Review

Insulin action and insulin resistance in human skeletal muscle

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Introduction

The pivotal role in vivo of insulin is to control fuel homeostasis. Insulin exerts its effect on three main target tissues: liver, fat and skeletal muscle. Resistance to the normal action of insulin contributes to the pathogenesis of a number of common human disorders, including Type I (insulin-dependent) and Type II (non-insulin-dependent) diabetes mellitus, hypertension and the metabolic syndrome x, thus constituting a major public health problem. Environmental factors such as poor nutrition (high-fat diet) or inactivity (lower oxygen consumption i.e. $\text{VO}_{2\text{max}}$) or both also seem to have a major effect on the development of peripheral insulin resistance [1–4]. Although the primary defect in the pathogenesis of Type II diabetes is not known, a combination of genetic and environmental factors probably contribute to the manifestation of this progressive metabolic disorder, which is usually not apparent clinically until mid-life. Type II diabetic patients are characterised by fasting hyperglycaemia and by increased, normal or low concentrations of insulin. Any one of these could be a cause or consequence of defects in insulin secretion from the beta cell or peripheral insulin resistance in skeletal muscle or adipose tissue. Defects in insulin

secretion have been noted in lean or normal weight patients with Type II diabetes [5, 6] and insulin resistance in skeletal muscle has been reported in Type II diabetic patients regardless of weight [7]. Skeletal muscle is the principal tissue responsible for insulin-stimulated glucose disposal [8] and the major site of peripheral insulin resistance [9]. Intense interest has been focused on explaining the molecular basis for the development of insulin resistance in skeletal muscle in an effort to control and improve whole-body glucose homeostasis. This review is focused on current understanding of the molecular mechanisms regulating insulin action and the factors contributing to insulin resistance in human skeletal muscle.

Whole-body insulin resistance in Type II diabetic patients

Most of our current understanding of the mechanisms involved in development of insulin resistance has evolved from in vivo studies of insulin action in diabetic animals and humans using the euglycaemic hyperinsulinaemic clamp technique, with or without radioactive tracers, developed nearly 20 years ago by DeFronzo and colleagues [9, 10]. In vivo studies show that skeletal muscle is the principal site of glucose uptake under insulin-stimulated conditions, accounting for approximately 75 % of glucose disposal after glucose infusion [8, 9, 11]. In the postprandial state, insulin-mediated glucose uptake and utilisation is greatly impaired in Type II diabetic patients [9, 12, 13] and in glucose tolerant first-degree relatives [12, 14]. From in vivo tracer studies, the defect in whole-body glucose uptake in Type II diabetic patients has been localised to the non-oxidative pathway for glucose metabolism [8, 13, 15, 16]. Thus, in vivo methods have provided valuable, yet indirect evidence, to suggest that the reduced rate of non-oxidative glucose

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Abbreviations: IR, Insulin receptor; IRS, insulin receptor substrate; PI, phosphatidylinositol; PKC, protein kinase C; DAG, diacylglyceride; ATB-BMPA, 2-N-4-(1-azido-2,2,2-trifluoroethyl)benzoyl-1,3-bis(d-mannose-4-yloxy)-2-propylamine; GK, Goto-Kakizaki; MAP, mitogen-activated protein; HK, hexokinase.

metabolism observed in Type II diabetic patients results from a defect at the level of glucose transport, glucose phosphorylation or glycogen synthase. In the past decade, several key proteins have been identified which are believed to be important for insulin signal transduction to metabolic and mitogenic responses. Consequently, it is now possible to study cellular, as well as whole-body systems, to explain the molecular basis for the development of peripheral insulin resistance.

Regulation of glucose transport: insulin signal transduction

Initial events related to tyrosine phosphorylation. After insulin binds to the insulin receptor (Fig. 1), the tyrosine kinase activity of the insulin receptor (IR) β -subunit is activated and several key proteins within the cell are phosphorylated, including the insulin receptor substrates (IRS) and Shc [17–19]. To date, four different IRS molecules have been cloned [19]. Evidence is emerging that the different IRS molecules have specialised roles in mediating metabolic and mitogenic effects of insulin [20, 21]. In skeletal muscle, IRS-1 and IRS-2 appear to be the predominant isoforms expressed. Insulin receptor substrate proteins act as docking proteins for downstream signalling molecules containing Src homology 2 domains, including the 85 000 M_r regulatory subunit of phosphatidylinositol (PI) 3-kinase [22]. Phosphatidylinositol 3-kinase has been implicated as a key signalling molecule in insulin-mediated glucose transport in skeletal muscle and adipocytes [23–25]. In 3T3-L1 adipocytes, IRS-1/2 and PI 3-kinase are targeted to intracellular locations, such as the actin cytoskeleton [26, 27]. Thus, signals for glucose transport seem to be compartmentalised within the cell. Recent studies provide evidence for several PI 3-kinase adapter subunit variants in skeletal muscle that have

different insulin-induced PI 3-kinase responses [28–31]. Insulin can recruit multiple adapter subunit isoforms of PI-3 kinase in human skeletal muscle [31]. Four different adapter subunit isoforms contribute to insulin-dependent PI-3 kinase signalling pathways in human skeletal muscle. Similar observations have been made in rodent skeletal muscle [28–30]. The different isoforms of the regulatory subunits have different insulin-induced responses that increase PI 3-kinase and this could provide mechanisms by which specificity is achieved in insulin signalling. The molecular link between PI 3-kinase and glucose transport is not known. Thus, enormous efforts are now being placed on identifying the molecules downstream of PI 3-kinase that are linked to glucose transport.

