

The Stimulatory Effect of Globular Adiponectin on Insulin-Stimulated Glucose Uptake and Fatty Acid Oxidation Is Impaired in Skeletal Muscle From Obese Subjects

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Adiponectin is an adipose-derived hormone that plays an important role in regulating insulin sensitivity in rodents. However, little is known regarding the effect of adiponectin on metabolism in human skeletal muscle. Therefore, we examined whether the globular head of adiponectin, gAcrp30, acutely activates fatty acid oxidation and glucose uptake in isolated human skeletal muscle. Furthermore, we aimed to determine whether these effects would differ in muscle from lean versus obese individuals. Treatment with gAcrp30 (2.5 $\mu\text{g/ml}$) increased fatty acid oxidation in lean muscle (70%, $P < 0.0001$) and to a lesser extent in obese muscle (30%, $P < 0.01$). In the absence of insulin, gAcrp30 increased glucose uptake 37% in lean ($P < 0.05$) and 33% in obese muscle ($P < 0.05$). Combined exposure of insulin and gAcrp30 demonstrated an additive effect on glucose uptake in lean and obese individuals, but this effect was reduced by 50% in obese muscle ($P < 0.05$). These metabolic effects were attributable to an increase in AMP-activated protein kinase- $\alpha 1$ (AMPK $\alpha 1$) and AMPK $\alpha 2$ activity. However, in obese muscle the activation of AMPK $\alpha 2$ by gAcrp30 was blunted. This study provides evidence that gAcrp30 plays a role in regulating fatty acid and glucose metabolism in human skeletal muscle. However, the effects are blunted in obesity, indicating the possible development of adiponectin resistance. *Diabetes* 54:3154–3160, 2005

Adiponectin is a 30-kDa adipose-derived hormone that appears to play an important role in regulating energy homeostasis and insulin sensitivity (1). The mRNA expression of adiponectin is reduced in obese and diabetic mice (2), and plasma levels are suppressed in conditions of obesity, insulin resistance, and type 2 diabetes (3,4). Administration of the globular head group of adiponectin (gAcrp30) reduces plasma glucose levels and ameliorates insulin resistance in

mice (5–7). In addition, mice lacking adiponectin exhibit insulin resistance and diabetes (8,9). Recent studies have also shown that treatment of isolated rodent skeletal muscle with gAcrp30 stimulates glucose uptake (10). The insulin-sensitizing effect of gAcrp30 appears to be mediated by an increase in fatty acid oxidation (1,10), leading to a reduction in muscle lipid content (6).

The molecular mechanism responsible for the stimulation of fatty acid oxidation and glucose uptake appears to be attributable to the activation of AMP-activated protein kinase (AMPK) via specific receptor signaling. Adiponectin receptor-1 is abundantly expressed in skeletal muscle and is a high-affinity receptor for gAcrp30 (11). On binding to its receptor, gAcrp30 activates AMPK, leading to subsequent inhibition of acetyl-CoA carboxylase (1,10). This reduces malonyl-CoA content and promotes mitochondrial fatty acid oxidation by diminishing the inhibitory effect of malonyl CoA on carnitine palmitoyltransferase-1. Adiponectin has no effect on fatty acid oxidation or glucose uptake when AMPK activity is blocked by dominant-negative AMPK expression, demonstrating that the metabolic effects of gAcrp30 occur through activation of AMPK (1).

Despite the growing body of evidence indicating that gAcrp30 is an antidiabetic hormone in rodents, directly regulating fatty acid and glucose metabolism, few studies have examined the role of gAcrp30 in regulating substrate metabolism in human skeletal muscle. Therefore, in the present study, we have used an isolated human skeletal muscle preparation to examine the effect of gAcrp30 on basal and insulin-stimulated glucose uptake, fatty acid oxidation, fatty acid partitioning into intramyocellular lipid pools, and AMPK signaling. Because it has recently been proposed that obesity is associated with adiponectin resistance (12,13), we hypothesized that the metabolic effects of gAcrp30 would be blunted in skeletal muscle from obese individuals because of impaired activation of AMPK.

RESEARCH DESIGN AND METHODS

The participants were 15 lean (BMI $\leq 27 \text{ kg/m}^2$; mean $24.7 \pm 0.6 \text{ kg/m}^2$) and 12 obese ($\geq 30.0 \text{ kg/m}^2$; $31.8 \pm 1.1 \text{ kg/m}^2$) nondiabetic women (Table 1). Subjects were admitted to McMaster Health Sciences Center for a variety of abdominal surgical interventions, and they gave informed written consent before participating. The majority of the subjects were admitted for hysterectomy procedures. Experimental protocols were approved by University of Guelph and McMaster University ethics committees. None of the subjects had any significant diseases or were taking any medications in the 6 months before surgery. All subjects had maintained a constant body mass during the year preceding surgery.

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AICAR, 5-amino-imidazole carboxamide riboside; AMPK, AMP-activated protein kinase; DAG, diacylglycerol; HAD, hydroxy-acyl-CoA dehydrogenase; TAG, triacylglycerol.

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TABLE 1
Subject characteristics

Characteristics	Lean	Obese
Age (years)	47 ± 2	47 ± 3
Body mass (kg)	66.4 ± 1.1	84.5 ± 3.7†
BMI (kg/m ²)	24.7 ± 0.6	31.8 ± 1.1†
Fasting blood glucose (mmol/l)	4.0 ± 0.2	4.2 ± 0.1
Fasting plasma insulin (pmol/l)	141.5 ± 4.6	151.0 ± 4.8
Fasting free fatty acid (mmol/l)	0.96 ± 0.10	0.90 ± 0.10
Fasting plasma adiponectin (µg/ml)	12.6 ± 2.4	4.4 ± 0.6*
Citrate synthase (µmol · min ⁻¹ · g dry wt ⁻¹)	40.3 ± 3.5	38.5 ± 3.8
β-HAD (µmol · min ⁻¹ · g dry wt ⁻¹)	20.7 ± 1.9	19.7 ± 1.2

Data are the means ± SE. *Significantly different from lean ($P < 0.05$); †significantly different from lean ($P < 0.0001$).

