In vitro simulation of calorie restriction-induced decline in glucose and insulin leads to increased insulin-stimulated glucose transport in rat skeletal muscle

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Arias EB, Cartee GD. In vitro simulation of calorie restriction-induced decline in glucose and insulin leads to increased insulin-stimulated glucose transport in rat skeletal muscle. Am J Physiol Endocrinol Metab 293: E1782–E1788, 2007. First published October 9, 2007; doi:10.1152/ajpendo.00531.2007.—In vivo calorie restriction [CR; consuming 60% of ad libitum (AL) intake] induces elevated insulin-stimulated glucose transport (GT) in skeletal muscle. The mechanisms triggering this adaptation are unknown. The aim of this study was to determine whether physiological reductions in extracellular glucose and/or insulin, similar to those found with in vivo CR, were sufficient to elevate GT in isolated muscles. Epitrochlearis muscles dissected from rats were incubated for 24 h in media with glucose (8 mM) and insulin (20 μU/ml) at levels similar to plasma values of AL-fed rats and compared with muscles incubated with glucose (5.5 mM) and/or insulin (20 μU/ml) at levels similar to plasma values of CR rats. Muscles incubated with CR levels of glucose and insulin for 24 h had a subsequently greater (P < 0.005) GT with 80 μU/ml insulin and 8 mM [3H]-3-O-methylglucose but unchanged GT without insulin. Reducing only glucose or insulin for 24 h or both glucose and insulin for 6 h did not induce altered GT. Increased GT after 24-h incubation with CR levels of glucose and insulin was not attributable to increased insulin receptor tyrosine phosphorylation, Akt serine phosphorylation, or Akt substrate of 160 kDa phosphorylation. Nor did 24-h incubation with CR levels of glucose and insulin for 24 h had a subsequently greater (P < 0.005) GT with 80 μU/ml insulin and 8 mM [3H]-3-O-methylglucose but unchanged GT without insulin. Reducing only glucose or insulin for 24 h or both glucose and insulin for 6 h did not induce altered GT. Increased GT after 24-h incubation with CR levels of glucose and insulin was not attributable to increased insulin receptor tyrosine phosphorylation, Akt serine phosphorylation, or Akt substrate of 160 kDa phosphorylation. Nor did 24-h incubation with CR levels of glucose and insulin for 24 h had a subsequently greater (P < 0.005) GT with 80 μU/ml insulin and 8 mM [3H]-3-O-methylglucose but unchanged GT without insulin. Reducing only glucose or insulin for 24 h or both glucose and insulin for 6 h did not induce altered GT. Increased GT after 24-h incubation with CR levels of glucose and insulin was not attributable to increased insulin receptor tyrosine phosphorylation, Akt serine phosphorylation, or Akt substrate of 160 kDa phosphorylation. Nor did 24-h incubation with CR levels of glucose and insulin for 24 h had a subsequently greater (P < 0.005) GT with 80 μU/ml insulin and 8 mM [3H]-3-O-methylglucose but unchanged GT without insulin.

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ALTERED SKELETAL MUSCLE GLUCOSE transport can contribute to important systemic changes in glucose homeostasis. For example, skeletal muscle insulin resistance for glucose uptake is an early and essential defect for the subsequent development of the metabolic syndrome and type 2 diabetes mellitus (19, 20). Previous studies have demonstrated that prior exposure of skeletal muscle to large changes in extracellular glucose and insulin concentrations over a period of hours can lead to altered glucose transport. However, only a few studies have characterized the effects of moderate physiological changes in glucose on glucose transport. In cultured myocytes, increasing glucose from ~5 to ~8–10 mM for 24 h did not markedly reduce glucose transport (31, 38). In isolated rat soleus muscles, there was also little difference in glucose uptake without insulin after 3 h of incubation with ~5 compared with ~8 mM glucose (38), but it is possible that a more prolonged incubation would reveal an effect in this range of glucose concentrations.

