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Acute exposure to rosiglitazone does not affect glucose transport in intact human skeletal muscle

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Abstract

Thiazolidinediones (TZDs) such as rosiglitazone are widely used as antidiabetic drugs. Animal studies suggest that TZDs may have direct metabolic actions in skeletal muscle. Here, we examined if acute exposure to rosiglitazone stimulates glucose transport rate and affects proximal insulin signaling in isolated skeletal muscle strips from nondiabetic men. Open muscle biopsies were obtained from musculus vastus lateralis from 15 nondiabetic men (50 ± 3 years old, 26.9 ± 1.1 kg/m^2). Skeletal muscle strips were isolated and exposed to rosiglitazone (1 or 10 μmol/L), 5-aminoimidazole-4-carboxamide 1-β-D-ribonucleoside (1 mmol/L), insulin (120 nmol/L), or a combination of insulin (120 nmol/L) and rosiglitazone (10 μmol/L) in vitro for 1 hour. Glucose transport was analyzed by accumulation of intracellular 3-*O*-methyl [^{3}H] glucose; phosphorylation of Akt-Ser^{473} and Akt-Thr^{308} and phosphorylation of acetyl coenzyme A carboxylase β were determined using phosphospecific antibodies. 5-Aminoimidazole-4-carboxamide 1-β-D-ribonucleoside and insulin increased glucose transport rate 1.5-fold ($P < .05$) and 1.7-fold ($P < .01$) in isolated muscle strips, respectively. Exposure to rosiglitazone transiently increased phosphorylation of acetyl coenzyme A carboxylase β, with a maximum effect at 15 minutes and return to baseline at 60 minutes. However, rosiglitazone did not affect basal or insulin-stimulated glucose transport rate, or phosphorylation of Akt-Ser^{473} or Akt-Thr^{308} in isolated muscle strips. In conclusion, acute exposure to rosiglitazone does not affect glucose transport in human skeletal muscle.

1. Introduction

Skeletal muscle is the main site of resistance to insulin-stimulated glucose metabolism in type 2 diabetes mellitus [1], and defects at the level of muscle glucose metabolism can be observed years before overt type 2 diabetes mellitus develops [2,3]. Therefore, interventions to improve muscle glucose metabolism would be beneficial in the prevention and treatment of type 2 diabetes mellitus. Thiazolidinediones (TZDs), such as rosiglitazone or pioglitazone, are widely used as antidiabetic drugs [4]. Thiazolidinediones improve whole-body insulin sensitivity by targeting peroxisome proliferator–activated receptor–γ (PPARγ), a transcription factor highly expressed in adipocytes [5]. Activation of PPARγ leads to transcriptional changes in a number of genes regulating adipogenesis and fatty acid uptake and storage. This leads to repartition of lipids from circulation, muscle, and liver into adipose tissue, resulting in improved insulin sensitivity [5,6]. Indeed, a number of clinical studies provide evidence that treatment with TZDs improves insulin sensitivity in insulin-resistant subjects such as people with obesity, type 2 diabetes mellitus, polycystic ovary disease, or impaired glucose tolerance (IGT) [7-9].

The PPARγ expression in adipocytes is viewed as the primary target of TZD action [5]. However, it is possible that TZDs have direct metabolic effects on other tissues such as skeletal muscle and liver, which may be independent of PPARγ. Transgenic mice with no adipose tissue or with a muscle-specific PPARγ deletion improve their insulin sensitivity in response to TZDs [10,11], and acute exposure to TZDs directly activates adenosine monophosphate–activated protein kinase (AMPK) and increases glucose uptake and palmitate oxidation in intact skeletal muscle from rodents [12]. Clinically, full therapeutic effect is reached

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after treatment with TZDs for several weeks to months, demonstrating a delayed onset of action [13-15]. However, LeBrasseur et al [12] demonstrated that an exposure for as short as 30 minutes to troglitazone increased glucose uptake in isolated rat skeletal muscle. It is not known if TZDs exert acute direct metabolic effects in intact human skeletal muscle. Therefore, we examined whether rosiglitazone affects glucose transport and proximal insulin-signaling events in isolated skeletal muscle strips from nondiabetic men. Because we have previously shown that an acute activation of AMPK by the nucleoside analog 5-aminoimidazole-4-carboxamide 1-β-D-ribonucleoside (AICAR) increases glucose transport rate in isolated human skeletal muscle strips [16], we also included AICAR exposure in our experiments as a positive control.

