Insulin Signaling and Glucose Uptake in the Soleus Muscle of 30-Month-Old Rats After Calorie Restriction With or Without Acute Exercise

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Abstract

Exercise and calorie restriction (CR) can each improve insulin sensitivity in older individuals, but benefits of combining these treatments on skeletal muscle insulin signaling and glucose uptake are poorly understood, especially in predominantly slow-twitch muscles (eg, soleus). Accordingly, our purpose was to determine independent and combined effects of prior acute exercise and CR (beginning at 14 weeks old) on insulin signaling and glucose uptake in insulin-stimulated soleus muscles of 30-month-old rats. CR alone (but not exercise alone) versus ad libitum sedentary controls induced greater insulin-stimulated glucose uptake. There was a main effect of diet (CR > ad libitum) for insulin-stimulated AktSer473 and AktThr308 phosphorylation. CR alone versus ad libitum sedentary increased Akt substrate of 160 kDa (AS160) Ser588 phosphorylation and TBC1D1 Thr596, but not AS160 Thr642 phosphorylation or abundance of GLUT4, GLUT1, or hexokinase II proteins. Combined CR and exercise versus CR alone did not further increase insulin-stimulated glucose uptake although phosphorylation of AktSer473, AktThr308, TBC1D1 Thr596, and AMPKThr172 for the combined group exceeded values for CR and/or exercise alone. These results revealed that although the soleus was highly responsive to a CR-induced enhancement of insulin-stimulated glucose uptake, the exercise protocol did not elevate insulin-stimulated glucose uptake, either alone or when combined with CR.

Key Words: Glucose transport—Glucose transporter—Dietary restriction—Insulin resistance—Physical activity

Whole-body insulin resistance is predictive of the subsequent development of many age-related diseases (1). Because skeletal muscle accounts for the largest portion of insulin-mediated glucose uptake (2), elucidating interventions that increase skeletal muscle glucose uptake is highly relevant for improving health with advancing age. Either exercise or moderate calorie restriction (CR; consuming ~20%–40% below ad libitum [AL] intake) can independently elevate insulin-mediated glucose uptake in either predominantly fast-twitch or predominantly slow-twitch skeletal muscle of rats up to 24 months old (3–7). A crucial aspect of the current study was the inclusion of (i) a sedentary and AL control group that was not subjected to either CR or exercise; (ii) a CR only group; (iii) an exercise only group; and (iv) a combined CR and exercise group. Including all four groups is essential to assess the independent and combined effects of CR and exercise. To the best of our knowledge, both insulin signaling and glucose uptake by skeletal muscle have been previously reported for all four of these groups only in our recent study (8). Separate benefits of CR or acute exercise were observed in the predominantly fast-twitch epitrochlearis muscle from 30-month-old rats, and CR combined with exercise produced even greater insulin-stimulated glucose uptake compared with exercise or CR alone in the epitrochlearis (8).
Physiological responses to diet and exercise can diverge for muscles with different fiber-type profiles. Thus, a second crucial aspect of this study was that it focused on the predominantly slow-twitch soleus from the same rats that provided the fast-twitch epitrochlearis in our earlier study (8) with the aim of determining the independent and combined effects of acute exercise and chronic CR on insulin-stimulated glucose uptake in the predominantly slow-twitch soleus. Using the same rats provides an advantage when comparing the results from the two muscles.

The second aim of the current study was to elucidate potential mechanisms for CR and/or exercise effects on insulin-stimulated glucose uptake by the soleus. Isolated soleus muscles from 30-month-old rats were analyzed to ascertain the effects of chronic CR (initiated at 14 weeks old) and/or acute exercise on (i) insulin-stimulated glucose uptake; (ii) activation of key insulin signaling steps that regulate glucose uptake (insulin receptor Tyr1146 phosphorylation, Akt Ser473 and Thr308 phosphorylation, AS160 Ser474 and Thr450 phosphorylation, and TBC1D1 Thr796 phosphorylation); (iii) abundance of GLUT4, GLUT1, and hexokinase II (proteins that control glucose transport and phosphorylation); (iv) Ser211 phosphorylation of Filamin-C (FLNC); and (v) Thr722 phosphorylation of AMPK. We hypothesized that CR and exercise would each lead to increased insulin-stimulated glucose uptake and that a further increase would occur with combined CR and exercise. We also hypothesized that the elevated insulin-stimulated glucose uptake with the respective treatments would be accompanied by increased Akt phosphorylation.

