

INTRAVENOUS GLUCOSE TOLERANCE TEST AND GLUCOSE KINETIC PARAMETERS IN RABBITS

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Summary

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The purpose of this study was to determine the normal range and test variability for intravenous glucose tolerance test (IVGTT) in rabbits. Fifty one clinically healthy New Zealand white rabbits of both sexes (31 female and 20 male), 3.5–4 months of age and weighing 2.8 to 3.7 kg were used. The IVGTT was performed after an overnight fasting period of 12 h by intravenous injection of 40% glucose (0.5 g/kg body weight) as a bolus dose over a period of 60 s. Blood samples were collected prior to (0 min) and at 5, 10, 20, 30, 45, 60, 75 and 90 min after glucose administration. The following kinetic parameters of glucose were calculated: half-life time for blood glucose disappearance ($t_{1/2\beta}$ glucose, min), glucose elimination rate constant (K_{el} glucose, min^{-1}), area under the blood glucose concentration curve versus time ($\text{AUC}_{\text{glucose } 0-90 \text{ min}}$; mmol/L.min). The test was repeated after an interval of 3 weeks in 15 rabbits and coefficients of variation (CV) were calculated by standard methods. The ranges for blood glucose concentration (mmol/L) at 0, 5, 10, 20, 30, 45, 60, 75 and 90 min after IVGTT were 3.8–8.8, 12.8–26.0, 11.5–21.3, 8.9–18.5, 6.6–15.9, 6.0–12.4, 4.8–10.6, 5.3–8.8 and 4.3–8.5, respectively. Means \pm SD (range) for K_{el} glucose, $t_{1/2\beta}$ glucose, $\text{AUC}_{\text{glucose } 0-90 \text{ min}}$ during IVGTT were $0.013 \pm 0.004 \text{ min}^{-1}$ ($0.0044-0.021 \text{ min}^{-1}$), $57.5 \pm 16.2 \text{ min}$ (20–95 min); $922 \pm 117 \text{ mmol/L.min}$ (642–1202 mmol/L.min), respectively. Mean values did not differ between the two tests performed 3 weeks apart and the intra-individual coefficients of variation for K_{el} glucose, $t_{1/2\beta}$ glucose, $\text{AUC}_{\text{glucose } 0-90 \text{ min}}$ were 16%, 18%, and 17%, respectively, indicating satisfactory reproducibility for these variables with acceptable variation in individual rabbits. Normal ranges for blood glucose concentration at different time points and glucose kinetic parameters after an intravenous glucose tolerance test established in the present study may contribute to better interpretation of test results and provide reliable criteria for the evaluation of normal and impaired glucose tolerance in rabbits.

Key words: intravenous glucose tolerance test, rabbits, glucose kinetic parameters

INTRODUCTION

Diabetes mellitus (DM) is a complex and frequent disease in humans and in both cats and dogs as well (Höppener *et al.*, 2000; Hoening, 2002; O'Brien, 2002; Rand

et al., 2004). Although in herbivores DM is rarely encountered, spontaneous cases in New Zealand white (NZW) rabbits have been reported (Roth *et al.*, 1980;

Taylor *et al.*, 1980; Conoway *et al.*, 1981). The DM in the cats is similar to human type 2 DM (DMT2) (Hoenig, 2002; Hoenig *et al.*, 2002; O'Brien, 2002), while in dogs the type 1 DM is predominant (Lendrum *et al.*, 1976; Palmer *et al.*, 1983; Rand *et al.*, 2004). However, many aspects concerning the etiology and the pathogenesis of DM in domestic animals are not clear and remain to be elucidated. Although not many studies are available in animals, it is generally assumed that DMT2 is associated with chronic insulin resistance and progressive β -cells dysfunction, obesity and advanced age being identified as the major risk factors (Hoenig, 2002; O'Brien, 2002; Thiess *et al.*, 2004).

The primary metabolic abnormality in DMT2 is hyperglycemia as a result of impaired insulin secretion and action (Nelson *et al.*, 1990; Kirk *et al.*, 1993; Cavaghan *et al.*, 2000; Appleton *et al.*, 2005). However it remains unclear what is the relative contribution of insulin resistance and β -cell dysfunction in the development and progression of DMT2.