After an overnight fast (12–18 h), general anesthesia was induced with a short-acting barbiturate and maintained by a fentanyl and rocuronium volatile anesthetic mixture. Venous blood samples were then collected in 5-ml heparinized tubes during anesthesia. Muscle strip preparation was as previously described (14,15), with minor modifications. A biopsy of 3 × 2 × 1 cm from the rectus abdominus muscle was excised perpendicular to the direction of the muscle fibers, clipped at resting length, and placed in oxygenated ice-cold medium 199 (Sigma, Oakville, ON, Canada) for transport to the laboratory (~5 min). Then, 8–12 muscle strips weighing ~25 mg were separated from the muscle sample and clipped at resting length.

Muscle viability. In preliminary experiments, ATP and phosphocreatine were measured spectrophotometrically (16) to ensure muscle viability. Muscle strips were rapidly frozen in liquid N₂ immediately after excision (0 min) or after a 120-min incubation.

Glucose uptake. Muscle strips were preincubated in a shaking water bath at 30°C for 30 min in pregassed (95% O₂/5% CO₂) Krebs-Henseleit buffer containing 8 mmol/l glucose and 32 mmol/l mannitol. Thereafter, muscle specimens went through three different incubation phases: phase 1 to equilibrate the muscle, phase 2 to wash glucose from the interstitial space, and phase 3 to determine glucose uptake into muscle. During phase 1, muscle strips were incubated for 20 min in the absence (basal) or presence of insulin (120 nmol/l). The concentration of insulin was maintained throughout all remaining incubation steps. In the second phase, muscle specimens underwent two 10-min incubations at 30°C in glucose-free Krebs-Henseleit buffer containing 4 mmol/l pyruvate in the absence or presence of recombinant human gAcrp30 (2.5 µg/ml; Peprotech, Ottawa, ON, Canada). The gAcrp30 concentration was maintained for the remaining incubations. Osmolarity was maintained by the addition of 36 mmol/l mannitol to the media. During phase 3, muscle strips were incubated for 20 min (insulin-stimulated) or 40 min (basal) in Krebs-Henseleit buffer containing 8 mmol/l 3-O-[³H]methyl-D-glucose (800 µCi/mmol) and 32 mmol/l [¹⁴C]mannitol (53 µCi/mmol). After incubation, muscles were blotted of excess fluid, frozen in liquid nitrogen, and stored at -80°C until analysis. Glucose uptake was analyzed as the accumulation of intracellular 3-O-[³H]methyl-D-glucose, as described previously (17).

Skeletal muscle fatty acid oxidation. Muscle strips were placed in a 20-ml glass scintillation vial containing 2 ml of warmed (30°C) pregassed (95% O₂/5% CO₂, pH 7.4) medium 199 containing 4% fatty acid-free BSA (ICN Biomedicals), 1.0 mmol/l palmitate, and 5.5 mmol/l glucose. Throughout the experiment, vials were gently shaken in a water bath. After a preincubation period of 30 min, muscle samples were incubated with or without gAcrp30 (2.5 µg/ml) for 1 h in the same medium specified above, with the addition of 0.5 µCi/ml of [1-¹⁴C]palmitate (Amersham, Oakville, ON, Canada). This permitted the monitoring of exogenous palmitate oxidation and incorporation of palmitate into endogenous lipid pools.

Gaseous ¹⁴CO₂ produced from the exogenous oxidation of [1-¹⁴C]palmitate during incubation was measured by transferring 1.0 ml of the incubation medium to a 20-ml glass scintillation vial containing 1.0 ml of 1 mol/l H₂SO₄ and a 0.5-ml Fisher microcentrifuge tube containing 1 mol/l benzethonium hydroxide. Liberated ¹⁴CO₂ was trapped in the benzethonium hydroxide over 60 min, the microcentrifuge tube containing trapped ¹⁴CO₂ was placed in a scintillation vial, and radioactivity was counted.

Extraction of muscle lipids. Muscles were placed in 13-ml plastic centrifuge tubes containing 5.0 ml of ice-cold 2:1 chloroform-methanol (vol/vol), and they were homogenized using a polytron (Brinkman Instruments, Mississauga, ON, Canada). After homogenization, samples were centrifuged at 2,000g (4°C) for 10 min. The supernatant was removed with a glass Pasteur pipette and

transferred to a clean centrifuge tube. Distilled water (2.0 ml) was added, and samples were shaken for 10 min and centrifuged as before to separate the aqueous and lipophilic phases. Then, 500 µl of the aqueous phase was quantified by liquid scintillation counting to determine the amount of ¹⁴C-labeled oxidative intermediates resulting from isotopic fixation. This represented a twofold correction factor for exogenous [¹⁴C]palmitate oxidation, as previously described (18). The chloroform phase, which contains the total lipids extracted from muscle, was gently evaporated under a stream of N₂ and redissolved in 100 µl of 2:1 chloroform-methanol. Small amounts of dipalmitin and tripalmitin (Sigma Chemical, St. Louis, MO) were added to the 2:1 chloroform-methanol to facilitate the identification of lipid bands on the silica gel plates. We spotted 50 µl of each sample on an oven-dried silica gel plate (Fisher Scientific Canada, Mississauga, ON). Silica gel plates were placed in a sealed tank containing solvent (60:40:3 heptane-isopropyl ether-acetic acid) for 40 min. Plates were then dried, sprayed with dichlorofluorescein dye (0.02% wt/vol in ethanol), and visualized under longwave ultraviolet light. The individual lipid bands were marked on the plate with a scalpel and scraped into vials for liquid scintillation counting.

