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SKELETAL MUSCLE AND INSULIN SENSITIVITY: PATHOPHYSIOLOGICAL ALTERATIONS

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1. ABSTRACT

Resistance to the normal action of insulin contributes to the pathogenesis of a number of common human disorders, Type II (non-insulin-dependent) diabetes mellitus. This review is focused on current understanding of the molecular mechanisms regulating insulin action and the factors contributing to insulin resistance in skeletal muscle. Since skeletal muscle is considered the major organ responsible for glucose uptake under insulin-stimulated conditions, defects in this target tissue are likely to contribute to metabolic disregulation in Type II diabetes mellitus. Defects in insulin signal transduction through the insulin-receptor substrate-1/phosphatidylinositol 3-kinase pathway is associated with reduced insulin-stimulated glucose transport activity in skeletal muscle from Type II diabetic patients. Glucose transport, the rate limiting step in glucose metabolism, is mediated by glucose transporter 4 (GLUT4) translocation and can be activated in skeletal muscle by two separate and distinct signaling pathways; one stimulated by insulin and the second by muscle contractions. Level of physical exercise has been linked to improved glucose homeostasis and enhanced insulin sensitivity. Understanding the molecular mechanism for the activation of signal transduction pathways by which insulin and muscle contraction increase glucose transport will provide a link to defining new strategies to enhance glucose metabolism in the diabetic patient.

2. INTRODUCTION

Type II diabetes (non-insulin dependent diabetes mellitus, NIDDM) is a progressive metabolic disorder characterized by chronic hyperglycemia. If gone unchecked Type II diabetes leads to numerous complications including retinopathy (1), cardiovascular disease (2), or renal failure (3). Peripheral insulin resistance in skeletal muscle is likely a major contributor to the development of overt Type II diabetes mellitus. This tissue accounts for ~80% of total glucose disposal under insulin-stimulated conditions (4), and defects in insulin action in skeletal muscle precede the clinical diagnosis of the disease (5). Thanks to advances in molecular biology, studies using cell culture systems have lead the way in unraveling the complexities governing the cellular events that regulate glucose transport in response to insulin. Nevertheless, in order to understand the underlying cause of impaired insulin action in diabetic muscle, knowledge gained from cell culture systems needs to be extended to examine insulin action in skeletal muscle from animal models of diabetes, as well as in muscle from diabetic patients. This review is focused on the current knowledge of factors that negatively or positively control insulin action in skeletal muscle.

3. GLUCOSE TRANSPORT REGULATION: INSULIN SIGNAL TRANSDUCTION AND GLUT4 VESICLE TRAFFIC

Insulin signal transduction is initiated by insulin binding to the extracellular domain of the insulin receptor (IR) (Figure 1). This in turn activates intracellular tyrosine kinase activity within the IR beta-subunit (6). The primary targets of this kinase activity include the insulin receptor substrate-1 (IRS-1) through 4 (7-10), and Shc (11). However, in skeletal muscle IRS expression appears to be restricted to IRS-1 and IRS-2 (7-10). Following

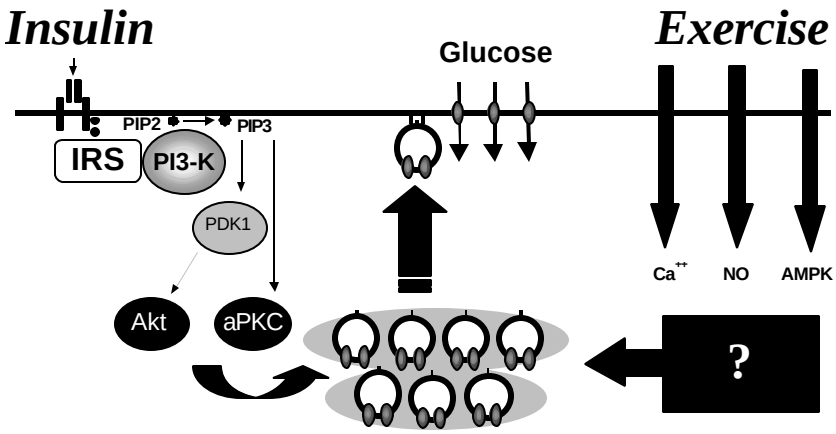

Figure 1. Proposed scheme of regulation of glucose transport in skeletal muscle in response to insulin or exercise (muscle contraction).

