

## RESEARCH ARTICLE

# A simple formula to correct for the effects of storage time and temperature on the insulin concentration

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## Abstract

**Objective:** To investigate the influence of storage time and temperature on plasma insulin levels and to establish a correction formula.

**Methods:** Venous blood samples were taken from 20 volunteers and processed as follows: whole blood samples, centrifuged samples, and separated plasma samples were stored at 4°C or 25°C. Insulin levels were determined by direct chemiluminescence at 0, 0.5, 1, 2, 4, and 8 hours. According to the correlation between the insulin concentration ratio and storage time, correction formulas for the insulin concentration were established. To verify the test, the venous blood samples of another 33 volunteers were processed in the same way. The insulin levels of the samples were corrected after 3, 6, 12, and 24 hours and compared with the value at 0 hours to verify the feasibility of the corrected formula.

**Results:** With the prolongation of storage time, the insulin levels of the whole blood samples at 4°C or 25°C and of the centrifuged samples at 25°C decreased gradually ( $P < .001$ ), and the insulin level correction formulas were  $C_{\text{correction}} = C_{\text{determination}} / 0.991e^{-0.069x}$ ,  $C_{\text{correction}} = C_{\text{determination}} / 1.048e^{-0.126x}$ , and  $C_{\text{correction}} = C_{\text{determination}} / [-0.068\ln(x) + 0.9242]$ . There was no significant difference between the corrected insulin results and the original results at any time within 12 hours ( $P > .05$ ).

**Conclusions:** The insulin levels of the whole blood samples at 4°C or 25°C and of the plasma samples at 25°C gradually decreased with storage time. The effect of storage time on the insulin level can be reduced with the correction formulas.

## KEYWORDS

correction formula, insulin, temperature, time

## 1 | INTRODUCTION

Diabetes is a type of metabolic disease that is characterized by hyperglycemia caused by insufficient insulin secretion from islet  $\beta$

cells, impaired insulin action, or both.<sup>1</sup> In diabetes mellitus, long-term disruption of blood glucose metabolism can lead to the injury and dysfunction of various organs, especially the kidneys, eyes, heart, nerves, and blood vessels.<sup>2</sup> According to the literature,

Zeng and Chen contributed equally to this work.

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due to the impacts of high-sugar, high-calorie diets and lifestyle, there were approximately 451 million diabetic patients worldwide in 2017, at which time the adult prevalence rate was as high as 8.4%; however, it is expected to reach 9.9% by 2045.<sup>3</sup> The mortality rate of patients with diabetes has also increased annually.<sup>4</sup> Therefore, early screening and timely treatment of diabetes are of great significance.

Insulin is a protein hormone with hypoglycemic function that is secreted by islet  $\beta$  cells. Insulin is involved not only in the regulation of glucose metabolism but also in the metabolism of fat and protein and is closely related to human health.<sup>5,6</sup> As insulin research has progressed, the clinical role of insulin has become more well known. Insulin secretion can be used to evaluate the function of islet  $\beta$  cells. Studies have shown that at the time of the diabetes diagnosis, islet  $\beta$ -cell function in patients is decreased by approximately half, which means that islet  $\beta$ -cell function is impaired long before the onset of diabetes.<sup>7</sup> Research on the role of islet  $\beta$ -cell function is beneficial toward effectively treating diabetes.<sup>8</sup> The oral glucose tolerance test (OGTT) is commonly used to diagnose diabetes in the clinic and measures glucose concentrations at 0, 0.5, 1, 2, and 3 hours after oral glucose administration. The simple insulin release test is performed by following the same procedure to determine the insulin concentration. The combination of these factors is conducive to the diagnosis, classification, staging, and treatment guidance of diabetes.<sup>9,10</sup> Insulin can be used to assess insulin resistance.<sup>11,12</sup> The assessment of insulin resistance can increase the early diagnosis of type 2 diabetes or the predictiveness of cardiovascular risk factors noted in many studies.<sup>13-16</sup> Insulin measurements can also be used in the diagnosis of spontaneous hypoglycemia.<sup>17</sup> Therefore, the accuracy of insulin data is very important in clinical practice. However, insulin determinations are limited by the pre-analytical processing of samples.<sup>18</sup> Moreover, because of the existence of insulin-degrading enzymes, the determination of insulin in nonhemolytic samples is affected by time and temperature.<sup>19,20</sup> In clinical practice, the number of patients in the hospital is large. The process for blood sampling, sample transfer, and final examination is not only limited by time but also by the fact that most samples may not be handled. The results of insulin measurements indicate that insulin levels are relatively low. Numerous studies have indicated that the stability of insulin in nonhemolytic specimens is mainly affected by time and temperature, but these

