

INTRAVENOUS GLUCOSE TOLERANCE TEST IN DOGS WITH EXPERIMENTAL *STAPHYLOCOCCUS* INFECTION

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Summary

Andonova, M. Y., E. P. Slavov, P. V. Djelebov, V. S. Urumova, K. M. Ivanova & G. M. Sarov, 2006. Intravenous glucose tolerance test in dogs with experimental *Staphylococcus* infection, *Bulg. J. Vet. Med.*, 9, No 2, 123–131.

Experimental *Staphylococcus* infection was induced in non-obese male dogs (group A, n=6, initial body weight 13.65±3.32 kg) and subjected to overfeeding (obese) male dogs (group B, n=6, initial body weight – 12.87±1.43 kg). In group B the body weight increased to 16.54±1.67 kg after 90 days of overfeeding on a high-fat diet.

At the end of the 90-day period of overfeeding of group B, *Staphylococcus* infection was caused by subcutaneous application of 5 mL bacterial suspension (10⁹ CFU/mL) of *Staphylococcus intermedius* in the lumbar region of dogs from groups A and B.

Some clinical signs – body temperature, heart rate, respiratory rate were detected before the infection in groups A and B as well as on post infection hours 3, 24, 48, 72 and day 7 and 14. In all infected animals the site of inoculation of *Staphylococcus intermedius* was painful, swollen and with elevated temperature. Later, an abscess developed, that turned to a skin erosion of 5–8 cm. Between post infection hours 3 and 72, body temperature, heart and respiratory rates increased in both non-obese dogs (group A) and obese dogs (group B).

The intravenous glucose tolerance test (IVGTT) was performed by infusion of 40% glucose solution at 0.3 mL/kg for a period of 2 min using pumps in the cephalic vein. Blood samples were collected from the cephalic vein of the opposite forelimb 10 min before infusion (min –10), right before infusion (min 0) and at post infusion min 3, 5, 30 and 60.

The experimental groups were submitted to IVGTT prior to infection and four days after it. The infection in dogs with normal condition (group A) did not have any significant effects on glucose tolerance (GT), while in obese dogs (group B), infection resulted in a significantly increased blood glucose levels by min 30 (7.6 ± 0.4 mmol/L), as compared to blood glucose levels in group A (4.93 ± 0.18 mmol/L) on the same minute.

It is concluded that in our experiment neither obesity nor infection had a significant impact on GT, but the combination of those two factors led to suppressed glucose utilization, that might be indicative of decreased insulin sensitivity.

Key words: dogs, infection, insulin resistance, intravenous glucose tolerance test, *Staphylococcus intermedius*

INTRODUCTION

In human medicine, the intravenous glucose tolerance test (IVGTT) is among the most commonly used functional tests in

carbohydrate metabolism studies (Jelinger *et al.*, 1978; Bor *et al.*, 1980; Ader *et al.*, 1985; Weber *et al.*, 1989; Tarello, 2001;

Ionut *et al.*, 2004). In veterinary medical studies, its application depends on several factors – animal species, feeding regimen, glucose clearance. This explains the variety of glucose doses, used in dogs and cats – from 0.12 to 1.25 g/kg (Bello *et al.*, 1976; Eigenmann *et al.*, 1977; Toffolo *et al.*, 1980; Ishioka *et al.*, 2005). It must be stated that these two species had evolved as obligate carnivores and their natural diet contains many proteins and few carbohydrates.

IVGGT has a number of advantages to the oral test – the effect of intestinal absorption is excluded, its reproducibility is very high (Church, 1980; Irvine *et al.*, 2002). It allows to detect troubles in carbohydrate tolerance and permits to analyze insulin resistance. The latter is a state of incapability of insulin to perform its biological effect in physiological concentrations. It could be related to impaired synthesis of insulin receptors as well as to their reduction in tissues (mainly fat and muscle tissues) as well as to impaired synthesis of insulin from pancreatic β -cells.

Obesity (Ishioka *et al.*, 2005), as well as acute and chronic infections are frequently accompanied by insulin resistance (Martin *et al.*, 1991).

