylation and, consequently, inhibition of lipolysis (Xue et al., 2001). Therefore, it needs to be investigated whether this antilipolytic effect of BAF is partly explained by its effect on Ca2+–flux in human adipocytes (Mauvezin et al., 2015).

CQ is a clinically relevant autophagy inhibitor that is widely used as...
an anti-malarial agent and debated for its potential use in COVID-19 treatment (Yang et al., 2013; Zou et al., 2020). CQ is a lysosomotropic weak base that neutralizes intra-lysosomal acidity and hence arrests autophagy by preventing the fusion of the autophagosome with lysosome (Galluzzi et al., 2009; Mauthe et al., 2018). As BAF influences the Ca2+–flux and CQ lowers the pH of lysosomes, a partly different effect on lipolysis is not surprising given the mode of action. In contrast to BAF, some (Galluzzi et al., 2009; Mauthe et al., 2018). As BAF influences the weak base that neutralizes intra-lysosomal acidity and hence arrests autophagy the weak base that neutralizes intra-lysosomal acidity and hence arrests autophagy, whereas CQ might inhibit other forms of autophagy like CMA. Early attempts to study acid lipids through the use of CQ suggested that lysosomal enzymes contribute to the lipolysis of intracellular lipids in hepatocytes (Debeer et al., 1979; Duee et al., 1985), but the condition may differ for adipocytes. It may also be that in human adipocytes lipases for lipid hydrolysis in lysosomes are not pH-dependent or that autophagy indirectly contributes to the regulation of cytosolic lipolysis via lysosomal/proteasomal-mediated PLIN1 degradation, as was recently been shown for the TFN-alpha mediated increase in basal lipolysis (Ju et al., 2019).

Finally, we investigated the effect of L-stat, a potent and selective lysosomal lipase blocker (Hamilton et al., 2012). In our study, L-stat was found to severely decrease lipolysis (around 50%) in both starvation- and ISO-stimulated adipocytes at a dose of 10 μM without affecting the autophagic flux marker LC3. This is rather surprising given the fact that cytosolic lipolysis contributes to around 90% of total lipolysis in human adipocytes (Jocken et al., 2015), therefore we suspected there might be an inhibiting effect of L-stat on cytosolic lipolysis/lipases. However, immunoblotting showed a massive increase of serine phosphorylated HSL despite a 50% reduction in glycerol release. We hypothesized that this activated pHSL was unable to access the lipid droplet and subsequently hydrolyze the stored triglycerides. In the adipocyte triglycerides are stored in droplets surrounded by a membrane coated by e.g. PLIN1 that limits the access of lipases (Brasaemle et al., 2000; Brasaemle, 2007). For allowing HSL to exert its activity PLIN1 needs to be phosphorylated and detached from the droplets in order to provide access to pHSL (Szتلary and Brasaemle, 2017; Choi et al., 2010). However, L-stat treatment did not affect phosphorylation nor translocation of PLIN1 from the droplet membrane to the cytosol. We suggest that L-stat inhibits phosphorylation (e.g. via binding to serine sites) and subsequent translocation of PLIN1 and thereby prevents cytosolic lipolysis in differentiated hMADs, which might partly explain the reduced lipolytic response. Of interest, L-stat was mentioned before to bind covalently to serine at the active site of lysosomal acid lipase LIPA and thereby to inhibit lysosomal lipase activity in murine cells (Tuohetahuntila et al., 2017; Schlager et al., 2017). However, it is unclear whether L-stat binds also to other serine residues of cytosolic lipolytic proteins (including ATGL, HSL and PLIN1) and whether L-stat might be of potential use for the treatment of obesity and related metabolic disorders type 2 Diabetes.

In summary, in differentiated hMADs, 3MA increases glycerol release whereas BAF and L-stat reduce the eflux of glycerol. BAF and CQ both blocked the autophagic flux in a time- and dose-dependent manner, as indicated by an increasing LC3II/LC3I without affecting cytosolic lipases (e.g., HSL). Taken together these data suggest that the autophagy-lysosomal pathway could contribute to triglyceride breakdown in human adipocytes, although to a limited extent (10–15%). Further research is needed to reveal the detailed mechanisms of these pharmacological compounds and their interplay with cytosolic lipid breakdown in human adipocytes.

Conflicts of interest

All authors contributed significantly to the study and approved the manuscript for submission. None of the authors declare a conflict of interest in this manuscript.

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CRediT authorship contribution statement

Qing Xu: Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Funding acquisition. Edwin CM. Mariman: Supervision, Writing – review & editing. Ellen E. Blaak: Supervision, Writing – review & editing. Johan WE. Jocken: Conceptualization, Methodology, Resources, Project administration, Funding acquisition, All authors read and approved the final version of the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mce.2022.111555.

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