

Hyperinsulinemia and Oxidative Stress

P. KYSELOVÁ, M. ŽOUREK, Z. RUŠAVÝ, L. TREFIL¹, J. RACEK¹

First Department of Internal Medicine and ¹Department of Clinical and Experimental Biochemistry, University Hospital, Pilsen, Czech Republic

Received November 29, 2001

Accepted April 12, 2002

Summary

The aim of the study was to compare the effect of short-term hyperglycemia and short-term hyperinsulinemia on parameters of oxidative stress in Wistar rats. Twenty male rats (aged 3 months, average body weight 325 g) were tested by hyperinsulinemic clamp (100 IU/l) at two different glycemia levels (6 and 12 mmol/l). Further 20 rats were used as a control group infused with normal saline (instead of insulin) and 30 % glucose simultaneously. Measured parameters of oxidative stress were malondialdehyde (MDA), reduced glutathione (GSH) and total antioxidant capacity (AOC). AOC remained unchanged during hyperglycemia and hyperinsulinemia. Malondialdehyde (as a marker of lipid peroxidation) decreased significantly ($p < 0.05$) during the euglycemic hyperinsulinemic clamp, and increased significantly during isolated hyperglycemia without hyperinsulinemia. Reduced glutathione decreased significantly ($p < 0.05$) during hyperglycemia without hyperinsulinemia. These results suggest that the short-term exogenous hyperinsulinemia reduced the production of reactive oxygen species (ROS) during hyperglycemia in an animal model compared with the control group.

Key words

Oxidative stress • Hyperglycemia • Hyperinsulinemia • Rat

Introduction

Oxidative stress, a potentially harmful imbalance between the level of pro-oxidants and anti-oxidants in favor of the former, is thought to be an important source of vascular injury (Halliwell 1993). All oxidative reactions are a continuous source of potentially cytotoxic reactive oxygen species (ROS), which play an important role in living systems both through their beneficial and detrimental effects (Halliwell and Gutteridge 1999). Under physiological conditions, ROS are fully inactivated by an elaborate cellular and extracellular antioxidant defense system (Yu 1994). However, under certain conditions increased generation of ROS and/or

reduction of the antioxidant capacity leads to enhanced ROS activity and oxidative stress. Oxidative stress can cause cellular injury and tissue damage by promoting several cellular reactions, e.g. lipid peroxidation, DNA damage etc. (Yu 1994, Maxwell 1995).

Diabetes mellitus is characterized by increased production of ROS, sharp reduction in antioxidant defense and altered cellular redox status (Mullarkey *et al.* 1990, West 2000). Hyperglycemia may lead to increased generation of free radicals *via* multiple mechanisms. Patients with diabetes may be especially prone to acute and chronic oxidative stress which enhances the development of late diabetic complications. The chronic presence of higher glucose levels enhances the production of ROS from glucose glucooxidation, protein glycation

and glycooxidation. Lipid peroxidation (Kawamura *et al.* 1994) and lipid peroxides may increase the participation of advanced glycation end-products in the development of chronic vascular complications (Cerami *et al.* 1988). A decrease of oxidative stress in diabetes may diminish not only glycooxidation and lipid peroxidation, but also the risk of cardiovascular disease (Reaven *et al.* 1995). The rapid and large postprandial increase in blood glucose concentration is a frequent event in the life of diabetic patients (Ceriello *et al.* 1993, Ceriello 1997). The possible impacts of postprandial glycemie spiking on the pathogenesis of diabetic complications are studied currently (DECODE study 1999).

Many prospective studies suggest that hyperinsulinemia may be an important risk factor for ischemic heart disease. However, it has not been determined whether plasma insulin levels are independently related to ischemic heart disease after adjustment for other risk factors, including plasma lipoprotein levels (Despres *et al.* 1996, Lamarche *et al.* 1998).

It has not yet been decided whether to treat the badly controlled type 2 diabetes patients with insulin or whether to use peroral antidiabetic agents for the treatment despite the fact that endogenous insulin production can be exaggerated and lead to deterioration of their compensation.

The aim of our pilot study was to compare the effects of short-term hyperinsulinemia on parameters of oxidative stress (total antioxidant capacity, malondialdehyde, glutathione) in an animal model.

Methods

Forty male Wistar rats with an average body weight of 325 g (Charles River Breeding Laboratories), aged 3 months, were housed under standard laboratory conditions (temperature 23 ± 1 °C, 12-h light-dark cycle), drank tap water and were fed a standard diet *ad libitum*.

