REACTIVE OXYGEN SPECIES AND INSULIN RESISTANCE ASSOCIATED TO GLUCOSE INTOLERANCE STATES

Octavian Savu

"N. C. Paulescu" National Institute of Diabetes, Nutrition and Metabolic Diseases, Bucharest

Abstract

Type 2 diabetes mellitus is a heterogeneous disease where a combination of beta-cell dysfunction and insulin resistance is usually present. Both impaired glucose tolerance and impaired fasting glucose represent intermediate metabolic states between normal and diabetic glucose homeostasis. Oxidative stress is defined as tissue injury resulting from a disturbance in the equilibrium between the production of reactive oxygen species, also known as free radicals, and antioxidant defense mechanisms. In this review, we consider how oxidative stress interferes with insulin resistance in different glucose intolerance states, including type 2 diabetes mellitus.

Key words: oxidative stress, glucose intolerance, insulin resistance.

Type 2 diabetes mellitus (type 2 DM) is a heterogeneous disease usually associated with a combination of beta-cell dysfunction and insulin resistance. Normal beta-cells compensate for insulin resistance by an increasing insulin secretion or beta-cell mass, whereas insufficient compensation leads to the onset of glucose intolerance. Once hyperglycemia becomes apparent, the beta-cell function further deteriorates.

Insulin resistance (IR) is the"condition in which normal amounts of insulin are inadequate to produce a normal insulin response". IR is a cardinal feature of type 2

DM, especially when is associated with fatty phenotype. It also occurs in a wide range of other clinical settings (1), and in approximately 25% of nonobese individuals with normal oral glucose tolerance (2, 3).

Both impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) represent intermediate metabolic states between normal and diabetic glucose homeostasis.

Oxidative stress implications in these pre-diabetic clinical settings are still debated. In a very recent study (4) increased oxidative stress and impaired endothelial function were found in early states of diabetes, i.e. both IGT and IFG. Brachial artery flow-mediated dilatation (FMD), nitrate-induced dilatation, plasma malondialdehyde (MDA) and superoxide dismutase (SOD) activity were measured in 133 subjects with carbohydrate abnormalities (45 IGT, 44 IFG and 44 Type 2 DM) and in 46 subjects with normal glucose tolerance (NGT). The conclusions of this study were summarized by the authors as the following new findings: (a) compared with control subjects, subjects with IFG and IGT showed an impaired FMD, more remarkable in subjects with T2DM than those with IFG and IGT; (b) FMD was inversely and strongly related to plasma glucose level and (c) low SOD was a potential early marker of susceptibility to diabetic vascular disease.

Studies investigating IR and insulin secretion in IFG or IGT states have conflicting results. Recently, Festa and colab. found that subjects with IGT may be more insulin resistant and have higher levels of common cardiovascular risk factors than those with isolated IFG. (5). The patients were selected from IRAS (Insulin Resistance Atherosclerosis Study) nondiabetics cohort and the assessment of glucose tolerance and insulin sensitivity was performed by a standard 75-g oral glucose tolerance test (OGTT) and a frequently sampled intravenous glucose tolerance test (FSIGTT) with minimal-model analysis. The difference in IR was slightly attenuated after adjusting for waist. Assuming that waist circumference reflects abdominal/visceral adiposity, these findings indicate that higher insulin resistance in isolated IGT may be partially explained by increased abdominal/visceral obesity in these individuals. Moreover, acute insulin response (AIR), reflecting first-phase insulin secretion, was lower in IFG versus IGT. Hence, it has been suggested that post challenge (and potentially postprandial) hyperglycemia is the result of increased IR rather than impaired insulin secretion, whereas fasting hyperglycemia reflects defective beta-cell function. No differences in major metabolic cardiovascular risk factors (total, LDL, and HDL cholesterol, blood pressure) were detected between the two groups. However, individuals with isolated IGT (versus isolated IFG) had higher triglyceride, and CRP and lower proinsulin levels.

