Exercise Effects of Muscle Insulin Signaling and Action
Invited Review: Exercise training-induced changes in insulin signaling in skeletal muscle

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Zierath, Juleen R. Invited Review: Exercise training-induced changes in insulin signaling in skeletal muscle. J Appl Physiol 93: 773–781, 2002;10.1152/japplphysiol.00126.2002.—This review will provide insight on the current understanding of the intracellular signaling mechanisms by which exercise training increases glucose metabolism and gene expression in skeletal muscle. Participation in regular exercise programs can have important clinical implications, leading to improved health in insulin-resistant persons. Evidence is emerging that insulin signal transduction at the level of insulin receptor substrates 1 and 2, as well as phosphatidylinositol 3-kinase, is enhanced in skeletal muscle after exercise training. This is clinically relevant because insulin signaling is impaired in skeletal muscle from insulin-resistant Type 2 diabetic and obese humans. The molecular mechanism for enhanced insulin-stimulated glucose uptake after exercise training may be partly related to increased expression and activity of key proteins known to regulate glucose metabolism in skeletal muscle. Exercise also leads to an insulin-independent increase in glucose transport, mediated in part by AMP-activated protein kinase. Changes in protein expression may be related to increased signal transduction through the mitogen-activated protein kinase signaling cascades, a pathway known to regulate transcriptional activity. Understanding the molecular mechanism for the activation of insulin signal transduction pathways after exercise training may provide novel entry points for new strategies to enhance glucose metabolism and for improved health in the general population.

AMP-activated protein kinase; diabetes; gene expression; insulin receptor substrates; mitogen-activated protein kinase; phosphatidylinositol 3-kinase

EXERCISE TRAINING: A PHYSIOLOGICAL TOOL TO ENHANCE INSULIN ACTION

People with non-insulin-dependent Type 2 diabetes mellitus are characterized by impaired insulin action on whole body glucose uptake, partly owing to impaired insulin-stimulated glucose transport in skeletal muscle (98, 99). Defects in insulin action on glucose uptake in skeletal muscle from Type 2 diabetic patients have been linked to impaired signal transduction (7, 43). Insulin sensitivity has been shown to be related to the degree of physical activity (64); therefore, physical training programs may offer a physiological means to improve insulin action in some insulin-resistant people. Exercise training improves glucose tolerance and insulin action in insulin-resistant humans (35, 59) or Type 2 diabetic patients (63, 74). The molecular mechanism for enhanced glucose uptake with exercise training may be related to increased expression and/or activity of key signaling proteins involved in the regulation of glucose uptake and metabolism in skeletal muscle (Fig. 1). For example, exercise training leads to increased expression of glucose transporter 4 (GLUT-4) content in skeletal muscle, and this has been
correlated with improved insulin action on glucose metabolism (10, 14, 29, 35, 57). However, emerging evidence suggests that these exercise-training-induced improvements in glucose uptake are not limited to changes in GLUT-4 expression. The improvements in insulin sensitivity after exercise training may be related to changes in expression and/or activity of proteins involved in insulin signal transduction in skeletal muscle. Acute exercise also increases AMP-activated protein kinase (AMPK) activity, leading to changes in glucose uptake and gene expression. Exercise training is associated with changes in mRNA of several components of insulin and MAPK signaling cascades. The “master regulator(s)” of exercise-responses on gene expression has not been completely defined.

**EARLY STEPS IN INSULIN SIGNAL TRANSDUCTION**

The insulin receptor is a heterotetrameric membrane glycoprotein composed of two \( \alpha \)-subunits and two \( \beta \)-subunits, linked together by disulfide bonds (reviewed in Ref. 77). Insulin binds to the extracellular \( \alpha \)-subunits, and this leads to activation of the transmembrane \( \beta \)-subunits and autophosphorylation of the receptor. Multiple tyrosine phosphorylation sites present on the \( \beta \)-subunit of the insulin receptor play important functional roles in promoting receptor kinase activity, mediating differential responses along mitogenic and metabolic pathways, and facilitating the interaction between the receptor and intracellular substrates. In recent years, research efforts have largely moved from studies designed to characterize insulin binding and receptor function to studies oriented toward the identification and characterization of postreceptor molecular targets that regulate insulin signal transduction to different metabolic and mitogenic responses. Although the picture is far from complete, some important early steps in insulin signaling have emerged.

