Does tumour necrosis factor α influence insulin sensitivity in skeletal muscle?

Skeletal muscle is the primary site for insulin-stimulated glucose uptake. Insulin resistance, and in particular resistance to the actions of insulin in skeletal muscle, is a major factor in the pathogenesis of Type II diabetes. Insulin increases glucose transport in skeletal muscle by inducing the translocation of GLUT4 (the major insulin-regulated glucose transporter) from intracellular vesicles to the plasma membrane. Although expression of the GLUT4 gene is normal in skeletal muscle from patients with Type II diabetes, defective insulin action is associated with impaired trafficking and function of GLUT4. Insulin signalling involves a cascade of events initiated by insulin binding to its cell-surface receptor. This is followed by receptor autophosphorylation and activation of receptor tyrosine kinases, which result in tyrosine phosphorylation of insulin receptor substrates (IRSs), including IRS-1, IRS-2, IRS-3, IRS-4, Gab1 and Shc. Binding of IRSs to the regulatory subunit of phosphoinositide 3-kinase (PI 3-kinase) at Src homology 2 (SH2) domains results in activation of PI 3-kinase, which is necessary for insulin action on glucose transport. PI 3-kinase activation is responsible, at least in part, for insulin stimulation of GLUT4 translocation from intracellular vesicles to the plasma membrane.

Insulin-stimulated PI 3-kinase activity is decreased in lean subjects with Type II diabetes, suggesting that a defect in insulin signalling could contribute to impaired GLUT4 translocation and insulin resistance. However, the mechanism(s) contributing to reduced insulin-mediated glucose uptake in people with Type II diabetes and insulin resistance are poorly understood. Despite a marked increase in our knowledge about insulin signalling mechanisms, the downstream pathways by which impaired insulin-stimulated activation of PI 3-kinase results in decreased GLUT4 translocation are poorly understood.

Obesity (body mass index > 30 kg/m²) is associated with a substantial increase in the relative risk of developing diabetes. Many cytokines are expressed by adipose tissue, and because some of these cytokines, such as tumour necrosis factor α (TNFα), have been shown to reduce insulin-stimulated glucose uptake in adipocytes, it has been suggested that increased cytokine production may contribute to insulin resistance in Type II diabetes. Although there is a body of evidence showing that TNFα reduces insulin-stimulated glucose uptake, and also increases lipolysis in adipose tissue, there is less convincing evidence to support a causal relationship between TNFα and insulin resistance in skeletal muscle. Since reduced insulin-mediated glucose uptake in skeletal muscle is a cardinal feature of insulin resistance in Type II diabetes, it is important to determine whether TNFα causes insulin resistance in skeletal muscle.

Investigators have used both human skeletal muscle cells and rat myoblasts to determine whether TNFα causes resistance to glucose uptake stimulated by insulin signalling pathways in skeletal muscle. Rat L6 myoblasts contain receptors for TNFα, and it has been shown, perhaps surprisingly, that TNFα weakly stimulated glucose uptake (by 3-fold) after 48 h of incubation [1]. By comparison, insulin-like growth factor-1 (IGF-1) maximally stimulated glucose uptake by 7-fold after a 24 h incubation. Furthermore, the IGF-1 receptor β-subunit, IRS-1 and mitogen-activated protein (MAP) kinase were all phosphorylated in response to IGF-1 after only 10 min of incubation. Whereas, in contrast, incubation with TNFα failed to phosphorylate either the IGF-1 receptor β-subunit or IRS-1, but did phosphorylate MAP kinase to the same extent as did IGF-1. Despite similar TNFα-induced MAP kinase phosphorylation (compared with IGF-1-induced MAP kinase phosphorylation), TNFα stimulated glucose uptake less than did IGF-1. Thus these results suggest that MAP kinase phosphorylation is not sufficient for glucose uptake in L6 myoblasts, and that TNFα-stimulated glucose uptake may utilize an alternative pathway (compared with IGF-1).

