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Insulin, Catecholamines, Glucose and Antioxidant Enzymes in Oxidative Damage During Different Loads in Healthy Humans

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Summary

Exercise, insulin-induced hypoglycemia and oral glucose loads (50 g and 100 g) were used to compare the production of malondialdehyde and the activity of antioxidant enzymes in healthy subjects. Twenty male volunteers participated in the study. Exercise consisted of three consecutive work loads on a bicycle ergometer of graded intensity (1.5, 2.0, and 2.5 W/kg, 6 min each). Hypoglycemia was induced by insulin (Actrapid MC Novo, 0.1 IU/kg, i.v.). Oral administration of 50 g and 100 g of glucose was given to elevate plasma glucose. The activity of superoxide dismutase (SOD) was determined in red blood cells, whereas glutathione peroxidase (GSH-Px) activity was measured in whole blood. The concentration of malondialdehyde (MDA) was determined by HPLC, catecholamines were assessed radioenzymatically and glucose was measured by the glucose-oxidase method. Exercise increased MDA concentrations, GSH-Px and SOD activities as well as plasma noradrenaline and adrenaline levels. Insulin hypoglycemia increased plasma adrenaline levels, but the concentrations of MDA and the activities of GSH-Px and SOD were decreased. Hyperglycemia increased plasma MDA concentrations, but the activities of GSH-Px and SOD were significantly higher after a larger dose of glucose only. Plasma catecholamines were unchanged. These results indicate that the transient increase of plasma catecholamine and insulin concentrations did not induce oxidative damage, while glucose already in the low dose was an important triggering factor for oxidative stress.

Key words

Glucose • Insulin • Catecholamines • Malondialdehyde • Antioxidant enzymes

Introduction

Free radicals and their metabolites, reactive oxygen species, are constantly formed in aerobic organisms (Roberfroid and Calderon 1995). Under some circumstances, they are supposed to play an important role in the destruction of cell membranes, proteins and nucleic acids. Free radicals first react with the lipid component of cell membrane inducing lipid peroxidation and its consequences range from increased membrane permeability to cell lysis or death. In general, the organism has an adequate antioxidant system to cope

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with the production of radical oxygen species. This system consists of antioxidant vitamins, glutathione and thiols, and antioxidant enzymes (Ji 1995). The participation of increased generation of free radicals and reactive oxygen species, which is not counteracted by sufficient antioxidant balance, is supposed to be involved in the pathogenesis of atherosclerosis and its complications, in diabetes, autoimmune diseases, cancer, aging, etc. (Halliwell *et al.* 1992).

Among the physiological states of enhanced free radical production, strenuous physical exercise (Aruoma 1994, Ji 1995), hyperglycemia (Wolff and Dean 1984, Nadler and Winer 1996), hyperinsulinemia (Habib *et al.* 1994, Paolisso and Giugliano 1996) and enhanced lipid mobilization (Toborek and Hennig 1994) seem to play an important role. Catecholamine metabolism, which is increased in exercise and hypoglycemia, may also participate in the formation of lipid peroxides (Singal *et al.* 1983). The aim of the present study was to examine the effects of changes in plasma glucose, catecholamine or insulin concentrations on the activation of antioxidant enzymes during exercise, hypoglycemia and hyperglycemia in young healthy subjects. The obtained results indicate a close relationship of the activity of antioxidant enzymes to plasma glucose concentrations, but no association with plasma insulin or catecholamine concentrations.

Subjects and Methods

Twenty healthy males gave their informed written consent to participate in the study, which was approved by the Ethics Committee of the Institute of Experimental Endocrinology, Slovak Academy of Sciences.

Starting on the evening before the trial, the volunteers were asked to fast, to restrain from tobacco and alcohol and to restrict their physical activity to a minimum. After arrival to the laboratory between 07:30 and 08:00 h, an indwelling catheter was placed in the antecubital vein and the subjects were asked to rest in a comfortable armchair for 30 min. Thereafter, blood pressure was measured using a Dinamap Vital Signs Monitor (model 845 XT; Critikon Inc., Tampa, Florida, USA) and blood samples were withdrawn.