**Insulin receptor substrates.** Insulin signaling is a complex series of events involving multiple effector proteins that orchestrate diverse cellular responses. Importantly, insulin signaling pathways are not necessarily linear, as there is a high degree of cross talk between the signal transducers. Insulin receptor substrate isoforms (IRS-1 to -4) (46, 47, 69, 70), Gab-1 (30), and Cbl (58) link the initial event of insulin receptor signaling cascade to downstream events. IRS molecules contain multiple tyrosine phosphorylation sites that become phosphorylated after insulin stimulation (reviewed in Refs. 77, 79) and bind downstream signaling molecules containing src homology 2 domains. IRS-1 and IRS-2 play selective roles in the regulation of metabolic and mitogenic responses in insulin-sensitive tissues, including skeletal muscle, adipose tissue, and liver. IRS-3 and IRS-4 are not expressed in skeletal muscle; therefore, these substrates will not be reviewed. Likewise, because of the paucity of data concerning the role of Gab-1 and Cbl in mediating insulin signaling to glucose transport after exercise in skeletal muscle, these substrates will not be reviewed.

Tissue-specific roles of IRS-1 and IRS-2 have been elucidated through studies performed with different knockout strategies in mice. IRS-1 appears to be the predominant isoform mediating signal transduction in skeletal muscle (2, 71), whereas IRS-2 appears to be important in \( \beta \)-cell development (80). Both isoforms are important for regulation of metabolism in liver (36). Although different IRS proteins clearly have selective roles in mediating many of metabolic and mitogenic responses, a degree of redundancy in the function may exist. For example, in skeletal muscle and adipose tissue from Type 2 diabetic subjects, insulin-mediated tyrosine phosphorylation of IRS-1 is impaired, whereas IRS-2 phosphorylation is normal (7, 43, 60). Thus IRS...
molecules are likely to play complementary roles in the mediation of insulin action.

**Phosphatidylinositol 3-kinase and downstream effectors.** Phosphatidylinositol 3-kinase (PI3-kinase) is one of the most characterized intermediate effector molecules that associate with IRSs. PI3-kinase associates with tyrosine phosphorylated IRSs after insulin stimulation and catalyzes the formation of phosphatidylinositol-3,4,5-trisphosphate, which serves as an allosteric regulator of phosphoinositide-dependent kinase (1). PI3-kinase plays an important role in the acute effect of insulin on glucose transport and GLUT-4 translocation in skeletal muscle (49, 65, 92). Because several reviews in this series will consider molecular mechanisms by which insulin or exercise mediate GLUT-4 translocation and glucose transport, this aspect will not be considered in depth in the present review. The downstream effectors of PI3-kinase that signal to glucose transport have not been fully elucidated. PI3-kinase presumably mediates glucose transport via signaling to protein kinase B (PKB)/Akt and/or protein kinase C (PKC)-ζ (reviewed in Ref. 77). Tissue culture systems or animal models in which either signaling via AKT/PKB (11) or PKC-ζ (4, 41) has been disrupted suggest that these targets partly contribute to the regulation of glucose uptake, although other intermediates are likely to participate (58). Through comparative genomics and pathway analysis, new downstream components of the insulin pathway are likely to be identified.