2. Methods

The study protocol was reviewed and approved by the Ethical Committee of the Department of Medicine, Helsinki University Central Hospital; and written informed consent was obtained from all subjects before participation. Physiologic characteristics of the study subjects (15 nondiabetic men) are presented in Table 1. Six of the men had no family history of diabetes, whereas 9 men had first- and/or second-degree relatives with type 2 diabetes mellitus. Oral glucose tolerance test (75 g) was performed in all subjects. Nine men had normal glucose tolerance (NGT), 3 men had impaired fasting glucose (IFG), and 3 men had IGT. None of the men was taking any regular medications. One man was a smoker. All subjects were instructed to avoid strenuous exercise for 72 hours before the muscle biopsy. The subjects were examined after an overnight fast.

Table 1
Subject characteristics and clinical chemistry

N	15
Age (y)	50 ± 3
BMI (kg/m^2)	26.9 ± 1.1
Waist (cm)	97 ± 3
Hip (cm)	100 ± 2
Waist-to-hip ratio	0.97 ± 0.02
Body fat %	22.8 ± 1.5
Vo_{2max} (mL kg^{-1} min^{-1})	35.8 ± 2.9
Systolic blood pressure (mm Hg)	146 ± 5
Diastolic blood pressure (mm Hg)	89 ± 3
Blood glucose (mmol/L)	5.1 ± 0.1
HbA_{1c} (%)	5.2 ± 0.1
Serum cholesterol (mmol/L)	4.7 ± 0.2
Serum triacylglycerol (mmol/L)	1.6 ± 0.2
Serum HDL cholesterol (mmol/L)	1.30 ± 0.08
Serum LDL cholesterol (mmol/L)	2.7 ± 0.2
Fasting blood glucose at OGTT (mmol/L)	5.3 ± 0.1
2-h blood glucose at OGTT (mmol/L)	5.3 ± 0.4

Blood samples were obtained for clinical chemistry analysis from fasted men. Results are means ± SE. N = 15 except for body fat percentage (n = 11) and Vo_{2max} (n = 10). HbA_{1c} indicates hemoglobin A_{1c}; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OGTT, oral glucose tolerance test.

2.1. Open muscle biopsy

Open biopsies were taken from the vastus lateralis muscle under local anesthesia (lidocaine hydrochloride, 5 mg/mL), as previously described [16,17]. A 4-cm incision was made 15 cm above the proximal border of patella, and the muscle fascia was exposed. Thereafter, 4 to 5 muscle fiber bundles were excised and placed in oxygenated Krebs-Henseleit buffer (KHB), which contained 5 mmol/L HEPES, 5 mmol/L glucose, 15 mmol/L mannitol, and 0.1% bovine serum albumin (radioimmunoassay grade; Sigma, St Louis, MO). Smaller skeletal muscle strips were dissected from the muscle biopsy specimen, mounted on Plexiglass clamps, and incubated in vitro in pregassed (95% O_2 and 5% CO_2) KHB in shaking water bath at 35°C. The gas phase in the vials was maintained during the incubation procedure. After 30 minutes of incubation in KHB, skeletal muscle strips were incubated for 30 minutes at 35°C without (basal) or with 1 or 10 μmol/L rosiglitazone (Alexis Biochemicals, Lausen, Switzerland), 120 nmol/L insulin (Actrapid; Novo Nordisk A/S, Bagsværd, Denmark), a combination of 10 μmol/L rosiglitazone and insulin, or 1 mmol/L AICAR (Sigma). These concentrations were maintained throughout the subsequent incubation procedures. As rosiglitazone was dissolved in methanol, equal amounts of methanol were added to the media to maintain concentration at 0.1% in all conditions.

2.2. Glucose transport

Skeletal muscle strips were transferred to fresh KHB containing 20 mmol/L mannitol and incubated for 10 minutes. Subsequently, muscles were incubated for 20 minutes in KHB containing 5 mmol/L 3-*O*-methyl [^{3}H] glucose (800 μCi/mmol) and 15 mmol/L [^{14}C] mannitol (53 μCi/mmol) (GE Healthcare, Life Sciences, Whitchurch, Cardiff, United Kingdom). Muscle strips were blotted of excess fluid, frozen in liquid nitrogen, and stored at −80°C until further analysis. Glucose transport was determined by the accumulation of intracellular 3-*O*-methyl [^{3}H] glucose, as described [17].

