Asymmetrical dimethylarginine triggers lipolysis and inflammatory response via induction of endoplasmic reticulum stress in cultured adipocytes

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Zhou QG, Zhou M, Hou FF, Peng X. Asymmetrical dimethylarginine triggers lipolysis and inflammatory response via induction of endoplasmic reticulum stress in cultured adipocytes. Am J Physiol Endocrinol Metab 296: E869–E878, 2009. First published February 10, 2009; doi:10.1152/ajpendo.91011.2008.—Protein energy wasting, a state of decreased stores of body protein and fat, is a risk factor for mortality in advanced chronic kidney disease (CKD). Little is known about the mechanism underlying loss of fat in CKD. Accumulation of asymmetric dimethylarginine (ADMA) is prevalent in advanced CKD. Here we assessed the effect of ADMA on cellular perturbation in cultured 3T3-L1 adipocytes. Exposure of adipocytes to ADMA induced lipolysis and decreased perilipin A, with no alteration of lipases expression or activity. ADMA treatment also upregulated the expression of inflammatory adipocytokines via activation of nuclear factor-κB (NF-κB). Blocking the inflammatory responses with NF-κB inhibitor partly inhibited the ADMA-induced lipolysis. Furthermore, ADMA treatment triggered endoplasmic reticulum (ER) stress, revealed by phosphorylation of PKR-like eukaryotic initiation factor 2α kinase, eukaryotic translational initiation factor 2α, c-Jun NH2-terminal kinase, and overexpression of glucose-regulated protein 78. Treatment with ER stress inhibitor completely abolished the ADMA-induced lipolysis and inflammatory responses. Moreover, conditioned medium from the ADMA-treated adipocytes increased protein degradation in cultured C2C12 myotubes, suggesting that the ADMA-induced adipocyte perturbation may promote skeletal muscle proteolysis. These data suggest that elevated ADMA promoted the adipocyte perturbation through induction of ER stress, which might have implication for protein energy wasting in CKD.

asymmetric dimethylarginine; adipocytes; lipolysis; inflammatory response; endoplasmic reticulum stress; protein energy wasting

PROTEIN ENERGY WASTING (PEW), a state of decreased stores of body protein and fat, characterized by malnutrition and inflammation, is a common manifestation in advanced chronic kidney disease (CKD) and has been linked to increased mortality in patients with CKD (11). Muscle wasting associated with PEW has been intensively studied and is attributable to activation of the ubiquitin proteasome pathway because of acidosis, insulin resistance, and inflammation (28). Although loss of body fat has been identified as an independent risk factor for the increased mortality in patients with advanced CKD (15), little is known about the underlying mechanisms.

Adipocytokines play a pivotal role in the regulation of whole body energy homeostasis and lipid metabolism through lipogenesis and lipolysis (37). Adipocytokines, a number of biological active molecules secreted by adipocytes, have been shown to be involved in the regulation of many processes, including energy homeostasis (27, 34). Emerging evidence has shown that adipocyte dysfunction and adipocytokine deregulation in CKD occur in both animal models and humans (3, 15, 43). However, relatively little information is available about factors that may induce or promote adipocyte perturbation in CKD.

Asymmetric dimethylarginine (ADMA) is the metabolite of methylated arginine residues on proteins. Plasma level of ADMA significantly increases in patients with CKD, probably because of the increased synthesis and impaired degradation (22). Accumulation of ADMA increases with deterioration of renal function and has been linked with the increased mortality in this population (47). Numerous studies have demonstrated that elevated ADMA disturbs the functions of macrophages, vascular endothelial cells, and smooth muscle cells (32, 33, 40), suggesting that accumulation of ADMA might be biological toxic for different cells. A close relationship between increased ADMA, low serum albumin, and inflammation in CKD patients has been observed (7, 46, 47), suggesting that ADMA accumulation might be involved in the pathogenesis of PEW.