**EFFECTS OF EXERCISE TRAINING ON INSULIN SIGNALING**

Immediately after an acute bout of exercise, glucose transport in skeletal muscle is increased through an insulin-independent translocation of GLUT-4 to the cell surface (18, 42, 49). Thus immediate effects of acute exercise on glucose homeostasis occur primarily at the level of GLUT-4 traffic rather than through enhanced insulin signaling at the level of the insulin receptor, IRS-1, IRS-2, or PI3-kinase (34, 48, 49, 66, 73, 86–88, 92, 97). Several hours after acute exercise, a persistent increase in insulin sensitivity of glucose transport occurs in skeletal muscle. Effects of exercise can be observed even <16 h after the last exercise session (10, 57). Measurements made at this time may reflect changes in protein expression (enhanced or suppressed) that occur in response to the exercise bout. Exercise training increases insulin-mediated whole body glucose disposal (15, 16, 32, 35). This effect is correlated with increased protein expression of GLUT-4 (10, 15, 32, 35, 56, 57, 94), as well as with adaptive responses in expression and function of key insulin-signaling molecules (10, 33, 40, 94). Although our understanding of the signaling pathways regulating glucose metabolism is limited, studies designed to examine the effects of exercise training on known constituents of the insulin signaling pathway are emerging.

**Insulin receptor substrates.** IRS-1 and IRS-2 are important signal transducers in skeletal muscle. Exercise training-induced effects on IRSs have been elucidated. In rodents, long-term endurance training (5 bouts/wk for 9 wk) increased insulin receptor and IRS-1 mRNA in skeletal muscle 48 h after the last bout of exercise (37). In contrast, insulin receptor and IRS-1 mRNA was not altered after short-term endurance training in humans (60 min/day for 9 days) (78). However, complementary studies of protein expression were not performed in either of these studies (37, 78). Consistent with this finding in humans, IRS-1 protein expression is not increased 16 h after short-term endurance training in rats (6 h/day for 1 or 5 days) (10). In this model, insulin-stimulated tyrosine phosphorylation of IRS-1 tended to be increased after 1 day of exercise. The increase in IRS-1 tyrosine phosphorylation correlated with increased insulin receptor tyrosine phosphorylation (10). Surprisingly, IRS-1 protein expression was reduced 16 h after 5 days of exercise, despite a profound increase in insulin-stimulated IRS-1 tyrosine phosphorylation. The reduction in IRS-1 protein expression in exercise-trained rodents is similar to the >55% reduction in IRS-1 protein expression in skeletal muscle obtained 48 h after exercise from subjects engaged in habitual training programs (running ~50 km/wk for >2 mo) (94). Major effects of exercise training on insulin signaling do not include transcriptional activation of the IRS-1 gene. Rather, improvements in insulin action after exercise training are likely to occur from more efficient signaling per molecule of IRS-1, leading to increased signal transduction to downstream substrates.

Exercise training has differential effects on protein expression of IRS-1 and IRS-2. In rat epitrochlearis muscle, 16 h after an acute 6-h swim bout, IRS-2 expression is increased threefold (10). In this model, IRS-2 expression is restored to pretraining levels in muscle studied 16 h after 5 days of repeated 6-h swim bouts. Thus increased IRS-2 protein expression partly accounts for increased insulin action in skeletal muscle after exercise. In support of this hypothesis, mRNA levels of IRS-2 in human skeletal muscle increase transiently 3 h after a single exercise bout, but this effect is diminished after short-term (9 days) endurance training (78). The initial observation that exercise increases insulin action at the level of IRS-2 was confirmed with IRS-2 knockout mice (34). In wild-type mice, insulin-mediated IRS-2 tyrosine phosphorylation was increased in skeletal muscle immediately after exercise, with no effect noted in IRS-2 null mice (34). Although IRS-2 protein expression was not assessed, increased protein expression of IRS-2 is not likely to account for enhanced tyrosine phosphorylation after exercise. Thus exercise has multiple effects on IRS-2 that involve changes in signal transduction and protein expression. Immediately after exercise, insulin-mediated IRS-2 tyrosine phosphorylation is enhanced. In the hours after an acute exercise bout, IRS-2 undergoes a rapid upregulation at the level of mRNA and protein. The enhanced insulin action on IRS-2 is maintained for at least 16 h after exercise. Detailed time-course studies of the effects of exercise on either signal transduc-
tion or protein expression of IRS-2 have not been performed in human subjects or in rodents. However, in people engaged in habitual exercise (long-distance running) programs, IRS-2 protein expression in skeletal muscle obtained 48 h after the last bout of exercise is decreased compared with levels measured in sedentary individuals (94). Thus repeated exercise may be associated with either increased degradation or decreased synthesis of IRS-2. The physiological role for IRS-2 in mediating insulin signaling in skeletal muscle after exercise is unknown.