2.3. Tissue processing

Muscle strips were homogenized in ice-cold homogenization buffer (90 μL/μg dry weight muscle) (20 mmol/L Tris [pH 7.8], 137 mmol/L NaCl, 2.7 mmol/L KCl, 1 mmol/L $MgCl_2$,, 1% Triton X-100, 10% [wt/vol] glycerol, 10 mmol/L NaF, 0.5 mmol/L Na_3VO_4, 1 μg/mL leupeptin, 0.2 mmol/L phenylmethyl sulfonyl fluoride, 1 μg/mL aprotinin). Homogenates were rotated for 60 minutes at 4°C. Subsequently, homogenates were subjected to centrifugation (12 000*g* for 10 minutes at 4°C); and protein concentration was determined using a bicinchoninic acid protein assay (Pierce, Rockford, IL). An aliquot of homogenate was mixed

with Laemmli buffer containing β-mercaptoethanol and heated (95°C) for 5 minutes.

2.4. Western blot analysis

Total and phosphorylated proteins were determined with commercially available antibodies: anti–phospho-Akt (Ser^{473}) (catalogue #9271), anti–phospho-Akt (Thr^{308}) (catalogue #9275), anti-Akt (catalogue #9272), anti-desmin (catalogue #04-585), and anti–phospho-ACCβ (Ser^{79}) (catalogue #07-303) (Upstate Biotechnology, Lake Placid, NY). Proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoride membranes, and blocked with Tris-buffered saline with 0.02% Tween containing 2.5% bovine serum albumin for 3 hours (pAkt-Ser^{473} and pAkt-Thr^{308}) or 7.5% milk for 2 hours (desmin, total Akt, and pACCβ-Ser^{79}). Membranes were incubated overnight at 4°C with primary antibodies (1:500 for Akt and desmin, 1:1000 for pACCβ). Membranes were washed in Tris-buffered saline with 0.02% Tween and incubated with appropriate secondary horseradish peroxidase-conjugated antibodies (Bio-Rad, Richmond, CA). Immunoreactive proteins were visualized by enhanced chemiluminescence (ECL plus; Amersham, Arlington Heights, IL) and quantified by densitometry using Molecular Analyst Software (Bio-Rad).

2.5. Clinical chemistry

Blood glucose concentration was analyzed by glucose dehydrogenase method (Precision-G Blood Testing System; Medisense, Abbott Park, IL). Hemoglobin A_{1c} was determined by an immunological method (DCA 2000+; Bayer Healthcare, Elkhart, IN). Serum total and high-density lipoprotein cholesterol, and triglyceride concentrations were analyzed enzymatically by an automated analyzer (Konelab 60i; Thermo Electron Oy, Clinical Chemistry and Automation Systems, Vantaa, Finland). Low-density lipoprotein cholesterol concentration was calculated by using the formula of Friedewald et al [18].

2.6. Body composition and maximal oxygen uptake

Waist circumference was measured midway between the lower rib margin and the iliac crest, and hip circumference was measured at the level of the trochanters with the use of a soft measuring tape. Body fat percentage was measured by dual-energy x-ray absorptiometry (Hologic Discovery A; Fenno Medical, Vantaa, Finland) (n = 11), and maximal oxygen uptake (Vo_{2max}) was determined by using a bicycle ergometer (900 ERG Ergometer; Marquette Hellige, Marquette Medical Systems, Milwaukee, WI) and a breath-by-breath gas exchange analysis system (Vmax 229, Sensormedics, Homestead, FL) (n = 10).

2.7. Statistics

Data are presented as mean ± SE. Normal distribution of the variables was verified by Kolmogorov-Smirnov test. Paired Student *t* test was used in the analysis of paired data. All statistical analyses were performed with SPSS statistical package (version 16.0; SPSS, Chicago, IL). Two-tailed *P* value < .05 was considered statistically significant.

