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Hydrogen peroxide: An oxidant stress indicator in type 2 diabetes

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ABSTRACT

Objective: The aim of the present study was to evaluate the indicative role of hydrogen peroxide (H_2O_2) in oxidative stress. **Patients and methods**: 200 type 2 diabetes were included and 200 controls. Hydrogen peroxide (H_2O_2), glycated hemoglobin (HbA1c), free fatty acids (FFA) and homocysteine were determined by commercial kits. Quantitative insulin sensitivity check index (QUIKI) was calculated. **Results:** H_2O_2 concentration was increased fourfold in type 2 diabetes compared to controls. 6 correlations were found: between H_2O_2 and HbA1c ($r = 0.85$, $p<10^{-3}$), between H_2O_2 and FFA ($r = 0.9$, $p<10^{-3}$), between H_2O_2 and homocysteine ($r = 0, 5$, $p<10^{-3}$), between H_2O_2 and IS ($r = -0.92$, $p<10^{-3}$), between the presence of H_2O_2 and arterial hypertension ($t = -4$, $p<10^{-3}$) and between arterial hypertension and homocysteine ($t = -7$, $p<10^{-3}$). **Conclusion:** The overproduction of H_2O_2 generated by hyperglycemia, increased dose of FFA and hyperhomocysteinemia, amplify the insulin resistance and induce arterial hypertension.

KEYWORDS

H_2O_2;
HbA_{1c};
FFA;
Homocysteine;
Type-2-diabetes.

INTRODUCTION

Systemic glucose homeostasis in type 2 diabetes is a key physiological function and needs glucoregulation. Despite medicines, treatments and dietary measures in diabetic patients, it's difficult to control blood glucose levels for them. This glycemic instability and chronic hyperglycemia promote the development of oxidative stress and generate many complications in diabetic patients.

Brownlee proposed an explanation for the pathogenesis of vascular complications of diabetes. Oxidative stress is the important metabolic pathway involved in the development of diabetes. By interaction with other pathways, it is responsible for all complications[1]. Increased glucose levels in endothelial cells induce an excessive substrate production to mitochondria. The overproduction of reactive oxygen species (ROS) via oxidative phosphorylation, induces an oxidative stress and then an endothelial dysfunction.

Several mechanisms, activated by hyperglycemia, involved in the generation of ROS causing vascular and kidney damage. The most important mechanisms are the increased polyol and hexosamine pathways, the over activation of the transcription factor NFkappaB, the angiotensin 2 synthesis stimulation, the protein kinase C activation, the overproduction of advanced glycation end products and the excessive NADH, H^+ and $FADH_2$ supplying the respiratory chain[2].

In order to demonstrate that oxidative stress is associated with chronic hyperglycemia, several biochemical parameters must be analyzed. The most important marker of oxidative stress establishment remains so far unclear. The aim of the present study was to evaluate the indicative role of hydrogen peroxide (H_2O_2) in oxidative stress.

MATERIALS AND METHODS

Study population

After hospital approvals were obtained, 200 confirmed type 2 diabetes patients were ascertained from the Endocrinology Department at CHU Farhat Hached Sousse, with no previous diagnosis of thyroid, adrenal or renal failure. A total of 200 controls were recruited among blood donors in regional blood transfusion center of Sousse. Controls were restricted to peoples having a body mass index under 27. Patients and controls were matched on age and sex. All participants have signed consent. Clinical data and hypoglycemic treatment are presented in TABLE 1.

Methods

Two blood samples were collected from each fasted participant. In order to determinate the insulin and homocysteine concentrations, the first sample is carried out on lithium heparin. The second was performed on EDTA to assess glycated hemoglobin (HbA1c), hydrogen peroxide (H_2O_2) and free fatty acids (FFA).