**PI3-kinase.** Insulin-stimulated PI3-kinase activity is impaired in skeletal muscle from Type 2 diabetic and obese insulin-resistant subjects (7, 24, 39, 43), thus constituting a pivotal site of insulin resistance. Several hours after acute exercise, a persistent increase in insulin sensitivity of glucose transport occurs in skeletal muscle. Enhanced phosphotyrosine-associated PI3-kinase activity (34, 88, 97) in the hours after exercise may partly contribute to the persistent increase in glucose uptake after exercise. Regular exercise training enhances insulin-stimulated PI3-kinase activity in skeletal muscle (10, 33, 40). Because PI3-kinase is an important regulatory step for glucose transport, increased signal transduction at this key step after exercise training may contribute to the exercise-associated increase in insulin action in skeletal muscle. Increased mRNA levels of the p85 alpha-subunit of PI3-kinase have been noted in rodents and humans engaged in acute (78) or long-term (38) exercise training; however, the physiological significance of this is unknown, because overexpression of the p85 alpha-subunit in L6 myotubes is associated with decreased, rather than increased, insulin-stimulated glucose uptake (75). Thus enhanced insulin-stimulated PI3-kinase activity, rather than changes in expression of the subunits of the enzyme, is likely to account for enhanced glucose metabolism after exercise.

Several studies have now been performed to determine the effects of exercise training on insulin-mediated PI3-kinase activity in skeletal muscle. In rodents, insulin-stimulated IRS-associated PI3-kinase activity is markedly increased in isolated epitrochlearis muscle studied 16 h after 1 or 5 days of prior exercise (6-h swim bouts) (10). This positive effect on insulin signaling occurs despite reduced IRS-1 protein expression. Importantly, the increased insulin-stimulated IRS-1-associated PI3-kinase activity after exercise training corresponds with an increase in insulin-stimulated 3-O-methylglucose transport activity (10). These findings in rodents can be translated to humans. In healthy young, but sedentary, men, 7 days of exercise training [60 min/day at 75% maximal oxygen consumption (V\textsubscript{O\textsubscript{2} max})] is associated with increased insulin sensitivity and enhanced insulin-stimulated phosphotyrosine-associated PI3-kinase activity in skeletal muscle (33). Because time course studies reveal that both insulin action on anti-phosphotyrosine- and insulin action on IRS-1-associated PI3-kinase activity occur in parallel (43), IRS-1 is likely to be the predominant tyrosine-phosphorylated molecule transmitting this exercise-mediated change in insulin signaling to PI3-kinase in human skeletal muscle. Consequently, changes in phosphotyrosine-associated PI3-kinase activity in human muscle after exercise training (33) are likely to represent increased IRS-1-associated PI3-kinase activity. Consistent with this observation, a subsequent study provided evidence that insulin-stimulated IRS-1-associated PI3-kinase activity is greater in skeletal muscle from subjects engaged in habitual exercise training programs (V\textsubscript{O\textsubscript{2} max} of 56.1 ± 2.5 ml/kg) compared with sedentary subjects (V\textsubscript{O\textsubscript{2} max} of 44.4 ± 2.7 ml/kg) (40). When exercise-trained and sedentary subjects were compared together, PI3-kinase activation was correlated with both glucose disposal and V\textsubscript{O\textsubscript{2} max} (40). Collectively, these results are consistent with the notion that regular exercise training leads to improvements in glucose disposal through enhanced insulin signaling at the level of PI3-kinase.