phosphorylation by the insulin receptor, IRS proteins act as docking proteins for downstream signaling molecules containing Src homology 2 (SH2) domains, including the 85-kDa regulatory subunit of phosphatidylinositol (PI) 3-kinase (12). PI 3-kinase as been implicated as an essential signaling intermediate for insulin-stimulated glucose transport based on investigations using the pharmacological inhibitor of PI 3-kinase, wortmannin. First in isolated apidocytes (13, 14), and soon thereafter in skeletal muscle (15-17), wortmannin was shown to completely inhibit insulin-stimulated PI 3-kinase activity and glucose transport. To date, the molecular link between PI 3-kinase and glucose transport has yet to be clearly established. Thus, intense research interest has been placed on identifying the molecules downstream of PI 3-kinase that are involved in signal transduction to glucose transport.

The serine/threonine kinase Akt has been identified as one such downstream target of PI 3-kinase (18, 19). Activation of Akt by insulin appears to involve direct activation by 3-phosphoinositide dependent protein kinase 1 (PDK1) (20). Expression of constitutively active forms of Akt in 3T3-L1 adipocytes (21), primary rat adipocytes (22), or L6 myotubes (23) stimulates glucose transporter 4 (GLUT4) translocation and glucose transport. Thus, these investigations are in accordance with Akt playing an active role in stimulation of glucose transport by insulin. However, conflicting reports from investigations using various dominant negative forms of Akt justify further examination into the role of Akt. Overexpression of Akt with mutations targeted to the phosphorylation sites has been reported to inhibit insulin-stimulated protein synthesis and p70S6-kinase activity in 3T3-L1 adipocytes, without effecting insulin-stimulated glucose uptake (24). However, overexpression of dominant-negative forms of Akt with mutations within the catalytic site, without (22) or with (25) co-mutation of the phosphorylation sites, causes inhibition of insulin-stimulated GLUT4 translocation in rat adipocytes and L6 myotubes, respectively. In light of these conflicting reports, the role of Akt in terms of signaling to glucose transport remains a point of debate. Nevertheless, activation of Akt by insulin has been demonstrated in both rat (26, 27) and human (28-31) skeletal muscle. This activation occurs rapidly (within 6 to 8 min) and remains persistently activated for up to 40 min (26, 31). Furthermore, the activation of Akt in skeletal muscle occurs in a PI 3-kinase dependent manner (28).

Additional downstream effectors of PI 3-kinase which may account for insulin-stimulated glucose transport include specific members of the protein kinase C (PKC) family. In 3T3-L1 cell culture, members of the atypical class of PKC, PKC-zeta and PKC-lambda, have been reported to be activated by insulin through a PI 3-kinase-dependent mechanism (32, 33), and have been implicated to play a role in insulin-stimulated glucose transport (34-36). Adenovirus-overexpression of a dominant-negative PKC-lambda inhibits insulin-stimulated glucose transport in 3T3-L1 adipocytes (34) and L6 myotubes (36), whereas overexpression of wild-type PKC-lambda was found to enhance insulin-stimulated glucose uptake in L6 cells (36). Likewise, overexpression of wild-type PKC-zeta increased basal and insulin-stimulated glucose transport in 3T3-L1 fibroblasts and adipocytes, with notable decreases reported following dominant-negative overexpression (35). Furthermore, overexpression of an atypical PKC isotype-specific interacting protein was reported to inhibit insulin-stimulated glucose uptake (37). Thus, signal transduction via the atypical PKC isoforms appears to mediate insulin-stimulated glucose transport. Nevertheless, the involvement of PKC-zeta or lambda in regulating insulin-stimulated glucose uptake in primary skeletal muscle has yet to be addressed.