FFA concentration was measured by an enzymatic method (Randox, Antrim, UK). HbA1c was determined by immunoturbidimetric (Roche Diagnostics, Mannheim, Germany) and total hemoglobin by colorimetry. The two concentrations were measured after blood hemolysis collected on anticoagulant; their ratio provided the percentage of HbA1c.

H_2O_2 was determined by the colorimetric technique (PerOx, Immune diagnostik, Wiesenstr, Bensheim). It was incubated with peroxidase in microlitic wells followed by conversion of the TMB into a colored product. H_2O_2 concentration was obtained using a microtiter plate reader Σ 960 (Metertech) at 450 nm. Homocysteine was determined by an immunological method of fluorescence polarization controller (AXSYM, Abbott, Wiesbaden, Germany). Linked homocysteine (oxidized form) is reduced to free homocysteine. Even and in response to the effect of dithiothreitol, homocystine, disulfides forms and mixed protein-homocystine are reduced to free homocysteine. A result of high levels of adenosine, free homocysteine is converted to S-adenosyl-L-homocysteine. Insulin was measured by an immunoradiometric sandwich type. Noncompetitive mouse monoclonal antibodies were directed toward two different epitopes of insulin. Samples, controls and calibrators were incubated in tubes with the first monoclonal antibody and a second one labeled with iodine 125. After incubation, the tubes were devoid of their contents and rinsed to remove unbound labeled antibodies; the radioactivity was measured by gamma counter PC-RIA-MAS (Stratec). Unknown values are determined by interpolation using the standard curve; radioactivity is directly proportional to insulin concentration in the sample. Insulin resistance was assessed by calculating the Quantitative Insulin

TABLE 1 : Clinical data of diabetes type 2 patients and controls.

	Controls n=200	Patients n=200
Age (X±σ ; year)	50±7	56±11
Diabetes during (X±σ ; year)	----	10,5±6,5
Sex : Man (%)	50	48
Women (%)	50	51
Menopausal women (%)	10	15
AH (%)	----	18
Smoking (%)	10	19,5
BMI (X±σ ; kg/m^2)	24±2	27±6
Personal history		
Retinopathy (%)	12	25
Neuropathy (%)	7	50
AH (%)	2	21
LVH (%)		5
Family history		
Type 2 diabetes (%)		51
AH (%)		22
Nephropathy (%)		5
Hypoglycemic		
Metformine (%)		26
Sulfonamide (%)		20
Metformine+Sulfonamide (%)		40
Insulin (%)		14

BMI : body mass index; A H: arteriel hypertension; Neuropathies : mononeuritis, polyneuritis; LVH : Left Ventricular Hypertrophy mesured by electrocardiogram; *X* : average; σ : deviation.

sensitivity Check Index (QUIKI) according to the formula of Perseghin et al.[3]

1/Log (insulin) + Log (glucose) + Log (FFA).

Statistical treatment

For the purposes of this analysis, we used the statistical software SPSS 10.0. The data were assessed using the correlation coefficient of Pearson for continuous variables and the correlation coefficient of Spearman for non-continuous variables.

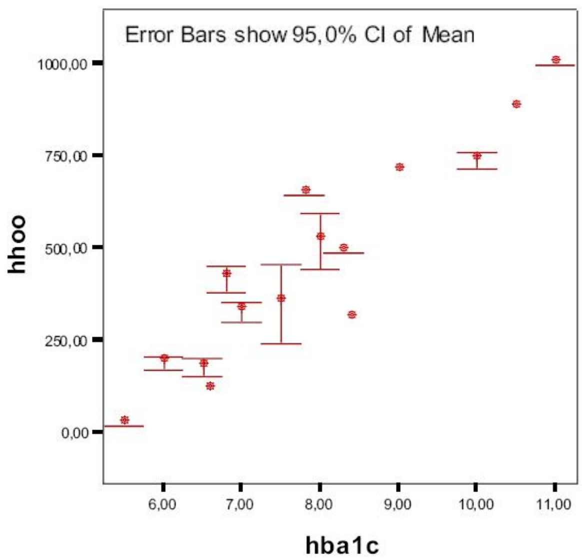

Figure 1: Positive correlation between glycated hemoglobin (HbA1c) and hydrogen peroxide concentration (hhoo) ; r = 0,85 and p<10^{-3}.

