Exercise Effects on Muscle Insulin Signaling and Action
Invited Review: Intracellular signaling in contracting skeletal muscle

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Sakamoto, Kei, and Laurie J. Goodyear. Invited Review: Intracellular signaling in contracting skeletal muscle. J Appl Physiol 93: 369–383, 2002; 10.1152/japplphysiol.00167.2002.—Physical exercise is a significant stimulus for the regulation of multiple metabolic and transcriptional processes in skeletal muscle. For example, exercise increases skeletal muscle glucose uptake, and, after exercise, there are increases in the rates of both glucose uptake and glycogen synthesis. A single bout of exercise can also induce transient changes in skeletal muscle gene transcription and can alter rates of protein metabolism, both of which may be mechanisms for chronic adaptations to repeated bouts of exercise. A central issue in exercise biology is to elucidate the underlying molecular signaling mechanisms that regulate these important metabolic and transcriptional events in skeletal muscle. In this review, we summarize research from the past several years that has demonstrated that physical exercise can regulate multiple intracellular signaling cascades in skeletal muscle. It is now well established that physical exercise or muscle contractile activity can activate three of the mitogen-activated protein kinase signaling pathways, including the extracellular signal-regulated kinase 1 and 2, the c-Jun NH2-terminal kinase, and the p38. Exercise can also robustly increase activity of the AMP-activated protein kinase, as well as several additional molecules, including glycogen synthase kinase 3, Akt, and the p70 S6 kinase. A fundamental goal of signaling research is to determine the biological consequences of exercise-induced signaling through these molecules, and this review also provides an update of progress in this area.

exercise; insulin signaling; mitogen-activated protein kinase; AMP-activated protein kinase; phosphatidylinositol 3-kinase

A SINGLE BOUT OF EXERCISE can increase skeletal muscle glucose uptake and metabolism and can also have profound effects on glycogen metabolism with increased rates of glycogenolysis during exercise, followed by a rapid resynthesis of glycogen in the postexercise state (12). These metabolic changes can result in improved glucose homeostasis in individuals with insulin-resistant diseases such as Type 2 diabetes and may also be responsible for the ability of exercise to prevent or delay the onset of Type 2 diabetes. Other benefits of regular physical exercise result from the structural remodeling of the skeletal muscle tissue. These changes are caused by alterations in the expression of critical muscle proteins, due to changes in gene transcription (96) and alterations in protein synthesis in skeletal muscle in the postexercise period (16).

A central issue in understanding these biological effects of exercise is elucidation of the intracellular signaling mechanisms that enable muscle cells to decipher and respond to the contractile stimulus. During the past decade, there has been a tremendous effort to define intracellular signaling mechanisms in a wide range of cell types. However, until recently, little of
this information has been incorporated into understanding the molecular basis for the clinically important adaptations that occur in skeletal muscle in response to exercise. The overall purpose of this highlighted topic series is to explore the interaction of exercise and insulin signaling in skeletal muscle. Here, we will begin the series by providing an update of the literature pertaining to the effects of exercise on muscle cell signaling mechanisms, independent of insulin. Most of this minireview will focus on the discoveries of the past several years demonstrating that exercise can signal through the mitogen-activated protein kinase (MAP kinase) signaling cascades and regulate activity of the AMP-activated protein kinase (AMP kinase). We will also briefly discuss evidence for contraction signaling via several other established signaling molecules in adult skeletal muscle. This review will not focus on Ca²⁺-regulated signaling in skeletal muscle [e.g., protein kinase C (PKC), calcineurin]; instead, the reader is referred to two recent reviews on this topic (9, 98).

**PUTATIVE MESSENGERS FOR THE EXERCISE RESPONSE**

Elucidating the specific molecular mechanisms that result in exercise-induced cellular responses has been complicated by the fact that during contraction the muscle fibers are exposed to numerous metabolic and mechanical stimuli. Changes in intracellular pH, a shift in the ATP-to-ADP ratio, and changes in the intracellular concentration of Ca²⁺ and other metabolites could act as second messengers for the regulation of cellular functions with exercise. In the case of Ca²⁺, this molecule is involved in the regulation of numerous intracellular proteins including calmodulin kinase, PKC, and calcineurin, important intermediaries in cellular signal transduction. The generation of mechanical forces by contraction is another mechanism for activating cellular responses. For example, the extracellular matrix, which carries the external mechanical force of muscle contraction to the cell surface, has been shown to interact with cytoskeletal integrins, resulting in the activation of stretch-dependent signaling pathways (88, 115).

The effects of exercise to regulate skeletal muscle growth and metabolism could also be mediated through receptor-linked signaling pathways. Exercise can cause potent systemic responses (e.g., hormones, catecholamines), which in turn could result in the activation of specific cell surface receptors. A good example of this type of mechanism is the epinephrine-mediated stimulation of β-adrenergic receptors that leads to the activation of cAMP and protein kinase A. The neurotransmitters calcitonin gene-related peptide, ciliary neurotrophic factor, and neuregulin are examples of extracellular messengers that are released by the motor nerve and may act to stimulate intracellular signaling pathways (125).

