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Glucose infusion causes insulin resistance in skeletal muscle of rats without changes in Akt and AS160 phosphorylation

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Hoy AJ, Bruce CR, Cederberg A, Turner N, James DE, Cooney GJ, Kraegen EW. Glucose infusion causes insulin resistance in skeletal muscle of rats without changes in Akt and AS160 phosphorylation. *Am J Physiol Endocrinol Metab* 293: E1358–E1364, 2007. First published September 4, 2007; doi:10.1152/ajpendo.00133.2007.—Hyperglycemia is a defining feature of Type 1 and 2 diabetes. Hyperglycemia also causes insulin resistance, and our group (Kraegen EW, Saha AK, Preston E, Wilks D, Hoy AJ, Cooney GJ, Ruderman NB. *Am J Physiol Endocrinol Metab* 290: E471–E479, 2006) has recently demonstrated that hyperglycemia generated by glucose infusion results in insulin resistance after 5 h but not after 3 h. The aim of this study was to investigate possible mechanism(s) by which glucose infusion causes insulin resistance in skeletal muscle and in particular to examine whether this was associated with changes in insulin signaling. Hyperglycemia (~10 mM) was produced in cannulated male Wistar rats for up to 5 h. The glucose infusion rate required to maintain this hyperglycemia progressively lessened over 5 h (by 25%, $P < 0.0001$ at 5 h) without any alteration in plasma insulin levels consistent with the development of insulin resistance. Muscle glucose uptake in vivo (44%; $P < 0.05$) and glycogen synthesis rate (52%; $P < 0.001$) were reduced after 5 h compared with after 3 h of infusion. Despite these changes, there was no decrease in the phosphorylation state of multiple insulin signaling intermediates [insulin receptor, Akt, AS160 (Akt substrate of 160 kDa), glycogen synthase kinase-3 β] over the same time course. In isolated soleus strips taken from control or 1- or 5-h glucose-infused animals, insulin-stimulated 2-deoxyglucose transport was similar, but glycogen synthesis was significantly reduced in the 5-h muscle sample (68% vs. 1-h sample; $P < 0.001$). These results suggest that the reduced muscle glucose uptake in rats after 5 h of acute hyperglycemia is due more to the metabolic effects of excess glycogen storage than to a defect in insulin signaling or glucose transport.

glucotoxicity; glycogen; hyperglycemia; in vivo metabolism; soleus muscle

HYPERGLYCEMIA IS A DEFINING characteristic of Type 1 and 2 diabetes, resulting in hepatic and skeletal muscle insulin resistance (56). Glucose-induced insulin resistance, also known as “glucose toxicity,” has been demonstrated in rodents infused with glucose for 1–7 days, manifesting as impaired insulin action in skeletal muscle (17, 19, 28, 38). It has also been demonstrated in perfused rat hindlimbs (46, 47) and isolated muscles incubated in vitro (22, 25, 36). Our group (34) has recently reported that a doubling of blood glucose to 10 mM by

acute infusion of glucose into rodents results in insulin resistance after 5 h and is associated with an increase in muscle diacylglycerol (DAG) content, which was not present after 3 h of infusion. DAG accumulation has been proposed to impinge on insulin signaling by PKC-dependent and -independent mechanisms (20, 21, 55). However, the role of insulin signaling defects in acute glucose infusion-induced insulin resistance was not examined in our previous study.

Insulin resistance has been suggested to be a consequence of defects in the insulin signaling cascade (45). This defect is thought to be due to serine/threonine phosphorylation of insulin receptor substrate-1 (IRS-1) by various kinases such as PKCs (48) and other serine/threonine kinases [see review (16)] that are activated by lipid metabolites such as DAGs and ceramides. Serine/threonine phosphorylation of IRS-1 results in reduced tyrosine phosphorylation and increased proteosomal degradation of the protein. Insulin signal transduction through phosphatidylinositol 3-kinase (PI3-kinase) and Akt is thereby reduced, resulting in decreased glucose transporter translocation to the plasma membrane (9, 16). However, recent work showing that shRNA-mediated decrease in IRS-1 in mouse skeletal muscle had no effect on insulin-stimulated glucose uptake (4) suggests that other nodes in the insulin-signaling cascade may be important. Several studies have suggested that inhibition of Akt activity may be the primary site for impaired insulin signaling in skeletal muscle (49, 52, 53); however, this is also contentious (32, 42). The recently identified RAB-GTPase-activating protein Akt substrate of 160 kDa (AS160), which modulates 5-aminoimidazole-4-carboxamide-1- β -D-ribofuranoside (an AMP-activated protein kinase agonist), exercise, and insulin-stimulated GLUT4 translocation (35), represents another potential site for reduced insulin signal transduction, as it has been shown to be impaired in muscle from individuals with Type 2 diabetes (27).

