Lipolysis Response to Endoplasmic Reticulum Stress in Adipose Cells*

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In obesity and diabetes, adipocytes show significant endoplasmic reticulum (ER) stress, which triggers a series of responses. This study aimed to investigate the lipolysis response to ER stress in rat adipocytes. Thapsigargin, tunicamycin, and brefeldin A, which induce ER stress through different pathways, efficiently activated a time-dependent lipolytic reaction. The lipolytic effect of ER stress occurred with elevated cAMP production and protein kinase A (PKA) activity. Inhibition of PKA reduced PKA phosphosubstrates and attenuated the lipolysis. Although both ERK1/2 and JNK are activated during ER stress, lipolysis is partially suppressed by inhibiting ERK1/2 but not JNK and p38 MAPK and PKC. Thus, ER stress induces lipolysis by activating cAMP/PKA and ERK1/2. In the downstream lipolytic cascade, phosphorylation of lipid droplet-associated protein perilipin A was significantly promoted during ER stress but attenuated on PKA inhibition. Furthermore, ER stress stimuli did not alter the levels of hormone-sensitive lipase and adipose triglyceride lipase but caused Ser-563 and Ser-660 phosphorylation of hormone-sensitive lipase and moderately elevated its translocation from the cytosol to lipid droplets. Accompanying these changes, total activity of cellular lipases was promoted to confer the lipolysis. These findings suggest a novel pathway of the lipolysis response to ER stress in adipocytes. This lipolytic activation may be an adaptive response that regulates energy homeostasis but with sustained ER stress challenge could contribute to lipotoxicity, dyslipidemia, and insulin resistance because of persistently accelerated free fatty acid efflux from adipocytes to the bloodstream and other tissues.

In mammals, fatty acids stored as triacylglycerols in adipose cells constitute the primary energy reserves. Triacylglycerol hydrolysis in adipocytes, termed lipolysis, produces glycerol and free fatty acids (FFAs). Because of the paucity of glycerol in normal adipocytes, glycerol is rarely re-utilized for triglyceride resynthesis; rather, it is released in the plasma along with FFAs to supply energy to various tissues. Adipose lipolysis is an important process that controls circulating FFA concentrations and governs energy homeostasis through the Randle glucose-fatty acid cycle. Dysregulation of the lipolysis pathway may result in elevated levels of circulating FFAs, which is the major basis for the development of insulin resistance in obesity and diabetes mellitus.

Background: Dysregulation of endoplasmic reticulum homeostasis elicits various stress responses.

Results: Endoplasmic reticulum stress activates lipolytic cascade in rat adipocytes.

Conclusion: The lipolysis response to endoplasmic reticulum stress is mediated via cAMP/PKA and ERK1/2 signaling.

Significance: Increased lipolysis promotes fatty acid efflux from adipocytes to other tissues and thus may contribute to lipotoxicity and insulin resistance in obesity and diabetes.

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Lipolysis is stimulated by various hormones and effectors. Catecholamines are major hormones that stimulate acute lipolysis. Thyronines, glucocorticoids (5), TNF-α (6–8), and lipopolysaccharides (9) induce a chronic lipolytic reaction. The lipolysis is closely associated with the production of cAMP and activation of cAMP-dependent protein kinase A (PKA) (2, 10). cAMP/PKA along with extracellular signal-regulated kinase-1/2 (ERK1/2) (6, 11) are the major early lipolytic signals. PKC may modulate lipolysis through a different pathway (12). In the downstream lipolytic cascade, the phosphorylation of both lipid droplet-associated protein perilipin A (13–17) and hormone-sensitive lipase (HSL) and the subsequent translocation of HSL from the cytosol to the lipid droplet surface are critical to confer full activation of HSL during PKA-stimulated lipolysis (14, 18, 19). Adipose triglyceride lipase (ATGL) is another important enzyme (20) that predominantly modulates basal lipolysis (21, 22) and may be indirectly activated during PKA-regulated lipolysis.