**Research Design and Methods**

**Materials**

All of the chemicals were from Sigma-Aldrich (St. Louis, MO) or Fisher Scientific (Hanover Park, IL) or unless otherwise noted. The apparatus and reagents for sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunoblotting were obtained from Bio-Rad Laboratories (Hercules, CA). Pierce MemCode Reversible Protein Stain Kit and bicinchoninic acid were purchased from Kinasource (Dundee, Scotland, UK). [3H]-2-deoxy-D-glucose ([3H]-2-DG), and d-[14C]-mannitol were purchased from PerkinElmer (Boston, MA).

**Animal Treatment**

Animal care procedures were approved by the University of Michigan Committee on Use and Care of Animals. AL and CR male Fischer-344 x Brown Norway (FBN) rats were obtained from National Institute of Aging (NIA) Calorie Restricted Rodent Colony at approximately 29 months old. Calorie restriction for the CR group was begun in 14-week-old rats when they were housed at the NIA. Animals were individually housed at the University of Michigan in specific pathogen-free conditions in shoebox cages (12-12 hour light-dark cycle; lights out at 17:00 hours) for approximately 1 month prior to the terminal experiment. AL rats were given NIH31 chow and CR rats were provided with NIH31/NIA-fortified chow including sufficient vitamin supplementation for the CR animals to ingest vitamins at levels comparable with the AL controls eating NIH31 chow. The CR rats were provided 60%–65% of AL daily intake. Animals were fed between 15:30 and 16:30 pm daily, and food intake was determined each day. The terminal experiment was performed when the animals were ~30 months old. The animals were fasted at approximately 19:00 on the night before the experiment. At approximately 07:00 on the next morning, the rats in the exercise groups swam in a barrel filled with water (35°C; 45 cm depth; three AL and three CR rats). The exercise was nine swimming bouts (10 minutes per bout) with 10-minute rest periods between each bout. Following 90 minutes of exercise, rats were dried and returned to their cages without food. Soleus muscles were dissected from anesthetized time-matched sedentary and exercised animals at 3–4 hours after the conclusion of exercise.

**Muscle Dissection and Incubation**

When rats were deeply anesthetized by an intraperitoneal injection of sodium pentobarbital (50 mg/kg), soleus muscles were dissected out and rapidly rinsed in Krebs–Henseleit buffer. Muscles were longitudinally split into four strips that were placed in vials including the appropriate media, shaken at 45 revolutions per minute while continuously gassed (95% O2–5% CO2) in a heated (35°C) water bath. Krebs–Henseleit buffer (2 mL) supplemented with bovine serum albumin (0.1%), 2 mM sodium pyruvate, 6 mM mannitol, and no insulin (basal) or a submaximally effective insulin dose (0.6 mU/ml was included in the vials during the initial incubation step (30 minutes). Muscles were subsequently transferred for 20 minutes to a vial containing 2 mL Krebs–Henseleit buffer/bovine serum albumin, the same insulin dose, 0.1 mU 2-DG (with a final specific activity of 2.25 mCi/mmol [3H]-2-DG), and 5.9 mM mannitol (with a final specific activity of 0.022 mCi/mmol [14C]-mannitol). Following this step, muscles were blotted on filter paper moistened with ice-cold Krebs–Henseleit buffer, trimmed, freeze-clamped using aluminum tongs cooled in liquid nitrogen, and stored at ~80°C for subsequent processing and analysis.