Hence, the aim of the present study was to test the hypothesis that glucose infusion induces insulin resistance in skeletal muscle via antagonism of the insulin signaling cascade.

RESEARCH DESIGN AND METHODS

Animals

All surgical and experimental procedures performed were approved by the Animal Experimentation Ethics Committee (Garvan Institute/

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St. Vincent's Hospital) and were in accordance with the National Health and Medical Research Council of Australia's guidelines on animal experimentation.

Adult male Wistar rats (Animal Resources Centre, Perth, Australia) were communally housed at $22 \pm 0.5^\circ\text{C}$ with a controlled 12:12-h light-dark cycle (lights on from 0700 to 1900). Rats were fed ad libitum a standard chow diet (Rat Maintenance Diet; Gordons Specialty Feeds, Sydney, Australia) containing 10% fat, 69% carbohydrate, and 21% (wt/wt) protein plus fiber, vitamins, and minerals. After 1 wk of acclimatization, rats were anesthetized with a combination of Ketalar (ketamine, 80 mg/kg) and Ilium Xylazil (xylazine, 20 mg/kg) injected intraperitoneally. Cannulae were implanted into the right and left jugular veins under aseptic conditions. Catheters were exteriorized at the back of the neck. Rats were housed individually after surgery and handled daily to minimize stress. Average body weight on the experimental day was 300–350 g.

Glucose Infusion

Seven days after cannulation surgery, rats were randomly divided into treatment groups. Rats were infused with either 50% glucose or with 0.9% saline (control) for 0 min, 30 min, 1 h, 3 h, or 5 h. Acute infusion was performed by use of a peristaltic roller pump (101U/R; Watson-Marlow, Falmouth, UK). A blood sample (300 μl) was collected 10 min before the infusion to determine basal levels of whole blood glucose and plasma glucose, insulin, and nonesterified fatty acids (NEFA).

A blood sample was taken every 30 min, and the glucose infusion rate was adjusted to maintain a whole blood glucose concentration of 10 mM (corresponding to a plasma glucose level of 16–17 mM). Red blood cells from each sample were resuspended in heparinized saline (10 U/ml) and returned to the rat. In one cohort, 2-deoxy-D-[2,6- ^3H]glucose (^3H]2DG) and [U- ^{14}C]glucose (Amersham Biosciences, Buckinghamshire, UK) were administered as an intravenous bolus 60 min before euthanasia. Blood samples (200 μl) were taken at 2, 15, 30, 45, and 60 min after administration of the bolus for estimation of plasma tracer and glucose concentration. After this, animals were euthanized with an overdose of pentobarbital sodium (Nembutal; Abbott Laboratories, Sydney, Australia). Tissues were rapidly removed, freeze-clamped, and stored at -80°C for subsequent determination of glucose clearance into specific tissues, glycogen content, and [^{14}C]glucose incorporation into glycogen. Analyses were performed in red quadriceps (RQ) muscle. In another cohort, tracers were not administered and soleus muscles were removed for *in vitro* studies as subsequently described.

Analytic Methods

Blood and plasma glucose levels were determined by an immobilized glucose oxidase method (YSI 2300; Yellow Springs Instruments, Yellow Springs, OH) using 25- μl samples. Plasma insulin was measured by radioimmunoassay kit (Linco, St. Louis, MO). Plasma NEFA levels were determined spectrophotometrically with a commercially available kit (NEFA-C; WAKO Pure Chemical Industries, Osaka, Japan).

Plasma and tissue levels of ^3H - and ^{14}C -labeled tracers were measured as described previously to calculate whole body uptake rate (R_d) and muscle glucose uptake, as were tissue glycogen concentration and [^{14}C]glucose incorporation rates into glycogen (23). Tissue glucose-6-phosphate (G-6-P) was measured in PCA extract by use of enzymatic fluorometric methods (40). Muscle glycogen synthase activity was measured in the presence or absence of 10 mM of G-6-P using a previously described method (44).