Knowledge is also limited about prolonged exposure to different physiological insulin levels. When rat epitrochlearis muscles were incubated for 5 h with a physiological insulin dose and a range of glucose levels (1–36 mM), there was a glucose concentration-dependent decline in glucose transport (41). Muscles exposed to differing glucose concentrations (0, 5, or 25 mM) with physiological insulin during a 2-h hindlimb perfusion had less of a reduction in subsequent glucose transport than muscles exposed to the same glucose with supra-physiological insulin (26). At least for moderate vs. very high insulin, prolonged exposure to higher insulin exacerbates glucose-induced decrements in glucose uptake.

Hyperglycemia and hyperinsulinemia can clearly lead to impaired glucose uptake, but it is also valuable to understand the effects of reducing glucose and/or insulin moderately below usual levels. Calorie restriction (CR; consuming ~60% of ad libitum intake), which results in small reductions in plasma glucose (~10–25%) and somewhat greater decrements in plasma insulin (~40–80%) (29, 30), can improve insulin sensitivity in many species, including humans (1) and rats (25). The enhanced in vivo insulin action is attributable, in part, to elevated insulin-stimulated glucose transport with unaltered basal glucose transport in skeletal muscle (11, 12, 15, 16, 23, 32–34).

The major goal of this study was to determine whether changes in glucose and/or insulin similar to those found with CR are sufficient to induce an increase in insulin-stimulated glucose transport of skeletal muscle. Epitrochlearis muscles from rats underwent a prolonged (up to 24 h) exposure to physiologically relevant concentrations of glucose (5.5 or 8 mM) and insulin (20 or 80 μU/ml) before the measurement of glucose transport. We hypothesized that exposing muscles to reductions in only insulin or only glucose would not alter subsequent glucose transport. We also hypothesized that exposing muscles to reductions in both insulin and glucose would result in subsequently increased insulin-stimulated, but not basal, glucose transport. The in vivo CR-induced increase in insulin-stimulated glucose transport appears to be partly attributable to increased Akt phosphorylation (32–34) despite unaltered GLUT4 glucose transporter abundance (12, 22). There-
fore, we further hypothesized that the enhanced insulin action in muscles that were incubated with reduced glucose and insulin would be accompanied by elevated insulin-stimulated Akt phosphorylation but not increased GLUT4 abundance.

**METHODS**

**Materials.** Unless otherwise noted, all chemicals were purchased from Sigma Chemical (St. Louis, MO) or Fisher Scientific (Hanover Park, IL). Human recombinant insulin was obtained from Eli Lilly (Indianapolis, IN). Reagents and apparatus for SDS-PAGE and immunoblotting were from Bio-Rad Laboratories (Richmond, CA). Protein concentration was measured using the bicinchoninic acid method (Pierce Biotechnology, Rockford, IL). Enhanced chemiluminescence (ECL; West Dura Extended Duration Substrate, Pierce, no. 9271), anti-GLUT4, and anti-phospho-(Ser/Thr) Akt substrate (Boston, MA), and [14C]mannitol was from Amersham Pharmacia Biotech (Charlottesville, VA). 3-O-methyl-[3H]glucose ([1H3]-MG) was from New England Nuclear Life Science Products (Boston, MA), and [14C]mannitol was from Amersham Pharmacia Biotech (Piscataway, NJ). Dulbecco’s modified Eagle’s medium (DMEM; no. 31600), gentamycin, streptomycin, and penicillin were purchased from Invitrogen (Carlsbad, CA). Total insulin receptor (IR) abundance (no. KHR9111) and IR tyrosine phosphorylation [pYpY1162/1163] (no. KHR9131) ELISA kits were purchased from BioSource International (Camarillo, CA). Teflon air filters (no. DDF02T1750) were purchased from GE Osmonics (Minnetonka, MN). Culture media filters were purchased from Nalg Nunc International (Rockefeller, NY).