The endoplasmic reticulum (ER) is an organelle that functions to synthesize, fold, and transport proteins. Dysregulation of ER homeostasis leads to accumulation of misfolded proteins in the ER lumen (23). Under stress conditions, cells activate the
unfolded protein response, which includes transcriptional induction of ER chaperones and translational attenuation, thus evoking a series of ER stress responses (23, 24). Recent studies reveal that ER stress occurs in pancreatic β-cells (25–27), hepatocytes (28), and adipocytes (28–30) in obesity and diabetes and is one of the mechanisms involved in triggering insulin resistance. The ER is also the site of triglyceride synthesis and nascent lipid droplet formation, although the process is far from clarified. One prevalent model proposes that the triglyceride droplet originates between the two leaflets of the ER membrane and then buds or patches to the cytoplasm, with a phospholipid monolayer along with resident proteins of the ER on the droplet surface (31). An alternative model assumes that the triglyceride droplet forms alongside the ER membrane but with the ER facilitating biosynthesis and holding the droplet like an egg (32). Regardless of the model, morphological evidence suggests that ER bilayers wrap closely around the phospholipid monolayer surface of lipid droplets (31–34). Thus, given the ER origin of lipid droplets, the close association of the two organelles, and the occurrence of lipolysis at the lipid droplet surface, we were interested in investigating whether ER stress involves modulation of the lipolysis of triglyceride droplets in adipocytes.

This study aimed to investigate the lipolysis in rat adipocytes stimulated by ER stress inducers. We revealed that the lipolysis action of ER stress was mediated by cAMP/PKA and ERK1/2 signaling and was accompanied by phosphorylation of perilipin and HSL and activation of cellular lipases. Our findings suggest a novel pathway of lipolysis response to ER stress that accelerates FFA efflux from adipose cells to the bloodstream and other tissues and thereby may contribute to lipotoxicity, dyslipidemia, and insulin resistance in obesity and diabetes.

**EXPERIMENTAL PROCEDURES**

**Materials**—Tunicamycin and H89 were from Alexis Biochemicals (San Diego), and thapsigargin, PD98059, U0126, and phenol red-free DMEM were from Sigma. Polyclonal antibody against rat perilipin was a gift from C. Londos (National Institutes of Health, Bethesda). Antibodies recognizing HSL, ATGL, protein kinase-like eIF2α kinase (PERK), eIF2α, and their phosphorylated species were from Cell Signaling Technology (Boston, MA). Defatted bovine serum albumin, modified Lowry protein assay kit, and enhanced chemiluminescence (ECL) detection reagent were from Applygen Technologies.

**Culture of Primary and Differentiating Rat Adipocytes**—Primary adipocytes were isolated from epididymal fat pads of normal male Sprague-Dawley rats (160–180 g) according to our laboratory method (9, 15, 35). The minced fat pads were digested in Krebs-Ringer solution containing 0.75 mg/ml type I collagenase, 200 μM adenosine, 25 mM Hepes, pH 7.4, and 1% FFA-free BSA. After digestion, primary adipocytes floating in the tube were collected, washed, and packed by centrifugation at 200 × g for 3 min for determining the packed cell volume of adipocytes (15). Adipocytes were preincubated in phenol red-free and serum-free DMEM in an atmosphere of 5% CO₂ at 37°C for 1 h before treatments.

After the isolation of primary adipocytes, rat preadipocytes residing in the digestion mixture were collected by centrifugation at 800 × g for 10 min and then plated and differentiated into adipocytes for 3 days in serum-free DMEM/F-12 (1:1) supplemented with 5 μg/ml insulin, 33 μM biotin, and 200 pm triiodothyronine, as we described previously (15, 35). At day 4, the differentiating adipocytes were transferred to phenol red-free and serum-free DMEM and incubated for 24 h before experiments.

**Evaluation of Lipolysis by Glycerol Assay**—Full hydrolysis of one triglyceride in adipocytes produces three fatty acids and one glycerol molecule, which are then released into the medium. Thus, we measured glycerol content in the medium as an index of lipolytic reaction (5, 9, 15). Adipocytes or minced fat tissues were incubated in serum-free and phenol red-free DMEM with or without the agents. The culture medium was collected and heated at 70°C for 10 min to inactivate residual lipase activity. Glycerol was determined by the enzyme-coupled colorimetric assay (GPO Trinder reaction) from the absorption of 550 nm (5), with use of a colorimetric assay kit (Applygen Technologies).