Exercise

Physical exercise was performed in 12 males aged 19 to 32 years (mean age 23 years) with body mass index (BMI) of 19.4-24.8 kg/m^2 (mean value 22.1 kg/m^2). Using a bicycle ergometer they were subjected to three consecutive work loads of graded intensity (1.5, 2.0 and 2.5 W/kg body weight) at a constant speed of 60 rev/min. Each period lasted 6 min, followed by 1 min of rest, in which blood pressure was measured. Electrocardiogram and heart rate were monitored continuously throughout the trial. The criteria for discontinuing the exercise included systolic blood pressure over 240 mm Hg, diastolic blood pressure over 130 mm Hg, leg fatigue, shortness of breath and significant electrocardiographic signs of ischemia. Blood was withdrawn and blood pressure measurements were taken immediately after the end of exercise, and then after 10, 30 and 60 min of rest in an armchair.

Hypoglycemia

Hypoglycemia was induced by short-acting insulin (0.1 IU/kg body weight, Actrapid MC NOVO) injected intravenously in the same group of volunteers. Blood samples were taken before and 30, 45, 60 and 90 min after insulin injection.

Hyperglycemia

Postprandial hyperglycemia was induced twice in 8 males aged 19 to 26 years (mean age 23 years) with BMI of 20.0-25.0 kg/m^2 (mean value 22.4 kg/m^2) to whom 50 g or 100 g of glucose were administered in an aqueous solution (400 ml). Subjects underwent tests in random order with 4-7 days apart.

Blood was collected into cooled polyethylene tubes using heparin as an anticoagulant. The samples were centrifuged at 4 °C, and the aliquots of plasma were frozen (–70 °C) after separation until analyzed.

Analytical methods

All samples were measured in duplicates. Enzyme activity was measured photometrically using commercial kits (Randox, Crumling, UK). Superoxide dismutase activity was measured in erythrocytes, whereas glutathione peroxidase activity was determined in whole blood. Both enzyme activities were expressed in terms of hemoglobin concentration.

The plasma malondialdehyde concentration was determined according to the method of Wong *et al.* (1987). This assay involves the hydrolysis of 50 µl plasma in diluted phosphoric acid (0.44 mol/l) at 100 °C, the reaction of malondialdehyde, a hydrolysis product, with thiobarbituric acid (42 mmol/l), methanol

precipitation of plasma proteins, fractionation of the protein-free extract on a C_{18} pre-column (Delta-Pak, 100 Å, Waters Company, Milford, Ma., USA) and C_{18} column (Waters Radial-Pak, Cartridge 8NVC18) equilibrated by pumping a potassium phosphate buffer (50 mmol/l, pH 6.8) at 2 ml/min and quantification by fluorescence of the malondialdehyde-thiobarbituric acid adduct (wavelength 515-550 nm, Waters pump 510, autosampler 717, fluorescence detector 470, using software baseline 820).

