GLUCOSE-BASED PERITONEAL DIALYSIS SOLUTION SUPPRESSES ADIPONECTIN SYNTHESIS THROUGH OXIDATIVE STRESS IN AN EXPERIMENTAL MODEL OF PERITONEAL DIALYSIS

Joo Young Huh,¹ Eun-Young Seo,¹ Hi Bahl Lee,² and Hunjoo Ha¹

Division of Life and Pharmaceutical Sciences,¹ Department of Bioinspired Science, College of Pharmacy, Ewha Womans University, Seoul; Kim's Clinic and Dialysis Unit,² Miryang, South Korea

◆ **Objective:** Accumulation of visceral fat is one of the major risk factors for the development of cardiovascular disease in peritoneal dialysis (PD) patients. Adiponectin, an adipokine commonly regarded as a negative indicator of metabolic disease, is reported to be downregulated in its gene level in end-stage renal disease patients. Since excessive fat deposit is involved in increased reactive oxygen species (ROS), PD solution (PDS) may contribute to ROS production, resulting in dysregulation of adiponectin. In this study, we tested our hypothesis that oxidative stress induced by PDS may play a role in the regulation of adiponectin.

◆ **Methods:** Commercial PDS containing 3.86% glucose (20 – 30 mL) was administered to SD rats for 12 weeks with and without N-acetylcysteine (NAC; 10 mmol/L). ELISA was used to quantify adiponectin in plasma and spent dialysate. For *in vitro* studies, fully differentiated 3T3-L1 adipocytes and adipocytes isolated from abdominal fat were treated with a high glucose solution, PDS, and H₂O₂. Adiponectin levels in the conditioned media were measured by ELISA and immunoblot assays. The mRNA levels of adiponectin in mature adipocytes were examined using real-time RT-PCR.

◆ **Results:** The levels of adiponectin in plasma and spent dialysate were significantly downregulated by PDS and this effect was suppressed by NAC. In 3T3-L1 adipocytes, adiponectin secretion was inhibited by 50 mmol/L glucose, PDS diluted 2-fold, and H₂O₂ (200 μmol/L). In addition, H₂O₂ downregulated expression of adiponectin mRNA and secretion of adiponectin oligomer complexes.

◆ **Conclusions:** Our data suggest that ROS induced by conventional glucose-based PDS may contribute to pathophysiological changes in abdominal fat and downregulate adiponectin secreted from adipocytes during long-term PD.

KEY WORDS: Adiponectin; N-acetylcysteine; reactive oxygen species.

Peritoneal dialysis (PD) patients are characterized by accumulation of intra-abdominal fat (1,2). Studies have highlighted that visceral adipose tissue produces large amounts of proinflammatory cytokines, such as interleukin-6, which in turn are linked to increased incidence of cardiovascular disease (CVD) and death (3,4). Since elevated serum levels of proinflammatory cytokines have been demonstrated to be associated with increased mortality in dialysis patients (5–8), accumulation of visceral fat could be considered one of the major risk factors for development of CVD in PD patients.

Adiponectin is a 244 amino acid, 30 kDa protein encoded by the *apM1* gene and belongs to a group of adipocyte-specific secretory proteins known as “adipokines” (9). Adiponectin circulates in human plasma in three major oligomeric forms: a low-molecular weight (LMW) trimer, a middle-molecular weight (MMW) hexamer, and high-molecular weight (HMW) 12- to 18-mers (10). In obese and type 2 diabetic mice and humans, plasma adiponectin levels are reported to be significantly decreased, whereas other inflammatory adipokines, such as tumor necrosis factor-α and plasminogen activator inhibitor-1, are increased (11,12). Therefore, reduction in circulating adiponectin level is commonly regarded as an indicator of metabolic diseases such as insulin resistance, hypertension, and CVD. Moreover, it is thought that the HMW adiponectin complex is the active form of this protein and that the ratio of HMW to total adiponectin (HMW + LMW) is more accurate than total adiponectin level in predicting insulin sensitivity, inflammation, and atherosclerosis (13–15).

During dialysis, the solutes contained in PD fluids are transferred by passive diffusion through the peritoneal membrane and make contact with intra-abdominal adipose tissue (16). It has been reported that adiponectin gene expression in fat tissue is significantly

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Correspondence to: H. Ha, Department of Bioinspired Science, Division of Life and Pharmaceutical Sciences, and College of Pharmacy, Ewha Womans University, 11-1 Daehyun-Dong, Seodaemun-Gu, Seoul 120-750, Korea.
hha@ewha.ac.kr

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downregulated in end-stage renal disease (ESRD) patients compared to healthy control patients, although circulating adiponectin is increased, possibly due to decreased renal excretion (17,18). Moreover, the level of plasma adiponectin is reduced after initiation of PD (19). It is therefore important to understand the mechanisms involved in abnormal regulation of adiponectin during PD in order to provide a novel strategy to prevent cardiovascular morbidity and mortality in PD patients.

