Bulgarian Journal of Veterinary Medicine (2005), **8**, No 3, 157–161

CHANGES IN BLOOD GLUCOSE, TRIGLYCERIDES AND LIPID PEROXIDATION PRODUCTS IN RABBITS AFTER HANGING FIXATION

G. M. SAROV[1] & T. I. VLAYKOVA[2]

[1]Department of Pathophysiology, [2]Department of Biochemistry,
Medical Faculty, Trakia University, Stara Zagora, Bulgaria

Summary

Sarov, G. M. & T. I. Vlaykova, 2005. Changes in blood glucose, triglycerides and lipid peroxidation products in rabbits after hanging fixation. *Bulg. J. Vet. Med.*, **8**, No 3, 157–161.

Psycho-emotional stress is one of the risk factors for metabolic syndrome and related diseases. We decided to investigate the changes in glucose and lipids levels, as well as oxidative stress, generated after hanging fixation in rabbits. We used 12 male New Zealand White rabbits (2.5–3.5 kg body weight): 6 animals for hanging fixation and another 6 animals for fixation in box. Blood samples were taken before hanging and at min 15, 30, 90, 180 and serum levels of blood glucose and triglycerides (TG) were measured by commercially available kits. Lipid peroxidation products in plasma were evaluated before hanging and at minute 180 using the thiobarbituric acid method. Blood glucose level rose sharply after hanging (P=0.008) and remained at this level to the end of the experiment. TG also increased significantly after hanging (P=0.007), remained higher up to minute 90 and at minute 180 dropped down to the basal level (P=0.744). Significant increase in malonedialdehyde (MDA) after hanging fixation was also found (P=0.028). Hanging fixation appears to produce significant increase in MDA, blood glucose and triglyceride levels by a not well-established mechanism. Further research is needed to reveal these underlying mechanisms and the rationality of the use of the hanging fixation for investigation of stress-induced homeostatic alterations.

Key words: hanging fixation, malondialdehyde, serum glucose, serum triglycerides

INTRODUCTION

Psycho-emotional stress induces release of steroid and adrenergetic hormones that activate gluconeogenesis and glycogenolysis in liver, which increase blood glucose and suppress insulin secretion. It was recently found that both acute (Kosoa *et al.*, 2000) and chronic (Hunt *et al.*, 1988; Gumieniczek *et al.*, 2002; Kyselova *et al.*, 2002) hyperglycaemias increase production of reactive oxygen species (ROS). Various mechanisms have been suggested to contribute to the formation of these reactive oxygen-free radicals and glucose oxidation is believed to be the main source of free radicals (Kyselova *et al.*, 2002). Psycho-emotional stress induced by immobilization, chronic (Gumuslu *et al.*, 2002) and acute (Dede *et al.*, 2002) cold or by physical exercises (Kosoa *et al.*, 2000), was also found to increase ROS generation. One of the most prominent indicators of the presence of oxidative stress is the increased level of lipid peroxidation products (in particular malondialdehyde - MDA). Furthermore, Catherwood *et al.*, (2002) suggested that plasma lipid peroxidation was a good indicator for glucose-induced oxidative stress.

This study reports data coming from our attempts to establish a method for rabbit fixation providing stable glucose levels and easy manipulations. When we tried hanging fixation we found fast and significant increase in blood glucose and decided to prove whether lipid and oxidative status are also affected by this procedure.

MATERIALS AND METHODS

Twelve random selected male New Zealand rabbits (2.5–3.5 kg b.w.) were housed individually in stainless steel cages kept in a room at 22 ± 2 ^{0}C with 12:12-h light-dark cycle. Rabbits had free access to tap water and standard rabbits chow (Provimi, SZ Bg). The animals were carried for and handled in conformance with the Guidelines for Breeding and Care of Laboratory Animals (Veterinary Public Health Reports, 1994) and the Institutional Animal Care Committee of Trakia University, that approved the study protocols.

The chow was removed 4 h before fixing. Six rabbits were fixed in bag and hanged (HG) at a height of 1.50 m from the ground for 3 h (Fig. 1). Another six rabbits were fixed in boxes (control group – CG). Blood samples were obtained before (time zero) and at minutes 15, 30, 90, 180 after fixation from both the hanged and control groups.

Serum glucose and triglyceride (TG) levels were determined by commercially available kits (Human Gesellschaft für Biochemica und Diagnostica mbH, Germany). The products of lipid peroxidation in the serum were measured by the thiobarbituric acid method, based on assessment of malondialdehyde products (MDA) (Plazer *et al.*, 1966; Hristozov *et al.*, 2001).