3. Results

3.1. Glucose transport

Isolated muscle strips were incubated for 60 minutes in the absence (basal) or in the presence of rosiglitazone (1 or 10 μmol/L), AICAR (1 mmol/L), insulin (120 nmol/L), or a combination of rosiglitazone (10 μmol/L) and insulin (120 nmol/L) (Fig. 1). There was no effect of 1 μmol/L rosiglitazone on glucose transport rate in intact skeletal muscle. Exposure to 10 μmol/L rosiglitazone tended to increase glucose transport rate, but this was not statistically significant ($P = .182$) (Fig. 1). 5-Aminoimidazole-4-carboxamide 1-β-D-ribonucleoside and insulin increased glucose transport rate 1.5-fold ($P < .05$) and 1.7-fold ($P < .01$), respectively. A combined exposure to both rosiglitazone and insulin did not have any effect on glucose transport rate compared with insulin alone ($P = .258$) (Fig. 1).

To examine if clinical characteristics of the study cohort affect glucose transport responses to rosiglitazone, we next analyzed glucose transport data according to glucose tolerance of the subjects. Men with NGT (47 ± 4 years old; body mass index [BMI], 25.1 ± 1.3 kg/m^2; n = 9) and men with IFG or IGT (54 ± 4 years old; BMI, 29.5 ± 1.5 kg/m^2; n = 6) had similar age ($P = .28$), whereas men with IFG/IGT had a higher BMI ($P < .05$). Exposure to 1 or 10 μmol/L rosiglitazone did not affect glucose transport rate in intact skeletal muscle in either men with NGT or men with IFG/IGT (Table 2). We next divided the subjects into 2 groups based

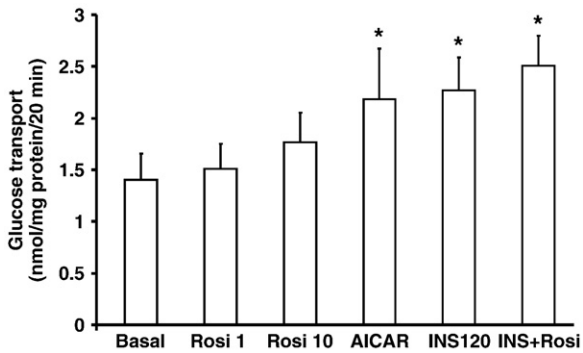

Fig. 1. Glucose transport. Open muscle biopsies were obtained surgically from vastus lateralis muscle from 15 nondiabetic men, as described in "Methods." Muscle strips were isolated and exposed to 1 or 10 μmol/L rosiglitazone, AICAR (1 mmol/L), insulin (120 nmol/L), or a combination of 10 μmol/L rosiglitazone and insulin (120 nmol/L) for 60 minutes. Glucose transport was analyzed by the accumulation of intracellular 3-*O*-methyl [^{3}H] glucose. Exposure to insulin and AICAR increased glucose transport, whereas exposure to rosiglitazone did not modify basal or insulin-stimulated glucose transport rate. *$P < .05$ vs basal. Data are presented as means ± SE. n = 9 to 15 per condition. Rosi 1 indicates 1 μmol/L rosiglitazone; Rosi 10, 10 μmol/L rosiglitazone.

Table 2
Glucose transport rate (in nanomoles per milligram protein per 20 minutes) in isolated skeletal muscle strips after 60-minute exposure to rosiglitazone according to glucose tolerance status, median BMI, median body fat percentage, or median Vo_{2max}

	Basal	Rosiglitazone 1 μmol/L	Rosiglitazone 10 μmol/L
NGT (n = 9)	1.4 ± 0.4	1.4 ± 0.3	1.8 ± 0.4
IFG/IGT (n = 6)	1.3 ± 0.4	1.7 ± 0.4	1.7 ± 0.5
BMI <27 kg/m^2 (n = 7)	1.2 ± 0.3	1.4 ± 0.2	1.8 ± 0.4
BMI ≥27 kg/m^2 (n = 8)	1.6 ± 0.4	1.6 ± 0.4	1.7 ± 0.4
BF <24.6% (n = 5)	1.2 ± 0.5	1.3 ± 0.4	1.9 ± 0.6
BF ≥24.6% (n = 6)	1.8 ± 0.5	2.0 ± 0.5	2.2 ± 0.4
Vo_{2max} <35 mL min^{-1}·kg^{-1} (n = 4)	1.7 ± 0.4	2.1 ± 0.6	2.2 ± 0.5
Vo_{2max} ≥35 mL·min^{-1}·kg^{-1} (n = 6)	1.2 ± 0.5	1.3 ± 0.3	1.8 ± 0.5

Body fat percentage was determined in 11 men; and Vo_{2max}, in 10 men. Data are given as means ± SE. BF indicates body fat percentage.

on the median BMI, body fat percentage, or Vo_{2max} of the whole cohort: (1) subjects with BMI, body fat percentage, or Vo_{2max} less than the median value and (2) subjects with BMI, body fat percentage, or Vo_{2max} equal to or higher than the median value. Rosiglitazone did not affect glucose transport rates in any of these subgroups (Table 2).