Serine phosphorylation downstream of PI 3-kinase. The serine/threonine kinase Akt is a downstream target of PI 3-kinase [32–34] and has been implicated as a component of the insulin-signalling pathway to glucose transport [35–39]. Whether Akt is directly coupled to glucose transport is still a topic of debate. Overexpression of an Akt mutant in which the two phosphorylation sites targeted by growth factors are mutated leads to inhibition of both insulin-induced activation of Akt and insulin-stimulated protein synthesis [40] but has no effect on insulin-stimulated glucose uptake in 3T3-L1 adipocytes [40] and L6 muscle myoblasts [39]. When the catalytic site of Akt was, however, also mutated, generating a triple Akt mutant, insulin-mediated GLUT4 translocation was inhibited [39]. These data provide strong evidence that both the phosphorylation sites and the catalytic sites are important for sending the insulin signal downstream from Akt to glucose transport.

Other downstream candidates in the insulin-signalling pathway to glucose transport included members of the protein kinase C (PKC) family. The PKC family consists of at least 12 serine/threonine kinase isoforms that phosphorylate and activate multiple proteins. The conventional PKC isoforms include α , β , and γ and are activated by Ca^{2+} , phosphatidylserine and diacylglyceride (DAG); the *novel* isoforms include δ , ϵ , η and θ and are Ca^{2+} -independent and activated by phosphatidylserine and DAG; and the atypical PKC isoforms include ζ and λ and are insensitive to Ca^{2+} and DAG [41]. In several different cell culture systems, PKCs have been shown to be important regulators of cell growth, differentiation and metabolism [42, 43]. Isoforms of PKC differ substantially in terms of structure, regulation, tissue distribution, expression throughout development, and substrate specificity. The basic mechanism of action for most PKC isoforms involves translocation from cytosol to cellular membranes and a subsequent response to changes in intracellular Ca^{2+} or 1,2-DAG concentration [42, 43]. The exact mechanism regulating iso-

Fig. 1. Simplified outline of insulin signalling in skeletal muscle. PKB, protein kinase B

form-specific PKC activation in skeletal muscle is poorly defined. It is believed that PKCs stimulate glucose transport in skeletal muscle by a mechanism distinct from the insulin and hypoxia pathways [44]. In cell culture systems, two atypical protein kinase C isoforms, PKC λ and PKC- ζ , have been reported to be activated by insulin through a PI 3-kinase-dependent mechanism [45, 46], and have been implicated as having a role in insulin-stimulated glucose transport. Adenovirus-mediated overexpression of a dominant-negative kinase dead mutant of PKC λ inhibited insulin-stimulated glucose transport by 60% in 3T3-L1 adipocytes [47]. Similarly, stable overexpression of wild-type PKC- ζ increased and a dominant-negative mutant form of PKC- ζ decreased basal and insulin-stimulated glucose transport in 3T3-L1 fibroblasts and adipocytes [48]. Thus, atypical PKC isoforms seem to mediate insulin signal transduction downstream of PI 3-kinase. Nevertheless, the physiological role of PKC ζ or λ has not yet been shown in human skeletal muscle. Data on the expression and regulation of the different PKC isoforms in human skeletal muscle is lacking.

Final biological event of glucose transport. Multiple lines of evidence suggest that PI 3-kinase has a central role in mediating insulin signalling to glucose transport in skeletal muscle [23–25]. Insulin stimulates glucose uptake in skeletal muscle primarily by eliciting translocation of GLUT4 from an intracellular pool to the plasma membrane [49–51]. Glucose transporter 4 is the predominant glucose transporter isoform expressed in skeletal muscle [52–54] and it catalyses this rate-limiting step for glucose uptake and metabolism [55–57]. Its translocation is a complex process involving membrane vesicle trafficking and sorting machinery (Fig. 2). The steps in the GLUT4-translocation process include budding or release of GLUT4 from an intracellular reservoir compartment,

transit to the plasma membrane and docking and fusion with the plasma membrane [58]. Thus, there are many potential steps at which GLUT4 translocation could be impaired in diabetic skeletal muscle. In human skeletal muscle, insulin elicits a translocation of GLUT4 from internal membranes to the plasma membrane [51], a finding consistent with studies using rat skeletal muscle [49–51]. Because the majority of the studies to assess signal transduction and glucose transport have been done in cell culture systems or in diabetic animals, little is known of the regulation of these processes under physiological conditions in Type II diabetic patients.