Reactive oxygen species (ROS) induced by glucose-based conventional PD solution play an important role in structural and functional alterations in peritoneal membrane during long-term PD (20,21). In our previous study, we demonstrated that ROS generated by glucose-containing conventional PD solution are responsible for peritoneal fibrosis and membrane hyperpermeability in a rat model where the peritoneum was exposed to PD solution for over 12 weeks (22). On the other hand, excessive fat accumulation is involved in increased oxidative stress, which results in dysregulation of adipokines such as adiponectin (23). Several clinical studies have elucidated the relationship between PD and adiponectin levels in chronic kidney disease (CKD) and ESRD patients (19). Since renal function in these patients is severely damaged, it is hard to delineate the direct effect of PD on the regulation of adiponectin. Therefore, in this study we used a rat PD model with normal functioning kidney to exclude the confounding effect of renal secretion in regulating adiponectin levels. We further examined the direct effect of PD solution on adiponectin synthesis using a differentiated 3T3-L1 adipocyte model and adipocyte fraction isolated from abdominal fat pads. We tested our hypothesis that PD solution contributes to pathophysiological changes in abdominal fat and that ROS may play an important role in downregulation of adiponectin.

MATERIALS AND METHODS

Unless otherwise stated, all chemicals and materials were obtained from Sigma Chemical Company (St. Louis, MO, USA) and Nunc (Rochester, NY, USA).

ANIMALS AND TREATMENT

The rat PD model that we previously reported (22) was utilized. Male Sprague-Dawley rats weighing 200 g were randomized to three groups ($n = 8$ for each group): control, PD solution, and PD solution with the antioxidant N-acetylcysteine (NAC). Commercial PD solution containing 3.86% glucose (Dianeal PD-2; Baxter Korea, Seoul, Korea) was administered intraperitoneally (20 – 30

mL, depending on body weight changes over the study period) with or without NAC (10 mmol/L) twice per day for 12 weeks. At the end of 12 weeks, body weights of control rats, PD solution-injected rats, and PD solution with NAC injected rats were 440 ± 28 g, 405 ± 32 g, and 423 ± 25 g. pH of PD solution was neutralized to 7.4 with NaOH to exclude possible detrimental effects of low pH (24). The control group received a sham injection (puncture of the peritoneal cavity without PD solution) twice per day for 12 weeks. Blood and spent dialysate were collected and frozen until analysis. Peritoneal equilibrium tests were performed using 30 mL 3.86% glucose PD solution. After 4 hours, a tail blood sample and a dialysate sample were obtained. Drained volume was calculated as the sum of peritoneal fluid aspirated and the weight of (wet – dry) gauze at 4 hours. Glucose was measured using a glucose oxidase method.

ADIPOCYTE DIFFERENTIATION AND TREATMENT

Murine 3T3-L1 preadipocytes were obtained from ATCC (American Type Culture Collection, Manassas, VA, USA). 3T3-L1 cells were maintained in Dulbecco's modified Eagle medium (DMEM; Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 g/mL streptomycin (complete medium) at 37°C in 5% CO₂. Two days post-confluent (day 0) cells were induced to differentiate by addition of a hormonal cocktail (0.5 mmol/L 3-isobutyl-1-methyl-xanthine, 1 µmol/L dexamethasone, and 5 µg/mL insulin) for 2 days and then incubated for an additional 2 days with only 5 µg/mL insulin added to the complete medium. From day 4, the cells were maintained in a complete medium until further experiments. Images of cells were captured using an EVOS microscope (AMG, Mill Creek, WA, USA) up to day 10. To test the secretion of adiponectin, differentiated 3T3-L1 adipocytes were treated with high D-glucose (30 and 50 mmol/L), mannitol (50 mmol/L), fresh PD solution diluted twofold with DMEM, and H₂O₂ (200 and 500 µmol/L) up to 24 hours, and then the cell culture media were collected and frozen until analysis.