Muscle contractile activity may also activate receptor-mediated cell signaling pathways through autocrine and/or paracrine mechanisms. Studies in cultured cells have suggested that stretch can activate intracellular signaling pathways by releasing growth factors in an autocrine fashion, which in turn stimulates their specific receptors and subsequently second messenger systems (90, 106, 109, 133). For example, increased cell signaling after stretching of cardiac myocytes in culture is mediated in part by angiotensin II that is secreted from the stretched myocytes (109, 154, 155). Insulin-like growth factor I and fibroblast growth factor may provide an autocrine mechanism for the activation of signaling pathways by exercise in skeletal muscle, since these factors are known to be increased in mechanically stimulated skeletal muscle (35, 90) and have been shown to be potent stimulators of tyrosine kinase receptor-mediated signaling in cultured cells (45). Nitric oxide is another molecule that may act as a messenger in skeletal muscle. Nitric oxide is released in skeletal muscle contracted in vitro, and inhibition of nitric oxide synthase has been demonstrated to decrease basal rates of muscle glucose transport (8), an effect that may be regulated by a cGMP-mediated mechanism (7). Thus exercise is a complex stimulus that can regulate numerous mechanical and metabolic factors that have the potential to regulate multiple intracellular signal transduction systems within skeletal muscle.

**MAP KINASE SIGNALING**

A growing body of evidence suggests that the MAP kinase signal transduction pathways play an important role in exercise signaling. The MAP kinase family of intracellular signaling cascades are expressed in all eukaryotic cells and include the extracellular signal-regulated kinase 1 and 2 (ERK1/2), the c-Jun NH₂-terminal kinase (JNK), p38, and the extracellular signal-regulated kinase 5 (ERK5 or big MAP kinase, BMK1). These pathways are stimulated by a wide variety of environmental stressors and growth factors (40, 74) and have been implicated in a large number of physiological processes, including cell proliferation, differentiation, hypertrophy, inflammation, apoptosis, carbohydrate metabolism, and gene transcription (40, 74, 128). Thus MAP kinase proteins exert a profound effect on cellular function.

Signaling within the MAP kinase pathways involves the sequential phosphorylation and activation of a MAP kinase kinase kinase (MAPKKK), a MAP kinase (MAPKK), and a MAP kinase (Fig. 1). The MAPKKKs are regulated by membrane recruitment, oligomerization, and phosphorylation. The MAPKKs are dual-specificity kinases, functioning to phosphorylate the MAP kinase proteins on a conserved Thr-X-Tyr motif in the activation loop of the kinase subdomain VIII.

The ERK1/2 cascade, the first MAP kinase signaling pathway to be characterized in mammalian cells, is activated primarily by growth factors (40), including insulin (124), epidermal growth factor (89), nerve growth factor (153), and other factors that promote cellular differentiation and growth. Upstream signaling to ERK1/2 involves several potential MAPKKK

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molecules, including Raf, the most well-defined MAPKKK for ERK1/2. After activation by a small GTP binding protein, Raf can phosphorylate and activate MEK1 and/or MEK2 (MAPKKks), which leads to the phosphorylation and activation of ERK1 and/or ERK2. Activated ERKs can then phosphorylate downstream kinases in the cytoplasm such as the p90 ribosomal S6 kinase 2 (RSK2) and/or the mitogen and stress-activated kinase 1/2 (MSK1/2). ERK1 and ERK2 can also translocate to the nucleus, where they can phosphorylate a variety of transcription factors.

The JNK signaling cascade can also be activated by growth factors; however, this cascade is activated most robustly by environmental stressors, including ultraviolet light (34), proinflammatory cytokines (75, 119), osmotic shock (43), and stretch (51, 81). MAP kinase kinase 4 and MAP kinase kinase 7 (MKK4/7) are known upstream kinases of JNK. Three JNK isoforms (JNK1, JNK2, and JNK3) have been identified, and each isoform has multiple splicing variants (30). The p38 signaling pathway is stimulated by many of the same stressors as the JNK cascade, such as proinflammatory cytokines (52, 102) and osmotic shock (52). The p38 cascade consists of a distinct series of proteins, including a variety of MAPKKKs, MAP kinase kinase 3 (MKK3) (34) and MAP kinase kinase 6 (MKK6) (53), and four isoforms of p38 (α, β, γ, and δ) (52, 68, 69, 86). ERK5 has been reported to be activated by serum, oxidative stress, and hyperosmolarity (74), and MEK5 has been identified as a selective upstream kinase of ERK5 (70). In contrast to the ERK, JNK, and p38 signaling cascades, much less information exists regarding the molecules that comprise the ERK5 signaling pathway (74).

Effects of Exercise on MAP Kinase Signaling

In 1996, it was first reported that exercise activates ERK1/2, JNK, and p38 signaling in skeletal muscle (46). In recent years, there has been intense interest in the regulation of these pathways in skeletal muscle. Much of this work has focused on exercise and muscle contraction regulation of the MAP kinases, and in this section we present a brief summary of these studies.

**ERK1/2 signaling.** Activation of ERK1/2 signaling has been reported in rat skeletal muscle with treadmill running (46, 95), in vitro contraction (57, 107, 146, 150, 151), and stretch (19); in mouse muscle in response to treadmill running (36); and in human skeletal muscle in response to cycle ergometer exercise (3, 99, 138) and marathon running (159). Upstream of ERK1/2, both MEK1/2 (3, 99, 117) and Raf1 (2, 3, 117) activities are increased by exercise and contraction. Molecules downstream of ERK1/2 that have been shown to be activated by exercise include RSK2 (3, 46, 72, 99, 107, 117, 159) and MSK1/2 (107, 159). MEK1/2 activation is necessary for ERK1/2 activation because the MEK1/2 inhibitor PD-98059 inhibits muscle contraction-induced ERK1/2 phosphorylation (57, 107, 146) and its downstream substrates RSK2 (57) and MSK1 (107).

