Globular Adiponectin Enhances Muscle Insulin Action via Microvascular Recruitment and Increased Insulin Delivery

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ABSTRACT

Rationale: Adiponectin enhances insulin action and induces nitric oxide-dependent vasodilatation. Insulin delivery to muscle microcirculation and trans-endothelial transport are two discrete steps that limit insulin’s action. We have shown that expansion of muscle microvascular surface area increases muscle insulin delivery and action.

Objective: To examine whether adiponectin modulates muscle microvascular recruitment thus insulin delivery and action in vivo.

Methods and Results: Overnight fasted, adult male rats were studied. We determined the effects of adiponectin on muscle microvascular recruitment using contrast-enhanced ultrasound (CEU), on insulin-mediated microvascular recruitment and whole body glucose disposal using CEU and insulin clamp, and on muscle insulin clearance and uptake with \(^{125}\)I-insulin. Globular adiponectin (gAd) potently increased muscle microvascular blood volume without altering microvascular blood flow velocity, leading to a significantly increased microvascular blood flow. This was paralleled by a ~30-40% increase in muscle insulin uptake and clearance, and ~30% increase in insulin-stimulated whole body glucose disposal. Inhibition of endothelial nitric oxide synthase (eNOS) abolished gAd-mediated muscle microvascular recruitment and insulin uptake. In cultured endothelial cells, gAd dose-dependently increased eNOS phosphorylation but had no effect on endothelial cell internalization of insulin.

Conclusions: gAd increases muscle insulin uptake by recruiting muscle microvasculature, which contributes to its insulin-sensitizing action.

Keywords: Adiponectin, endothelium, microcirculation, muscle, insulin delivery

Nonstandard Abbreviations:
CEU  Contrast-enhanced ultrasound
fAd  Full length adiponectin
gAd  Globular adiponectin
MBV  Microvascular blood volume
MFV  Microvascular blood flow velocity
MBF  Microvascular blood flow

INTRODUCTION

Adiponectin circulates as both a full-length form (fAd) and a C-terminal globular domain (gAd) that bind to adiponectin receptors 1 and 2 (AdipoR1 and AdipoR2) to exert many distinct effects. Ample evidence has confirmed that adiponectin has insulin sensitizing properties and hypo-adiponectinemia is linked to the development of insulin resistance and type 2 diabetes in humans. Indeed, plasma levels of adiponectin are decreased in the insulin resistant states and replenishment of adiponectin ameliorates insulin resistance in liver, adipose tissue and skeletal muscle. In Wistar rats, adenovirus-mediated over-expression of adiponectin augments skeletal muscle insulin sensitivity. The mechanisms underlying the insulin sensitizing effect of adiponectin have been extensively studied but remain to be defined. Adiponectin activates AMP-activated protein kinase (AMPK) and PPAR\(\alpha\), and increases fatty acid oxidation and glucose uptake, each of which can contribute to increased insulin...
sensitivity 1-3. In addition, adiponectin has been shown to increase muscle insulin signaling by reducing p70 S6 kinase-mediated serine phosphorylation of insulin receptor substrate (IRS)-1 11.

Adiponectin also is a potent vasodilator 12, 13. This effect is mediated through a nitric oxide (NO)-dependent pathway as inhibition of NO synthase blocks adiponectin’s vasodilatory effect and in cultured endothelial cells adiponectin directly increases NO production 14-16. Conversely, hypoadiponectinemia is associated with impaired vasoreactivity in humans 17, which could contribute to the development of cardiovascular complications of diabetes.

We and others have shown that factors that increase endothelial production of NO, such as systemic administration of insulin 18, 19, glucagon-like peptide 1 20 or an angiotensin II type 1 receptor blocker 21, 22 all potently vasodilate the muscle pre-capillary arterioles to increase microvascular perfusion and this action is associated with increased insulin action in muscle. As muscle microvasculature provides the principal endothelial surface area for substrate exchange between plasma and muscle interstitium and resting blood flow in muscle is relatively low, even a small increase in the muscle microvascular blood volume (MBV) can significantly increase substrate and hormonal exchanges, including that of insulin, resulting in increased insulin delivery and action in muscle. Indeed, insulin’s microvascular action is closely coupled with its metabolic action and inhibition of insulin-mediated microvascular recruitment with NO synthase inhibitor decreases insulin-stimulated glucose disposal by ~40% 18, 19.

