Insulin thermostability in a real-world setting

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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

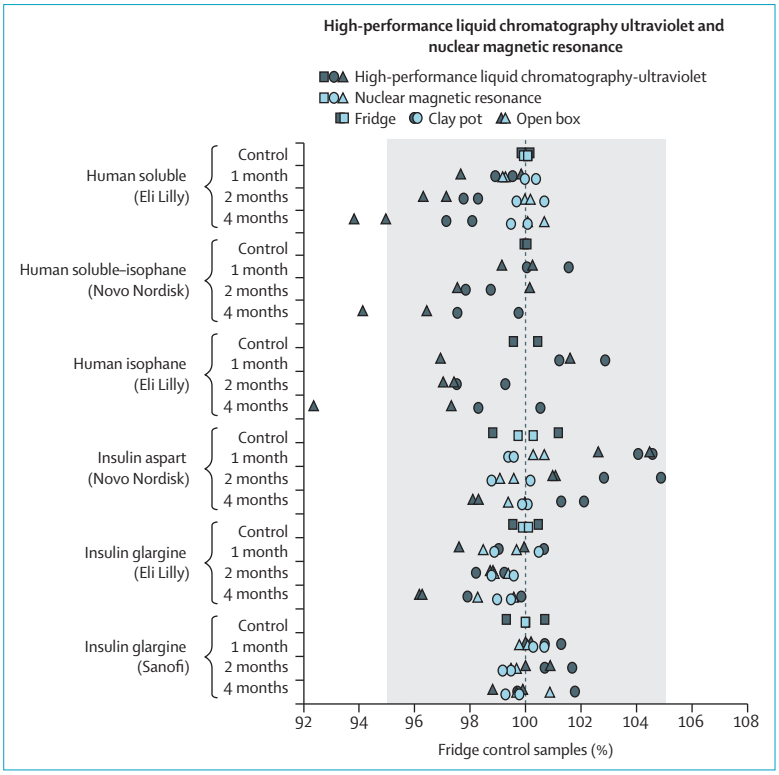

Figure: **Relative potency and total concentration of insulins at each timepoint**
Relative potency measured by high-performance liquid chromatography-ultraviolet (shaded in grey), and relative concentration measured by nuclear magnetic resonance (shaded in blue), of the control insulins after storage 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.

See Online for appendix

(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 time-points. 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 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 benefical 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.

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

type 1 diabetes prevention and reversal (not directly insulin thermostability), as well as receiving an independent award from the Novo Nordisk Foundation. MAA is also President of Insulin for Life USA. All other authors declare no competing interests.

*Sanket Pendsey, Steven James, Timothy J Garrett, Anders Bay Nord, Sharad Pendsey, Daniel Malmodin, Göran Karlsson, Jayanthi Maniam, Mark A Atkinson, Gun Forsander, *Graham D Ogle*

gogle@diabetesaustralia.com.au

Diabetes Research Education and Management Trust, Nagpur, India (SaP, ShP); Life for a Child, Diabetes NSW & ACT, Sydney, NSW 2037, Australia (SJ, JM, GDO); University of Florida, Gainesville, FL, USA (TJG, MAA); University of Gothenburg, Gothenburg, Sweden (ABN, DM, GK, GF), University of the Sunshine Coast, Petrie, QLD, Australia (SJ); Queen Silvia Children's Hospital, Gothenburg, Sweden (GF); Science for Life Laboratory, Gothenburg, Sweden (GK)

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On the occasion of International Women's Day

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I am a woman, a wife, a mother, and an Indian. I am also a physician treating diabetes, a researcher, and the Managing Director of one of the world's largest chains of diabetes centres. As someone who has been involved in diabetes care and research for the past 20 plus years, I have had the opportunity to make a difference to the lives of tens of thousands of people in this vast and diverse country. This is not a privilege given to many people, let alone many women. Upon reflecting on medical care in general, and diabetes in particular, I am struck by the low representation of women among the key medical opinion leaders and researchers in India. Although there is no disparity in the sex ratio among medical students in my country, a largely patriarchal society, the competing demands made on a woman's time mean that many female doctors are forced to compromise some of their professional commitments, hampering their rise up the ladder.

I am fortunate to have been born in a community that, unlike most of India, follows a matrilineal system of inheritance. This means that women in my community have enjoyed educational and employment opportunities and autonomy in professional and life choices far beyond what has been allowed for my counterparts in the rest of the country. I was also blessed to have, as my role model, one of the strongest women I have had the privilege to know: my mother, Dr Rema Mohan. My mother's pioneering work on diabetic eye diseases inspired me to become a doctor and help underprivileged communities. My father, Dr Viswanathan Mohan, is one of India's leading diabetologists and his work on diabetes has gained international renown. He always made me feel that being a girl would never stand in the way of achieving whatever I wanted to in life. To him, I owe my initiation into diabetology and my passion for research. My journey thus far has emphasised to me the importance of role models, as well as a supportive home environment (with a big shoutout to my husband, son, and the rest of my family and friends).

Notwithstanding these advantages, my foray into diabetes research has been a steep and hard climb. Challenges in resource availability (both technical and financial), intellectual bandwidth, and infrastructure are common issues in a low-income and middle-income country (LMIC), and often get further compounded when novel ideas and strategic and technological solutions originate from women. Collaborators and colleagues have constantly told me that I should not aim too high, or attempt to combine research, patient care, and administration. They have told me that I should not worry my pretty young head with work. However, as more and more research proposals are funded and the translation of my group's research continues to change clinical practice, there is a sense of gratification and reassurance that makes the struggle seem worthwhile.

What have I learnt from my two decades as a female diabetes clinician, researcher, and entrepreneur in a LMIC? First, it is necessary to prioritise and be pragmatic. Make practical lists of short-term tasks and long-term goals and review them regularly. Second, it is important to dare to aim high. Have faith in your own abilities and do not let anyone discourage you. Third, surround yourself with positive and supportive people who believe in you. Finally, if you are a mother, instil in your son(s) a sense of respect and appreciation for women, and in your daughter(s) the confidence that nothing is impossible. After all, there is always a strong woman behind every successful person, be it a man or a woman.

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R M Anjana

dranjana@drmohans.com

Dr Mohan's Diabetes Specialities Centre and Madras Diabetes Research Foundation, Chennai 600 086, India