

SHORT COMMUNICATION

**INTRAVENOUS GLUCOSE TOLERANCE TEST IN
MACACA RADIATA RADIATA**

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Abstract : A reliable method for performing sensitive intravenous glucose tolerance tests in monkeys has been standardized. This helps in assessment of beta cell function. A normal curve for glucose disposal is constructed. A high variability in insulin levels is also documented.

Key words : IVGTT (Intravenous glucose tolerance test)
fasting glucose peak glucose insulin levels

INTRODUCTION

In view of the fact that *Macaca radiata radiata* species of monkeys is being increasingly used in studies on diabetes, we felt the need to expand the database by giving a normal curve for intravenous glucose tolerance tests in these animals. Also, the technique of IVGTT described in this paper has given consistent results.

METHODS

Five animals belonging to the family *Macaca radiata radiata* were employed in the study. The animals were of both sexes and weighed between 3 and 7 kg. They were maintained in individual cages in our animal house for a minimum of 10 days before experimentation. They were fed twice a day with a natural diet and had free access to water.

The test animal was fasted overnight. (The day before the experiment, food was removed

from the cage at 5 pm). The animal however, had free access to water.

Between 8 and 9 am on the day of experiment, the animal was anaesthetized with nembotal, 35 mg/kg body weight, given intraperitoneally. The procedure was begun one hour after the animal lost consciousness. The monkey had to be anaesthetized because quick blood sampling in rapid succession during an IVGTT would be extremely difficult in a conscious monkey.

Procedure

The saphenous vein was chosen for blood collection. The thigh and leg were splinted and a paediatric blood pressure cuff was wrapped around the thigh to serve as a tourniquet. A scalp vein set (18 G or 19 G - to avoid hemolysis) was placed in the leg vein (such that the needle tip was towards the foot) and sealed with heparinized saline. The length of the scalp vein tube was reduced to be about 10 cm.

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The cephalic vein was chosen for injecting the glucose bolus and a scalp vein set of any available size (usually 21 G or 22 G) was fixed and locked with heparinized saline.

A fasting blood sample (0 min) was collected from the leg vein. A glucose bolus of 0.5 g/kg body weight as a 25% dextrose solution was injected into the arm vein as a slow i.v. injection lasting for a min. The post injection period was accurately timed and blood samples were collected from the leg vein at 3, 5, 10, 20, 30, 40, 60 and 90 min after the glucose injection into tubes containing fluoride EDTA as the anticoagulant.

Method of blood collection : Exactly 20 seconds before the due time, the blood pressure cuff on the thigh was inflated to 40-60 mm Hg to ensure free flow, and the syringe sealing the scalp vein tube removed 5 seconds before the due time. The first 5-6 drops of blood were discarded to avoid dilution of the sample with heparin.

Blood collection in the test tubes began exactly at the due time and lasted for 20-30 seconds. About 1.5 to 2 ml was collected each time. The pressure in the cuff was then released to reduce flow, the scalp vein tube was flushed with heparin saline and resealed with the syringe. (Aspiration with a syringe was avoided because the flow was never as good as with the above method).

Collection of plasma : The tubes were centrifuged at 2,500 RPM for 10 min. Centrifuging was always done within half an hour after blood collection. The plasma was transferred to clean test tubes. The samples did not show any hemolysis.

Glucose estimation in the samples was done on the day of the experiment. Boehringer Mannheim kits which employ the glucose oxidase method were used (1).

Insulin estimation in the samples : The plasma samples were frozen at -18°C for radioimmunoassay of insulin. RIA was done at a later date using kits from Bhabha Atomic Research Centre (BARC) using human insulin standards. The assay was done in duplicate.

RESULTS

Fasting plasma glucose in normal monkeys : The mean value of fasting blood glucose in normal monkeys was found to be 52 ± 6 mg/dl.

Normal pattern of glucose disposal during an IVGTT (Table I): The peak level of glucose was attained within 3 min.

After the 3 minute peak, there was a steady decrease in glucose levels. This decrease followed exponential decay pattern (with a high rate of disposal initially and lower rates in the later stages). The overall rate of glucose disposal as given by the K value is -2.35 ± 0.15 S.D. (% decrease in glucose per min) K value was calculated by the method of least squares to give the disposal rate from the peak glucose to 90 minute value. By 90 min, the glucose load is seen to be completely disposed.

Pattern of insulin secretion during an IVGTT (Table II): Insulin levels were estimated in samples taken from 4 animals. The fasting insulin value was around 40 $\mu\text{U/ml}$ in 3 animals and 15 $\mu\text{U/ml}$ in one. There was high variability in the insulin levels at other points in time

TABLE I : Mean plasma glucose levels during an IVGTT in normal monkeys.

Glucose mg/dl \pm S.D.	Time (min)								
	0	3	5	10	20	30	40	60	90
	52 \pm 6	257 \pm 28	226 \pm 23	185 \pm 18	147 \pm 18	111 \pm 34	82 \pm 32	53 \pm 31	46 \pm 13

TABLE II: Insulin levels during an intravenous glucose tolerance test in four animals which showed normal glucose tolerance.

Monkey No	Plasma insulin ($\mu\text{U/ml}$)							
	Fasting	3 min	5 min	10 min	20 min	30 min	40 min	60 min
1	47	208	168	260	205	168	100	27
2	40	242	269	530	845	195	85	38
3	40	168	195	329	383	90	42	26
4	15	180	220	490	308	221	50	36

during the IVGTT. However, the peak value was consistently attained between 10 and 20 min. This value ranged from 260-845 $\mu\text{U/ml}$.

DISCUSSION

Though the intravenous GTT is unphysiologic, it was selected in preference to oral GTT, for the following reasons :

1. Oral gavaging of monkeys with the glucose bolus is difficult.
2. The variation in absorption rates of glucose from the intestine and the interference by intestinal stimuli and intestinal hormonal secretion may affect the outcome (2, 3).
3. The rapidity of insulin release in response to glucose load can be assessed in this method.

A normal glucose disposal curve for intravenous glucose tolerance test in *Macaca radiata radiata* is documented in this study.

The fasting plasma glucose level in *Macaca radiata radiata* (52 ± 6 mg/dl) is lower than in human beings (4). Also, the glucose disposal rate calculated as K value after the initial peak is higher in this species (-2.35 ± 0.15 mg/100 ml/min) as compared to humans in whom it averages at -1.21 (5) and to cynomolgus monkeys in which it is -1.86 ± 0.3 (6). In spite of the very high insulin level in one monkey, (Table I) its glucose disposal was not any different from the rest of the animals. It could be because of an increased peripheral resistance to insulin in this animal, compared to the others. In fact this monkey was the heaviest in the study group. It has been documented that obese individuals have exaggerated insulin responses to glucose (6), the reason being peripheral antagonism to insulin action (7, 8). It is also interesting to note that the smallest monkey had the lowest insulin levels. The other monkeys had intermediate ranges of insulin secretion.

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