Commentary

MOLECULAR METABOLISM

AMPK is required for exercise to enhance insulin sensitivity in skeletal muscles

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The signaling mechanisms by which exercise improves muscle insulin sensitivity seem even harder to solve than to get people to exercise. Exercise (muscle contraction) has two diverse effects on muscle glucose metabolism. Firstly, acute exercise stimulates glucose uptake in skeletal muscles via translocation of GLUT4 translocation. This effect is insulin independent, and glucose uptake remains elevated a couple of hours after termination of exercise. Secondly, exercise increases insulin sensitivity in skeletal muscles. This latter effect remains for many hours after cessation of exercise, and is obviously insulin dependent. Indeed, candidates have been abundant for both effects of exercise, but convincing signalling mechanisms have not emerged [1]. From a health perspective, the improved muscle insulin sensitivity in the period after exercise improves metabolic regulation. The observation that muscle contraction increases insulin action in prior active muscle was reported in 1982 by Richter et al. [2]. Until now, the most important finding on the mechanisms governing insulin action after exercise is the reports from Holloszy's laboratory in the 1980s describing that the glycogen content in muscles determines insulin sensitivity after exercise. These studies showed that carbohydrate feeding reduced insulin sensitivity in muscles, whereas insulin sensitivity remained elevated when muscle glycogen content was kept low [3,4]. Indeed, glycogen has kept its central position in regulation of insulin action and capacity to store glucose in muscles [5,6], but little progress has occurred on the mechanisms for elevated muscle insulin sensitivity after exercise. It was obvious to look for enhanced activation of the insulin signaling pathway, but this research has been discouraging. In fact, we found that exercise reduced insulin-stimulated IRS-1 associated PI-3 kinase activity [7]. Other studies, including studies of man, have also found that increased insulin-stimulated glucose uptake following exercise is not associated with enhanced activation of the proximal insulin signalling pathway [8]. The limited progress in our understanding of the mechanisms regulating insulin sensitivity may result from most researchers' focus on enhanced activation of insulin signaling rather than other signaling mechanisms.

In a ground-breaking paper published in *Diabetes*, Kjøbsted et al. link AMPK to improved insulin sensitivity after exercise [9]. Professor Jørgen Wojtaszewski's group shows that AMPK activity is required for muscle contraction to increase insulin sensitivity as deletion of the two catalytic subunits (α_1 and α_2) prevented the ability of exercise to increase insulin sensitivity. Kjøbsted et al. also highlight another well-known problem in the AMPK field; the Thr^{172} phosphorylation of AMPKα is not a sensitive method to judge AMPK activation. So, despite no detectable increase in AMPK Thr^{172} phosphorylation, activity of the AMPKγ3 complex was elevated 3 h after muscle contraction concomitant with elevated insulin sensitivity. This is a significant finding, and measurements of activity in various AMPK complexes may provide important information about regulation AMPK in the future.

AMPK has long been a "dark horse" in regulation of glucose metabolism. Initially, great enthusiasm occurred because AICAR, an activator of AMPK, increased glucose uptake in skeletal muscles. However, as various AMPK-deficient models occurred, it became clear that AMPK was not necessary for exercise to stimulate glucose uptake. However, several gain of function mutations in the AMPK subunits cause accumulation of glycogen in muscle and heart [10,11], but the mechanisms are not understood. Wojtaszewski's group showed last year that prior AICAR stimulation increases muscle insulin sensitivity in an AMPK-dependent manner [12]. The fact that activation of AMPK increases insulin sensitivity raises the possibility that the elevated glycogen content in muscles with AMPK gain of function mutations results from increased insulin sensitivity. This agrees with the requirement of AMPK for exercise to increase insulin sensitivity

But where to go from AMPK to elevated insulin sensitivity? TBC1D4 is an obvious candidate as both insulin and exercise stimulates TBC1D4 phosphorylation [13], and elevated TBC1D4 phosphorylation has been link to improved insulin action [14]. Kjøbsted et al. reported elevated insulin-stimulated TBC1D4 phosphorylation in exercised muscles and suggest this as the mechanism for elevated insulin sensitivity [9]. This explanation seems likely as truncated mutation in TBC1D4 is linked to insulin resistance in the Greenlandic population [15]. But how can insulin-stimulated TBC1D4 phosphorylation be elevated after exercise when activation of the proximal insulin signalling pathway was unchanged? Kjøbsted et al. suggest that the elevated AMPKγ3 activity 3 h after contraction primes a pool of TBC1D4 for phosphorylation by PKB during insulin stimulation [9]. Future research is required to investigate this possibility, but the elevated activity of the AMPKγ3 several hours after cessation of exercise raises unexpected research aspects on the mechanism regulating insulin sensitivity.

So what now? Is there a link between the important findings by the groups of Holloszy and Wojtaszewski more than 25 years apart? AMPK is normally considered a stress sensor aiming to restore energy homeostasis by promoting catabolic and inhibiting anabolic

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pathways [16]. However, the AMPKβ subunits have a glycogen binding domain, and it is well documented that AMPK activation after muscle contraction is much higher when glycogen is low [17]. Does AMPK function as a glycogen sensor? This could also explain why insulin action is elevated when glycogen content is low.

Do we need to change our view of AMPK? From an evolutionary perspective, glycogen synthesis may be necessary to restore energy homeostasis — with help from AMPK. Indeed, glycogen degradation contributes to ATP synthesis, but glycogen degradation may be considered a disturbed energy homeostasis, and muscle glycogen must be replenished to optimize success during upcoming "flight-or-fight" situations. In support of this idea, muscle glycogen (in humans) is maintained during 72 h fasting [18]. Thus, increased muscle insulin sensitivity after exercise may be a question of survival (directing glucose to muscle glycogen) rather than a question of metabolic health. Still, we may be able to use this knowledge in promoting exercise and developing treatments.

Is the mechanism by which exercise improves muscle insulin sensitivity finally to be solved? Indeed, there are still questions to be addressed. It will be important to clarify if the same mechanism is responsible for improving insulin sensitivity after exercise in insulin resistant muscles or whether AMPK independent mechanisms mediate the effect in insulin resistant muscles. Furthermore, it will be important to investigate if AMPK is required to increase insulin sensitivity after all types of exercise.

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