In Vitro Muscle Incubation Analysis

Maximal insulin-stimulated glucose transport capacity. The soleus muscle was carefully dissected into longitudinal strips from tendon to

tendon by use of a 25-gauge needle. Two strips were utilized from each soleus muscle. Glucose transport in isolated soleus muscle was assayed in sealed vials containing pre-gassed (95% O_2 -5% CO_2) Krebs-Henseleit bicarbonate buffer supplemented with 4 mM sodium pyruvate, 8 mM mannitol, and 0.1% wt/vol BSA at 30°C . Muscles were preincubated for 30 min and were then incubated with or without insulin for 30 min. Maximal insulin-stimulated glucose transport capacity was then assayed for 15 min using [^3H]2DG (1 mM, 0.128 $\mu\text{Ci/ml}$) in the presence or absence of 1 mU/ml insulin as described previously (54).

Glucose oxidation and glucose incorporation into glycogen. After the preincubation phase, muscles were transferred to buffer containing 5 mM glucose and 2 μCi of [U- ^{14}C]glucose to monitor glucose oxidation and incorporation into glycogen. To determine glucose incorporation into glycogen, muscles were heated at 70°C in 1 M KOH for 15 min to digest the tissue. Glycogen was precipitated from the KOH digest (54). The glycogen pellet was digested with amyloglucosidase for 2 h at 37°C in 1 ml of 0.25 M acetate buffer. The [U- ^{14}C]glucose incorporated into glycogen was quantified in 0.8 ml of the amyloglucosidase digest. The remaining digest was used for quantification of total glucosyl unit content of glycogen by means of a glucose oxidase spectrophotometric assay.

Gaseous $^{14}\text{CO}_2$ produced from the exogenous oxidation of [U- ^{14}C]glucose during the incubation was measured by transferring 1 ml of the incubation medium to a 20-ml glass scintillation vial containing 1 ml of 70% PCA with a 0.5-ml microcentrifuge tube containing 1 M NaOH. Liberated $^{14}\text{CO}_2$ was trapped in the NaOH over 60 min, the microcentrifuge tube containing trapped $^{14}\text{CO}_2$ was placed in a scintillation vial, and radioactivity was counted.

Western Blot Analysis

Protein extraction. Skeletal muscle was homogenized in an ice-cold solubilization buffer containing 65 mM Tris (pH 7.4), 150 mM NaCl, 5 mM EDTA, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 10% glycerol, 1 $\mu\text{g/ml}$ aprotinin, 1 $\mu\text{g/ml}$ leupeptin, 10 mM sodium fluoride, 1 mM Na_3VO_4 , and 1 mM PMSF. The homogenate was incubated for 2 h at 4°C and then centrifuged at 12,000 g for 15 min to remove insoluble material. The protein concentration of the supernatants was determined with the Bio-Rad protein assay (Bio-Rad Laboratories).

Immunoblot analyses. Tissue lysates (15–30 μg) were subjected to SDS-PAGE, transferred to PVDF membranes, and then immunoblotted with antibodies for anti-insulin receptor- β obtained from BD Biosciences (San Jose, CA), anti-phospho-Tyr $^{1162/1163}$ insulin receptor from BioSource International (Camarillo, CA), anti-Akt, anti-phospho-Ser 473 Akt, anti-glycogen synthase kinase-3 β (GSK3 β), anti-phospho-Ser $^{21/9}$ GSK3 α/β from Cell Signaling Technology (Danvers, MA), anti-AS160 from Upstate Biotechnology (Lake Placid, NY); and anti-phospho-Ser 588 AS160 and anti-phospho-Thr 642 AS160 were gifts from Symansis (Auckland, New Zealand).

Statistical Analysis

Statistical analyses were performed with the use of a statistics package (StatView; Abacus Concepts, Berkeley, CA). Differences among relevant groups were assessed by unpaired Student's *t*-test or ANOVA using Fisher's protected least significant difference post hoc tests as appropriate. $P < 0.05$ was considered significant. Data are reported as means \pm SE.

RESULTS

Plasma Parameters Before and After Chronic Infusions of Glucose or Saline

Blood glucose, plasma insulin, and NEFA levels did not differ among the groups in the basal state (data not shown).

Blood glucose was clamped at 11.3 ± 0.1 mM (Fig. 1A) with glucose infusion, and this resulted in a plasma insulin concentration of 275 ± 13 mU/l (Fig. 1B). Basal plasma NEFA levels were 0.54 ± 0.05 mM and were suppressed after 30 min of glucose infusion to 0.27 ± 0.01 mM ($P < 0.001$) and remained stable.