Clearly, there remains considerable uncertainty surrounding the downstream steps involved in the signaling cascade that mediates insulin-stimulated glucose transport. Nevertheless, it is clear that the final event required for skeletal muscle glucose transport involves the trafficking of GLUT4, the predominant glucose transporter isoform expressed in skeletal muscle (38-40). Insulin stimulates glucose uptake in skeletal muscle primarily by eliciting

translocation of GLUT4 from an intracellular pool to the plasma membrane (41, 42). Once fully integrated into the plasma membrane, GLUT4 mediates the transport of glucose into the cell. The importance of GLUT4 for insulin-stimulated glucose transport is underscored in studies utilizing muscle specific GLUT4-knockout mice. In isolated soleus or extensor digitorum longus (EDL) muscle, insulin failed to stimulate glucose transport activity, suggesting that GLUT4 plays an essential role in mediating the glucose transport process in muscle (43). This report is in partial disagreement with earlier studies using whole-body GLUT4-knockout mice (44). While insulin was ineffective in stimulating glucose transport in EDL muscle from whole-body GLUT4 knockout mice, a blunted, but significant effect of insulin was reported in soleus muscle from female GLUT4-knockout mice (44). However, this finding may be attributed to a compensatory adaptation, which may or may not be a part of the normal response in skeletal muscle. Nevertheless, GLUT4 was established as the primary mediator of skeletal muscle glucose transport.

The translocation of GLUT4 is a multi-step process that includes budding or release of GLUT4 containing vesicles from an intracellular storage compartment, movement to the plasma membrane, followed by docking and fusion with the plasma membrane (45). Thus, there are many potential points at which GLUT4 translocation may be impaired in diabetic skeletal muscle. Sub-cellular fractionation studies have shown that insulin stimulates a redistribution of GLUT4 within skeletal muscle fibers from internal membranes to the plasma membrane (41, 42). A more recent advance in the study of GLUT4 traffic in skeletal muscle involves the use of a sensitive exofacial bis-mannose photolabeling technique (46). This technique utilizes a membrane impermeable glucose transporter label, thus allowing for more quantitative studies of GLUT4 translocation to be performed in skeletal muscle. Studies implementing this technological advance have reported that maximal insulin stimulation increased cell surface GLUT4 levels and glucose transport with similar magnitudes (46-48). Furthermore, the calculated turnover rate of GLUT4 is not affected by insulin (48). Thus, the major mechanism for insulin-stimulated glucose transport in skeletal muscle is the translocation of GLUT4 to the plasma membrane rather than modification of catalytic activity.

4. DEFECTS IN INSULIN SIGNAL TRANSDUCTION AND GLUT4 TRANSLOCATION

Reduced insulin-stimulated glucose transport into skeletal muscle of Type II diabetic patients has been clearly established (49-51). Therefore, identifying methods to enhance insulin action in this tissue are likely to result in improved whole-body glucose homeostasis in the diabetic patient. However, if effective strategies for the treatment of skeletal muscle insulin resistance are to be developed, a clear understanding of the underlying cause(s) of reduced insulin-stimulated glucose transport in diabetic muscle must be reached. Certainly this is no easy task given the complexity, and incomplete picture, of the cellular signaling and GLUT4 vesicle trafficking mechanisms that govern glucose transport. Nevertheless, intense research interest has been geared towards identifying diabetes-associated defects in insulin action.

Insulin action at the level the IR, IRS-1, and PI 3-kinase are prime candidates for defects leading to reduced glucose transport in skeletal muscle from Type II diabetic patients, given that these signaling molecules have a well established role in regulating glucose transport. In morbidly obese humans, impaired insulin-stimulated glucose transport in skeletal muscle is associated with decreased autophosphorylation of the IR, impaired IRS-1 phosphorylation, and reduced PI 3-kinase activity (52). However, the downregulation of insulin signaling in obese insulin resistant individuals may be a consequence of a reduction in IR protein expression in muscle from these subjects (52). In skeletal muscle from non-obese Type II diabetic subjects, IR phosphorylation has been reported to be either reduced (53) or unchanged (31, 54) compared to non-diabetic control subjects. In lean to moderately obese Type II diabetic subjects, defects at the levels of IRS-1 and PI 3-kinase have been reported (55). In this study IRS-1 tyrosine phosphorylation was increased 6-fold in skeletal muscle from control subjects following 40 min of *in vivo* hyperinsulinemia, whereas a similar level of insulin exposure was insufficient to stimulate phosphorylation of IRS-1 in Type II diabetic subjects. This absence of IRS-1 phosphorylation could not be attributed to a reduction in IRS-1 protein expression. When PI 3-kinase activity was considered, hyperinsulinemia resulted in a 2-fold stimulation of IRS-1-associated PI 3-kinase activity in muscle from control subjects, with no increase observed in Type II diabetic subjects. Thus, suppression of insulin action at the level of IR, IRS-1, or PI 3-kinase is likely to contribute to reduced glucose transport in skeletal muscle from diabetic patients.

