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AMPK-TBC1D4–Dependent Mechanism for Increasing Insulin Sensitivity of Skeletal Muscle

Diabetes 2015;64:1901–1903 | DOI: 10.2337/db15-0010

Insulin resistance in skeletal muscle, the major site of insulin-stimulated blood glucose clearance, is a primary defect in the development of type 2 diabetes. Accordingly, there is great interest in interventions that improve muscle insulin sensitivity. A single exercise session can substantially enhance muscle insulin sensitivity for up to 24–48 h postexercise (1–11), but full discernment of the specific processes responsible for this health benefit remains elusive. Illuminating these mechanisms may be helpful for designing optimally effective exercise protocols. Furthermore, because of the very limited exercise capacity of many insulin-resistant people, these insights may enable the development of other therapies that improve insulin sensitivity by engaging the exercise-induced mechanisms.

In vivo exercise influences many physiological systems, making it challenging to isolate the specific events that account for improved insulin sensitivity. A strategy to eliminate systemic exercise responses is to electrically stimulate the contraction of isolated rodent skeletal muscles. Using this approach, Gao et al. (5) discovered that ex vivo contractions could induce subsequently greater insulin-stimulated glucose uptake. Although ex vivo contraction is much less complicated than in vivo exercise, it still produces complex effects on calcium, tension, high-energy phosphates, glycogen and lipids, metabolic intermediates, redox potential, reactive oxygen and nitrogen species, and many enzymes.

An even simpler strategy is to incubate an isolated muscle with a chemical that triggers a limited subset of exercise's numerous consequences without causing muscle contraction. AMPK, which is stimulated by exercise/contraction, is also stimulated by AICAR. Fisher et al. (12) found that incubation of isolated rat muscles with AICAR produced a subsequent increase in insulin-stimulated glucose uptake, suggesting that AMPK activation can lead to increased insulin sensitivity in muscle.

Although much is unknown about the mechanisms for improved insulin sensitivity after exercise, elevated phosphorylation of the Rab-GTPase protein known as TBC1D4 (also called Akt substrate of 160 kDa, AS160) has emerged as an attractive candidate to participate in the persistent increase in insulin-stimulated glucose uptake. Enhanced TBC1D4 phosphorylation, which is crucial for insulin-stimulated GLUT4 translocation (13), tracks closely with the increased glucose uptake by insulin-stimulated muscle during the hours after acute exercise (7–11,14–16). If prior AICAR treatment is found to also produce greater TBC1D4 phosphorylation in insulin-stimulated muscles, it would suggest that AICAR and exercise may share a common mechanism to improve insulin sensitivity.

In the current issue of *Diabetes*, Kjøbsted et al. (17) aim to determine if AMPK activation is essential for the AICAR-induced increase in muscle insulin sensitivity. Isolated extensor digitorum longus (EDL) and soleus muscles from mice were studied 4–6 h after 50 min of AICAR incubation. Prior AICAR exposure caused greater insulin-stimulated glucose uptake in the EDL, but not the soleus. To learn if AMPK activation was required for improved insulin sensitivity, additional experiments used EDL muscles from three different genetically modified mouse models in which muscle AMPK activity was greatly reduced. Prior AICAR exposure led to greater insulin-stimulated glucose uptake in muscles from wild-type mice with normal AMPK activity, but not in AMPK-deficient muscles. Furthermore, for insulin-stimulated muscles from wild-type mice, but not insulin-stimulated muscles from AMPK-deficient mice, prior AICAR stimulation led to greater TBC1D4 phosphorylation on Thr^{649} and Ser^{711}. In tibialis anterior muscles expressing TBC1D4 that was mutated so that it could not be phosphorylated on Ser^{711}, there was an attenuated insulin effect on TBC1D4 Thr^{649} phosphorylation. Given that AMPK can phosphorylate TBC1D4 on Ser^{711} (18) and that Thr^{649} phosphorylation is crucial

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See accompanying article, p. 2042.