In Vivo Responses

Whole body. The glucose infusion rate required to maintain hyperglycemia remained stable for the first 3 h of glucose infusion but was significantly less after 4 and 5 h compared with after 3 h of glucose infusion (Fig. 1C). Similarly, R_d increased 10-fold (62.3 ± 3.8 mg·min⁻¹·kg⁻¹) after 3 h of hyperglycemia compared with that shown in basal animals. After 5 h of hyperglycemia, there was a trend for a reduction in R_d (50.9 ± 3.8 mg·min⁻¹·kg⁻¹; $P = 0.083$ vs. 3 h). These changes were associated with stable plasma insulin levels.

Skeletal muscle insulin sensitivity. Glucose uptake into individual tissues (derived from [³H]2DG uptake) was measured in RQ muscle during the final 60 min of the glucose infusion. Reflecting whole body glucose flux, glucose uptake into skeletal muscle was increased by 10-fold after 3 h of glucose infusion. The ability of insulin to stimulate glucose uptake in RQ muscle was significantly reduced after 5 h of glucose infusion compared with that after 3 h (40%; $P = 0.04$; Fig. 1D). These data all indicate that insulin resistance developed between 3 and 5 h of glucose infusion.

Skeletal muscle insulin signaling. To determine whether any changes in insulin signaling accompanied the development of insulin resistance, we examined several components of the insulin signaling pathway for evidence of a decline in activation despite the continuing stimulus of hyperinsulinemia during the glucose infusion. Phosphorylation of insulin receptor (Tyr^{1162/1163}) was increased and remained elevated with glucose infusion [phosphorylated insulin receptor/total insulin receptor: 0.39 ± 0.12 arbitrary units (AU) for basal, 2.41 ± 0.50 AU for 3 h, 2.31 ± 0.88 AU for 5 h; $n = 5-7$]. Phosphorylation of Akt (Ser⁴⁷³; Fig. 2A) and its downstream targets GSK3 β (Fig. 2B) and AS160 (Fig. 2, C and D) were increased and maintained with prolonged glucose infusion. There was no observed change in total protein expression of these components, and densitometry measurements demonstrate that there was no significant difference in phosphorylation state between animals infused with glucose for either 3 or 5 h, despite the significant impairment in glucose uptake observed during this period (Fig. 1D).

Skeletal muscle glucose metabolism. Because there was no apparent difference in insulin signaling, we further examined parameters of glucose metabolism in skeletal muscle to determine whether changes in these variables may contribute to the decrease in glucose uptake. Glycogen content increased in RQ after 3 h of glucose infusion, but no further increase after 5 h was observed (Fig. 3A). Consistent with this, in vivo rates of glycogen synthesis in muscle were significantly reduced in the 5-h glucose-infused group compared with that in the 3-h group (Fig. 3B) despite no change in glycogen synthase activity (ratio $-/+$ G-6-P: 0.31 ± 0.03 for basal group, 0.28 ± 0.04 for 3-h group, and 0.27 ± 0.03 for 5-h group; $n = 5-7$). RQ G-6-P increased in a time-dependent manner up to 3 h (Fig. 3C) and then was reduced after 5 h of glucose infusion compared with