studies have not provided information on the degree of influence of either factor.<sup>21-25</sup> To determine a more realistic insulin value, this study examined the changes in the insulin concentrations of samples exposed to common handling methods in clinical laboratories at different times and temperatures and aimed to establish corresponding correction formulas.

## 2 | MATERIALS AND METHODS

### 2.1 | Specimen collection and processing

Twenty volunteers, including 5 males and 15 females, aged 21-26 years, were recruited. Approximately 32 mL of venous blood was drawn from each individual, with his or her informed consent. The blood was collected into 32 heparin tubes at approximately 1 mL per tube, and one tube was used for the determination of the hemoglobin (Hb) concentration. Another tube was centrifuged, and the insulin concentration was measured immediately (0 hour). The remaining 30 tubes were classified into one of the following three groups and stored at 4°C or 25°C: whole blood, centrifuged blood (After blood centrifugation, the centrifugal state of plasma and red blood cells in the same tube without absorbing plasma), and separated plasma. The centrifuged blood and separated plasma were centrifuged at 2586 g for 5 minutes before storage, and all of the stored specimens were centrifuged at 2586 g for 5 minutes before measurements. The appearance of the specimens was not characterized by jaundice, lipids, or hemolysis.

### 2.2 | Insulin measurement

The insulin concentrations of the whole blood samples, centrifuged samples, and separated plasma samples were measured at 0.5, 1, 2, 4, and 8 hours by direct chemiluminescence (Centaur XP, Siemens).

### 2.3 | Hemoglobin measurement

The Hb concentration of venous blood of 20 volunteers was measured by colorimetry (BC6800, Mindary).

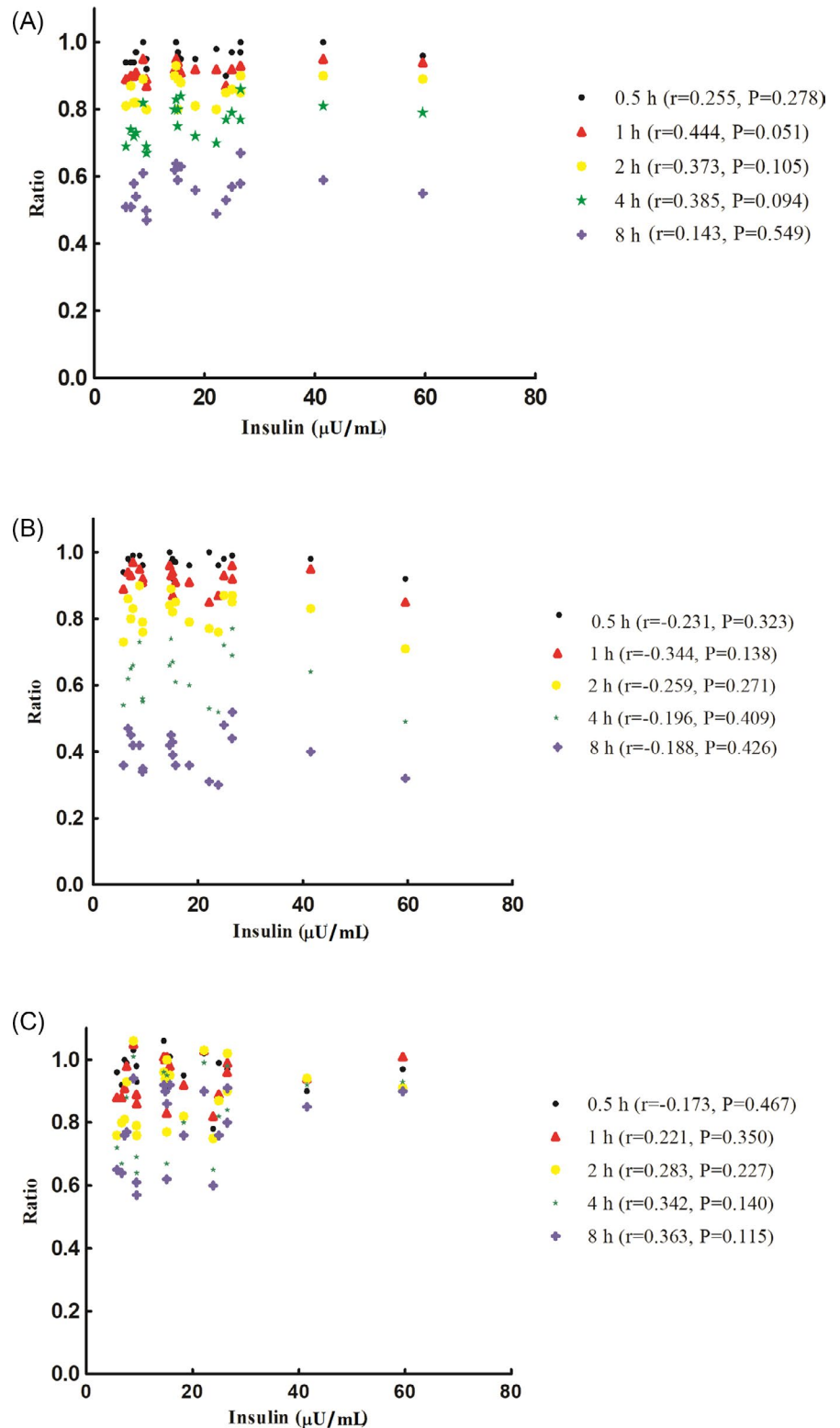
**TABLE 1** Relationship between insulin ratios and preservation time under different temperatures and preservation conditions ( $\bar{x} \pm s$ )

