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Short communication

A CASE WITH UNUSUAL BLOOD CHEMISTRY PARAMETERS FOLLOWING A GLUCOSE TOLERANCE TEST AND STRESS

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The type II diabetes affects about 3% of human population. Its pathogenesis is not fully elucidated and thus, appropriate animal models for chronic experiments aiming to study the mechanisms controlling glucose homeostasis are needed. In the literature, models using mice and rats are reported, but they are not adequate for collection of sufficient amounts of blood during a chronic experiment. Therefore, our interest was directed towards another animal species, that is sometimes cited in studies on glucose homeostasis – the New Zealand white rabbit (NZW) (Kosoa *et al.*, 2000). A group of NZW rabbits was submitted to various immobilization procedures and to routine intravenous glucose tolerance test (IGTT) (Quon *et al.*, 1994; Hermans *et al.*, 1999) that allows to assess both the insulin resistance and the pancreatic insulin secretion.

Stress is an important risk factor for insulin sensitivity disturbance because of the resulting endogenic hyperglycaemia. Recently it was found out that immobilization stress, chronic (Gumuslu *et al.*, 2002) and acute (Dede *et al.*, 2002) exposure to cold as well as exercise (Kosoa *et al.*, 2000) increased the oxidative stress. Some investigators consider that the plasma lipid peroxidation is a suitable indicator of diabetes-induced oxidative stress in NZW rabbits (Davis *et al.*, 2000) and of occurring of glucose-induced oxidative stress (Catherwood *et al.*, 2002). That is why, we decided to investigate the effect of a stressful immobilization upon blood concentrations of glucose, triacylglycerols and the products of lipid peroxidation. In this communication, we present data obtained in a single animal, that strongly differed from others and analysed the significance of this case in explaining the impaired glucose tolerance in NZW rabbits.

Ten male NZW rabbits, weighing 2.5–3 kg, were submitted to two consecutive experiments with a 10–day interval between. In the first experiment, the rabbits were fixed to the cage using an original approach of ours – via a clamp fixing the head of the animal to the feeding-trough. One hour after the fixation, an intravenous glucose tolerance test (IGTT) was performed via infusion of 0.3 mL/kg 40% glucose for 2 min. Blood samples of 0.4 mL were obtained prior to (min 0) and by post infusion min 3, 5, 10, 20, 30, 45, 60 from the auricular vein. After separation of the serum, glucose concentrations were assayed by the glucose oxidase method and insulin – by immunoradiometric assay (DiaSorin). Ten days later, we reproduced a stress model via fixation in a bag for 3 h (Sarov, 2004). Blood samples (0.4 mL) for glucose and trigly-

cerides determinations were obtained prior to the fixation and 15, 30, 90 and 180 min after that. The blood specimens (1.5 mL) for peroxidation products determination were collected prior to and 3 h after the fixation. The plasma glucose concentrations were assayed as described, triacylglycerols levels – with a commercial kit of Human (Germany), and the total amount of plasma products of lipid peroxidation – using the thiobarbituric acid method by spectrophotometric measurement of malondialdehyde (Plazer *et al.*, 1966).

The rabbit designated as R1, was an animal from the studied group and was submitted to identical stress procedures. The remaining rabbits that showed almost undiversified reactions to experimental procedures, were accepted as controls. Their data were submitted to analysis of variance and compared to R1.

The experiments were performed according to the provisions of Council directive of 24 November 1986 on approximation of laws regulations and administrative provisions of the Member States regarding the protection of animals used for experimentation and other scientific purposes (86/609/EEC).

The R1 rabbit's results were noticeably different from those of the other animals in the group. In the IGTT, serum glucose concentrations by min 0, 5, 10, 30, 45 and 60 were higher than the mean values plus SD of controls, and insulin levels by min 3, 5, 20, 30, 45 and 60 – lower than mean insulin levels minus SD in controls (Table 1). This is indicative for an impaired glucose tolerance, due to hypoinsulinaemic response to the glucose challenge.

Under fixation stress, R1 exhibited a significantly higher endogenic hyperglycaemia by min 15, 30, 90 and 180 compared to the mean values plus SD of controls as well as much higher MDA levels by min 0 and 180, compared to mean values plus SD of controls (Table 2) – an indirect indication for the presence of oxidative stress in basic conditions and its significant increase under the influence of fixation stress.