Although a clear involvement of Akt in mediating the effects of insulin on glucose transport activity has yet to be established, it remains an attractive candidate for the development of insulin resistance. In skeletal muscle from Type II diabetic subjects, *in vitro* exposure to maximal insulin concentrations (60 nM) reveals a defect in Akt activation (31). However, when a lower concentration of insulin was used (2.4 nM), Akt activation in diabetic muscle strips was found to be normal. Thus, normal sub-maximal activation of Akt occurs in diabetic muscle despite dramatically reduced levels of glucose transport under identical conditions. Likewise, *in vivo* insulin infusion in type II diabetic subjects is accompanied by normal levels of Akt phosphorylation (56) and kinase activity (30) within skeletal muscle, despite reduced whole-body glucose utilization. In light of these results, Akt has been suggested to have a limited role in insulin resistance in Type II diabetic humans (30). However, Akt2 has been shown to associate with the GLUT4 vesicle in 3T3-L1 adipocytes in response to insulin (57). Therefore, in addition to measurements of total kinase activity, studies addressing the trafficking of this signaling molecule in healthy and diabetic muscle are warranted in order to determine its relevance to the development of insulin resistance.

While no studies thus far have addressed the issue of insulin-stimulated activation of the atypical PKC isoforms (downstream targets of PI 3-kinase) in diabetic skeletal muscle, several studies provide evidence that alterations in the cellular localization of PKC isoforms occur in muscle from diabetic animals (58). Some of these changes may be chronic, occurring in response to elevated levels of glucose, insulin, and/or lipids. Changes in PKC-theta and PKC-epsilon are evident in skeletal muscle from dietary-induced (high-fat-fed) insulin resistant rats and these changes appear to be related to muscle tri- and diacyglycerol levels (59). In the non-obese Goto-Kakizaki (GK) diabetic rat (60), PKC enzyme activity and levels of PKC-alpha, PKC-beta, PKC-epsilon, and PKC-delta are increased in membrane fractions and decreased cytosolic fractions of soleus muscle, relative to control Wistar rats. In addition, PKC-theta levels in GK soleus muscles are decreased in both membrane and cytosol fractions. Changes in the compartmentalization and/or expression of the PKC isoforms may occur in response to an acute increase in glucose availability. Acute exposure of isolated soleus muscle from Wistar rats to 25 mmol/l glucose increased the total membrane content of PKC-beta$_2$ and PKC-zeta by 3- and 1.4-fold, respectively, (61). While the effect of these glucose-induced changes in PKC distribution on skeletal muscle insulin action is not clear, a role for PKC-beta (1 and/or 2) as a modulator of insulin sensitivity in muscle has been proposed. Recently engineered PKC-beta knockout mice display enhanced glucose transport activity in adipose tissue and skeletal muscle suggesting that PKC-beta 1 and/or 2 play a negative role in the regulation of insulin action (62). Furthermore, the ablation of PKC-beta resulted in enhanced glucose transport activity without affecting IRS1-associated PI 3-kinase activity, suggesting that PKC-beta affects insulin action at a level distal to PI 3-kinase.

In addition to impaired insulin signal transduction, defects in GLUT4 expression or vesicle trafficking may contribute to reduced skeletal muscle glucose uptake. In morbidly obese individuals, reduced skeletal muscle expression of GLUT4 may partially account for the insulin resistant state (63), however this does not appear to be the case in lean diabetic subjects where GLUT4 expression has been reported to be normal (64, 65). Nevertheless, defects in GLUT4 vesicle traffic may indeed contribute to insulin resistance in diabetic individuals. For instance, under non-stimulated conditions, the basal pool of GLUT4 in skeletal muscle from insulin resistant individuals was found to sediment to a denser sucrose gradient fraction than that of control subjects (66). This abnormal GLUT4 distribution was associated with reduced GLUT4 translocation following *in vivo* insulin stimulation in diabetic muscle. This suggests that defects in GLUT4 trafficking and translocation can also contribute to insulin resistance in skeletal muscle. Recently, bis-mannose photolabeling was implemented to quantify the magnitude of the insulin response on GLUT4 translocation in skeletal muscle from Type II diabetic patients (67). As observed previously (48), insulin stimulated 3-O-methylglucose transport and cell surface GLUT4 content were tightly correlated in skeletal muscle from healthy individuals. When diabetic muscle strips were examined, glucose transport and cell surface GLUT4 were reduced 40 and 71%, respectively compared to control individuals. Therefore, insulin resistance can be accounted for by reduced cell surface GLUT4 in response to insulin.