**cAMP Radioimmunoassay**—According to our previous method (5, 8), adipocytes were lysed in 150 μl of ice-cold buffer containing 50 mM Tris-HCl, pH 7.4, and 1 mM EDTA. The lysate was centrifuged at 12,000 × g for 20 min at 4°C. The cytosol fraction was collected from below the solidified fat cake in the tube, mixed with ½ volume of 40% trichloroacetic acid, and further cleaned by centrifugation. The supernatant was collected and used for cAMP assay by use of a commercial 125I radioimmunoassay kit (Isotope Laboratory of Shanghai University of Chinese Medicine).

**Immunoblotting**—Adipocytes were lysed in sample buffer containing 62 mM Tris-HCl, pH 6.8, 2% SDS, 0.1 mM sodium orthovanadate, and 50 mM sodium fluoride. The protein content was determined by the Lowry protein assay. Equal amounts of proteins were loaded and separated by SDS-PAGE. For most immunoblot assays, the traditional polyacrylamide gels were used. For detection of phosphorylated perilipin with the anti-perilipin antibody, we used a low-bis concentration polyacrylamide gel prepared with 10% acrylamide and 0.07% N,N,N′-methylenebisacrylamide (at a ratio of 142:1 versus 39:1 in the traditional gel), because this gel system provides maximal resolution of proteins in the 60–70-kDa range (9, 15, 16, 36). The proteins transferred on membranes were recognized with primary antibodies and horseradish peroxidase-conjugated secondary antibodies. The blots were developed by use of ECL reagents. If required, the antibodies bound to membranes were removed by a commercial stripping solution (Applygen Technologies), and the blots were reprobed with use of other antibodies.

**Lipase Activity**—Lipase activity was assayed by determining the rate of hydrolysis of emulsified triolein substrates by endogenous lipases of primary adipocytes as described previously (37) with modifications as described previously (5, 8). Adipocyte lysates were centrifuged at 12,000 × g for 15 min at 4°C. The infranatant phase below the fat cake fraction was transferred to a new tube and further cleaned by centrifuging at 12,000 × g for 15 min at 4°C. The supernatant was added to an emulsified triolein substrate solution (reaction A) and in parallel to a control solution without triolein substrates (reaction B). The reac-
Lipolysis Response to ER Stress

**Lipolysis of Adipocytes Is Activated in Response to ER Stress**—To investigate whether lipolysis is activated on ER stress challenge, we measured the glycerol release from differentiated or primary rat adipocytes and minced epididymal adipose tissues stimulated with ER stress inducers. Three agents, thapsigargin, tunicamycin, and brefeldin A, which elicit ER stress through different pathways (23), effectively elevated lipolytic reaction in adipocytes (Fig. 2A). The magnitude of glycerol release was lower in thapsigargin-stressed adipocytes than in adipocytes stimulated with isoproterenol, a known adrenergic lipolysis activator (Fig. 2A, inset). The lipolysis in differentiated adipocytes was time-dependently increased on stimulation with 1 μM thapsigargin (Fig. 2B). Similarly, glycerol level was increased in isolated primary adipocytes 2 h after ER stress induction (Fig. 2C). To evaluate *ex vivo* lipolytic action, minced epididymal adipose tissue was treated with 1 μM thapsigargin for 8 or 24 h. The 1-h glycerol release in the freshly changed medium significantly increased, which indicates lipolytic activation *ex vivo* in adipose tissues in response to ER stress (Fig. 2D).

Evaluation of cell viability by LDH and MTT assay indicated no significant LDH leakage into culture medium from rat adipocytes during an 8-h incubation with thapsigargin at 0.5 and 1 μM or tunicamycin at 5 μg/ml (Table 1). Only tunicamycin at 10 μg/ml conferred cytotoxicity in adipocytes. Therefore, the agents at the tested concentrations did not affect adipocyte viability.

**Lipolysis Response to ER Stress Is Mediated by Activation of cAMP/PKA Signaling**—cAMP and PKA are the major signals for controlling lipolysis (2). We determined cAMP content in adipocytes by 125I radioimmunoassay (5, 9). Incubation with 1 μM thapsigargin for 1 h increased cAMP production by 68%, which persisted during the 8-h stimulation (Fig. 3A). As a positive control, the classical lipolytic agent isoproterenol caused a similar increase in cellular cAMP level (Fig. 3A). Furthermore, PKA activity was analyzed by immunoblotting with a primary antibody against a specific motif (RRX(S/T)) of PKA phosphosubstrates (9). Stimulation with 1 μM thapsigargin for various times significantly promoted PKA activity (Fig. 3B), although this effect was relatively weaker than that induced by isoproterenol (Fig. 3C). Preincubation with the PKA inhibitor H89 diminished PKA phosphosubstrate level (Fig. 3C) and greatly attenuated the lipolysis stimulation of thapsigargin (Fig. 3D) or isoproterenol (data not shown). These data suggest that lipolysis response to ER stress was mediated mainly by activating cAMP/PKA signals in adipocytes.