**Muscle Processing**

Frozen muscles were weighed and then homogenized in ice-cold lysis buffer (1 mL) using a TissueLyser II homogenizer (Qiagen, Valencia, CA). The lysis buffer included T-PER Tissue Protein Extraction Reagent (#PI-78510; Thermo Scientific, Rockford, IL) along with 1 mM ethylenediaminetetraacetic acid, 1 mM ethylene glycol tetraacetic acid, 2.5 mM sodium pyrophosphate, 1 mM sodium vanadate, 1 mM β-glycerophosphate, 1 μg/mL leupeptin, and 1 mM phenylmethylsulfonyl fluoride. Lysates were rotated for 1 hour (4°C).
prior to centrifugation (15,000g for 15 minutes at 4°C). The supernatants were transferred to microfuge tubes and stored at −80°C for subsequent analyses. Protein concentration was determined using the bicinchoninic acid procedure (Pierce Biotechnology, Rockford, IL; #23225).

2-Deoxy-β-Glucose Uptake

Aliquots of the supernatants from muscle lysates were pipetted in a vial along with scintillation cocktail (Research Products International, Mount Prospect, IL). A scintillation counter (PerkinElmer) determined the 3H and 14C disintegrations per minute. 2-DG uptake was calculated as previously described (9–11).

Immunoblotting

An equal amount of protein from muscle lysates was combined with 6x Laemmli buffer, boiled (5 minutes), separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (7% resolving gel), and transferred to polyvinyl difluoride membranes. Equal loading was confirmed using the MemCode protein stain. Bovine serum albumin (5% in TBST; Tris-buffered saline, pH 7.5 plus 0.1% Tween-20) was used for blocking (1 hour at room temperature). Membranes were then washed (3 times 5 minutes in TBST) and subsequently incubated with primary antibody (in TBST plus 5% bovine serum albumin) overnight (4°C) and washed (3 times 5 minutes in TBST). Next, membranes were incubated with secondary antibody (1 hour at room temperature) and washed (3 times for 5 minutes in TBST) followed by washing (3 times for 5 minutes) in TBS. Enhanced chemiluminescence (Luminata Forte Western HRP Substrate; #WBLUF0100; Millipore) was used to visualize the protein bands that were quantified via densitometry (AlphaEase FC; Alpha Innotech, San Leandro, CA). Results were expressed relative to the normalized mean of all the samples on the blot.

Statistical Analysis

Two-way analysis of variance was performed for each insulin dose (0 or 0.6 nM), and the two independent factors were diet (the two levels for diet were AL or CR) and exercise (the two levels for exercise were sedentary or 3 hours post-exercise). The interaction effect (Diet x Exercise) was also determined, and the Tukey test was used for post hoc analysis to identify the source of significant variance for main effects or interaction effects (SigmaPlot version 11.0; Systat Software, San Jose, CA). When data were not characterized by normal distribution and/or equal variance, they were transformed to attain normality and equal variance before performing two-way analysis of variance. Kruskal–Wallis one-way analysis of variance on ranks was used if transformation was unable to normalize the data or equalize the variance, and post hoc analysis used Dunn’s method. Spearman’s rank order correlation was used to test associations between two parameters.

Results

2-Deoxy-β-Glucose Uptake

For 2-DG uptake in muscles incubated without insulin (Figure 1), there was a significant $p < .01$ main effect of diet (CR > AL). Post hoc analysis revealed the sedentary and CR (SED-CR) group exceeded the sedentary and AL (SED-AL) group ($p < .001$), the 3hPEX-CR group was greater than 3hPEX-AL groups ($p < .001$).

Immunoblotting

Equal loading of samples was confirmed based on the MemCode results (8, 12). For all of the phosphorylated proteins, the data were expressed as a ratio of the phosphorylated-to-total protein values. Expressing the results as phosphorylated-to-total protein ratio rather than as the phosphorylated protein without dividing by the total protein value did not change the interpretation of the results for any of the proteins that were assessed.