In NZW rabbit populations, an impaired glucose tolerance, reduced insulin secretion (Taylor *et al.*, 1980) and even a

Table 1. Serum glucose and insulin concentrations during IGTT in control rabbits and in the R1 rabbit

Time*	Serum glucose, mmol/L		Serum insulin μU/L	
	Controls (mean±SD)	R1	Controls (mean ± SD)	R1
0	5.90 ± 1.07	8.59	7.39 ± 2.23	6.27
3	13.14 ± 2.81	13.54	98.64 ± 68.11	4.02
5	11.39 ± 1.80	13.39	98.06 ± 64.49	10.50
10	10.02 ± 1.52	12.23	59.96 ± 59.47	5.93
20	10.80 ± 3.11	10.43	68.47 ± 50.16	10.50
30	8.21 ± 1.50	10.31	26.84 ± 12.25	5.67
45	7.07 ± 1.24	10.46	24.47 ± 15.00	5.39
60	7.49 ± 1.40	9.30	19.54 ± 13.44	5.00

* min after the beginning of glucose infusion.

Table 2. Plasma glucose, tryglycerides and malondialdehyde concentrations following a fixation stress in control rabbits and in the R1 rabbit

Time*	Plasma glucose, mmol/L		Plasma triglycerides, mmol/L		Plasma malon-dialdehyde, μmol/L	
	Controls (mean±SD)	R1	Controls (mean ± SD)	R1	Controls (mean ± SD)	R1
0	9.75 ± 0.47	6.46	1.57 ± 0.73	1.62	0.56 ± 0.34	1.23
15	12.70 ± 4.30	19.20	1.85 ± 0.81	1.87		
30	15.04 ± 4.60	26.10	2.04 ± 0.74	2.14		
90	12.57 ± 2.36	22.66	2.08 ± 0.49	2.52		
180	11.99 ± 2.74	26.17	1.41 ± 0.25	2.29	1.68 ± 0.45	4.26

* min after fixation.

spontaneous diabetes (Roth *et al.*, 1980) have been reported. Considering the parameters in R1, it probably refers to this subgroup. We have however established also that R1 was more sensitive to stress procedures. As stress is related to adrenergic and corticosteroid-mediated hyperglycaemia and adrenergic suppression of the insular apparatus, the pathogenesis of the impaired glucose tolerance observed in NZW rabbits could be due to the involvement of contrainsular homones, released by fixation stress and the experimental handling procedures.

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,** No 2, 599–608.

Davis, R. L., C. L. Lavine, M. A. Arredondo, P. McMahon & T. E. Jr. Tenner, 2002. Differential indicators of diabetes-induced oxidative stress in New Zealand White rabbits: Role of dietary vitamin E supplementation. *International Journal of Experimental Diabetes Research,* **3**, No 3, 185–192.

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.

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.

Hermans, M., C. J. Levy, R. J. Morris & C. Robert, 1999. Comparison of tests of b-cell function across a range of glucose tolerance from normal to diabetes. *Diabetes,* **48**, 1779.

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.

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

Quon, M. J., C. Cochran, S. I. Taylor & R. C. Eastman, 1994. Direct comparison of standard and insulin modified protocols for

minimal model estimation of insulin sensitivity in normal subjects. *Diabetes Research,* **25**, No 4, 139–149.

Roth, S. I., H. H. Conaway, L. L. Sanders, R. E. Casali & A. E. Boyd 3rd, 1980. Spontaneous diabetes mellitus in the New Zealand white rabbit: Preliminary morphologic characterization. *Laboratory Investigation*, **42**, No 5, 571–579.

Sarov, G. M. & T. I. Vlaykova, 2004. Changes in blood glucose, lipids and lipid peroxidation products in rabbits after hanging fixation. *Bulgarian Journal of Veterinary Medicine* (in press).

Taylor, R. L., H. H. Conaway & S. I. Roth, 1980. Depression of leucine and isoproterenol induced insulin secretions in the spontaneously diabetic New Zealand white rabbit. *Endocrine Research Communications,* **7**, No 2, 121–136.

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