**Animal treatment.** Procedures for animal care were approved by the University of Michigan Committee on Use and Care of Animals. Female Fischer 344 rats (7–8 wk old, ∼170 g; Harlan, Indianapolis, IN) were provided with Purina rat chow (no. 5001) and water ad libitum. On loss of pedal reflexes, epitrochlearis muscles were quickly excised and transferred into vials containing the appropriate incubation media.

**In vitro muscle incubations.** The incubation procedures summarized in Fig. 1 are modifications of those previously used with rat epitrochlearis muscles incubated for up to 9 h (24) or 19 h (5). Following dissection, muscles were incubated for 1, 6, or 24 h (incubation step 1) in flasks containing 2 ml of DMEM supplemented with 2.54 mM CaCl2, 25 mM NaHCO3, 0.1% bovine serum albumin (BSA), glucose (5.5 or 8 mM), insulin (20 or 80 μU/ml), and antibiotics (100 μg/ml penicillin, 100 μg/ml streptomycin, and 50 μg/ml gentamycin). All flasks and water used during muscle incubation steps were autoclaved. Previous results indicated that it is important that media include branched chain amino acids (in DMEM) and a physiologic insulin concentration when rat epitrochlearis muscles undergo prolonged in vitro incubations to avoid a large increase in basal glucose transport (24). Flasks were placed in a shaking water bath at 35°C and continuously gassed with 95% O2–5% CO2 throughout the experiment. Teflon air filters (Osmonics) were used to minimize aerosol contaminants.

**Five different incubation protocols were studied.** Each protocol included four incubation steps, and the only procedural differences among the protocols occurred during the first incubation step (Fig. 1). Paired epitrochlearis muscles were dissected from each rat. Within each protocol, comparisons were made between paired muscles that were treated identically except for a single aspect of the incubation conditions during step 1 (i.e., duration of incubation, glucose and/or insulin concentration). The purpose of Protocol 1 was to determine whether the glucose transport rate in a muscle after a relatively brief initial incubation step (1 h) would be maintained by its contralateral muscle after a prolonged initial incubation step (24 h). All muscles in this protocol were incubated with 8 mM glucose and 80 μU/ml insulin during incubation step 1. These glucose and insulin concentrations are within the range of values found in the plasma of ad libitum-fed rats (29, 30, 35, 36, 39). The groups were designated as simulated ad libitum values of glucose and insulin for 1 h (sALgi-1h) or 24 h (sALgi-24h).

For all muscles studied in Protocol 2, the duration of the initial incubation step was 24 h. The purpose of this protocol was to determine the effect of altering only the concentration of glucose during step 1 on subsequent glucose transport rate. One of the paired muscles was incubated as described above for the sALgi-24h treatment. The contralateral muscle was incubated identically, except that during incubation step 1, the glucose concentration was 5.5 mM. This glucose concentration is within the range of plasma values found in rats that are undergoing moderate CR, i.e., that have been consuming ∼60% of ad libitum food intake (29, 30, 35, 36, 39). The group with a lower glucose concentration during step 1 was designated as simulated CR values of glucose for 24 h (sCRg-24h).

Protocol 3 compared the effects of altering only insulin during incubation step 1 on subsequent glucose transport. One of the paired muscles was incubated as described above for the sALgi-24h treatment. The contralateral muscle was incubated identically, except the insulin concentration during incubation step 1 was 20 μU/ml. This insulin concentration is within the range of plasma values found in CR rats (29, 30, 35, 36, 39). The group with a lower insulin concentration during step 1 was designated as simulated CR values of insulin for 24 h (sCRi-24h).

Protocol 4 determined the effects of reducing both insulin and glucose concentrations during incubation step 1 on subsequent glucose transport. One of the paired muscles was incubated as described above for the sALgi-24h treatment. The contralateral muscle was incubated identically, except the insulin concentration during incubation step 1 was 20 μU/ml. This insulin concentration is within the range of plasma values found in CR rats (29, 30, 35, 36, 39). The group with a lower insulin concentration during step 1 was designated as simulated CR values of insulin for 24 h (sCRi-24h).