ISOLATION OF ADIPOCYTE FRACTION AND EX VIVO ANALYSIS

Adipocytes were isolated from the abdominal (epididymal + omental) fat pads of mice using collagenase by a modified method (25). For fractionation of adipocytes from stromal vascular cells, fat pads were placed in HEPES-buffered DMEM containing 1 mg/mL collagenase type I (Worthington, Lakewood, NJ, USA) and minced with gentle agitation for 30 minutes. The suspension was then passed through a sterile 250-µm nylon mesh

and centrifuged at 500g for 10 minutes. The floating cells were collected as adipocytes for the *ex vivo* experiments. Adipocyte fractions from each mouse were separated into two groups and incubated for 15 hours in DMEM with or without H₂O₂ (200 µmol/L).

OIL RED O STAINING

Oil Red O staining was performed to monitor the progression of adipocyte differentiation. Briefly, on days 0, 4, and 10, cells were washed with PBS and then fixed using 10% formaldehyde for 1 hour. Oil Red O (0.3%) was prepared in isopropanol, mixed with water at a 3:2 ratio, and filtered through a 0.22-µm filter (Millipore, Bedford, MA, USA). The fixed cells were incubated with Oil Red O for 10 minutes at room temperature and then washed with water. The stained fat droplets in the cells were visualized by light microscopy and photographed.

ADIPONECTIN PROTEIN QUANTITATION IN PLASMA, SPENT DIALYSATE, AND CELL CULTURE MEDIA

A commercial ELISA kit for rat adiponectin (R&D Systems, Minneapolis, MN, USA) was used to quantify adiponectin in plasma and spent dialysate and a kit for mouse adiponectin (R&D Systems) was used for 3T3-L1 adipocyte culture media according to manufacturers' instructions. Confirmed by the manufacturer, the ELISA kit for adiponectin measures all oligomeric forms of adiponectin, including HMW adiponectin. Intra-assay coefficients of variance (CV) were 5.8% – 6.6% (*n* = 20) and interassay CVs were 5.0% – 6.4% (*n* = 23).

WESTERN BLOT ANALYSIS

Multimer adiponectin was analyzed under non-reducing conditions. The media collected from differentiated 3T3-L1 cells were centrifuged at 13 000 rpm for 5 minutes to remove cell debris. The media were mixed with sample buffer (100 mmol/L Tris-HCl, pH 6.8, 4% SDS, 20% glycerol, and 1% bromophenol blue) and incubated at room temperature for 10 minutes. Protein content was determined from cell lysates using the Bradford method (Bio-Rad, Hercules, CA, USA); media equivalent to 20 µg of cell lysates were loaded. Proteins were separated on NuPAGE 4% – 12% Bis-Tris gel (Invitrogen) and transferred to a nitrocellulose membrane. The blots were blocked with 5% skim milk in Tris-buffered saline with 0.1% Tween 20 (TBST) and incubated with adiponectin antibody (Adipogen, Incheon, Korea), at a dilution of 1:1000 in TBST with 1% bovine serum albumin, at 4°C overnight. Subsequently, blots

were washed with TBST and incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG (Santa Cruz Biotechnology, Santa Cruz, CA, USA), at a dilution of 1:2000 in TBST with 5% skim milk, for 1 hour at room temperature. Immunoreactive proteins were detected and visualized using enhanced chemiluminescence detection reagents (Amersham Biosciences, Buckinghamshire, UK).

REVERSE TRANSCRIPTION (RT)-PCR AND REAL-TIME PCR

Total RNA was prepared from 3T3-L1 adipocytes and isolated adipocyte fraction using Trizol reagent (Invitrogen) following the manufacturer's protocol. Total RNA (1 µg) was reverse-transcribed with 0.5 µg oligo dT₁₂₋₁₈ primers (Invitrogen), 1 mmol/L dNTP mix, 0.01 mol/L DTT, ribonuclease inhibitor (RNaseOUT), and RevertAid M-MuLV reverse-transcriptase (Fermentas, Glen Burnie, MD, USA). 3T3-L1 cDNA transcripts were used for PCR amplification using the Accupower HF PCR kit (Bioneer, Seoul, Korea) and iCycler (Bio-Rad). The sequences for the primers are as follow: adiponectin forward 5'-AAG GACAAGGCCGTTCTCT-3' and reverse 5'-ATGGGTAGTTGC AGTCAGTTGG-3'; β-actin forward 5'-AGGTCATCACTA TTGGCAAC-3' and reverse 5'-ACTCATCGTACTCCTGCTTG-3'; GAPDH forward 5'-TGCACCACCAACTGCTTAG-3' and reverse 5'-GGATGCAGGGATGATGTTC-3'. The amplification profile involved 25 cycles of denaturation at 95°C for 20 seconds, primer annealing at 56°C (adiponectin and β-actin) or 53°C (GAPDH) for 20 seconds, and extension at 72°C for 20 seconds. Final PCR products were electrophoresed in 1% agarose gel containing 0.2 µg/mL ethidium bromide. Quantification of mRNA bands was performed by densitometry using the TINA 2.0 Image program.