As the first step toward testing the hypothesis, we examined whether increased levels of ADMA affected lipolysis and the expression of proinflammatory adipocytokines in cultured adipocytes. Our data showed that ADMA induced lipolysis, which was associated with decreased perilipin A. Increased levels of ADMA also upregulated the expression of proinflammatory adipocytokines through activation of nuclear factor-κB (NF-κB), which was partly involved in the ADMA-induced lipolysis. Furthermore, we found that the ADMA-induced adipocyte perturbation was dependent on activation of endoplasmic reticulum (ER) stress. Conditioned medium from the ADMA-treated adipocytes increased protein breakdown in cultured myotubes. These data provided new information for the potential involvement of ADMA accumulation in adipocyte perturbation and CKD-related complications such as PEW.

MATERIALS AND METHODS

Culture of Adipocytes

Mouse 3T3-L1 preadipocytes (American Type Culture Collection, Rockville, MD) at passages 3 to 8 were grown and maintained in DMEM (Invitrogen, Carlsbad, CA) containing 10% bovine calf serum (HyClone, Logan, UT), penicillin (200 U/ml), and streptomycin (200 μg/ml) in a humidified atmosphere (95% air and 5% CO2). Differentiation was initiated by the adipogenic agents (1 μM dexamethasone, 10 μg/ml insulin, and 0.5 mM 3-isobutyl-1-methylxanthine) in DMEM...
containing 10% FBS after 2 days complete confluence (45). After 48 h, cells were switched to postdifferentiation medium and grown in DMEM containing 10 μg/ml insulin and 10% FBS. The media was changed every other day until cells were shown to be maximally morphologically differentiated (above 90% of cells), which commonly occurred at days 8–10. All experiments were performed using the differentiated adipocytes.

Adipocyte integrity during incubation with ADMA was assessed by measuring the activity of lactate dehydrogenase in the media using a spectrophotometric assay kit (Sigma, St. Louis, MO).

**Lipolysis Assay**

Lipolysis was assessed by measurement of the amount of glycerol released in the medium as previously described with some modifications (19). Overnight serum-deprived adipocytes were incubated in serum-free DMEM containing 2% fatty acid-free BSA (used as a fatty acid acceptor) in the presence or absence of ADMA for the indicated time. Aliquots of the medium were collected and centrifuged at 3,000 g for 10 min at 4°C. Glycerol content in the supernatants was measured using a colorimetric assay by free glycerol determination (Sigma) on a microplate reader at 540 nm. Results were normalized by cellular protein determined by the bicinchoninic acid protein assay kit (Pierce, Rockford, IL).

**Western Blot Analysis**

Western blot analyses were carried out as described previously (45). Briefly, cells were lysed with the radioimmunoprecipitation assay buffer, and the lysates were separated by SDS-PAGE. The membrane blots were first probed with a primary antibody normalized by cellular protein determined by the bicinchoninic acid assay buffer, and the lysates were separated by SDS-PAGE. The Western blot analyses were carried out as described previously (45).

**Cellular Lipase Activity**

Cellular lipase activity was determined as described previously (19). For the preparation of cell extracts, adipocytes were disrupted in lysis buffer [0.25 M sucrose, 1 mM EDTA, 1 mM diithiothreitol, 20 mg/ml leupeptin, 2 mg/ml antipain, and 1 mg/ml pepstatin (pH 7.0)]. Cellular homogenates were obtained by centrifugation at 18,000 g at 4°C for 10 min and subjected to triglyceride (TG) hydrolase assay. For determination of the cellular lipase activity, 50 μg (protein) of cell extracts in a total volume of 100 μl lysis buffer were incubated with equal volume of TG standard (1 mM) in a water bath at 37°C for 30 min. After incubation, the reaction was stopped and subjected to analysis for glycerol by the method mentioned above.

**Adipocytokines Enzyme-Linked Immunosorbent Assay**

Concentrations of tumor necrosis factor (TNF-α), interleukin (IL)-6, and monocyte chemotactic protein-1 (MCP-1) in the supernatants were measured using mouse-specific enzyme-linked immunosorbent assay kits (R&D, Minneapolis, MN) according to the manufacturer’s protocols. Results were normalized by cellular protein levels.