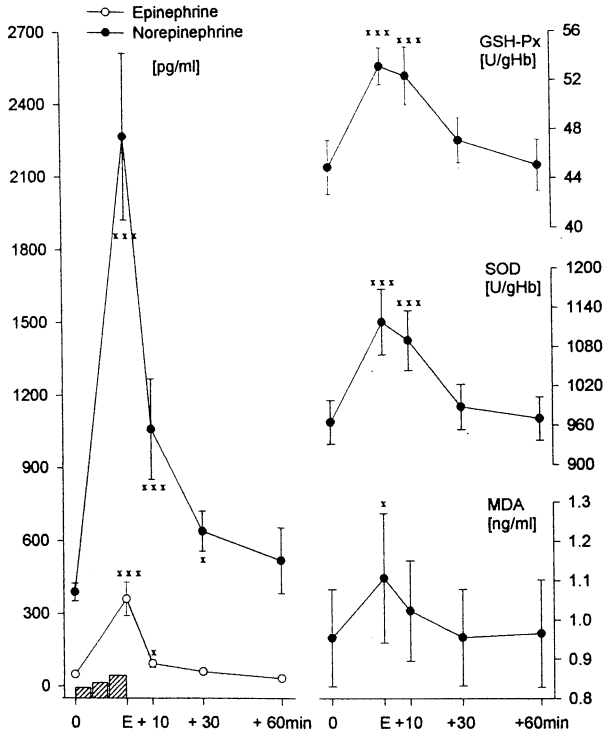

Fig. 1. *Plasma concentrations of catecholamines and MDA, activity of SOD and GSH-Px in healthy subjects during submaximal physical exercise. Significantly different from control samples (time 0): $^{x}p<0.05$, $^{xx}p<0.01$, $^{xxx}p<0.001$.*

Plasma glucose was analyzed using the glucose-oxidase method (Boehringer, Mannheim, Germany). Catecholamines were determined by the radioenzymatic method described by Peuler and Johnson (1977). Radioimmunoassays were carried out in order to determine insulin concentrations in the plasma using commercial kits from DPC (Los Angeles, Ca, USA).

Statistical analysis was performed using the SIGMASTAT 2.0 program (Jandel Scientific, San Rafael, Ca, USA). The Mann-Whitney U test and Fischer's exact test were used for evaluating differences between the two groups with respect to the clinical and laboratory parameters at baseline. Two-way analysis of the variance for repeated measurements with consecutive post-hoc tests was used to determine 1) the differences from the baseline within each group, and 2) the differences between the groups within one investigation. All data were expressed as means ± S.E.M. $P<0.05$ (two-sided) was considered as significant.

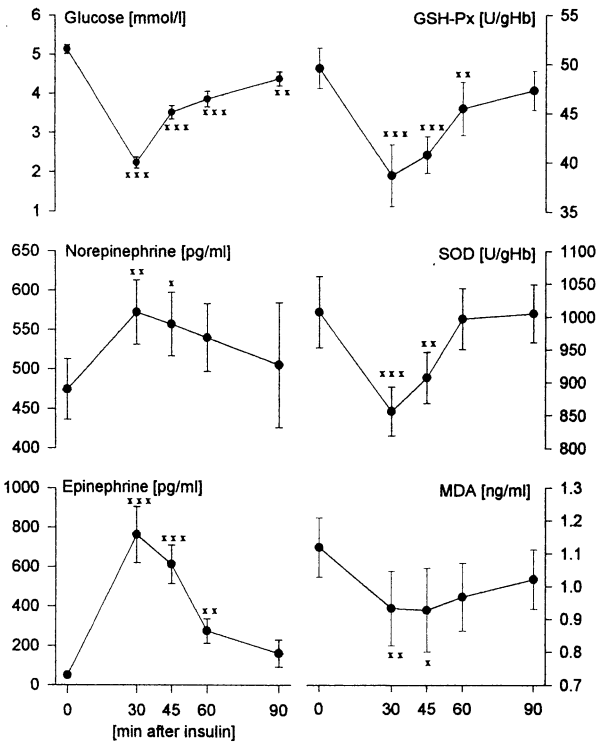

Fig. 2. *Plasma concentrations of catecholamines, glucose and MDA, activity of SOD and GSH-Px in healthy males during insulin-induced hypoglycemia. Significantly different from control samples (time 0): $^{x}p<0.05$, $^{xx}p<0.01$, $^{xxx}p<0.001$.*

Results

Exercise

As expected, plasma concentrations of the two catecholamines rose significantly towards the end of the exercise with a predominating norepinephrine response ($p<0.001$, Fig. 1). The concentrations of glucose and insulin were not changed. At the end of the exercise, MDA concentrations and enzyme activities were also elevated (MDA: $p<0.01$, GSH-Px: $p<0.05$, SOD: $p<0.01$).