Insulin resistance in skeletal muscle from Type II diabetic patients

Techniques to study human skeletal muscle in vitro. Since the initial observation that skeletal muscle is quantitatively an important site of insulin resistance in Type II diabetic patients [9], the molecular basis of peripheral insulin resistance has been a major challenge to analyse in the intact human. Clearly, whole-body studies of glucose metabolism in humans are essential to further our understanding of metabolic disorders associated with alterations in glucose homeostasis. In vitro methods are, however, also required to study insulin action directly in the muscle tissue. In 1998 a new surgical biopsy procedure to obtain rectus abdominal skeletal muscle specimens under general anaesthesia was presented [59]. Muscle strips were incubated in vitro in the presence of pharmacological concentrations of insulin and glucose transport was reported to be greatly impaired in morbidly obese (BMI ~ 50 kg/m²) people with or without Type II diabetes. With this methodological advance it is now possible to do detailed studies of insulin action on intracellular events in diabetic human skeletal muscle.

Fig. 2. Working hypothesis of cellular GLUT4 traffic. GLUT4 translocates from an intracellular storage site to the plasma membrane. The GLUT4 vesicle is believed to dock, fuse with the plasma membrane and undergo a transition from an occluded to a fully functionally active state to facilitate the glucose transport into the cell. TCA, trichloroacetic acid

Glucose transport in skeletal muscle from Type II diabetic patients. Defects in insulin-stimulated glucose transport are not limited to morbidly obese people [60]. In rectus abdominal skeletal muscle biopsy specimens from lean to moderately overweight Type II diabetic patients, a profound reduction in insulin-stimulated 3-O-methylglucose transport was noted at physiological and pharmacological insulin concentrations (Fig. 3). In these studies, 3-O-methylglucose, a glucose analogue that is transported into the cell by the glucose transporters but is not further metabolised, was used to assess glucose transport. The rate of intracellular accumulation of 3-O-methylglucose is an index of the rate of glucose transport and is not influenced by subsequent steps in glucose metabolism [61]. This suggests that insulin resistance in skeletal muscle from Type II diabetic patient is due to defects

Fig. 3. Insulin-stimulated glucose transport in human skeletal muscle. Basal and insulin-stimulated 3-*O*-methylglucose glucose transport was assessed in isolated rectus abdominus skeletal muscle obtained from age- and weight-matched healthy and Type II diabetic subjects. Reproduced from Andreasson et al. [60] with permission from Acta Physiologica Scandinavica

in glucose transport. Reduced insulin-mediated glucose transport is not limited to Type II diabetic patients because similar defects are noted in muscle from insulin-resistant rodents [62–69].

Comparison between whole-body and cellular studies of glucose uptake. In vitro [3, 59, 60, 70, 71] and in vivo [16, 72] studies in rodents and humans show that the decrease in insulin-mediated peripheral glucose uptake can be localised to a defect in the mechanism involved in insulin action at the level of glucose transport in skeletal muscle. Strikingly, regardless of technique (in vitro vs in vivo), insulin increased glucose transport twofold to fivefold in skeletal muscle from control subjects, with minimal effects noted in Type II diabetic patients. Furthermore, using both techniques, the rate of insulin-stimulated glucose transport in skeletal muscle is reduced by about 50% in Type II diabetic patients [16, 60, 71, 73]. Thus, insulin action on glucose transport seems to be the first step in peripheral glucose utilisation that is impaired in Type II diabetic patients. Whether this is a permanent defect or a short-term down-regulation secondary to the diabetic state is, however, still needs to be explained.

Additional methods to study human skeletal muscle. Despite the indisputable novelty of the rectus abdominal muscle preparation, there are caveats associated with this procedure. For example, general anaesthesia is required during the surgery and this could alter insulin action or glucose metabolism in skeletal muscle. In addition, surgical trauma is asso-

ciated with altered insulin action and reduced glucose transport in skeletal muscle [74]. With the rectus abdominal muscle preparation, surgical stress is impossible to avoid and the extent to which this alters glucose metabolism in the muscle biopsy specimen is not known. Finally, from a practical point of view, there is an inherent dependency upon choosing subjects from a limited number of patients undergoing abdominal surgery, making it a challenge to carefully match study groups. Thus, we have developed an open biopsy procedure to obtain vastus lateralis skeletal muscle under local anaesthesia. One advantage of the open muscle biopsy technique is that the investigator is not limited to choosing patients undergoing surgery, thus giving greater freedom to select the patient group and control subjects. Furthermore, surgical trauma and general anaesthesia can be avoided. Finally, subjects can be matched for age, physical activity, BMI and various other characteristics known to influence insulin sensitivity.

Studies of GLUT4 traffic in skeletal muscle. Using a sensitive exofacial bis-mannose photolabelling technique (ATB[2-³H]BMPA bis-mannose), stimulation of vastus lateralis muscle from healthy young subjects leads to an increase in the cell surface content of GLUT4 [75]. Maximum insulin stimulation increased cell surface GLUT4 contents and glucose transport twofold. Importantly, insulin-stimulated 3-*O*-methylglucose is strongly correlated with cell surface GLUT4 content. Furthermore, the calculated turnover rate of GLUT4 is not affected by insulin. Thus, the major mechanism for insulin-stimulated glucose transport in human skeletal muscle is GLUT4 translocation to the plasma membrane. With the use of new bis-mannose photolabels [76] it is now possible to quantify the magnitude of the insulin response on GLUT4 translocation in skeletal muscle from Type II diabetic patients. Furthermore, using this technique, dose-response relations for insulin-stimulated glucose transport and cell surface GLUT4 content can be assessed in skeletal muscle from the same subject. Studies of the regulation of GLUT4 traffic are essential to our understanding the underlying mechanism for the development of insulin resistance, which is associated with many disease states including diabetes and obesity. Overcoming defects in the insulin signal transduction pathway leading to GLUT4 translocation is likely to improve glucose homeostasis in the diabetic patient.