Thirty-six hours before the experiment the rats were anesthetized with ketamin (100 mg/kg i.p.) + xylazine (16 mg/kg i.p.) and the carotid artery and jugular vein were catheterized. Insulin-mediated whole body glucose uptake was measured in awake, unstressed, chronically catheterized rats by using the euglycemic hyperinsulinemic clamp (EHC) and hyperglycemic hyperinsulinemic clamp (HHC) as was previously described by De Fronzo *et al.* (1979).

Briefly, 20 rats received an infusion of insulin (100 IU/l) and 30 % glucose simultaneously during the hyperinsulinemic clamp at two different glycemia levels: 6 mmol/l (EHC) and 12 mmol/l (HHC). Another 20 rats

were used as controls and were given an infusion of normal saline (instead of insulin) and 30 % glucose simultaneously as described above. The clamp lasted for 180 min and each glycemia was maintained for 90 min. Three blood samples were withdrawn from each clamped rat (zero time, 90 and 180 min later). The measured markers of oxidative stress were malondialdehyde (MDA), glutathione (GSH) and total antioxidative capacity (AOC). Blood glucose was determined by using a B-glucose analyzer (HemoCue Sweden), AOC by TAS (Randox, United Kingdom) measured by a Hitachi 717 analyzer ($\lambda=600\text{nm}$), GSH by Bioxytech GSH-400 (OXIS, USA) measured by Clinicon ($\lambda=400\text{nm}$) and MDA after extraction with n-butanol measured on a Elisa-reader ($\lambda=532\text{nm}$). Insulinemia was measured by CIS Biointernational, France. The M-value (a numeric expression of peripheral tissue insulin resistance) was calculated to observe a glucose disposal rate.

One-way analysis of variance (ANOVA) was used in statistical analysis of the data, which are presented as mean \pm S.D. $P<0.05$ was considered significant.

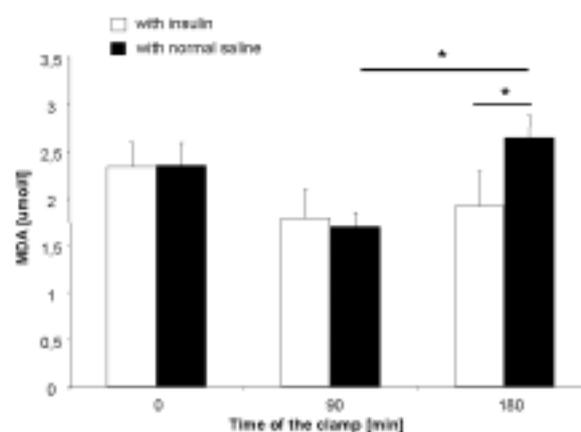


Fig. 1. Plasma concentration of malondialdehyde during euglycemic hyperinsulinemic and hyperglycemic hyperinsulinemic clamp in rats infused either with insulin (empty columns) or with saline (black columns). The data are means \pm S.D. * $p<0.05$.

Results

The concentration of malondialdehyde was the same in the two groups at the beginning of the clamp (2.34 ± 0.26 vs 2.35 ± 0.24 $\mu\text{mol/l}$). There was a non-significant decrease of malondialdehyde by the 90th min (1.79 ± 0.31 vs 1.70 ± 0.15 $\mu\text{mol/l}$) in the two groups, which might be caused by plasma dilution during blood sampling. During the following 90 min of hyperglycemic clamp (180th min of the whole clamp) there was an increase of malondialdehyde plasma concentration only

in the group infused with normal saline (1.92 ± 0.37 and $2.65 \pm 0.23 \mu\text{mol/l}$, respectively) (Fig. 1).

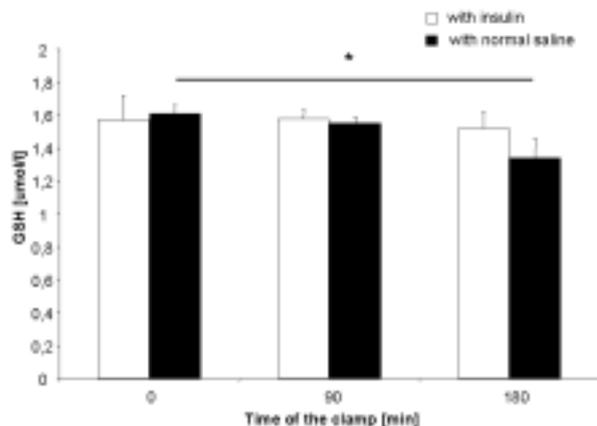


Fig. 2. Plasma concentration of reduced glutathione during euglycemic hyperinsulinemic and hyperglycemic hyperinsulinemic clamp in rats infused either with insulin (empty columns) or with saline (black columns). The data are means \pm S.D. * $p < 0.05$.