**RT-PCR**

For semiquantitatively measuring mRNA expression of the adipocytokines, total RNA was extracted from the 3T3-L1 adipocytes using Trizol reagent (Invitrogen). RT-PCR was performed as described previously (45). Briefly, 2 μg of each RNA sample were converted into cDNA using the RT-PCR Kit (Invitrogen, La Jolla, CA). Equal amounts of cDNA were subsequently amplified by PCR reactions using gene-specific primer pairs. PCR products that were resolved in 2% agarose gels were photographed under ultraviolet light. Densities of bands were measured by scanning densitometry with UVIBAND version 99 Software (UVI, SJ, Cambridge, UK). For each sample, 18S rRNA was used as an internal control. Primers for specific genes were designed to cross intron-exon boundaries and used to generate amplicons in their linear ranges as follows: TNF-α (464-bp product), forward, 5'-CAAGTCTGATGCACTTCACC-3' and reverse, 5'-GAGGAGTACGCTGGAGCATC-3'; IL-6 (285-bp product), forward, 5'-AACGATGATGCACTTGGCAGA-3' and reverse, 5'-GAGGTATTGGATAATGTTG-3'; MCP-1 (411-bp product), forward, 5'-ATGGAAGATGCTGATTTGG-3' and reverse, 5'-GGGAGCTGGAGAAACGCC-3'; and reverse, 5'-GGGAGCTGGAGAAACGCC-3'.

**Protein Degradation of Skeletal Muscle Cells**

**Culture of skeletal muscle myoblasts**. Mice skeletal muscle C2C12 myoblast cells (American Type Culture Collection) at passage 3–8 were differentiated and labeled in 2% horse serum DMEM containing 10% FBS, 4.5 g/l glucose, 100 U/ml penicillin, 100 μg/ml streptomycin, and 4 mm glutamine in a 95% O2,5% CO2 humidified atmosphere. Cell differentiation by myotubes was induced by growing myoblasts to 70–80% confluence and replacing the growth medium with DMEM supplemented with 2% horse serum as described previously (45).

**Protein degradation**. Protein degradation in cultured myotubes was measured using cells prelabeled with L-[U-14C]phenylalanine as described previously (45). Briefly, C2C12 cells at 70–80% confluence were differentiated and labeled in 2% horse serum DMEM containing L-[U-14C]phenylalanine for 72 h. After the labeling period, all cells were subjected to a 2-h incubation in DMEM containing 2% horse serum and 2 mM nonradioactive L-phenylalanine (to minimize reutilization of released L-[U-14C]phenylalanine) to remove L-[14C]phenylalanine released from short-lived proteins. The myotubes were incubated with 3 ml of conditioned medium for 24 h. L-[14C]phenylalanine released from labeled proteins was measured after precipitation of proteins with TCA (10% vol/vol). At the end of the sampling period, cell monolayers were solubilized in 1% SDS to determine the radioactivity remaining in the cells, and the rate of protein degradation was determined by calculating the slope of the logarithm of [14C]phenylalanine remaining in cell protein vs. time points.

Protein degradation was also determined in myotubes treated with conditioned medium in the presence or absence of the neutralizing antibodies against TNF-α (the maximal neutralizing activity, 5 μg/ml), IL-6 (the maximal neutralizing activity, 1 μg/ml), or MCP-1 (the maximal neutralizing activity, 30 μg/ml) (R&D).

**Analysis of actin cleavage**. Protein was harvested in hypotonic buffer for Western blots to detect the 14-kDa actin fragment (45). Myotubes were suspended in buffer consisting of 20 mM HEPES (pH 7.5), 10 mM MgCl2, 1 mM EDTA, 1 mM EGTA, 1 mM diithiothreitol containing 0.1 mM phenylmethylsulfonyl fluoride, 5 μg/ml antipain, 5 μg/ml leupeptin, and 5 μg/ml aprotinin. The cells were kept on ice for 30 min. The swollen cells were gently homogenized, and the nuclei and debris were removed by centrifugation. The actin cleavage was measured by Western blotting with an anti-actin antibody that recognizes the carboxy-terminal 11 amino acids of α-actin (Sigma).
Statistical Analysis

Data were presented as means ± SD of three independent experiments. All data were analyzed with SPSS 11.0 for Windows. Difference in mean values between groups was tested using one-way ANOVA. To identify significant difference between two groups, comparisons were made using Student’s t-test. P values <0.05 were considered significant.