**ERK1/2 Activation Accounts for ER Stress-induced Lipolysis**—In addition to cAMP/PKA, ERK1/2 participates in lipolysis regulation (7, 11). Immunoblotting results showed that thapsigargin time-dependently promoted the phosphorylation of Raf-1 (Fig. 4A), an upstream Ser/Thr kinase in the MAPK cascade. In addition, phosphorylation of ERK1/2 was induced rapidly after a 0.5-h exposure to thapsigargin and was sustained at a high level during an 8-h stimulation (Fig. 4A). Tunicamycin and dithiothreitol, two other ER stress inducers, also stimulated significant phosphorylation of ERK1/2 in adipocytes (Fig. 4B). Preincubation with the ERK1/2 inhibitor U0126 eliminated ERK1/2 phosphorylation stimulated by thapsigargin or a con-

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**FIGURE 1.** ER stress is induced by thapsigargin in rat adipocytes. Rat differentiated adipocytes were incubated for 0.5 to 8 h with or without 1 μM Thaps. Protein levels of GRP78, PERK, or its Thr-980 phosphorylation form, eIF2α, or its Ser-51 phosphorylation species, with actin as a loading control, were detected by immunoblotting analysis. The blots are representative of three separate experiments.
To regulate ER stress-induced lipolysis. After incubation with 1 mM thapsigargin was inhibited by 50 and 42%, respectively (Fig. 4), which suggests that PKA and ERK1/2 cooperate partially suppressed the ER stress-responsive lipolysis. How- ever, U0126 decreased the lipolysis of TNF-α by 78% (Fig. 5A), but phosphorylation of p38 MAPK was undetectable (data not shown). However, preincubation for 1 h with the JNK inhibitor SP600125 did not alter glycerol release stimulated by thapsigargin (Fig. 5B). Also, the lipolysis activation was not affected by the p38 inhibitor SB203580 (Fig. 5C) or PKC inhibitor Ro-31-8220 (Fig. 5D). Therefore, ERK1/2 but not JNK, p38 MAPK, or PKC is involved in lipolytic regulation in ER-stressed adipocytes.

Enhanced Phosphorylation of Perilipin on ER Stress—Perilipsins are the most abundant proteins located at the surface of lipid droplets in adipocytes (16, 36). Phosphorylation (13–15, 17) or down-regulation (9) of perilipins facilitate lipolysis. Because a specific antibody against phosphorylated perilipin is not available, we performed two protocols for immunodetection of perilipin phosphorylation. First, adipocyte extracts were separated on low-bis SDS-polyacrylamide gels (10: 0.07% acrylamide/bisacrylamide), because the gel system allows better resolution of the 67-kDa phosphorylated perilipin A (9, 15, 16). Immunoblot analysis with an anti-perilipin antibody revealed that thapsigargin at 1 μM caused time-

![FIGURE 2. Lipolysis is activated in response to ER stress in rat adipose cells or tissues.](http://www.jbc.org/)

**TABLE 1**

Effect of tunicamycin and thapsigargin on cell viability of adipocytes

|                  | MTT assay cell viability | LDH assay | LDH leakage |
|------------------|--------------------------|-----------|-------------|
|                  | % control (%)            | % total   | % total     |
| Control          | 100.0 ± 0.0              | 10.4 ± 0.7| 9.6 ± 0.5   |
| Thaps (0.5 μM)   | 98.7 ± 1.5               | 9.2 ± 0.6 | 9.2 ± 0.3   |
| Thaps (1 μM)     | 95.0 ± 7.2               | 10.0 ± 0.3| 12.7 ± 0.6  |
| Tun (5 μg/ml)    | 92.2 ± 6.0               | 12.7 ± 0.6| 18.5 ± 0.3  |
| Tun (10 μg/ml)   | 82.6 ± 0.5*              |           |             |