3.2. Akt phosphorylation at Ser^{473} and Thr^{308}

Insulin increased phosphorylation of Akt-Ser^{473} (P < .001) and Akt-Thr^{308} (P < .01). Rosiglitazone did not affect phosphorylation of Akt either at Ser^{473} or Thr^{308}, and it did not modify insulin-stimulated phosphorylation of Akt at Ser^{473} and Thr^{308} (Figs. 2 and 3).

3.3. Phosphorylation of acetyl–coenzyme A carboxylase

Activation of AMPK leads to phosphorylation of its downstream target, acetyl–coenzyme A (CoA) carboxylase

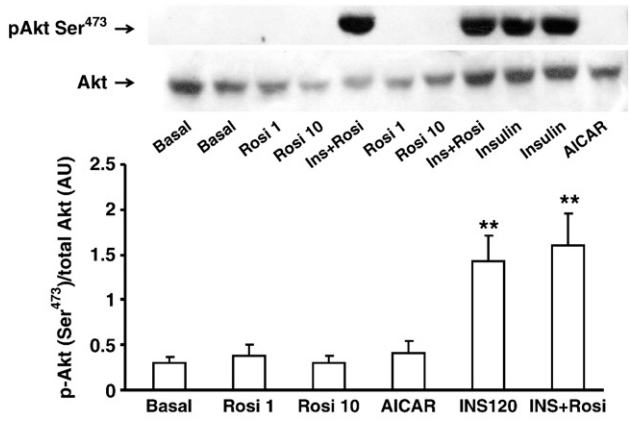

Fig. 2. Phosphorylation of Akt-Ser^{473}. Insulin increased phosphorylation of Akt-Ser^{473} in nondiabetic muscle. Rosiglitazone did not affect basal or insulin-stimulated phosphorylation of Akt-Ser^{473}. Because of variability in total Akt, phospho-Akt-Ser^{473} data have been expressed in relation to total Akt expression. **P < .01 vs basal. Data are presented as means ± SE. n = 9 to 15 per condition. AU indicates arbitrary units.

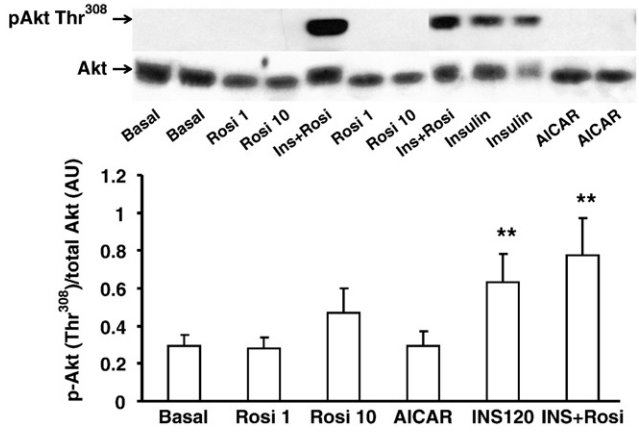

Fig. 3. Phosphorylation of Akt-Thr^{308}. Insulin increased phosphorylation of Akt-Thr^{308} in skeletal muscle from nondiabetic men. Rosiglitazone did not affect phosphorylation of Akt-Thr^{308} in basal or insulin-stimulated state. Because of variability in total Akt, phospho-Akt-Thr^{308} data have been expressed in relation to total Akt expression. **P < .01 vs basal. Data are presented as means ± SE. n = 9 to 15 per condition.

(ACC) [19]. Thus, we determined whether exposure to 10 μmol/L rosiglitazone modifies ACCβ phosphorylation in intact skeletal muscle. The ACCβ phosphorylation was transiently increased in response to rosiglitazone. Phosphorylation reached a peak at 15 minutes (P = .051) and was restored to basal levels after 60 minutes of exposure to rosiglitazone (Fig. 4). Equal protein loading was confirmed by unchanged desmin expression.