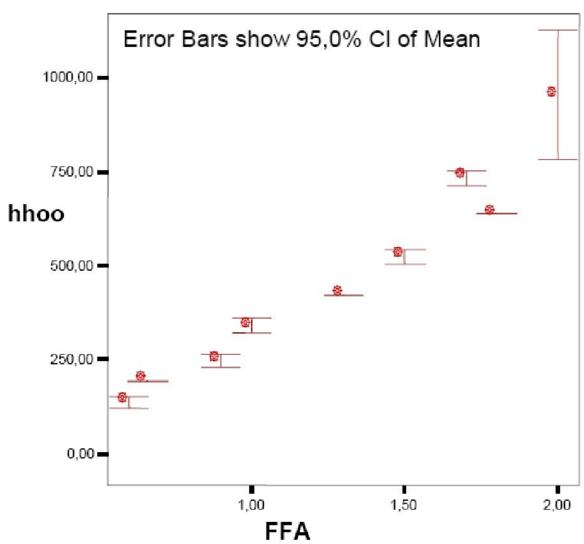

Figure 2 : Positive correlation between free fatty acids concentration (FFA) and hydrogen peroxide concentration (hhoo) ; r =0,9 and p<10^{-3}.

RESULTS

The H_2O_2 concentration determined was four times higher in type 2 diabetes compared with controls. A positive correlation was found between HbA1c and H_2O_2 concentration (r = 0.85, p <10^{-3}, Figure 1). An other positive correlation was observed between FFA concentration and H_2O_2 concentration (r = 0.9, p <10^{-3}, Figure 2). As shown in Figure 3, there is a positive correlation between homocysteine concentration and H_2O_2 concentration (r = 0.5, p <10^{-3}). We also found a

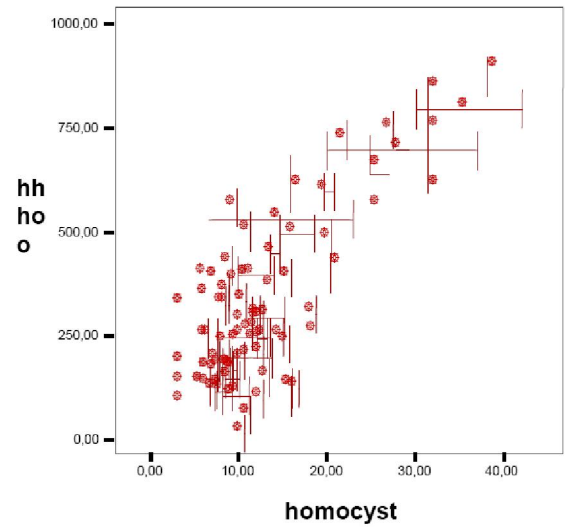

Figure 3 : Positive correlation between homocystéine concentration (homocst) and hydrogen peroxide concentration (hhoo); r=0,5 and p<10^{-3}.

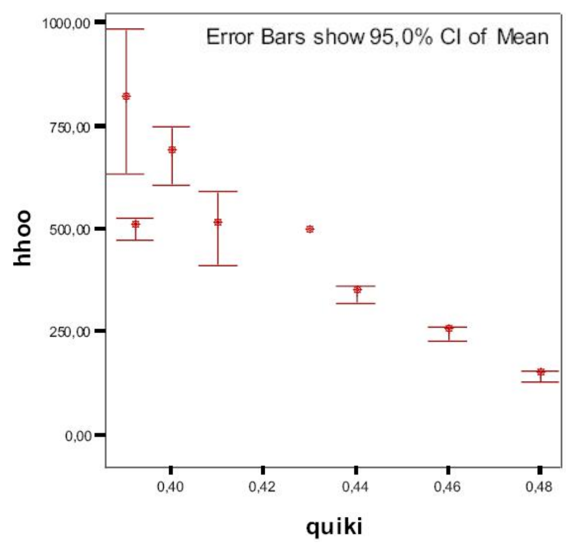

Figure 4 : Negative correlation between quantitative insulin sensitivity check index (quiki) and hydrogen peroxide concentration (hhoo); r=-0,92 and p<10^{-3}.