Despite the fact that in dogs, staphylococci and particularly *Staphylococcus intermedius* are the main etiological agent for a number of septic states (Bohach *et al.*, 1990; Opal & Cohen, 1999; Tarello, 2001), abscesses (Brook, 2002), wound infections and dermatitis (Allaker *et al.*, 1991; Day, 1994) there are no data about the effect of infections caused by these agents on systemic glucose tolerance. Also, there is no much information about the simultaneous effect upon glucose homeostasis of factors such as obesity and infection, which independently are able to produce insulin resistance.

The aim of the present study was to evaluate the effects of *Staphylococcus intermedius* infection in dogs in normal condition and obese dogs upon the insulin sensitivity.

MATERIALS AND METHODS

Animals

Twelve male, clinically healthy adult mongrel dogs aged 4–7 years were used. They were housed in individual metal cages with wooden floor, at an ambient temperature of 20–22 °C and air humidity of 50–60%. The animals were walked on a leash for 15 min twice a day. The dogs were treated against parasites with Prazimec – D (Biovet, Peshtera, Bulgaria) at a dose of 1 tablet/10 kg. Also, they were treated against ectoparasites with antiparasite shampoo, Ectomin and Tapilan (Dorvet, Israel). The period of adaptation of dogs prior to the experiment was 1 month.

Experimental design

The experimental animals were divided into 2 groups:

- Group A – (control non-obese dogs). It consisted of 6 dogs with initial body weight of 13.65 ± 3.32 kg. Their daily ration included 310 g commercial dry food (Jambo dog, Gallisman S.A., Bulgaria), containing extruded grain, vegetable protein, fat, dehydrated poultry meat, amino acids, edible chestnut extract (tannin), plant extracts, vitamins and trace elements, minerals and antioxidants. The food analytical content was as followed: crude protein – min. 17 %, crude ash – 8 %, crude fibre – max. 4%, crude fat – min. 8%, phosphorus – 0.75%–0.95%, calcium – 0.95%–1.30%, zinc – 35

mg/kg, chlorides – 0.95%, sodium – 0.4%, magnesium – 50 mg/kg, copper – 9 mg/kg, vitamin E – min. 200 mg/kg, vitamin A – 11 000 UI/kg.

- Group B (subjected to overfeeding, obese dogs). It included 6 dogs with initial body weight 12.87 ± 1.43 kg. The animals were maintained for 90 days with 400 g daily commercial dry food (Jambo dog, Gallisman S.A., Bulgaria), supplemented with 10 g/kg fat in order to induce obesity. The body weight of this group at the end of feeding period with the high calorie diet was 16.54 ± 1.67 kg.

At the end of the 90-day period of overfeeding, in groups A and B, experimental staphylococcal infection was produced by subcutaneous application of 24-hour broth culture of *Staphylococcus intermedius* with a density of 10^9 colony forming units per mL (CFU/mL), at a dose of 5 mL/dog in the lumbar region.

After the infection, all animals – both non-obese (group A) and obese (group B) received dry food in an amount, stated for group A. Each dog had free access to drinking water.

Intravenous glucose tolerance test (IVGTT)

IVGTT was performed in dogs from both groups on post infection day 4. For comparative purposes, the results of the same test performed in animals from group A prior to the experiment and the dogs from group B after getting overweight but prior to the infection, were used.

IVGTT was performed in the morning, after 12 h fasting, via infusion of 40% glucose solution at 0.3 mL/kg for a period of 2 min using pumps in the cephalic vein. Blood samples were collected from the opposite cephalic vein 10 min before infusion (min –10), right before infusion (min

0) and on post infusion minutes 3, 5, 30 and 60.

Blood glucose concentrations were assayed by an express glucose oxidase method using Glucometer Elite (Bayer®).

Bacterial strain

A field *Staphylococcus intermedius* strain isolated from uroculture of a dog with clinical and microbiologically proven urinary infection was used. The strain was isolated in the Department of Veterinary Microbiology, Infectious and Parasitic Diseases – Trakia University, and its typification was done by a semi-automatic identification system BD BBL Crystal Gram Positive ID System.

Clinical status

Experimental dogs were weighed and the rectal temperature, heart rate, respiratory rate, colour of conjunctives, behaviour and appetite were determined prior to *S. intermedius* infection and at post infection hours 3, 24, 48, 72 and post infection days 7 and 14. The changes in hair and skin at the site of infective agent inoculation were monitored visually and by palpation.