With one-legged cycling exercise in human subjects, activation of ERK1/2 (3, 138) and its downstream targets (3, 72) is observed in the muscle from the exercised leg but not in the resting leg. These data suggest that stimulation of ERK1/2 signaling in response to exercise in skeletal muscle is due to primarily a local, tissue-specific phenomenon, rather than a systemic effect. The molecules involved in this tissue-specific stimulation are still elusive. What is known is that neurotransmitter release from motor nerve neurons (57) and conventional PKCs (57, 107) are not likely mechanisms because inhibitors to these molecules do not diminish contraction-stimulated ERK1/2 activation. Moreover, She and Grb2 phosphorylation and increases in Sbc-Grb2 and insulin receptor substrate (IRS)-Grb2 association are not seen with in situ muscle contraction, indicating that the classical receptor tyrosine kinase pathway can be eliminated as an upstream signal regulating not only the ERK1/2 pathway but also JNK and p38 signaling (57).

**JNK signaling.** Activation of JNK signaling has been reported in rat skeletal muscle in response to in vitro contractions (19), in situ contractions (2, 83), treadmill running exercise (46), muscle overload (23), and me-
mechanical stretch (19). In human subjects, JNK is activated in response to cycle ergometer exercise (1), knee extensions resulting in concentric and eccentric contractions of the quadriceps muscles (17), and marathon running (18). Upstream of JNK, exercise increases MEKK1 (2) and MKK4 (2, 17, 18, 138) in skeletal muscle. In contrast to ERK1/2 signaling, activation of the JNK cascade is sustained during in situ muscle contractions, whereas the activation of the ERK cascade is more rapid and transient. This suggests that the upstream proteins that regulate the JNK signaling cascade are distinct from that of ERK1/2 (2).

p38 signaling. Activation of p38 has been reported in rat skeletal muscle in response to treadmill running (46, 95), in vitro contractions (19, 107, 120, 150, 151), in situ contractions (95), muscle overload (23), and mechanical stretch (19). It has also been shown that p38 signaling is increased in humans during cycle ergometer exercise (138) and marathon running (18, 159). Phosphorylation of MAP kinase-activated protein kinase (MAPKAPK-2), a downstream substrate for ERK1/2, increased in response to exercise (1) and contraction (146) of mouse skeletal muscle, suggesting that JNK is not involved in the regulation of contraction-stimulated glucose uptake; in a subsequent study, which utilized a hindlimb perfusion system, contraction-stimulated glucose uptake was not inhibited in the presence of PD-98059 in red and white rat gastrocnemius muscles (146). Similarly, insulin-stimulated glucose uptake in cultured adipocytes (56, 129), isolated skeletal muscles (57), and hindlimb skeletal muscles in situ (146) is not attenuated in the presence of PD-98059. Thus ERK1/2 signaling is not necessary for the regulation of either contraction- or insulin-stimulated glucose uptake in skeletal muscle.

The ERK1/2 signaling cascade has also been proposed to regulate glycogen synthase activity. This hypothesis was based on the finding that RSK2 could phosphorylate and inactivate glycogen synthase kinase-3 (GSK3) and phosphorylate and activate the glycogen-bound form of type 1 protein phosphatase (PP1G) in vitro, two reported regulators of insulin-stimulated glycogen synthase activity (32, 126). However, for contraction-stimulated regulation of glycogen metabolism, glycogen synthase activity was not attenuated in the presence of PD-98059 in isolated rat muscles (57). Furthermore, PD-98059 did not decrease glycogen synthase activity in response to in situ muscle contraction (146). Similarly both pharmacological inhibition of ERK signaling and studies with RSK2 knock-out mice demonstrated that ERK1/2 signaling is not necessary for insulin-stimulated glycogen synthase activity (36, 76). These results demonstrate that ERK1/2 signaling is not part of the mechanism for the regulation of contraction- or insulin-stimulated glucose uptake and glycogen synthase activation in skeletal muscle.

There have been few studies examining the effects of JNK activation in the regulation of carbohydrate metabolism in skeletal muscle. One study demonstrated that activation of JNK by anisomycin, a protein synthesis inhibitor, mimics insulin’s action to stimulate glycogen synthesis in mouse skeletal muscle in vivo (91). On the basis of their findings, this group concluded that JNK stimulates glycogen synthase activity through the regulation of RSK3 and GSK3. Because exercise and contraction robustly activate JNK activity, JNK may be involved in the regulation of contraction-stimulated glycogen synthase activity. In preliminary studies, overexpression of wild-type JNK1 in skeletal muscle in vivo by DNA injection followed by electroporation dramatically increased basal and contraction-stimulated JNK activity. However, this increase in JNK activity did not enhance basal and contraction-stimulated glycogen synthase activity in mouse skeletal muscle, suggesting that JNK is not involved in the regulation of contraction-stimulated glycogen synthase activity (41). Whether JNK is involved in glucose transport regulation in muscle is not known. Recently, selective cell-permeable inhibitors of JNK signaling have been developed (15), and these may be important tools in future studies aimed at elucidating the physiological function of JNK in contracting skeletal muscle.
Recent studies have provided evidence that p38 is involved in the regulation of contraction-stimulated glucose uptake in skeletal muscle. Glucose uptake and p38 activity were increased in isolated extensor digitorum longus (EDL) muscles with contraction in vitro (120), and the p38 antagonist SB-203580 abolished the activation of p38 and reduced contraction-stimulated glucose uptake by 40–50%. However, inhibition of p38 was observed when SB-203580 was added directly to the kinase assay, and it is still unclear whether the attenuation of glucose uptake was due to inhibition of contraction-stimulated p38 activity or a direct effect of the compound on glucose uptake (120). Recently, p38 has been implicated in the regulation of glucose transport (152) (see also subsequent section), whereas the involvement of p38 signaling in glycogen metabolism has not been investigated.

