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ORIGINAL ARTICLE

Adiponectin protects endothelial cells from the damages induced by the intermittent high level of glucose

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Abstract Globular adiponectin (gAd) has anti-atherogenic effects on the vascular wall. Intermittent hyperglycemia induces endothelial cells (ECs) injury but the physiological factors that may protect against ECs damage are largely unknown. In the present study, we investigated the effect of gAd on ECs dysfunction induced by intermittent high glucose. The gAd significantly attenuated intermittent high glucose-induced apoptosis and oxidative stress in human umbilical vein endothelial cells. This was achieved by decreasing caspase-3 and 3-nitrotyrosine protein expression, increasing nitric oxide (NO) secretion and phosphorylation of Akt, AMPK, and endothelial nitric oxide synthase protein expression. Pretreatment with a phosphatidylinositol 3' kinase (PI3K) inhibitor, LY294002, partly reversed adiponectin's anti-apoptotic effect. Taken together, our results indicate that gAd acts as a critical physiological factor which protects against fluctuating high glucose-induced endothelial damage. It may act via attenuating apoptosis and increasing synthesis of NO through both the PI3K/AKT and AMPK signaling pathway to reduce oxidative stress and cell apoptosis.

Keywords Globular adiponectin · Endothelial cell · Intermittent high glucose · Apoptosis · Oxidative stress

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Introduction

Endothelial cells (ECs) play a critical role in the maintenance of vascular homeostasis. Hyperglycemia has been reported to trigger and accelerate apoptosis in ECs [1]. Apoptosis in ECs is a vital event in diabetes-associated vascular diseases, and is particularly prominent in endothelial injuries caused by fluctuating hyperglycemia [2–4]. Although the protective effect of adiponectin in ECs against constant hyperglycemia is well known, the effect of adiponectin in ECs against the fluctuating glucose levels remains to be fully established.

Adipose tissue secretes a variety of adipokines to modulate endothelial function [5]. Among them, adiponectin is known to be anti-atherogenic [6]. Adiponectin is present in human plasma in two different forms: full length and globular form. Both the full-length and the C-terminal globular forms have been shown to be anti-atherogenic through protecting the cellular components of blood vessels [7]. Decreased plasma level of adiponectin is associated with endothelial dysfunction seen in type 2 diabetes mellitus (T2DM), obesity, and metabolic syndrome [8]. Circulating adiponectin level is negatively correlated with the risk level of developing coronary artery disease in type 1 diabetes mellitus (T1DM), suggesting that adiponectin protects vessels from hyperglycemia [9]. Adiponectin has been shown to oppose the adverse effects of angiotensin II (Ang II), TNF-α, and high glucose levels in induction of vascular dysfunction. [10, 11]. Although a number of studies have examined the protective role of adiponectin against the apoptosis induced by the serum starvation or Ang II in ECs [12, 13], the role of adiponectin in the fluctuating high glucose-induced damage in ECs has not been established yet. The purpose of this study is to examine the effect of gAd in the development of

endothelial dysfunction caused by the intermittent high level of glucose.

Materials and methods

Reagents

Recombinant gAD and nitrotyrosine ELISA kit were obtained from R&D system (Minneapolis, MN). LY294002 was purchased from Sigma Chemical (St. Louis, MO). Antibodies against Akt, phospho-Akt (Ser473), eNOS, phospho-eNOS (Ser113), AMPK, and phospho-AMPK (Thr172) were from Cell Signal Technology (Beverly, MA). Antibodies against β-actin were from Santa Cruz Biotechnology (Santa Cruz, CA). Caspase-3 activity assay kit, NO assay kit, and Annexin V-FITC/PI apoptosis kit were purchased from Biyuntian Company (Beijing, China).

Cell culture and treatments

Human umbilical vein endothelial cells (HUVECs) were obtained from China Center for Type Culture Collection (CCTCC). HUVECs were cultured as previously described [14]. Cells were cultured in endothelial cell basal medium-2 supplemented with 10% fetal bovine serum (FBS), 100 IU/ml penicillin, 0.1 mg/ml streptomycin, and 20 mmol/l L-glutamine at 37°C and 5% CO2. Cells in passages 4–5 were used. As noted, cells were incubated for 48 h with different glucose concentrations: normal glucose (5 mmol), constant high glucose (25 mmol), and intermittent high glucose (switching from 5 mmol to 25 mmol and back and forth every 8 h). Recombinant human gAd (1 μg/ml) was added as indicated. Equal amount of mannitol (Mann) was used as an osmotic control. Some HUVECs were pretreated with PI3 k inhibitor LY294002 (40 μmol/l) for 1 h and then incubated with intermittent high glucose together with gAd (1 μg/ml) for 48 h as noted.

MTT assay

Cellular viability was measured by methyl thiazolyl tetrazolium (MTT). HUVECs (20,000/well) were incubated in 96-well culture plates with or without gAd as indicated. Four hours before the end of the treatment, 10 μl of MTT stock solution (12 mM in phosphate buffered saline) was added to each well. At the end of the treatment, the incubation medium was removed and the formazan crystals were dissolved in 100 μl of solution of dimethyl sulfoxide (DMSO). MTT reduction was quantified by measuring the light absorbance with a multi-label plate reader at 570 nm.