Defects in insulin signal transduction skeletal muscle

Early steps in the insulin-signalling cascade. Intense interest has now focused on whether the reduced insulin-mediated glucose transport in muscle from

Table 1. Regulation of insulin signalling in skeletal muscle from insulin resistant and Type II diabetic subjects

	Insulin receptor		IRS-1		PI 3-kinase		Glucose transport	
	Binding or protein	Phosphorylation or activity	Protein	Phosphorylation	Protein	Activity	Protein (GLUT4)	Activity
Non-obese diabetic	↔ ^{81,90} ↓ ⁸⁷	↔ ^{81,88} ↓ ^{87,90}	↔ ^{77,81}	↓ ^{77,81}	↔ ⁸¹	↓ ^{77,81}	↔ ^{105,106}	↓ ^{60,70–73,77}
Obese Type II diabetic	↔ ⁹⁰	↓ ^{79,90}				↓ ⁹¹	↔ ^{105,106} ↓ ^{104^a}	↓ ^{59^a,104^a}
Obese	↔ ⁹⁰ ↓ ^{79^a}	↔ ⁷⁹ ↓ ^{78^a,90}	↓ ^{78^a}	↓ ^{78^a}	↓ ^{78^a}	↔ ⁹¹ ↓ ^{78^a}	↔ ^{105,106} ↓ ^{104^a}	↓ ^{59^a,78^a}

^a Decreases in muscle from morbidly obese subjects

people with Type II diabetes results from alterations in the insulin signal transduction pathway [19, 71, 77–81] or from alterations in either traffic or translocation of GLUT4 to the plasma membrane [82, 83]. Evidence from animal studies suggests that insulin-signalling defects in muscle are associated with altered whole-body glucose homeostasis [64, 69, 84–86]. Early and intermediate steps in the insulin signalling cascade including the IR, IRS-1, and PI 3-kinase are candidates for defects leading to reduced glucose transport in skeletal muscle from obese insulin-resistant subjects and Type II diabetic patients (Table 1). In morbidly obese humans [78] and obese rodents [64, 69, 84–86], impaired insulin-stimulated glucose transport in skeletal muscle is associated with decreased IR and IRS-1 protein content, decreased IR and IRS-1 phosphorylation and reduced PI 3-kinase activity. Recently, evidence has been provided for altered protein expression of the regulatory subunit of PI 3-kinase in muscle and liver of Zucker Fatty rats [30]. In contrast to the obese state, in hyperglycaemic, hypoinsulinaemic states, such as in the streptozotocin-induced diabetic rat, IR and IRS-1 phosphorylation and PI 3-kinase activity are enhanced in skeletal muscle [84, 86], despite reduced insulin-stimulated glucose transport [66]. The extent to which these signalling defects are, however, causative in the development of insulin resistance or secondary to the altered metabolic state associated with Type II diabetes, is currently not known.

In skeletal muscle from non-obese Type II diabetic subjects, IR phosphorylation is reduced [87] or unchanged [81, 88] compared with non-diabetic subjects. In lean to moderately obese Type II diabetic subjects, reduced IRS-1 tyrosine phosphorylation and PI 3-kinase activity has been observed in skeletal muscle [77]. Specifically, a rise in serum insulin concentrations from approximately 60 to approximately 650 pmol/l increased IRS-1 tyrosine phosphorylation sixfold over basal concentrations in control subjects, whereas no significant increase was noted in Type II diabetic subjects (Fig. 4). The reduced IRS-1 phosphorylation in skeletal muscle from Type II diabetic patients was not related to changes in IRS-1 protein content [77, 89]. Physiological hyperinsulinaemia increased PI 3-kinase activity

Fig. 4A, B. Insulin-receptor substrate-1 tyrosine phosphorylation in human skeletal muscle. Muscle biopsy specimens were obtained from healthy and Type II diabetic subjects, before and after euglycaemic hyperinsulinaemic clamp. **A** shows basal or insulin-stimulated IRS-1 tyrosine phosphorylation in skeletal muscle from control and Type II diabetic subjects. **B** shows tyrosine phosphorylation of IRS-1 assessed in basal (□) or insulin-stimulated (■) (~700 pmol/l) skeletal muscle [77]. Reproduced from Björnholm et al. [77] with permission from Diabetes

in control muscle twofold, whereas no increase was noted in skeletal muscle from Type II diabetic subjects. Furthermore, 3-*O*-methylglucose transport stimulated by insulin in vitro was 40% lower in isolated muscle from Type II diabetic patients. These findings couple both reduced insulin-stimulated IRS-1 tyrosine phosphorylation and PI 3-kinase activity to impaired insulin-stimulated glucose transport in skeletal muscle from lean to moderately obese Type II diabetic subjects. Thus, in addition to reduced IR-kinase activity [79, 87, 90], defects in insulin-signalling through IRS-1 and PI 3-kinase [77, 81, 91] could contribute to impaired glucose transport [3, 60, 70, 71] and reduced insulin-stimulated GLUT4 translocation in muscle from Type II diabetic patients [92]. Future studies performed in first-degree relatives of Type II diabetic patients could show whether these signalling defects are a primary cause of insulin resistance or secondary to an altered metabolic milieu.

