

Evaluation of the Effect of Temperature on the Potency of Isophane Insulin in a Rat Model

Sir,

Being proteinaceous, insulin undergoes degradation. Potency of insulin refers to the amount needed to reduce blood glucose. Insulin's potency is affected by exposure to light, vibration, storage temperature, and duration of storage.^[1-4] Most studies on insulin stability were done in Western countries, where room temperature is relatively low.^[5] Many parts of India experience a temperature of 30°C–40°C and over 40°C during summer. Poor people often are unable to store insulin as per the manufacturer's recommendations. There is also ignorance of proper storage requirements. Patients from rural areas are advised to store insulin in mud pots where the inside temperature is a little lower than room temperature. However, during summer, the inside pot temperature also may rise above 35°C. Hence, even insulin in a mud pot can be affected. This study investigated the effect of temperature and duration of storage on potency of isophane insulin, a combination of two types of insulin in a fixed proportion – fast- and short-acting regular insulin 30% (the short-acting phase) and intermediate- and long-acting NPH insulin 70% (the long-acting phase).

The study was conducted on 20–22-week-old normal male Wistar rats weighing 250–300 g procured from the Institutional Animal House. In addition to diabetic animals, insulin is also known to reduce blood glucose in normal animals.^[4] A validation study was done using six rats to determine the dose of isophane insulin and time points of blood sampling for the main study. There are no reliable references for time points of blood collection in rats after administration of isophane insulin. To determine the time points for blood sampling, consecutive blood sampling was done at ½ h intervals for the initial 2.5 h (duration of action of short-acting phase), followed by 1 h intervals for 10 h (duration of action of long-acting phase). Blood sampling was done on days 1, 7, 21, and 35. The insulin tolerance test was done at various time points as determined by the validation study. During validation, the peak effect of short-acting regular phase of isophane insulin was observed between 0.5–1.5 h and that of the long-acting phase was between 4–8 h. Therefore, blood sampling was done at 0.5, 1, 1.5 and 4, 6, and 8 h after the administration of insulin. The study was approved by the Institutional Review Board and Animal Ethics Committee (IRB Minute No. 9074, dated 06.10.2014). Rats were individually housed in polypropylene cages containing standard bedding material and given free access to water and rat chow and provided with 12 h of light/dark cycles and maintained at 23°C ± 2°C temperature and a humidity of 50% ± 5%.

Eighteen male Wistar rats were selected for the final study. They were equally distributed to the three different temperature study groups, i.e., 5, 25, and 40°C. Isophane insulin was stored at three different temperatures, i.e., 5°C (to simulate the refrigerator since most patients keep insulin in the lower refrigerator compartment), 25°C (to simulate an air-conditioned room since the package insert for isophane insulin recommends this temperature), and 40°C (to simulate a hot air oven since in many places in India this temperature is reached during summer). The rats in the control group were administered normal saline.

Each rat group served as its own control since each rat group received both isophane insulin and normal saline during the 1st week and the last (6th) week. Each rat was injected intraperitoneally insulin 0.25 U/kg body weight (dose predetermined from the validation study) or an equivalent quantity of normal saline (control) stored at the same temperature. The Hamilton microsyringe was used for injection. Each rat received 1 dose of insulin (0.25 U/kg) on each blood sampling day (days 1, 7, 21, and 35). Hence, each rat received four doses of insulin. Then, each rat was returned to a cage. Food was prohibited during the 8 h of blood sampling.

The baseline blood glucose level was measured in each rat after overnight fasting, before injecting insulin, with the help of a commercially available glucometer. Blood from each rat was obtained from the tail tip. A 1–2 mm of tissue was incised distal to the bone using a scalpel. One µl of blood was collected by direct flow or by gently massaging the tail. Successive samples were obtained by repeating the massaging procedure or by gently removing the scab. The insulin vials and saline were opened on day 1 and stored at their respective temperatures till the end of the study (day 35). The control arm intervention with saline injection was done only with the first and last (35th) day of sampling. The data were expressed as the % reduction in blood glucose level from the fasting state (after overnight fasting for 12 h). For comparing the changes between different days of storage (days 1, 7, 21, and 35) at a particular temperature, repeated measure analysis of variance was used, which was followed by *post hoc* analysis (pairwise Bonferroni test).

