Insulin signal transduction in human skeletal muscle: identifying the defects in Type II diabetes

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Abstract
Type II diabetes is characterized by defects in insulin action on peripheral tissues, such as skeletal muscle, adipose tissue and liver and pancreatic β-cell defects. Since the skeletal muscle accounts for approx. 75% of whole body insulin-stimulated glucose uptake, defects in this tissue play a major role in the impaired glucose homeostasis in Type II diabetic patients. Thus identifying defective steps in this process may reveal attractive targets for drug development to combat insulin resistance and Type II diabetes. This review will describe the effects of insulin on glucose transport and other metabolic events in skeletal muscle that are mediated by intracellular signalling cascades. Evidence for impaired activation of the insulin receptor signalling cascade and defective glucose transporter 4 translocation in the skeletal muscle from Type II diabetic patients will be presented. Through the identification of the intracellular defects in insulin action that control glucose homeostasis, a better understanding of the disease pathogenesis can be gained and strategies for intervention may be developed.

Insulin resistance and Type II diabetes
Insulin resistance is defined as a failure to respond to normal concentrations of circulating insulin [1]. This can be associated with Type II diabetes, obesity, aging, physical inactivity and a genetic predisposition. Insulin resistance is characterized by a decreased ability of insulin to act on peripheral tissues, such as skeletal muscle and adipose tissue, and a failure of insulin to inhibit hepatic glucose output [2]. The fundamental defects in the pathogenesis of Type II diabetes mellitus are insulin resistance in skeletal muscle and liver. Moreover, in frank Type II diabetes, the insulin-secreting pancreas is unable to compensate for the relative insulin deficiency caused by the insulin resistance. Hyperglycaemia is associated with a number of complications, such as cardiovascular disease, renal failure, retinopathy and neuropathy. The primary defect contributing to Type II diabetes is unknown, but a combination of genetic and environmental factors is supposed to play a role in the development of the disease. The most important parts of any treatment programme for Type II diabetic subjects are diet, exercise and weight loss [3]. There are now different classes of oral drugs to treat Type II diabetes (reviewed in [4]). However, currently available drugs are often incompletely effective and the treatment goals in many Type II diabetic patients are difficult to achieve. New molecular targets need to be identified for the development of new drugs to combat insulin resistance and Type II diabetes. Since skeletal muscle glucose transport, a process that normally accounts for approx. 75% of whole body insulin-stimulated glucose uptake, is impaired in Type II diabetic subjects, identifying defective steps in this pathway may reveal attractive targets for drug development.

Early steps in insulin signal transduction
The effects of insulin on glucose transport and other metabolic events in skeletal muscle are mediated by intracellular signalling cascades (Figure 1). Initial tyrosine phosphorylation of the receptor and downstream substrates signals to a series of phosphorylation/dephosphorylation events of tyrosine and serine/threonine kinases. These kinases transmit the insulin signal to metabolic events within the cell.

Insulin receptor
Insulin initiates its action by binding to the insulin receptor. The insulin receptor belongs to a family of growth factor receptors, all with protein tyrosine kinase activity (reviewed in [5]). The insulin receptor is a heterotetrameric membrane glycoprotein consisting of two α-subunits and two β-subunits. Insulin binds to the extracellular α-subunit of the receptor and induces a conformational change that brings the α-subunits closer together. This leads to a rapid auto-phosphorylation of the receptor. Multiple tyrosine sites are phosphorylated on the activated insulin receptor. Phosphorylation of Tyr626 creates a recognition motif for the phosphotyrosine-binding domain of the IRSs (insulin receptor substrates) [6]. Results from studies of insulin receptor phosphorylation in the skeletal muscle from Type II diabetic subjects are contradictory. Insulin receptor phosphorylation appears to be normal or reduced in non-obese Type II diabetics [7].

IRSs
At least 12 substrates of the insulin receptor have been identified so far: IRS-1, IRS-2, IRS-3, IRS-4, IRS-5, IRS-6, Gab-1,
Insulin signalling pathway to metabolic and gene regulatory events in the skeletal muscle

Insulin signalling downstream of PI3K

Intracellular serine/threonine kinases, including Akt, PKC and p70S6 kinase are activated by the formation of lipid products of PI3K. These kinases require the involvement of IRS-1 to transmit the insulin signal to downstream biological events [11,23].