Storage conditions	0.5 h	1 h	2 h	4 h	8 h	F	P
Whole blood at 4°C	0.960 ± 0.028	0.916 ± 0.025	0.854 ± 0.043	0.764 ± 0.055	0.569 ± 0.056	255.732	.000
Centrifuged blood at 4°C	0.950 ± 0.084	0.940 ± 0.098	0.936 ± 0.117	0.918 ± 0.124	0.891 ± 0.125	0.862	.490
Separated plasma at 4°C	0.951 ± 0.058	0.950 ± 0.060	0.955 ± 0.065	0.950 ± 0.070	0.965 ± 0.059	0.290	.933
Whole blood at 25°C	0.970 ± 0.023	0.918 ± 0.036	0.817 ± 0.052	0.631 ± 0.080	0.399 ± 0.061	372.856	.000
Centrifuged blood at 25°C	0.963 ± 0.061	0.942 ± 0.069	0.889 ± 0.100	0.836 ± 0.123	0.782 ± 0.123	11.002	.000
Separated plasma at 25°C	0.925 ± 0.059	0.938 ± 0.071	0.915 ± 0.084	0.893 ± 0.016	0.893 ± 0.016	1.042	.390

## 2.4 | Establish correction formula

We collected samples from 20 volunteers. A correction formula was established according to the relationship between insulin

concentration ratio (insulin concentration at each time point/0 hours insulin concentration) and time at different times under different storage conditions (0.5, 1, 2, 4, 8 hours). The time was taken as the X axis and the insulin ratio as the Y axis.