Measurement of cellular apoptosis

HUVECs were cultured until 80% confluence, and were then treated with constant or intermittent high glucose in the presence or absence of gAd. Cells on glass coverslips were rinsed, fixed, and stained with fluorescence dyes using an annexin V-FITC/propidium iodide (PI) kit according to the manufacturer's instructions. Cells were visualized and photographed by using a Nikon fluorescence microscope. The rate of apoptosis was determined by the percentage of cells with hypodiploid DNA stained with PI. After centrifugation, HUVECs were gently resuspended in 1 ml of hypotonic fluorochrome solution (50 μg/ml PI, 3.4 mM sodium citrate, 1 mM Tris, 0.1 mM EDTA and 0.1% Triton X-100) and incubated in the dark at 4°C overnight before analyzed by flow cytometry (Beckerman, coulter). Apoptotic HUVECs nuclei were distinguished by their hypodiploid DNA content from the diploid DNA content of normal HUVECs nuclei. All measurements were performed under the same instrument settings.

Caspase-3 activity assay

Caspase-3 activity was measured using a commercial kit (Biyuntian Company, Beijing) according to the manufacture's instructions. HUVECs were cultured in 10 cm dishes and treated with the indicated concentrations of glucose and gAd for 48 h. Cells were dissolved in the lysis buffer and supernatants were collected to incubate with *N*-acetyl-Asp-Glu-Val-Asp-p-nitroanilide (Ac-DEVD-pNA, 200 μM) at 37°C for 2 h. The release of the chromophore *p*-nitroaniline was measured at 405 nm. Caspase-3 activity was presented as percentage of enzyme activity compared to the normal glucose group.

Measurement of NO production

HUVECs were grown in 6-well dishes until 80% confluence. Cells were treated with the indicated concentrations of glucose and gAd, and the supernatants were collected after 48 h. Levels of nitrite/nitrate and the stable end products of NO were measured using a commercial kit. In brief, nitrate was converted to nitrite with nitrite reductase, and the total nitrite was measured with the Griess reagents. The absorbance was determined at 550 nm. Cells were dissolved in 1% sodium dodecyl sulfate (SDS) and used for protein assay (BCA protein assay kit). Nitrite levels were normalized by protein contents and the results were presented as μmol per gram protein.

Nitrotyrosine measurement

Nitrotyrosine is an indirect indicator of oxidative stress. Nitrotyrosine content was evaluated by ELISA as previously reported [15]. An identical amount of protein from cell supernatant (50 µl) was applied to ELISA plates together with nitrated BSA standard and allowed to bind overnight at 4°C. After blocking, wells were incubated at 37°C for 1 h with a mouse monoclonal antibody against nitrotyrosine (5 µg/ml) and incubated for 45 min at 37°C with a peroxidase-conjugated goat anti-mouse IgG secondary antibody diluted at 1:1,000. After washing, peroxidase reaction product was generated using tetramethylbenzidine (TMB) peroxidase substrate.

Western blotting

Cells were harvested and lysed in RIPA buffer containing 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris–HCl (pH 7.4), 50 mM glycerophosphate, 20 mM NaF, 20 mM EGTA, 1 mM DTT, 1 mM Na_3VO_4, 1 mM phenylmethylsulfonyl fluoride, and protease inhibitors. Equal amounts of proteins (40 µg) were separated by SDS-PAGE (10%) and transferred to a nitrocellulose membrane. After blocking with 0.5% skimmed milk, the membrane was incubated with primary rabbit polyclonal IgG anti-total- or anti-phospho-Akt or eNOS, anti-total-AMPK or anti-phospho-AMPK, or monoclonal IgG anti-phospho-eNOS (1:1,000 dilution) at 4°C overnight. After washing, the membrane was incubated with a horseradish peroxidase-conjugated secondary antibody (1:2,000 dilution). Bands were detected by enhanced chemiluminescence (ECL) reagents (Amersham, Piscataway, NJ) and exposure to X-ray film.

Statistical analysis

Data are presented as the mean ± SD. Statistical analysis was based on one-way ANOVA analysis of variance for multiple comparisons. A value of $P < 0.05$ was considered as significant.

Results

Adiponectin protects ECs from high glucose-induced cytotoxicity

HUVECs were incubated with different levels of glucose in the presence or absence of gAd for 48 h, followed by the evaluation of cellular viability. As shown in Fig. 1a, both the constant and intermittent high levels of glucose caused a significant decrease in HUVEC viability in comparison to the normal glucose level ($P < 0.01$). The presence of gAd ameliorated the effect of the intermittent high glucose level in cell viability ($P < 0.05$), and the effect of gAd was not significantly altered by LY294002 (Fig. 1a). Adiponectin tended to reverse the reduction of cell viability caused by the constant high level of glucose without reaching statistical significance.