In the current study, we hypothesized that adiponectin enhances muscle insulin action by regulating muscle microvascular recruitment and muscle insulin uptake in vivo. Our results indicate that gAd potently recruits muscle microvasculature via a NO-dependent pathway by expanding muscle MBV, which leads to increased muscle insulin delivery and insulin-stimulated whole body glucose disposal.

METHODS

Animal preparations and experimental protocols.

Overnight fasted adult male Sprague-Dawley rats (250-360 g, Charles River Laboratories, Wilmington, MA) were studied. Rats were housed at 22 ± 2°C on a 12-hr light-dark cycle and fed standard laboratory chow and water ad libitum prior to the study. After being anesthetized with pentobarbital sodium (50 mg/kg i.p., Abbott Laboratories, North Chicago, IL), rats were placed in a supine position on a heating pad to ensure euthermia and intubated to maintain a patent airway. The carotid artery and the jugular vein were cannulated with PE50 polyethylene tubing (Fisher Scientific, Newark, DE) for arterial blood pressure monitoring, arterial blood sampling, and various infusions. After a 30 - 45 min baseline period to assure hemodynamic stability and stable anesthesia, rats were studied under the following three protocols (Fig. 1).

In Protocol 1 (Fig. 1, top panel), rats received an i.p. injection of either full length adiponectin (fAd, 0.2 µg/g body weight) or globular adiponectin (gAd, 0.2 or 0.4 µg/g body weight), or saline 30 min after initiation of systemic infusion of either N\(^{\text{G}}\)-nitro-L-arginine methyl ester (L-NAME, 50 µg/kg/min, Sigma-Aldrich, St. Louis, MO) or saline. Both fAd and gAd were obtained from Biovision Inc. (Mountain view, CA). Hindlimb skeletal muscle MBV and microvascular blood flow velocity (MFV) were measured before and then every 30 min after i.p. injection using contrast-enhanced ultrasound (CEU), as previously described 18, 20, 22-24. Microvascular blood flow (MBF) was calculated as the product of MBV and MFV. Plasma NO concentrations were determined at time 0, 30, 60, 90 and 120 min, as described below.
In protocol 2 (Fig. 1, middle panel), rats received a 2-hr infusion of insulin (3 mU/kg/min) after i.p. injection of either saline or gAd (0.4 µg/g body weight) in the presence or absence of L-NAME infusion (50 µg/kg/min). Hindlimb skeletal muscle MBV and MFV were measured before and then every 30 min after i.p. injection using CEU. Arterial blood glucose was determined every 10 min using an Accu-Chek Advantage glucometer (Roche Diagnostics, Indianapolis, IN), and 30% dextrose (30% wt/vol) was infused at a variable rate to maintain blood glucose within 10% of basal 25, 26. Steady-state whole body glucose disposal rates were calculated. Skeletal muscle MBV, MFV and MBF were determined at time 0, 30, 60, and 120 min. Rats were then sacrificed, gastrocnemius muscle freeze-clamped for later measurement of Akt and ERK1/2 phosphorylation using Western blotting, as described previously 25, 26.

In protocol 3 (Fig. 1, lower panel), rats received a systemic infusion of either saline or L-NAME for 60 min (from -30 to 30 min). At time 0, gAd (0.4 µg/g body weight) or saline was injected intraperitoneally. Each rat then received a bolus i.v. injection of $^{125}$I-insulin (1.5 µCi, PerkinElmer Inc, Boston, MA) 25 min later and was sacrificed at 30 min. Blood and gastrocnemius were obtained for determination of muscle clearance of insulin and muscle $^{125}$I-insulin uptake.

Throughout the study, mean arterial blood pressure (MAP) was monitored via a sensor connected to the carotid arterial catheter (Harvard Apparatus, Holliston, MA, and AD Instruments, Inc., Colorado Springs, CO). Pentobarbital sodium was infused at a variable rate to maintain steady levels of anesthesia and blood pressure throughout the study. Adiponectin was given as a single dose intraperitoneal injection because of its long plasma half-life of ~ 3-6 hours 27, 28. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (Publication No. 85–23, revised 1996). The study protocols were approved by the Animal Care and Use Committee of the University of Virginia.

**Determination of hindleg glucose uptake.**
Carotid arterial and femoral venous blood glucose concentrations were determined using an Accu-Chek Advantage blood glucometer (Roche) as previously described 20, 22 and hindleg glucose uptake (mg/dL) was calculated as the arterial-venous glucose differences.