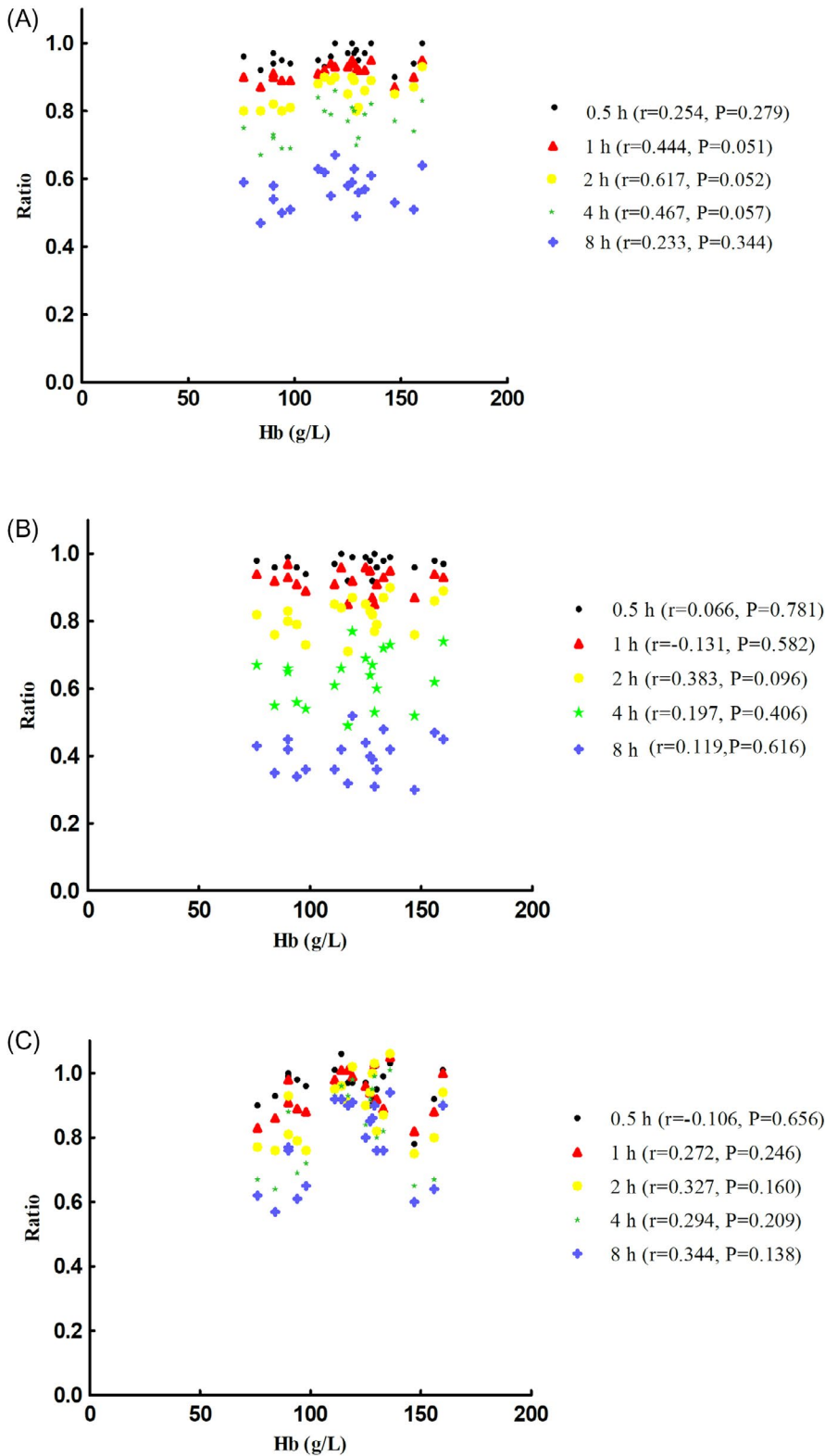


**FIGURE 1** The correlation between insulin concentration and insulin ratio at different times. A, Whole blood samples were stored at  $4^{\circ}\text{C}$ ; B, whole blood samples were stored at  $25^{\circ}\text{C}$ ; C, centrifuged samples were stored at  $25^{\circ}\text{C}$ . All  $P$  values  $>0.05$ , this indicated that there were no correlations between the initial concentration of insulin and the degradation of insulin in different sample processing conditions

## 2.5 | Verification of correction formulas

Thirteen milliliters of venous blood was drawn from 33 volunteers after informed consent was obtained, and the samples were

processed in the same way. The insulin levels of the specimens at 3, 6, 12, and 24 hours were measured and corrected according to the correction formulas. The feasibility of the correction formulas was verified by comparing the corrected values with the original values.