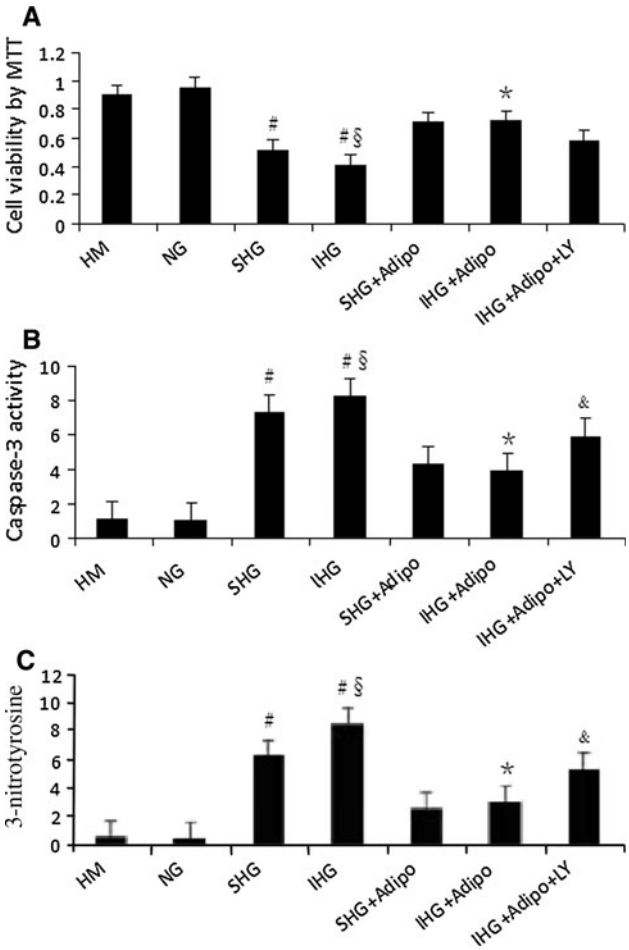

Fig. 1 Adiponectin protects HUVECs from the high glucose-induced apoptosis. HUVECs were treated with normal (5 mmol/l), high (25 mmol/l), or alternating normal/high glucose concentrations, and 1 µg/ml gAd for 48 h. **a** Cell viability assay by MTT. **b** Caspase-3 activity analysis. **c** Adiponectin inhibits oxidative stress. 3-nitrotyrosine in the media was measured by ELISA. Data represent mean ± SD from three independent experiments: $^{\#}P < 0.01$ compared with normal glucose group; $^{\S}P < 0.05$ compared with group SHG; $^{*}P < 0.05$ compared with group IHG.; $^{\&}P < 0.05$ compared with group IHG + Adipo. *HM* hyperosmotic mannitol (25 mmol/l) *NG* normal glucose (5 mmol/l) *SHG* stable high glucose (25 mmol/l) *IHG* intermittent high glucose (alternating 5 or 25 mmol/l per 8 h) Adipo adiponectin LY LY294002

Adiponectin protects HUVECs from high glucose-induced apoptosis

HUVECs were incubated with different levels of glucose in the presence or absence of gAd for 48 h, followed by