Insulin Receptor

For total IR abundance, there was a moderate (~19%), but significant effect of diet (AL > CR) either without insulin ($p < .05$) or with insulin ($p < .05$; data not shown). Post hoc analysis indicated that in the absence of insulin, SED-CR exceeded SED-AL values ($p < .05$). In the presence of insulin, 3hPEX-CR values were greater than 3hPEX-AL values ($p < .01$; data not shown). For pIR$^{TYR1146}$/IR ratio in the absence of insulin, there were significant effects of diet (AL > CR; $p < .001$) and exercise (SED > 3hPEX; $p < .001$; Figure 2). Post hoc analysis indicated that SED-AL values exceeded both SED-CR ($p < .001$) and 3hPEX-AL values ($p < .01$), 3hPEX-AL ($p < .001$) and CR-SED values ($p < .05$) were each greater than 3hPEX-CR values. For pIR$^{TYR1146}$/IR ratio in the presence of insulin, there was no significant effect of diet and exercise (Figure 2).

Akt and Akt2

There was no significant effect of diet or exercise on total Akt or total Akt2 abundance (data not shown). For pAkt$^{Thr^{173}}$/Akt ratio in the absence of insulin, there was significant $p < .01$ main effect
of diet (CR > AL; Figure 3A). Post hoc analysis revealed that the SED-CR group was greater than the SED-AL group \((p < .05)\) and the 3hPEX-CR exceeded the 3hPEX-AL group \((p < .05); \) Figure 3A). For \(p\text{Akt}^{\text{Thr308}}/\text{Akt}\) ratio in the presence of insulin, there were significant effects of diet (CR > AL; \(p < .01\)) and exercise (3hPEX > SED; \(p < .05\); Figure 3A). Post hoc analysis indicated that the 3hPEX-CR group was greater than the 3hPEX-AL group \((p < .01)\). For the \(p\text{Akt}^{\text{Ser473}}/\text{Akt}\) ratio in the absence of insulin, the SED-CR exceeded the SED-AL group \((p < .01; \) Figure 3B). In the presence of insulin, there were significant main effects of diet (CR > AL; \(p < .01\)) and exercise (3hPEX > SED; \(p < .05\)) on the \(p\text{Akt}^{\text{Ser473}}/\text{Akt}\) ratio (Figure 3B). Post hoc analysis demonstrated that the 3hPEX-CR group exceeded both the 3hPEX-AL \((p < .01)\) and the SED-CR \((p < .05)\) groups. For the \(p\text{Akt}^{\text{Ser473}}/\text{Akt2}\) ratio in the absence of insulin, there was a significant effect of diet (CR > AL; \(p < .05\); Supplementary Figure 1). In the presence of insulin, there were significant effects of diet (CR > AL; \(p < .001\)) and exercise (3hPEX > SED; \(p < .05\)), as well as a significant Diet \(\times\) Exercise interaction \((p < .05)\). Post hoc analysis indicated that the 3hPEX-CR group exceeded both the 3hPEX-AL and SED-CR groups \((p < .01)\). There was a significant \((p < .0001)\) correlation \((R = .891)\) between \(p\text{Akt}^{\text{Ser473}}/\text{Akt2}\) and \(p\text{Akt}^{\text{Ser473}}/\text{Akt}\).

**AS160**

There was no significant effect of diet or exercise on AS160 total abundance (data not shown). For the ratio of \(p\text{AS160}^{\text{Ser474}}/\text{AS160}\) in the absence of insulin, there were no significant effects of diet or exercise, but there was a significant Diet \(\times\) Exercise interaction \((p < .05; \) Figure 4A). Post hoc analysis revealed that 3hPEX-CR values exceeded 3hPEX-AL values \((p < .05)\). For the ratio of \(p\text{AS160}^{\text{Thr642}}/\text{AS160}\) in the presence of insulin, there was a significant effect of diet (CR > AL; \(p < .01\); Figure 4A). Post hoc analysis revealed that SED-CR values exceeded SED-AL values \((p < .01)\). For the ratio of \(p\text{AS160}^{\text{Ser588}}/\text{AS160}\), there were no significant effects of diet or exercise, either in the absence or presence of insulin (Figure 4B).