The attention is mostly directed to the pre-diabetic states called impaired glucose tolerance (IGT). The main feature of IGT is the decreased elimination rate of glucose after oral or intravenous administration as a consequence of insulin resistance (IR) of target cells (muscles, adipose tissues and liver).

Usually, during the early stages of IGT the fasting blood glucose concentration is within the upper limits of the reference values or slightly elevated. That is because the β -cells secretion capacity is not yet affected and insulin resistant individuals compensate for IR by enhancement of the insulin secretion (Cavaghan *et al.*, 2000; Kahn, 2001). Therefore, it is impossible to early distinguish the animals with glucose homeostasis deteriorations

only on the basis of fasting blood glucose concentration.

The final stage of DMT2 is characterized by failure and progressive loss of pancreatic β -cell function subsequent to the deterioration of glucose control (Kirk *et al.*, 1993; Hermans *et al.*, 1999).

New Zealand white rabbits are often used as experimental subjects because they are reported as appropriate animals in chronic experiments on glucose homeostasis and insulin resistance (Kitajima *et al.*, 2004; Sarov *et al.*, 2004) and for both testing of the effect of newly synthesized medical products on the blood glucose concentration and the secretion of insulin (Sharma *et al.*, 2003; Sepici *et al.*, 2004; Dastmalchi *et al.*, 2005; Gupta *et al.*, 2005; Kesari *et al.*, 2005). In addition, rabbits have a lipid profile (LDL mammals) similar to that of humans (de Roos *et al.*, 2001; Kitajima *et al.*, 2004). Hence, they could be also used as experimental animals to study the effect of some drugs influencing lipid metabolism, because disturbed lipid metabolism is often involved in deterioration of insulin sensitivity and/or glucose homeostasis.

In human beings different methods have been validated to assess β -cell function and peripheral insulin sensitivity, such as fasting blood glucose level, oral glucose tolerance test, insulin tolerance test, insulin sensitivity test, euglycemic hyperinsulinemic clamps, etc. (Jones *et al.*, 1999; Piche *et al.*, 2004). In humans glucose tolerance can be assessed on the basis of blood glucose concentration at 0; 60; 90 and 120 min following a standardized oral glucose tolerance test and glucose half-life ($t_{1/2\beta}$ glucose) and the rate constant of disappearance for glucose (K_{el} glucose) (Haris, 1995).

However, in domestic animals including rabbits, there are relatively few studi-

es in this field, which makes the elaboration of exact criteria to distinguish the animals with normal glucose tolerance, impaired glucose tolerance and overt diabetes difficult.

Therefore, the purpose of this study was to determine a normal range and test variability for blood glucose concentration at different time points and glucose kinetic parameters during intravenous glucose tolerance test in rabbits.

MATERIALS AND METHODS

Test animals

Fifty one clinically healthy NZW rabbits, originating from the Research University Station, Stara Zagora, Bulgaria, of both sexes (31 female and 20 male) were used. They were 3.5–4 months of age, weighing between 2.8 to 3.7 kg. For the duration of their adaptation and experimental period, the recommendations of caring and treatment of rabbits reared as experimental animals were followed (Boers *et al.*, 2002). The trials were performed during the spring season from March to June. The animals were housed in individual metal cages (80×60×40 cm) in a temperature-controlled room (18–20°C). The light/dark regime corresponded to the circadian cycle. They were fed with a commercially available pellet diet for adult rabbits (Agricultural Institute, Stara Zagora, Bulgaria), containing dry matter – 888 g/kg, crude protein – 183 g/kg, fat – 35 g/kg, gross energy – 2556.7 kcal/kg, fibres – 12.9 g/kg and minerals – 245.1 mg/kg, and had free access to water. The rabbits were left undisturbed under those conditions for 3 weeks prior to the beginning of the experiment.

Rabbits were determined to be clinically healthy on the basis of results of

routine physical examination and daily monitoring of their behaviour, food and water intakes, and the consistency of their faeces.

Experimental procedures

In order to avoid a stress reaction due to glucose injection and blood sample collection during the IVGTT, rabbits were submitted to a handling procedure of fixation on 3 consecutive days for 5 min before the experiments were started.