AMPK activity. AMPKα1 and -α2 activities were examined after the 30-min preincubation (0 min) and after 20 or 60 min of gAcrp30 treatment. Briefly, muscle strips were incubated in medium 199 (5.5 mmol/l glucose) containing 4% fatty acid-free BSA, 5.5 mmol/l glucose, and 1.0 mmol/l palmitate with or without the addition of 2.5 µg/ml gAcrp30. At the end of the incubation period, the muscle strips were quickly freeze-clamped and stored in liquid nitrogen until subsequent analysis.

To determine AMPK activity, muscle strips (~20 mg) were homogenized in buffer (50 mmol/l Tris · HCl, pH 7.5, 1 mmol/l EDTA, 1 mmol/l EGTA, 1 mmol/l dithiothreitol, 50 mmol/l NaF, 5 mmol/l Na pyrophosphate, 10% glycerol, 1% Triton X-100, 10 µg/ml trypsin inhibitor, 2 µg/ml aprotinin, 1 mmol/l benzamidine, and 1 mmol/l phenylmethylsulfonyl fluoride). The homogenates were incubated with AMPKα1 and -α2 (Upstate Scientific, Charlottesville, VA) antibody-bound protein A beads (Sigma, St. Louis, MO) each for 2 h at 4°C. Immunocomplexes were washed with PBS and suspended in 60 µl dilution buffer (50 mmol/l Tris, pH 7.5, 1 mmol/l dithiothreitol, 10% glycerol, and 0.1% Triton-X) for AMPK activity assay (19). Briefly, 20 µl of the sample was combined with 20 µl of reagent mixture (5 mmol/l HEPES, pH 7.5, 1 mmol/l MgCl₂, 0.5% glycerol, 1 mmol/l dithiothreitol, and 100 µmol/l SAMS peptide; Upstate Scientific), 250 µmol/l ATP with ³²P-γ-ATP (Amersham Biosciences, QC, Canada) and 100 µmol/l AMP. The reaction proceeded for 15 min, after which 23 µl of reaction mixture was spotted onto p81 filter paper (Upstate Scientific) and washed three times in 1% phosphoric acid. Filter papers were dried and placed in organic scintillant for counting.

Citrate synthase and β-hydroxy-acyl-CoA dehydrogenase activity. Citrate synthase and β-hydroxy-acyl-CoA dehydrogenase (HAD) activity was assayed in a portion of muscle (~10 mg) that was rapidly frozen in liquid N₂ immediately after excision. Muscle was homogenized in 100 vol/wt of a 100-mmol/l potassium phosphate buffer solution, and citrate synthase activity was assayed spectrophotometrically at 37°C (20). β-HAD activity was assayed spectrophotometrically at 37°C by measuring the disappearance of NADH, using the whole-muscle homogenate as for citrate synthase (21).

Calculations and statistics. All data are the means ± SE. Differences within a group were determined using paired *t* tests. To examine differences between the lean and obese groups, unpaired *t* tests were used. Statistical significance was accepted at $P < 0.05$. Total palmitate uptake by the muscle strips was calculated by summing the incorporation of labeled palmitate into lipid pools plus oxidation.

RESULTS

Subject characteristics are shown in Table 1. Obese individuals had significantly greater body mass (+27%, $P < 0.0001$) and BMI (+29%, $P < 0.0001$) compared with lean subjects. Fasting levels of plasma glucose, free fatty acids, and insulin were not significantly different between lean and obese women ($P > 0.05$). Adiponectin levels were significantly decreased (-65%, $P < 0.05$) in obese individuals. The activities of citrate synthase and β-HAD were not different between lean and obese subjects (Table 1) and were comparable to the activities of citrate synthase and β-HAD reported in the vastus lateralis (22), which is a more commonly sampled human muscle.

Muscle strip preparation viability. Muscle viability was preserved during incubations based on maintenance of ATP (0 min, 20.7 ± 0.8; 120 min, 19.1 ± 0.8 µmol/g dry wt)

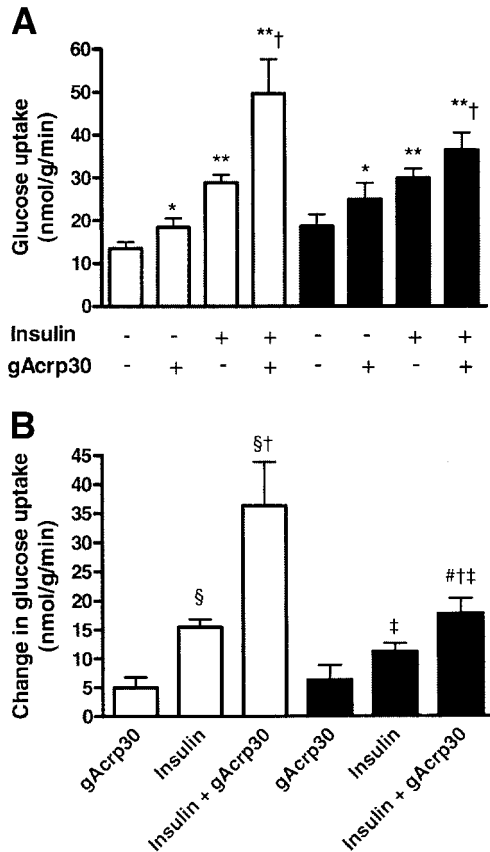


FIG. 1. Effects of gAcrp30 or insulin on glucose uptake in skeletal muscle from lean (□; $n = 8$) and obese (■; $n = 8$) subjects. **A:** Muscle strips were exposed and incubated in the absence or presence of 2.5 $\mu\text{g/ml}$ gAcrp30, 120 nmol/l insulin, or a combination of 2.5 $\mu\text{g/ml}$ gAcrp30 and 120 nmol/l insulin as described in RESEARCH DESIGN AND METHODS. **B:** The individual change in glucose uptake rate in response to gAcrp30 or insulin. Data were calculated by subtracting respective rate of basal glucose uptake from the gAcrp30 or insulin response. Data are the means \pm SE. * $P < 0.05$ vs. basal; ** $P < 0.01$ vs. basal; † $P < 0.05$ vs. corresponding insulin-stimulated value; ‡ $P < 0.05$ vs. corresponding gAcrp30 value; § $P < 0.01$ vs. corresponding gAcrp30 value; # $P < 0.05$ vs. corresponding insulin-stimulated value; †† $P < 0.05$ vs. lean.

and phosphocreatine content (0 min, 77.5 ± 2.9 ; 120 min, 70.2 ± 3.2 $\mu\text{mol/g}$ dry wt).