**TBC1D1**

There was no significant effect of diet or exercise on total TBC1D1 abundance (data not shown). For the ratio of \(p\text{TBC1D1}^{\text{Thr596}}/\text{TBC1D1}\)
TBC1D1 in the absence of insulin, there were no significant effects of either diet or exercise. For the ratio of pTBC1D1Thr596/TBC1D1 in the presence of insulin, there was a significant effect of diet (CR > AL; \(p < .001\); Figure 4D). Post hoc analysis indicated that SED-CR exceeded SED-AL (\(p < .001\)), and 3hPEX-CR was greater than 3hPEX-AL (\(p < .001\)).

Filamin-C
For FLNc in the absence of insulin, there was a small (~10%), but significant diet effect on total abundance (AL > CR; \(p < .01\); data not shown), and post hoc analysis indicated that SED-AL values were greater than SED-CR values (\(p < .05\) for FLNcSer2231; \(p < .001\) for pFLNcThr172). There was a small (~11%), but significant (\(p < .01\)) exercise effect on GLUT1 abundance (SED > 3hPEX; Supplementary Figure 3). There were no significant effects of diet or exercise on hexokinase II abundance (Supplementary Figure 4).

AMPK
For total AMPK abundance, there was a small (~12%), but significant effect of diet (AL > CR) without insulin (\(p < .05\); data not shown). Post hoc analysis indicated no further significant effect. For pAMPKThr172/AMPK ratio in the absence of insulin, there were significant effects of exercise (3hPEX > SED; \(p < .05\); Figure 6). Post hoc analysis indicated no further significant effect. For pAMPKThr172/AMPK ratio in the presence of insulin, there were significant effects of exercise (3hPEX > SED; \(p < .05\); Figure 6). Post hoc analysis revealed that 3hPEX-CR values were greater than both 3hPEX-AL (\(p < .05\)) and SED-CR values (\(p < .01\)).

Discussion
This study was the first to evaluate the combined effects of CR and acute exercise on insulin-stimulated glucose uptake and insulin signaling in a predominantly slow-twitch muscle. The most important new functional results were that insulin-stimulated glucose uptake by the soleus of 30-month-old rats (i) was significantly increased by CR alone; (ii) not significantly increased by acute exercise alone; and (iii) was not greater for combined CR and acute exercise versus CR alone. By evaluating key insulin signaling proteins and the proteins that regulate glucose transport and phosphorylation, the current
study also provided novel insights into the potential mechanisms for these functional outcomes.

No animal model exactly replicates human biology, but the male FBN rat is a preferred model for elucidating aging effects on skeletal muscle with potential relevance for humans (13). Focusing first on the effects of CR alone, this study extended the observations from earlier research that demonstrated CR caused greater insulin-stimulated glucose uptake in the predominantly slow-twitch soleus of 24-month-old male FBN rats (5). For male FBN rats, 24 months corresponds with ~90% survival and 30 months corresponds to ~70% survival (14). For men in the U.S. population, 90% survival corresponds to ~55 years old, and 70% survival corresponds to ~70 years old (15). Although modest age-related changes are evident in the soleus of 30-month-old FBN rats (eg, reduced citrate synthase and lactate dehydrogenase activities), soleus mass does not markedly decline until later in life (16, 17). Previous research indicated that CR produced increased insulin-stimulated glucose uptake in the predominantly fast-twitch epitrochlearis muscle at 30 months old (8). The consistent effect of CR on insulin-stimulated glucose in the soleus and epitrochlearis from older rats corresponds with the results of many studies with younger rodents that have found that CR can improve insulin-stimulated glucose uptake in muscles with differing fiber-type profiles (18-20). Taken together, these studies have revealed CR’s very robust effect on insulin-stimulated glucose uptake in isolated muscles of diverse fiber-type compositions across a wide range of the adult life span.