It seemed possible that reducing insulin and glucose concentrations for <24 h might be sufficient to alter glucose transport. Accordingly, Protocol 5 was identical to Protocol 4, except that the duration of incubation step 1 was 6 h. The groups with glucose and insulin similar to AL and CR values were designated at sALgi-6h and sCRgi-6h, respectively.

Following incubation step 1, muscles from all protocols were rinsed in Krebs-Henseleit buffer supplemented with 0.1% BSA, 2 mM sodium pyruvate, and 5 mM mannitol for two 30-min periods (incubation steps 2 and 3) at 30°C. During steps 2 and 3 for all protocols,
the Krebs-Henseleit buffer included either no insulin (basal) or 80 μU/ml insulin. The insulin concentration during steps 2 and 3 were identical for paired muscles (i.e., either both muscles from each rat were incubated without insulin or both with 80 μU/ml insulin).

**Incubation with 3-MG.** In the final incubation step (step 4) for every protocol, muscles were incubated at 30°C for 15 min in 1.5 ml of Krebs-Henseleit buffer containing 0.1% BSA, 8 mM 3-MG (including 0.25 μCi/mmol [3H]-3-MG), and 2 mM mannitol (including 0.1 μCi/mmol [3H]-mannitol). The insulin concentration remained the same as in steps 2 and 3 (0 or 80 μU/ml insulin). Muscles were then rapidly blotted, trimmed, clamp frozen with aluminum tongs cooled to the temperature of liquid N2, and stored at −80°C until further processing.

**Muscle lysate preparation.** Frozen muscles were weighed, transferred to prechilled glass tissue grinding tubes (Kontes, Vineland, NJ), and homogenized in ice-cold lysis buffer (1 ml/muscle) containing 20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% Triton X-100 (vol/vol), 1 mM EDTA, 1 mM EGTA, 2.5 mM sodium pyrophosphate, 10 mM sodium fluoride, 1 mM sodium vanadate, 1 mM β-glycerophosphate, 1 μg/ml leupeptin, and 1 mM phenylmethylsulfonyl fluoride. Homogenates were transferred to microfuge tubes, rotated for 1–2 h at 4°C, and then centrifuged at 15,000 g for 10 min at 4°C. The supernatants were transferred to microfuge tubes, rotated for 1–2 h at 4°C, and then centrifuged at 15,000 g for 10 min at 4°C. The supernatants were solubilized in SDS sample buffer, heated at 95°C for 3 min, and subjected to SDS-PAGE. Resolved proteins were transferred to nitrocellulose paper overnight at a constant current of 100 mA/transfer apparatus in electrotransfer buffer (20 mM Tris, pH 8.0, 150 mM NaCl, 1% Triton X-100). Nitrocellulose blots were blocked in 5% milk in TBST (0.1% Tween 20 in Tris-buffered saline, pH 7.5) for 1 h at room temperature and transferred to 5% BSA-TBST with primary antibody for 3 h at room temperature or overnight at 4°C. Blots were then washed and incubated in anti-rabbit IgG horse-radish peroxidase (1:20,000) for 1 h at room temperature. Blots were washed of excess antibody and subjected to ECL, and immunoreactive protein was quantified by densitometry.

**Statistical analysis.** All data are expressed as means ± SE. Differences between paired muscles were determined by paired t-test using SigmaSTAT (SPSS, Chicago, IL). P ≤ 0.05 was considered statistically significant.

**RESULTS**

**3-MG transport.** In Protocol 1, basal glucose transport was not different between muscles with an initial incubation period of 1 h compared with muscles undergoing a 24-h initial incubation step (data not shown). Glucose transport determined with insulin was ~31% greater (P < 0.05) for muscles in the sALgi-24h group compared with paired muscles in the sALgi-1h group (data not shown).

