The Effect of high temperature on the stability of basal insulin in a pen: a randomized controlled, crossover, equivalence trial

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ABSTRACT

Introduction Insulin is an essential medicine in the management of diabetes. When stored at high temperatures (HTs), its efficacy could rapidly decline. Therefore, appropriate storage of in-use insulin is necessary to achieve its maximum therapeutic effects. However, the ambient temperature in tropical countries is normally relatively high. This study aimed to compare the efficacies of basal insulin in a pen previously kept at 37°C for 21 days and basal insulin in a refrigerated pen (2°C–8°C). Continuous glucose monitoring (CGM) was used to evaluate daily mean glucose levels (MGLs).

Research design and methods This randomized controlled, crossover, equivalence trial recruited adults with type 2 diabetes mellitus and glycated hemoglobin levels <8% who had used insulin glargine for >3 months. Subjects were randomized for sequential use of refrigerated basal insulin followed by basal insulin kept at HT, with a 2-week washout between phases. The HT insulin pens were stored in a 37°C incubator for 21 days before use, while the refrigerated insulin pens were stored at 2°C–8°C. Study patients received 7-day CGM. The primary outcome was the difference in the groups’ MGLs. The secondary outcome parameters were glucose variability represented by the standard deviation (SD), mean amplitude of glycemic excursion (MAGE), and percentage of time in range (TIR). The remaining quantity of insulin was evaluated by ultrahigh-performance liquid chromatography (UHPLC) assay.

Results Forty patients completed the study. The MGL was 158.7±30.5 mg/dL and 157.0±40.9 mg/dL in the HT and refrigerated insulin pen groups, respectively (p=0.72). The groups had no significant differences in MAGE, SD, percentage of TIR, carryover period, or treatment effects (all p>0.05). There was also no significant difference in the remaining quantity of insulin evaluated by UHPLC (p=0.97).

Conclusions HT basal insulin pens retain their potency and have biological activity comparable to that of refrigerated pens.

Trial registration number TCTR20210611002.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Insulin efficacy decreases rapidly when exposed to high temperatures.

⇒ Insulin manufacturers recommend keeping in-use insulin pens at room temperatures not exceeding 30°C (86°F) at all times.

⇒ Insulin stored at temperatures above 30°C should be discarded; however, the average ambient temperature in tropical countries is higher than 30°C.

WHAT THIS STUDY ADDS

⇒ This study showed, both in vitro and in vivo (human study), that basal insulin pens stored at temperatures as high as 37°C (98.6°F) for 21 days and refrigerated basal insulin pens have comparable insulin efficacies.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Further studies are warranted on the potency and structure of insulin exposed to higher ambient temperatures when refrigeration is unavailable.

INTRODUCTION

Diabetes is an important and evolving non-communicable disease. In 2021, it was estimated to affect 537 million adults worldwide, and the number was projected to increase to 783 million by 2045.1 Good glycemic control has been shown to reduce the incidence and slow the progression of diabetic complications.2 3

Insulin injections are one of the essential medicines for the management of diabetes. Many people with diabetes require insulin for glycemic control. Administration of insulin therapy must have a predictable potency; otherwise, unexpected hypoglycemia or hyperglycemia can occur. Among several factors influencing insulin efficacy, insulin must be stored correctly and at recommended temperatures. At room temperature (RT), insulin degrades almost linearly. At elevated temperatures, the loss of insulin potency...
accelerates, leading to a reduction in efficacy and poor therapeutic results. Thus, insulin should be routinely stored under recommended conditions to maintain its maximum potency and reduce the likelihood of unexpected blood glucose variability. More specifically, unopened insulin pens or vials should be refrigerated (2°C–8°C), whereas opened insulin can be kept at room temperature (below 30°C) for up to 28 days, depending on the brand, formulation, and container.6

The American Diabetes Association and insulin pen manufacturers recommend that in-use insulin pens (defined as insulin pens that have already been opened) should be stored at RTs not exceeding 30°C.7,8 However, the ambient temperatures in tropical countries are typically higher than those found in countries at other latitudes, and these higher temperatures may adversely affect insulin potency. Global concern about this issue has increased, especially in low-income and middle-income countries, where the prevalence of diabetes is increasing.9 In low-resource countries, at least 33% of people with diabetes treated with insulin do not have a home refrigerator,9 meaning they cannot appropriately store their in-use insulin when ambient temperatures exceed 30°C. The potency of the incorrectly stored insulin may therefore be diminished. As a result, these patients might experience poor glycemic control and need to increase their insulin dosage to lower their blood glucose levels.