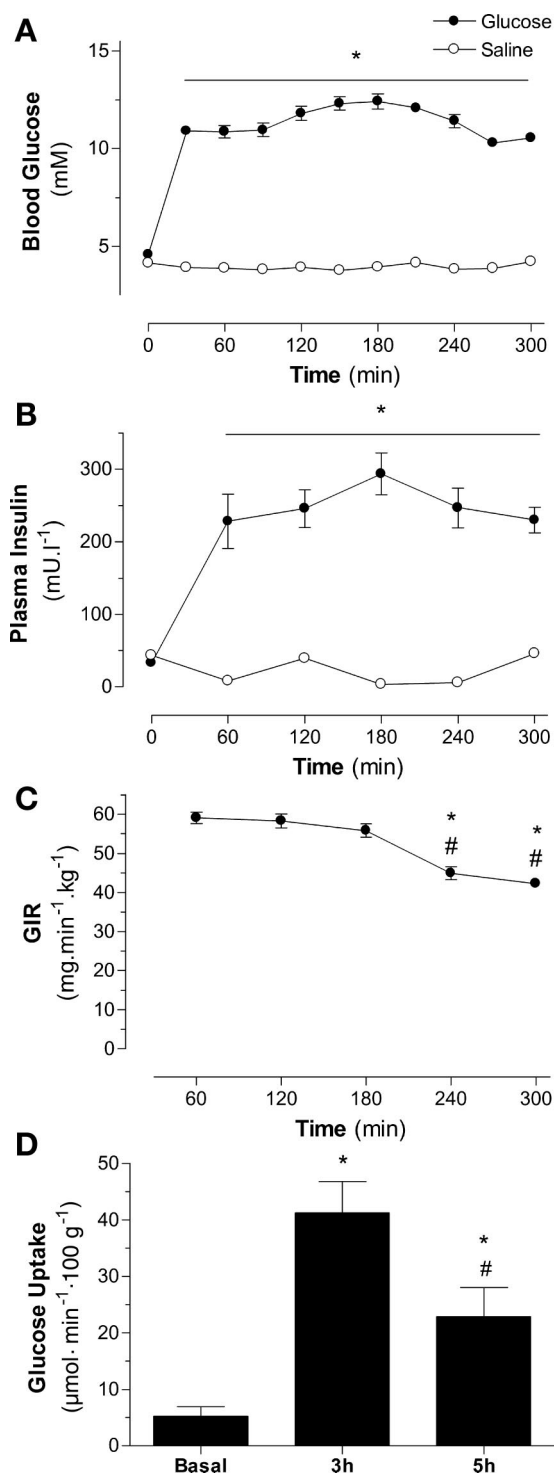


Fig. 1. Blood glucose (A) and plasma insulin (B) in rats infused with glucose (●) or saline (○) for 5 h. C: glucose infusion rate (GIR) required to maintain 10 mM blood glucose. D: red quadriceps glucose uptake for basal animals and in animals after 3 and 5 h of glucose infusion. Data are means \pm SE; $n = 5-7$ rats per group. * $P < 0.05$ vs. saline control (A and B) with Student's *t*-test or 60 min of glucose infusion (C) or basal (D) with ANOVA; # $P < 0.05$ vs. 3-h glucose infusion with ANOVA.

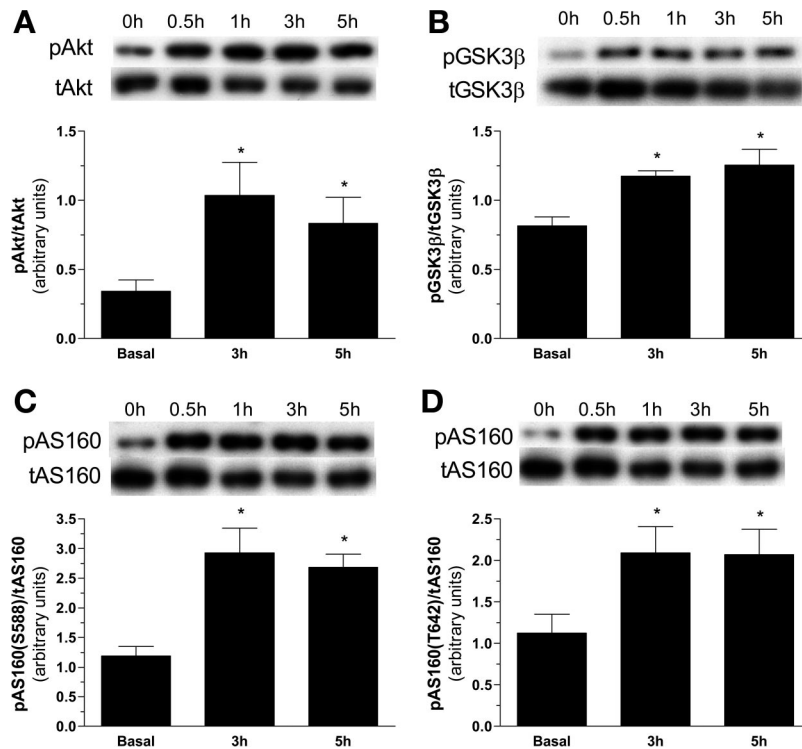


Fig. 2. Effect of glucose infusion on insulin signaling intermediates in red quadriceps; measurements were at basal state and after 3 or 5 h of glucose infusion. *A*: Akt (Ser⁴⁷³). *B*: glycogen synthase kinase-3β (GSK3β; Ser²¹). *C*: Akt substrate of 160 kDa (AS160; Ser⁵⁸⁸). *D*: AS160 (Thr⁶⁴²). Data are means \pm SE; $n = 5-7$ rats per group. p, Phosphorylated; t, total. * $P < 0.05$ vs. basal with ANOVA.

that after 3 h ($P = 0.018$). There was no difference between saline-infused and basal (no infusion) animals in glycogen content, glycogen synthesis rate, and G-6-P.