In Protocol 2, incubating muscles for 24 h with only glucose reduced (sCRg-24h) compared with controls (sALgi-24h) did not significantly alter subsequent glucose uptake measured with or without insulin (Fig. 2). Similarly, in Protocol 3, reducing only insulin for 24 h (sCRi-24h) did not alter subsequent glucose transport measured with or without insulin (Fig. 3). However, in Protocol 4, when both glucose and insulin were reduced for 24 h, subsequent glucose transport with insulin was increased ~29% in sCRgi-24h compared with sAL-24h (P < 0.005) muscles, whereas basal glucose transport was unaffected (Fig. 4). In Protocol 5, incubation with reduced glucose and insulin for 6 h (sCRgi-6h) compared with controls (sALgi-6h) was insufficient to alter glucose transport without or with insulin (data not shown).

**ELISA.** IR abundance and IR tyrosine phosphorylation in muscle homogenates were measured using Total IR (no. KHR9111) and pY-IR (no. KHR9131) ELISA kits, respectively, according to the manufacturer’s instructions (BioSource International).

**Immunoblotting.** Aliquots of supernatants (40–60 μg protein) were solubilized in SDS sample buffer, heated at 95°C for 3 min, and subjected to 10% SDS-PAGE. Resolved proteins were transferred to nitrocellulose paper overnight at a constant current of 100 mA/transfer apparatus in electrotransfer buffer (20 mM Tris, pH 8.0, 150 mM NaCl, 2% SDS, and 20% methanol). Nitrocellulose blots were blocked in 5% milk in TBST (0.1% Tween 20 in Tris-buffered saline, pH 7.5) for 1 h at room temperature and transferred to 5% BSA-TBST with primary antibody for 3 h at room temperature or overnight at 4°C. Blots were then washed and incubated in anti-rabbit IgG horse-radish peroxidase (1:20,000) for 1 h at room temperature. Blots were washed of excess antibody and subjected to ECL, and immunoreactive protein was quantified by densitometry.

Muscle IRS-1 abundance was not significantly different between paired muscles incubated with both glucose and insulin reduced for 24 h (sCRgi-24h) and sALgi-24h controls (Fig. 5A). The glucose and insulin concentrations during the initial incubation step did not alter the subsequent level of IR tyrosine phosphorylation for sCRgi-24h compared with sALgi-24h groups under basal or insulin-stimulated conditions (Fig. 5B).

**DISCUSSION**

The most important new findings of this study are the following: 1) exposure of isolated rat skeletal muscle for 24 h to moderate reductions in both extracellular glucose and insulin, comparable to changes found with in vivo CR, can trigger an increase in insulin-stimulated glucose transport with unaltered basal glucose transport; 2) reduction of only glucose or insulin was insufficient for this adaptation; 3) reduction of glucose and insulin for only 6 h was also insufficient for...
elevated insulin-stimulated glucose transport; 4) the increased glucose transport was not attributable to increased tyrosine phosphorylation of the insulin receptor or increased GLUT4 abundance; and 5) the mechanism for the improved glucose transport appeared to be distinct from that after in vivo CR, as pSerAkt of insulin-stimulated muscles was unaffected.

The absence of a significant effect of reducing extracellular glucose from 8 to 5.5 mM glucose on basal glucose transport in the present study is similar to the small or absent effects previously reported for intact skeletal muscle, in which the difference in glucose concentration was 5 vs. 10 mM (38). The present results extend the earlier findings because the duration of glucose exposure was considerably longer (24 compared with 3 h). Furthermore, unlike the earlier study (38), the present study included measurement of insulin-stimulated glucose transport, which was not found to differ for muscles with prolonged exposure at these levels of glucose. In the absence of changes in insulin concentration, there appears to be little effect on glucose transport when glucose is modulated within this physiological range, at least for glucose exposures of up to 24 h.

Glucose transport was also unaltered after 24-h incubation with 20 compared with 80 μU/ml insulin in the presence of 8 mM glucose. These results also indicate that, in the presence of a normal extracellular glucose concentration, a 75% reduction in insulin concentration within the physiological range did not modify subsequent glucose transport. These findings are in contrast to data from rat hindlimbs perfused for 2 h with 5 mM glucose and 50 vs. 20,000 μU/ml insulin (26). Under those conditions, prior perfusion with supraphysiological compared with physiological insulin was characterized by reduced glucose uptake. Alterations in muscle glucose uptake after exposure to extreme differences in insulin concentration (no insulin vs. physiological insulin levels or physiological vs. supraphysiological insulin levels) cannot be considered predictive of the outcome after prolonged exposure to differing insulin concentrations within the normal physiological range.