Fig. 1. Photograph of the method of hanging fixation.

The data were statistically analysed using the StatView^{TM} package for Windows, v.4.53 (Abacus Concepts, Berkeley CA, USA). Basic descriptive statistics were applied to calculate mean values and standard deviations (SD). A paired *t*-test was used for comparison of values at different time points. When $P<0.05$, differences were considered to be statistically significant.

RESULTS

Hanging induced significant increases in blood glucose at min 15 (14.19±4.25 mmol/L vs 6.51±0.59 mmol/L at min 0; $P=0.008$) that remained significantly elevated to the end of experiment – 17.53±5.7 mmol/L, 16.32±6.13 mmol/L, 17.11±8.24 mmol/L at min 30, 90 and 180, respectively ($P<0.05$) (Fig. 2A). Analogous results, displaying dynamics of a significant increase in triglycerides compared to the baseline level were also present: 1.45±0.65 mmol/L; 1.78±0.65 mmol/L; 2.0±0.6 mmol/L; 2.06±0.5 mmol/L; 1.55±0.41mmol/L at min 0, 15, 30, 90, respectively ($P<0.05$). Only at the end of the next period (min 180), the level of TG dropped to the baseline (1.55±0.41

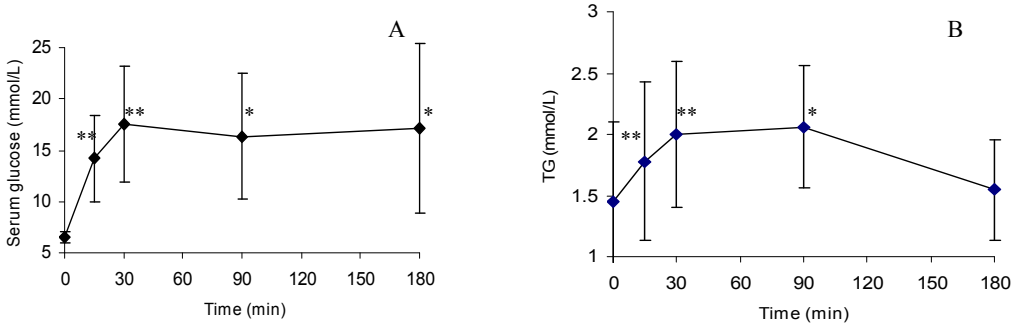

Fig. 2. Dynamics of serum glucose (A) and triglycerides (TG) (B) after hanging fixation of rabbits; * P<0.05; ** P<0.01 compared to baseline levels.

mmol/L, P=0.744) (Fig. 2B). The levels of lipid peroxidation products (MDA) were significantly increased at the end of the study period compared to the pre-treatment ones (2.24±1.12 μmol/L vs. 0.68±0.35 μmol/L, P=0.028). In control group, blood glucose, triglycerides and MDA levels remained unaltered during the experiment (P>0.05).

DISCUSSION

The increase in blood glucose, triglycerides and MDA after hanging fixation might be due to stress produced by the procedure. There are different methods of stress induction in cats (Feldhahn *et al.*, 1999), rats (Gumuslu *et al.*, 2002) and humans (Kosoa *et al.*, 2000) but rabbits are seldom used and we did not find any reports on hanging fixation. The precise mechanism of stress induction by hanging could not be determined at this stage of investigations. We suppose that the fear of height and/or sensation of body tightening by gravity may contribute to the observed effects. Nevertheless, stress explains agreeably the observed changes of blood biochemical indices. Since the catecholamines induce glycogenolysis in liver, the early increase in blood glucose might be due to the effect of epinephrine and norepinephrine. The gluconeogenetic activity of corticosteroids might be responsible for the later elevation in glucose level.

It is well-known that catecholamines activate lipolysis in adipose tissue and increase the free fatty acid flow to the liver where increased triglyceride synthesis and secretion occurs. Thus, we suppose that the observed increased level of triglycerides after the hanging could be attributed to the catecholamine effects.