**FIGURE 2** The correlation between Hb concentration and insulin ratio at different times. A, Whole blood samples were stored at 4°C; B, whole blood samples were stored at 25°C; C, centrifuged samples were stored at 25°C. All  $P$  values  $>.05$ , this indicated that there were no correlations between the concentration of Hb and the degradation of insulin in different sample processing conditions

## 2.6 | Statistical methods

SPSS 22.0 software was used for data analysis. The insulin ratio, which was the ratio of the insulin level at a specific point time to the insulin level at 0 hour with the same treatment, was expressed as the mean  $\pm$  standard deviation ( $\bar{x} \pm SD$ ), and analysis of variance was performed for group comparisons. *t* tests were used for comparisons of insulin concentration ratios at different temperatures. Pearson's correlation analysis was performed to analyze the correlation between the original insulin concentration or Hb level and the insulin concentration ratio at each storage time. The rank-sum test was used to compare the corrected value of insulin with the original value. Regression analysis was used to analyze the correlation between the insulin concentration ratios and time variation.  $P < .05$  was considered statistically significant.

## 3 | RESULTS

### 3.1 | The relationship between insulin concentration and processing methods, storage time, and temperature

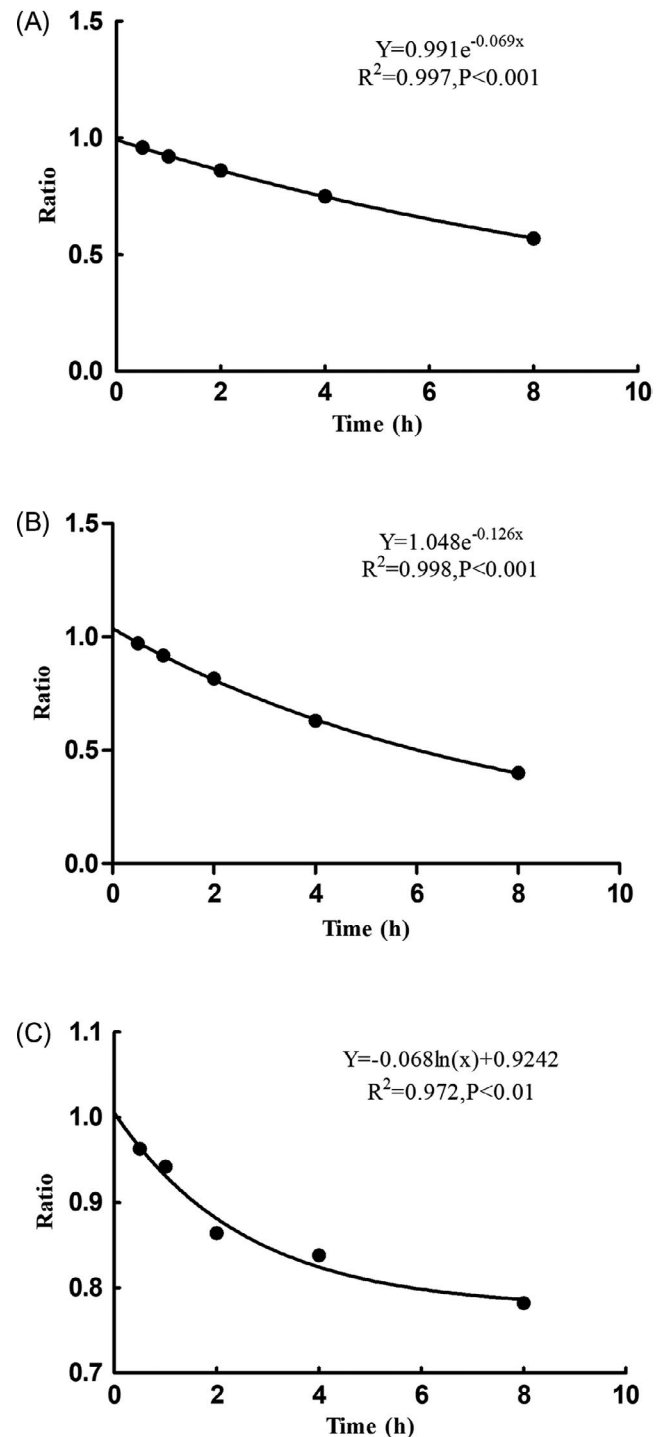
As Table 1 shows, there were no significant differences in the insulin ratios of the plasma samples that were stored 4°C or 25°C and the centrifuged samples that were stored 4°C within 8 hours ( $P > .05$ ). With the prolongation of storage time, the insulin levels of the whole blood samples at 4°C or 25°C and of the centrifuged samples at 25°C decreased gradually ( $P < .001$ ), and the degree of the reduction in the whole blood samples at 4°C was lower than that of the whole blood samples at 25°C but higher than that of the centrifuged samples at 25°C ( $P < .001$ ).