**Measurement of Plasma NO, insulin and adiponectin levels.**
Plasma NO levels were measured using 280i Nitric Oxide Analyzer (GE Analytical Instruments), according to the manufacturer’s instructions. In brief, ice-cold ethanol was mixed with plasma samples at a ratio of 2:1, kept at 0°C for 30 min, and then centrifuged at ~14,000 RPM for 5 min. The supernatant was then used for NO analysis based on a gas-phase chemiluminescent reaction between NO and ozone. Plasma insulin concentrations were determined using a rat insulin ELISA assay kit (Mercodia AB, Uppsala, Sweden). Plasma adiponectin was measured using an ab108784 Adiponectin Rat ELISA kit (Abcam, San Francisco, CA) which recognizes both full length and goliublar adiponectin.

**Determination of muscle uptake of $^{125}$I-insulin.**
$^{125}$I-insulin was used as tracer to track the muscle clearance and uptake of native insulin as described previously 20, 21, 24. Five minutes after a bolus i.v. injection of 1.5 µCi $^{125}$I-insulin, blood sample was collected and each rat was then flushed with 120 mL ice-cold saline (10 mL/min) via the carotid artery catheter. Gastrocnemius muscle was then dissected from the right hindlimb. Protein-bound $^{125}$I-iodine in blood and muscle samples was precipitated with 30% trichloroacetic acid and radioactivity was measured using a gamma-counter. Muscle clearance of $^{125}$I-insulin [muscle $^{125}$I-insulin (DPM/g dry weight)/blood $^{125}$I-insulin (DPM/mL)/5 min] and muscle $^{125}$I-insulin uptake [muscle $^{125}$I-insulin (DPM/g dry weight)/5 min] were calculated.
Culture of endothelial cells and determination of endothelial insulin uptake.
Endothelial cell insulin uptake was assessed using $^{125}$I-insulin as previously reported $^{29-32}$. In brief, bovine aortic endothelial cells (bAECs) in primary culture were purchased from Lonza (Walkersville, MD). Cells between passages 3 to 6 were cultured in 6-well plates until 80% confluence, serum starved for 18 - 22 hrs and then incubated with pre-warmed N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES)-binding buffer (HBB) (0.1 M HEPES, 0.12 M NaCl, 5 mM KCl, 1.2 mM MgSO$_4$, 8 mM glucose, and 1% bovine serum albumin; pH 7.8) containing 200 pM $^{125}$I-insulin in the presence or absence of unlabelled regular insulin (2 µM) or various concentrations of gAd (0.1, 1, 2, 3 µg/mL) at 37°C for 15 min. The reaction was stopped by transferring the culture plates onto ice. Cells were washed once with ice-cold HBB buffer, then twice with ice-cold acid solution (0.5 M NaCl, 0.2 M acetic acid, pH 3.0) to remove surface bound $^{125}$I-insulin, and then lysed with 0.5 mL 1 M NaOH on ice for 1 hr. Aliquots of cell lysate were used for protein content determination and radioactivity quantification using a gamma counter. After subtracting the non-specific binding, endothelial insulin uptake was calculated and expressed as cpm/µg protein.

Determination of protein phosphorylation in muscle and endothelial cells.
Phosphorylation of eNOS, Akt, ERK1/2 and AMPK in muscle and/or cultured endothelial cells were determined by Western blotting, as described previously $^{21, 33, 34}$. Primary antibodies against phospho-eNOS (Ser$^{1177}$), total eNOS, phospho-Akt (Ser$^{473}$), total Akt, phospho-ERK (Thr$^{202}$/Tyr$^{204}$), total ERK1/2, phospho-AMPK (Thr$^{172}$) and total AMPK were obtained from Cell Signaling Technology (Beverly, MA). All blots were developed using enhanced chemiluminescence (GE Healthcare Bio-Sciences Corp, Piscataway, NJ). Autoradiographic films were scanned densitometrically and quantified using ImageQuant 3.3 software. Both the total and phosphospecific densities were quantified and the ratios of phosphospecific to total density calculated.

Statistical analysis.
All data are presented as mean ± SEM. Statistical analyses were performed with SigmaStat 3.1.1 software (Systat Software, Inc), using student t-test or ANOVA with post-hoc analysis as appropriate. A p-value of < 0.05 was considered statistically significant.

RESULTS
Adiponectin increases skeletal muscle microvascular perfusion via a NO-dependent pathway.