Glucose uptake in isolated skeletal muscle. Basal glucose uptake was not different between lean and obese subjects (Fig. 1A). Treatment with gAcrp30 increased glucose uptake 37% ($P < 0.05$) in lean subjects and 33% ($P < 0.05$) in obese subjects. Insulin increased glucose uptake 110% ($P < 0.01$) in control subjects and 60% ($P < 0.01$) in obese subjects (Fig. 1A). The absolute change in glucose uptake in response to insulin was 28% lower in obese subjects ($P < 0.05$) (Fig. 1B). Combined exposure of insulin and gAcrp30 increased glucose uptake 270% ($P < 0.01$) in control subjects and 90% ($P < 0.01$) in obese subjects (Fig. 1A). The additive effect of gAcrp30 and insulin on glucose uptake was 50% lower ($P < 0.05$) in obese subjects (Fig. 1B).

Skeletal muscle fatty acid metabolism. In the absence of gAcrp30, fatty acid oxidation was not different between lean and obese subjects (Fig. 2A). Fatty acid oxidation was significantly increased with gAcrp30 treatment in both lean (+69%, $P < 0.001$) and obese (+30%, $P < 0.01$) subjects (Fig. 2A). The absolute change in fatty acid oxidation in response to gAcrp30 was 58% lower in obese subjects ($P < 0.05$) (Fig. 2B). Fatty acid esterification into

diacylglycerol (DAG) was not different between lean and obese subjects and was unaffected by gAcrp30 (Fig. 2C). However, fatty acid esterification into triacylglycerol (TAG) was greater in obese subjects (+69%, $P < 0.05$) (Fig. 2D). Fatty acid esterification into TAG was not reduced in either lean or obese muscle in the presence of gAcrp30. Basal rates of total palmitate uptake were also greater in obese subjects (+25%, $P < 0.05$) (Table 2). Treatment with gAcrp30 increased total palmitate uptake in lean muscle (+38%, $P < 0.05$) (Table 2). The ratio of palmitate esterification (DAG and TAG) to oxidation was significantly elevated in obese muscle (+34%, $P < 0.05$), indicating a partitioning of incorporated fatty acid toward storage (Fig. 2E). The presence of gAcrp30 resulted in a significant reduction in the fatty acid esterification-to-oxidation ratio in lean muscle (-28%, $P < 0.05$) (Fig. 2E) and obese muscle (-23%, $P < 0.01$) (Fig. 2E), with the absolute change being similar in the lean and obese subjects (Fig. 2F).

Effect of gAcrp30 on AMPK activity. In the absence of gAcrp30, AMPK α 1 activity remained stable in both the lean muscle (0.78 ± 0.10 , 0.66 ± 0.10 , and 0.73 ± 0.12 pmol \cdot mg protein $^{-1} \cdot$ min $^{-1}$ for 0, 20, and 60 min, respectively) and obese muscle (0.51 ± 0.10 , 0.54 ± 0.12 , and 0.70 ± 0.15 , respectively). After 60 min of exposure to gAcrp30, AMPK α 1 activity was elevated in muscle from both groups (Fig. 3A). AMPK α 1 activity was lower in muscle from the obese patients at 0 min ($P < 0.05$) (Fig. 3A) and remained suppressed after 20 min of gAcrp30 treatment ($P < 0.05$) (Fig. 3A). After 60 min of gAcrp30 treatment, AMPK α 1 activity tended to be reduced in the obese muscle ($P = 0.06$) (Fig. 3A). Basal AMPK α 2 activity was not different between groups (Fig. 3B). Throughout the incubation period, AMPK α 2 activity was constant in the untreated control strips from both lean (0.31 ± 0.03 , 0.28 ± 0.03 , and 0.32 ± 0.05 pmol \cdot mg protein $^{-1} \cdot$ min $^{-1}$ for 0, 20, and 60 min, respectively) and obese (0.30 ± 0.04 , 0.33 ± 0.09 , and 0.36 ± 0.07 , respectively) patients. Treatment with gAcrp30 for 20 min increased AMPK α 2 activity in muscle from lean and obese individuals (Fig. 3B), whereas AMPK α 2 remained elevated after 60 min of gAcrp30 treatment only in lean muscle (Fig. 3B). However, there was a trend for AMPK α 2 activity to be elevated in obese muscle after 60 min of treatment with gAcrp30 ($P = 0.06$) (Fig. 3B). At that time, AMPK α 2 activity was reduced in the obese compared with the lean subjects ($P < 0.01$) (Fig. 3B).

DISCUSSION

Adiponectin is a recently discovered adipokine that can exert systemic effects on insulin sensitivity and lipid metabolism in rodents (1). Although low levels of adiponectin are associated with obesity and type 2 diabetes (3,4), suggesting an important role for adiponectin in regulating insulin sensitivity, there is little information on the direct effects of adiponectin on skeletal muscle metabolism in humans. Therefore, in this study we used an isolated human skeletal muscle strip preparation to examine the effects of the globular head of adiponectin, gAcrp30, on glucose uptake and fatty acid metabolism. The biological significance of gAcrp30 is unclear. It has previously been reported that \sim 1% of total circulating adiponectin is in the globular form (5); the fact that the concentration of adiponectin is \sim 100-fold greater than other cytokines suggests that this fraction may be of biological significance. However, other studies have not detected the presence of gAcrp, indicating that it is the high-molecular weight forms of