Table 1 gives the results of potency of isophane insulin after storage for 1, 7, 21, and 35 days at 5, 25, and 40°C. The potency of insulin stored at 40°C was significantly lower than insulin stored at 25°C after 1 and 7 days of storage. The potency of insulin stored at 40°C was significantly lower than insulin stored at 5°C after 35 days of storage. As shown, the potency of the short-acting phase of isophane insulin

was significantly lower even after 24 h of storage. Figure 1 is a box and whisker plot showing the variation in the % reduction in blood glucose levels across different time points over 1–35 days in rats in the 40°C temperature group. There was greater variation in the % reduction in blood glucose levels in the short-acting phase of isophane insulin, since the greatest variation occurred within the first 4 h. This suggests that there was a loss of potency in the short-acting phase of the insulin stored at 40°C. On days 1, 7, and 35, there was a significant difference in potency of insulin (short-acting

Table 1: Potency of isophane insulin after 1, 7, and 35 days of storage at different temperatures

Temperatures	Short acting phase*		Long acting phase*	
	25°C (P)	40°C (P)	25°C (P)	40°C (P)
Day 1				
5°C	0.615	0.197	0.09	0.671
25°C		0.036+		0.358
Day 7				
5°C	0.913	0.051	0.588	0.753
25°C		0.023+	0.959	
Day 21				
5°C	0.564	0.991	0.345	0.164
25°C		0.642		0.786
Day 35				
5°C	0.292	0.008+	0.526	0.272
25°C		0.153		0.87

Figures given are *P* values of comparison of potency of insulin stored at temperatures on the Y axis with insulin stored at temperatures on the X axis. *Statistically significant difference, *Based on data obtained from validation study

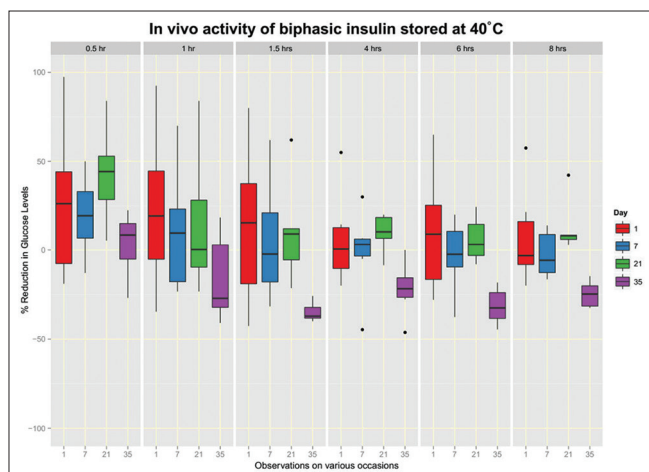


Figure 1: Box and whisker plot showing the variation in the percentage reduction in the blood glucose levels across different time points over 1–35 days (days: 1, 7, 21, 35) in rats in the 40°C group. The time points mentioned at the top denote time after administration of insulin to rats

phase) stored at 40°C [Table 1]. Hence, even 1 day of exposure to a temperature like 40°C affected insulin potency. The long-acting part did not show any significant difference in potency. This could be due to physiological variation in the rats due to prolonged fasting till the completion of the experiment, i.e., 8 h and compensatory glucose production. Storing isophane insulin at 5 and 25°C up to 35 days did not result in loss of potency.

Vimalavathini and Gitanjali^[4] found that there was no difference in insulin potency stored at 25°C and 26°C compared to 5°C. Moreover, they showed that there was no loss of insulin potency stored at 25°C and 26°C after 28 days. However, at 32°C and 37°C, there was 14%–18% decrease in regular and biphasic insulin potency after 28 days. Storvick and Henry^[6] evaluated the effect of storage temperature (5°C, 25°C, 37°C, and 50°C) on stability of many commercial insulin preparations for 36 months. They found that for any type of insulin at 5°C, there was no significant loss of potency. However, when stored at higher temperatures, insulin potency was lost depending on the insulin type and storage duration. In general, when compared to regular insulin, all modified insulin preparations were more stable.

In conclusion, we have shown that there was a decrease in potency of isophane insulin stored at 40°C for 24 h, whereas insulin stored at 5 and 25°C did not lose its potency over 35 days. Hence, insulin must be stored at the proper temperature to maintain its potency.

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Conflicts of interest

There are no conflicts of interest.

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