Fig. 5. Time course of Akt phosphorylation. Isolated vastus lateralis skeletal muscle from healthy subjects incubated in vitro in the absence or presence of 60 nmol/l insulin for 4 to 40 min. Muscle lysates were subjected to SDS-polyacrylamide gel electrophoresis and immunoblotted with a phosphospecific polyclonal anti-Akt antibody as described [74]. Results are presented as means $\pm$ SEM

Defects in intermediate steps in the insulin-signalling cascade. The serine/threonine kinase Akt is stimulated by receptor tyrosine kinases and is a downstream target of PI 3-kinase [32–34, 93], through activation of the phosphatidylinositol 3,4,5-trisphosphate-dependent protein kinase [94]. Akt is stimulated in human skeletal muscle in response to in vitro [71, 95] or in vivo [91] insulin exposure. In human skeletal muscle, Akt is rapidly activated by insulin within 8 min (Fig. 5), and activation is sustained during 40 min of insulin stimulation. Insulin-stimulated (60 nmol/l) Akt phosphorylation is reduced in skeletal muscle from Type II diabetic patients compared with control subjects [71]. At lower, more physiological insulin concentrations, Akt activity is, however, similar between control and Type II diabetic subjects. Akt phosphorylation was determined in skeletal muscle after insulin stimulation in vivo (Fig. 6). A modification of the hyperinsulinaemic clamp procedure was used in conjunction with a needle biopsy to obtain vastus lateralis muscle. Muscle biopsy specimens were obtained under local anaesthesia, 30 min after a glucose-priming period. Thereafter, a bolus injection of insulin was given ($18.4 \text{ nmol} \times \text{kg}^{-1} \times \text{min}^{-1}$) for 4 min and hyperinsulinaemia was maintained by a continuous insulin infusion ($5.8 \text{ nmol} \times \text{kg}^{-1} \times \text{min}^{-1}$). At 40 min after the onset of the insulin infusion, a second biopsy specimen was obtained. Serum insulin concentrations at the time of the muscle biopsy were 40 ± 4 and $118 \pm 36 \text{ pmol/l}$ under basal conditions ($p = 0.07$) and 1774 ± 253 and $2080 \pm 242 \text{ pmol/l}$ (NS) under insulin-stimulated conditions for four control and five Type II diabetic subjects, respective-

Fig. 6 A,B. Phosphorylation of Akt in response to in vivo insulin infusion. Vastus lateralis muscle biopsy specimens were obtained from healthy or Type II diabetic subjects before (B) or after a 40 min insulin infusion (I). Muscle lysates were analysed for Akt phosphorylation as described in Fig. 5. **A** Representative immunoblot. **B** Results are presented as means $\pm$ SEM

ly. When skeletal muscle was stimulated in vivo by means of a hyperinsulinaemic clamp, Akt phosphorylation was similar between moderately overweight Type II diabetic patients and control subjects (Fig. 6). These findings are consistent with a previous report showing normal insulin action on Akt in obese subjects with or without Type II diabetes [91]. Furthermore, insulin-mediated activation of the three Akt isoforms was normal in skeletal muscle from obese Type II diabetic patients despite reduced IRS-1-associated and IRS-2-associated PI 3-kinase activity [91]. Thus insulin resistance at the level of Akt does not appear to account for the impaired insulin stimulation of glucose transport noted in skeletal muscle from obese or Type II diabetic subjects.

Several studies provide evidence that alterations in the distribution of PKC isoforms occur in skeletal muscle from diabetic animals [96]. Some of these changes could be long-term, occurring in response to increased concentrations of glucose, insulin, or lipids. Changes in PKC θ and PKC ϵ are evident in skeletal muscle from dietary-induced (high-fat fed) insulin-resistant rats and these changes seem to be related to muscle triglyceride and diglyceride concentrations [97]. In the non-obese Goto-Kakizaki (GK) diabetic rat [98], PKC enzyme activity and the content of PKC α , PKC β , PKC ϵ and PKC δ is increased in membrane fractions and decreased cytosolic fractions of