**Gene regulation.** Regulation of transcriptional events is an established function of MAP kinase signaling, although little of this work has been directed toward the study of adult skeletal muscle. With the use of various cell systems, activated MAP kinases have been shown to translocate to the nucleus and phosphorylate numerous transcription factors such as cAMP-dependent response element-binding protein, Elk-1, activating transcription factor 2, c-Jun, c-Myc, myocyte enhancer binding factor 2C, and CCAAT/enhancer-binding protein (C/EBP) homologous protein (61, 74) (Fig. 2). Not only can MAP kinases regulate gene transcription by direct interaction with transcription factors, they can also activate other downstream substrates such as RSK2, MAPKAPK-2/3, and MSK1/2 that can translocate to the nucleus and phosphorylate numerous transcription factors.

Physical activity level has a profound impact on gene expression in skeletal muscle. On the basis of the fact that exercise stimulates multiple MAP kinase signaling pathways, these signaling proteins have been postulated to play a critical role in the transcriptional regulation of muscle genes in response to exercise (139). For example, activation of ERK1/2 and JNK by exercise and contraction is associated with the rapid induction of immediate early genes such as c-fos (117) and c-jun (1, 2). These data are only correlative in nature, and at this time there is no direct evidence linking MAP kinases and gene transcription in contracting skeletal muscle.

**AMP KINASE SIGNALING**

Studies implicating AMP kinase as a critical signaling molecule for the regulation of multiple metabolic and growth processes in contracting skeletal muscle has been an exciting advance in the field of skeletal muscle biology (Fig. 3). AMP kinase is a member of a metabolite-sensing protein kinase family that acts as a fuel gauge monitoring cellular energy levels and is the mammalian homolog of the SNF-1 protein kinase in yeast, which is critical for the adaptation of yeast to nutrient stress (54). When AMP kinase “senses” decreased energy storage, it acts to switch off ATP-consuming pathways and switch on alternative pathways for ATP regeneration. AMP kinase is a heterotrimeric protein consisting of one catalytic subunit (α) and two noncatalytic subunits (β, γ) (54). The noncatalytic subunits are essential for optimum enzyme activity and may participate in substrate targeting. Several isoforms for each subunit have been identified, and the contribution of each isoform to the AMP kinase heterotrimer varies in different tissues. The α1- and α2-isoforms share 90% amino acid sequence identity for the catalytic core but only 60% identity outside the catalytic core (121). There is some evidence for differences in subcellular localization and substrate specificity between α1- and α2-complexes, implying that the

![Fig. 2. Contraction effects on MAP kinase signaling pathways in skeletal muscle. Physical exercise and muscle contraction activate the c-Jun NH2-terminal kinase (JNK), extracellular signal-regulated kinase (ERK), and p38 signaling cascades in rodent and human skeletal muscle. The mechanism(s) that leads to activation of MAP kinase signaling with exercise could involve mechanical, systemic, autocrine, or paracrine factors, as well as a change in the energy status of the contracting muscle fibers. Established and putative cytosolic and nuclear substrates of MAP kinases in skeletal muscle are shown. RSK, 90-kDa ribosomal S6 kinase; MSK, mitogen and stress-activated protein kinase; MNK, MAP kinase-interacting kinase; ATF-2, activating transcription factor 2; CHOP, C/EBP homologous protein; CREB, cAMP-dependent response element-binding protein; MEF2, myocyte enhancer-binding factor 2.](image-url)
two catalytic isoforms may have distinct functions (114).

AMP kinase is rapidly activated in tissues and cells under several conditions, including ischemia, hypoxia, inhibition of glycolysis, uncouplers of oxidative phosphorylation, and heat shock (54). AMP kinase is activated by an increase in the AMP-to-ATP and creatine-to-phosphocreatine ratios via a complex mechanism that involves allosteric modification, phosphorylation by an AMP kinase kinase, and decreases in phosphatase activities. Substrates for AMP kinase in vitro or in vivo in various tissues include acetyl-CoA carboxylase (ACC), 3-hydroxy-3-methylglutaryl-CoA reductase, glycerophosphate acyltransferase, endothelial nitric oxide synthase, 6-phosphofructo-2-kinase, hormone-sensitive lipase, and IRS-1 (55, 67).

**Muscle Contraction and Physical Exercise Increase AMP Kinase Activity**

Contractile activity alters the fuel status of skeletal muscle, and depending on the intensity of the contractions there can be significant decreases in both phosphocreatine and ATP concentrations. Thus exercise is a likely physiological stimulus to elicit AMP kinase activation in working skeletal muscles. In the rat, exercise in vivo (103, 104), sciatic nerve-stimulated muscle contractions in situ (65, 133, 135), and contraction of isolated muscles in vitro in the absence of systemic factors (58, 59, 66) all significantly increase AMP kinase activity. The greater the force production generated by contraction (66), or the greater the intensity of treadmill running exercise (104), the greater the activation of AMP kinase. In humans, moderate-intensity cycle exercise (42, 122, 147), as well as high-intensity "sprint" exercise (24), will increase skeletal muscle AMP kinase activity and/or phosphorylation. Interestingly, in human subjects with Type 2 diabetes where there is impaired insulin signaling in skeletal muscle, exercise results in normal activation of AMP kinase (93).

