

# OBESITY AND THE ADIPOCYTE

## Studies of the mechanism of inhibition of insulin signaling by tumor necrosis factor- $\alpha$

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Insulin resistance is defined as a smaller than normal response to a given dose of insulin. It is also a ubiquitous correlate of obesity and a central component of non-insulin dependent diabetes mellitus. Insulin resistance causes a wide range of pathological disorders such as dyslipidemia, arteriosclerosis, and cardiovascular disorders. Several lines of evidence indicate that tumor necrosis factor (TNF)- $\alpha$  plays a central role in the insulin resistance observed in obesity. Indeed, it has been observed in animals (Hotamisligil *et al.* 1993, Hofmann *et al.* 1994, Hamann *et al.* 1995), and more recently in humans (Hotamisligil *et al.* 1995, Kern *et al.* 1995), that obesity is linked to an overexpression of TNF- $\alpha$  in adipocytes. TNF- $\alpha$  plays a causal role in the insulin-resistant state of experimental animals since neutralization of TNF- $\alpha$  in obese rats increases their insulin sensitivity (Hotamisligil *et al.* 1993), probably due to the concomitant increase in the tyrosine kinase activity of the insulin receptor (IR) in adipose tissue and muscle (Hotamisligil *et al.* 1994a). In cell culture, TNF- $\alpha$  interferes with insulin signaling by inhibiting IR tyrosine kinase activity, and tyrosine phosphorylation of one of its substrates, IRS-1. This effect is observed in various cell lines such as adipocytes, fibroblasts, hepatocytes and myeloid cells (Feinstein *et al.* 1993, Hotamisligil *et al.* 1994b, Peraldi *et al.* 1996). At the molecular level, TNF- $\alpha$  induces serine phosphorylation of IRS-1 (Kanety *et al.* 1995, Hotamisligil *et al.* 1996), and this modified form of IRS-1 can function as an inhibitor of the IR tyrosine kinase activity *in vitro* and in intact cells (Hotamisligil *et al.* 1996). This effect is dependent upon the phosphorylation of IRS-1 and is reversible by dephosphorylation of IRS-1 by alkaline phosphatase. Two pieces of evidence indicate that this mechanism is the one by which TNF- $\alpha$  induces insulin resistance in animals. First, as compared with IRS-1 from lean animals, IRS-1 obtained from adipocytes and muscles of obese rats was also found to inhibit insulin receptor autophosphorylation. Secondly, in intact cells, the presence of IRS-1 seems to be crucial for TNF- $\alpha$  mediated insulin receptor inhibition. Indeed, 32D cells which lack endogenous IRS-1 are resistant to the effect of TNF- $\alpha$  on insulin

receptor phosphorylation. When IRS-1 is expressed ectopically in these cells, insulin-mediated insulin receptor phosphorylation becomes very sensitive to TNF- $\alpha$ .

TNF- $\alpha$  binds with high affinity to two receptors which, besides their ligand binding domain, exhibit no homology. These receptors, p55TNFR and p75TNFR, are glycoproteins with a single transmembrane domain. Both proteins are devoid of any enzymatic activity, but associate with several different intracellular proteins (Vandenabeele *et al.* 1995). Stimulation of p55TNFR alone is sufficient to inhibit IR and IRS-1 tyrosine phosphorylation with the same potency of stimulation of both TNF receptors. However, there is some reason to believe that p75TNFR could play some role *in vivo*. First, stimulation of p75TNFR alone induces some inhibition of insulin signaling (although this effect is much smaller than the effect observed after stimulation of p55TNFR). Secondly, p75TNFR binds TNF- $\alpha$  with a higher affinity and a higher dissociation rate than p55TNFR, so that at low TNF- $\alpha$  concentration p75TNFR can 'concentrate' locally the ligand and make it available for p55TNFR according to the 'ligand passing model'. An inhibition of IR and IRS-1 tyrosine phosphorylation is also observed after treatment of the cells with sphingomyelinase and synthetic analogs of ceramide (Peraldi *et al.* 1996), mediators that have been linked to p55TNFR. This suggests that activation of sphingomyelinase and production of ceramides is likely to be a major pathway used by p55TNFR to inhibit insulin signaling. Ceramides directly activate various enzymes such as PKC- $\zeta$ , a membrane-associated kinase which phosphorylates and activates Raf-1, and a ceramide-activated protein phosphatase which is a subtype of heterotrimeric phosphatase 2A. This leads to the activation of a cascade of phosphorylation/dephosphorylation events. It is likely that stimulation of these enzymes leads to modification of IRS-1 and subsequent inhibition of the insulin receptor. Several questions remained to be asked: (i) what is the physiological stimulator of TNF- $\alpha$  production by adipocyte during obesity; (ii) what is the mode of action of TNF- $\alpha$  (autocrine, paracrine, endocrine); (iii) which kinase phosphorylates IRS-1 in response to TNF- $\alpha$ ; and

(iv) by which mechanism does IRS-1 inhibit the tyrosine kinase activity of the insulin receptor after TNF- $\alpha$  treatment of the cells? A better understanding of the connection(s) between the TNF- $\alpha$  and the insulin signaling pathways could be important to find a cure for the state of insulin resistance observed during obesity.

## References

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