soleus muscle, relative to control Wistar rats. In addition, PKC ϑ content in GK soleus muscles is decreased in both membrane and cytosol fractions, whereas PKC- ζ content is not changed in either fraction. Changes in the distribution or expression or both of the PKC isoforms could occur in response to a short-term increase in glucose or lipid availability. Short-term exposure of isolated soleus muscle from Wistar rats to 25 mmol/l glucose increased the total membrane content of PKC β_2 and PKC ζ threefold and 1.4-fold, respectively, whereas membrane content of PKC α , β_1 , δ , ϵ and ϑ were unchanged [99]. The effect of these glucose-induced changes in PKC distribution on intracellular events in skeletal muscle is, however, currently not known. High extracellular glucose concentrations have been reported to inhibit insulin action by inducing serine phosphorylation of the insulin receptor through a PKC-mediated mechanism [100]. In addition, increases in circulating concentrations of non-esterified fatty acids (NEFA) lead to an activation of PKC ϑ and a reduction in insulin-stimulated PI 3-kinase activity associated with IRS-1 [101]. Increased membrane-associated PKC activity leads to reduced insulin action in freshly isolated circulating mononuclear cells, liver and skeletal muscle in obese people with and without Type II diabetes [102, 103]. Because hyperglycaemia or increased concentrations of NEFA or both can have a pronounced effect on whole-body glucose homeostasis, activation of the PKCs by high glucose or increased concentrations of NEFA could be linked to impaired insulin signal transduction and reduced glucose transport in Type II diabetic patients.

Defects in GLUT4 traffic. Defects in the GLUT4 translocation machinery could contribute to impaired insulin-stimulated, whole-body glucose uptake [82, 83, 92]. This could be a consequence of impaired insulin signal transduction or a defect(s) in GLUT4 traffic or both. In morbidly obese people, the reduced insulin-stimulated glucose transport could also be explained in part from reduced GLUT4 expression in skeletal muscle [104]. In lean Type II diabetic patients, total GLUT4 protein expression in skeletal muscle is, however, not altered [105, 106]. Thus, impaired function or distribution of GLUT4 probably accounts for reduced glucose uptake [92]. Nevertheless, GLUT4 has an abnormal subcellular localisation in skeletal muscle from insulin-resistant subjects without or with overt Type II diabetes [82]. This suggests that defects in GLUT4 trafficking and translocation are a cause of insulin resistance in skeletal muscle. Thus, insulin resistance could be partly due to a failure of GLUT4 vesicles to translocate, dock or fully fuse with the plasma membrane (Fig. 2).

In rodents the reduced insulin-stimulated glucose transport in soleus muscle observed after high-fat feeding [62], can be accounted for by a decrease

Fig. 7. Correlation between cell-surface GLUT4 and glucose transport. Comparison between basal and insulin-stimulated (2000 μ U/ml) 3-O-methylglucose transport ($\square$) and cell-surface GLUT4 content as assessed by photolabelling ($\blacksquare$) in isolated soleus muscle from mice fed a high-fat or standard rodent chow. * $p < 0.05$, ** $p < 0.01$ fat-fed vs chow-fed mice. Reproduced from Zierath et al. [62] with permission from Diabetes

in cell surface GLUT4 content as assessed by exofacial labelling with ATB[2- 3 H]BMPA bis-mannose (Fig. 7). Thus, insulin resistance associated with high-fat feeding results from impaired insulin-induced translocation or fusion of GLUT4 with the plasma membrane or both. This could result from an acquired defect in the activation of PI 3-kinase by insulin because PI 3-kinase associated with IRS-1 is also reduced in skeletal muscle after high-fat feeding [62]. In the same study, okadaic acid-stimulated glucose transport was, however, reduced in skeletal muscle after high-fat feeding. Because okadaic acid seems to activate glucose transport at a point downstream from PI 3-kinase [107], this compound could also affect proteins involved in the traffic of GLUT4 vesicles to the plasma membrane. Thus, reduced insulin-stimulated glucose transport could result from defects in early components of the insulin signalling cascade, as well as from signalling defects which alter GLUT4 exocytosis from an intracellular compartment to the plasma membrane. A critical undefined, downstream signalling intermediate could, however, be severely impaired in diabetic skeletal muscle and this could account for the reduced glucose transport.

Hyperglycaemia as a negative regulator of glucose transport

Chronic hyperglycaemia is a characteristic feature of Type II diabetes and could be one factor leading to the development of peripheral insulin resistance in skeletal muscle. In diabetic rodents, substantial evidence has accumulated to suggest that chronic hyperglycaemia can directly contribute to the development

of peripheral insulin resistance [67, 68, 108–110]. The precise sensing mechanism by which hyperglycaemia leads to impaired insulin action is not fully known. The effects of hyperglycaemia are mediated in part by metabolites of the hexosamine pathway. Increased biosynthetic activity within the hexosamine pathway is associated with the development of insulin resistance [110]. In partially pancreatectomised diabetic rats, correction of hyperglycaemia by phlorizin treatment restored insulin-stimulated glucose transport in isolated adipocytes and improved whole-body insulin sensitivity [108–110]. In Type II diabetic patients, restoration of euglycaemia after weight loss [111, 112], sulphonylurea treatment [113, 114] or insulin therapy [115, 116] enhances insulin sensitivity in adipocytes and skeletal muscle. We have shown that insulin-stimulated glucose transport is fully restored to normal in isolated skeletal muscle from Type II diabetic patients after a 2-h exposure *in vitro* to media containing low (4 mmol/l), but not high (8 mmol/l) glucose [73]. Furthermore, restoration of blood glucose concentrations in patients with poorly controlled Type I diabetes [117] is associated with improved insulin-mediated, whole-body glucose uptake. Although restoration of glycaemia is associated with improved insulin action on glucose uptake, the extent to which restoring glycaemia to normal can improve impaired insulin signalling in skeletal muscle from insulin-resistant animals or humans is not fully known.