**AMP Kinase as a Mediator of Contraction-Stimulated Glucose Transport**

Initial evidence in support of a role for AMP kinase in contraction-stimulated glucose transport came from studies that used 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR). AICAR is a compound that is taken up into skeletal muscle and metabolized by adenosine kinase to form ZMP, the monophosphorylated derivative that mimics the effects of AMP on AMP kinase (59, 85). AICAR infusion enhances insulin-stimulated glucose transport in the perfused rat hindlimb skeletal muscle (85). With the use of the isolated intact rat epitrochlearis muscle, AICAR can stimulate glucose transport in the absence of insulin, similar to the effects of contraction (11, 59). Insulin, muscle contraction and AICAR all robustly increase 3-O-methylglucose transport in rat epitrochlearis muscles incubated in vitro, and, similar to contraction-stimulated transport, AICAR-stimulated transport is not inhibited by wortmannin, the pharmacological inhibitor of phosphatidylinositol 3-kinase (PI3-kinase). Furthermore, the increase in glucose transport with the combination of maximal AICAR plus maximal insulin treatments is partially additive, although there is no additive effect on glucose transport with the combination of AICAR plus contraction (59). Studies with the adenosine receptor antagonist 8-(p-sulfophenyl)-theophylline show that AICAR effects on glucose transport are likely due to activation of AMP kinase and are not mediated through adenosine receptors (11). Short-term infusion of rats with AICAR (and glucose to maintain euglycemia) also increases 2-deoxyglucose transport in multiple muscle types. Hindlimb perfusion studies reveal that AICAR-induced increases in skeletal muscle glucose transport are mediated by the translocation of the GLUT-4 glucose transporter to the plasma membrane (73), comparable to the effects of exercise and muscle contraction on GLUT-4 translocation (60). Although experiments with AICAR as an
AMP kinase activator have generated important information for the function of AMP kinase, there are limitations to this approach, as AICAR does not have strict specificity to AMP kinase (48, 136, 158). Specific activation and inhibition of AMP kinase by pharmacological agents would be a valuable approach to more clearly define the role of AMP kinase in glucose uptake and other metabolic effects, but, unfortunately, such compounds are not currently available. Two putative AMP kinase inhibitors, i.e., idotubercidin and araA, are not specific to AMP kinase, and recent studies show that, although these compounds inhibit AICAR-induced activation of AMP kinase, they fail to inhibit contraction-induced AMP kinase activation in skeletal muscle (94). A very valuable approach to elucidating the functions of skeletal muscle AMP kinase in vivo has been to generate transgenic mice overexpressing an inactive (dominant-negative) AMP kinase protein. Interestingly, glucose transport in response to electrically stimulated contractions of hindlimb muscles was reduced by only 30% in these mice (92). This important study suggests that AMP kinase may only be part of the mechanism leading to contraction-stimulated glucose transport. Dissociation of AMP kinase and glucose transport in contracting muscle was also demonstrated where glycogen-supercompensated rat soleus muscles had normal contraction-stimulated glucose transport in the absence of an increase in total AMP kinase activation (33). These studies suggest that there are additional signaling mechanisms that regulate contraction-stimulated glucose transport in skeletal muscle.

The molecules downstream of AMP kinase leading to the regulation of contraction-stimulated glucose transport are not known. However, a recent report utilizing clone 9 cells has provided evidence that p38 is downstream of AMP kinase in the regulation of glucose transport. AICAR-stimulated glucose transport is inhibited in the presence of the p38 inhibitor compound SB-203580 or when a dominant-negative p38 protein is overexpressed (152). Together, these studies, along with work showing that exercise increases p38 activity, raise the possibility that p38 may be a downstream component of AMP kinase signaling in exercise-stimulated glucose uptake in skeletal muscle. At this time, no study has directly assessed this hypothesis.

It is well established that insulin sensitivity for glucose transport is enhanced after exercise in skeletal muscle. The molecular mechanisms responsible for this enhanced glucose uptake in the postexercise period has been the focus of numerous studies but has remained elusive. Recently, contraction, AICAR, and hypoxia were shown to enhance insulin-stimulated glucose transport in isolated epitrochlearis muscles 3.5 h after stimulation (39). Because all three of these stimuli activate AMP kinase, this study suggests that the AMP kinase may be a key mechanism involved in the initiation of the signaling process that results in enhanced insulin sensitivity after exercise. Much more work on the role of AMP kinase in contraction-stimulated glucose transport and insulin sensitivity will be necessary to understand the precise role of this important signaling molecule in skeletal muscle.

**AMP Kinase and Glycogen Metabolism**

Although the involvement of AMP kinase in the regulation of glucose uptake is now well established, the role of AMP kinase in glycogen metabolism is less well understood. In vitro, AMP kinase can phosphorylate proteins involved in glycogen metabolism including Ser7 on glycogen synthase in vitro (22), which would be expected to inhibit glycogen synthesis (118), and phosphorylase kinase (22), the immediate upstream effector of glycogen phosphorylase. On the basis of these studies, a putative role for AMP kinase in contracting muscle would be to promote glycogen degradation and inhibit glycogen synthesis. This is a logical hypothesis because in many cell systems AMP kinase accelerates fuel catabolism to buffer cellular ATP levels. Consistent with this concept is a study showing that a single amino acid mutation in the γ3-subunit of AMP kinase of Hampshire pigs results in reduced AMP kinase activity and that this mutation is associated with increased glycogen content in skeletal muscle (87). Furthermore, perfusion of hindlimb muscles with AICAR leads to phosphorylation and deactivation of glycogen synthase in rat soleus and red and white gastrocnemius muscles, which was correlated with the degree of AMP kinase α2 activity (145).