Statistical analysis

The data were statistically processed by one-way ANOVA at $P < 0.05$ simultaneously with the Tukey's test (Stat Most version 2.50 for Windows 1994/1995). They were presented as mean \pm SEM.

RESULTS

Identification of S. intermedius

The determination of morphological and biological properties of the strain showed that it was a catalase-positive, oxidase-negative, plasmocoagulase- and deoxyribo-

Table 1. Clinical parameters in dogs with experimental staphylococcal infection (*Staphylococcus intermedius*, 24-hour broth culture, 10^9 CFU/mL), applied s.c at 5 mL/dog (mean \pm SEM)

	Prior to infection		Time after experimental infection ***											
	before getting overweight*	after getting overweight**	hour 3	hour 24	hour 48	hour 72	day 7	day 14	hour 3	hour 24	hour 48	hour 72	day 7	day 14
Group A (non-obese dogs)	Body temperature (°C)	38.7 \pm 0.06	39.2 \pm 0.1 ^{a1}	39.7 \pm 0.17 ^{a2}	39.1 \pm 0.1 ^{a1}	38.9 \pm 0.18	38.4 \pm 0.12 ^{c2, d3, e2, f1}	38.6 \pm 0.09 ^{e1, d2, e1}	91 \pm 7.56	104 \pm 5.26 ^{a1}	93 \pm 6.17	89 \pm 7.3	85 \pm 2.16	83 \pm 3.5
	Heart rate (min ⁻¹)	89 \pm 2.6	91 \pm 7.56	104 \pm 5.26 ^{a1}	93 \pm 6.17	89 \pm 7.3	85 \pm 2.16	83 \pm 3.5	29 \pm 2.15	39 \pm 2.73 ^{a2}	38 \pm 1.66	34 \pm 1.20	28.8 \pm 0.91	28.17 \pm 1.66
	Respiratory rate (min ⁻¹)	26 \pm 0.93	29 \pm 2.15	39 \pm 2.73 ^{a2}	38 \pm 1.66	34 \pm 1.20	28.8 \pm 0.91	28.17 \pm 1.66	38.8 \pm 0.12	38.9 \pm 0.17	39.8 \pm 0.31 ^{b2, c1}	39.5 \pm 0.33 ^{b1}	39.2 \pm 0.8	39.4 \pm 0.35 ^{d3, e2,}
Group B (obese dogs)	Body temperature (°C)	38.6 \pm 0.09	38.9 \pm 0.17	39.8 \pm 0.31 ^{b2, c1}	39.5 \pm 0.33 ^{b1}	39.2 \pm 0.8	39.4 \pm 0.35 ^{d3, e2,}	38.5 \pm 0.23	89 \pm 3	107 \pm 5.9	95 \pm 2.65	90 \pm 2.78	83 \pm 2.15	91 \pm 2.95
	Heart rate (min ⁻¹)	91 \pm 2.94	95 \pm 4.80	107 \pm 5.9	95 \pm 2.65	90 \pm 2.78	83 \pm 2.15	91 \pm 2.95	28 \pm 0.58	31 \pm 1.66	27 \pm 1.34	29 \pm 2.15	27 \pm 1.66	26 \pm 1.57
	Respiratory rate (min ⁻¹)	26.0 \pm 0.73	29 \pm 2.15	31 \pm 1.66	27 \pm 1.34	29 \pm 2.15	27 \pm 1.66	26 \pm 1.57	26.0 \pm 0.73	31 \pm 1.66	27 \pm 1.34	29 \pm 2.15	27 \pm 1.66	26 \pm 1.57

* baseline A (for group A); ** baseline B (for group B); *** the data for groups A and B are referring to non-overweight and overweight dogs, respectively. Statistical significance: a1 – P<0.05, a2 – P<0.01 vs baseline A; b1 – P<0.05, b2 – P<0.01 vs baseline B; c1 – P<0.05, c2 – P<0.01 vs hour 3; d2 – P<0.01, d3 – P<0.001 vs hour 24; e1 – P<0.05, e2 – P<0.01 vs hour 48; f1 – P<0.05 vs hour 72.

nuclease-producing *Staphylococcus intermedius*.