Isolated Skeletal Muscle

To further elucidate the mechanism for glucose infusion-induced reduction in skeletal muscle glucose uptake, we utilized isolated soleus muscle strips from glucose-infused rats to measure various steps of glucose metabolism. Soleus glycogen content was determined in the muscle strips used to measure glycogen synthesis rate. Glycogen content remained significantly increased (30%; $P < 0.05$ vs. basal) after 1 h of glucose infusion and was further increased (57%; $P < 0.0001$ vs. 1 h) after 5 h, which is similar to our *in vivo* observations (Fig. 4A). Glycogen synthesis rate was significantly reduced after the 5-h glucose infusion with and without insulin (Fig. 4B), as was observed *in vivo* in RQ (Fig. 3B). Maximal insulin-stimulated glucose transport capacity in isolated soleus muscle strips from glucose-infused rats remained the same compared with nonglucose-infused animals (Fig. 4C). There was no significant difference in noninsulin-stimulated glucose oxidation after 1 h (Fig. 4D); however, there was a trend toward increased insulin-stimulated glucose oxidation after the 5-h glucose infusion compared with that shown in basal animals ($P = 0.067$). There was no difference between basal animals (no infusion) and saline-infused animals in insulin-stimulated glucose transport capacity, glycogen synthesis rate, and glucose oxidation (data not shown).

DISCUSSION

We have previously shown that 5 h of glucose infusion induces skeletal muscle insulin resistance, and this coincides with an increase in DAG and malonyl CoA levels in skeletal

muscle (34). Because accumulation of metabolically active metabolites such as DAGs have been shown to impair insulin signaling [see review (48)], we therefore examined whether the development of insulin resistance in this model was associated with changes in insulin signaling. The major findings of the present study were that, contrary to our original hypothesis, there was no alteration in insulin signaling in skeletal muscle at the level of insulin receptor Akt or its downstream targets AS160 and GSK3β, despite the presence of reduced glucose uptake. Furthermore, in muscle strips isolated from 5-h glucose-infused rats, *in vitro* maximal insulin stimulated glucose transport capacity was not impaired, although glycogen synthesis was reduced, suggesting that diminished glucose metabolism or storage may underpin glucose infusion-induced insulin resistance. These results demonstrate a disassociation of insulin action and insulin signaling in an *in vivo* model of acute glucose toxicity.

Insulin-stimulated glucose transport occurs via a PI3-kinase-Akt-dependent pathway, resulting in translocation of the glucose transporter GLUT4 to the plasma membrane to mediate glucose uptake (58). Changes in glucose transport are thought to be the rate-limiting step in glucose uptake, and this is thought to result from reduced activation of the insulin signaling cascade in insulin resistance (9). Reduced insulin signal transduction in skeletal muscle has been demonstrated in a variety of insulin-resistant rodent models (3, 12, 15, 50). In isolated muscle incubated in high glucose for 4 h, insulin-stimulated Akt activation is impaired, resulting in reduced glucose transport (36). Contrary to this, Kawanaka et al. (28) reported that Akt activation was not different between isolated muscles incubated in 36 mM glucose for 5 h vs. those incubated in 5 mM. In the present study, we observed reduced glucose uptake after 5 h of glucose infusion compared with that