Insulin-stimulated IRS-2 associated PI3-kinase is also increased after exercise training. However, IRS-1 and IRS-2 undergo differential regulation in skeletal muscle in response to exercise (10). The increase in IRS-2 protein expression in rat epitrochlearis muscle noted 16 h after a 6-h exercise bout was associated with enhanced basal and insulin-stimulated IRS-2-associated PI3-kinase activity. In contrast to findings for IRS-1, in which protein levels were decreased with exercise training, IRS-2 protein expression and IRS-2-associated PI3-kinase activity normalized to sedentary levels after 5 days of exercise (10). Thus IRS-1 and IRS-2 are likely to have specialized rather than redundant roles in mediating insulin signal transduction in skeletal muscle in response to exercise training. This finding is further reinforced in studies whereby insulin signaling immediately after exercise has been examined in IRS-2 knockout mice (34). In IRS-2-deficient mice, the increase in insulin-stimulated phosphotyrosine-associated PI3-kinase activity immediately after an acute treadmill running was attenuated compared with that in wild-type mice, suggesting that IRS-2 signaling can partly account for the increase in phosphotyrosine-associated PI3-kinase activity after exercise. However, insulin-stimulated 2-deoxyglucose uptake in skeletal muscle measured after exercise was not different between IRS-2-deficient and wild-type mice. Thus the exercise effect on IRS-2 may be masked in the presence of normal levels of IRS-1. Alternatively, another undefined tyrosine-phosphorylated protein may contribute to insulin-mediated glucose uptake in IRS-2-deficient skeletal muscle (34). The physiological significance of the exercise-induced IRS-2 signaling awaits further elucidation.

**AMP-ACTIVATED PROTEIN KINASE**

AMP-activated protein kinase (AMPK) has been implicated as an important mediator of muscle contraction-induced glucose transport (84) and a target for pharmacological intervention to treat altered glucose
homeostasis associated with Type 2 diabetes and obesity (51). AMPK is a heterotrimeric protein, composed of one catalytic (α) and two noncatalytic (β and γ) subunits (84) and is activated by cellular stress associated with ATP depletion (26). Although AMPK activity does not appear to be increased in response to insulin, some discussion of this kinase is warranted in the present review as it has been implicated to be one of several critical regulators of mitogenic and metabolic events in response to exercise in skeletal muscle. For example, an increase in AMPK activity in response to muscle contraction or exercise has been correlated with GLUT-4 translocation and glucose transport in skeletal muscle (5, 6, 26, 27, 45, 50). Furthermore, increased AMPK activity has also been correlated with increased free fatty acid oxidation in skeletal muscle (6), decreased lipogenesis and lipolysis in adipocytes (68), and decreased free fatty acid and cholesterol synthesis in hepatocytes (28). Thus recent evidence is consistent with the hypothesis that AMPK plays a central role in the regulation of glucose homeostasis in response to exercise.

Exercise-mediated changes in AMPK activity. Isoform-specific and exercise-intensity-dependent changes in AMPK activity have been observed in skeletal muscle (21, 89). Low- to moderate-intensity aerobic exercise induces an isoform-specific and intensity-dependent increase in AMPK α2 but not in AMPK α1 activity in moderately trained subjects (21, 89). However, in response to anaerobic sprint exercise, activity of AMPK α1 and α2 are both increased (9). These exercise-intensity differences may be related to the finding that AMPK complexes containing the α2-isoform rather than the α1-isoform have a greater dependence on AMP (9, 62). Although these studies do not directly link activation of AMPK to increased glucose uptake, direct evidence can be acquired from studies in transgenic animal models. Transgenic overexpression of a dominant inhibitory mutant of AMPK in skeletal muscle completely blocks the ability of hypoxia to activate glucose uptake, whereas only partially reducing contraction-stimulated glucose uptake (52). Thus AMPK-dependent and AMPK-independent pathways contribute to the regulation of glucose uptake in skeletal muscle in response to exercise. For example, in rats, glucose transport in slow-twitch muscle can be markedly activated in response to contraction, without measurable changes in AMPK activity (17). Collectively, these studies illustrate the complexity in identifying the precise role of the AMPK pathway in regulating metabolic events, and they strongly suggest that additional factors contribute to the regulation of exercise-mediated glucose uptake. However, the latter studies do not distract from the attractiveness of AMPK as a target for exercise-induced glucose transport and a candidate for pharmacological intervention to improve glucose homeostasis.