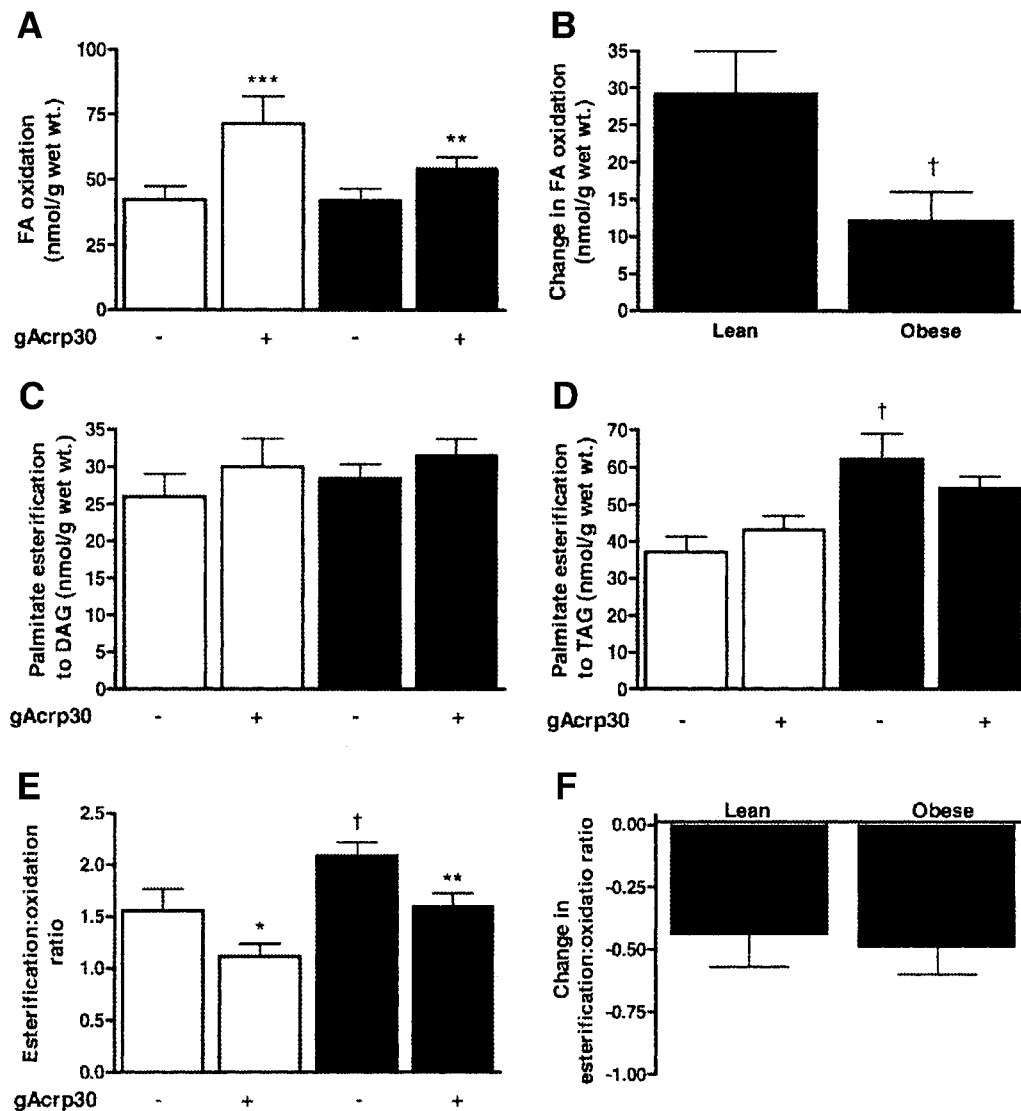


FIG. 2. The effect of gAcrp30 on fatty acid (FA) metabolism in skeletal muscle from lean (\square ; $n = 8$) and obese (\blacksquare ; $n = 9$) subjects. *A*: The rate of fatty acid oxidation in muscle strips in the absence or presence of gAcrp30 (2.5 $\mu\text{g/ml}$). *B*: The individual change in fatty acid oxidation in response to gAcrp30. Data were calculated by subtracting the respective rate of basal fatty acid oxidation from the gAcrp30 response. *C*: Palmitate incorporation into DAG. *D*: Palmitate incorporation into TAG. *E*: Fatty acid partitioning (esterification-to-oxidation) ratio of incorporated palmitate. *F*: The individual change in the fatty acid partitioning ratio of incorporated palmitate. Data are the means \pm SE. * $P < 0.05$ vs. control; ** $P < 0.01$ vs. control; *** $P < 0.001$ vs. control; † $P < 0.05$ vs. lean.

adiponectin that are biologically important (23,24). Nevertheless, the effect of globular adiponectin on muscle metabolism and insulin sensitivity is certainly of therapeutic significance. To our knowledge, this is the first time that gAcrp30 has been shown to increase glucose uptake in human skeletal muscle. Furthermore, we demonstrate that gAcrp30 had an additive effect on insulin-stimulated glucose uptake. We have also confirmed recent findings (12) that gAcrp30 stimulates fatty acid oxidation in human skeletal muscle. Our results suggest

that gAcrp30 stimulates glucose uptake and fatty acid oxidation by activating AMPK. Of particular interest is the finding that the effects of gAcrp30 on insulin-stimulated glucose uptake and fatty acid oxidation are blunted in skeletal muscle of obese individuals, which appears to be due to impaired activation of AMPK.