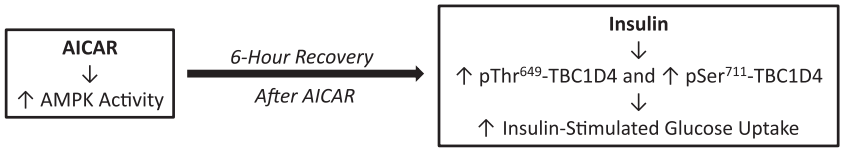

Figure 1—AMPK activity was increased in isolated muscles after brief (50 min) AICAR incubation, and AMPK was previously shown to phosphorylate TBC1D4 on Ser^{711}. Six hours after AICAR treatment, insulin produced greater TBC1D4 Ser^{711} and Thr^{649} phosphorylation ($pSer^{711}$-TBC1D4 and $pThr^{649}$-TBC1D4, respectively) along with increased glucose uptake compared with insulin-stimulated muscles without AICAR pretreatment. Muscles expressing TBC1D4 that was mutated to prevent phosphorylation on Ser^{711} had attenuated insulin-stimulated $pThr^{649}$-TBC1D4 (a site known to be crucial for insulin-stimulated glucose uptake). Prior effects of AICAR on TBC1D4 phosphorylation and insulin-stimulated glucose uptake were absent in genetically modified muscles that were AMPK deficient. The working hypothesis is that AICAR activation of AMPK leads to greater $pSer^{711}$-TBC1D4, which facilitates elevated $pThr^{649}$-TBC1D4 in subsequently insulin-stimulated muscles, leading to greater insulin-mediated glucose uptake.

for TBC1D4 regulation of insulin-stimulated glucose uptake (13,19), the authors speculate that the ability of AICAR to elevate TBC1D4 Ser^{711} phosphorylation may play a role in AICAR's AMPK-dependent effect on insulin sensitivity (Fig. 1).

The key and novel aspect of the experimental design of the current study was the use of muscles with normal AMPK activity and muscles that were AMPK deficient. In addition, the effect of AICAR on the activity of multiple AMPK isoforms was examined, and the greatest response was found for the $\alpha2\beta2\gamma3$ heterotrimer. Furthermore, prior AICAR exposure led to greater insulin-stimulated glucose uptake concomitant with elevated TBC1D4 phosphorylation on Thr^{649} and Ser^{711}. The results for TBC1D4 phosphorylation and insulin-stimulated glucose uptake were reminiscent of earlier observations after acute exercise (8–11,15).

This study raises new questions that will require further research. First, AICAR treatment activated AMPK without enhancing subsequent insulin sensitivity in the soleus. This observation differs from the results of earlier research in which prior exercise led to enhanced insulin-stimulated glucose uptake in the soleus of either mice or rats (11,20). AICAR is a useful, but complementary, tool rather than a substitute for directly studying exercise. It would now be valuable to use AMPK-deficient models to test if normal AMPK activation is essential for the postexercise effect on insulin-stimulated glucose uptake by multiple muscles, including the soleus. Second, insulin-stimulated TBC1D4 Thr^{649} phosphorylation was attenuated in tibialis anterior muscles expressing TBC1D4 that was mutated to prevent Ser^{711} phosphorylation, but insulin-stimulated glucose uptake was not assessed, nor was the effect of AICAR pretreatment evaluated. Furthermore, the tibialis anterior was stimulated with insulin in vivo, in contrast to the isolated EDL that was used for other experiments. Future research should determine if mutation of TBC1D4 Ser^{711} eliminates the effects of AICAR or exercise on insulin-stimulated glucose uptake. Last, prior exercise leads to greater insulin-stimulated glucose uptake concomitant with elevated TBC1D4 phosphorylation in insulin-resistant muscle (10,15). Does prior AICAR treatment also elevate TBC1D4 phosphorylation and insulin sensitivity in insulin-resistant muscle?

Kjøbsted et al. (17) provide compelling evidence that AMPK activation is essential for AICAR-induced elevation of muscle insulin sensitivity. The effects of prior AICAR exposure on TBC1D4 phosphorylation and glucose uptake in insulin-stimulated muscle were reminiscent of the effects of in vivo exercise on these outcomes. Together with earlier research demonstrating that acute exercise activates AMPK and leads to a subsequent increase in insulin sensitivity, the current observations provide the basis for the working hypothesis that the exercise-induced increase in insulin sensitivity is regulated by an AMPK-TBC1D4 signaling mechanism.

Funding. G.D.C. was supported by a grant from the National Institutes of Health (R01-DK-071771).

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

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