AMPK and metabolic disease. Because AMPK appears to increase glucose metabolism by insulin-independent signaling cascades (27), activation of this pathway provides an alternative strategy to increase glucose transport in insulin-resistant skeletal muscle. An obvious hypothesis to consider is whether pharmacological intervention of AMPK with compounds designed to mimic the exercise response on glucose uptake or fatty acid oxidation may be efficacious in the management of metabolic abnormalities associated with Type 2 diabetes mellitus. One compound commonly utilized to test this hypothesis is 5-aminooimidazole-4-carboxamide ribonucleoside (AICAR). AICAR is an adenosine analog that can be taken up into intact hepatocytes, adipocytes, and skeletal muscle and can be phosphorylated to form 5-aminooimidazole-4-carboxamide ribonucleotide, the monophosphorylated derivative that mimics the effects of AMP on AMPK without affecting ATP or ADP content. In isolated epitrochlearis muscle incubated in serum, AICAR exposure leads to an increase in insulin sensitivity that appears to mimic an exercise response (20).

AICAR effects on whole body glucose homeostasis have been determined in diabetic rodents. Treatment of diabetic ob/ob (67) or KKAy-CETP (19) mice with AICAR lowers blood glucose and insulin concentration and improves glucose tolerance. Furthermore, in vitro exposure of isolated skeletal muscle to AICAR elicits a normal increase in glucose transport in insulin-resistant ob/ob mice (67). This is consistent with studies in Type 2 diabetic subjects whereby exercise is reported to elicit a normal increase in AMPK α2 activity in skeletal muscle (53). These studies provide evidence to suggest that exercise-induced AMPK activity and AICAR-induced AMPK activity are not impaired in insulin-resistant skeletal muscle. However, AICAR treatment of ob/ob (67) and KKAy-CETP (19) mice is associated with a worsening of the blood lipid profile. Because AICAR is a nonspecific AMPK activator (12, 76), long-term exposure to AICAR may trigger effects other than activation of AMPK in either liver or adipose tissue and this may influence plasma lipid mobilization. In this respect, the recent work from Moller and colleagues (95) is important to emphasize, as they have identified AMPK as the elusive target of metformin, further highlighting the importance of AMPK in the regulation of glucose homeostasis and providing “proof of concept” that activation of this target can enhance insulin sensitivity, as metformin is a widely used drug for treatment of Type 2 diabetes mellitus. Through use of a novel AMPK inhibitor, AMPK activation was shown to be required for metformin’s inhibitory effect on glucose production by hepatocytes. Furthermore, incubation of isolated epitrochlearis muscle with metformin resulted in an increase in the activity of both catalytic subunits of AMPK, coincident with an increase in glucose uptake. These findings (95) have important clinical implications because metformin also increases insulin-stimulated glucose transport in skeletal muscle from Type 2 diabetic subjects (22, 23).

MECHANISMS FOR INCREASED PROTEIN EXPRESSION IN SKELETAL MUSCLE AFTER EXERCISE

Mitogen-activated protein kinase signaling. One future direction will be the identification of pathways
that regulate gene expression in skeletal muscle after exercise. Clearly, multiple mechanisms contribute to the regulation of insulin action and protein expression. Recent evidence suggests that mitogen-activated protein kinase (MAPK) signaling cascades may constitute one important cellular signaling mechanism mediating exercise-induced adaptations in skeletal muscle. MAPK activation has been implicated as an important mechanism governing cellular proliferation and differentiation in many cell types (reviewed in Ref. 55). Although the possible involvement of MAPK signal transduction pathways in exercise-mediated regulation of gene expression in skeletal muscle has been considered in detail (reviewed in Ref. 82), a brief review is warranted.