Effect of gAcrp30 on skeletal muscle glucose uptake. Studies in rodents have reported that gAcrp30 increases glucose uptake in skeletal muscle (1,10). However, no studies have examined the effect of gAcrp30 on glucose uptake in human muscle. Therefore, a major finding of the present study was that gAcrp30 increased glucose uptake in the absence of insulin in skeletal muscle from both lean and obese individuals. Importantly, we also demonstrate that gAcrp30 had an additive effect on insulin-stimulated glucose uptake in skeletal muscle from both lean and obese subjects. Similar observations have recently been reported in L6 myotubes. Ceddia et al. (25) found that gAcrp30 increased basal rates of glucose uptake via trans-

TABLE 2
Total palmitate uptake

Uptake (nmol/g wet wt)	-gAcrp30	+gAcrp30
Lean	105.5 \pm 13.3	145.5 \pm 15.7*
Obese	131.5 \pm 7.1†	142.7 \pm 10.8

Data are the means \pm SE. $n = 8-9$ per group. *Significantly different from -gAcrp30 ($P < 0.05$); †significantly different from lean ($P < 0.05$).

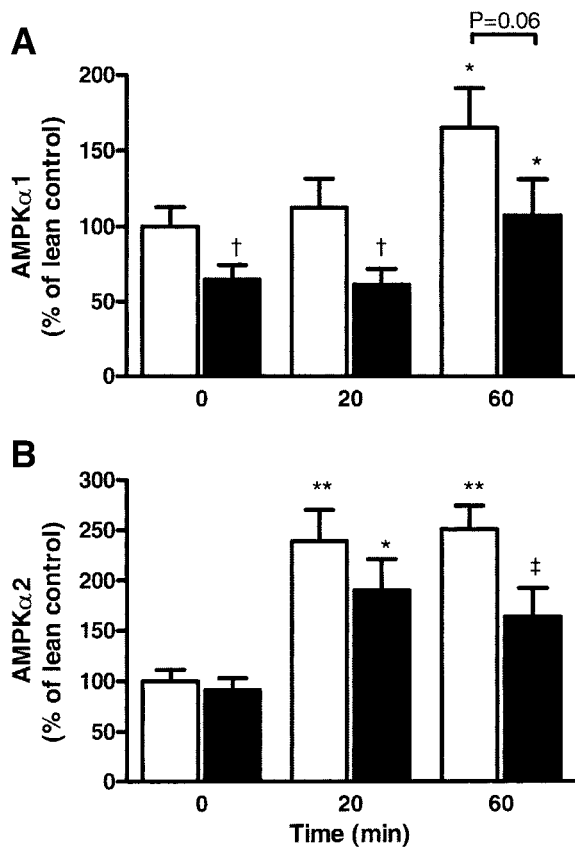


FIG. 3. Time course of changes in AMPK α 1 (A) and AMPK α 2 (B) activity in muscle from lean (□) and obese (■) subjects incubated with gAcrp30. Data are the means \pm SE ($n = 6-10$ per group) and are expressed relative to the untreated control strip from the lean group at the same time point. * $P < 0.05$ vs. control; ** $P < 0.001$ vs. control; † $P < 0.05$ vs. lean; ‡ $P < 0.01$ vs. lean.

location of GLUT4 to the cell surface. Furthermore, when used in combination with insulin, gAcrp30 exerted an additive effect on glucose uptake and GLUT4 translocation (25). It should be noted that the pharmacological insulin concentration used in the present study was chosen because this is known to maximally stimulate glucose uptake in an incubation model. Thus, it must be acknowledged that the high level of insulin, particularly in the absence of other hormones and peptides normally present in the circulation, makes it impossible to directly extrapolate our findings to the in vivo condition. Taken together, the results of the current study and those of Ceddia et al. (25) suggest that like muscle contraction and 5-aminoimidazole carboxamide riboside (AICAR), gAcrp30 increases glucose uptake via an insulin-independent mechanism, possibly involving AMPK. It is also noteworthy that in the current study, the stimulation of glucose uptake in the presence of gAcrp30 and insulin was actually greater than that which would be predicted by adding the individual responses to these hormones, i.e., a greater than additive effect. This may imply that adiponectin also increases insulin sensitivity in muscle.

A novel finding of the current study was that the additive effect of gAcrp30 and insulin on glucose uptake was blunted in the muscle from obese patients. Recently, it has been proposed that in animal models of obesity and human type 2 diabetes, adiponectin resistance may develop, resulting in a decrease in the adiponectin effects on fatty acid oxidation and AMPK activation (12,13). Interest-

ingly, in muscle from the obese individuals, the increase in basal glucose uptake in response to gAcrp30 was not impaired, despite reduced activation of AMPK compared with lean muscle. Therefore, these results suggest that the reduction in insulin-stimulated glucose uptake in the muscle from obese individuals may be responsible for the impairment in glucose uptake in response to a combination of gAcrp30 and insulin. Surprisingly, the fasting levels of blood glucose and insulin were not different between the lean and obese groups (Table 1), suggesting that whole-body insulin sensitivity was not actually impaired in the obese subjects. However, it should be noted that these measurements were made after a prolonged fast (12–18 h) before surgery, and that a more dynamic test, such as a euglycemic-hyperinsulinemic clamp or an oral glucose tolerance test, may have revealed a difference in insulin sensitivity between the groups.

Effect of gAcrp30 on skeletal muscle fatty acid metabolism. The insulin-sensitizing effect of gAcrp30 has been suggested to be mediated by an increase in fatty acid oxidation (1,10), leading to a reduction in muscle lipid content (6). In the current study, we demonstrated that gAcrp30 increases fatty acid oxidation in skeletal muscle from both lean and obese subjects and that this is associated with enhanced insulin sensitivity. However, similar to the effect on insulin-stimulated glucose uptake, the stimulatory effect of gAcrp30 on fatty acid oxidation is blunted in skeletal muscle from obese individuals. This is consistent with the finding of impaired activation of AMPK in muscle from obese subjects. Our data are in agreement with the recent study of Chen et al. (12), who report that gAcrp30 stimulates fatty acid oxidation in cultured human myotubes and that this response is impaired in obese and obese type 2 diabetic myotubes.