IVGTT was performed as previously described (Kitajima *et al.*, 2004; Gonzales *et al.*, 2005) after an overnight fasting period of 12 h (rabbits were however not deprived of water). The test was completed on the next morning through intravenous infusion into the marginal ear vein of 40% glucose (0.5 g glucose/kg body weight) as a bolus dose over a period of 60 s using a 22 G needle. Blood samples were collected prior to (0 min) and 5, 10, 20, 30, 45, 60, 75 and 90 minutes after glucose administration from the opposite ear vein using sterile lancets (Vitrex Medical Aps, Denmark).

Whole blood glucose concentration was measured immediately after collection by means of a glucose meter (Home Diagnostics, Inc., USA), using the glucose oxidase assay method using one drop of whole blood.

The test was repeated after an interval of 3 weeks in 15 rabbits and coefficients of variation (CV) were calculated by standard methods.

Determination of the kinetic parameters of glucose

Using a non-compartmental analysis, based on the statistical moment theory (Gibaldi, 1984; Martinez, 1998) of the individual readings of glucose after IVGTT, selected model-independent kinetic pa-

rameters, indicative for the "fate" of glucose within the test rabbits' organisms were calculated. Software from WinNonlin Professional 4.0.1 (Pharsight Corporation, 800 West El Camino Real, Mountain View, CA, USA) was used (Riviere, 1999). The area under the concentration-time curve ($AUC_{\text{glucose } 0-90 \text{ min}}$, mmol/L.min) was calculated by the trapezoidal rule. The following kinetic parameters were also calculated: biological half-life (elimination half-life) of blood glucose ($t_{1/2\beta \text{ glucose}}$, min), the elimination rate constant of blood glucose ($K_{\text{el glucose}}$, min^{-1}). $K_{\text{el glucose}}$ and $t_{1/2\beta \text{ glucose}}$ were calculated by linear regression analysis of the semilogarithmic plot of glucose concentration versus time between min 5 and 90 after glucose administration.

Statistical analysis

The statistical processing of data was performed by ANOVA (Statistica for Windows, StatSoft Ins., USA, 1993). All data were presented as mean values \pm standard deviation (mean \pm SD). The statistical significance of differences in the values of glucose before and after injection were determined by the LSD test and considered significant if $P < 0.05$. The effect of sex was evaluated by use of one-way ANOVA (Statistica for Windows, StatSoft Ins., USA, 1993). The data were preliminarily tested for outliers and then analyzed for normality by the use of the Shapiro-Wilk test (Statistica for Windows, StatSoft Ins., USA, 1993). Normal ranges of glucose at different time points after glucose administration and glucose kinetic parameters were established by the calculation of tolerance intervals containing results for 95% of the population with a probability of 0.90 as previously described (Lumsden & Mullen, 1978). Results of the test repeated at a 3-week interval in 15 rabbits

were compared by use of a one-way repeated measure elements ANOVA (Statistica for Windows, StatSoft Ins., USA, 1993). Coefficients of variation (CV) between results of both tests performed 3 weeks apart were calculated as the standard deviation divided by the mean then multiplied by 100. Correlations between $t_{1/2\beta \text{ glucose}}$, $K_{\text{el glucose}}$ and glucose concentration at 0, 5, 10, 20, 30, 45, 60, 75 and 90 min were also calculated.

RESULTS

The concentration of blood glucose (Fig. 1) increased very quickly ($P < 0.001$) after the intravenous administration of glucose and reached highest values (19.4 ± 2.9 mmol/L) 5 min after glucose administration. Then, it decreased gradually. The values of glucose at 60 min were within the physiological range and returned to the initial levels after 90 min. There was no effect of sex on glucose concentration at different time points and glucose kinetic parameters. The ranges for blood glucose concentration (mmol/L) at 0, 5, 10, 20, 30, 45, 60, 75 and 90 min after IVGTT were 3.8–8.8, 12.8–26.0, 11.5–21.3, 8.9–18.5, 6.6–15.9, 6.0–12.4, 4.8–10.6, 5.3–8.8 and 4.3–8.5, respectively.

