Review Article

Reactive oxygen species and the role of inflammatory markers (IL-6 and TNF-α) in the causation of insulin resistance in type 2 obese diabetics

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ABSTRACT

Oxidative stress is increased in diabetes and the overproduction of the reactive oxygen species in diabetes is a direct consequence of hyperglycemia. Inflammatory cytokines such as tumor necrosis factor (TNF-α) and interleukin (IL-6), and free radicals are believed to play key roles in the causation of insulin resistance in obese type 2 diabetes subjects. Free radicals are formed disproportionately in diabetes by glucose oxidation, nonenzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins. Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes. Different vascular cells like vascular smooth muscle, adipose tissue, renal cells, and endothelial cells are able to produce ROS under hyperglycemic condition. Both NADPH oxidase and mitochondrial electron gradient play roles in hyperglycemia induced ROS generation with increased lipid peroxidation, and production of inflammatory markers (IL-6 and Tnf-α) leading to the development of insulin resistance. Cytokines produced by high fat in adipocytes acts as a signaling molecule for the activation of signal transduction cascades in which activateIKK- inhibitor of nF-κB kinase and nuclear factor-κB (IKKβ/nF-κB) and Janus kinases (JNK) pathways in adipocytes, hepatocytes, mesangial cells and associated macrophages. These factors activate transcription factors leading to the transcriptional activation of the inflammatory cytokines TNF-α, which impairs insulin signaling through serine phosphorylation of IRS-1 and can reduce GLUT4 gene expression, plausible cellular basis for inflammatory markers as a mediator of insulin resistance in type 2 obese diabetes subjects.

Abbreviations:
ROS: Reactive oxygen species, IL-6: Interleukin-6, Tnf-α: Tumor necrosis factor alpha, IR: Insulin resistance, SOCS: suppressor of cytokine signaling, nF-κB: nuclear factor-κB, IKK: inhibitor of nF-κB kinase, JNK: Janus kinases, FABPs: fatty acid–binding proteins, AP-1: activator protein, LXR: liver X receptor, PPAR: Peroxisome proliferator activated receptor γ,
IRS: Insulin receptor substrate, GLUT4: Glucose transporter-IV, ER: Endoplasmic reticulum

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**Introduction**

The world is facing a twin epidemic of diabetes and obesity, together termed as Diabesity. Obesity particularly abdominal obesity is not only a major risk factor for type 2 diabetes but also for other non-communicable diseases such as cardiovascular diseases. Obesity is characterized by pathological accumulation of fat molecules in adipose tissue thereby promoting insulin resistance in muscles, liver and other tissues. (1) Studies of twins have clearly attributed the variation in BMI to genetic factors, obesity is a clear reflection of an interaction of development and environment with genotype.

Obesity and insulin resistance are associated with ectopic lipid accumulation suggesting an insufficient uptake and storage of lipids in the adipose tissue which plays an important role as an endocrine organ secreting different hormones and cytokines that can augment or impair whole-body insulin sensitivity (2).

Chronic inflammation in type 2 diabetes activate adipose tissue to synthesize and release the main pro-inflammatory cytokines, tumor necrosis factor-alpha (TNF-a), interleukin-1 (IL-1) and interleukin-6 (IL-6), which are associated with increased body fat mass. Pro-inflammatory cytokines and acute phase reactants are involved in multiple metabolic pathways relevant to insulin resistance, including insulin regulation, reactive oxygen species formation, lipoprotein lipase action and adipocyte function (3). Therefore, obesity, activated innate immunity and inflammation are relevant factors for the pathogenesis of type 2 diabetes mellitus (3, 4).

**Inflammation in obesity correlating oxidative stress**

Increased oxidative stress is seen in both obese animal and human models of insulin resistance. At the cellular level, increased oxidative stress induces insulin resistance via inhibition of components of the insulin-signaling pathway (5) and decreasing GLUT4 expression and translocation to the cellular membrane (6). Sources of reactive oxygen species (ROS) in the vessel wall include both mitochondrial sources and cytosolic sources as a result of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (7) and dysfunctional endothelial nitric oxide synthase (eNOS), which generates superoxide ions rather than NO in the presence of insufficient amounts of its critical cofactor, tetrahydrobiopterin (8). Superoxide production by NADPH oxidase impairs endothelial-dependent vasodilatation produced during oxidative stress (9).

The increased delivery of glucose to adipose tissue, endothelial cells in the fat pad may take up increasing amounts of glucose through their constitutive glucose transporters. Increased glucose uptake by endothelial cells in hyperglycemic conditions causes excess production of ROS in mitochondria, which inflicts oxidative damage and activates inflammatory signaling cascades inside endothelial cells (10). Endothelial injury in the adipose tissue might attract inflammatory cells such as macrophages to this site and further exacerbate the local inflammation. Hyperglycemia also stimulates ROS production in adipocytes, which leads to
increased production of proinflammatory cytokines (11). In overweight and obese subjects, serum levels of TNF-α were significantly higher than those in lean subjects. Plasma TNF-α was negatively correlated with HDL cholesterol, glycated hemoglobin, and serum insulin concentrations (12). This rise in plasma cytokine levels was completely abrogated by simultaneous administration of antioxidant glutathione, suggesting a role for an oxidative mechanism (13). Hyperglycemia stimulates generation of reactive oxygen species by leukocytes in humans and decreases vitamin E levels, suggesting oxidative mechanisms (14). High-calorie diet rich in carbohydrates, fats (especially saturated and trans-fats), or protein stimulates the production of reactive oxygen species (15) generating the free radicals which enhances NADPH oxidase activity indicating an increase in oxidative stress and target-organ damage along with the production of IL-6, TNF-α, and CRP causing in insulin resistance in type 2 diabetes mellitus in obese subjects (16).