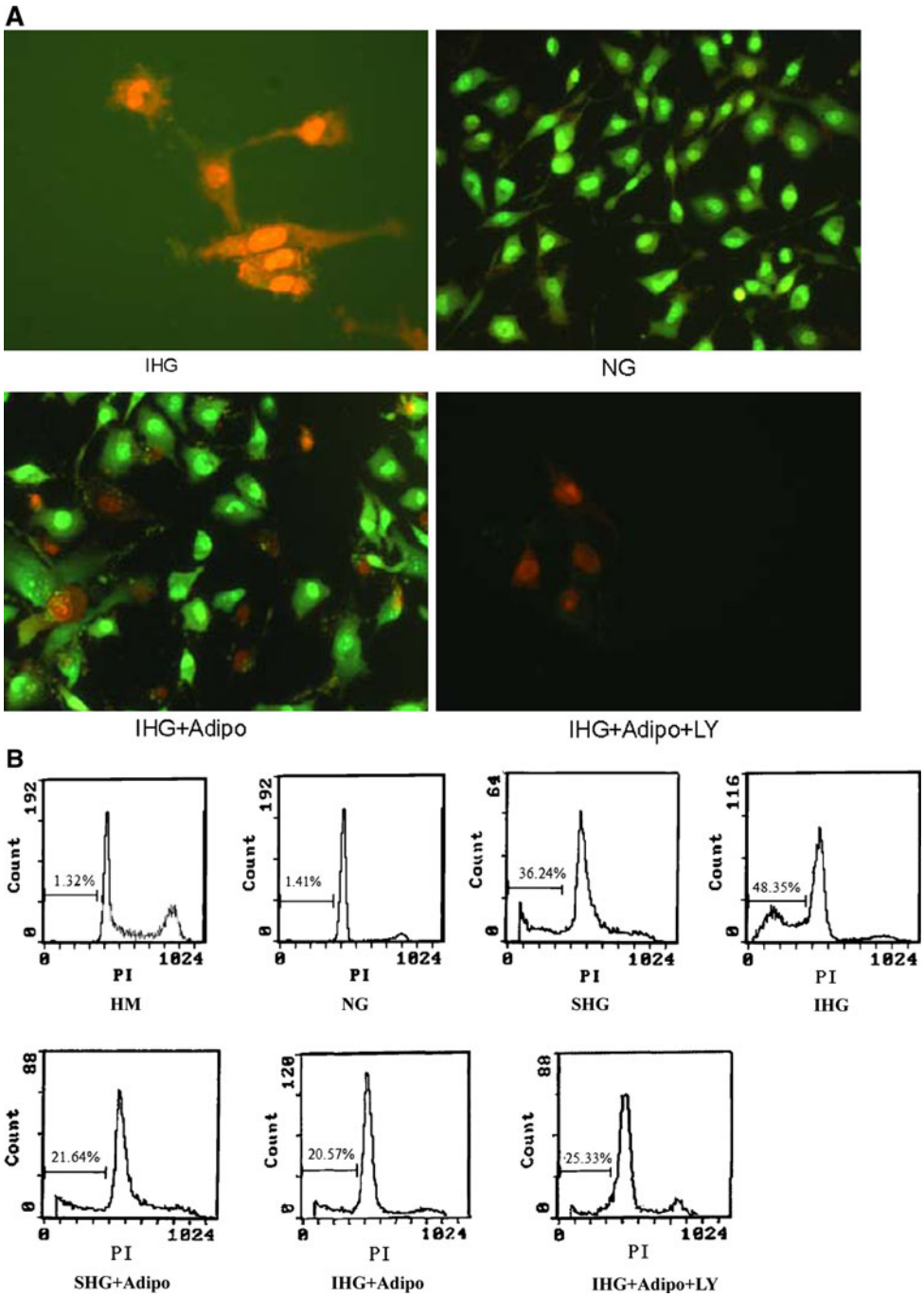

Fig. 2 Adiponectin inhibits HUVECs apoptosis. **a** Apoptosis was detected by fluorescence microscopy analysis. Apoptotic cells were observed by fluorescence microscopy (magnification × 100) analysis using propidium iodide (PI) or annexin V staining. The number of apoptotic HUVECs was increased by the intermittent high level of glucose. **b** The percentage of apoptotic cells was measured by flow cytometry. Apoptosis was assessed according to the percent of cells with hypodiploid DNA by flow cytometry as described in Materials and methods. Values of percentage of apoptotic cells represent the average of three experiments. The apoptosis rate of HUVECs treated with the intermittent high level of glucose was 48.35%, and the presence of gAd reversed the apoptosis rate to 20.57%. *HM* hyperosmotic mannitol (25 mmol/l) *NG* normal glucose (5 mmol/l) *SHG* stable high glucose (25 mmol/l) *IHG* intermittent high glucose (alternating 5 or 25 mmol/l per 8 h) Adipo adiponectin LY LY294002

quantifying the numbers of apoptotic cells. Apoptotic cells numbers were determined by Annexin-V-FITC, propidium iodide staining, fluorescence microscopy, and flow cytometry. As shown in Fig. 2a, the number of apoptotic HUVECs was increased by the intermittent high level of glucose. Furthermore, the apoptosis rate of HUVECs treated with the intermittent high level of glucose was 48.35% (Fig. 2b), and the presence of gAd reversed the apoptosis rate to 20.57%.

To determine whether or not the protective effect of gAd against intermittent high glucose level is caspase-dependent, we examined the effect of gAd on caspase-3 activity, an early signal of apoptosis. As shown in Fig. 1b, caspase-3 activity was markedly increased in cells incubated with intermittent high glucose ($P < 0.001$). However, the presence of gAd reversed the caspase-3 activity significantly ($P < 0.01$). LY294002 partially neutralized the effect of gAd ($P < 0.05$).

Adiponectin inhibits oxidative stress

To determine whether or not the observed antiapoptotic effect of gAd is due to the reduction of oxidative stress, we investigated the effect of gAd on 3-nitrotyrosine generation in HUVECs. As shown in Fig. 1c, 3-nitrotyrosine level was increased in HUVECs incubated with intermittent high glucose compared to groups with either normal or constant high glucose ($P < 0.001$ and $P < 0.05$, respectively). Co-incubation of gAd prevented the effect of intermittent high glucose in 3-nitrotyrosine production ($P < 0.01$). Pre-incubation with LY294002 partially neutralized the effect of gAd in the intermittent high glucose-induced 3-nitrotyrosine generation ($P < 0.05$).

Adiponectin enhances NO production in ECs

HUVECs were incubated with different levels of glucose in the presence or absence of gAd for 48 h, followed by the evaluation of NO production. Intermittent high glucose significantly decreased NO production in HUVECs compared with normal glucose ($P < 0.01$). The presence of gAd abolished the effect of intermittent high glucose in NO production ($P < 0.05$) (Fig. 3a).

Adiponectin stimulates phosphorylation of Akt, AMPK, and eNOS

To investigate the underlying mechanisms of gAd against the intermittent high glucose-induced reduction in NO production, levels of phosphorylated-Akt, phosphorylated-AMPK, and phosphorylated-eNOS were detected by Western blotting. As shown in Fig. 3b and c, the constant and intermittent high glucose reduced levels of both phospho-Akt, phospho-AMPK, and phospho-eNOS in HUVECs. Co-incubation with gAd markedly prevented the reduction of phospho-Akt, phospho-AMPK, and phospho-eNOS caused by the intermittent level of glucose. Pre-incubation with LY294002 partially neutralized the effect of gAd in high glucose-induced reduction in phosphorylations of Akt and eNOS.

Discussion

In the present study using an in vitro model, we have observed that gAd protects HUVECs from damages caused by the intermittent high glucose. Specifically, our results indicate that intermittent high glucose level led to HUVECs morphological and biochemical features of apoptosis, overproduction of reactive oxygen species, and activation of caspase-3, and presence of gAd prevents the effects of intermittent high glucose.