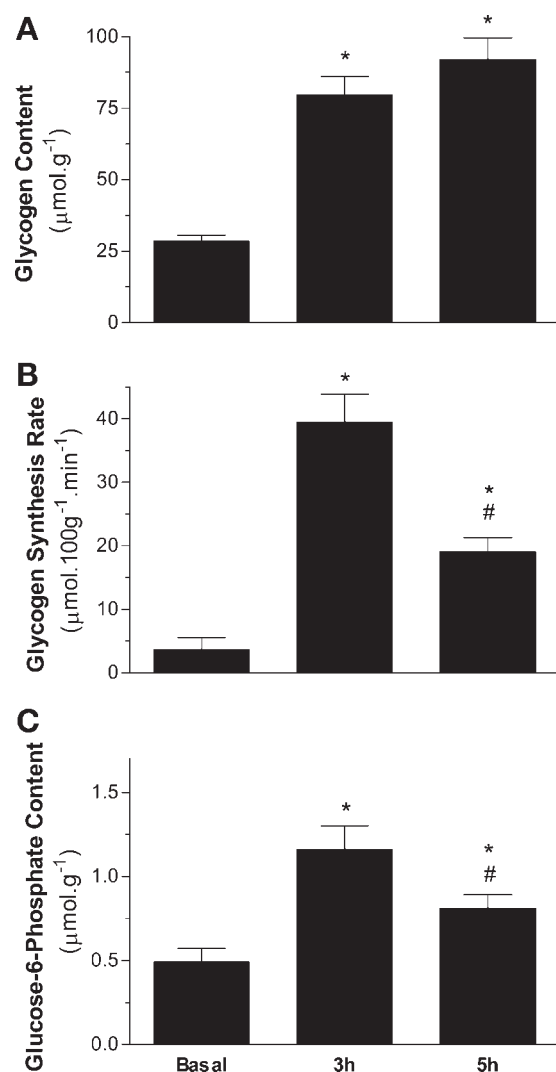


Fig. 3. Effect of glucose infusion on glucose metabolism in red quadriceps; measurements were at basal state and 3 or 5 h after glucose infusion. A: glycogen content. B: glycogen synthesis rate. C: glucose-6-phosphate content. Data are means \pm SE; $n = 5-7$ rats per group. * $P < 0.05$ vs. basal; # $P < 0.05$ vs. 3-h glucose infusion with ANOVA.

shown after 3 h in vivo (Fig. 1) despite maintained insulin receptor and Akt phosphorylation (Fig. 2). Furthermore, downstream targets of Akt involved in insulin signal transduction to glucose transport (AS160) and glucose metabolism (GSK3 β) remained activated even when skeletal muscle glucose uptake was significantly reduced.

The lack of a role for changes in insulin signaling during acute hyperglycemia has support in the literature. In a similar model, Steiler et al. (51) demonstrated that, with 3-h hyperglycemia via glucose infusion to raise blood glucose to 20 mM combined with infusion of somatostatin to suppress insulin levels, in vivo insulin-stimulated PI3-kinase activity, Akt, and ERK phosphorylation were not impaired. However, this study did not report any measure of insulin action or glucose uptake at either the whole body level or at the tissue level. It can be concluded from the study of Steiler et al. and the present study that defects in insulin signaling, via Akt and AS160, are unlikely to be the primary cause of acute glucose infusion-

induced insulin resistance in skeletal muscle in vivo. However, we cannot rule out the possibility that other signaling pathways may be involved.

Because our results indicate that the initiation of insulin resistance induced by glucose infusion occurs independent of aberrant insulin signaling, we examined other factors involved in glucose metabolism that may play a role. It has been