Since GLUT4 translocation occurs downstream of insulin signaling, the impairment observed in skeletal muscle from Type II diabetic patients may be due to a defect in either signaling and/or GLUT4 vesicle traffic. A means of addressing this issue is to utilize stimuli that act upon GLUT4 translocation in a manner independent of insulin action. In skeletal muscle hypoxia stimulates glucose transport and GLUT4 translocation by a mechanism independent of insulin signaling (16, 17). In skeletal muscle from Type II diabetic subjects, hypoxia-stimulated GLUT4 translocation was 50% lower versus that observed in healthy subjects (67). Thus, insulin resistance may be partly due to a failure of GLUT4 vesicles to translocate, dock, or fully fuse with the plasma membrane. However, as the defect in insulin-stimulated GLUT4 translocation was greater than that observed following exposure to hypoxia, lesions in signal transduction as well as GLUT4 traffic likely contribute to the insulin resistant state in Type II diabetic muscle.

5. ALTERED METABOLIC STATES CAN SUPPRESS INSULIN ACTION IN SKELETAL MUSCLE

Investigations into the underlying cause(s) of skeletal muscle insulin resistance have uncovered multiple molecular candidates to account for reduced insulin-stimulated glucose transport in diabetic muscle. However there still remains considerable uncertainty why these defects in signaling and/or membrane trafficking exist. Current evidence suggests that the altered metabolic milieu that is associated with the diabetic state may itself constitute a contributing factor to peripheral insulin resistance. For example, hyperglycemia, a characteristic feature of Type II diabetes, appears to be one factor leading to insulin resistance in skeletal muscle. Under *in vitro* conditions, insulin-stimulated glucose transport is fully normalized in isolated skeletal muscle from Type II diabetic patients after a two hour incubation in media containing low (4 mM), but not high (8 mM) glucose (68). Furthermore, in diabetic rodent models, *in vivo* correction of hyperglycemia by phlorizin-treatment improves whole-body insulin sensitivity and restores insulin-stimulated glucose transport in isolated adipocytes and skeletal muscle (27, 69-71). The molecular mechanism by which restoration of glycemia improves insulin action in skeletal muscle appears to involve normalization at the level of Akt (27). Restoration of glycemia by phlorizin treatment in diabetic GK rats partially restored glucose tolerance and fully restored insulin-stimulated Akt activity and glucose transport (27). These improvements in insulin action were independent from insulin action at the level of PI 3-kinase. Similarly, incubation of non-diabetic rat EDL muscle with 25 mM glucose was reported to inhibit insulin action at the level of Akt, but not PI 3-kinase (72). The precise mechanism by which hyperglycemia downregulates insulin action is not fully known. However, the effects of

hyperglycemia may be mediated in part by metabolites of the hexosamine pathway. Increased biosynthetic activity within the hexosamine pathway is associated with the development of insulin resistance (73). However, unlike the effects of high glucose, two hours of glucosamine infusion in the conscious rat was reported to down-regulate insulin signaling at the level of PI 3-kinase activity, whereas activation of Akt was unaltered (74). In any case, these studies are consistent with the notion that hyperglycemia negatively impacts insulin signaling in skeletal muscle.