Members of the MAPK family form at least three parallel signaling cascades that include the extracellular-regulated kinase (ERK1/2 or p42 and p44 MAPK), p38 MAPK, and c-Jun NH2 kinase. Evidence is emerging that MAPK signaling pathways are directly activated in human skeletal muscle in response to acute, short-term exercise (3, 44, 81, 83) or endurance running (8, 93). Activity of several downstream substrates of ERK and p38 MAPK signaling cascades, such as MAPK-activated protein kinase (MAPKAPK) 1 and 2, as well as the mitogen and stress-activated kinase (MSK) 1 and 2, are increased immediately after acute sprint (44) or endurance exercise (93). Substrate specificity for MAPK signaling cascades has been determined with an ex vivo system to achieve contraction (electrical stimulation) of isolated rat epitrochlearis muscle, combined with the use of chemical inhibitors of ERK and p38 MAPK (61). Thus contraction-induced inductions of MAPKAPK1 and MAPKAPK2 occur via separate pathways, reflecting ERK and p38 MAPK stimulation, respectively. In contrast, induction of MSK1 and MSK2 requires simultaneous activation of ERK and p38 MAPK (61). The direct link between MAPK activation and changes in gene expression in skeletal muscle after exercise has yet to be established, as the majority of studies to address this point have been correlative (reviewed in Ref. 82). Future work directed toward understanding whether exercise-induced MAPK signaling directly suppresses or enhances gene expression is necessary.

**AMPK signaling.** AMPK has been proposed to regulate gene expression (25). This may be partly through direct targeting of AMPK complexes containing the \( \alpha_\text{2} \)-isoform to the nucleus (62). AMPK is involved in transcriptional regulation by repressing genes involved in glucose signaling in hepatocytes (62, 90) and upregulating genes involved in glucose uptake and substrate metabolism in skeletal muscle (31, 54, 85). For example, activation of AMPK mimics several classic exercise-mediated responses on gene expression, including increases in GLUT-4 mRNA and protein content, hexokinase II mRNA and activity, uncoupling protein-3 mRNA, mitochondrial enzymes, and glycogen content in skeletal muscle (31, 54, 85, 96). These changes can also be observed in skeletal muscle from diabetic rodents. Hexokinase II and GLUT-4 protein expressions, as well as in vitro MEF2 sequence-specific binding activity, are increased in skeletal muscle from lean and ob/ob mice after 7 days of AICAR treatment (67), presumably through increased AMPK activity. A similar increase in MEF2 sequence-specific binding activity has also been observed in human skeletal muscle after marathon running (93). Thus increased MEF2 sequence-specific binding activity may confer exercise-specific changes in gene expression. Consistent with this hypothesis, the MEF2 site appears to be essential for GLUT-4 expression, because deletions or point mutations within the MEF2 consensus binding sequence of the human GLUT-4 promoter completely prevent tissue-specific and hormonal/metabolic regulation of GLUT-4 (72).

**SUMMARY AND FUTURE DIRECTIONS**

Exercise training appears to enhance insulin sensitivity by increased postreceptor insulin signaling. Increased insulin-mediated glucose transport appears to be related to enhanced signal transduction at the level of IRS proteins and PI3-kinase. These findings are clinically relevant because insulin-stimulated tyrosine phosphorylation of IRS-1 and activity of PI3-kinase are reduced in skeletal muscle from Type 2 diabetic patients (7, 13, 43). Thus exercise training may be one therapeutic strategy to restore impaired insulin signal transduction in skeletal muscle from Type 2 diabetic patients.

Because the insulin-signaling pathway(s) to glucose transport has not been fully elucidated, a more complete mapping of the necessary and required components of this network is required. Identification of intermediates in the insulin signaling pathway may be achieved through comparative genomics, using genetically modified model organisms, combined with bioinformatic approaches to identify mammalian homologues for pathway analysis. Studies with ex vivo models and chemical inhibitors may directly link insulin signaling and MAPK or AMPK pathways to changes in gene expression in response to exercise.
training. Transgenic and knockout mice in which components of insulin signaling and MAPK or AMPK cascades have been overexpressed or ablated will reveal the requirements for these signaling intermediates in exercise-mediated responses. Knowledge of the human genome sequence, used in concert with gene and/or protein array technology, will provide a powerful means to facilitate efforts in revealing molecular targets that regulate glucose homeostasis in response to exercise training. This will also offer quicker ways forward to identifying gene expression profiles in insulin-sensitive and insulin-resistant human tissue and may by useful to identify biochemical entry points for drug intervention to improve glucose homeostasis.

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