The molecular mechanism by which restoration of glycaemia improves insulin action in skeletal muscle could involve improved insulin signal transduction at the level of IRS-1, PI 3-kinase, Akt kinase, or GLUT4 translocation. Functional defects have been reported at each of these steps in skeletal muscle from diabetic rodents [30, 64, 69, 84–86] and humans [71, 77, 78, 91, 118]. Effects of hyperglycaemia on insulin signal transduction and glucose transport have been assessed in the spontaneously diabetic GK rat, a non-obese model of Type II diabetes [67, 68]. This animal model of diabetes is developed by selective breeding of glucose-intolerant Wistar rats over several generations [119]. Restoration of glycaemia by phlorizin treatment in GK rats partly restored glucose tolerance and fully restored insulin-stimulated signal transduction at the level of Akt and glucose transport [67, 68]. The improvements in insulin action were completely disassociated from insulin action at the level of PI 3-kinase [68]. Similarly, incubation of non-diabetic rat soleus muscle with 25 mmol/l glucose has been found to inhibit insulin action on glycogen synthase and Akt but not PI 3-kinase [120]. Thus, it could be that full activation of PI 3-kinase is not necessary for full activation of glucose transport. Furthermore, there are probably other signalling pathways mediating insulin action, glucose transport and glycogen synthesis. Collectively, these data are consistent with the notion that chronic hyperglycaemia

has deleterious effects on the intermediate and final components of the insulin signalling pathways in skeletal muscle.

Muscle contraction as a positive regulator of glucose transport

In skeletal muscle, glucose transport can be activated by at least two separate pathways, one stimulated by insulin, insulin mimicking agents and insulin-like growth factors, and one activated by muscle contraction/exercise, hypoxia [2, 3, 23–25, 62, 121–126]. Phosphatidylinositol 3-kinase is involved in insulin-stimulated but not contraction-stimulated glucose transport and GLUT4 translocation [23–25]. Whereas, 5'-AMP-activated kinase [127, 128], as well as increases in cytoplasmic calcium concentrations [129, 130], could be involved in the contraction response because they both lead to insulin-independent increases in glucose transport. Reduced glucose transport activity in skeletal muscle from Type II diabetic patients can occur from defects in insulin signalling or GLUT4 vesicle traffic to the plasma membrane. A means of addressing whether a trafficking impairment contributes to the insulin resistance in Type II diabetes is to examine the translocation of GLUT4 in response to stimuli other than insulin. Exposure of isolated skeletal muscle to hypoxia leads to an insulin-independent increase in glucose transport and GLUT4 translocation [62, 125, 126]. Hypoxia and exercise induce GLUT4 recruitment to the cell surface and increase glucose transport [62, 125, 126, 131]. Therefore, perturbing the muscle contraction/hypoxia pathway is a method to show whether the insulin resistance associated with Type II diabetes is limited to the insulin-signalling cascade or is a consequence of generalised resistance in the mechanism(s) involved in GLUT4 translocation. In skeletal muscle from diabetic rats, muscle contraction stimulates glucose transport despite severe insulin resistance of the glucose transport process [2, 121, 132, 133]. 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 [62]. Thus, activation of the muscle contraction pathway is a powerful stimulator of glucose transport and GLUT4 translocation and could be one alternative means to activate glucose transport in insulin-resistant conditions.

Exercise as a therapeutic intervention in the management of Type II diabetes

For the well being of the patient and from a public health care perspective, effective intervention strategies are essential to develop to reduce the incidence

of Type II diabetes and the resulting complications. In healthy, non-diabetic people, long-term exercise has very little effect on blood glucose concentrations. In healthy subjects and Type II diabetic patients, short-term exercise has, however, long been known to increase glucose uptake in skeletal muscle [134]. Recently, short-term exercise has been shown to increase glucose transport in isolated skeletal muscle from healthy subjects (Fig. 8). In human skeletal muscle, exercise is a potent stimulator of glucose transport [3]. The last observation provides direct evidence that in humans, exercise is a potent regulator of glucose transport activity [3]. Furthermore, the combined effect of insulin and exercise are additive on glucose transport activity [3], a finding which is consistent with studies using isolated rat epitrochlearis muscle [121, 133].

Effects of short-term exercise on glucose transport. Short-term exercise could be one strategy to improve glucose homeostasis in Type II diabetic patients. For example, exercise of moderate to heavy intensity is usually associated with a decrease in blood glucose concentration in Type II diabetic patients. Thus, a single bout of exercise often leads to a decrease in plasma glucose concentrations [135–137] and this persists into the post-exercise period [135, 138]. The effect of exercise in lowering blood glucose in Type II diabetic patients could be explained by the insulin-independent activation of glucose transport and GLUT4 by exercise [132] as well as by increased insulin sensitivity [139]. Activation of this alternative pathway could be one way to improve glucose homeostasis by increasing glucose uptake and lowering blood glucose concentrations in Type II diabetic patients. A preliminary report provides evidence that 45–60 min of cycle exercise at 60–70 % $\text{VO}_{2\text{max}}$ led to a similar mean increase in plasma membrane GLUT4 content ($\sim 70\%$ above basal contents) in skeletal muscle from healthy subjects and Type II diabetic patients [140]. Although the plasma membrane content was about 30 % lower in the Type II diabetic patients at rest and after exercise, this difference was not statistically significant. These studies highlight the potential importance of physical exercise in maintaining glucose homeostasis in Type II diabetic patients.