negative correlation between QUIKI and H_2O_2 ($r = -0.92$, $p < 10^{-3}$, Figure 4). Finally, two additional correlations were found; the first one between arterial hypertension and the concentration of H_2O_2 ($t = -4$, $p < 10^{-3}$) and the second one between arterial hypertension and homocysteine ($t = -7$ $p < 10^{-3}$).

DISCUSSION

The H_2O_2 concentration determined was increased fourfold in type 2 diabetes compared with controls. Our results are similar to those found in a study that showed an increased mitochondrial H_2O_2 production in hyperglycemic mice[4].

The positive correlation founded between the HbA1c and the H_2O_2 concentrations reflects that ROS production is as intensive as glycemia imbalance. In fact, some studies have shown that glucose metabolization, via pentose pathway, is less than twice in cells with overglucosed medium (33 mM). They concluded that increased H_2O_2 in diabetics is due to the pentose pathway reduction[5]. Other studies have reported that reaction between AMADORI products such as HbA1c and molecular oxygen produce superoxide anion ($O_2^{\bullet}$) or H_2O_2[6].

We have identified a positive correlation between FFA concentration and H_2O_2 concentration (Figure 2, $r = 0.9$, $p < 10^{-3}$). The correlation between FFA and H_2O_2 shows FFA decoupling effect on mitochondria. In one hand this leads to ATP decrease, partially responsible on insulin secretion inhibition verified by low insulin concentration in our diabetes. In the other hand FFA decoupling effect increases anion superoxide production which is dismuted in H_2O_2. Other studies have shown that increased FFA in type 2 diabetes induce the long-chain acyl-CoA accumulation and diacylglycerols which are a potent activators of protein kinase C isoforms and the kappaB nuclear factor[7,8]. All mechanisms of kinase activation may explain the ROS formation mediated by FFA[9]. Other studies have shown that prolonged exposure of islets to fatty acids induce the production of peroxynitrite (ONOO>) which bind to cytochrome C oxidase and inhibit the respiratory chain. Permeability transition pore is open which causes a proton leakage and an ATP hydrolysis. Mitochondria swell and release the cytochrome C, which activates cytoplasmic proteases called caspases. These are responsible for proteolysis stimulation and cell death[10]. However, a recent study suggests that beta mitochondrial oxidation cannot fight against the FFA increasing, in particular palmitic acid which is associated with obesity and type 2 diabetes. This overload is conveyed to the peroxisomal beta-oxidation leading to the H_2O_2 production[11].

In this study we found a positive correlation between homocysteine and H_2O_2 (Figure 3, $r = 0.5$, $p < 10^{-3}$). Peyrin-Biroulet et *al*. showed that homocysteine has in vitro a pro-oxidant action; the thiol group is oxidized to form ROS[12]. At high concentrations, homocysteine undergoes auto-oxidation producing homocysteine and superoxide anion ($O_2^{>}$)[13]. This activates the superoxide dismutase and reduced to H_2O_2. Moreover, homocysteine bind to proteins, producing reactive oxygen species. These changes can affect LDL protein contributing to their retention in the intima. Recently, it has been shown that homocysteine increases mitochondrial H_2O_2 production kidneys[14].