During IVGTT the average half-life of glucose in blood, measured by $t_{1/2\beta \text{ glucose}}$ was 57.5 ± 16.2 min (range 20–95 min), mean $K_{\text{el glucose}}$ that reflect the glucose elimination rate from the central compartment, was $0.013 \pm 0.004 \text{ min}^{-1}$ (range 0.0044 – 0.021 min^{-1}). The mean values of $AUC_{0-90 \text{ min}}$ were 922 ± 117 mmol.min/L (range 642–1202 mmol.min/L).

Glucose half life, $K_{\text{el glucose}}$, $AUC_{\text{glucose } 0-90 \text{ min}}$, and blood glucose concentration at all time points did not differ significantly ($P > 0.05$) between the two tests performed 3 weeks (21 days) apart (Fig. 2). Coeffi-

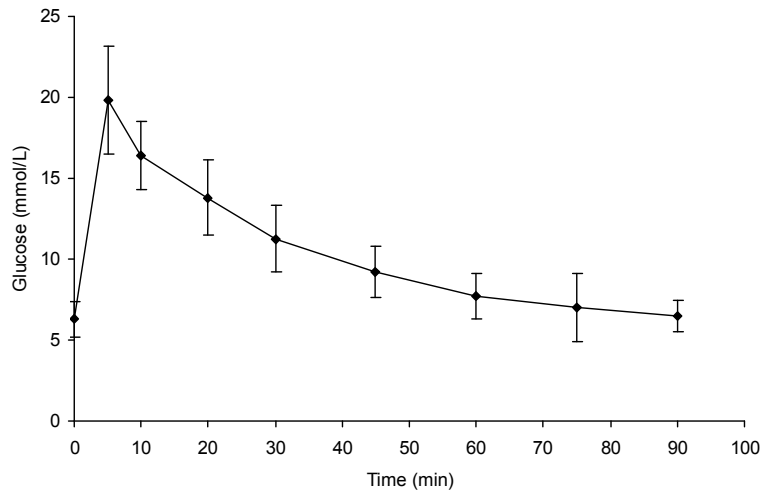


Fig. 1. Total blood glucose concentration (mean \pm SD) determined during intravenous glucose tolerance test, performed by injection of 40% glucose (0.5 g/kg body weight) in 51 clinically healthy rabbits.

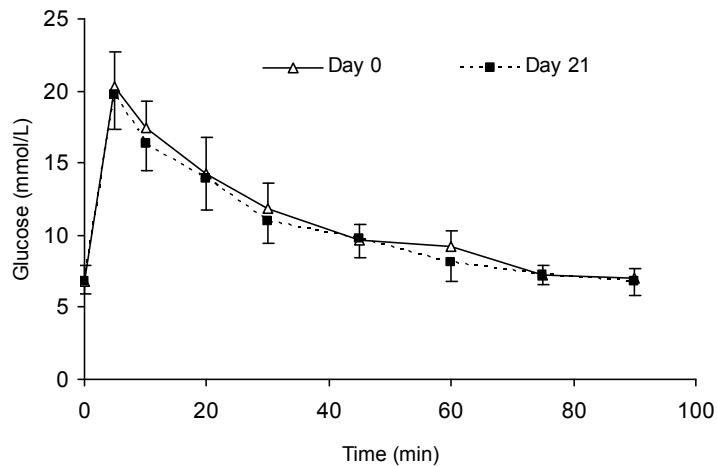


Fig. 2. Total blood glucose concentration (mean \pm SD) determined during 2 intravenous glucose tolerance tests, performed 3 weeks (21 days) apart in 15 clinically healthy rabbits.

coefficients of variation (CV) for $t_{1/2\text{glucose}}$, K_{glucose} , and $\text{AUC}_{\text{glucose } 0-90 \text{ min}}$ were 18%, 16% and 17%, respectively. CV for glucose concentration at different time points ranged from 9% to 16%.

There was a weak but statistically significant ($P < 0.05$) correlation between 60-

minute glucose concentrations and $t_{1/2\beta\text{glucose}}$ ($r = 0.42$) and $K_{\text{el glucose}}$ ($r = -0.36$).