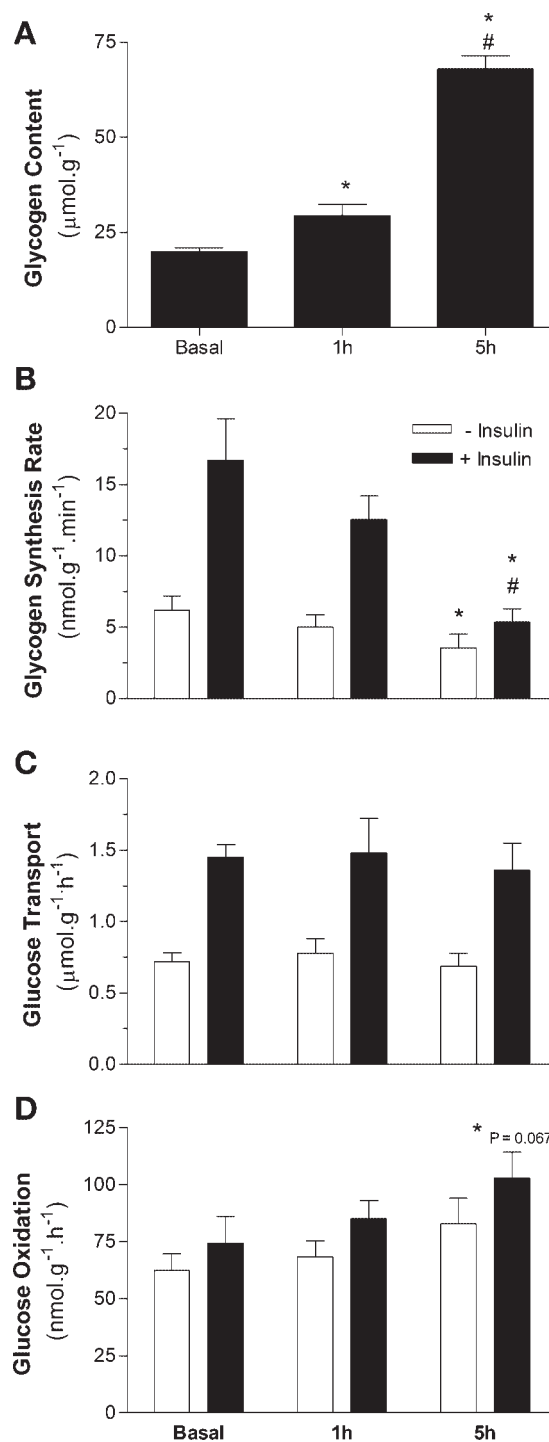


Fig. 4. Effect of glucose infusion on glycogen content (A), glycogen synthesis (B), glucose transport (C), and glucose oxidation (D) at basal state and after 1 or 5 h of glucose infusion in isolated soleus muscle. Data are means \pm SE; $n = 5-7$. * $P < 0.05$ vs. basal; # $P < 0.05$ vs. 1-h glucose infusion with ANOVA.

postulated that an impairment in glucose metabolism plays a role in the development of skeletal muscle insulin resistance (30). Increased glycogen stores have been correlated with reduced glucose uptake in a variety of studies, including glucose-induced insulin resistance (1, 8, 17, 24, 25, 29, 37). This is thought to be due to direct feedback inhibition of glycogen on glucose transport or the activity of hexokinase or glycogen synthase (2, 5–7, 18). These effects have been shown to occur independently of impaired insulin signaling in fasting/refeeding studies (25, 26) but not in the context of hyperglycemia. Increased glycogen content has been suggested to reduce glucose transport via association of GLUT4-containing vesicles with glycogen to inhibit translocation to the plasma membrane (6). Alternatively, there is a growing body of evidence that, during hyperglycemia, the rate-limiting step in glucose uptake shifts from transport to phosphorylation [see review (57)]. Glucose phosphorylation by hexokinase can be inhibited by increased G-6-P levels (41), or hexokinase itself becomes rate-limiting when transport is maximal (13, 14). Finally, reduced glycogen synthase activity may contribute to glucose infusion-induced skeletal muscle insulin resistance, as glycogen content strongly regulates insulin-stimulated glycogen synthase activity in skeletal muscle (43). Decreased glycogen synthesis rate has been demonstrated to result in reduced glucose uptake (10) and increased glucose oxidative and non-oxidative metabolism (37). However, other studies have demonstrated that increased glycogen content, by either overexpression of glycogen synthase or fasting/refeeding, does not decrease basal and insulin-stimulated glucose uptake (8, 11, 25).

We observed in vivo that skeletal muscle glycogen content increased linearly with time of glucose infusion up to 3 h with no further significant increase after 5 h. Glycogen synthesis rate and G-6-P were significantly decreased after 5 h of glucose infusion vs. after 3 h. Thus it is plausible that the reduced glucose uptake that we observed in this time frame may have resulted from reduced flux through glycogen synthesis pathway independently of changes in glycogen synthase activity. The in vitro studies with skeletal muscle from glucose-infused rats showed that maximal insulin-stimulated glucose transport capacity was unaffected by increased glycogen content, whereas rates of basal and insulin-stimulated glycogen synthesis were significantly reduced. Furthermore, after 5 h of glucose infusion, there was a trend for an increase in glucose shunting toward oxidation, as indicated by a trend for an upregulation of insulin-stimulated glucose oxidation.

The hypothesis that impairment in glucose metabolism is the early cause of insulin resistance has been demonstrated in a number of studies by Youn and colleagues in a variety of models of insulin resistance (3, 30, 31, 33). These studies have reported that reduced glucose oxidation and glycolysis precede insulin resistance. The data presented in the present study support these studies of Youn and colleagues and extend our group's previous investigation (34). This impairment in glucose metabolism arising from acute glucose infusion leads to metabolite accumulation, which may then lead to adaptations such as decreased AMP-activated protein kinase activation (34), increased PKC activation (39), and reduced insulin signaling (19) that impinge further on glucose uptake and insulin action such as reduced plasma membrane-bound GLUT4 protein content after 24-h glucose infusion (28).

In summary, this study reports that the onset of insulin resistance in skeletal muscle by glucose infusion was not accompanied by defects in insulin signaling or insulin-stimulated glucose transport capacity. However, there was reduced flux through the glycogen synthesis pathway, suggesting that the rate-limiting step had shifted from transport to potentially glucose phosphorylation.

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