Another common feature of Type II diabetes, especially when obesity related, is elevated levels of circulating free fatty acids (FFA) (75, 76). Like hyperglycemia, FFA may also contribute to the insulin resistant state. A two hour *in vitro* exposure of rat soleus muscle to high levels of oleate was shown to inhibit 2-deoxyglucose uptake, thus demonstrating a direct role of FFA for development of insulin resistance (77). Furthermore, FFA-induced insulin resistance has been demonstrated *in vivo*, as reduced whole-body glucose utilization has been observed in both rodents (78, 79) and humans (80, 81). In healthy human subjects, a five hour triglyceride/heparin infusion to raise FFA levels is sufficient to reduce insulin-stimulated glucose utilization and muscle glycogen accumulation by as much as 50% (81). These findings are paralleled by a 90% reduction in IRS1-associated PI 3-kinase activity. Similar findings have been reported in rat skeletal muscle following chronic triglyceride/heparin infusion, with defects noted at the level of insulin-stimulated IRS1 phosphorylation, IRS1-associated PI 3-kinase activity, and 2-deoxyglucose uptake (79). Additionally, these defects were also associated with a dramatic shift of PKC-theta localization from the cytosol to the membrane fraction. Thus, while the molecular basis linking FFA to impaired insulin action is still unknown, the role PKC-theta can be considered.

6. SKELETAL MUSCLE FIBER-TYPE: A SPECIAL CONSIDERATION FOR INSULIN ACTION

Based on myosin ATPase staining or enzymatic analysis, skeletal muscle fibers can be classified into three distinct categories: type I (slow-twitch-oxidative), type IIa (fast-twitch-oxidative-glycolytic) and type IIb (fast-twitch-glycolytic) (82). In addition to exhibiting diverse contractile and enzymatic properties, specificity of insulin action occurs in a fiber-type manner. For instance, insulin-stimulated glucose transport is greater in type I oxidative *versus* type IIa or IIb glycolytic muscle (83, 84). This fiber-specific difference upon glucose transport may be of clinical relevance to the development of insulin resistance. In humans, whole-body glucose uptake and muscle glucose transport is positively correlated with type I muscle fibers (85) and negatively correlated with type IIb muscle fibers (86). Furthermore, the percentage of type I muscle fibers are reduced with extreme obesity (85) and inactivity due to paralysis (87), and therefore may be a contributing factor to the development of insulin resistance. Futures strategies to target increased expression of type I skeletal muscle fibers may improve insulin sensitivity in insulin resistant individuals.

The superior glucose transport capacity displayed in type I muscle fibers may be in part due to greater levels of GLUT4 expression, as GLUT4 expression is positively correlated to the percentage of type I fibers (88). However, differences in expression and function of key signaling proteins also influence fiber-type specificity of insulin action (26, 84). Fiber-type specific differences include increased insulin action at the level of IR binding (84), tyrosine phosphorylation of IR, IRS-1 and IRS-2, PI 3-kinase activity, and Akt phosphorylation in rodent skeletal muscle composed predominantly of type I oxidative skeletal muscle fibers (26). These functional differences are associated with increased protein expression of p85α subunit of PI 3-kinase, and Akt kinase, with no reported difference in IR, IRS-1 or IRS-2 between oxidative and glycolytic muscles (26). Therefore, expression and/or function of all identified steps in the insulin signaling pathway to glucose transport are effected by the fiber-type composition of the muscle.

7. EXERCISE: A MEANS TO IMPROVE GLUCOSE HOMEOSTASIS

In skeletal muscle, glucose transport can be acutely activated by at least two separate pathways, one stimulated by insulin, and another activated by muscle contraction/exercise (89-93) (Figure 1). While PI 3-kinase is required for insulin-stimulated glucose transport, inhibition of PI 3-kinase activity does not impair exercise/muscle contraction stimulated glucose transport (15-17). Furthermore, exercise stimulates glucose transport without increasing tyrosine phosphorylation of the IR or IRS1, or kinase activity of PI 3-kinase or Akt (94, 95). The mechanism behind activation of exercise induced glucose transport is unclear, but may be linked to release of calcium from the sarcoplasmic reticulum (96), activation of nitric oxide synthase (97), and/or stimulation of AMP-activated protein kinase (98). Although the precise signaling pathway to exercise-induced glucose transport has not been definitively established, activation of this insulin-independent glucose transport pathway may be one alternative means to activate glucose transport, and thereby improve whole-body glucose homeostasis in insulin-resistant individuals. In skeletal muscle from diabetic rats, muscle contraction stimulates glucose transport despite severe insulin resistance of the glucose transport process (93, 99). Even in muscle from animals with dietary induced insulin resistance, perturbing the muscle contraction pathway with *in vitro* hypoxia or through incubation with W-7, fully activates glucose transport (47). Importantly, a recent report provides evidence that 45-60 min of cycle exercise is sufficient to stimulate GLUT4 translocation in skeletal muscle from healthy subjects and Type II diabetic patients (100). Although the plasma membrane content was ~30% lower in the Type II diabetic patients at rest and after exercise, this difference was not significant. These studies highlight the potential importance of physical exercise in maintaining glucose homeostasis in Type II diabetic patients.