Reductions in both glucose and insulin were required for the elevated insulin-stimulated glucose transport. One interpretation of the requirement for lowering both glucose and insulin concentrations to achieve increased insulin sensitivity is that the mechanism is not simply a direct effect of reduced insulin...
signaling but rather is secondary, at least in part, to reduced insulin-stimulated glucose flux into the cell because of decrements in both glucose and insulin concentrations.

The prolonged incubation with reduced glucose and insulin concentrations recapitulated the effect of in vivo CR on glucose transport, i.e., glucose transport was elevated in skeletal muscle stimulated with submaximally effective insulin, and basal glucose transport was unaltered. The magnitude of the increase in glucose transport observed (29% above controls) compares with increments of ~20–100% in earlier studies of isolated epitrochlearis muscles from in vivo CR vs. ad libitum-fed rats (11, 12, 15–17, 33, 34). Thus the relative magnitude of the adaptation with the present model overlaps with the lower range of effects reported after in vivo CR.

Although the change in glucose transport was similar with the in vitro model compared with earlier studies with in vivo CR, the lack of an increase in Akt phosphorylation differs from our previous finding that in vivo CR leads to increased Akt phosphorylation in rodent muscle stimulated with submaximally effective insulin concentrations (32–34). Data from mice that are null for Akt2, the isoform that is important for insulin-stimulated glucose transport (6), indicate that Akt2 is essential for the full effect of in vivo CR on insulin-stimulated glucose transport in skeletal muscle (32). AS160 is the most distal insulin signaling protein that is known to link insulin’s activation of Akt to GLUT4 translocation (9, 13, 27, 28, 37). The lack of a difference in PAS-160kDa for the sALgi-24h compared with sCRgi-24h groups is consistent with the observation of no difference in Akt phosphorylation between these groups. The effect of in vivo CR on insulin-stimulated AS160 phosphorylation has not been reported. However, because in vivo CR leads to an increase in insulin-stimulated Akt phosphorylation (32–34), it seems reasonable to hypothesize that AS160 phosphorylation may be greater for insulin-stimulated muscles from CR compared with ad libitum-fed rats.

Previous research using Akt2-null mice suggested that improved Akt2 phosphorylation was not likely the only mechanism for improved insulin sensitivity after in vivo CR (32). The Akt2-independent mechanism whereby CR can improve insulin sensitivity has not been identified, but it is conceivable that there is an enhancement in an insulin signaling step that has not yet been evaluated with CR, e.g., atypical protein kinase C. Another possibility is that there is a modification of the molecular machinery that performs GLUT4 trafficking downstream of the insulin signaling cascade. Regardless, it is unclear whether the Akt-independent mechanism with the present model is also playing any role in the improved insulin action found with in vivo CR.

Fig. 6. Akt serine phosphorylation \([p\text{Akt}(S473)]\) determined for epitrochlearis muscles in Protocol 4. Paired muscles were initially incubated with 8 mM glucose + 80 \(\mu\)U/ml insulin (sALgi-24h) or 5.5 mM glucose + 20 \(\mu\)U/ml insulin (sCRgi-24h) for 24 h. Subsequently, both muscles from each rat were incubated either without insulin or with 80 \(\mu\)U/ml insulin for 1.25 h. Data are means ± SE for 15 (basal) or 14 (insulin-stimulated) muscles per group. S473, Ser473. There were no statistically significant differences between the sALgi-24h and sCRgi-24h groups.