RESULTS

ADMA Increased Lipolysis in Association with Decreased Perilipin A

Lipolysis is a catabolic process leading to breakdown of TGs into nonesterified fatty acids and glycerol. Thus the level of glycerol released by adipocytes can serve as a marker of lipolysis (9). As shown in Fig. 1, A and B, exposure of adipocytes to ADMA induced glycerol release in a dose- and time-dependent manner. To determine the mechanisms underlying the ADMA-induced lipolysis, we examined the expression of HSL and ATGL, the essential regulators of lipolysis (29), and the total cellular lipase activity. The expression of perilipin A, the most abundant protein that coats the surfaces of lipid droplets and regulates the rate of basal lipolysis in adipocytes (6), was also examined. Treatment with ADMA (5 and 40 μM, 24 h) had no effect either on the expression of HSL or ATGL (Fig. 1, C and D) or on the total cellular lipases activity (Fig. 1E). However, the expression of perilipin A was decreased significantly in ADMA-treated cells (Fig. 1D). Adipocyte integrity, assessed by lactate dehydrogenase activity, was not affected when cells were incubated with ADMA at the concentration used in the study (data not shown).

ADMA Induced Inflammatory Responses Through Activation of NF-κB

Inflammatory responses, such as overexpression of adipocytokines, have been demonstrated in adipocytes under certain stimulation (24). In addition, proinflammatory cytokines, such as TNF-α and IL-6, have been demonstrated to induce lipolysis (35, 41). We therefore examined the expression of proinflammatory adipocytokines in the ADMA-treated adipocytes. The adipocytes were incubated with ADMA (5 and 40 μM) for 24 h. As shown in Fig. 2, A–C, treatment with ADMA induced overexpression of TNF-α, IL-6, and MCP-1 at both mRNA and protein levels.
Because NF-κB is the major signal mediator that plays a critical role in the regulation of inflammatory cytokine expression (20), we examined whether NF-κB activation is involved in the ADMA-induced inflammatory responses in adipocytes. The cells were incubated with ADMA (5 and 40 μM) for 24 h. As presented in Fig. 2, ADMA stimulation increased phosphorylation of IkBα, decreased the abundance of IkBα (Fig. 2D), and increased phosphorylation of IKK (Fig. 2E) and NF-κB p65 (Fig. 2F). These data indicate that ADMA triggered activation of NF-κB in adipocytes.

To further determine the relationship between the activation of NF-κB and the overexpression of TNF-α, IL-6, and MCP-1, adipocytes were incubated with ADMA (40 μM) for 24 h in the presence or absence of NF-κB SN50, a cell-permeable specific inhibitor of NF-κB translocation (21). SN50 inactive peptide (control SN50) was used as the control. As shown in Fig. 2G, incubation of adipocytes with NF-κB SN50, but not control SN50, completely abolished the ADMA-induced overexpression of the proinflammatory adipocytokines at both mRNA (Fig. 2G) and protein levels (Fig. 2H), suggesting that induction of the adipocytokines is dependent on activation of NF-κB.

Previous studies have shown that inflammatory cytokines such as TNF-α and IL-6 induce lipolysis (35, 41). To test whether ADMA-induced lipolysis was mediated by the NF-κB activation-associated adipocytokines, the glycerol levels in the supernatants were determined following incubation of adipocytes with ADMA (40 μM) in the presence or absence of NF-κB SN50. As shown in Fig. 2I, addition of NF-κB inhibitor partly inhibited the ADMA-induced glycerol release, indicating that the inflammatory responses might be partially involved in the ADMA-induced lipolysis. Because activation of extra-cellular signal-related kinase (ERK) has been shown to be involved in IL-6- and TNF-α-induced lipolysis (39, 41), we examined the glycerol release in adipocytes incubated with ADMA in the presence or absence of PD-98059, a specific inhibitor of ERK. As shown in Fig. 2I, treatment with PD-98059 partly inhibited the ADMA-induced glycerol release, indicating that activation of ERK might be partially involved in the ADMA-induced lipolysis.

ADMA Triggered Adipocyte Perturbation Through Induction of ER Stress

There is increasing evidence showing that ER stress is linked to adipocyte dysfunctions (13). We next examined whether ER stress was induced in ADMA-challenged adipocytes. Cells were incubated with ADMA (5 and 40 μM) for 12 h. The ER stress markers (13, 25), PERK, eIF2α, and JNK, and expression of GRP78 were determined by Western blot. As shown in Fig. 3, the ER stress markers were increased significantly in ADMA-treated adipocytes compared with cells in medium alone, suggesting that ER stress was induced in ADMA-treated cells.