We first tested whether adiponectin exerts vasodilatory action in the muscle microvasculature and, if so, whether this effect was NO-dependent. We injected rats intraperitoneally with fAd (0.2 µg/g body weight) or gAd (0.2 or 0.4 µg/g body weight) and then measured muscle microvascular parameters every 30 min for 2 hrs. As shown in Fig. 2, both fAd and gAd recruited muscle microvasculature by increasing MBV without altering MFV. This resulted in an ~ 2-fold increase in muscle MBF (Fig. 2, panel C). At the dose of 0.2 µg/g body weight, gAd appeared to be more effective than fAd in inducing muscle microvascular recruitment and this effect lasted for 90 min. At higher dose (0.4 µg/g body weight), gAd-induced microvascular recruitment lasted for the entire 2 hrs. As such, we used gAd 0.4 µg/g body weight for all subsequent studies. The increase in muscle microvascular recruitment was paralleled by a significant increase in the plasma NO levels (Fig. 2, panel D). Neither fAd nor gAd at the doses selected affected blood glucose, blood pressure or plasma insulin concentrations throughout the study (Table 1). Simultaneous infusion of L-NAME increased MAP by ~ 20-30 mmHg without affecting blood glucose levels. However, it completely abolished the high dose gAd-induced microvascular recruitment.
Adiponectin enhances muscle metabolic responses to insulin.

As muscle microvascular recruitment is associated with increased insulin action in muscle, we next examined whether adiponectin could modulate insulin-mediated glucose disposal during insulin infusion. Insulin infusion raised plasma insulin concentrations from 113.1 ± 8.6 to 621.1 ± 42.9 pM in the saline group and from 118.4 ± 11.7 to 612.2 ± 43.7 pM in the gAd group (n=5, NS between groups). Administration of gAd prior to the initiation of insulin significantly increased insulin-mediated whole body glucose disposal by ~ 30% over the entire clamp period (p<0.001, ANOVA) and at steady-state (90-120 min, p<0.05) (Fig. 3A and B). Co-infusion of L-NAME completely abolished gAd-induced increases in insulin-stimulated glucose disposal. Infusion of gAd alone (0.4 µg/g body weight) did not significantly alter hindleg [A] – [V] glucose differences (3.5 ± 0.4, 4.9 ± 0.5, 4.2 ± 0.4, 4.6 ± 0.3, and 4.8 ± 0.4 mg/dL for baseline, 30 min, 60 min, 90 min and 120 min respectively, n=6, p=0.107, ANOVA). Neither blood glucose concentrations nor femoral blood flows changed during gAd alone infusion (p>0.05).

To further confirm that adiponectin indeed modulated insulin signaling in muscle, we examined the phosphorylation status of Akt and ERK1/2, two intermediate signaling molecules in the insulin signaling pathway, in biopsied gastrocnemius samples at the conclusion of insulin infusion (Fig. 4). Administration of gAd at either dose did not significantly increase muscle Akt phosphorylation. Insulin infusion alone increased muscle Akt phosphorylation by > 2-fold (p<0.05). In the presence of gAd, insulin-mediated Akt phosphorylation further increased to 3-fold of the control level (p<0.001). In contrast, gAd at 0.4 µg/g body weight enhanced muscle ERK1/2 phosphorylation to the extent seen with insulin alone (p<0.05, ANOVA) and the combination of gAd and insulin did not further increase the extent of ERK1/2 phosphorylation.

Adiponectin increases muscle 125I-insulin uptake via a NO-dependent pathway.

As insulin action in muscle is closely correlated with interstitium muscle insulin concentrations 35, we next examined whether the increased metabolic responses to insulin after gAd administration was secondary to increased muscle uptake of insulin. We used 125I-insulin to trace native insulin movement in vivo. As shown in Fig. 5, gAd administration significantly increased muscle clearance of insulin from blood (Fig. 5A) and uptake of insulin (Fig. 5B) and this effect was again completely abolished by the inhibition of NO production with L-NAME. These changes were clearly not due to differential degradation of 125I-insulin as evidenced by comparable fractions of intact 125I-insulin in both blood and muscle across all 3 groups (Fig. 5C).

Adiponectin increases muscle microvascular recruitment without affecting endothelial insulin uptake.

The increased muscle uptake of insulin could be secondary to increased insulin delivery to the muscle via microvascular recruitment and/or transendothelial insulin transport. Fig. 6 showed that combination of adiponectin and insulin recruited significantly more muscle microvasculature than insulin alone. Similar to previous report 21, insulin infusion alone increased both muscle MBV and MBF by ~ 60% (p<0.05) without affecting MFV. Administration of gAd prior to the initiation of insulin recruited significantly more microvasculature by expanding MBV (p<0.05) without altering MFV.