Concerning the observed elevated levels of lipid peroxidation products (MDA), we suggest that it might be due to blood glucose increase after hanging fixation. It is well-proven that hyperglycaemia is a key causative factor in oxidative stress in tissue sites for diabetic complications (Obrosova, 2002). The oxidative stress in diabetes mellitus is characterized by increased production of ROS, sharp reduction in antioxidant defense and altered cellular redox status (Kyselova *et al.*, 2002). These changes occur via multiple mechanisms including glucose glycoxidation, protein glycation and glycooxidation. The acute short-time hyperglycaemia has also been shown to produce

increased level of lipid peroxide end-products (MDA) (Kyselova *et al.*, 2002) and to reduce total plasma free radical trapping activity (Ceriello, 1997). Our finding of increased level of lipid peroxide end-product (MDA) is in agreement with the above mentioned works and may confirm the key role of hyperglycaemia in generating the disbalance between pro-oxidative reactions and anti-oxidative defense. The slight, but statistically significant sustained increased level of triglycerides found by us, may also contribute to the enhanced concentration of lipid peroxidation products, since the serum lipoproteins (especially VLDL and LDL) are highly susceptible to the attack of ROS and lipid peroxidation.

In conclusion, hanging fixation seemed to be stressful enough to produce changes in glucose, lipid metabolism and MDA levels. We still do not recommend hanging fixation for stress modeling, since the precise mechanism of observed homeostatic alterations remained to be elucidated in further studies.

ACKNOWLEDGEMENTS

The current work is a part of a project financed by the Faculty of Medicine, Trakia University, Stara Zagora. We would like deeply to thank Asoc. Prof. Veselina Gadjeva, PhD, for her kind permission to use the adapted method for MDA assesment.

REFERENCES

Catherwood, M. A., L. A. Powell, P. Anderson, D. McMaster, P. C. Sharpe & E. R. Trimble, 2002. Glucose-induced oxidative stress in mesangial cells. *Kidney International,* **61,** 599–608.

Ceriello, A., 1997. Acute hyperglycaemia and oxidative stress generation. *Diabetic Medicine*, **14**, S45–49.

Dede, S., Y. Deger & I. Meral, 2002. Effect of short-term hypothermia on lipid peroxidation and antioxidant enzyme activity in rats. *Journal of Veterinary Medicine Series A - Physiology Pathology Clinical Medicine*, **49**, No 6, 286–288.

Feldhahn, J. R., J. S. Rand & G. Martin, 1999. Insulin sensitivity in normal and diabetic cats. *Journal of Feline Medicine and Surgery,* **1**, No 2, 107–115.

Gumieniczek, A., H. Hopkala, Z. Wojtowicz & M. Wysocka, 2002. Changes in antioxidant status of lung tissue in experimental diabetes in rabbits. *Clinical Biochemistry*, **35**, 147–149.

Gumuslu, S., S. B. Sarikcioglu, E. Sahin, P. Yargicoglu & A. Agar, 2002. Influences of different stress models on the antioxidant status and lipid peroxidation in rat erythrocytes. *Free Radical Research*, **36**, No 12, 1277–1282.

Hristozov, D., V. Gadjeva, T. Vlaykova & G. Dimitrov, 2001. Evaluation of oxidative stress in patients with cancer. *Archives of Physiology and Biochemistry,* **109**, No 4, 331–336.

Hunt, J. V., R. T. Dean & S. P. Wolff, 1988. Hydroxyl radical production and autoxidative glycosylation. Glucose autoxidation as the cause of protein damage in the experimental glycation model of diabetes mellitus and ageing. *The Biochemical Journal,* **256**, 205–212.

Kosoa, J., P. Blazicek., M. M. Grna & J. D. Kvetnansky, 2000. Insulin, catecholamines, glucose and antioxidant enzymes in oxidative damage during different loads in healthy humans. *Physiological Research,* **49,** suppl. 1, S95–S100.

Kyselova, P., M. Zourek, Z. Rusavy, L. Trefil & J. Racek, 2002. Hyperinsulinemia and oxidative stress. *Physiological Research,* **51,** 591–595.

Obrosova, I. G., 2002, How does glucose generate oxidative stress in peripheral nerve? *International Review of Neurobiology,* **50,** 3–35.

Plazer, Z. A., L. L. Cushman & B. C. Johnson, 1966. Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. *Analytical Biochemistry,* **16,** 359–364.

Veterinary Public Health Reports, 1994. Guidelines for Breeding and Care of Laboratory Animals, WHO/FAO Collaboration Centre for Research and Training in Veterinary Public Health.

Paper received 25.09.2003; accepted for publication 15.04.2004

Correspondence:

Dr. G. M. Sarov
Section of Pathophysiology,
Faculty of Medicine,
Trakia University,
11, Armeiska str.
6010 Stara Zagora, Bulgaria