To test the role of ER stress in the ADMA-induced lipolysis and inflammatory responses in ADMA-challenged adipocytes, we first examined the time course of ER stress (represented by phosphorylation of PERK), lipolysis (represented by glycerol release), and expression of adipocytokines (represented by protein levels of TNF-α, IL-6, and MCP-1). The results showed that 1 h treatment with ADMA was sufficient to induce a statistically significant increase in phosphorylation of PERK. This kinase phosphorylation was progressively increased over time and remained stable after 3 h (Fig. 4A). In contrast, a significant increase in lipolysis appeared after 6 h of ADMA treatment (Fig. 4B). Similar results were observed for the expression of TNF-α, IL-6, and MCP-1 (Fig. 4C). These data suggested that ER stress preceded the increase in lipolysis and expression of the adipocytokines induced by ADMA.

To further confirm the causative role of ER stress in the ADMA-induced lipolysis and inflammatory responses, we evaluated the glycerol release and expression of TNF-α, IL-6, and MCP-1 following incubation of adipocytes with ADMA in the presence or absence of tauroursodeoxycholic acid (TUDCA), a chemical chaperone that has been demonstrated to suppress the ER stress in cultured adipocyte and in a diabetic model (26). Treatment with TUDCA completely blocked the ADMA-induced expression of TNF-α, IL-6, and MCP-1 at both mRNA (Fig. 4D) and protein (Fig. 4E) levels. Addition of TUDCA also suppressed the ADMA-triggered phosphorylation of IκB and IKK (Fig. 4, F and G) but preserved the abundance of IκB (Fig. 4F). Furthermore, treatment with TUDCA prevented the ADMA-induced decrease in perilipin A (Fig. 4H) and inhibited the ADMA-induced glycerol release (Fig. 4I). These data suggested that the ADMA-induced lipolysis and inflammatory responses might be mediated by induction of ER stress.

Conditioned Medium From ADMA-Challenged Adipocytes Induced Protein Degradation in Cultured Myotubes

It has been demonstrated that there is cross talk between adipocytes and skeletal muscle cells (30, 31, 45). To explore whether the ADMA-induced adipocyte perturbation affected the skeletal muscle protein metabolism, we examined the effect of conditioned medium from the ADMA-challenged adipocytes on protein degradation in cultured myotubes. Adipocytes were incubated with ADMA (5 and 40 μM) for 24 h. The cells were washed and then incubated with fresh DMEM without ADMA for 2 h. The supernatants were harvested and served as the conditioned medium. The supernatants collected from adipocytes cultured in medium alone served as the controls. As shown in Fig. 5A, the conditioned medium from both 5 and 40 μM ADMA-treated adipocytes significantly increased protein degradation in cultured myotubes. Furthermore, the marker of 
proteolytic process in muscle catabolism, a 14-kDa actin band (28), was also significantly increased in conditioned medium from 5 and 40 µM ADMA-treated myotubes (Fig. 5, B and C).

To further confirm the effect of ADMA-induced adipocyte perturbation on protein metabolism of myotubes, protein degradation was determined in myotubes treated with conditioned medium from adipocytes incubated with ADMA for 1–6 h. As shown in Fig. 5D, protein degradation of myotubes was increased significantly in cells treated with conditioned medium of 6 h ADMA exposure, but not affected by conditioned medium from adipocytes incubated with ADMA for shorter than 6 h.

Protein degradation was also determined in myotubes treated with conditioned medium from 40 µM ADMA in the presence or absence of various neutralizing antibodies against individual adipocytokines. Inhibition of TNF-α, IL-6, and MCP-1 with their neutralizing antibodies decreased the protein degradation by 28.6, 22.6, and 18.8%, respectively (Fig. 5E), suggesting that the ADMA-induced adipocytokines expression was partly involved in the increased protein degradation of myotubes. Taken together, these data indicate that the ADMA-induced adipocyte perturbation might contribute to protein degradation in myotubes.