4. Discussion

Thiazolidinediones are widely used antidiabetic drugs [4]. However, the molecular details of how TZD treatment improves insulin sensitivity are still incompletely resolved.

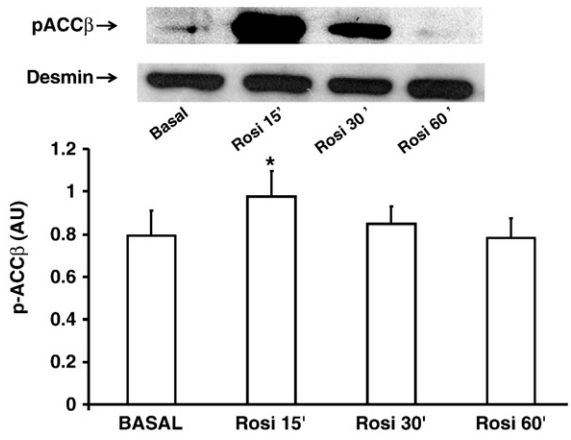

Fig. 4. Phosphorylation of ACCβ. Exposure of nondiabetic muscle to 10 μmol/L rosiglitazone resulted in a transient increase in phosphorylation of ACCβ. Equal protein loading was confirmed by unchanged desmin expression (P = .782, Friedman test). *P = .051 vs basal. Data are presented as means ± SE. n = 5.

Thiazolidinediones are high-affinity ligands for PPARγ, a transcription factor highly expressed in adipocytes and also to some extent in other tissues such as liver and skeletal muscle [20,21]. Peroxisome proliferator–activated receptor–γ controls expression of several genes involved in adipose tissue differentiation, glucose, and lipid metabolism [5]. It has been suggested that TZDs induce "lipid steal" by enhancing sequestration of fatty acids in adipose tissue, resulting in diminished ectopic fat content in skeletal muscle and liver and, consequently, improved insulin action [22]. This concept is supported by clinical studies using a combination of insulin clamp technique and magnetic resonance imaging, which demonstrate enhanced whole-body insulin action and reduced ectopic fat content in skeletal muscle and liver in response to therapy with TZDs [8,23-25]. Moreover, the insulin-sensitizing effect of TZD treatment is associated with improved insulin signaling via IRS1/PI3-kinase/Akt pathway in skeletal muscle from obese type 2 diabetes mellitus patients [26,27].

The effects of TZDs on PPARγ and adipocytes do not preclude the possibility that TZD action might also involve other target tissues and signaling mechanisms. Thiazolidinediones, like metformin, reduce the activity of respiratory complex I in skeletal muscle; and direct exposure for 24 hours to TZDs elevates lactate release and reduces glucose and palmitate oxidation in isolated rat skeletal muscles [28]. Although 24 hours of exposure may be long enough to mediate some of the effects via gene regulatory changes, short-term exposure is likely to mediate its effect via signaling pathways independent of PPARγ-mediated gene expression. This concept is supported by data showing that 5-hour exposure of rat muscle to troglitazone reduces oxidative metabolism, an effect not affected by exposure to inhibitors of transcription (actinomycin D) and protein synthesis (cycloheximide) [29]. Additional support for direct metabolic effects of TZDs comes from LeBrasseur et al [12] who show that acute exposure of rat EDL muscle to troglitazone increases 2-deoxyglucose uptake and palmitate oxidation, and activates AMPK. Because long-term treatment with TZDs improves insulin action in human insulin resistance [8,9,25], these acute exposure studies in rodent muscle lead us to examine whether TZDs have an acute effect directly in intact human skeletal muscle. Skeletal muscle biopsies were obtained from a cohort of nondiabetic men; small muscle strips were isolated and stimulated in vitro for 60 minutes. Acute exposure to rosiglitazone did not modify basal or insulin-stimulated glucose transport. In subgroup analysis, the absence or presence of abnormal glucose metabolism, obesity, or poorer physical fitness did not differentially modify the glucose transport responses to rosiglitazone (Table 2). Moreover, exposure to rosiglitazone did not affect proximal insulin-signaling events, as reflected by unchanged basal or insulin-stimulated phosphorylation of Akt at Ser^{473} and Thr^{308}. Although we did not see a statistically significant improvement in glucose transport, there was a tendency for increased glucose transport rate in response to higher rosiglitazone concentration (10 μmol/L). It is thus possible that longer exposure to rosiglitazone than 60 minutes would have been needed to observe an effect, although exposure of isolated mouse muscle for 5 hours to TZD does not modify insulin-stimulated 2-deoxyglucose uptake [30]. Our data are in agreement with lack of an acute stimulation of 3-*O*-methyl glucose transport in L6GLUT4-myc myotubes [31]. Interestingly, 2-deoxyglucose uptake was increased in response to troglitazone because of stimulation of 2-deoxyglucose phosphorylation in L6GLUT4myc myotubes, suggesting that hexokinase flux was increased. In the current study, we determined muscle 3-*O*-methyl glucose accumulation that reflects only the glucose transport step because 3-*O*-methyl glucose cannot be phosphorylated by hexokinase. In contrast, 2-deoxyglucose uptake reflects both glucose transport and phosphorylation steps. Therefore, the lack of an acute effect on glucose transport does not exclude the possibility that rosiglitazone still might acutely stimulate glucose fluxes downstream of glucose transport step in human skeletal muscle.