The concentration of reduced glutathione was the same in the two groups at the beginning of the clamp (1.57 ± 0.15 vs $1.61 \pm 0.05 \mu\text{mol/l}$). There was no change of this parameter in rats infused with insulin during the whole clamp. In contrast, a small but significant ($p < 0.05$) reduction was evident in the saline-infused group between basal values and the concentration at the end of the clamp ($1.34 \pm 0.12 \mu\text{mol/l}$ in the 180th min) (Fig. 2).

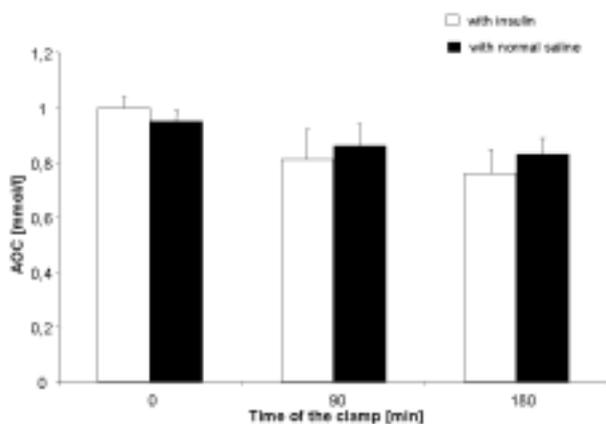


Fig. 3. Total antioxidant capacity during euglycemic hyperinsulinemic and hyperglycemic hyperinsulinemic clamp in rats infused either with insulin (empty columns) or with saline (black columns). The data are means \pm S.D. * $p < 0.05$.

The total antioxidant capacity of plasma remained unchanged during the whole clamp without any significant changes within the two groups (1.00 ± 0.04 vs

$0.95 \pm 0.04 \text{ mmol/l}$ at zero time, 0.81 ± 0.11 vs $0.86 \pm 0.08 \text{ mmol/l}$ in the 90th min, 0.76 ± 0.09 vs $0.83 \pm 0.06 \text{ mmol/l}$ in the 180th min) (Fig. 3).

Discussion

It is interesting that Haffner *et al.* (1995) has recently reported that the plasma of type 2 diabetic patients from the San Antonio Heart Study was significantly more susceptible to lipid peroxidation with a free radical initiator than the plasma of subjects with either normal or impaired glucose tolerance. In their view, hyperglycemia rather than insulin resistance caused the increased susceptibility to oxidation (Giugliano *et al.* 1996). Ceriello *et al.* (1997) found that acute hyperglycemia provokes oxidative stress which leads to depletion of antioxidant capacity. This finding has been related to the physiological situation in a study looking at the effect of a standard meal on the antioxidant capacity in diabetic and non-diabetic subjects. The total plasma free radical trapping activity was significantly reduced during short-term hyperglycemia in patients with type 2 diabetes mellitus and also in healthy individuals (Ceriello *et al.* 1997). Our findings are in agreement with this suggestion. This situation may be explained by the depletion of serum albumin during the clamp due to frequent blood sample withdrawal and simultaneous consumption of plasma antioxidants stimulated by acute hyperglycemia and production of ROS. Plasma albumin, via its thiol groups, is the main extracellular antioxidant molecule (Jones *et al.* 1988). Many glucose oxidation pathways generate reactive oxygen species. Therefore, glucose levels were more than sufficient to induce the generation of O_2^- in the presence of traces of transition metal cations. The glucose residues on glycated protein may donate electrons and form hydrogen peroxide. The gluconolactone product of glucose autooxidation binds to protein amino groups considerably more readily than does the unmodified glucose (Lindsay *et al.* 1997).

Insulin resistance and increased oxidative stress have been observed in obese type 2 diabetic patients (Paglioso *et al.* 1994, Škrha *et al.* 1996). Our finding of an inverse relationship between plasma MDA concentration and reduced GSH concentration glucose disposal rate during acute hyperglycemia without hyperinsulinemia in the control group is in disagreement with this suggestion. A decrease of oxidative stress could therefore improve insulin action in subjects with insulin resistance.

The results of published studies provide the evidence that acute hyperglycemia induces oxidative stress (Ceriello *et al.* 1997). The recognition of a connection between acute hyperglycemia, acute hyperinsulinemia and oxidative stress may contribute to the debate on the management of diabetes mellitus.

In conclusion, our results suggest that the short-term exogenous hyperinsulinemia reduced the production of reactive oxygen species during hyperglycemia in an animal model compared with the control group.