DISCUSSION

Loss of body fat and deregulation of adipocytokines have been demonstrated in both CKD animal models and CKD patients (3, 15, 43). Increased recognition of the involvement of adipose tissue in energy metabolism has highlighted the importance of determining the role of adipocyte perturbation in the pathogenesis of fat catabolism and its underlying mechanisms in CKD. Here we provided the first evidence demonstrating that ADMA challenge triggered the lipolysis and inflammatory responses through induction of ER stress in adipocytes.

The concentration of ADMA employed in this experiment was higher than that seen in CKD. However, chronic exposure to the lower levels of ADMA observed in disease states appears to have similar effects on cellular biology as does short-term exposure to the higher dose used in several in vitro studies, which ranged from 10 to 100 µM (5, 32, 33, 38, 40). It has also been shown that the intracellular ADMA levels are 10-fold higher than the reported range for plasma values (5).

An important finding of the study was that the ADMA-induced lipolysis was neither associated with altered expression of HSL or ATGL, nor with total cellular lipase activity, indicating that the underlying mechanisms for the ADMA-induced lipolysis were different from that seen in other catabolic condition such as cancer cachexia, in which loss of fat mass is associated with enhanced activity of HSL (1). Our results showed that the ADMA-induced increase in lipolysis was accompanied by decreased perilipin A, a lipid droplet-associated protein that is involved in regulation of basal lipolysis of adipocyte (6). Restoring the expression of perilipin A by inhibition of ER stress completely prevented the ADMA-induced glycerol release, suggesting that the increased lipolysis might be associated with decreased perilipin A. Perilipin A functions as a “barrier” for preventing cellular lipases from interacting with their substrates (23). A previous study has also demonstrated that nelfinavir, an inhibitor of human immunodeficiency virus protease, increases adipocyte lipolysis due to decreased perilipin A (17). Although the underlying mechanism for the ADMA-induced reduction in perilipin A remains to be investigated, the present study suggests that ADMA may affect lipid homeostasis through increasing adipocyte lipolysis.
ADMA-induced adipocyte perturbation was dependent on activation of ER stress. A–C: adipocytes were incubated with ADMA (40 μM) for the indicated time. Time courses of ADMA-induced phosphorylation of PERK (A), glycerol release (B), and expression of adipocytokines (C) are shown. D–I: adipocytes were treated with ADMA (40 μM) for 24 h in the presence or absence of ER stress inhibitor tauroursodeoxycholic acid (TUDCA, 1 mM). Addition of TUDCA completely blocked ADMA-induced overexpression of the adipocytokines at both mRNA (D) and protein (E) levels. TUDCA treatment abolished ADMA-triggered phosphorylation of IκBα and preserved the abundance of IκBα (F), blocked ADMA-triggered phosphorylation of IKKα/β (G), prevented the ADMA-induced decrease in perilipin A (H), and blocked ADMA-induced lipolysis (I). Data are expressed as means ± SD of three independent experiments. *P < 0.01 and **P < 0.05 vs. control. #P < 0.01 vs. ADMA-treated group.

