## Insulin thermostability in a real-world setting

Various forms of diabetes care necessitate exogenous insulin replacement. Administered insulin must have predictable potency to avoid potentially dangerous glycaemic excursions. However, insulin is temperature sensitive, with potency reduced through rising temperatures.[1–3] Insulin manufacturers and regulatory agencies direct that insulin be refrigerated (at 4–6°C), never frozen, and with a maximum usage or storage period of approximately 1 month at standard room temperatures (20–25°C).[3] This requirement is especially challenging in hot climate settings if home refrigeration is unavailable, which is the case for some 770 million people.[4,5] Furthermore, in some situations, insulin must also be stored for a few months due to clinic visit frequency, travel costs, and intermittent pharmacy supplies.[4,6] In response to this, many families use evaporative cooling with clay pots to assist in reducing insulin storage temperatures, by storing insulin within an air-filled space or a sealed bag in clay pots.[6]

Data regarding insulin stability outside the recommendations are scarce. In 1968, Storvick and Henry[1] reported that animal-derived isophane insulin retained 95% or more of its potency for 12 months at 25°C. In 1972, Pingel and Volund[2] found that similar isophane and soluble insulins retained 95% or more of their potency for at least 5 months at temperatures of up to 30°C. A study using human insulin showed loss of some potency during storage at 32–37°C for 28 days,[7] whereas a recent study showed that potency of human and analogue insulin remained unchanged at oscillating temperatures of 25–37°C for 28 days and 12 weeks.[8]

In this pilot study, we aimed to identify: (1) the potency of six 100 IU/mL insulins (vials of human soluble; Eli Lilly), human isophane (Eli Lilly), and human soluble–isophane (30:70; Novo Nordisk); and 3 mL cartridges of insulin aspart (Novo Nordisk), and two preparations of insulin glargine (Sanofi and Eli Lilly) stored unopened for 1–4 months in non-refrigerated conditions in a real-world setting during the summer in India, all compared with control samples of each insulin, which remained refrigerated; and (2) whether there was any difference in storage temperatures and changes in potency between samples stored within and outside clay pots. These insulins were chosen to include commonly used insulins.

Six families with a person younger than 25 years with insulin-requiring diabetes attending the Diabetes Research Education and Management (DREAM) Trust in Nagpur, India, between March and June, 2021, participated in this study. Each received two different types of insulin. Insulin vials were stored in watertight bags. Bags were placed in either an open plastic container stored on a high shelf or in a cupboard, or in clay pots with a separate water compartment. All storage containers were placed in shaded areas (appendix). Control samples of all insulins were also refrigerated at the DREAM Trust, and these samples were used to measure relative potency of unrefrigerated samples. The temperature was measured every 15 min by electronic data loggers. The methodology of this study is described in detail in the appendix (pp 1–5).

The monthly mean open box temperature across the six families ranged from 29·4°C to 32·0°C, with mean maximum temperatures of 30·4–34·9°C and minimum temperatures of 28·3–29·8°C (appendix pp 6–7). Compared with the open boxes, the clay pots significantly reduced temperatures by mean 2·6°C (SD 1·8, range 0·4–5·7, p<0·0001) in ten of the 12 clay pots (appendix p 8).

Insulin analyses were done at the University of Florida, FL, USA, with high-performance liquid chromatography, and at the University of Gothenburg, Gothenburg, Sweden, with nuclear magnetic resonance spectroscopy (appendix pp 8–21). The potency was measured with US Pharmacopeia monographs for each insulin as described in the appendix (pp 8–11). Potency is defined in terms of insulin units per millilitre (IU/mL) and be around 100 IU/mL (±5%).[9]

In the analysis at the University of Florida, all human insulin samples maintained 95% or more of the refrigerated potency except for one vial each of human soluble, human soluble–isophane (30:70), and human isophane (range 92·4–94·1%), all at 4 months (figure). Human soluble and human soluble–isophane (70:30) relative potencies drifted downward over the 4-month period. The analogue insulins (Aspart [Kalundborg, Denmark], Glargine [Basaglar, Indianapolis, IN, USA], and Glargine [Lantus, Frankfurt, Germany]), tested all assayed 95% or more relative potency at all timepoints. All relative and raw sample measurements are included in the appendix (pp 8–11). Clay pot storage resulted in less decline in relative potency compared with refrigerated samples at 4 months than open box storage (0·5% *vs* 3·6%, p=0·001; appendix p 20).

In the analysis done at the University of Gothenburg, there was an apparent subtle line width increase and slight peak shifts in nuclear magnetic resonance spectra, which correlated with storage time for non-refrigerated samples (appendix pp 16-17). This is consistent with subtle, well known changes due to altered conformation (eg, relaxed to tense transition of the insulin hexamer) or multimerisation (eg, formation of dimers of insulin hexamer) in a minor fraction of the insulin molecules. A decrease in total concentration in any of the insulin types was not observed as being larger than the precision of measurements (<1%), regardless of storage time or type of storage (appendix pp 12–14).