In addition to the beneficial effects of acute exercise, chronic exercise training improves whole-body glucose homeostasis and insulin-stimulated glucose transport (87, 101, 102). Furthermore, physical training improves whole-

body insulin sensitivity in insulin resistant individuals (103, 104). Therefore, improved insulin action through regular exercise training may be one means of overcoming defects in insulin signal transduction in diabetic muscle. The molecular basis for enhanced glucose uptake with chronic exercise training may be related in part to increased expression and/or activity of key proteins involved in the regulation of glucose uptake and metabolism in skeletal muscle. Such candidates include GLUT4 (101, 105), hexokinase II (87, 106), and glycogen synthase (87, 106), proteins known to be upregulated by exercise training.

Since exercise training is associated with upregulation of multiple genes, it is difficult to determine which of the proteins effected by exercise are responsible for enhanced insulin action. Nevertheless, insight into the roles of GLUT4, hexokinase II and glycogen synthase as mediators of training-induced upregulation of glucose transport have been obtained through the use of transgenic mice. The role for GLUT4 is clear, as several investigations have shown that transgenic overexpression of the GLUT4 protein in skeletal muscle results in significant increases in both insulin- and contraction-stimulated glucose uptake (107-110). Furthermore, transgenic overexpression of GLUT4 is sufficient to overcome many insulin resistant states such as those associated with streptozotocin treatment (111), high-fat feeding (112), and obesity (113). Unlike GLUT4, the role of hexokinase II is less clear. Initially, overexpression of hexokinase II was reported to enhance insulin-stimulated glucose transport (114), suggesting that hexokinase activity may be rate-limiting for glucose uptake. However, a separate recent report disputes this finding (115). In this latter report, skeletal muscle glucose uptake was not improved by overexpression of hexokinase II. Furthermore, overexpression of hexokinase in GLUT1 transgenic mice did not further enhance glucose uptake *versus* mice overexpressing GLUT1 alone. Therefore, results from this study indicate that upregulation of hexokinase activity does not enhance glucose transport even when the flux of glucose into the muscle cell is great. Similarly, a minimal role for glycogen synthase in regulating the rate of glucose uptake has been proposed (116). Despite a dramatically enhanced capacity to store glucose as muscle glycogen, overexpression of glycogen synthase does not enhance glucose transport capacity in skeletal muscle.

While exercise-training studies have highlighted the importance of increased GLUT4 for enhanced insulin-stimulated glucose transport, recent investigations have examined the involvement of insulin signal transduction following exercise training (117, 118). For instance, insulin-action at the level of the IR, IRS-1, PI 3-kinase, and Akt are all upregulated within five days of swim training in rats (118). Furthermore, in young healthy humans, seven days of exercise training is sufficient to improve insulin-stimulated PI 3-kinase activity (117). These studies demonstrate the importance of exercise training to enhance insulin signal transduction in skeletal muscle, nevertheless studies examining the potential for such training regimes to improve insulin signal transduction in diabetic muscle are lacking.

8. PERSPECTIVE

Reduced insulin-stimulated glucose transport in Type II diabetes may occur in response to defects in signal transduction and/or GLUT4 translocation in skeletal muscle. While defects in signal transduction have been identified in skeletal muscle from Type II diabetic patients, the degree to which these defects actually contribute to the insulin resistant state is still unclear. Furthermore, relatively little is understood about the nature of the underlying cause(s) of impaired insulin signal transduction and GLUT4 vesicle trafficking in type II diabetic muscle. Future studies geared towards addressing this issue are likely to give rise to new therapeutic strategies for treating insulin resistance. Exercise has been identified as a physiological means to activate glucose transport as well as enhance insulin-stimulated glucose transport in skeletal muscle. Thus, exercise may have important therapeutic implications for controlling glucose homeostasis in insulin resistant diabetic patients in a non-pharmacological manner. Nevertheless, the extent to which exercise can be used to reverse insulin resistance warrants further investigation.

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