PDK (phosphoinositide-dependent protein kinase)/Akt

Akt (protein kinase B, c-Akt) is one of the serine/threonine kinases downstream of PI3K. Akt was originally implicated in cancer development, promoting cell proliferation and inhibition of apoptosis. Insulin and other growth factors acutely activate Akt [24]. Three isoforms of Akt have been identified: Akt1, Akt2 and Akt3, all of which are ubiquitously expressed. Full activation of Akt1 requires phosphorylation of two specific sites, Thr308 and Ser473 [25]. Akt1 is activated by phosphorylation of Thr308 by PDK-1. The mechanism for phosphorylation of Ser473 is unclear, but PDK-2 is believed to be involved. Both Akt1 and Akt2 are involved in insulin signal transduction in skeletal muscle and adipose tissue. In contrast, Akt3 is not activated by insulin in liver, muscle or adipose tissue [26].

The importance of this pathway is unknown in human insulin-sensitive tissues and the role of this pathway in the skeletal muscle is not clear [22].

PI3K

PI3K is activated by insulin, insulin-like growth factor-1 and other growth factors. PI3K is a heterodimeric lipid kinase with a broad range of cellular functions, including growth and differentiation, synthesis and degradation of carbohydrates, proteins and lipids, and membrane trafficking (reviewed in [18]). PI3K consists of a regulatory subunit that associates with a catalytic subunit. The regulatory subunit binds the IRSs, whereas the catalytic subunit phosphorylates phosphatidylinositol in the membrane. PI3K is supposed to phosphorylate phosphatidylinositol 4,5-bisphosphate at position 3 of the inositol ring to generate the putative lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate [19]. Inhibition of PI3K by either Wortmannin or LY-294002 blocks the formation of phosphatidylinositol 3,4,5-trisphosphate within the cell and leads to the inhibition of several intracellular events, most importantly GLUT4 (glucose transporter 4) translocation, thereby inhibiting insulin-stimulated glucose transport in the skeletal muscle [20]. Activation of PI3K with insulin is insufficient for insulin-stimulated glucose transport [21]. A new pathway suggests that CAP (Cbl-associated protein)/Cbl may play a role in glucose uptake. CAP/Cbl is recruited to the insulin receptor in 3T3-L1 adipocytes and disruption of this interaction attenuates insulin-stimulated glucose transport [21].

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Glucose transport
The rate-limiting step in whole body glucose metabolism under normoglycaemic conditions is the transport of glucose into the skeletal muscle cells [28]. Glucose enters the cell by facilitated diffusion mediated by a group of structurally related GLUT proteins. To date, at least 12 GLUTs have been described [29]. In skeletal muscle and adipose tissue, GLUT1 mediates basal glucose transport, whereas GLUT4 is responsible for insulin-mediated glucose uptake [30]. Exercise, independently from insulin, also promotes GLUT4 translocation and glucose transport in the skeletal muscle [31]. Insulin-stimulated glucose transport is reduced in the skeletal muscle from Type II diabetic subjects. Whereas total GLUT4 protein expression is unchanged in these subjects, cell surface GLUT4 content is reduced under insulin-stimulated conditions [7].

Mechanisms impairing insulin receptor/IRS/PI3K activation
Dysregulation of the insulin receptor or IRSs constitutes a common feature in insulin resistance. Mechanisms that could explain how this dysregulation might occur include TNFα (tumour necrosis factor α)-mediated down-regulation of mRNA transcription, kinase-mediated serine/threonine phosphorylation, proteosome-mediated degradation and phosphatase-mediated dephosphorylation.

The cytokine TNFα is produced by adipocytes, and expression at both mRNA and protein level is increased in most animal models of obesity and obese humans [32]. However, the role of TNFα in insulin resistance is controversial [32,33]. Incubation of 3T3-L1 adipocytes with TNFα reduces insulin-stimulated glucose transport, which could be a consequence of reduced insulin receptor, IRS-1 and GLUT4 protein expression [34]. PKC is another serine/threonine kinase downstream of PI3K. Insulin activates PKC isoforms in the skeletal muscle, and dysregulation of PKC action has been linked to the regulation of glucose transport [35]. PKCs are believed to induce serine phosphorylation of the insulin receptor and IRS-1. Incubation of cells with activators of PKC is associated with increased phosphorylation of Ser612 on IRS-1 [36].