Moreover, a previous study revealed that approximately 40% of people with diabetes treated with insulin were advised to store their in-use insulin pens in a cooling device when traveling to protect the insulin from being exposed to high temperatures.10 Most people with diabetes reported that it was inconvenient to carry their in-use insulin in a cooling device when outside their homes. Consequently, many decided to leave their insulin at home and miss their injection. This problem can significantly compromise diabetes management in tropical countries or low-resource settings.

In 2009, regular and biphasic insulin formulations were investigated for their stability after storage in high temperature conditions.11 Vimalavathini and Gitanjali11 reported that insulin potency, as determined by high-performance liquid chromatography (HPLC), was decreased by 14% and 18% after exposure to isothermal temperatures of 32°C and 37°C, respectively, for 28 days. In that same study, insulin stored at 32°C and 37°C was intraperitoneally injected into rabbits. The rabbits receiving insulin stored at 32°C and 37°C did not exhibit a significant reduction in blood glucose levels compared with those administered insulin kept at 5°C. Based on their findings, the authors concluded that insulin should be used within 2 weeks after opening in settings where insulin cannot be stored at the recommended temperature. In 2019, Kongmalai et al12 investigated the stability of in-use basal insulin in pen devices (glargine and three commercialized formulations of Neutral Protamine Hagedorn (NPH)). Comparisons were made over 28 days for three conditions: RT (range: 25.5°C–37.1°C), refrigerator storage (range: 2°C–8°C), and incubator storage (37°C). Their results revealed no significant differences in the stability of the three groups. However, the biological activity of insulin was not evaluated in this study. To address this issue, Kaufmann et al13 investigated the heat stability of human insulin formulations (rapid, NPH, and mixed insulin) and four analog insulin formulations (lispro, aspart, glargine, and mixed lispro analog) administered as vials and cartridges. They found that insulin exposed to temperatures fluctuating between 25°C and 37°C remained stable for 4 weeks, as evaluated by HPLC. The bioactivity of mixed insulin was also assessed in the hepatocyte cell line. The results showed that mixed insulin stored at the same oscillating temperature maintained its bioactivity.

Although the thermostability of insulin has previously been investigated, no study has evaluated the biological activity of insulin exposed to high temperatures in people with diabetes. Accordingly, this study aimed to determine the daily mean glucose levels (MGLs) achieved by basal insulin in pens stored at 37°C for 21 days and in refrigerated pens. The MGLs were assessed via continuous glucose monitoring (CGM). This study’s findings will help clarify the temperature-related risks associated with in-use insulin pen storage and will help improve management strategies and compliance among people with diabetes treated with insulin.

**MATERIALS AND METHODS**

**Study design**

This was a 4-week, randomized, single-blind, crossover, controlled, equivalence trial. Using CGM, it compared the stability and biological activity of basal insulin in a pen that had been stored at high temperature (37°C for 21 days) with the corresponding values of refrigerated basal insulin. The study followed the Consolidated Standards of Reporting Trials guidelines and was registered at the Thai Clinical Trials Registry (TCTR20210611002).

**Participants and eligibility criteria**

The patient population was sourced from the outpatient department of Siriraj Hospital, Bangkok, Thailand, from April 2019 to March 2021. To be eligible for inclusion, patients must have met all of the following criteria: older than 18, diagnosed with type 2 diabetes mellitus, had been taking a stable dose of insulin glargine for at least 3 months, and had a glycated hemoglobin (HbA1c) level less than 8% (64 mmol/mol). Candidates meeting any of the following criteria were excluded: HbA1c ≥8% (64 mmol/mol); use of insulin other than insulin glargine; history of severe hypoglycemia within 1 month or ischemic stroke or acute myocardial infarction within 6 months; estimated glomerular filtration rate <30 mL/min/1.73 m²; psychiatric problems; or receiving a drug that affects plasma glucose, such as thiazide, glucocorticoid, or contraceptive pill. Patients who had taken drugs...
or consumed foods that potentially affected the level of interstitial glucose measured by CGM, such as paracetamol, atenolol, albuterol, lisinopril, and red wine, were also excluded. All eligible and willing participants provided written informed consent to participate.