The central importance in the activation of inflammatory pathways in obesity is due to increase the oxidative stress on the ER of adipose tissue, which undergoes severe changes in tissue architecture, increases in protein and lipid synthesis, and perturbations in intracellular nutrient and energy fluxes which leads to activation of JNK and nF-kβ pathway producing cytokines to cause insulin resistance (17, 18). ER stress also activates IKK signaling cascade for the activation of important signaling pathway by inflammatory genes which down regulate insulin signaling cascade decreasing insulin sensitivity (19).

**Figure 1:** Pathways correlating reactive oxygen species production, metabolism and inflammatory signaling in adipocytes or macrophages.

**Figure 2:** Major causes of insulin resistance in key target organs, such as adipose tissue, liver, muscle, and brain.
Adipose tissue, inflammation and type 2 diabetes

Adipose tissue releases adipokines and adipocyte derived hormones have specific cellular effects that mediate insulin resistance and atherosclerosis. Proinflammatory immune mediators TNF-α and IL-6 can directly interfere with insulin signaling (30), and the binding of these cytokines to their cognate receptor on muscle cells or hepatocytes has been shown to induce an intracellular response that interferes with the ability of the insulin receptor to phosphorylate its intracellular targets decreasing cellular response to insulin. One well-described example of this is the induction of the protein suppressor of cytokine signalling-3 (SOCS-3) by IL-6 in hepatocytes. This protein associates with the insulin receptor and suppresses insulin dependent receptor autophosphorylation and IRS-1 phosphorylation (31). SOCS-3 also binds to IRS-1 and IRS-2, leading to their ubiquitination and proteasomal degradation. Immune mediators also affect insulin sensitivity indirectly by modulating the regulatory function of fat, nerve or other cells, e.g. by influencing the release of leptin or by activating the hypothalamic–pituitary–adrenal axis (32).

Insulin resistance and inflammation in obese Type 2 diabetes mellitus

Obesity is characterized by macrophage accumulation in white adipose which is responsible for the development of adipose tissue inflammation due to production of inflammatory cytokines either alone or in concert with adipocytes, which suggests a potentially important influence of macrophages in promoting insulin resistance. (20, 21). The chronic activation of the innate immune system reduces insulin sensitivity and precedes the development of type II diabetes mellitus (22). Adiponectin levels also inversely correlate with insulin resistance, atherosclerosis, and obesity (23). Adiponectin may act locally, potentially by reducing foam cell formation, affecting plasma triglyceride levels through increased VLDL-triglyceride catabolism and a reduction in adiponectin levels augments insulin resistance and hyperglycemia, promoting a cycle of enhanced diabetes and lesion development (24, 25).

A molecular marker of inflammation in type 2 diabetes mellitus also includes acute-phase response proteins sialic acid, glycoprotein, serum amyloid A, C-reactive protein, cortisol, and cytokine IL-6, a key mediator of the inflammatory response produced in adipocytes along with immune cells, fibroblasts, endothelial cells, and monocytes are considered as the major sources, of inflammation. IL-6 levels increases with several measures of obesity, including body mass index (BMI), waist to hip ratio, and increased body fat in type 2 diabetes mellitus and insulin-resistant states (26).

Insulin affects cells through binding to its receptor on the surface of insulin-responsive cells. The stimulated insulin receptor phosphorylates itself and several substrates, including members of the insulin receptor substrate (IRS) family, thus initiating downstream signaling events (27,28). The inhibition of signaling downstream of the insulin receptor is a primary mechanism through which inflammatory signaling leads to insulin resistance. Exposure of cells to TNF-α or elevated levels of free fatty acids stimulates inhibitory phosphorylation of serine residues of IRS-1. This phosphorylation reduces both tyrosine phosphorylation of IRS-1 in response to insulin and the ability of IRS-1 to associate with the insulin receptor and thereby inhibits downstream signaling and insulin action decreasing insulin sensitivity (29).
Conclusion

Obesity leading to insulin resistance and type 2 diabetes is a long-recognized phenomenon with fundamentally important scientific and clinical implications. The enhanced glucose uptake by adipocyte produces ROS in mitochondria which is responsible for endothelial cell dysfunction and production of various inflammatory markers (IL-6 and TNF-α). High blood glucose and decreased insulin level also depends upon β-cell function of pancreas and the level of inflammatory markers produced by different macrophages, monocytes, cytokines and activated T-cells.

Type 2 diabetes, obesity and cardiovascular disease share a metabolic milieu characterized by insulin resistance and chronic subacute inflammation. Insulin receptor signaling pathways is a central mechanism through which inflammatory and oxidative stress results in insulin resistance. Inflammatory markers, IL-6 and Tnf-α produced by adipocytes during oxidative stress and due to increase level of FFA in blood causes insulin resistance in type –II obese diabetics. The administration of drugs to the patients that increase the transcription factor PPARγ receptors in the nucleus that brings about the suppression of Tnf-α and activation of the enzyme responsible for the formation of Glycerol-3 phosphate in adipose tissue promoting FFA in blood esterification, forming TG which stored in adipose tissue will help to increase the insulin sensitivity.

References