The most consistent CR effect on insulin signaling in muscle is increased insulin-stimulated Akt phosphorylation. In the current study, there was a significant main effect of diet (CR > AL) for Akt phosphorylation on both Ser473 and Thr308. Earlier research in the soleus of 24-month-old rats also demonstrated greater insulin-stimulated Akt phosphorylation on both Ser473 and Thr308 (5). In the soleus of ~2 to 3-month-old mice, CR resulted in greater Akt Ser473 and Thr308 phosphorylation (18,21). Several studies have found that CR causes elevated Akt phosphorylation on Ser473 and Thr308 in epitrochlearis muscles of ~5 to 9-month-old rats (20,22-25). The current study was the first to demonstrate CR leads to greater Akt2 phosphorylation in muscle from old animals. This result is significant because it confirms earlier results for CR in young animals (18,22,24) and because Akt2 is the isoform that regulates insulin-stimulated glucose transport (26). A previous study demonstrated that in 9-month-old rats, a selective Akt inhibitor prevented CR’s enhancement of both Akt phosphorylation and insulin-stimulated glucose uptake in the predominantly fast-twitch epitrochlearis muscle (24). It would be valuable to use a similar approach to learn if CR effects on Akt phosphorylation are also crucial for increased insulin-stimulated glucose uptake in the soleus.

Compelling evidence links site-specific phosphorylation of AS160 on Ser473 and Thr308 in the epitrochlearis muscle at ~5 to 9-month-old rats (20,22-25). The current study included the novel observation that CR led to greater pTBC1D1 phosphorylation on both Ser473 and Thr308 (5). Earlier research demonstrated that in the epitrochlearis from 9-month-old rats, CR induces greater AS160 phosphorylation on both Thr308 and Ser318 (20). However, analysis of the soleus from 9- and 24-month-old rats revealed that CR did not elevate insulin-stimulated AS160 phosphorylation on either site (5,20,34). Taken together, these results suggest that the CR-induced enhancement of insulin-stimulated glucose uptake in the soleus may involve Akt substrates other than AS160.

Therefore, we evaluated the phosphorylation of TBC1D1, a paralog protein of AS160 that is also an Akt substrate (28,35). TBC1D1 Thr596, which corresponds to Thr442 in AS160, becomes phosphorylated in response to insulin treatment (28,36,37). The current study included the novel observation that CR led to greater pTBC1D1 Thr596 in insulin-stimulated muscle. Convincing evidence links phosphorylation of AS160 on Ser318 and Thr442 to insulin-stimulated GLUT4 translocation and glucose transport (28), but the role of
Ser588 phosphorylation for insulin-stimulated glucose transport in muscle is uncertain in light of the report that insulin-stimulated glucose uptake was unaffected in muscle overexpressing TBC1D1 that was mutated to prevent Thr596 phosphorylation (38). However, it is possible that the endogenous, nonmutated TBC1D1 in the muscle of this earlier study may have been sufficient for the normal glucose uptake (38).

FLNc is another interesting protein because it is an insulin-regulated Akt substrate and actin-binding protein that is selectively expressed in skeletal muscle (39, 40), and actin remodeling has been linked to the subcellular localization of insulin signaling proteins and GLUT4 (41). In addition, the abundance of GLUT4 is positively correlated to the abundance of FLNc in single fibers from rat skeletal muscle (42). Furthermore, FLNc becomes Ser422 phosphorylated in response to insulin stimulation (24, 43). However, in the current study, CR did not alter FLNcSer422 phosphorylation. This outcome differs from earlier results for the epitrochlearis of 9-, 24-, and 30-month-old rats in which CR resulted in greater insulin-stimulated FLNcSer422 phosphorylation (8, 24, 43). The current data indicate that in the soleus, greater FLNc phosphorylation was not essential for enhanced insulin-stimulated glucose uptake.

The combined CR and exercise group was characterized by elevated pAMPK Thr172 in insulin-stimulated muscles. However, glucose uptake for the combined CR and exercise group did not exceed glucose uptake for CR alone. Elevated AMPK phosphorylation in the combined group may have some functional consequences because AMPK has a vast number of biological effects, including altered lipid metabolism, protein metabolism, and gene expression (44).

There was no evidence that greater GLUT4 or hexokinase II abundance was important for the CR-induced increase in glucose uptake by the soleus, consistent with earlier results in the soleus of 24-month-old rats (5). Furthermore, neither GLUT4 nor hexokinase II levels were elevated by CR in the predominantly fast-twitch epitrochlearis of 30-month-old rats (8). Previous research in the epitrochlearis of young rats demonstrated that CR can induce greater insulin-stimulated GLUT4 translocation (45), and it seems reasonable to predict a similar result for the soleus of older animals.