We found a negative correlation between QUIKI and H_2O_2 (Figure 4, $r = 0.5$, $p < 10^{-3}$). This reflects that chronic hyperglycemia increases the resistance of peripheral tissues to insulin via H_2O_2 in type 2 diabetes. One study has shown that insulin resistance is associated with an increased capacity for mitochondrial H_2O_2 release in Zucker diabetic fatty rats[15]. Recently, a study has discerned that insulin increased ROS production by pancreatic beta cells and the H_2O_2 effect. These effects were accentuated by the inhibition of receptor signaling to insulin, which indicated an independent effect of the waterfall insulin receptors. This study concluded that high levels of insulin may exacerbate cell death induced by H_2O_2 and other apoptosis inducers[16]. However, several studies on cell lines in vitro have shown that oxidative stress inhibits signal transduction of insulin. H_2O_2 micromolar concentrations inhibit the auto-phosphorylation of insulin receptor, the insulin receptor substrate-1 (IRS-1) and the downstream events of (IRS-1) such as the activation of phosphatidylinositol 3-kinase and glucose transport, further the activation of mitogen-activated protein kinases (MAPK)[17].

Oxidative stress inhibits the glucose transporter GLUT4 translocation[18] and the protein kinase C activation[19] stimulated by insulin in fat cells. A recent study showed that exposure of cell cultures for 2 h at 60-90 uM of H_2O_2 caused a significant loss of insulin stimulation in both proximal (IR tyrosine phosphorylation) and distal (Akt and GSK-3β phosphorylation of serine) elements of insulin signaling and glucose transport activity[20]. Exposure of isolated soleus muscle for 4 h at the same concentration of H_2O_2 was associated with a selective loss of IRS-1 and IRS-2, exacerbating the loss of insulin in response to oxidative stress[21].

We found 2 additional correlations: a correlation between H_2O_2 concentration and arterial hypertension ($t = -4$, $p < 10^{-3}$) and a correlation between homocysteine and arterial hypertension ($t = -7$ $p < 10^{-3}$). These results allow assuming that homocysteine affect endothelial cell function via H_2O_2. Overproduction of H_2O_2 may change the signal transduction machinery and the regulation of gene expression, causing an imbalance between proliferation, hypertrophy and apoptosis of smooth muscle cells and endothelial dysfunction involved in the pathogenesis of atherosclerosis and restenosis. Our results are partially consistent with those of Framingham Heart Study 5[22]. In fact, they are in favor of a link between homocysteine concentrations and increased cardiovascular risk. This association was more pronounced among women. On the other side, no relationship was found between homocysteine and arterial hypertension. Many experimental and clinical studies have also shown a major role of the deficiency in omega-3 and excess saturated fatty acids, oxidative stress including cell membranes and disorders of methylation reactions resulting in hyperhomocysteinemia[23]. One study showed that mice with type 2 diabetes have significantly increased the production of H_2O_2 in the arteriolar wall[24]. Besides, other than its inhibitory action on endothelial NO production, H_2O_2 can actively participate in the mechanisms of endothelium-dependent vasodilation in type 2 diabetes. The mechanism of H_2O_2-mediated dilation is not fully understood, but studies have shown that stimulating potassium channels by calcium, H_2O_2 hyperpolarizes the vascular smooth muscle cells and acts as a potential EDHF[25-27]. Other studies have shown that the vasodilation, induced by H_2O_2, is mediated by endothelial NO release[28]. In vitro, studies have found that during the exposure of human endothelial cells from umbilical veins at low glucose concentrations (up to 0.4 g / l); NO levels lowered quickly in response to decreased synthesis, by eNOS, and accelerated degradation. This was accompanied by activation of ($O_2^{\bullet}$) and H_2O_2 production, which are coupled by the mitochondrial membrane hyperpolarization due to the lack of NO[29].

CONFLICT OF INTERESTS

None.

CONCLUSION

The overproduction of H_2O_2 generated by hyperglycemia, increased dose of FFA and hyperhomocysteinemia, amplify the insulin resistance and induce arterial hypertension.

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