We finally assessed in cultured endothelial cells whether gAd treatment alters the uptake of insulin which is the first step in transendothelial insulin transport as this process can also limit muscle uptake of insulin and signaling proteins that could contribute to this process. As shown in Fig. 7, similar to insulin (10 nM), gAd significantly increased the phosphorylation of both Akt (Ser473) and eNOS (Ser1177) (p<0.05, ANOVA). Treatment of cells with both insulin and gAd further increased Akt and eNOS phosphorylation over insulin alone (p<0.05), suggesting an additive effect. There was no additive...
effect on AMPK phosphorylation. However, gAd had no effect on endothelial uptake of $^{125}$I-insulin ($p=0.217$, ANOVA).

**DISCUSSION**

Diabetes is associated with hypoadiponectinemia and adiponectin has been shown to improve insulin sensitivity. Muscle microvasculature provides endothelial exchange surface area to facilitate nutrient and hormonal exchanges between plasma and muscle interstitium. In the current study, we used a combination of the insulin clamp and CEU techniques and found that gAd potently recruited muscle microvasculature, which was associated with increased muscle insulin uptake and action. Inasmuch as adiponectin has both insulin sensitizing and vasodilatory effects (in the conduit arteries and resistance arterioles), we tested whether adiponectin’s vascular actions contributed directly to its insulin sensitizing actions. Our results show for the first time that adiponectin vasodilates pre-capillary arterioles to increase muscle microvascular perfusion and thereby endothelial exchange surface area. This action improved insulin and glucose delivery to muscle capillary exchange surfaces and allowed increased glucose and insulin uptake. These findings provide the first demonstration that a significant component of the insulin sensitizing effect of adiponectin derives from its vascular effects.

For insulin to act on muscle cells, it has to be first delivered to muscle microcirculation and then muscle interstitium via trans-endothelial transport, two discrete steps that limit insulin’s action $^{36, 37}$. We and others have shown that multiple interventions that increase muscle’s endothelial exchange surface area also increase muscle insulin uptake $^{20, 21, 24, 38}$. Our current findings again indicate that the muscle microvasculature plays an important role in the regulation of muscle insulin action. We found a close coupling of gAd-induced microvascular recruitment and increased insulin uptake and action in muscle. Inasmuch as both insulin delivery to the microcirculation and insulin transendothelial transport can limit muscle insulin uptake and action, the absence of an effect of gAd on endothelial cells insulin uptake suggests that gAd’s insulin sensitizing action in muscle is due to recruiting microvasculature and expanding the exchange surface area.

Adiponectin exerts pleiotrophic actions on the vasculature via direct actions on almost all the major cell types present in blood vessels and protects against endothelial dysfunction, atherosclerosis, and hypertension $^{39}$. Unlike previous reports of adiponectin vasodilating larger vessels (e.g. aorta and mesenteric arteries $^{12, 13}$), we found that adiponectin has a potent vasodilatory effect on the microvasculature even in the absence of increases in total leg blood flow. This is of particular significance as this microvascular effect can account for some of the effects of adiponectin to regulate tissue/organ substrate metabolism $^{3, 39-43}$. It is thus not surprising that hypoadiponectinemia is not only associated with endothelial dysfunction and hypertension, but also insulin resistance and diabetes.

Our study suggests that gAd is more potent than fAd in recruiting muscle microvasculature. Indeed, at a dose of 0.2 µg/g body weight, fAd only increased muscle MBV at 60 min despite persistently elevated plasma concentrations while gAd potently increased muscle MBV at 30 min and this effect lasted for 90 min. At 0.4 µg/g body weight, gAd’s microvascular effect lasted for the entire 2 hours. This is not surprising as both AdipoR1 and AdipoR2 exhibit 3-4-fold higher binding affinity for gAd than for fAd $^{1}$. Although endothelial cells and microvessels express both AdipoR1 and AdipoR2 $^{16, 44}$, it is possible that gAd may have acted via the adipoR1 as it is the most abundant isoform in the skeletal muscle and AdipoR2 is more prevalent in the liver $^{1}$. It is of interest to note that in cultured muscle cells gAd has also been shown to directly increase GLUT4 translocation and glucose uptake $^{45}$. Taken together, our in vivo study results and the previously reported in vitro muscle cell findings suggest that gAd may act on both...
endothelium (to increase muscle perfusion and insulin uptake) and muscle cells (to directly increase GLUT4 translocation) to enhance muscle glucose utilization.