Experimental staphylococcal infection

Clinically, the experimental staphylococcal infection was manifested in dogs from groups A and B between post infection hours 3 and 72. Non-specific signs, common for a number of infectious diseases (restricted locomotor activity, decreased appetite, fever, enhanced heart and respiratory rates) were observed.

In non-obese dogs (group A – Table 1), the studied clinical parameters peaked by hour 24 when rectal body temperature was increased to 39.7 ± 0.17 °C ($p < 0.01$) vs 38.7 ± 0.06 °C at baseline, the heart rate became 104 ± 5.26 min⁻¹ compared to 89 ± 2.6 min⁻¹ ($p < 0.05$) initially and the respiratory rate – 39 ± 2.73 min⁻¹ vs 26 ± 0.93 min⁻¹ ($p < 0.01$) at baseline.

Obese dogs, following the 90-day high caloric diet (group B) weighing 16.54 ± 1.67 kg at the time of inoculation, also reacted with increased values of clinical parameters with maximum by post infection hour 24 when the body temperature was 39.8 ± 0.31 °C; the heart rate 107 ± 5.9 min⁻¹ and the respiratory rate – 31 ± 1.66 min⁻¹.

In all dogs, the site of inoculation by post infection hour 24 was painful, swollen and temperate. Later, abscesses as well as tissue erosions with a size of 5–8 cm developed.

Glucose tolerance test

The blood glucose concentrations after the IVGTT, performed prior to and after infection of non-obese and obese dogs, are shown on Fig. 1. It could be seen that the dynamics of blood glucose changes in non-obese infected and obese non-infected dogs was similar. A sharp increase occurred by min 3 when maximum

levels occurred in all dogs. The maximum was the best manifested in obese dogs whereas the lowest initial values were measured in non-obese infected dogs. Afterwards, blood glucose levels decreased. By the 30th minute, in all animals excluding obese infected ones, the parameter returned either to or under the initial levels.

Statistically significant differences were observed only by min 30 in obese and infected dogs vs non-obese, non-infected animals ($P < 0.05$). They were manifested with persistence of high glucose levels – 7.6 ± 0.4 mmol/L, that were higher than those in non-obese dogs prior to (5.18 ± 0.28 mmol/L) and after (4.93 ± 0.18 mmol/L) infection, as well as than those at the background of obese dogs prior to infection (5.29 ± 0.36 mmol/L). By min 60, blood glucose levels in obese infected dogs did not return back to initial concentrations yet remained at a higher level.

DISCUSSION

Our experiment showed that in dogs under both normal condition and obesity, the subcutaneously inoculation of 5 mL *Staphylococcus intermedius* (1×10^9 CFU/mL), provoked an infection accompanied by local and general systemic signs. The observed fever, enhanced heart and respiratory rates by post infection hour 24 are an indication of a systemic non-specific response and signs of inflammation. As the maximum values of these clinical parameters were insignificantly higher than the reference ones for the species (Lineva, 2003; Bickhardt, 1992) it could be assumed that the inflammatory response in both normal and obese dogs was subacute. It is known that the time and degree of clinical manifestation of the infection re-