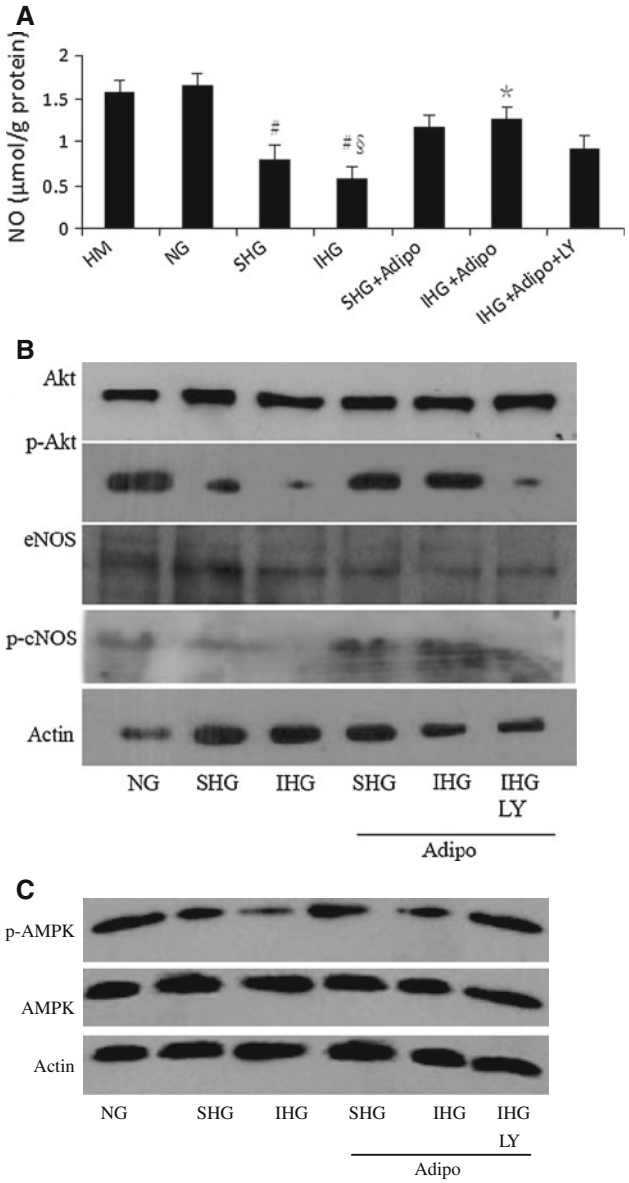

Fig. 3 Adiponectin enhances NO production in ECs and stimulates phosphorylation of Akt, AMPK, and eNOS. HUVECs were treated with normal (5 mmol/l), high (25 mmol/l), or alternating normal/high glucose concentrations, and 1 μg/ml gAd for 48 h. **a** The supernatants were collected for NO measurement. Data represent mean ± SD from three independent experiments. $^{\#}P < 0.01$ compared with normal glucose group; $^{\S}P < 0.05$ compared with group SHG; $^{*}P < 0.05$ compared with group IHG. **b** Expression of phosphorylated Akt and eNOS determined by Western blot. **c** Expression of phosphorylated AMPK determined by Western blot. *HM* hyperosmotic mannitol (25 mmol/l) *NG* normal glucose (5 mmol/l) *SHG* stable high glucose (25 mmol/l) *IHG* intermittent high glucose (alternating 5 or 25 mmol/l per 8 h) Adipo: adiponectin; LY: LY294002.

There are several evidences that suggest glucose variability may also be an independent risk factor for cardiovascular complications in diabetes [16–18]. New diabetes therapies focused on reducing postprandial hyperglycemia have become available and may benefit glycemic control

and cardiovascular disease risk factor levels [19]. Recent studies have shown that prolonged exposure to oscillating glucose generates a more detrimental condition in terms of ROS production, oxidative stress, and DNA damage [3–5, 20, 21], even leading to a memory effect of higher intensity than exposure to constant high glucose [22]. Moreover, p53 is overactivated by glucose oscillation, probably because of suboptimal feedback inhibition during the low glucose phases, and that its activation persists after glucose normalization [22].

Many evidences indicated that adiponectin plays an important role as a salutary, anti-diabetic, and insulin signaling-sensitizing factor with properties of anti-atherogenesis and inflammation in vasculatures [23, 24]. Adiponectin suppresses inflammatory signal generation triggered by high glucose at the level of IKKβ enzyme activation in ECs [25]. In addition, both AMP kinase and cAMP signaling play a role in the effects of adiponectin to block the rise in IKKβ activity induced by high glucose. Nevertheless, the cAMP pathway and AMP kinase signaling were both implicated in the effect of gAd on IKKβ activity induced by high glucose concentrations [25]. These results are of interest, since they support an important role for adiponectin in anti-inflammatory signaling in the vasculature and also imply that multiple pathways are involved in the cellular effects of adiponectin. Adiponectin is present as either a full-length protein or a fragment comprised of the C-terminal globular domain. In circulation, adiponectin can be present as a variety of different forms including multimers, trimers (low molecular weight, LMW), hexamers (medium molecular weight, MMW), 12-mers, and 18-mers (high molecular weight, HMW) [26]. There are evidences indicating that treatment of some cells with 1 μg/ml gAd increased proliferation, survival, motility, differentiation, and relevant signal pathway protein [27–30]. Different forms of adiponectin may play different physiological roles. Although it is still debatable [31, 32], many previous reports have shown that low plasma level of adiponectin is an independent risk predictor of cardiovascular diseases [33–36]. The discrepancy among different reports may be caused by the different functions of various forms of adiponectin [37, 38]. The high molecular weight oligomeric forms of adiponectin, but not the trimeric or hexameric forms of adiponectin, inhibit apoptosis and caspase-3 activity in HUVECs through activating the AMPK signalling pathway [12]. On the other hand, the globular domain of adiponectin dose-dependently inhibits Ang II-induced apoptosis in HUVECs [13]. Adiponectin is a unique cytokine that improves endothelial function by enhancing eNOS activity and attenuates oxidative/nitrative stress by blocking iNOS and NADPH oxidase expression, and ONOO– production [39]. In agreement, plasma level of adiponectin is reduced in type 2 diabetes. Intermittent high glucose markedly suppressed adiponectin mRNA expression and its protein secretion in adipocytes [40]. The reduced adiponectin level may contribute to the endothelial damages and dysfunction caused by the fluctuating hyperglycemia in diabetes. All these clinical and experimental studies strongly suggest that adiponectin is a critical vascular protective molecule whose reduction may contribute to vascular injury in diabetes.