The microvascular effect of gAd was clearly NO-dependent as plasma NO levels increased promptly after gAd administration and simultaneous infusion of NO synthase inhibitor L-NAME completely abolished gAd-stimulated microvascular perfusion and insulin uptake. This is consistent with our in vitro observation that gAd potently increased eNOS phosphorylation at Ser\textsuperscript{1177} and previous reports that adiponectin directly increases NO production by promoting eNOS phosphorylation (Ser\textsuperscript{1177}) and eNOS association with heat shock protein 90\textsuperscript{14-16} in cultured endothelial cells, an effect that is mediated through a multiple domain adaptor protein APPL1\textsuperscript{16}. Consistent with these in vitro findings, treatment of diet-induced obese rats with recombinant adiponectin increased eNOS activity, NO production, and endothelium-dependent relaxation of aortic rings\textsuperscript{46}. Similarly, in an isolated mesenteric artery preparation adiponectin-induced vasodilation was completely inhibitable by NOS inhibition\textsuperscript{13} and administration of adiponectin to \textit{db/db} diabetic mice reversed the impairment in endothelium-dependent relaxations in isolated coronary arterioles and aortae\textsuperscript{47}.

It appears that insulin and adiponectin have additive effect on recruiting muscle microvasculature. While insulin alone increased muscle MBV by ~60% and gAd increased MBV by 100%, combination of gAd and insulin increased MBV by >150%. This is not surprising as insulin and adiponectin act via different receptors to increase NO production, and neither insulin nor gAd appears to have maximal vasodilatory effect on the precapillary arterioles. We and others have previously shown that administration of either angiotensin II type 1 receptor losartan or glucagon-like peptide 1, or low frequency electric stimulation can increase muscle MBV by >3-fold\textsuperscript{20-24}. This is also consistent with our in vitro cell studies demonstrating that insulin and gAd exert an additive effect on eNOS phosphorylation. While insulin acts via its receptors to activate Akt and eNOS and recruit muscle microvasculature, gAd increases NO production via AdipoR1 and/or AdipoR2. However, there is clearly a crosstalk between the two systems and APPL1 has been shown to counteract obesity-induced vascular insulin resistance and endothelial dysfunction by modulating the endothelial production of NO and endothelial-1 \textsuperscript{48}. This is potentially very important in the state of insulin resistance as insulin facilitates its own delivery and action in the insulin sensitive state and insulin resistance blunts this action. Thus, gAd could increase insulin action in the insulin resistant state by acting on AdipoR1 and/or AdipoR2 to increase insulin delivery, uptake and action in muscle.

In conclusion, gAd recruits muscle microvasculature and expands endothelial exchange surface area via a NO-dependent mechanism which contributes to increased muscle insulin delivery, uptake and action. Our results provide new understanding of the mechanisms underlying the insulin sensitizing action of gAd and suggest that both hypoadiponectinemia and muscle microvasculature could be important therapeutic targets in the prevention and treatment of insulin resistance and diabetes.

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**DISCLOSURES**
None.
REFERENCES


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Table 1. Changes in blood pressure, blood glucose levels and plasma insulin and adiponectin concentrations.

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<td>131.0±0.9*</td>
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| **Blood Glucose Concentrations (mg/dL)** | |
| Saline                           | 92.4±5.3 | 94.3±2.8 | 93.3±3.3 | 98.5±4.0 | 96.6±5.5 |
| fAd 0.2                          | 93.7±8.4 | 96.3±9.8 | 97.8±10.1 | 99.0±6.8 | 98.5±7.2 |
| gAd 0.2                          | 96.0±5.7 | 97.2±2.7 | 95.5±4.1 | 97.3±5.43 | 93.8±3.3 |
| gAd 0.4                          | 86.6±6.6 | 87.0±10.5 | 86.3±11.0 | 92.3±11.0 | 88.3±10.5 |
| L-NAME + Saline                  | 89.0±5.0 | 93.2±4.7 | 97.5±5.9 | 94.6±6.1 | 95.0±4.6 |
| L-NAME + gAd 0.4                 | 93.0±1.0 | 97.7±4.0 | 95.6±5.5 | 92.6±6.7 | 93.0±4.6 |