Effects of exercise training on glucose transport. Improvements in insulin sensitivity through regular exercise could overcome defects in insulin signal transduction noted in muscle from Type II diabetic patients. Importantly, exercise training improves glucose tolerance and insulin action in insulin-resistant humans [141, 142]. The molecular mechanism for enhanced glucose uptake with long-term exercise training could be related in part to increased expression and activity of key proteins involved in the regulation of glucose uptake and metabolism in skeletal muscle. Molecular

Fig. 8. Exercise-induced increase in glucose transport in human skeletal muscle. The separate or combined effects of exercise (bicycle exercise to exhaustion) or maximum insulin (1000 $\mu\text{U}/\text{ml}$) on 3-*O*-methylglucose transport in human skeletal muscle. Muscle biopsy specimens were obtained from vastus lateralis muscle. Reproduced from Zierath [3] with permission from Acta Physiologica Scandinavica

candidates for improved glucose homeostasis in connection with exercise include GLUT4 [142–149], hexokinase (HK) II [149–151] and glycogen synthase [149, 152–154]. Muscle contraction/exercise directly leads to the activation of several mitogen-activated protein (MAP) kinase cascades [155–159]. The MAP kinase family forms a major and ubiquitous intracellular signalling system, related to cell growth, differentiation and survival [160]. Extracellular-regulated kinase 1/2 phosphorylation and p38 MAP kinase phosphorylation are increased in response to forced contraction of isolated rat skeletal muscle by electrical stimulation [159] and through a more physiological means to achieve muscle contraction by short-term (30 min) bicycle exercise in humans [158]. Activation of the MAP kinase cascade could provide a mechanism by which muscle contraction alters gene expression. Clearly additional studies are warranted to link the MAP kinase signalling cascade to changes in protein expression in skeletal muscle after exercise.

Glucose transporter 4 has been identified as a key player in the regulation of glucose transport. Thus, an exercise training-induced overexpression of GLUT4 could improve glucose transport in diabetic muscle. A direct connection between increased GLUT4 protein expression and increased basal and insulin-stimulated glucose transport and metabolism has been made from GLUT4-transgenic mouse models [126, 161–164]. Several groups have successfully shown overexpression of GLUT4 in skeletal muscle can prevent impaired whole-body glucose homeostasis associated with various states of insulin resistance. For example, modest overexpression (twofold) of GLUT4 in heart, skeletal muscle and adipose tis-

sue in transgenic mice prevented the impairment of glycaemic control and the accompanying hyperglycaemia caused by high-fat feeding [165]. Furthermore, GLUT4 overexpression in skeletal muscle considerably improved whole-body insulin action and reduced blood glucose concentrations in streptozotocin-induced diabetic [166] and homozygous genetically predisposed diabetic (*db/db*) mice [167]. Although glucose transport is generally believed to be rate limiting for insulin-mediated glucose metabolism in muscle [55, 168], under hyperglycaemic or hyperinsulinaemic conditions, the rate-limiting step could shift beyond transport [169]. Note, transgenic overexpression of HK II, the predominant HK isoform in skeletal muscle, leads to increased basal and insulin-stimulated glucose uptake [170].

Perturbations which alter neuromuscular activity patterns, including physical exercise or long-term electrical stimulation, are physiological means to achieve protein overexpression of key genes involved in maintaining glucose homeostasis [142–149]. Exercise training leads to a twofold to fourfold increase in GLUT4 protein expression in human skeletal muscle and improves glucose intolerance and insulin action [142, 146, 148, 149, 171]. Muscle contraction improves insulin action on whole-body and cellular glucose uptake in cervical-cord-injured people through a major increase in protein expression of key genes involved in the regulation of glucose metabolism [149]. Furthermore, exercise training leads to an increase in basal and insulin-stimulated glucose transport in human skeletal muscle [149]. Nevertheless, adaptive responses to prior exercise are not limited to changes in GLUT4 expression, as increased expression or function of proteins involved in insulin-signal transduction could occur [172, 173]. For example, insulin-stimulated receptor phosphorylation, IRS-1 tyrosine phosphorylation and PI 3-kinase activity associated with IRS-1 are increased 16 h after 1 or 5 days of swimming training in rats [173]. Future studies are warranted to fully ascertain the extent to which the exercise training-induced increase in GLUT4 protein content can improve defects in glucose transport in skeletal muscle from Type II diabetic patients. Collectively, these studies underscore the importance of exercise training as a therapeutic strategy to maintain whole-body glucose homeostasis. Importantly, they provide molecular evidence that exercise leads to changes in activity and expression of proteins known to regulate cell growth and glucose homeostasis.

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