For mouse adipocyte fraction analysis, real-time PCR was performed using ABI7300 (Applied Biosystems, Carlsbad, CA, USA) with a 20-µL reaction volume consisting of cDNA transcripts, primer pairs as described above, and the SYBR Green PCR Master Mix (Applied Biosystems). For each run, the samples underwent 40 amplification cycles and fluorescence was acquired each cycle at 60°C during the amplification step. Quantifications were normalized to β-actin in each reaction.

STATISTICAL ANALYSES

The mean values obtained from each group were compared by analysis of variance with subsequent Fisher's significant difference method. Nonparametric analyses were also used where appropriate. Data are presented as mean ± SE. A *p* value of < 0.05 was used as the criterion for a statistically significant difference.

RESULTS

PLASMA AND SPENT DIALYSATE ADIPONECTIN LEVELS IN RATS THAT UNDERWENT EXPERIMENTAL PD

To examine the effect of PD solution on adiponectin level in an *in vivo* model, rats were treated with PD solution for 12 weeks as previously described (22). At the end of 12 weeks, rats treated with PD solution had significantly lower drain volumes compared to control rats; addition of NAC (10 mmol/L) prevented the decrease in drain volumes [Figure 1(a)]. Plasma glucose levels were not significantly different among the three groups [Figure 1(b)]. Plasma adiponectin levels were significantly reduced in the PD solution-injected group compared to the sham group; this PD-induced downregulation of adiponectin was effectively suppressed by NAC [Figure 1(c)]. Adiponectin levels in spent dialysate were also significantly reduced by PD; NAC effectively suppressed this downregulation [Figure 1(d)].

ADIPONECTIN SECRETION IN DIFFERENTIATED 3T3-L1 ADIPOCYTES

To study the direct effects of PD solution on adipocytes, we used the murine 3T3-L1 preadipocyte, which is one

of the most commonly used *in vitro* models for studying the development of adipocytes and the pathogenesis of obesity (26). 3T3-L1 preadipocytes were differentiated as described in Methods. Differentiation status was closely monitored, stained with Oil Red O, and photographed on days 0, 4, and 10 [Figure 2(a)]. By day 8, more than 90% of preadipocytes had differentiated into mature adipocytes. By day 10, the adipocytes had become larger and there were more lipid droplets in the cytoplasm. Adiponectin mRNA was expressed from day 2 and increased gradually until day 6. The expression level of adiponectin mRNA was maintained up to day 10 [Figure 2(b)]. To evaluate the effect of high glucose, PD solution, and ROS on adiponectin secretion, mature 3T3-L1 adipocytes were treated with D-glucose, mannitol, fresh PD solution diluted twofold with DMEM, and H_2O_2 for 24 hours. PD solution was diluted to mimic the equilibrium that occurs shortly after infusion into the peritoneal cavity (27) and to avoid toxicity during cell culture (24). Results of ELISA showed that adiponectin secreted in the medium was significantly inhibited by D-glucose and H_2O_2 in a dose-dependent manner. Twofold-diluted PD solution also downregulated adiponectin secretion from 3T3-L1 adipocytes. In contrast, there was no change in 50 mmol/L mannitol-treated cells, suggesting that high

Figure 1 — Effect of peritoneal dialysis solution (PDS) with and without N-acetylcysteine (NAC) on drain volume (A), plasma glucose (B), and adiponectin levels in plasma (C) and spent dialysate (D) in rat. After 12 weeks of PD, a peritoneal equilibrium test using 3.86% glucose PDS was performed immediately before the rats were sacrificed; plasma and spent dialysate were collected and analyzed. Values are mean $\pm$ SE of 8 animals. * p < 0.05 versus sham, † p < 0.05 versus PDS-treated rats.