The effects of TNFα on glucose uptake and glycogen synthase activity have also been studied in human skeletal muscle cell cultures from both non-diabetic subjects and subjects with Type II diabetes [2]. In muscle cells from non-diabetic subjects, acute (90-min) exposure to TNFα (5 ng/ml), also perhaps surprisingly, stimulated glucose uptake (73±14% increase) to a greater extent than did insulin (37±4% increase; P < 0.02). The acute uptake response to TNFα in cells from subjects with diabetes (51±6% increase) was also greater than that to insulin (31±3% increase; P < 0.05). Prolonged (24-h) exposure of cells from non-diabetic subjects to TNFα resulted in a further stimulation of uptake (152±31%; P < 0.05), whereas the increase in cells from subjects with Type II diabetes was not significant compared with that in cells receiving acute treatment. After TNFα treatment, the level of GLUT1 protein was elevated in cells from non-diabetic subjects (4.6-fold increase) and in those from subjects with Type II diabetes (1.7-fold increase). In contrast, acute TNFα treatment had no effect on the fractional velocity of glycogen synthase in cells from either non-diabetic subjects or those with Type II
diabetes, whereas prolonged exposure reduced the glyco-
gen synthase fractional velocity in cells from both
groups of subjects. Therefore, in summary, both acute
and prolonged treatment with TNF-α up-regulates

glucose uptake activity in cultured human muscle cells,
but reduces glycogen synthase activity.

In this issue of Clinical Science, Patiag et al. [3] have
studied the effects of TNF-α and inhibition of protein

kinase C on insulin-stimulated glucose uptake. In con-
trast with other investigators, Patiag et al. [3] investigated
the effects of TNF-α on: (a) basal glucose uptake and (b)

glucose uptake in the presence of increasing concentra-
tions of insulin in L6 myoblasts. They concluded that

TNF-α does not affect insulin-independent glucose up-
take, because they show no change in glucose uptake in
the basal state. Furthermore, Patiag et al. [3] conclude that

insulin-stimulated glucose uptake was also not affected.

Insulin sensitivity was examined in the presence of high
(10 ng/ml) and low (1 ng/ml) concentrations of TNF-α.

The authors calculated the insulin concentrations re-
quired to increase glucose uptake by 50% in the presence
of either concentration of TNF-α. Their conclusions were
that insulin sensitivity was unaffected by either TNF-α
concentration, suggesting that TNF-α does not affect

insulin-stimulated glucose uptake and thereby does not
cause insulin resistance in skeletal muscle cells. Patiag et
al. [3] determined whether various concentrations of

insulin increased glucose uptake in the presence of high
or low concentrations of TNF-α. Incubation with

high TNF-α levels produced an increase (albeit non-
significant) in insulin sensitivity (lower insulin con-
centrations were required to increase glucose uptake
by 50%). However, the standard error around the mean
was large with the high concentration of TNF-α

[(4.37 ± 4.0) x 10^-8 M, compared with (7.08 ± 1.3) x

10^-8 M for the control]. Thus the large variation around
the mean may have produced a type 2 statistical error.

What can we learn from this work? Importantly,
Patiag et al. [3] provide convincing evidence that TNF-α
does not decrease insulin sensitivity. Non-specific in-
hibition of protein kinase C markedly attenuated glucose
uptake, but there was no effect of TNF-α. Taking the
work of Patiag et al. [3] in conjunction with the published
evidence, we can tentatively conclude that TNF-α is

expressed in adipocytes and induces insulin resistance in
adipocytes. However, in skeletal muscle, both acute and
prolonged treatment with TNF-α has no effect, or perhaps
even increases glucose uptake. It is possible to speculate
that increased skeletal muscle glucose uptake mediated
by TNF-α could be a compensatory mechanism that
attenuates the impact of reduced insulin-stimulated

glucose uptake in Type II diabetes.

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Endocrinology 139, 4793–4800


Effects of tumour necrosis factor-α and inhibition of


Sci. 99, 303–307