**Study drug**
The basal insulin pen was investigated for two reasons. First, it is typically used once daily. Second, almost half of insulin-treated patients with diabetes store in-use insulin at RT at home; this insulin is likely to be exposed to high temperatures once opened. In addition, insulin glargine (Lantus; Sanofi SA, Paris, France) was studied because a previous publication demonstrated that it had the best stability after storage at a high temperature compared with other basal insulins. For the high temperature (HT) insulin pen group, the pens were stored in an incubator at 37°C for 21 days before being used. As for the refrigerated insulin pen group, unopened pens were kept in a refrigerator at 2°C–8°C until their first use. For each trial period, two insulin pens from the same batch (batch number 8F5613A) were used. The first pen was used during days 1–3 and the second pen was used during days 4–7.

**Baseline assessment at screening**
Before being randomized, all participants underwent baseline assessments. These included demographic information, a detailed medical history (underlying conditions and the dosage and type of antidiabetic drugs), a physical examination, and a laboratory investigation (HbA1c level and fasting plasma glucose within 3 months).

**Randomization and allocation concealment**
Participants who fulfilled the enrollment criteria were randomly assigned, in a 1:1 ratio using a computer-generated randomization number, to receive either an HT insulin pen or a refrigerated insulin pen in the first phase (days 1–7) and the second phase (days 22–28). TK generated and concealed the allocation sequence until the interventions were assigned. PO enrolled the participants, and PC assigned the participants to their groups. The HT insulin pen and refrigerated insulin pen were virtually identical in appearance. The participants and ultrahigh-performance liquid chromatography (UHPLC) investigators were blinded to the treatment allocations throughout the experiment. The trial dataset was locked and directly analyzed by the study statistician using a prespecified data analysis plan.

**Study visits**
A total of four visits to our laboratory department were required. At the first appointment, each participant was given a 7-day CGM using a Medtronic iPro2 system (Medtronic, Dublin, Ireland) that included an Enlite glucose sensor and an iPro2 digital recorder. The subjects were instructed on how to use the glucometer. Self-monitoring of blood glucose was required to calibrate the CGM at least twice daily using an Accu-Chek Performa Glucometer (Roche Diagnostics, Basel, Switzerland). Throughout the experiment, the participants were asked to balance their dietary intake and daily activities and to document them in a record book. At the second visit, held 1 week later, the CGM device was removed. The digital recorder was connected to the iPro2 docking station to upload its data for analysis by the CareLink iPro software program. The participants were checked to determine whether they were strictly following the trial protocol. This discussion explored any medication changes or adverse events, documenting their dietary intake and activities, control of insulin injections, and self-monitoring of blood glucose. To rule out a carryover effect, a washout period of 2 weeks (days 8–21) was applied between the two study phases. During the washout interval, the subjects resumed using their own insulin, which was kept in a refrigerator. After the 2-week washout, individuals were given the opposite kind of insulin, per the crossover design (online supplemental figure S1). The third and final visits were identical to the first two visits, except that the opposite type of insulin was administered.

Although the participants could continue their previous medications, the dosages of oral glucose-lowering drugs and insulin were required to remain unchanged throughout the study period. Subjects were immediately excluded from the study if they experienced hypoglycemia (capillary blood glucose less than 40 mg/dL with or without symptoms) or hyperglycemia (capillary blood glucose more than 250 mg/dL on two consecutive occasions). At the end of each study phase, the remaining insulin in the pens was maintained at −20°C until the amount of insulin in each pen could be quantitated by the Department of Biochemistry, Faculty of Medicine Siriraj Hospital, Mahidol University.

**Primary and secondary outcomes**
The glucose data obtained from CGM were used to calculate the glucometrics, including the MGLs and glucose variability. The study’s primary outcome was the difference in the MGLs of the groups. The secondary outcome parameters were glucose variability represented by the coefficient of variation (CV), standard deviation (SD), mean amplitude of glycemic excursion (MAGE), percentage of time in range (TIR), time above range (TAR), time below range (TBR), and quantity of the remaining insulin evaluated by UHPLC assay. MAGE was calculated using a computerized calculator (GlyCulator2 program) developed by Pagacz et al.