Fig. 7. Phosphorylated protein of apparent molecular weight of 160 kDa identified using the PAS antibody (PAS-160kDa) determined for epitrochlearis muscles in Protocol 4. Paired muscles were initially incubated with 8 mM glucose + 80 \(\mu\)U/ml insulin (sALgi-24h) or 5.5 mM glucose + 20 \(\mu\)U/ml insulin (sCRgi-24h) for 24 h. Subsequently, both muscles from each rat were incubated either without insulin or with 80 \(\mu\)U/ml insulin for 1.25 h. Data are means ± SE for 12 (basal) or 10 (insulin-stimulated) muscles per group. There were no statistically significant differences between the sALgi-24h and sCRgi-24h groups.

Fig. 8. GLUT4 abundance was determined for epitrochlearis muscles in Protocol 4. Paired muscles were initially incubated with 8 mM glucose + 80 \(\mu\)U/ml insulin (sALgi-24h) or 5.5 mM glucose + 20 \(\mu\)U/ml insulin (sCRgi-24h) for 24 h. There was no statistically significant difference between the sALgi-24h and sCRgi-24h groups for GLUT4 abundance. Data are means ± SE for 29 muscles per group.
Previous research that quantified insulin receptors in skeletal muscle using various methods, including Western blotting (2–4, 42) and insulin receptor binding (7, 14), has usually found no effect of CR, consistent with the present results, although Wang et al. (39) reported increased insulin receptor binding in rat skeletal muscle after CR. Two studies have indicated that insulin receptor tyrosine phosphorylation can be increased by CR in muscle (3, 18), whereas an equal number of studies have not found this effect of CR (2, 4). In addition, Zhu et al. (42, 43) found that CR led to increased insulin receptor tyrosine phosphorylation in muscle from old, but not young, rats. Thus the lack of an increase in insulin receptor tyrosine phosphorylation after exposure to reduced glucose and insulin is consistent with some, but not all, of the published effects of in vivo CR on insulin receptor tyrosine phosphorylation. We previously found that in vivo CR can lead to a reduction in rat and mouse skeletal muscle IRS1 abundance (16, 21, 34), although others did not find this result in mouse skeletal muscle (2–4), and we did not find this CR effect in muscle from nonhuman primates (22). In any case, a reduction in muscle IRS1 levels would not be expected to account for improved insulin sensitivity. GLUT4 abundance was not significantly altered in the muscles after prolonged incubation with reduced glucose and insulin. These results are consistent with most earlier research on in vivo CR, which indicates no effect of CR on skeletal muscle GLUT4 total abundance (2, 4, 12, 22, 39), although one previous study reported an increase in mouse muscle GLUT4 with CR (3). GLUT1 abundance was unaffected in the present study, consistent with the lack of an effect of in vivo CR on GLUT1 from rat soleus or white gastrocnemius muscles, although a CR-associated increase in GLUT1 was found in a mixed sample of whole gastrocnemius and quadriceps muscles (40). In skeletal muscle, GLUT1 is involved in basal rather than insulin-stimulated glucose transport, so the lack of altered GLUT1 abundance is consistent with the unaltered basal glucose transport in this model and with in vivo CR.

In conclusion, this study provides the proof of principle that moderate reductions in extracellular glucose and insulin, similar to those found with in vivo CR, are sufficient to induce an increase in insulin-stimulated glucose transport of muscle that is comparable to the increase found with in vivo CR. The results for insulin receptor, GLUT1, and GLUT4 abundance after incubating muscles with reduced extracellular glucose and insulin were consistent with the findings of most studies of in vivo CR. However, the absence of an increase in Akt phosphorylation in this in vitro model, as opposed to the increased Akt phosphorylation that has usually been observed with in vivo CR, suggests that the mechanisms for improved insulin sensitivity are distinct for the two conditions. The present study was an initial approach in which only extracellular glucose and insulin were manipulated. Modifying the model to include other CR-induced effects (e.g., a decrement in extracellular adiponectin), simulation of diurnal modulations of glucose and insulin, and extension of the experiment beyond 24 h would likely provide further insights into the mechanisms for improved insulin sensitivity with in vivo CR.

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REFERENCES