*p < 0.01 versus control.

c-Jun N-terminal Kinase (JNK) and p38 MAPK and PKC Do Not Regulate ER Stress-stimulated Lipolysis—JNK (7) and PKC (12) regulate lipolysis action of isoproterenol or TNF-α. JNK is activated by ER stress in adipocytes (29, 38). Therefore, we investigated whether JNK and PKC are involved in the lipolysis response to ER stress. Immunoblot analysis revealed that JNK phosphorylation was elevated 2 h after thapsigargin stimulation (Fig. 5A), but phosphorylation of p38 MAPK was undetectable (data not shown). However, preincubation for 1 h with the JNK inhibitor SP600125 did not alter glycerol release stimulated by thapsigargin (Fig. 5B). Also, the lipolysis activation was not affected by the p38 inhibitor SB203580 (Fig. 5C) or PKC inhibitor Ro-31-8220 (Fig. 5D). Therefore, ERK1/2 but not JNK, p38 MAPK, or PKC is involved in lipolytic regulation in ER-stressed adipocytes.

FIGURE 2. Lipolysis is activated in response to ER stress in rat adipose cells or tissues. Rat adipose cells or tissues were incubated in phenol red-free and serum-free DMEM. Glycerol content in the medium was assayed as an index of lipolysis. A, lipolysis responses to ER stress after rat differentiating adipocytes were incubated for 8 h with or without 1 μM Thaps, 5 μg/ml tunicamycin (Tun), or 0.5 μg/ml brefeldin A (BFA). Inset in A shows a positive stimulation of the lipolysis. After incubation with 1 μM Thaps or 100 μM ISO, an adrenergic lipolytic activator, 1-h glycerol release from the adipocytes, was assayed. B and C, time-dependent lipolysis in rat differentiated (B) and primary (C) adipocytes stimulated with 1 μM Thaps. D, ex vivo lipolysis. Minced rat epididymal adipose tissues were incubated with 1 μM Thaps for the indicated times and then 1-h glycerol release in freshly changed medium was determined. Lipolysis data are expressed as micromoles of glycerol/ml of packed cell volume (PCV) or milligram of cell proteins. Data are mean ± S.E. of at least three separate experiments performed in triplicate. *, p < 0.05; **, p < 0.01; and ***, p < 0.001 versus control.
dependent shifts of the perilipin A from the 65-kDa native form to the 67-kDa phosphorylated species, which indicates that phosphorylation of perilipin A occurs during ER stress (Fig. 6A). Such a migration shift was due to increased incorporation of phosphate in perilipin A proteins, as confirmed previously with $^{32}$P labeling of adipocytes and autoradiography (15, 16, 36).

As an alternative, adipocyte extracts were run on traditional polyacrylamide gels and underwent immunoblotting with an antibody against phospho-Ser/Thr PKA substrates, which was previously used to recognize phosphorylated perilipins (5, 8). The control test showed that the immunoprecipitants of primary adipocyte with anti-phospho-Ser/Thr antibodies were recognized by anti-perilipin antibodies to raise two concrete bands of phosphorylated perilipin A together with its truncated 46-kDa isoform perilipin B induced by isoproterenol (Fig. 6B, top image). Furthermore, immunoblot analysis confirmed that phosphorylation of perilipin A was effectively induced in adipocytes stimulated with thapsigargin, tunicamycin, or dithiothreitol (Fig. 6, B, top image, and C). Phosphorylation of the perilipin B isoform was also moderately promoted. However, the protein level of native perilipins was not altered (Fig. 6, A and bottom image in B). In the presence of the PKA inhibitor H89, phosphorylation of perilipin A (Fig. 6D) and the accompanying lipolysis action (data not shown) were reduced in adipocytes stimulated with thapsigargin or the positive lipolytic agent isoproterenol.

Regulation of Lipase Activity on ER Stress—HSL and ATGL are two major lipases in adipocytes. We assayed the hydrolysis rate of triolein substrates in the reaction with adipocyte extracts, which served as an index of lipase activity. Thapsigargin increased lipase activity by 44%, as compared with 80% with isoproterenol at 30 min (Fig. 7A). Protein levels of HSL and ATGL were not altered after thapsigargin treatment (Fig. 7B). However, thapsigargin promoted HSL phosphorylation at Ser-563 and Ser-660 residues (Fig. 7C), two critical phosphoserine sites for controlling HSL activity (19). Translocation of HSL from cytosol to lipid droplets is crucial for conferring a full lipolysis reaction (14, 18, 39). Immunofluorescent staining indicated that thapsigargin moderately increased HSL translocation to the lipid droplet surface, whereas isoproterenol significantly promoted this process (Fig. 7D).