**Insulin quantification using UHPLC**
Insulin glargine was analyzed using UHPLC techniques as previously described. Briefly, each insulin sample was diluted 1:50 in 0.01 N hydrochloric acid. Two microliters of the sample were then injected into an Agilent 1290 Infinity LC System equipped with an Agilent Poroshell 120 EC-C18 column (3.0×50 mm, 1.9 µm; Agilent Technologies, Santa Clara, California, USA). The separation
was performed at a column temperature of 40°C and a flow rate of 0.75 mL/min with isocratic elution using a mobile phase consisting of acetonitrile and solution A (26:74 v/v). Solution A was prepared by dissolving 28.4 g of anhydrous sodium sulfate in 1000 mL of water, and 2.7 mL of phosphoric acid was subsequently added. If necessary, the pH was adjusted to 2.3 with ethanolamine. The analysis was run for 7 min, and the eluted insulin was detected at a wavelength of 214 nm. Each sample was assayed in duplicate.

**Sample size calculation and statistical analysis**

The recommended clinically meaningful difference in treatment effect compared with standard treatment in diabetes was reported to be 0.3% for HbA1c and 14 mg/dL for mean plasma glucose. The sample size for this study was calculated using NCSS software (Kaysville, Utah, USA). Using a two-tailed α of 0.05, an equivalence margin of 10%, and an 80% power, a sample size of 20 patients for each phase of the study was needed to identify the minimum clinically important difference in mean plasma glucose. As the size was increased by 10% to compensate for dropouts for any reason, the final total sample size was 44 patients.

Consistent with the per-protocol analysis principle, only data from participants who completed the study protocol were included in the final analysis. Data were analyzed using NCSS software (V.10; NCSS Statistical Software) and Minitab (V.19; Minitab, State College, Pennsylvania, USA). For the primary outcome, a 2×2 crossover study and Schuirmann’s two one-sided t-tests were used. Secondary outcomes, including the differences between CV, SD, MAGE, and percentage of TIR, were analyzed by paired t-tests. Continuous data with a normal distribution pattern are presented as mean±SD, whereas non-normally distributed continuous data are presented as median and interquartile range (IQR). To evaluate differences between the percentages of TBR and TAR, both of which are non-normally distributed, the Wilcoxon signed-rank test was used. Frequencies and percentages were used to report categorical data. For all analyses, a p value <0.05 was considered statistically significant.
RESULTS
Forty-four patients were enrolled in this study. However, four patients were withdrawn: one accidentally disconnected the Enlite glucose sensor, one did not comply with the study protocol, and two experienced asymptomatic nocturnal hypoglycemia during the study’s first phase. Therefore, 40 patients (22 in sequence 1 and 18 in sequence 2) completed the trial (figure 1). The record books of the 40 participants were examined. None reported any adverse events, changed their diabetes or other medications, or had any other medical conditions that would have affected glucose control. The diets and physical activity levels were comparable across the two study periods.

Baseline characteristics
The baseline demographic and clinical characteristics of the study population are summarized in table 1. The mean age was 55.2 years, the mean fasting plasma glucose was 125.7 mg/dL, the mean HbA1c was 7.0% (53 mmol/mol), and the mean duration of diabetes was 10.9 years.

MGLs of HT and refrigerated insulin pen groups
Twenty-two subjects in sequence 1 (refrigerated insulin → HT insulin) and 18 subjects in sequence 2 (HT insulin → refrigerated insulin) completed the study protocol. The MGL was 158.7±30.5 mg/dL for the HT insulin pen group and 157.0±40.9 mg/dL for the refrigerated insulin pen group. The MGL of the HT insulin group was non-significantly higher, by 1.5 mg/dL, than that of the refrigerated insulin group (p=0.72). The result of the equivalence test for a 2×2 crossover design demonstrated that carryover and sequence of insulin treatment had no statistically significant effects (p=0.35 and p=0.92, respectively; table 2).

The equivalence plot showed that the 90% CI for equivalence (−5.5 to 8.5) fell entirely within the equivalence interval, defined by the lower equivalence limit (−14) and the upper equivalence limit (14). This finding indicates that the insulin of the two study groups was equally effective (online supplemental figure S2, online supplemental table S1).