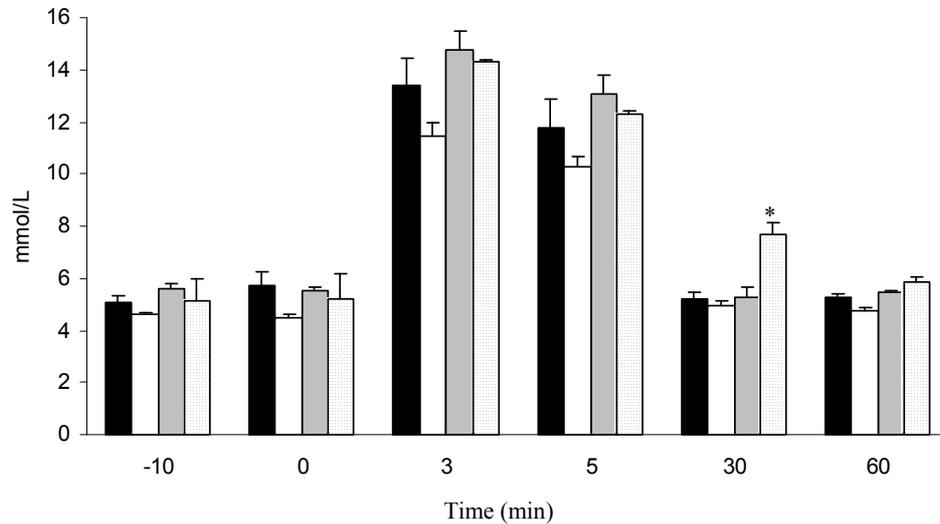


Fig. 1. Blood glucose levels after IVGTT in non-obese and obese dogs prior to and after experimental infection with *Staphylococcus intermedius*, 24-hour broth culture, 10^9 CFU/mL, applied s.c. at 5 mL/dog (mean \pm SEM); ■ – non-obese dogs (group A) prior to infection – baseline; □ – non-obese dogs (group A) after infection; ▒ – obese dogs (group B) prior to infection – baseline; ▨ – obese dogs (group B) after infection; * $P < 0.05$ vs non-obese and non-infected dogs.

sulting from the s.c. administration of staphylococci was directly related to the dose and the species of the infection agent, as evidenced by Dimitrova *et al.* (2003). After the use of another microbial species – *Staphylococcus aureus* at a dose different from our ($5 \text{ mL } 1 \times 10^7 \text{ CFU/mL}$), the most evident clinical changes in body temperature, heart and respiratory rates were observed by the 4th post infection hour.

In our experiments, the functional investigation of carbohydrate metabolism in healthy non-obese and obese dogs and dogs with staphylococcal infection was performed via an intravenous model of glucose tolerance test according to the feeding regimen and the biological species. The applied dose of glucose (0.12 g/kg) was chosen accounting for the fact that the high glucose concentration in dogs could cause endothelial damage,

glucosuria and to increase the risk of hypophosphataemia (Finco *et al.*, 1994; Festa *et al.*, 2000).

The lack of statistically significant differences in this test's results between non-obese infected and obese non-infected dogs suggested that the low-degree systemic stimuli used in the present experimental design (low dose of glucose loading, weakly manifested experimental infection, obesity caused by intake of high fat diet for 90 days) taken alone, did not result in alteration of systemic glucose tolerance. The combination of sub-threshold stimuli however resulted in severe pathological changes, manifested with impaired glucose tolerance (in obese and infected dogs). That is why, it could be concluded that weak stimuli could be as pathogenic as strong ones because of the possibility of combination of their systemic effects.

The infections, accompanied by deeply impaired hormonal and metabolic systemic state, result in insulin resistance. This is supported by the fact that the maintenance of blood glucose within the normal range is a consequence of an intricate homeostatic mechanism that is influenced by infectious agents in a variety of ways. It includes not only the functional state of the pancreas (Ahren & Pacini, 2004), but also the status of the liver (Bor *et al.*, 1980; Bradley & Bergman, 1992), the nervous system (Jelinger *et al.*, 1978; Rozman *et al.*, 2002), the thecretory function of the anterior pituitary gland (Court *et al.*, 1998), adrenal glands (Peterson *et al.*, 1984; Martin *et al.*, 1991; Fernandez-Real *et al.*, 2000, Fernandez-Real & Ricart, 2003). The intestinal absorption (Kohler *et al.*, 1992), the renal function and the degree of glucose utilization by tissues (Falholt *et al.*, 1985) are also supposed to play an important role in this connection.

In conclusion, the results of the present experiment suggested that independently, obesity and infection did not influence the glucose tolerance. This is substantiated by the changes in blood glucose curves in non-obese infected dogs and obese non-infected dogs (Fig. 1). Perhaps, both processes had autonomous effects upon insulin sensitivity, dependent on their intensity. Probably, their negative effects upon glucose homeostasis are added up throughout their combination and glucose utilization could be delayed, a fact that could be interpreted as a signs of insulin resistance.

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American Journal of Physiology, **256**, No 4 (1), E524–535.

* Author's translation

Paper received 24.10.2005; accepted for publication 20.04.2005

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