Hypoglycemia

Supraphysiological hyperinsulinemia after insulin administration induced a fall of glucose

concentration, reaching the minimum 30 min after the injection. The catecholamine response was characterized by more pronounced epinephrine and less pronounced norepinephrine release ($p<0.001$, $p<0.01$, respectively, Fig. 2). The concentration of MDA and activity of antioxidant enzymes decreased during hypoglycemia ($p<0.01$ and $p<0.001$, respectively, Fig. 2).

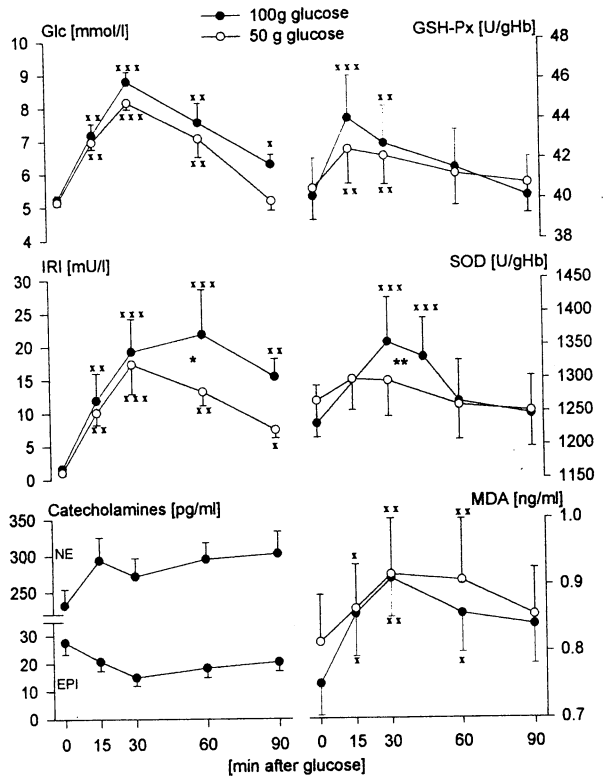

Fig. 3. *Variables after 50 g (empty circles) and 100 g (filled circles) glucose ingestion in healthy male subjects: plasma concentrations of glucose, insulin, MDA and catecholamines (only after 100 g), activity of SOD and GSH-Px. Significantly different from control samples (time 0): $^{x}p<0.05$, $^{xx}p<0.01$, $^{xxx}p<0.001$; significant difference between the two glucose doses: $*p<0.05$, $*p<0.01$, $***p<0.001$.*

Hyperglycemia

Glucose ingestion (both doses) induced a physiological increase of plasma insulin levels detected 30 min after its administration. After the higher glucose dose, the decline of insulinemia was more prolonged ($p<0.05$, Fig. 3). Plasma catecholamines were not significantly changed, although there was a tendency for increased norepinephrine and reduced epinephrine concentrations (Fig. 3). MDA concentration and GSH-Px activity were significantly elevated after both glucose doses (Fig. 3). Activity of SOD increased significantly only after 100 g glucose ingestion ($p<0.001$, Fig. 3). No significant dose-dependent differences in GSH-Px and MDA responses were observed (Fig. 3).

Discussion

Antioxidant defense mechanisms, important for protection of cells and tissues from oxidation damage, consist of non-enzymatic antioxidants and antioxidant enzymes, including SOD and GSH-Px. A balance is assumed to exist between free radicals and protective antioxidant reserves. When more free radicals are generated in certain physiological or pathological situations, greater activation of antioxidant enzymes and increased concentrations of toxic products (e.g. MDA) were observed (Roberfroid and Calderon 1995). In our investigation, free radical production was evaluated indirectly. Different mechanisms have been suggested to be operative in oxidative stress induced by muscle exercise, hyperinsulinemia or hyperglycemia. Increased activity of the sympathetic nervous system associated with oxidative pathways of catecholamines and mobilization of free fatty acids may also induce the formation of free radicals (Singal *et al.* 1983).