Glucose variability of HT and refrigerated insulin pen groups
Glucose variability was assessed by CV, SD, MAGE, and percentage of TIR at 7 days. The CV \(_{\text{TIR7day}}\) was 31.1±9.5% and 32.7±8.5% in the HT and refrigerated insulin pen groups, respectively (95% CI −2.0 to 5.1, p=0.37). The SD \(_{\text{TIR7day}}\) was 53.1±19.1 and 51.1±16.0 in the HT and refrigerated insulin groups, respectively (95% CI −8.4 to 4.3, p=0.52). The MAGE \(_{\text{TIR7day}}\) was 133.2±37.9 mg/dL and 134.5±39.7 mg/dL in the HT and refrigerated insulin pen groups, respectively (95% CI −1.7 to 14.8, p=0.87). The percentage of TIR \(_{\text{TIR7day}}\) was 64.2±23.5 and 64.5±19.7 in the HT and refrigerated insulin groups, respectively (95% CI −6.9 to 7.7, p=0.91; figure 2). There was also no statistically significant difference in the glucose variability of the groups. The median percentages of TAR \(_{\text{TIR7day}}\) and TBR \(_{\text{TIR7day}}\) are presented in table 3.

UHPLC quantification
The area under the curve (AUC) of the chromatogram represents insulin quantity. The mean±SD of the AUC in the HT insulin and refrigerated insulin pen groups was 579.3±14.1 mAU*s and 579.4±13.4 mAU*s, respectively (p=0.97; online supplemental table S2). An unopened insulin glargine pen stored under appropriate conditions was used as the control (AUC 568 mAU*s). The percentage amount of residual insulin in the HT and refrigerated insulin pen groups was 101.8±2.7 and 102.2±2.4, respectively (p=0.23).

DISCUSSION
Insulin and its various analogs are complex labile proteins that are susceptible to denaturation when...
Instability in these proteins is attributed to a differential level of changes in the protein structure. Denaturation can occur when they are exposed to any of several physiochemical changes in the environment. The degradation of insulin is widely classified into two types: physical and chemical. Physical degradation, such as adsorption or aggregation, refers to an irreversible change in the physical state of the protein without any change in its covalent structure. Chemical degradation refers to a change in the covalent structure of the protein. The susceptibility of insulin to chemical degradation depends on its thermodynamic properties and tendency to undergo a conformational change. Thermal denaturation is a complex process influenced by both time and temperature. An increase in temperature can disturb the native protein conformation, which promotes the unfolding of protein parts over time. Degradation of insulin can lead to loss of bioactivity in lowering blood glucose. Therefore, insulin and its various analogs should be stored correctly, per manufacturer recommendations, at all times to maintain potency and enable precise dosing for people with diabetes.

The present study set forth to assess the stability of basal insulin stored at HT in an insulin pen. A high ambient temperature reflects the environment in which in-use insulin pens are often used in regions where RTs are frequently higher than the maximum temperature recommended by manufacturers. According to the results of CGM, the current investigation showed equality in the MGLs achieved by basal insulin in pens stored at HT (37°C for 21 days) and by refrigerated basal insulin (2°C–8°C). In addition, no statistically significant differences were found in the glucose variability profiles, such as CV, SD, MAGE, percentage of TIR, TAR, and TBR, of the HT and refrigerated insulin groups. Moreover, the two groups demonstrated no statistically significant differences in Table 3.

**Table 3** Median percentages of TAR\(_{7\text{day}}\) and TBR\(_{7\text{day}}\) achieved by basal insulin in pens stored at high temperature (37°C for 21 days) and by refrigerated basal insulin