| **Plasma Insulin Concentrations (pM)** | |
| Saline                           | 104.4±9.4 | 87.8±7.1 | 93.3±7.1 | 99.9±6.7 | 98.9±7.4 |
| fAd 0.2                          | 104.2±7.1 | 111.5±11.1 | 88.2±7.2 | 83.9±6.6 | 104.3±6.6 |
| gAd 0.2                          | 111.1±11.6 | 122.6±12.5 | 91.1±12.0 | 103.0±7.8 | 106.1±6.5 |
| gAd 0.4                          | 108.1±10.8 | 114.3±2.2 | 103.8±9.5 | 103.0±7.6 | 102.7±9.3 |
| L-NAME + Saline                  | 127.9±23.0 | 118.2±4.8 | 99.3±14.5 | 117.2±9.1 | 118.6±13.4 |
| L-NAME + gAd 0.4                 | 109.0±16.8 | 113.2±12.9 | 113.7±11.6 | 133.3±17.0 | 112.1±12.3 |

| **Plasma Adiponectin Concentrations (µg/mL)** | |
| Saline                           | 5.7±0.12 | 6.06±0.14 | 5.21±0.41 | 5.55±0.23 | 5.45±0.47 |
| fAd 0.2                          | 5.81±0.74 | 6.97±0.50 | 7.69±0.25 | 8.49±0.77* | 8.61±0.71* |
| gAd 0.2                          | 5.66±0.68 | 7.74±0.46 | 7.59±0.80 | 7.91±0.79 | 7.81±0.45 |
| gAd 0.4                          | 5.76±0.50 | 9.65±1.04* | 9.47±0.66* | 9.65±0.32* | 9.81±0.83* |

Values are mean ± SEM; MAP: mean arterial blood pressure; fAd 0.2: full length adiponectin 0.2 µg/g body weight; gAd 0.2: globular adiponectin 0.2 µg/g body weight; gAd 0.4: globular adiponectin 0.4 µg/g body weight; L-NAME: Nω-nitro-L-arginine methyl ester. n=5-6. Compared with saline group, * p<0.05.
FIGURE LEGENDS:

Figure 1. Experimental protocols.

Figure 2. Adiponectin increases skeletal muscle microvascular perfusion and plasma NO levels. Each rat was given an intraperitoneal injection of either fAd (0.2 µg/g body weight), gAd (0.2 or 0.4 µg/g body weight) or saline in the absence or presence of L-NAME infusion. CEU measurements were done before and then every 30 min after i.p. injection. A. MBV; B. MFV; C. MBF; D. Plasma NO levels. n=5-6. Compared with saline control * p<0.05 (ANOVA). fAd 0.2: full length adiponectin 0.2 µg/g body weight; gAd 0.2: globular adiponectin 0.2 µg/g body weight; gAd 0.4: globular adiponectin 0.4 µg/g body weight

Figure 3. Globular adiponectin augments insulin-stimulated whole body glucose disposal. Each rat received i.p. injection of saline or gAd (0.4 µg/g body weight) followed by a 2-hr euglycemic hyperinsulinemic clamp (3 mU/kg/min) in the presence or absence of L-NAME infusion. A. Time course of glucose infusion rate (GIR). Compared with saline group, p<0.001 (ANOVA); B. Steady-state GIR. Compared with saline control and L-NAME groups, ** p<0.01. n=5-6.

Figure 4. Effects of gAd on muscle Akt and ERK1/2 phosphorylation. A: Changes in muscle Akt phosphorylation. B: Changes in muscle ERK phosphorylation. Compared with saline control, * p<0.05, ** p<0.01. Compared with Insulin # p<0.05. n=3-4. gAd 0.2: globular adiponectin 0.2 µg/g body weight; gAd 0.4: globular adiponectin 0.4 µg/g body weight; insulin: 3 mU/kg/min; gAd+insulin: globular adiponectin 0.4 µg/g body weight + insulin 3 mU/kg/min;

Figure 5. Globular adiponectin increases muscle 125I-insulin uptake. Blood and skeletal muscle were collected 5 min after bolus i.v. injection of 125I-insulin (1.5 µCi). Intact 125I-insulin was determined after TCA (30%) precipitation. A. Muscle clearance of insulin; B. Muscle insulin uptake; C. Fraction of 125I-insulin in blood and in muscle. n=5-6. Compared with saline group, * p<0.05.