On the other hand, there is also compelling evidence that AMP kinase functions to increase glycogen synthesis, since chronic AICAR treatment for 5–28 days increases glycogen content in red, slow-twitch and white, fast-twitch quadriceps and gastrocnemius muscles (21, 142). Administration of AICAR to rats in vivo increases AMP kinase α2 activity in both red and white gastrocnemius muscles. Surprisingly, glycogen synthase activity increases in red gastrocnemius muscle and decreases in white gastrocnemius muscles (4). To determine whether AICAR has direct effects on glycogen metabolism, isolated fast-twitch and slow-twitch muscles were incubated with AICAR. Under these conditions, AICAR treatment had no effect on either glycogen synthase or glycogen phosphorylase in both muscle types (4). These results demonstrate that AMP kinase does not directly regulate glycogen synthase or glycogen phosphorylase in skeletal muscle. Consistent with these finding, an investigation with four different phosphorylase kinase substrates demonstrates that this protein is not a substrate of AMP kinase in vitro (13). Thus, because chronic AICAR treatment causes numerous metabolic responses as well as increased GLUT-4 content in skeletal muscle (142), the increased glycogen content observed in the studies mentioned above may be due to the effects of AICAR on metabolism rather than direct regulation of glycogen synthase or glycogen phosphorylase.
AMP Kinase as a Mediator of Contraction-Stimulated Fatty Acid Oxidation

The initial studies showing AMP kinase activation in skeletal muscle provided the first evidence that AMP kinase plays an important role in the regulation of fatty acid oxidation during exercise (65, 135, 140). This occurs through AMP kinase phosphorylation of the β isoform of ACC, resulting in ACC inactivation, a fall in malonyl-CoA content, and a subsequent increase in fatty acid oxidation after relieving inhibition of carnitine palmitoyltransferase I (65, 135, 140). Recent studies have shown that AMP kinase activation with physical exercise is associated with ACC phosphorylation (25, 122) and deactivation (31). Although a reduction of ACC activity would be expected to reduce the rate of malonyl-CoA synthesis during muscle contraction, there is another key molecule that can also decrease malonyl-CoA content. Malonyl-CoA decarboxylase (MCD) is thought to catalyze the major pathway for degradation of malonyl-CoA in muscle. Involvement of AMP kinase in the regulation of MCD activation has been suggested by studies demonstrating that in situ muscle contraction (111), treadmill exercise (110), and AICAR treatment in isolated EDL muscle in vitro (111) increased MCD activity. On the other hand, another group has reported that AMP kinase fails to alter purified hepatic MCD activity in vitro (37), and, consistent with this finding, contraction and AICAR treatment of isolated EDL muscles did not increase MCD activity (49). The reason for the differing results is not clear. A detailed discussion of the role of AMP kinase in the regulation of fatty acid oxidation in skeletal muscle can be found in two recent reviews (105, 141).

AMP Kinase and Gene Regulation

Studies of SNF-1, the AMP kinase homologue in yeast, have provided evidence that AMP kinase plays an important role in the regulation of gene transcription (54). In INS-1 cells, α2 is located in the cytosol and the nucleus, and it has been speculated that α2 contributes to transcriptional regulation (114). In BHK-21 cells, AMP kinase phosphorylates p300, a transcriptional coactivator that mediates the activity of many nuclear receptors including the peroxisome proliferator-activated receptors (156). Although the physiological role of this phosphorylation is p300 on Ser89 remains unknown, this indicates the direct involvement of AMP kinase in transcriptional regulation.

In support of the concept that AMP kinase is involved in gene regulation in adult skeletal muscle, chronic administration of AICAR via daily injection for 5–28 days significantly increases the expression of GLUT-4 and hexokinase in multiple muscles composed of different fiber types including epitrochlearis (14), gastrocnemius (63), and red and white quadriceps muscles (142). This is not due to systemic factors, since incubation of isolated epitrochlearis muscle for 18 h in the presence of AICAR also increases GLUT-4 protein expression and hexokinase activity (97). Chronic treatment with β-guanadino propionic acid for 9 wk, a creatine analog known to deplete cellular phosphocreatine and ATP, results in increased AMP kinase activity in rat hindlimb muscles. This was associated with increased cytochrome c content, increased mitochondrial density, and increased DNA binding activity of nuclear respiratory factor-1, a transcription factor that acts on a nuclear set of genes required for the transcription of respiratory proteins as well as mitochondrial transcription and replication (10). These effects of AICAR and chronic treatment with β-guanadino propionic acid are similar to the adaptive responses induced by endurance exercise training. Transcriptional regulation of GLUT-4 by AMP kinase activation (induced by AICAR injection) has been examined with transgenic mice expressing various regions of the proximal promoter of the human GLUT-4 gene controlling induction of the bacterial chloramphenicol acetyltransferase reporter gene (160). This study demonstrates that the region within 895 bp from the GLUT-4 transcription start site contains an element essential for induction of GLUT-4 mRNA by AICAR stimulation. In addition, a preliminary study reported that AICAR infusion resulted in significantly increased transcription of the genes encoding for uncoupling protein-3, heme oxygenase-1, hexokinase II, and GLUT-4 in rat gastrocnemius muscles (123). The mechanism by which AMP kinase modulates gene transcription remains unclear; however, these studies raise the possibility that AMP kinase is a key intermediary in the effects of a single bout of exercise to alter the induction of multiple genes. The accumulation of these individual effects of each exercise bout may lead to muscle adaptations to chronic exercise training.