<table>
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<tr>
<th></th>
<th>High temperature insulin</th>
<th>Refrigerated insulin</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAR(_{7\text{day}}) (IQR)</td>
<td>24.0 (13.4–75.0)</td>
<td>30.0 (12.0–37.8)</td>
<td>0.80</td>
</tr>
<tr>
<td>TBR(_{7\text{day}}) (IQR)</td>
<td>1.0 (0–7)</td>
<td>1.0 (0–6)</td>
<td>0.73</td>
</tr>
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P<0.05 indicates statistical significance.
TAR, time above range; TBR, time below range.
significant difference in their remaining quantities of insulin, assessed by UHPLC at the end of the study. This study’s in vitro and in vivo results affirm the outcome of our previous experiment, namely that basal insulin in a pen retains its potency and biological activity after being stored at temperatures as high as 37°C for 28 days. It found a significant loss of insulin potency after storage at 32°C and 37°C for 28 days, both in vitro and in vivo. This difference between studies could be due to differences in insulin formulation (regular and biphasic vs basal) and insulin container (vial vs cartridge). However, our study’s results are consistent with Kaufmann et al’s findings in 2021. They investigated the effects of oscillating temperature (range: 25°C–37°C) on the stability of various insulin types. The results demonstrated that insulin could be stored at oscillating ambient temperatures for 4 weeks. The outcome of the present study provides reassuring evidence that an in-use insulin pen can be stored in RTs as high as 37°C for 28 days. These results remove cold storage as a significant barrier to the safe and effective use of an insulin pen for up to 4 weeks. Moreover, our findings will improve drug compliance among people with diabetes who presently strictly follow storage guidelines for insulin. In situations where their insulin might become exposed to high temperatures, some people with diabetes currently elect to leave their insulin pen at home. Unfortunately, doing so results in their missing insulin injections. Therefore, the current study’s findings are of great value to insulin-treated patients with diabetes living in tropical or low-income countries.

According to several pharmacopeias and previous studies, HPLC is the gold standard for determining insulin potency. Hence, this study used HPLC to evaluate insulin potency. The results of our investigation are also consistent with those of many previous studies that found good correlations between insulin potency evaluated by HPLC and its residual bioactivity.

The present work has several strengths. This is the first study to investigate the heat stability of basal insulin in a pen device for people with diabetes. Second, according to our randomized controlled crossover trial design, each subject acted as his or her own control. Thus, other factors influencing glucose control, such as insulin resistance, were minimized. Moreover, the participants were instructed to balance their food intake and physical activity throughout the two study periods. They were required to record their daily activities, nutritional intake, and health issues in a booklet to reduce factors contributing to poor glycemic control. Third, CGM was used to measure participants’ glucose levels every 5 min throughout the study period. Interestingly, 2 of 44 subjects had nocturnal hypoglycemic unawareness detected by CGM. Therefore, physicians should raise their patients’ awareness of the condition when prescribing insulin, even if they report no hypoglycemia symptoms. Fourth, UHPLC was performed to evaluate the potency of the remaining insulin at the end of each study phase in both study groups. Fifth, unlike most previous studies that evaluated the stability of insulin vials, the present study focused on an insulin pen because it is currently more widely used. Insulin in a cartridge is expected to undergo more rigorous agitation and potential exposure to variable temperatures during use than insulin in a vial. Hence, in-use dating of cartridges takes into account the smaller volume and fewer total units of insulin compared with vials.

Limitations
This study has some notable limitations. First, it was conducted under an isothermal temperature condition rather than a cyclic temperature condition, and no insulin was withdrawn during the 21 days, which would more closely resemble a natural ambient environment and real-life practice. However, Kongmalai et al’s study to investigate the heat stability of basal insulin in a refrigerator (2°C–8°C). This critical finding overcomes concerns related to insulin storage in hot climate countries. The finding is particularly relevant to low-income situations where insulin-treated patients with diabetes might not have access to home refrigeration and hence cannot consistently keep their opened insulin pens below 30°C. The standard advice that heat-exposed insulin should be discarded needs to be reconsidered.

CONCLUSIONS
A basal insulin pen exposed to temperatures as high as 37°C for 21 days retains its potency and has a biological activity comparable with that of a newly opened insulin pen stored in a refrigerator (2°C–8°C). This critical finding overcomes concerns related to insulin storage in hot climate countries. The finding is particularly relevant to low-income situations where insulin-treated patients with diabetes might not have access to home refrigeration and hence cannot consistently keep their opened insulin pens below 30°C. The standard advice that heat-exposed insulin should be discarded needs to be reconsidered.

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Contributors TK, BD, and AS contributed to the study’s conceptualization and design. TK and PO conducted the study, data collection, analysis, and result interpretation. PC participated in the study’s conduction. SJ performed UHPLC, and CS verified the results. AS supervised the study protocol and findings. TK was the lead writer of the manuscript. AS and CS provided critical feedback and helped shape the analysis and manuscript. TK and AS are responsible for the overall content as the guarantor. All authors read and approved the final manuscript, and all authors were in agreement to submit this article for journal publication.

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Competing interests None declared.

Patient consent for publication Not required.

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Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. The raw data that support the findings of this study are available from the corresponding author upon reasonable request.

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