Short exercise at submaximal intensity in healthy volunteers was followed by increased plasma malondialdehyde concentrations. Exercise provides a useful model for studying oxidative processes in biological systems. Despite the limited direct evidence of free radical generation during exercise, there is an abundance of data providing indirect support for the hypothesis that oxidative processes occur at the cellular and molecular levels during prolonged aerobic exercise when tissue oxygen is markedly increased (Ji 1995, Aruomo 1994). A small part of oxygen which is not utilized by mitochondria, may be converted into several intermediates (O_2^-, H_2O_2 and OH^-), which subsequently leak out of the electron transport chain (Ji 1995). Using electron paramagnetic spectroscopy, Davies *et al.* (1982) demonstrated that free radical signals from tissues of rats running to exhaustion were intensified. The short-lasting submaximal work load applied in our investigations induced cardiovascular and catecholamine responses Vigaš *et al.* 1998, Križanová *et al.* 1998, Koška *et al.* 1999). A comparable work load was reported to increase oxygen consumption, glucose turnover and lipid mobilization (Kjaer *et al.* 1987). Submaximal exercise in our investigation was followed by increased

malondialdehyde concentrations and enhanced activity of the enzymatic antioxidant system. Increased oxidation of catecholamines at enhanced plasma adrenaline and noradrenaline levels may also participate in the mechanism of oxidative stress.

Insulin administration in the dose used in our study induces a short-lasting supraphysiological increase of plasma insulin concentrations up to 1000 μU/ml. This high insulin concentration induced hypoglycemia and high elevation of plasma adrenaline. Hyperinsulinemia has also been supposed to cause a rise in the concentration of plasma free radicals (Paolisso and Giugliano 1996). Krieger-Bauer and Kather (1992) demonstrated *in vitro* that insulin activated a plasma membrane-bound H_2O_2-generating system. However, insulin did not exhibit any such stimulating effect on the oxidative damage in our *in vivo* study. Moreover, the concentration of MDA and the activity of antioxidant enzymes were slightly diminished. The decreased formation of lipoperoxides in healthy men seems to be related to plasma glucose concentration rather than to hyperinsulinemia.

In healthy volunteers, glucose-induced hyperglycemia increased plasma C-peptide and insulin concentration, which was more than 20 times lower in comparison to the insulinemia after exogenous insulin administration during a hypoglycemic load. Increased MDA levels and enhanced activity of SOD and GSH-PX were observed in all subjects. This finding is in agreement with the assumption that hyperglycemia may be involved in the enhancement of plasma free radical production (Hunt *et al.* 1988, Ceriello *et al.* 1995, 1996). Glucose autoxidation represents a pathway by which glucose itself generates free oxygen radicals. The enediol form of glucose may be autoxidized to an enediol radical anion. The reduced oxygen products are superoxide radical anion (O_2^-), the hydroxyl radical (OH^-) and hydrogen peroxide (H_2O_2). The hydroxyl radicals produced specifically by glucose autoxidation were shown to damage proteins. Moreover, elevated glucose was also found to activate lipoxygenase enzymes (Nadler and Winer 1996) and to decrease the activity of NO synthase and glutathione reductase, resulting in increased susceptibility of endothelial cells to damage (Paoliso and Giugliano 1996). Interestingly, glucose ingestion was not followed by increased catecholamine release (Tse *et al.* 1983) and lipid mobilization (Vuorinen-Markkola *et al.* 1992) which can not thus participate in increasing the production of free radicals in hyperglycemia.

Our results indicate the presence of a clear-cut relation of plasma glucose concentration to oxidative damage and to an antioxidant response in healthy men. No relationship was observed among malondialdehyde concentration, antioxidant enzymes and plasma insulin, adrenaline, noradrenaline concentration, or the rate of glucose metabolism. In exercise-induced activation of antioxidant enzymes, however, other mechanisms than the glucose concentration were involved.

In conclusion, glucose induced the formation of lipoperoxides in a dose-dependent manner in healthy subjects. Hyperglycemia was involved in the mechanism of generation of oxidative stress in patients with NIDDM (Ceriello *et al.* 1995, 1996, Cominacini *et al.* 1996, 1997). Plasma glucose fluctuations should thus also be considered in studies evaluating oxidative damage in other diseases.

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