DISCUSSION

In this study, we demonstrated that ER stress stimuli induced lipolysis by activating cAMP/PKA and ERK1/2 signaling in adipocytes. This lipolytic activation is probably an adaptive response that regulates energy homeostasis but, with sustained ER stress, may contribute to lipotoxicity and impair insulin sensitivity because of persistently accelerated FFA efflux from adipocytes to the bloodstream and various tissues.

Significant ER stress occurs in adipocytes in obesity and diabetes (28–30), yet its origins are unknown. The agents that disrupt ER homeostasis allow for exploring ER stress responses.
Thapsigargin, tunicamycin, and brefeldin A trigger ER stress by inhibiting the ER Ca\(^{2+}\) pump and protein glycosylation or interfering with ER-Golgi protein trafficking, respectively (23, 38). With thapsigargin stimulation, we observed a rapid increase in GRP78 protein level and an induction of eIF2α and PERK phosphorylation, thus confirming that ER stress was evoked in rat adipocytes. Thapsigargin, tunicamycin, and brefeldin A, which elicit ER stress by different pathways, effectively activated significant lipolysis in rat adipocytes.

The mechanisms by which ER stress stimulates lipolysis may be multifactorial. cAMP and PKA are the major early signals that control lipolysis with catecholamine hormone stimulation (2). Our radioimmunoassay results revealed that cellular cAMP production increased by 68%, along with a significant elevation of PKA phosphosubstrates in thapsigargin-treated adipocytes. PKA inhibition with H89 decreased PKA phosphosubstrates and inhibited the lipolysis stimulation of thapsigargin. Recent reports show that the survival of INS-1 cells and rat pancreatic β-cells under thapsigargin-induced ER stress can be improved by the PKA activator forskolin or by the glucagon-like peptide-1 receptor agonist that may elevate cAMP. This protective effect was attenuated on PKA inhibition, which implicates an involvement of cAMP/PKA signals in modulating the unfolded protein response during ER stress (25, 40). Increased cAMP production might occur as an adaptive response to ER stress challenge, which then activates PKA to modulate several components in the ER stress cascade. However, to date, direct evaluation of cellular cAMP and PKA activation during ER stress is lacking. Our data reveal an activation of cAMP/PKA at least in ER-stressed adipocytes stimulated with thapsigargin. Although the mechanism needs to be clarified, the activation of cAMP/PKA is a major signaling event triggering the lipolytic cascade in ER-stressed adipocytes.

In addition to PKA, ERK1/2 participates in regulating chronic lipolysis stimulation of TNF-α or lipopolysaccharides (6–9). JNK (7) and PKC (12) may also modulate lipolysis.
Recent studies suggest that ERK1/2 and JNK are readily activated with ER stress. ERK1/2 activation protects cells against ER stress-induced death (41), but JNK activation impairs insulin action (38). We observed that thapsigargin, tunicamycin, and dithiothreitol promoted phosphorylation of Raf-1, an upstream kinase in the MAPK cascade, accompanied by rapid phosphorylation of both ERK1/2 and JNK. Nevertheless, inhibition of JNK and p38 MAPK or PKC failed to inhibit the lipolysis response to ER stress. Instead, preincubation with ERK1/2 kinase inhibitors abrogated ERK1/2 phosphorylation and moderately attenuated the lipolysis in ER-stressed adipocytes. Furthermore, when PKA and ERK1/2 were simultaneously inhibited with H89 plus U0126, lipolysis was completely abolished. Of note, PKA-independent effects of H89 may occur at high concentrations, although H89 is more selective for PKA inhibition. Davies et al. (42) reported that H89 at the concentration of $10^{-6}$ M, used in this study, can inhibit several other kinases such as AMPK, ERK2, and PKC by 81, 13, and 21%, respectively. Because these kinases may modulate lipolysis with different stimulations, the inhibitory effects of H89 on the lipolytic signaling in ER-stressed adipocytes should be discussed. AMPK was proposed to activate lipolysis (43), but several studies debated that AMPK has antilipolytic activity (44, 45). The latter view is supported by our previous work that antidiabetic biguanide metformin, which induces AMPK (44), can inhibit the lipolysis action of isoproterenol and TNF-α in primary rat adipocytes (46, 47). Thus, by preferring an antilipolytic action of AMPK, we speculate that AMPK inactivation of H89, although significant, may not account for the lipolytic inhibition of H89 in ER-stressed adipocytes. However, the slight suppression of H89 on ERK2 but not PKC could contribute in part
to the inhibitory effect of H89 on ER stress-mediated lipolytic signaling. Therefore, PKA and ERK1/2 may be the key signals that cooperatively regulate lipolysis in ER-stressed adipocytes. PKA may govern a rapid lipolytic action, and ERK1/2 may act in the chronic phase of the lipolysis response to ER stress.

