Insulin signaling under normal physiological conditions as well as its disruption in response to oxidative stress represents a field of intense research interest. It is generally accepted that insulin, on binding to its receptor, initiates the tyrosine phosphorylation of cellular substrates including the insulin receptor substrate (IRS) family members, which in turn modulate the activation of the phosphatidylinositol 3-kinase (PI 3-K) and downstream kinases such as PKCζ and Akt, which are responsible for initiating a number of cellular effects. It follows that defects in this signaling cascade lead to an altered cellular responsiveness to insulin and to insulin resistance, the earliest detectable abnormality in the development of diabetes.

See accompanying article on page 1355

Recently, a protein called TRIB3, a mammalian tribbles homolog also known as TRB3/NIPK, was reported to interfere with insulin signaling by binding to and inhibiting the serine kinase Akt. TRIB3 expression can be increased by several stimuli including starvation, PPAR-α activation, and chronic alcohol ingestion, and in all of the conditions listed is linked to insulin insensitivity. Moreover, a relatively frequent (minor allele frequency 15%) missense TRIB3 polymorphism has also been described in which a glutamine residue is substituted by an arginine at position 84 (Q84R), to result in a protein that more readily binds to the pleckstrin homology domain of Akt to prevent its plasma membrane association. Overexpression of this TRIB3 variant in a cell line moderately decreases (≈22%) the insulin-induced phosphorylation and activation of Akt.

In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Andreozzi et al report that human umbilical vein endothelial cells carrying different TRIB3 genotypes (QQ-QR- or RR) show a distinctly different sensitivity to insulin. Specifically, cells expressing the TRIB3 R84 (RR) variant demonstrate an attenuated insulin-induced NO production in vitro compared to the QQ-expressing cells. The Q84R substitution did not affect the insulin-induced phosphorylation of the insulin receptor or IRS-1 or the binding of IRS-1 to PI 3-K. However, expression of the Q84R variant was associated with a decrease in the insulin-induced phosphorylation of Akt on both of its regulatory sites, a finding that correlated with a moderately increased association of Q84R TRIB3 with the kinase.

As the activation of Akt has been linked to the enhanced phosphorylation (on Ser1177) of the endothelial NOS synthase (eNOS) as well as its activation, Andreozzi et al next assessed the insulin-induced activation of eNOS. In the same cultured endothelial cells the authors found that the expression of the R84 TRIB3 variant was linked to an attenuated insulin-induced phosphorylation of eNOS on Ser1177 and corresponding decrease in the dephosphorylation of the reciprocally regulated inhibitory site in the calmodulin-binding domain (Thr495). The latter response is generally attributed to an elevation in intracellular Ca²⁺, an effect that insulin does not elicit. However, the dephosphorylation of Thr495 can also be achieved by activation of the phosphatase PP1, and Andreozzi et al found that insulin elicits the Ca²⁺-independent dephosphorylation of Thr495 via the Akt-dependent activation of PP1. Indeed, the R84 TRIB3 variant, by preventing the activation of Akt, was associated with decreased PP1 activity and an attenuated dephosphorylation of the site.

So far so good; the next step was of course to demonstrate that the altered phosphorylation of eNOS is linked in some way to a change in NO production. The authors approached this by comparing insulin and ionomycin-induced changes in NO production (citrulline assay) and cyclic GMP levels in the genotyped endothelial cells. Consistent with the rest of the data presented the authors report that insulin stimulates an increase in NO and cyclic GMP production in cells expressing Q84 TRIB3 but only induces a small increase in endothelial cells expressing the R84 variant. Surprisingly, the data presented show that the activation of eNOS by insulin was greater than that elicited by ionomycin, but showed at most a 2-fold increase (ionomycin-induced increases of ×20 to ×30 are generally observed); also the cyclic GMP levels were lower than generally reported. To address the mechanisms underlying the Q84R TRIB3-mediated inhibition of Akt, the authors generated molecular models of the Q84 and R84 TRIB3 and report a significant difference in the structure of the two TRIB3 variants, which has potential consequences on the formation of intramolecular salt bridges and the interaction of the protein with Akt.

Insulin elicits vasodilatation in vivo, a response linked to a number of mechanisms including: an interaction with the sympathetic nervous system at the vascular level; the activation of ion channels; in particular ATP-dependent K⁺.
channels[13]; the release of adenosine[14,15]; and an increase in the generation of NO.[16,17] Although the latter mechanism can account for the finding that the insulin-induced vasodilation observed in vivo is sensitive to NO synthase (NOS) inhibitors,[17–19] the acute application of insulin to endothelial cells or isolated arteries does not generally elicit an immediate increase in NO production accompanied by relaxation.[20–22] Indeed, acute effects of insulin on vascular tone have also been attributed to endothelium-independent actions, related to alterations in smooth muscle Ca2+ channels,[12] or the activation of K+ channels,[12] and even the activation of a constitutively expressed NOS in smooth muscle cells.[24] In vitro, in cultured endothelial cells the situation is obviously different, and many groups have been able to demonstrate the acute insulin-induced phosphorylation and activation of eNOS,[25–28] albeit usually in passaged cells and the level of activation is generally low. In primary cultures of endothelial cells this phenomenon is not observed[29] a finding that seems to be linked to the insulin-induced phosphorylation of eNOS on a negative regulatory site ie, Tyr567.[30] The latter residue is phosphorylated by PYK2, the activity of which decreases in the presence of insulin.[30,31] Such findings account for some of the controversy in the literature and again highlight the complexity of eNOS regulation by its phosphorylation on a series of positive and negative-regulatory sites. Determining the relevance of the R84 TRIB3 variant for the regulation of eNOS activation in the native endothelium will have to await the in vivo analysis of insulin-induced vasodilation, studies that Andreozzi et al are in an ideal position to perform.

Leaving the controversy regarding the insulin-induced activation of eNOS to one side, it is clear that there are many Akt substrates in endothelial cells that could be affected by the Q84R mutation and Andreozzi et al were also able to show that the insulin-induced phosphorylation of the glyogen synthase kinase 3 was also slightly attenuated. Thus, the article highlighted here has made an important contribution by making intelligent use of endothelial cells carrying different TRIB3 genotypes to demonstrate the impact of a naturally occurring protein mutation on insulin-signaling. Unfortunately, the study focuses on the insulin-induced activation of eNOS without addressing the effects of other Akt-activating, eNOS-stimulating growth factors such as vascular endothelial growth factor (VEGF). It will certainly be interesting to determine the full extent of the role played by the Q84R TRIB3 protein in modifying Akt signaling in the vasculature and eventually also its role in determining the development of vascular disease.

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References


Double Tribble: Two TRIB3 Variants, Insulin, Akt, and eNOS
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