The mechanisms of gAd increases synthesis of NO in ECs are largely unknown. High molecular weight (HMW) adiponectin was observed to activate AMPK, thereby increasing the phosphorylation of eNOS and NO production in ECs [41].However, new evidence indicate that administration of gAd at physiological concentrations promoted endothelial progenitor cells (EPCs), migration and tube formation, and dose-dependently upregulated phosphorylation of eNOS, Akt, and augmented NO production. The gAd reversed the high glucose-impaired EPC functions through NO- and p38 MAPK-related mechanisms [42]. Our results show that gAd increases levels of phosphor-Akt, phospho-AMPK, and phosphor-eNOS, suggesting that Akt and AMPK are targets of gAd. Furthermore, we show that the specific PI3 K inhibitor LY294002 significantly inhibits gAd-induced phosphorylation of both Akt and eNOS. Consistent with activation of eNOS phosphorylation, exposing HUVECs to gAd significantly increased levels of nitrite, the stable end product of NO. In addition, our results show that intermittent high glucose may be stronger than constant high glucose level in causing endothelial dysfunction and oxidative stress, but gAd protects ECs from dysfunction caused either by the intermittent or constant high glucose level equally.

In conclusion, the present study provided the evidence that treatment with gAd significantly attenuates endothelial dysfunction associated with oscillating glucose and increases synthesis of NO through the PI3 K/AKT and AMPK signaling pathway. This result not only provides additional evidence that reduced adiponectin in metabolic disorders contributes to the development of endothelial dysfunction, but it also suggests that supplementation of gAd in patients with metabolic disorder may normalize endothelial function and prevent or reduce atherosclerosis. This result also raises the possibility that therapeutic application of gAd may be a useful treatment of metabolic disorders with vascular complication.

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References

1. M.L. Sheu, F.M. Ho, R.S. Yang, K.F. Chao, W.W. Lin, S.Y. Lin-Shiau, S.H. Liu, High glucose induces human endothelial cell