This study of insulin thermostability outside refrigeration, during the

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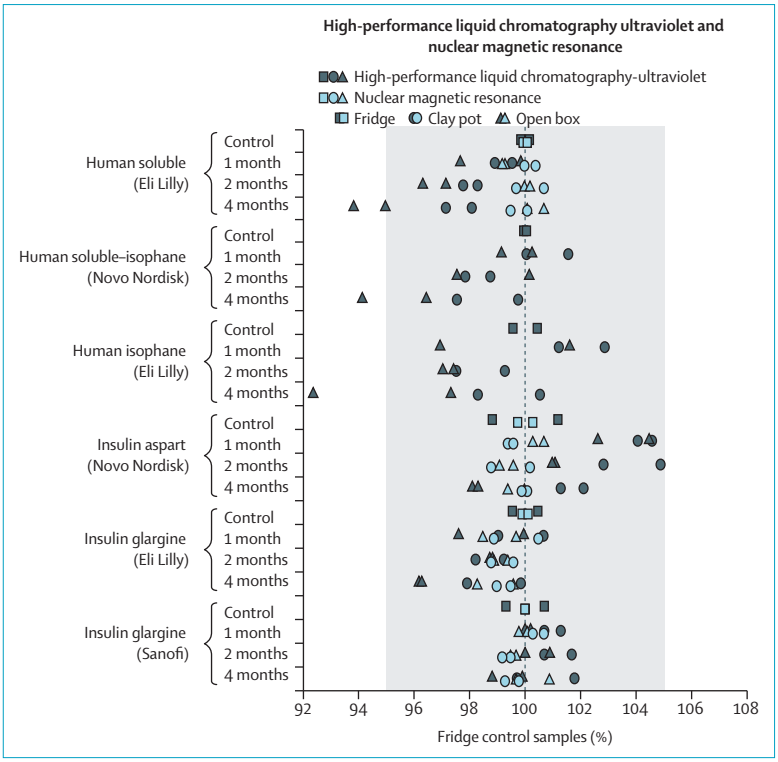


***Figure:*** **Relative potency and total concentration of insulins at each timepoint**
Relative potency measured by high-performance liquid chromatography-ultraviolet (shaded in black), and relative concentration measured by nuclear magnetic resonance (shaded in blue), of the control insulins after storage in refrigerator (squares), and insulins stored in clay pots (circles) or boxes (triangles) for 1, 2, or 4 months. Nuclear magnetic resonance analysis was done at the University of Gothernburg and liquid chromatography-ultraviolet analysis at the University of Florida.

summer in India, showed that acceptable insulin concentrations were maintained up to 2 months for all samples of all insulin preparations. At 4 months, all samples from three analogue insulin preparations and three of four samples for each of the human insulins also maintained a relative concentration of 95% or more. The US Pharmacopeia stipulates that insulin potency should be 100 IU/mL (±5%) to be safe to use.[9] No loss of relative concentration was found using nuclear magnetic resonance. These results are consistent with the findings of Kaufman and colleagues,[8] which noted preservation of insulin concentration of human and analogue insulins at 4 weeks and then 12 weeks, at cycled laboratory temperatures similar to the current study.

Study limitations include the small number of samples, and that the samples were re-refrigerated after the period in the open box or clay pot. Furthermore, insulin potency and concentration were only tested by analytical methods and not by changes in glucose concentration in vivo. The employed methods do not establish the exact nature of the observed insulin following this method of storage, so additional studies will be required to address this. The vials remained unused during the study, whereas in a real-world situation insulin would be withdrawn frequently. The sterility of vials stored in clay pots was not tested.

If these results are confirmed in real-world and laboratory studies with larger sample sizes, and the further in vivo and biochemical tests show reassuring findings, then regulatory agencies could be prompted to review the requirement to dispose of unrefrigerated insulin after 1 month or a similar period at room temperature (20–25°C).[6] Potentially, usage could be extended to 2–4 months in situations in which daily temperatures cycle from 25°C to 35°C when refrigeration is not available. This would reduce cost, waste, and family anxiety about whether the insulin is still effective and safe to use. In addition, it could help to provide health professionals with guidance and reassurance; and likely improve insulin access in under-resourced settings. Pande and Thakur[10] described the ethical dilemma commonly faced in these situations about what advice the doctor should provide about storing insulin according to the recommendations, in a situation where replacement insulin is unavailable or unaffordable. Clay pots and other traditional evaporative cooling techniques are used by families and recommended by many health professionals to keep insulin cool in several countries, and such devices have been shown to reduce storage temperatures by up to 8°C. Clay pots are especially effective in lower-humidity situations.[6] Our results strengthen evidence for the use of these basic devices by showing that clay pot storage can reduce the decline in insulin potency.

Finally, it would be beneficial if, concurrently, insulin manufacturers would release any other relevant data on potency and safety of insulin stored for periods and in temperatures beyond product guidelines, so this could be considered in guidelines for less-resourced situations. Notably, the European Medicines Agency has released a recommendation that permits storage of two human insulin preparations for 30 days at temperatures less than 30°C before use.

In conclusion, the results of this study suggest that unrefrigerated insulins might be sufficiently thermostable for 2 months, and possibly even up to 4 months. In the absence of refrigeration, clay pot storage appears to be effective in reducing storage temperatures and declines in insulin potency.

For more on the **European Medicines Agency recommendation** see https://www.ema.europa.eu/en/news/facilitating-global-access-diabetes-treatments-non-eu-patients

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