DISCUSSION

In this study we evaluated the glucose kinetic parameters during IVGTT in rab-

bits. For the IVGTT we used 40% glucose at a dose rate of 0.5 g/kg body weight. Because there are no data for glucose kidney threshold in these species we chose this dose of glucose which is commonly used in cats for the evaluation of glucose kinetic parameters during IVGTT (Link *et al.*, 1997; Link & Rand, 1998; Appleton *et al.*, 2001). Glucose homeostasis depends primarily on the relationship between three main factors: insulin secretion from the pancreatic β -cells, sensitivity and responsiveness of the target cells to insulin-dependent and insulin-independent uptake of glucose (Cavaghan *et al.*, 2000; Kahn *et al.*, 2001; Kahn, 2003). Impaired glucose tolerance, which is considered as early stage of DMT2 in both humans and animals (cats), has been intensively studied, but nevertheless, its molecular mechanisms are not yet fully elucidated. The differences in glucose dosage, glucose infusion rate and blood sample collection protocols make the comparison of reference values for glucose kinetics of our study with those in the literature difficult.

Rats have been the most often used model animals in this area, but the last few years revealed that their mechanisms of glucose concentration control partly differed from those in people and animals (McArthur *et al.*, 1999). This fact brings the need of looking for other experimental animals to study the mechanisms regulating glucose homeostasis and the relative contribution of β -cell dysfunction and peripheral insulin resistance for development of impaired glucose tolerance status.

To the best of our knowledge, this is the first study providing information on reference values of glucose tolerance test in healthy rabbits. Although similar dynamic of blood glucose in rabbits during IVGTT has been previously reported (Sarav *et al.*, 2004), reference values for

the IVGTT were not evaluated in their studies. In addition, glucose values at different time points in these studies were lower than in our study, possibly to the lower dose of glucose administered in IVGTT (0.3 g/kg vs. 0.5 g/kg in our study). In a study by Kitajima *et al.* (2004) in rabbits, glucose concentrations at each time point were higher than in our study and did not return to the basal levels after 120 min. This was likely and at least in part due to the higher dose used (0.6 g glucose/kg body weight) and the site of blood samples collection in their study (*a. auricularis*). On the basis of results from our study, we recommend the following normal values for IVGTT in healthy, 3.5–4 months old NZW rabbits when using 0.5 g glucose/kg body weight and measuring glucose concentration in whole blood with a glucose meter: concentration of glucose ≤ 8.8 mmol/L at 0 min, ≤ 15.9 mmol/L at 30 min, ≤ 10.6 at 60 min, ≤ 8.5 at 90 min and $t_{1/2\beta \text{ glucose}} \leq 95$ min, $K_{el \text{ glucose}} \geq 0.0044$ min^{-1} , and $\text{AUC}_{\text{glucose } 0-90 \text{ min}} \leq 1202$ mmol.min/L. As additional judgement significant correlations between 60-min glucose concentration and $t_{1/2\beta \text{ glucose}}$ and $K_{el \text{ glucose}}$ could be used.

The intra-individual coefficients of variation for glucose concentrations and glucose kinetic parameters determined from results of two glucose tolerance tests performed 3 weeks apart in 15 rabbits, ranged between 9 and 17%. This fact and the similar parameters of the GTT indicated satisfactory repeatability for these variables and with acceptable variation in each rabbits. These coefficients of variation were similar to those in cats (Appleton *et al.*, 2001), but lower than those reported in humans (Harding *et al.*, 1973; Ganda *et al.*, 1978). A standard procedure for the glucose tolerance indices was studied in cats. Our data for $t_{1/2\beta \text{ glucose}} (\leq 95$

min) agree with results reported by Link & Rand, 1998 ($t_{1/2\beta \text{ glucose}} \leq 94.7$ min) in cats during IVGTT indicating similar rates of glucose disposition in both rabbits and cats.

In conclusion, reference values for blood glucose concentration at different time points and glucose kinetic parameters after an intravenous glucose tolerance test established in the present study may contribute to better interpretation of test results and prove reliable criteria for the evaluation of normal and impaired glucose tolerance in rabbits when they are used as experimental animal model to investigate impairments in controlling mechanisms of glucose homeostasis and when testing the anti-diabetic effects of newly synthesized drug formulations.

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