The possible role of reactive oxygen species (ROS) in IR has previously been suggested on the basis of two types of indirect evidence. On one hand, there is extensive association of markers of oxidative stress with obesity and type 2 DM (6, 7). On the other hand, there are in vitro experiments showing that direct treatment of adipocytes (i.e. 3T3-L1 cell line) with high doses of hydrogen peroxide (one of the most potent ROS inductor) can induce IR (8, 9). However, none of the previously mentioned studies was able to show evidence that IR can be prevented to some degree by blocking the increase of ROS levels. The in vivo experiments proving that treatment of either rodents or humans with alpha-lipoic acid can partially improve insulin sensitivity are difficult to interpret because the action of alpha-lipoic acid is unclear: it can act either as an antioxidant or a pro-oxidant, and can directly stimulate insulin-dependent glucose uptake (10).

The causative role of ROS in IR is for the first time demonstrated by Houstis, Rosen and Lander (11) on cultured 3T3-L1 adipocytes exposed to TNF-alpha (4 ng/ml) or dexamethazone (20 nM). The cells became insulin resistant within several days, as assessed by the ability of insulin to stimulate glucose uptake. Increased levels of ROS in this model were certified in two different ways: a) by genome-wide gene expression analysis in TNF-treated, dexamethasone-treated and untreated adypocites; over expressed ROS-related genes by both treatments were then selected and compared; b) by direct estimation of ROS levels in treated and un-treated adipocytes by either measuring oxidation of a redox-sensitive dye dichlorofluorescein (DCF) or protein carbonyl levels. Next, the effect of antioxidants on insulin resistance was evaluated. Significant partial restoration of TNF-alpha or dexamethasone-induced IR was observed with

dose with either N-acetyl-cysteine (NAC) (0-1 mM) or MnTBAP [Mn(III)tetrakis(4-benzoic acid)porphyrin Chloride], an analogue of superoxide dismutase (SOD) (0-300 μM). Moreover, the same effect was obtained in 3T3-L1 cell lines over-expressing ROS-scavenging enzymes, including SOD (i.e. CuZnSOD, MnSOD) and catalases (i.e. peroxisomal and mitochondrion catalase). A decreased cellular IR in relation to reduced excessive ROS production shows the causal relationship between ROS and IR in these systems.

The in vitro observations were further extended in vivo in ob/ob mice (11). These mice are leptin-deficient and become extremely obese, developing significant IR and glucose intolerance by 8 weeks of age. Male ob/ob mice were treated with MnTBAP (at 2.5, 5 or 10 mg/kg body weight), the TZD rosiglitasone (3 mg/kg body weight) or placebo. Treatments were administered daily, beginning at 8 weeks of age and continuing for a period of 12 weeks. At the end of the study period, a glucose tolerance and insulin sensitivity test was performed. MnTBAP had no effect on the body weight but resulted in dose-dependent improvement of glucose tolerance and insulin sensitivity with dose (at 5 and 10 mg/kg body weight), with a maximum effect comparable to that of animals treated with rosiglitasone.

In summary, it can be postulated that oxidative stress implication in early phases of glucose intolerance (i.e. IGT or IFG) is still debated. This can be due to the fact that the concept of IR itself is controversial at this stage of glucose intolerance, despite new evidences showing increased oxidative stress in early endothelial dysfunction. Nevertheless, it became certain that reactive oxygen species have a causal role in multiple forms of IR, including type 2 DM. The downstream pathways translate elevated ROS levels into IR are not known, yet. ROS have been shown to induce various signaling pathways involving different proteins. The particular pathway activated will depend on the magnitude of oxidative stress, the specific type of ROS, the cell type, duration of exposure, and other factors. One attractive possibility is that ROS-induced IR is mediated by c-Jun N-terminal kinases/stress-activated protein kinase pathway (cJNK) (11 – 13). The source of ROS in IR is also unclear. One candidate for a common mechanism for both TNF-alpha and dexamethasone induced IR above mentioned (11) might be the sphingolipid ceramide (14).

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