PI3-KINASE-MEDIATED SIGNALING PROTEINS

In addition to MAP kinase and AMP kinase signaling, the effects of exercise on other established signaling systems that are regulated by hormone or growth factors have also been studied in recent years. Although, to this point, there has been less emphasis on these signaling proteins, they represent very attractive candidates for regulation by exercise.

Proximal Insulin Signaling Proteins

Insulin and contraction have many similar biological effects in skeletal muscle, as both stimuli can act to increase in glucose uptake, amino acid uptake, and glycogen synthesis. On the basis of these similarities, it was a logical hypothesis that insulin and exercise utilize similar signaling proteins in the regulation of these metabolic events. However, it is now established that an acute bout of physical exercise or muscle contraction via nerve stimulation do not increase tyrosine phosphorylation of the insulin receptor, IRS proteins (IRS-1, IRS-2), and Shc, the first two steps in insulin signaling (47, 64, 117, 144). Downstream of the IRS proteins in insulin signaling are the PI3-kinase molecules. Activity of the class IA PI3-kinases, measured in IRS-1, insulin receptor, phosphotyrosine, or p85 immunocomplexes, is not increased immediately after exer-
cise or contraction (47, 144, 148). The lack of activation of these molecules is consistent with findings that insulin and contraction utilize different signals leading to glucose uptake and glycogen synthesis in skeletal muscle and that contraction-stimulated glucose uptake and glycogen synthase activation occur through a PI3-kinase-independent mechanism (60, 77, 79, 112, 146, 157). Established signaling molecules downstream of PI3-kinase, which may be relevant for the biological effects of exercise, include Akt, GSK3, and 70-kDa S6 protein kinase (p70S6K).

**Akt/Protein Kinase B**

Akt is a serine/threonine kinase and is activated by a wide variety of growth factors by both PI3-kinase-dependent (134) and -independent (108, 113) mechanisms. A proposed function of Akt in skeletal muscle is to mediate many of the cellular effects of insulin, since overexpression of constitutively active forms of Akt in muscle cells mimics the actions of insulin (50, 132). Recent reports provide intriguing evidence that Akt functions to regulate skeletal muscle growth and metabolism. In Akt1-deficient mice, there is marked impairment in organism growth (27), whereas Akt2-deficient mice exhibit reduced insulin-stimulated glucose uptake in isolated EDL muscles (26). With the use of a DNA injection and electroporation technique to overexpress a constitutively active Akt in mouse skeletal muscle, Akt has been shown to have critical roles in hypertrophy and the prevention of muscle atrophy in vivo (14). These results raise the possibility that Akt could be an important signaling molecule in the exercise response.

Whether exercise activates Akt and utilizes this molecule as a signaling mediator has been controversial. Early studies reported no Akt activation or phosphorylation with in vitro and in situ muscle contraction (20, 80, 117) or physical exercise (82, 138, 144). In contrast, other studies have shown some degree of activation or phosphorylation of Akt in intact skeletal muscles in response to exercise (130) and in situ muscle contraction (95, 131). These discrepant findings suggest that contraction may regulate Akt in an intensity- and time-dependent manner, or perhaps Akt stimulation in muscle may be fiber-type specific. A detailed series of in situ and in vitro contraction studies has recently established that contraction regulates Akt activity in skeletal muscle (112). In situ contraction significantly increases Akt phosphorylation in five different hindlimb muscles, which is accompanied by the activation of all three isoforms of Akt activity. Akt activation and phosphorylation with muscle contraction are rapid and transient and are inhibited in the presence of wortmannin, a PI3-kinase inhibitor. Because class IA PI3-kinases associated with tyrosine-phosphorylated proteins do not increase with contraction (47, 144, 148), it is possible that class IB PI3-kinase, which has a distinct activation mechanism (116), may be upstream of contraction-stimulated Akt activation. The physiological function of Akt signaling with exercise is not known, but a role for Akt in regulating contraction-stimulated glycogen synthase has been ruled out (112).

**GSK3**

GSK3 is a serine/threonine kinase that was originally identified as the primary upstream kinase that phosphorylates and inactivates glycogen synthase. GSK3 is now known to play a role in a wide variety of cellular functions, including several components of fuel metabolism and transcriptional regulation (28). A major regulatory mechanism for GSK3α and GSK3β activity occurs via phosphorylation of serine residues near the amino terminus (Ser21 on GSK3α, Ser9 on GSK3β), which results in deactivation of the enzyme (28).

Most studies of GSK3 in skeletal muscle have focused on insulin regulation of this kinase (82, 143). As described in previous sections, insulin stimulation of PI3-kinase leads to Akt activation, and Akt can then phosphorylate GSK3 on Ser921, leading to deactivation of GSK3. Deactivation of GSK3 is then thought to promote activation of glycogen synthase (29).

