Editorial
PARAMETERS OF OXIDATIVE STRESS IN DIABETES

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Oxidative stress (OS) is defined as tissue injury resulting from a disturbance in the equilibrium between the production of reactive oxygen species (ROS), also known as main free radicals, and antioxidant defense mechanisms [1]. Under normal conditions, the antioxidant defenses are able to protect against the deleterious effects of free radicals, while were the free radical formation is increased or the antioxidant defenses are inactivated, accumulation of free radicals occurs, leading to cellular and tissue damages [1].

There is strong evidence that oxidative stress plays a role in the pathogenesis of different diseases [2], including diabetes mellitus (DM) and its complications [3].

The role of OS in the etiology of diabetes was long time back recognized, when streptozocin and alloxan [4-5] were used in experimental animals to induce diabetes. It has also been shown that the expression of proinflammatory cytokines (i.e. IL-1beta) is increased in the area of beta-cellular insulitis and correlates with the occurrence of clinical manifest type 1 DM [6].

Beta cellular dysfunction and insulin resistance (IR) defines type 2 DM. Glucotoxicity is essential for beta cellular dysfunction [7] and recent years revealed that oxidative stress is an essential mediator of this process [8]. IR is a cardinal feature of type 2 DM, especially when is associated with fatty phenotype. The causative role of ROS in IR is now demonstrated either *in vitro* (in adipocytes exposed to TNF-alpha or dexamethazone) or *in vivo* (in ob/ob mice treated with MnTBAP, an analogue of superoxide dismutase (SOD), rosiglitasone or placebo) [9].

Both the degree of glycemic control and the duration of DM predict the risk of diabetic complications [10-11]. These observations have given rise to the "glucose hypothesis," which suggests that glucose mediates many of the deleterious effects of the disease. Several pathogenic pathways are activated in diabetes and have been proposed to be responsible for the development of long-term complications of diabetes (increase polyol pathway flux, increased advanced glycation end products, activation of proteinkinase C, increased hexosamine pathway flux), including an overproduction of ROS by the mitochondria electron-transport chain, suggested to be a common mechanism for all the others [12]. Hypoxia, directly and through induction of ROS, has been recently suggested to have an important role in the development of chronic complications of diabetes [13-16].

To date, there is no direct measure of OS in biological systems [17]. This is why most of the reports evaluate the impact of oxidative stress by measuring one or more specific markers of oxidative damage along with one or more parameters of antioxidant capacity. The study published here by Onaca and colab. uses the same approach.

ROS can widely affect any molecule in humans, i.e. nucleic acids, proteins or lipids. Oxidative changes to DNA are repaired by

endonucleases/glycosylases-liberating deoxynucleotides and bases, respectively, which are excreted in urine. Of great interest is measuring of 8-hydroxy-2'deoxyguanosine (8-OHdG) and its free base 8-hydroxyguanine (8-OH-G) in blood cells or urine using different analytical methods, i.e. solid-phase extraction and HPLC with electrochemical detection, gas chromatography-mass spectrometry (GC-MS), sandwich ELISA and COMET assay [18-19]. The modifications of proteins alter secondary and tertiary structures either by direct or combinative effect of ROS with glucose which generates several markers of oxidative damage, i.e. nitrotyrosine [20] or carbonyl derivates [21]. Other enzymes or proteins involved in the defense against oxidative stress, such as human myeloperoxidase [22] and human plasma lactoferin [23] were also considered as good markers for oxidative damages. Lipid peroxidation is probably the most extensively investigated process induced by free radicals. The most studied are lipid peroxides using isoprostane assays [24] and bioactive aldehydes, i.e. malondialdehyde (MDA), thiobarbituric acid-reactive substances (TBARS), and acrolein [25]. More accurate methods, such as electron spin resonance (ESR)[26], are developed and perfected to measure OS more reliably.

In the present analysis, Onaca and colab. used two different markers of OS damages, i.e. MDA (as measure of lipid peroxidation) and carbonyl proteins (as indicators of protein oxidation). Both parameters increase in diabetic patients when compared with healthy controls, irrespective of type of diabetes, and negatively correlate with the degree of metabolic control. This in accordance with the majority of published data showing that oxidative stress increases with age, duration of diabetes and low degree of metabolic control.

Despite controversy, it is generally accepted that, at least in advanced DM, antioxidant defense is depressed. Intracellular antioxidant capacity is mainly enzymatic, while plasma especially, contains small active molecules, either water soluble, i.e. albumin, uric acid or bilirubine, or lipid soluble, i.e. tochopherols, ubiquinone, lycopene or carotinoids. Enzymes most widely investigated are SODs, a family of metalloenzymes that convert superoxide radical into hydrogen peroxide and molecular oxygen, and glutathione (GSH) and glutathione transferase (GST)-related enzymes (glutathione peroxidase, glutathione reductase), which form an important antioxidant system that functions directly in the pathways of elimination of toxic peroxides and aldehydes, and indirectly in maintaining vitamins C and E in their reduced functional forms [27]. The net antioxidant capacity of the plasma can be measured in two different ways, with several advantages and disadvantages [28]. One way is to use an agent acting as prooxidant on a specific substrate, typically generating peroxyl radicals, i.e. total peroxyl radical trapping, TRAP [29]. The other, is to neutralize a preformed radical which is decomposed with the amount of antioxidants in plasma sample, i.e. by measuring the decomposition rate of a cationic radical, ABTS at 734 nm [30]. To date, the measurement of plasma F2-isoprostane is considered the best measure of plasma OS, especially by GC-MS, or – with less sensitivity and specificity – by radioimmunoassay [31]. Lipid peroxides, i.e. ox-LDL, are also considered as other specific measures of oxidative damage in plasma [32-33]. Ceruloplasmin (Cp) is an acute-phase-responsive oxidase enzyme. Depending of the method used, circulating Cp is generally

increased in type 2 DM and controversial in type 1 DM [34]. Moreover, elevated circulating Cp is associated with cardio-vascular disease, due to its copper which can promote various negative vascular effects. Moreover, DM can augment these negative effects [35] due to an increased expression of ROS-generating enzymes, such is NADPH.

Onaca and colab. find an increased level of Cp in diabetic patients when compared with controls, which is in accordance with the data already mentioned. Cautions should be raised in respect with Cp values and the correlation between the OS and degree of metabolic control.

In conclusion, the present study is in accordance with the data published in the literature attesting an increased OS with age, duration of DM and poor metabolic control. Moreover, it supports the fact that circulating Cp is increased in diabetic patients.

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