Adenosine monophosphate–activated protein kinase is a central regulator of cellular energy status and various metabolic pathways [19,32]. An increase in the cellular AMP to adenosine triphosphate ratio activates AMPK by a number of allosteric and covalent mechanisms, including phosphorylation of the α-subunit on Thr^{172} by tumor suppressor LKB1 and calmodulin-dependent protein kinase kinase. Adenosine monophosphate–activated protein kinase activation by the nucleoside analog AICAR normalizes insulin-stimulated glucose transport by stimulating GLUT4 translocation to the cell surface in skeletal muscle from insulin-resistant type 2 diabetic men [16], highlighting AMPK as an attractive target to treat insulin resistance. Adenosine monophosphate–activated protein kinase activation also improves fatty acid oxidation. This is mediated by inhibitory phosphorylation of ACC and stimulatory phosphorylation of malonyl-CoA decarboxylase, which lead to a decrease in malonyl-CoA concentration, consequently activating carnitine palmitoyltransferase 1 and stimulating fatty acid oxidation [33,34]. Treatment for 4 days with TZDs ameliorated impaired fatty acid oxidation in cultured muscle cells from type 2 diabetes mellitus patients, concomitant with increased phosphorylation of ACC [35]. Emerging evidence suggests that one of the mechanisms whereby TZDs stimulate glucose uptake and lipid metabolism is through AMPK signaling cascade. Adenosine monophosphate–activated protein kinase activation by TZDs has been reported in H-$2K^b$ muscle cells [36] and in L6GLUT4myc myotubes [31]. Moreover, a decrease in the expression of AMPK by small interfering RNA inhibits the acute stimulation of 2-deoxyglucose uptake by troglitazone [31]. Recently, LeBrasseur et al [12] demonstrated that TZDs rapidly activated AMPK in intact skeletal muscle from rats; and this was associated with increased glucose uptake and palmitate oxidation. We analyzed in the current study whether rosiglitazone activates AMPK in intact human

skeletal muscle. Exposure to 10 μmol/L rosiglitazone induced a transient increase in ACC phosphorylation, with a peak at 15 minutes, suggesting AMPK activation by acute exposure to rosiglitazone. The time course is similar to rodent muscle, where a peak increase in AMPK activity and ACC phosphorylation after exposure to 5 μmol/L troglitazone occurs at 5 to 15 minutes, with a return to baseline by 60 minutes [12]. However, acute AMPK activation by rosiglitazone did not modify glucose transport in the current study. Because AMPK-ACC signaling pathway is central to the regulation of fatty acid oxidation [34], it is possible that rosiglitazone might acutely modify lipid metabolism in intact human skeletal muscle. This hypothesis needs to be tested in further experiments.

In summary, we demonstrate that acute exposure to rosiglitazone does not affect glucose transport and does not modify insulin-stimulated phosphorylation of Akt in intact human skeletal muscle from nondiabetic men. However, exposure to rosiglitazone leads to a transient activation of AMPK, as reflected by phosphorylation of ACC. Whether this affects fatty acid oxidation and glucose metabolism downstream of glucose transport step in human skeletal muscle needs to be explored in further studies.

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