Fig. 4. ADMA-induced adipocyte perturbation was dependent on activation of ER stress. A–C: adipocytes were incubated with ADMA (40 μM) for the indicated time. Time courses of ADMA-induced phosphorylation of PERK (A), glycerol release (B), and expression of adipocytokines (C) are shown. D–I: adipocytes were treated with ADMA (40 μM) for 24 h in the presence or absence of ER stress inhibitor tauroursodeoxycholic acid (TUDCA, 1 mM). Addition of TUDCA completely blocked ADMA-induced overexpression of the adipocytokines at both mRNA (D) and protein (E) levels. TUDCA treatment abolished ADMA-triggered phosphorylation of IκBα and preserved the abundance of IκBα (F), blocked ADMA-triggered phosphorylation of IKKα/β (G), prevented the ADMA-induced decrease in perilipin A (H), and blocked ADMA-induced lipolysis (I). Data are expressed as means ± SD of three independent experiments. *P < 0.01 and **P < 0.05 vs. control. #P < 0.01 vs. ADMA-treated group.
Another new finding of the study was that ADMA triggered the inflammatory responses in adipocytes, which was partly involved in the enhanced lipolysis. Exposure of adipocytes to ADMA significantly increased the expression of proinflammatory adipocytokines, such as TNF-α, IL-6, and MCP-1, at both mRNA and protein levels. ADMA-induced overexpression of the adipocytokines was mediated by activation of NF-κB, since inhibition of NF-κB completely blocked the inflammatory responses. It is interesting to note that inhibition of NF-κB activation significantly improved, but failed to completely restore, the ADMA-induced lipolysis. Moreover, inhibition of ERK activation, which has been demonstrated to mediate the TNF-α and IL-6-induced lipolysis (39, 41), also improved the ADMA-induced lipolysis. Consistent with our finding, previous studies demonstrate that proinflammatory cytokines, such as TNF-α and IL-6, increase lipolysis in cultured adipocytes (35, 41). The underlying mechanisms for TNF-α-induced lipolysis are multiple, including activation of protein kinase A, ERK, and inhibition of perilipin expression (8). The presence of low-grade inflammation, characterized by an increase in plasma proinflammatory cytokines, has been well documented in patients with CKD (18). The finding that ADMA triggered inflammatory responses in adipocytes supports the hypothesis that fat tissue might be a source of chronic inflammation in CKD (2). Given the fact that ADMA accumulation is closely correlated with chronic inflammation (7, 46), which has been demonstrated to contribute to the pathogenesis of PEW (11, 36), it seems reasonable to propose that ADMA accumulation might be involved in CKD-associated PEW through increasing lipolysis and promoting inflammation in adipocytes.

The mechanisms by which ADMA promotes lipolysis and inflammation in adipocytes remain to be studied. Here, we...
provided several lines of evidence demonstrating that ADMA triggered adipocyte perturbation through induction of ER stress. First, exposure of adipocytes to ADMA induced activation of the key processes of ER stress, including phosphorylation of PERK, eIF2α, and JNK, and upregulation of GRP78 expression, suggesting that ER stress was induced in the ADMA-challenged cells. Second, activation of ER stress preceded the increase in lipolysis and inflammatory responses in the ADMA-treated adipocytes, indicating that ER stress was the upstream event of the cell perturbation. Third, inhibition of ER stress completely blocked the ADMA-induced lipolysis and inflammatory responses, suggesting that ER stress played a central role in the ADMA-induced adipocyte perturbation.

The ER is a specialized cytosolic organelle in which various metabolic signals are integrated to regulate lipid, glucose, cholesterol, and protein metabolism. Certain pathological stimulation, such as hypoxia and lipid metabolism abnormalities, can disrupt ER homeostasis and trigger ER stress (14, 16, 44). ER stress has recently been shown to be involved in the inflammatory network (10, 12, 42) and linked to adipose tissue dysfunction (4, 13).

It has been demonstrated that adipose-muscle cross talk plays an important role in the regulation of muscle protein metabolism (30, 31, 45). To determine whether the ADMA-induced adipocyte perturbation affected muscle protein catabolism, we examined the effect of conditioned medium from ADMA-challenged adipocytes on protein degradation in cultured myotubes. Protein degradation was only observed in myotubes treated with conditioned media from adipocytes incubated with ADMA for at least 6 h, which was consistent with the time when the ADMA-induced inflammatory response and lipolysis occurred. These results suggest that molecules released by the ADMA-challenged adipocytes may increase skeletal muscle protein catabolism. Partial inhibition of protein degradation by neutralizing TNF-α, IL-6, or MCP-1 further supports involvement of the adipocytokines in myotube protein degradation. Given the early report that free fatty acid induced protein degradation in cultured myotubes (45), there is a possibility that release of free fatty acid during lipolysis might also play a role in the pathogenesis of protein degradation. Whether ADMA has direct effect on protein degradation of myotubes is an interesting and important issue that needs to be studied further.

In summary, elevated ADMA increased lipolysis and inflammatory responses via induction of ER stress in cultured adipocytes. The ADMA-induced adipocyte perturbation positively modulated protein catabolism of skeletal muscle cells. ADMA accumulation is prevalent in advanced CKD in which PEW is a common risk factor for mortality. Thus, identifying the effects of ADMA on abnormalities of adipocyte metabolism, which is linked to PEW in CKD, might be an important step toward understanding the pathobiological effects of ADMA and may provide new targets for intervention of PEW.

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