In the downstream lipolytic cascade, perilipin is one of the most abundant PKA phosphoproteins in adipocytes (16, 36). Unlike its less abundant splicing variant (46-kDa perilipin B), the full-length perilipin A (simply termed perilipin in this study) is the major isoform, and its function on lipolytic regulation has been firmly established. Perilipins coat lipid droplets as a barrier to prevent triglyceride hydrolysis by lipases (13). Phosphorylation of perilipin in at least three of the six PKA consensus site serines may impair its barrier function and enhance HSL translocation to the lipid droplet, hence facilitating lipolysis (13–18). We found that ER stress did not alter the level of perilipin proteins but increased the phosphorylation. When perilipin phosphorylation was attenuated on PKA inhibition, the lipolysis response ceased. HSL and ATGL are two major lipases that control ~95% of the triglyceride hydrolase activity in adipocytes (20, 21). HSL hydrolyzes both triglycerides and diglycerides, but the affinity for the latter is 10-fold higher (48). PKA-mediated phosphorylation of HSL at Ser-660, Ser-659, and Ser-563 is crucial for controlling lipolysis activity (19). The translocation of HSL from the cytosol to lipid droplets is an important step for PKA-regulated lipolysis (14, 18, 39), which requires the phosphorylation of both HSL (49) and perilipin (14, 15). Thapsigargin increased phosphorylation of HSL at Ser-660 and Ser-563 and moderately promoted its translocation to the lipid droplet surfaces, which indicates an activation of HSL during ER stress. Unlike HSL, ATGL predominantly hydrolyzes triglyceride but is not phosphorylated by PKA (20). A recent model proposes an indirect control of ATGL activity by PKA dependent on perilipin phosphorylation (50, 51). Perilipin binds the ATGL co-activator Abhd5/CGI-58 and thereby suppresses the interaction of the lipase with Abhd5. Phosphorylation of perilipin on Ser-492 or Ser-517 results in a release of sequestered Abhd5, hence allowing Abhd5 to interact with and activate ATGL (50, 51). In adipocytes stimulated with thapsigargin, protein levels of HSL and ATGL were unchanged, but total lipase activity was increased by 44%, which was associated with PKA activation and strong perilipin phosphorylation. Therefore, perilipin phosphorylation occurring during ER stress may indirectly promote ATGL activity by regulating accessibility to its coactivator Abhd5, although this speculation requires further investigation. Activated HSL and ATGL may cooperatively manipulate the hydrolysis of triglycerides and diglycerides in ER-stressed adipocytes.

In conclusion, we reveal a novel pathway of lipolysis response to ER stress in adipocytes. Obesity and diabetes mellitus are associated with increased adipocyte lipolysis and a high level of circulating FFA (3, 4), accompanied by significant ER stress in adipose cells (28–30). The lipolysis response to ER stress promotes FFA efflux from adipocytes to the plasma, which could be a cellular basis of lipotoxicity, dyslipidemia, and insulin resistance in these pathologies. Moreover, because fatty acids...
Lipolytic Response to ER Stress

...themselves can trigger ER stress in pancreatic β-cells, for example (26, 27), accelerated lipolysis and FFA efflux from already stressed adipocytes may produce a feed-forward machinery to further stimulate or worsen ER stress in other tissues. This phenomenon implicates a central role of adipose cells in the development of ER stress-related pathologies in various tissues in obesity and diabetes.

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