The modestly (~17%) greater basal glucose uptake for SED-CR versus SED-AL is similar to the trend from our earlier studies for ~15%–20% greater basal glucose uptake in the soleus of CR rats (5, 34). We assessed GLUT1 abundance, a glucose transporter that contributes to basal glucose uptake, but found no CR effect. AMPK activation is believed to trigger increased cell-surface GLUT4 content, leading to greater insulin-independent glucose uptake (46), but CR alone did not increase pAMPK Thr172. Additional research will be needed to determine if CR alters any of the various other signals that are proposed to elevate insulin-independent glucose uptake (46).

AMPK activation can also modulate insulin sensitivity (44, 47). Earlier research has differed with regard to CR’s effect on the activation of AMPK in muscle. In the current study, CR alone did not alter AMPKThr172 phosphorylation, consistent with results of most of the previous studies that tested for possible CR effects on AMPK in muscle (18, 20, 21, 48, 49). However, a smaller number of studies have found CR-related activation of muscle AMPK (50–52). The reason for the differing results is uncertain, but differences in the muscles studied may be a factor. Studies that reported no CR effect have included rat soleus and epitrochlearis (20) and mouse extensor digitorum longus, gastrocnemius, soleus, and unspecified muscles (18, 21, 48, 49). Studies reporting that CR increased AMPK activation were in mouse quadriceps, hindlimb muscles, and unspecified muscles (50–52).

Focusing on the independent effects of exercise, insulin-mediated glucose uptake was not significantly elevated in the current study. This observation differs from earlier results in 24-month-old rats, in which soleus glucose uptake was increased after a similar exercise protocol (4). It also differs from results for the epitrochlearis from the same rats (8). It is possible that with advancing age, the soleus becomes less responsive to exercise-induced improvements in insulin sensitivity. It is also possible that in the current study, the soleus was insufficiently recruited to enhance subsequent insulin-stimulated glucose uptake and that a different exercise protocol would be effective for increasing insulin-stimulated glucose uptake by the soleus of 30-month-old rats.

It is notable that the absence of an exercise effect on insulin-stimulated glucose uptake in the soleus was accompanied by no significant exercise effects on the phosphorylation of any of the signaling proteins that were studied (Akt, AS160, TBC1D1, FLNc, or AMPK). There was also no effect of exercise alone on GLUT4 or hexokinase II abundance in the soleus. The same exercise protocol in the same rats was previously found to elevate Akt Ser473 and Thr308 phosphorylation and FLNcSer422 phosphorylation in insulin-stimulated epitrochlearis concomitant with greater GLUT4 abundance (8). However, it is uncertain if any of these results can account for the difference between the soleus and the epitrochlearis for exercise effects on insulin-stimulated glucose uptake.

Changes in mitochondrial function and/or muscle lipids are associated with altered insulin sensitivity, so it is reasonable to consider possible relationships between these parameters and improved insulin sensitivity after CR and/or exercise. An advantage of assessing insulin sensitivity a few hours after acute exercise in this study was that it would not be expected to produce large changes in mitochondrial content. However, even acute exercise can transiently influence mitochondrial function. Accumulation of ceramides and/or diacylglycerols is associated with insulin resistance (53), but acute exercise caused increased insulin-stimulated glucose uptake by epitrochlearis from young rats with normal insulin sensitivity and insulin-resistant rats without lowering muscle ceramides or diacylglycerols (54). Reports indicate CR-induced improvement in mitochondrial function and/or content (55–59), but other studies have not supported this idea (60, 61). Reduced muscle ceramide levels are not required for improved insulin sensitivity in FBN rats with CR (62). The roles of mitochondria and/or lipids in the CR and exercise effects on insulin sensitivity have not been directly tested in old age.