Figure 6. Globular adiponectin enhances insulin-mediated muscle microvascular recruitment. Each rat received an i.p. injection of saline or gAd (0.4 µg/g body weight) followed by a 2-hr euglycemic hyperinsulinemic clamp (3 mU/kg/min). CEU measurements were done at the set time points. A. MBV; B. MFV; C. MBF; D. MAP. n=5-7. Compared with basal, * p<0.05, ** p<0.05; compared with insulin group, # p<0.05.

Figure 7. Globular adiponectin increases eNOS phosphorylation but not endothelial 125I-insulin uptake in cultured bAECs. A. Changes in Akt phosphorylation; B. Changes in AMPK phosphorylation; C. Changes in eNOS phosphorylation; D. Changes in endothelial 125I-insulin uptake. n=3-4. Compared with control, * p<0.05, ** p<0.01. Compared with insulin # p<0.05.
Novelty and Significance

What Is Known?

- Adiponectin enhances insulin action and induces nitric oxide-dependent vasodilatation.
- Insulin delivery to muscle microcirculation and trans-endothelial transport are two discrete steps that limit insulin’s action.
- Expansion of muscle microvascular surface area promotes muscle insulin delivery and action.

What New Information Does This Article Contribute?

- Adiponectin expands muscle microvascular blood volume and endothelial exchange surface area by dilating the precapillary arterioles thus recruiting muscle microvasculature via a nitric oxide-dependent mechanism.
- Globular adiponectin enhances insulin action in muscle by increasing muscle delivery of insulin.
- The actions of globular adiponectin and insulin on muscle microvasculature are additive.

While adiponectin has been shown to enhance the metabolic action of insulin and induce nitric oxide-dependent vasodilation, evidence linking these two effects is lacking. It has been shown previously that muscle microvasculature is a critically important target for insulin’s metabolic action as it provides the principal endothelial surface area for substrate exchange between plasma and muscle interstitium. We examined the effects of adiponectin on muscle microvasculature and insulin’s metabolic action. We found that globular adiponectin recruits muscle microvasculature and expands endothelial exchange surface area via a nitric oxide-dependent vasodilation of the precapillary arterioles. This action contributes to increased muscle insulin delivery, uptake and action. These results provide new understanding of the mechanisms underlying the insulin sensitizing action of globular adiponectin and suggest that both hypoadiponectinemia and muscle microvasculature could be important therapeutic targets in the prevention and treatment of insulin resistance and diabetes.
Protocol 1

-30 0 30 60 90 120 min

Saline infusion + i.p. of saline, fAd or gAd @ 0 min

L-NAME infusion + i.p. of saline or gAd @ 0 min

Protocol 2

-30 0 30 60 120 min

Saline or L-NAME infusion

Insulin clamp + i.p. of saline or gAd @ 0 min

Protocol 3

-30 0 25 30 min

Saline infusion + i.p. of saline or gAd @ 0 min

L-NAME infusion + i.p. of saline or gAd @ 0 min

125I-Insulin
Figure 3

A. Glucose infusion rate (mg/kg/min) over time:
- Saline + Ins
- gAd + Ins
- L-NAME + gAd + Ins

B. Steady state glucose infusion rate (mg/kg/min):
- Saline + Ins
- gAd + Ins
- L-NAME + gAd + Ins

** Indicates significant difference.
Figure 4

A. 

- p-Akt (Ser473) vs T-Akt
- * *

B. 

- p-ERK (Thr202/Tyr204) vs T-ERK
- * *

Columns: Saline, gAd 0.2, gAd 0.4, Insulin, gAd +insulin

Bar graphs showing fold of control for p-Akt/T-Akt and p-ERK/T-ERK.
Figure 5

A.

Muscle clearance of $^{125}$I-insulin (ml/g/5 min)

- Saline
- gAd
- L-NAME+gAd

B.

Muscle $^{125}$I-insulin uptake (dpm/mg dry tissue/5 min)

- Saline
- gAd
- L-NAME+gAd

C.

Fraction of intact $^{125}$I-insulin (%)

- Blood
- Muscle

- Saline
- gAd
- L-NAME+gAd
Figure 6

A. MBV (Fold of control)

B. MFV (1/Sec)

C. MBF (Fold of control in V/Sec)

D. MAP (mmHg)
Figure 7

**A.**

p-Akt (Ser473) vs T-Akt

**B.**

p-AMPK (Thr172) vs T-AMPK

**C.**

p-eNOS (Ser1177) vs T-eNOS

**D.**

125I-Insulin uptake (Fold of control)
Globular Adiponectin Enhances Muscle Insulin Action via Microvascular Recruitment and Increased Insulin Delivery
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