apoptosis through a phosphoinositide 3-kinase-regulated cyclooxygenase-2 pathway. Arterioscler. Thromb. Vasc. Biol. **25**, 539–545 (2005)
2. H. Nakagami, R. Morishita, K. Yamamoto, S.I. Yoshimura, Y. Taniyama, M. Aoki, H. Matsubara, S. Kim, Y. Kaneda, T. Ogihara, Phosphorylation of p38 mitogen-activated protein kinase downstream of bax-caspase-3 pathway leads to cell death induced by high D-glucose in human endothelial cells. Diabetes **50**, 1472–1481 (2001)
3. L. Quagliaro, L. Piconi, R. Assaloni, L. Martinelli, E. Motz, A. Ceriello, Intermittent high glucose enhances apoptosis related to oxidative stress in human umbilical vein endothelial cells: the role of protein kinase C and NAD(P)H-oxidase activation. Diabetes **52**, 2795–2804 (2003)
4. A. Risso, F. Mercuri, L. Quagliaro, G. Damante, A. Ceriello, Intermittent high glucose enhances apoptosis in human umbilical vein endothelial cells in culture. Am. J. Physiol. Endocrinol. Metab. **281**, E924–E930 (2001)
5. T. Ronti, G. Lupattelli, E. Mannarino, The endocrine function of adipose tissue: an update. Clin. Endocrinol. (Oxf). **64**, 355–365 (2006)
6. S.P. Marso, S.K. Mehta, A. Frutkin, J.A. House, J.R. McCrary, K.R. Kulkarni, Low adiponectin levels are associated with atherogenic dyslipidemia and lipid-rich plaque in nondiabetic coronary arteries. Diabetes Care **31**, 989–994 (2008)
7. N. Ouchi, S. Kihara, Y. Arita, Y. Okamoto, K. Maeda, H. Kuriyama, K. Hotta, M. Nishida, M. Takahashi, M. Muraguchi, Y. Ohmoto, T. Nakamura, S. Yamashita, T. Funahashi, Y. Matsuzawa, Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway. Circulation **102**, 1296–1301 (2000)
8. J.G. Yu, S. Javorschi, A.L. Hevener, Y.T. Kruszynska, R.A. Norman, M. Sinha, J.M. Olefsky, The effect of thiazolidinediones on plasma adiponectin levels in normal, obese, and type 2 diabetic subjects. Diabetes **51**, 2968–2974 (2002)
9. T. Costacou, J.C. Zgibor, R.W. Evans, J. Otvos, M.F. Lopes-Virella, R.P. Tracy, T.J. Orchard, The prospective association between adiponectin and coronary artery disease among individuals with type 1 diabetes. The Pittsburgh Epidemiology of Diabetes Complications Study. Diabetologia **48**, 41–48 (2005)
10. N. Kubota, Y. Terauchi, T. Yamauchi, T. Kubota, M. Moroi, J. Matsui, K. Eto, T. Yamashita, J. Kamon, H. Satoh, W. Yano, P. Froguel, R. Nagai, S. Kimura, T. Kadowaki, T. Noda, Disruption of adiponectin causes insulin resistance and neointimal formation. J. Biol. Chem. **277**, 25863–25866 (2002)
11. T.A. Hopkins, N. Ouchi, R. Shibata, K. Walsh, Adiponectin actions in the cardiovascular system. Cardiovasc. Res. **74**, 11–18 (2007)
12. H. Kobayashi, N. Ouchi, S. Kihara, K. Walsh, M. Kumada, Y. Abe, T. Funahashi, Y. Matsuzawa, Selective suppression of endothelial cell apoptosis by the high molecular weight form of adiponectin. Circ. Res. **94**, E27–E31 (2004)
13. L.Y. Lin, C.Y. Lin, T.C. Su, C.S. Liau, Angiotensin II-induced apoptosis in human endothelial cells is inhibited by adiponectin through restoration of the association between endothelial nitric oxide synthase and heat shock protein 90. FEBS Lett. **574**, 106–110 (2004)
14. R. Ouedraogo, X. Wu, S.Q. Xu, L. Fuchsel, H. Motoshima, K. Mahadev, K. Hough, R. Scalia, B.J. Goldstein, Adiponectin suppression of high glucose-induced reactive oxygen species in vascular endothelial cells evidence for involvement of a cAMP signaling pathway. Diabetes **55**, 1840–1846 (2006)
15. L. Piconi, L. Quagliaro, R. Assaloni, R. Da Ros, A. Maier, G. Zuodar, A. Ceriello, Constant and intermittent high glucose enhances endothelial cell apoptosis through mitochondrial superoxide overproduction. Diabetes Metab. Res. Rev. **22**, 198–203 (2006)
16. A. Ceriello, M. Ihnat, 'Glycemic variability': a new therapeutic challenge in diabetes and the critical care setting. Diabet. Med. **27**, 862–867 (2010)
17. A. Ceriello, Postprandial hyperglycemia and diabetes complications: is it time to treat? Diabetes **54**, 1–7 (2005)
18. L. Nalysnyk, M. Hernandez-Medina, G. Krishnarajah, Glycaemic variability and complications in patients with diabetes mellitus: evidence from a systematic review of the literature. Diabetes Obes. Metab. **12**, 288–298 (2010)
19. E.J. Bastyr, C.A. Stuart, R.G. Brodows, S. Schwartz, C.J. Graf, A. Zagar, K.E. Robertson, Therapy focused on lowering postprandial glucose, not fasting glucose, may be superior for lowering HbA1c: IOEZ Study Group. Diabetes Care. **23**, 1236–1241 (2000)
20. A. Ceriello, K. Esposito, L. Piconi, M.A. Ihnat, J.E. Thorpe, R. Testa, M. Boemi, D. Giugliano, Oscillating glucose is more deleterious to endothelial function and oxidative stress than mean glucose in normal and type 2 diabetic patients. Diabetes **57**, 1349–1354 (2008)
21. L. Quagliaro, L. Piconi, R. Assaloni, R. Da Ros, A. Maier, G. Zuodar, A. Ceriello, Intermittent high glucose enhances ICAM-1, VCAM-1 and E-selectin expression in human umbilical vein endothelial cells in culture: the distinct role of protein kinase C and mitochondrial superoxide production. Atherosclerosis **183**, 259–267 (2005)
22. B. Schisano, G. Tripathi, K. McGee, P.G. McTernan, A. Ceriello, Glucose oscillations, more than constant high glucose, induce p53 activation and a metabolic memory in human endothelial cells. Diabetologia **54**, 1219–1226 (2011)
23. B.J. Goldstein, R.G. Scalia, X.L. Ma, Protective vascular and myocardial effects of adiponectin. Nat. Clin. Pract. Cardiovasc. Med. **6**, 27–35 (2009)
24. Essick EE, Ouchi N, Wilson RM, Ohashi K, Ghobrial J, Shibata R, Pimentel DR, Sam F. Adiponectin mediates cardioprotection in oxidative stress-induced cardiac myocyte remodeling. Am. J. Physiol. Heart Circ. Physiol. 2011 10 (in press)
25. Wu X, Mahadev K, Fuchsel L, Ouedraogo R, Xu S-q, Goldstein BJ. Adiponectin suppresses IκB kinase activation induced by tumor necrosis factor-α or high glucose in endothelial cells: role of cAMP and AMP kinase signaling. Am. J. Physiol. Endocrinol. Metab. 293: E1836–E1844 (2007)
26. T.S. Tsao, H.E. Murrey, C. Hug, D.H. Lee, H.F. Lodish, Oligomerization state- dependent activation of NF-kappa B signaling pathway by adipocyte complement-related protein of 30 kDa (Acrp30). J. Biol. Chem. **277**, 29359–29362 (2002)
27. P.H. Park, H. Huang, M.R. McMullen, K. Bryan, L.E. Nagy, Activation of cyclic-AMP response element binding protein contributes to adiponectin-stimulated interleukin-10 expression in RAW 264.7 macrophages. J. Leukoc. Biol. **83**, 1258–1266 (2008)
28. T. Fiaschi, F.S. Tedesco, E. Giannoni, J. Diaz-Manera, M. Parri, G. Cossu, P. Chiarugi, Globular adiponectin as a complete mesoangioblast regulator: role in proliferation, survival, motility, and skeletal muscle differentiation. Mol. Biol. Cell **21**, 848–859 (2010)
29. F. Haugen, C.A. Drevon, Activation of nuclear factor-kappaB by high molecular weight and globular adiponectin. Endocrinology **148**, 5478–5486 (2007)
30. C. Wang, X. Xin, R. Xiang, F.J. Ramos, M. Liu, H.J. Lee, H. Chen, X. Mao, C.K. Kikani, F. Liu, L.Q. Dong, Yin-Yang regulation of adiponectin signaling by APPL isoforms in muscle cells. J. Biol. Chem. **284**, 31608–31615 (2009)
31. G.R. Hajer, Y. van der Graaf, J.K. Olijhoek, M. Edlinger, F.L. Visseren, Low plasma levels of adiponectin are associated with low risk for future cardiovascular events in patients with clinical evident vascular disease. Am. Heart J. **154**, E1–E7 (2007)
32. N. Sattar, G. Wannamethee, N. Sarwar, J. Tchernova, L. Cherry, A.M. Wallace, J. Danesh, P.H. Whincup, Adiponectin and