Insulin resistance is associated with accumulation of cytosolic lipids within muscle (26). It has been hypothesized that interventions that increase fatty acid oxidation will improve insulin sensitivity by reducing the accumulation of muscle lipids. Indeed, adiponectin treatment has been shown to improve insulin sensitivity by decreasing muscle triglyceride content (6). Therefore, we examined whether gAcrp30 treatment would affect fatty acid esterification into DAG and TAG pools. Interestingly, fatty acid esterification into DAG and TAG was unaffected by gAcrp30 treatment, despite the observed increase fatty acid oxidation. It is possible that our incubation period was too short in duration to see an effect of gAcrp30 on fatty acid esterification into the endogenous lipid pools. Furthermore, the effect of gAcrp30 on fatty acid esterification into the lipid pools may only become evident when in the presence of other hormones that stimulate lipogenesis, such as insulin. However, despite no changes in the esterification of fatty acids into the lipid pools, we were able to detect a significant reduction in the esterification-to-oxidation ratio in both lean and obese muscle, indicating a partitioning of fatty acid toward oxidation rather than storage.

Effect of gAcrp30 on AMPK activation. The signaling pathways regulated by adiponectin are just beginning to be characterized. On binding to its receptor, adiponectin receptor-1, gAcrp30 activates AMPK, which appears to mediate the metabolic effects of gAcrp30 (11). Previous studies using cell culture and isolated rodent muscle have shown that AMPK activity is rapidly and transiently activated by gAcrp30 (1,10). However, here we show that AMPK α 1 activity is only activated after 60 min of exposure to gAcrp30 in both lean and obese skeletal muscle. In

contrast, AMPK α 2 activity is increased after 20 min of incubation with gAcrp30 in muscle from lean and obese individuals. This effect is maintained in muscle from lean subjects after 60 min of gAcrp30, whereas there was a tendency for AMPK α 2 to remain elevated at this time point in muscle from obese subjects. Although we do not know the precise mechanisms underlying these differences, it is possible that species differences exist such that a more prolonged AMPK response is observed in human skeletal muscle after acute treatment with gAcrp30.

Interestingly, we found impaired stimulation of AMPK α 1 and α 2 by gAcrp30 in skeletal muscle of obese subjects. In fact, we found that the basal activity of AMPK α 1 is reduced in obese skeletal muscle. The metabolic significance of this reduction in AMPK α 1 activity is unknown because currently it appears that the α 2 isoform plays a more important role in regulating substrate metabolism (27,28). Indeed, AMPK α 2 knockout mice are resistant to AICAR-induced glucose uptake (27) and develop whole-body insulin resistance (28), whereas AMPK α 1 knockout mice appear normal (25). In this regard, the reduction in AMPK α 2 activity in the obese subjects may explain the impaired metabolic responses to gAcrp30. Furthermore, it has recently been reported that the mRNA expression of adiponectin receptor-1 is reduced in insulin-resistant rodents (13) and humans (29), which may result in decreased activation of AMPK by gAcrp30.

In conclusion, this is the first study to show that gAcrp30 acutely increases glucose uptake in human skeletal muscle. Furthermore, we show an additive effect of gAcrp30 on insulin-stimulated glucose transport in lean and obese skeletal muscle. These effects may be mediated by a shift in fatty acid partitioning toward oxidation and away from storage, which is likely due to activation of AMPK. Importantly, our data also show that the effects of gAcrp30 on insulin-stimulated glucose uptake and fatty acid oxidation are blunted in skeletal muscle from obese subjects because of impaired activation of AMPK. These results suggest that adiponectin resistance develops in obesity.

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REFERENCES

1. Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, Yamashita S, Noda M, Kita S, Ueki K, Eto K, Akanuma Y, Froguel P, Foufelle F, Ferre P, Carling D, Kimura S, Nagai R, Kahn BB, Kadowaki T: Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med* 8:1288–1295, 2002
2. Hu E, Liang P, Spiegelman BM: AdipoQ is a novel adipose-specific gene dysregulated in obesity. *J Biol Chem* 271:10697–10703, 1996
3. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y: Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* 257:79–83, 1999
4. Kern PA, Di Gregorio GB, Lu T, Rassouli N, Ranganathan G: Adiponectin expression from human adipose tissue: relation to obesity, insulin resistance, and tumor necrosis factor- α expression. *Diabetes* 52:1779–1785, 2003
5. Fruebis J, Tsao T-S, Javorschi S, Reed-Ebbets D, Erickson MRS, Yen FT, Bihain BE, Lodish HF: Proteolytic cleavage product of 30-kDa adipocyte

complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. *Proc Natl Acad Sci U S A* 98:2005–2010, 2001

6. Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki T: The fat-derived hormone adiponectin reverses insulin resistance associated with both lipotrophy and obesity. *Nat Med* 7:941–946, 2001
7. Berg AH, Combs TP, Du X, Brownlee M, Scherer PE: The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nat Med* 7:947–953, 2001
8. Kubota N, Terauchi Y, Yamauchi T, Kubota T, Moroi M, Matsui J, Eto K, Yamashita T, Kamon J, Satoh H, Yano W, Froguel P, Nagai R, Kimura S, Kadowaki T, Noda T: Disruption of adiponectin causes insulin resistance and neointimal formation. *J Biol Chem* 277:25863–25866, 2002
9. Maeda N, Shimomura I, Kishida K, Nishizawa H, Matsuda M, Nagaretani H, Furuyama N, Kondo H, Takahashi M, Arita Y, Komuro R, Ouchi N, Kihara S, Tochino Y, Okutomi K, Horie M, Takeda S, Aoyama T, Funahashi T, Matsuzawa Y: Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. *Nat Med* 8:731–737, 2002
10. Tomas E, Tsao T-S, Saha AK, Murrey HE, Zhang CC, Itani SI, Lodish HF, Ruderman NB: Enhanced muscle fat oxidation and glucose transport by ACP30 globular domain: acetyl-CoA carboxylase inhibition and AMP-activated protein kinase activation. *Proc Natl Acad Sci U S A* 99:16309–16313, 2002
11. Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, Sugiyama T, Miyagishi M, Hara K, Tsunoda M, Murakami K, Ohteki T, Uchida S, Takekawa S, Waki H, Tsuno NH, Shibata Y, Terauchi Y, Froguel P, Tobe K, Koyasu S, Taira K, Kitamura T, Shimizu T, Nagai R, Kadowaki T: Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature* 423:762–769, 2003
12. Chen MB, McAinch AJ, Macaulay SL, Castelli LA, O'Brien PE, Dixon JB, Cameron-Smith D, Kemp BE, Steinberg GR: Impaired activation of AMP-kinase and fatty acid oxidation by globular adiponectin in cultured human skeletal muscle from obese type 2 diabetics. *J Clin Endocrinol Metab* 90:3665–3672, 2005
13. Tsuchida A, Yamauchi T, Ito Y, Hada Y, Maki T, Takekawa S, Kamon J, Kobayashi M, Suzuki R, Hara K, Kubota N, Terauchi Y, Froguel P, Nakae K, Kasuga M, Accili D, Tobe K, Ueki K, Nagai R, Kadowaki T: Insulin/Foxo1 pathway regulates expression levels of adiponectin receptors and adiponectin sensitivity. *J Biol Chem* 279:30817–30822, 2004
14. Dohm GL, Tapscott EB, Pories WJ, Dabbs DJ, Flickinger EG, Meelheim D, Fushiki T, Atkinson SM, Elton CW, Caro JF: An in vitro human muscle preparation suitable for metabolic studies: decreased insulin stimulation of glucose transport in muscle from morbidly obese and diabetic subjects. *J Clin Invest* 82:486–494, 1988
15. Steinberg GR, Parolin ML, Heigenhauser GJF, Dyck DJ: Leptin increases FA oxidation in lean but not obese human skeletal muscle: evidence of peripheral leptin resistance. *Am J Physiol Endocrinol Metab* 283:E187–E192, 2002
16. Bergmeyer HU. *Methods of Enzymatic Analysis*. 2nd ed. New York, Academic, 1974
17. Wallberg-Henriksson H, Zetan N, Henriksson J: Reversibility of decreased insulin-stimulated glucose transport capacity in diabetic muscle with in vitro incubation: insulin is not required. *J Biol Chem* 262:7665–7671, 1987
18. Dyck DJ, Peters SJ, Glatz J, Gorski J, Keizer H, Kiens B, Liu S, Richter EA, Spriet LL, Van Der Vusse GJ, Bonen A: Functional differences in lipid metabolism in resting skeletal muscle of various fibre types. *Am J Physiol Endocrinol Metab* 272:E340–E351, 1997
19. Chen ZP, McConell GK, Michell BJ, Snow RJ, Canny BJ, Kemp BE: AMPK signaling in contracting human skeletal muscle: acetyl-CoA carboxylase and NO synthase phosphorylation. *Am J Physiol Endocrinol Metab* 279:E1202–E1206, 2000
20. Srere PA: Citrate synthase. *Methods Enzymol* 13:3–11, 1969
21. Lowry OH, Passonneau JV: *A Flexible System of Enzymatic Analysis*. New York, Academic, 1972
22. Helge JW, Wu BJ, Willer M, Dagaard JR, Storlien LH, Kiens B: Training affects muscle phospholipid fatty acid composition in humans. *J Appl Physiol* 90:670–677, 2001
23. Tonelli J, Li W, Kishore P, Pajvani UB, Kwon E, Weaver C, Scherer PE, Hawkins M: Mechanisms of early insulin-sensitizing effects of thiazolidinediones in type 2 diabetes. *Diabetes* 53:1621–1629, 2004 [erratum in *Diabetes* 54:587, 2005]
24. Pajvani UB, Hawkins M, Combs TP, Rajala MW, Doebber T, Berger JP, Wagner JA, Wu M, Knopps A, Xiang AH, Utzschneider KM, Kahn SE, Olefsky JM, Buchanan TA, Scherer PE: Complex distribution, not absolute

- amount of adiponectin, correlates with thiazolidinedione-mediated improvement in insulin sensitivity. *J Biol Chem* 279:12152–12162, 2004
25. Ceddia RB, Somwar R, Maida A, Fang X, Bikopoulos G, Sweeney G: Globular adiponectin increases GLUT4 translocation and glucose uptake but reduces glycogen synthesis in rat skeletal muscle. *Diabetologia* 48:132–139, 2005
26. Pan DA, Lillioja S, Kriketos AD, Milner MR, Baur LA, Bogardus C, Jenkins AB, Storlien LH: Skeletal muscle triglyceride levels are inversely related to insulin action. *Diabetes* 46:983–988, 1997
27. Jorgensen SB, Viollet B, Andreelli F, Frosig C, Birk JB, Schjerling P, Vaulont S, Richter EA, Wojtaszewski JF: Knockout of the $\alpha 2$ but not $\alpha 1$ 5'-AMP-activated protein kinase isoform abolishes 5-aminoimidazole-4-carboxamide-1- β -D-ribofuranoside but not contraction-induced glucose uptake in skeletal muscle. *J Biol Chem* 279:1070–1079, 2004
28. Viollet B, Andreelli F, Jorgensen SB, Perrin C, Geloën A, Flamez D, Mu J, Lenzner C, Baud O, Bennoun M, Gomas E, Nicolas G, Wojtaszewski JF, Kahn A, Carling D, Schuit FC, Birnbaum MJ, Richter EA, Burcelin R, Vaulont S: The AMP-activated protein kinase $\alpha 2$ catalytic subunit controls whole-body insulin sensitivity. *J Clin Invest* 111:91–98, 2003
29. Civitarese AE, Jenkinson CP, Richardson D, Bajaj M, Cusi K, Kashyap S, Berria R, Belfort R, DeFronzo RA, Mandarino LJ, Ravussin E: Adiponectin receptors gene expression and insulin sensitivity in non-diabetic Mexican Americans with or without a family history of type 2 diabetes. *Diabetologia* 47:816–820, 2004