Phosphorylation of Ser612 on IRS-1 has been proposed to inhibit insulin signalling, thereby providing a potential mechanism accounting for insulin resistance [36]. c-Jun N-terminal kinase has been hypothesized to interact at Ser507 on IRS-1 and down-regulate IRS-1 tyrosine phosphorylation in response to stimulation with TNFα [37]. This, together with the finding that an increase in phosphorylation of Ser507 on IRS-1 is observed in the skeletal muscle from healthy subjects after a euglycaemic hyperinsulinaemic clamp, suggests the relevance of this mechanism to human insulin resistance [38]. Especially important will be the investigation of this mechanism in the skeletal muscle from Type II diabetic subjects, where defects in the insulin signalling are due to functional defects in the insulin signalling cascade and are unrelated to alterations in protein expression. Insulin treatment of 3T3-L1 adipocytes increases the degradation rate of IRS-1, thereby reducing its expression [39]. This has been confirmed in cultured cell systems [40], and is specific to IRS-1, since IRS-2 expression is not affected. Several pathways for protein degradation have been identified. A further study proposes the involvement of PI3K, but not the mammalian target of rapamycin in the IRS-1 degradation pathway [41].

PTPs (protein-tyrosine phosphatases) have been implicated in the regulation of the insulin signalling pathway. PTP-1B interacts directly with the insulin receptor [42]. PTP-1B knockout mice have increased insulin receptor and IRS-1 tyrosine phosphorylation in the skeletal muscle, concomitant with enhanced insulin sensitivity [43]. On the basis of these results, inhibition of PTP-1B is suggested as a potential target for the treatment of insulin resistance. Unexpectedly, reduced PTP-1B expression and activity was noted in the skeletal muscle from Type II diabetic subjects [44], emphasizing the importance of early validation of drug targets in relevant human tissues. In obese non-diabetic people, PTP-1B expression and PTP activity are increased in the skeletal muscle [45], implicating differential regulation of PTP-1B in Type II diabetes and obesity. Collectively, these results suggest that inhibition of specific PTPs offers a potential strategy to improve insulin sensitivity in obesity and Type II diabetes.

Treatment of Type II diabetes
The ultimate goal for examining the insulin signalling pathway and glucose transport process in insulin-sensitive tissues in states of insulin resistance is to identify molecular targets that can be manipulated to reduce hyperglycaemia and improve glucose uptake in Type II diabetic subjects. Currently, drugs have been developed without the knowledge of the exact mechanism of action. Recently, an improved understanding of processes regulating glucose homoeostasis has developed; however, much is still unknown. One approach that has gained attention is the identification of a small molecule insulin-mimetic that can activate the insulin receptor and downstream signalling to glucose transport [46]. Oral administration of this insulin-mimetic to animal models of Type II diabetes proved to have blood glucose lowering effects. Biological validation of this approach in humans is currently lacking.

Many of the current therapeutic approaches are also under extensive biological re-evaluation. The mechanism by which the commonly used anti-diabetic therapy metformin inhibits hepatic glucose production and hepatic steatosis is incompletely resolved. AMPK (AMP-activated protein kinase) activation is required for part of the beneficial effects of metformin [47], which also has direct effect on stimulating glucose transport in isolated skeletal muscle [48]. Peroxisome-proliferator-activated receptor γ agonists constitute another widely studied class of compounds to treat Type II diabetes. Peroxisome-proliferator-activated receptor γ is the molecular target for thiazolidinediones. The thiazolidinedione rosiglitazone increases insulin sensitivity in peripheral adipocytes, which results in lower plasma fatty acids and a redistribution of intracellular lipid from skeletal muscle...
References


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and liver to peripheral adipocytes [49]. In addition, defective PKC/ζ activation in skeletal muscle and adipocytes is improved by resiglitazone treatment, leading to increased adipocyte glucose transport as a consequence [50].

Conclusion

Hyperglycaemia in Type II diabetes mellitus is a consequence of a reduction in the ability of the skeletal muscle to clear glucose from the blood. Insulin-stimulated glucose uptake, assessed under in vivo and in vitro conditions is decreased in the skeletal muscle from Type II diabetic patients and various animal models of the disease. Studies in the skeletal muscle from Type II diabetic patients demonstrate impaired insulin activation of the IRS-1/P13K signalling pathway to glucose transport. In addition, defective GLUT4 translocation in the skeletal muscle may also contribute to impaired glucose homeostasis in Type II diabetic patients. Through the identification of the intracellular defects in insulin action, a better understanding of the disease pathogenesis can be gained and strategies for intervention may be developed.

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