coronary heart disease: a prospective study and meta-analysis. Circulation **114**, 623–629 (2006)
33. R. Wolk, P. Berger, R.J. Lennon, E.S. Brilakis, D.E. Davison, V.K. Somers, Association between plasma adiponectin levels and unstable coronary syndromes. Eur. Heart J. **28**, 292–298 (2007)
34. T. Bouhali, D. Brisson, J. St-Pierre, G. Tremblay, P. Perron, C. Laprise, M.C. Vohl, M.N. Vissers, B.A. Hutten, J.P. Després, J.J. Kastelein, D. Gaudet, Low plasma adiponectin exacerbates the risk of premature coronary artery disease in familial hypercholesterolemia. Atherosclerosis **196**, 262–269 (2008)
35. M.P. Chen, J.C. Tsai, F.M. Chung, S.S. Yang, L.L. Hsing, S.J. Shin, Y.J. Lee, Hypoadiponectinemia is associated with ischemic cerebrovascular disease. Arterioscler. Thromb. Vasc. Bio. **25**, 821–826 (2005)
36. T. Pischon, C.J. Girman, G.S. Hotamisligil, N. Rifai, F.B. Hu, E.B. Rimm, Plasma adiponectin levels and risk of myocardial infarction in men. JAMA **291**, 1730–1737 (2004)
37. C. Lara-Castro, N. Luo, P. Wallace, R.L. Klein, W.T. Garvey, Adiponectin multimeric complexes and the metabolic syndrome trait cluster. Diabetes **55**, 249–259 (2006)
38. T. Yamauchi, J. Kamon, Y. Minokoshi, Y. Ito, H. Waki, S. Uchida, S. Yamashita, M. Noda, S. Kita, K. Ueki, K. Eto, Y. Akanuma, P. Froguel, F. Foufelle, P. Ferre, D. Carling, S. Kimura, R. Nagai, B.B. Kahn, T. Kadowaki, Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat. Med. **8**, 1288–1295 (2002)
39. R. Li, W.Q. Wang, H. Zhang, X. Yang, Q. Fan, T.A. Christopher, B.L. Lopez, L. Tao, B.J. Goldstein, F. Gao, X.L. Ma, Adiponectin improves endothelial function in hyperlipidemic rats by reducing oxidative/nitrative stress and differential regulation of eNOS/iNOS activity. Am. J. Physiol. Endocrinol. Metab. **293**, E1703–E1708 (2007)
40. J. Sun, Y. Xu, H. Deng, S. Sun, Z. Dai, Y. Sun, Intermittent high glucose exacerbates the aberrant production of adiponectin and resistin through mitochondrial superoxide overproduction in adipocytes. J. Mol. Endocrinol. **44**, 179–185 (2010)
41. Y. Hattori, Y. Nakano, S. Hattori, A. Tomizawa, K. Inukai, K. Kasai, High molecular weight adiponectin activates AMPK and suppresses cytokine-induced NF-kappaB activation in vascular endothelial cells. FEBS Lett. **582**, 1719–1724 (2008)
42. P.H. Huang, J.S. Chen, H.Y. Tsai, Y.H. Chen, F.Y. Lin, H.B. Leu, T.C. Wu, S.J. Lin, Chen JW. Globular adiponectin improves high glucose-suppressed endothelial progenitor cell function through endothelial nitric oxide synthase dependent mechanisms. J. Mol. Cell. Cardiol. **51**, 109–119 (2011)