References

- CERAMI A, VLASSARA H, BROWNLEE M: Role of advanced glycosylation products in complications of diabetes. *Diabetes Care* **11** (Suppl 1): 73-79, 1988.
- CERIELLO A: Acute hyperglycemia and oxidative stress generation. *Diabet Med* **14**: S45-S49, 1997.
- CERIELLO A, DELLO RUSSO P, CURCIO F, PEGORARO I, GIUGLIANO D, AMSTAD P, CERUTTI PA: Antioxidant defences are induced by hyperglycemia in cultured human endothelial cells. *Diabetologia* **36** (Suppl 1): 203A, 1993.
- DECODE STUDY: Consequences of the new diagnostic criteria for diabetes in older men and women. *Diabetes Care* **22**: 1667-1671, 1999.
- DE FRONZO RA, TOBIN JD, ANDREAS R: Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* **237**: E214-E223, 1979.
- DESPRES JP, BENOIT L, PASCALE M, BERNARD C, GILLES R: Hyperinsulinemia as an independent risk factor for ischemic heart disease. *N Engl J Med* **334**: 952-957, 1996.
- GIUGLIANO D, CERIELLO A, PAOGLIOSO G: Oxidative stress and diabetic vascular complications. *Diabetes Care* **19**: 257-267, 1996.
- HAFFNER SM, AGIL A, MYKKANEN L, STERN MP, JIALAL I: Plasma oxidizability in subjects with normal glucose tolerance, impaired glucose tolerance and NIDDM. *Diabetes Care* **18**: 646-653, 1995.
- HALLIWELL B: The role of oxygen radicals in human disease, with particular reference to the vascular system. *Haemostasis* **23** (Suppl 1): 118-126, 1993.
- HALLIWELL B, GUTTERIDGE JMC: *Free Radicals in Biology and Medicine*, Oxford University Press, 1999, pp 20-37.
- JONES AF, WINKLES JW, JENNINGS PE : Serum antioxidant activity in diabetes mellitus. *Diabetes Res* **7**: 89-92, 1988.
- KAWAMURA M, HEINECKE JW, CHAIT A: Pathophysiological concentrations of glucose promote oxidative modification of low density lipoprotein by a superoxide dependent pathway. *J Clin Invest* **94**: 771-778, 1994.
- LAMARCHE B, TCHERNOF A, MAURIEGE P, CANTIN B, DAGENAIS GRLUPIEN PJ, DESPRES JP: Fasting insulin and lipoprotein B levels and low-density lipoprotein particle size as risk factors for ischemic heart disease. *JAMA* **279**: 1955-1961, 1998.
- LINDSAY RM, SMITH W, LEE WK, DOMINICZAK MH, BAIRD JD: The effect of delta-gluconate, an oxidised analogue of glucose, on the nonenzymatic glycation of human and rat hemoglobin. *Clin Chim Acta* **263** : 239-247, 1997.
- MAXWELL SR: Prospects for the use of antioxidant therapies. *Drugs* **49**: 345-361, 1995.
- MULLARKEY CJ, EDELSTEIN D, BROWNLEE M: Free radical generation by early glycation products: a mechanism for accelerated atherogenesis in diabetes. *Biochem. Biophys Res Commun* **173**: 932-939, 1990.
- PAGLIOSO G, D'AMORE A, VOLPE C, QUATRARO A, LEFEBVRE PJ: Evidence for a relationship between oxidative stress and insulin action in non-insulin-dependent type II diabetic patients. *Metabolism* **43**: 1426-1429, 1994.

Acknowledgements

This study was supported by a research grant LFUK Plzeň CEZ: J13/98:111400001. We warmly thank I. Klimeš and E. Šeböková from the Diabetes and Nutrition Research Group of the Slovak Academy of Sciences in Bratislava for helpful comments concerning our experiments.

-
- REAVEN PD, HEROLD DA, BARNETT J, EDELMAN S: Effects of vitamin E on susceptibility of low-density lipoprotein and low-density lipoprotein subfractions to oxidation and protein glycation in NIDDM. *Diabetes Care* **18**: 807-816, 1995.
- ŠKRHA J, ŠINDELKA G, HAAS T, HILGERTO VÁ J, JUSTOVÁ V: Comparison of insulin sensitivity in patients with insulinoma and obese type II diabetes mellitus. *Horm Metab Res* **28**: 595-598, 1996.
- WEST IC: Radicals and oxidative stress in diabetes. *Diabet Med* **17**: 171-180, 2000.
- YU BP: Cellular defenses against damage from reactive oxygen species. *Physiol Rev* **74**: 139-162, 1994.
-

Reprint requests

Dr. P. Kyselová, Department of Internal Medicine, University Hospital, Alej Svobody 80, 304 60 Pilsen, Czech Republic. E-mail: Kyselova@fnplzen.cz