Figure 2 — Differentiation of 3T3-L1 preadipocytes. Images of mouse cells were captured on days 0, 4, and 10 by light microscope. Inset pictures represent Oil Red O staining. 3T3-L1 cells were fully differentiated at day 10 (A; right panel). Adiponectin mRNA expression level during 3T3-L1 cell differentiation was examined by RT-PCR (B). Adiponectin secretion in the media was measured by ELISA after 24-hour incubation under indicated experimental condition (C). Relative secretion was calculated as the ratio of adiponectin to protein ($\mu\text{g}/\text{mg}$). Basal adiponectin secreted in the media was $6.0 \pm 0.7 \mu\text{g}/\text{mg}$ protein. Values are mean $\pm$ SE of 4 – 5 independent experiments. * $p < 0.05$ versus control; † $p < 0.05$ versus H_2O_2 200 $\mu\text{mol}/\text{L}$. HG = high D-glucose; PDS = peritoneal dialysis solution diluted twofold with DMEM; RT-PCR = reverse-transcription polymerase chain reaction.

glucose downregulated adiponectin secretion at a cellular level independently of its osmolality [Figure 2(c)].

EFFECT OF ROS ON ADIPONECTIN SECRETION IN 3T3-L1 ADIPOCYTES

High-molecular weight adiponectin is known to be a more active form of adiponectin. Therefore, we studied secretion of adiponectin during the differentiation period using a nonreducing method. Adiponectin secretion level was highest at day 12 after differentiation of 3T3-L1 adipocytes. HMW, MMW, and LMW adiponectin secretion were all suppressed by 24-hour H_2O_2 treatment [Figure 3(a)]. The effect of oxidative stress on expression of adiponectin mRNA was measured at days 8, 10, and 12 after differentiation. The mRNA level was significantly decreased by H_2O_2 (200 $\mu\text{mol}/\text{L}$) treatment at days 10 and 12 compared to the control [Figure 3(b)]. These data suggest that ROS directly downregulate adiponectin protein and mRNA expression.

EFFECT OF ROS ON ADIPOCYTES FRACTIONATED FROM ABDOMINAL FAT

Although the 3T3-L1 cell line is an invaluable tool for *in vitro* systems, it cannot represent adipocytes in the abdominal tissue. To overcome this limitation of the *in vitro* system, adipocytes were isolated from epididymal and omental fat pads and used as an *ex vivo* strategy to evaluate the effect of ROS on secretion of adiponectin from adipocytes. Adiponectin mRNA levels were significantly downregulated by 200 $\mu\text{mol}/\text{L}$ H_2O_2 compared to the control [Figure 3(c)], which confirms the 3T3-L1 data that ROS stimulation directly reduced adiponectin expression.

DISCUSSION

The first significance of the present study is to provide an experimental PD model that supports decreased plasma adiponectin in PD patients (19). Plasma and spent

Figure 3 — Effect of H₂O₂ on adiponectin oligomer secretion (A), adiponectin mRNA expression during 3T3-L1 cell differentiation (B), and in adipocytes freshly isolated from mouse abdominal fat pads (C). The effect of H₂O₂ (H; 200 μ mol/L) on multimer, hexamer, and trimer adiponectin secretion was evaluated under nonreducing conditions. mRNA expression levels were examined by RT-PCR and real-time PCR. Values are mean $\pm$ SE of 3–4 independent experiments. * p < 0.05 versus control (C) at each time point. RT-PCR = reverse-transcription polymerase chain reaction; HMW = high-molecular weight 12- to 18-mer; MMW = middle-molecular weight hexamer; LMW = low-molecular weight trimer.

dialysate adiponectin concentrations were decreased in the animal PD model, where the peritoneum exhibited significantly lowered ultrafiltration as a result of exposure to conventional PD solution for 12 weeks.

Studies have shown that decreased adiponectin levels are inversely correlated to risk of CVD in patients with increased CVD morbidity and mortality, such as patients with dyslipidemia, essential hypertension, type 2 diabetes mellitus, and obesity (28). On the other hand, elevated plasma adiponectin levels have been demonstrated in ESRD patients before dialysis and in patients on maintenance dialysis (29–31). The reasons why ESRD patients with increased CVD morbidity and mortality have increased plasma adiponectin levels are not clear. However, one of the possibilities for CKD-associated hyperadiponectinemia could be related at least in part to reduced glomerular filtration rate and passive accumulation (32,33). This assumption is supported by the findings of significantly decreased plasma adiponectin concentrations after successful kidney transplantation

(34). Despite the elevated plasma adiponectin concentration in CKD stages 4 and 5 patients, it has been reported that adiponectin mRNA expression is decreased in these patients (17,18). Decreased plasma adiponectin in the present study can thus be partly explained by the fact that our model elucidates the effect of PD solution in the situation of normal renal function.