In a study that compared insulin and exercise effects on GSK3, insulin was shown to increase Akt phosphorylation and activity, which was closely matched by GSK3α Ser21 phosphorylation and a 40–60% decrease in GSK3α and GSK3β activity. Exercise also deactivated GSK3α and GSK3β activity to a similar degree as did insulin, but there was no detectable change in Ser21 phosphorylation in response to exercise (82). This study was the first to demonstrate that exercise alters the activity of GSK3 and suggested that there is an alternative mechanism to regulate GSK3 activity in skeletal muscle. In contrast to this finding, bicycle exercise in human subjects, performed at either low (~50% maximal O2 consumption) or high (~75% maximal O2 consumption) intensity, increased glycogen synthase activity immediately after exercise, with no detectable deactivation of both isoforms of GSK3 in vastus lateralis muscle (149). The reason for this discrepancy between rodent and human studies is not clear but may be a function of exercise intensity or duration. It is important to point out that this group also observed the tendency for a decrease in Akt Ser473 phosphorylation with exercise. This is in contrast to results showing that 60 min of bicycle exercise in humans increased Akt Ser473 phosphorylation in vastus lateralis muscle (130), which would be consistent with a deactivation of GSK3 activity (GSK3 activity was not measured).

The physiological consequences of GSK3 deactivation with exercise are not known. One function of decreased GSK3 activity may be to increase glycogen synthase activity with exercise, although this appears to be only part of the mechanism for glycogen synthase regulation (5, 112). It is likely that GSK3 functions in the regulation of additional metabolic and transcriptional processes. For example, resistance exercise increases eukaryotic translation initiation factor 2B (eIF2B) activity (38, 71), a key molecule involved in the
regulation of translation initiation. Because under basal conditions GSK3 suppresses eIF2B activity via phosphorylation of the e-subunit at Ser\(^{540}\) (137), it is possible that exercise stimulates translation initiation and net protein synthesis via inhibiting GSK3 phosphorylation of eIF2Be at Ser\(^{540}\). Another major function of GSK3 is to phosphorylate transcription factors, many of which are known to be regulated by exercise. The precise role of GSK3 in the regulation of carbohydrate and protein metabolism, as well as transcriptional regulation in contracting skeletal muscle, is clearly an important area for future investigation.

\(p70^{S6K}\)

The \(p70^{S6K}\) is thought to play a critical role in regulating the translation of a class of mRNA transcripts, which contain an oligopyrimidine tract at their transcriptional start site (78, 100). Blockade of \(p70^{S6K}\) activity results in significant inhibition of protein synthesis in response to insulin or serum treatment in multiple cell systems (101). Because resistance exercise training causes muscle hypertrophy and a single bout of resistance exercise increases protein synthesis, it has been hypothesized that exercise regulation of protein synthesis is mediated through the activation of \(p70^{S6K}\) in skeletal muscle. Several studies have reported no activation or phosphorylation of \(p70^{S6K}\) immediately or shortly after in vitro contraction (20), in situ contraction (117), or exercise (44) in rat skeletal muscles. On the other hand, several hours after in situ contraction (6, 95) and resistance exercise in vivo (62), there is increased \(p70^{S6K}\) activity in rat skeletal muscles. The increase in \(p70^{S6K}\) activity 6 h after high-force eccentric contractions tightly correlated with the percent change in muscle mass after 6 wk of training (6). In another study, overloading-induced muscle hypertrophy was shown to be abolished with rapamycin treatment, a selective inhibitor of the upstream regulator of \(p70^{S6K}\), mTOR (14), suggesting that this form of muscle hypertrophy by overloading is mediated through Akt, mTOR, and \(p70^{S6K}\) signaling. The signaling mechanism leading to the delayed increase in \(p70^{S6K}\) activity is unknown but may involve Akt and mTOR signaling (14).

Glycogen/Sarcoplasmic Reticulum-Associated Type-1 Protein Phosphatase

Muscle PP1G is composed of a 37-kDa catalytic subunit and a 160-kDa glycogen-targeting or regulatory (R\(_{GL}\)) subunit. PP1G was originally postulated to be the signal that mediates insulin’s control of glycogen synthesis because PP1G is activated by insulin and this enzyme can dephosphorylate all of the sites in glycogen synthase in vitro (76). However, insulin activates glycogen synthase in muscle of \(R_{GL}\) knockout mice similarly to wild-type animals (127), suggesting that PP1G is not essential for insulin regulation of glycogen synthesis. In contrast, treadmill exercise increases glycogen synthase activity in wild-type mice but does not result in glycogen synthase activation in the \(R_{GL}\) knockout mice (5). The \(R_{GL}\) knockout mice, which also had a reduced basal glycogen synthase activity associated with significantly reduced basal glycogen levels, exhibited a severely impaired maximal exercise capacity despite normal contraction-induced activation of glucose transport. These results indicate...
that PP1G is essential for regulation of glycogen metabolism under basal conditions and in response to contractile activity but not by insulin. Determination of exercise regulation of PP1G and subsequent modulation of glycogen synthase by PP1G will be critical for understanding the mechanisms of enhanced glycogen synthesis after exercise in skeletal muscle.

SUMMARY AND FUTURE DIRECTIONS

In the past several years, considerable progress has been made to elucidate intracellular signaling mechanisms in contracting skeletal muscle. It is now apparent that multiple messengers and signaling systems are activated (or deactivated) during physical exercise and that the degree of activation of each protein is dependent on multiple factors, including exercise intensity, duration, and the fiber-type composition of the working muscle. The major challenge now is to determine the biological consequences of increased signaling through these intracellular cascades (Fig. 4). Given that exercise results in a multitude of favorable adaptations to skeletal muscle, elucidating the specific signaling proteins that mediate these beneficial adaptations makes these molecules prime targets for drug development. For example, largely on the basis of studies aimed at elucidating the signaling mechanisms for the insulin-independent effects of contraction to increase glucose transport, AMP kinase is now a major target of AMPK activation in human skeletal muscle. J Appl Physiol 87: 1668–1673, 1999.

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