Rats and mice are often studied to identify mechanisms that regulate muscle insulin sensitivity. The soleus is composed of ~70% slow-twitch fibers in humans, ~80%–90% slow-twitch fibers in rats, and ~30%–50% slow-twitch fibers in mice (16, 63–69). AS160 abundance did not differ among the gastrocnemius, vastus lateralis, and soleus of humans (64). Similarly, in 12 different rat muscles with widely different fiber-type profiles (including the soleus), there were no significant differences for AS160 abundance (65). In contrast, for mice, AS160 abundance was ~10-fold greater for the soleus versus the extensor digitorum longus (63). Either human (64) or rat (70, 71) muscle studied immediately after exercise had significant increases in pAS160Ser588 and pAS160Thr522, whereas in mice, there were no significant increases in pAS160Ser588 and pAS160Thr522 in the extensor digitorum longus or soleus immediately post-exercise (71). These observations indicate several advantages for the rat as a model for understanding glucose uptake in human muscle.

What are the effects of CR and/or exercise on insulin sensitivity in humans? From ~3 to 48 hours after acute exercise by humans, whole-body insulin sensitivity is increased as determined by the
hyperinsulinemic glucose clamp method (72–74). In obese humans (53 years) with impaired glucose tolerance or mild type 2 diabetes, CR produced increased insulin-stimulated glucose disposal determined by the glucose clamp (75). In overweight humans (45–65 years), combined CR and chronic exercise compared with either CR or exercise alone produced a greater improvement in Matsuda insulin sensitivity index using the frequently sampled oral glucose tolerance test (76). In overweight humans (25–50 years), CR combined with chronic exercise versus CR alone produced a greater increase in insulin sensitivity determined by the insulin-modified frequently sampled intravenous glucose tolerance test (77). In nonobese older (~60 years) humans, diet-induced weight loss combined with chronic exercise was more effective than either treatment alone for lowering the insulin response to an oral glucose tolerance test (78,79). In earlier studies of chronic exercise in humans, insulin sensitivity was evaluated 12–48 hours after the last exercise session, when at least a portion of effects on glucose disposal were likely attributable to the final exercise bout (72,80). To summarize, the available evidence for humans indicates that CR or acute exercise alone can improve insulin sensitivity, and combined CR and chronic exercise produces greater benefits than either treatment alone.

What is known about the effects of CR and/or exercise on insulin signaling, GLUT4, and GLUT1 in human muscle? In humans (22–28 years), prior acute exercise did not alter insulin’s effects on proximal insulin signaling steps ranging from the insulin receptor to Akt (81,82), but it produced greater insulin-stimulated AS160 phosphorylation (83). In young rats after acute exercise, there were no effects on proximal insulin signaling from the insulin receptor to Akt, but there was increased AS160 phosphorylation (54,84). GLUT4 protein abundance in human muscle has been reported to increase a few hours after acute exercise, but other studies did not detect this increase (46). In humans with type 2 diabetes (51 years), CR had no effect on muscle insulin receptor binding or tyrosine kinase activity (85). We are unaware of published data on the effects of combined CR and exercise on insulin signaling or GLUT4 in human muscle. We do not know of studies that assessed GLUT1 protein in human muscle after CR and/or acute exercise. We are also unaware of human studies reporting CR and/or acute exercise effects on glucose uptake, insulin signaling, GLUT1, or GLUT4 protein in slow-twitch muscle. The current study provides valuable information that is absent in the human literature.

In conclusion, the current study revealed that the effect of CR alone on insulin-stimulated glucose uptake by the soleus from 30-month-old rats is similar to results from earlier research for the epimysialis from rats of the same age and for the epimysialis of the soleus from younger animals. The study also revealed that insulin-stimulated glucose uptake in the soleus was unresponsive to either acute exercise alone or combined CR and acute exercise. This outcome contrasted with the recent report of significant effects of identical interventions on insulin-stimulated glucose uptake by the epimysialis from the same 30-month-old rats (8). Future research should test in old rats (i) if CR-related enhancement in Akt activation is required for the CR effect on insulin-stimulated glucose uptake by the soleus and (ii) if the lack of effects of exercise alone or combined exercise and CR on insulin-stimulated glucose uptake were attributable to insufficient soleus recruitment during the exercise protocol.

Supplementary Material

Supplementary material can be found at: http://biomedgerontology.oxfordjournals.org/

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