Decreased adiponectin in spent dialysate may reflect the diffusion of lower levels of plasma adiponectin secreted from adipocytes. It is still unclear, however, whether cells other than adipocytes contribute to plasma adiponectin (35). Adiponectin mRNA is expressed in both mature adipocytes and nonfat fractions obtained by collagenase digestion of human omental adipose tissue, but not in preadipocytes. Since adiponectin mRNA expression in nonfat fractions was 1/20–1/60 of mature adipocytes, the question remains whether cells other than adipocytes contribute to circulating adiponectin in humans. To clarify the local effect of PD solution on adipocytes, *in vitro* studies were conducted. Our *in vitro*

studies using 3T3-L1 adipocytes provided a more direct effect of PD solution on adiponectin downregulation. Teta *et al.* (36) also reported decreased adiponectin expression in 3T3-L1 adipocytes exposed to a 50:50 mixture of glucose-based PD4 1.36% (Baxter Healthcare) and M199 containing 10% serum. However, another study by the same group demonstrated twofold diluted glucose-based PD solution did not affect adiponectin secretion (27). The reason for this discrepancy is not clear at this time.

The second significance of our study is to provide evidence that ROS induced by glucose-based PD solution may play a role in reducing adiponectin expression during PD. Our data show that NAC significantly suppressed the effect of PD solution *in vivo* and that secretion of adiponectin was significantly decreased not only by PD solution but also by high glucose and H₂O₂ *in vitro*. Furthermore, direct stimulation of 3T3-L1 adipocytes with H₂O₂ decreased the secretion of adiponectin protein oligomers as well as the level of mRNA expression, and this effect seemed to be more significant as the adipocytes matured. Although the 3T3-L1 cell line is a widely used system in studies investigating the role of adipokines, strictly speaking, it does not represent adipocytes derived from abdominal fat since it is derived from the Swiss 3T3 cell line, which originated from disaggregated mouse embryo. To overcome this limitation, we isolated adipocyte fractions from epididymal and omental fat pads. Results consistently showed that H₂O₂ downregulates adiponectin mRNA expression in adipocytes from mouse abdominal fat tissues. Therefore, our data imply that glucose-containing conventional PD solution has a role in dysregulation of adiponectin in adipocytes and that it could be in part through oxidative stress. In accordance with these data, Furukawa *et al.* (23) have reported the role of ROS in adiponectin gene expression in 3T3-L1 adipocytes. Also, in a recent cross-sectional study, Barazzoni *et al.* (18) demonstrated a link between increased oxidative stress and decreased adiponectin mRNA expression in adipocytes of CKD patients. Lower adiponectin mRNA was, in turn, notably associated with lower plasma adiponectin concentration, as well as lower tissue adiponectin protein content.

It is now well recognized that obesity is characterized by increased accumulation of oxidative stress and that this in turn can result in aberrant production of adipokines, but the mechanisms involved in this process remain to be elucidated. Recently, Chen *et al.* (37) showed that oxidative stress induces Akt and JAK/STAT but not the ERK1/2 pathway in downregulation of adiponectin expression. Also, Furukawa *et al.* (23) discussed that downregulation of adiponectin expression may be partially attributed to the decreased gene expression

and smaller amount of nuclear PPAR γ under conditions of oxidative stress. This implies that oxidative stress can indeed activate signaling pathways or transcription factors to regulate adiponectin levels.

It should be considered that glucose degradation products in glucose-based conventional PD solution can generate intracellular ROS and downstream signaling. It has been reported that methylglyoxal-induced heparin-binding epidermal growth factor-like growth factor mRNA expression in rat aortic smooth muscle cells was shown to be inhibited by NAC (38), and that acetaldehyde-induced α 1(I) procollagen mRNA expression in mouse hepatic stellate cells was blocked by catalase (39). Glucose degradation products are potent precursors of advanced glycation end products, which also induce cellular ROS (40,41). The relative importance of high glucose, glucose degradation products, and advanced glycation end products in PD solution-induced ROS and subsequent suppression of adiponectin expression in mature adipocytes remains to be further studied.

In conclusion, the present data suggest that oxidative stress induced by conventional glucose-based PD solutions may play an important role in downregulation of adiponectin secreted from adipocytes during long-term PD. The present *in vivo* and *in vitro* models will lead to further studies to elucidate the mechanisms of PD solution-induced adipokine dysregulation and provide a novel strategy for inhibiting CVD in long-term PD patients.

DISCLOSURES

The authors do not have any conflicts of interest to declare.

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