### 3.2 | Analysis of the correlation between insulin or Hb levels and insulin ratios

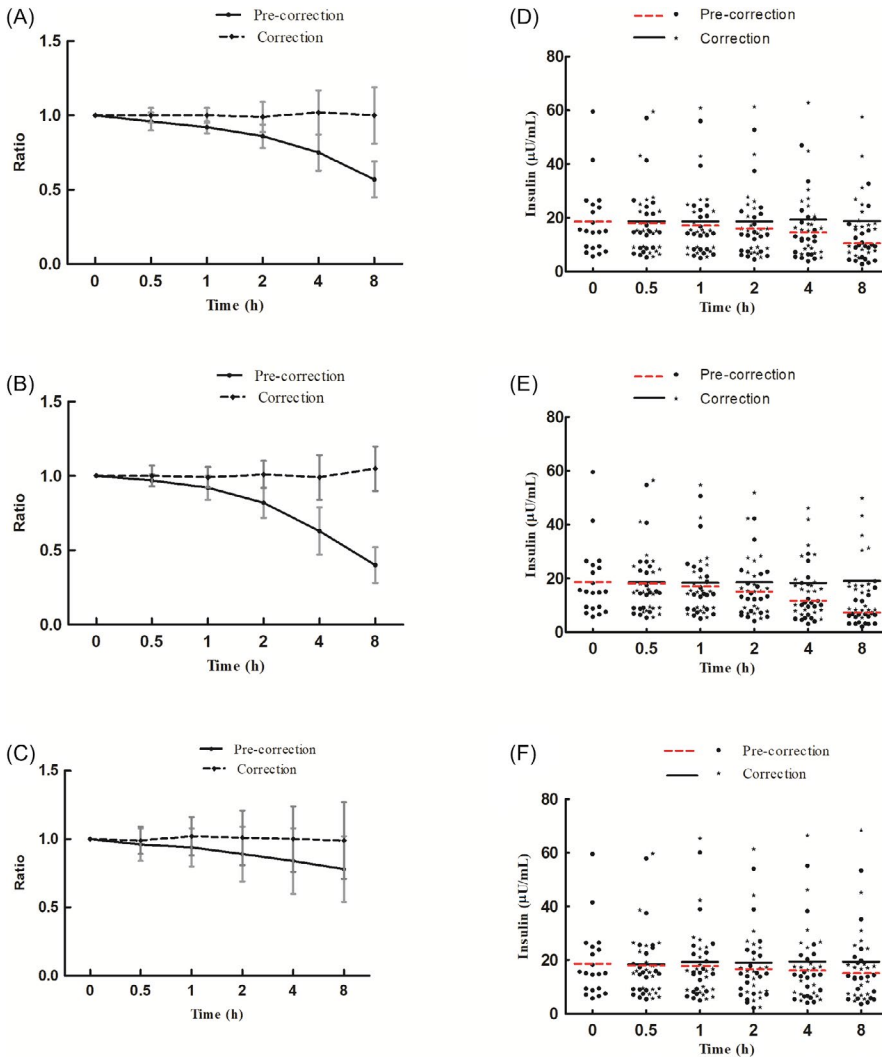
There was no correlation between the insulin ratios and the original insulin levels or Hb levels of the volunteers at different times ( $P > .05$ ; Figures 1 and 2).

### 3.3 | Establishment of insulin correction formulas

The insulin concentration in the whole blood samples at 4°C or 25°C and in the centrifuged samples at 25°C changed with time. According to the regression analysis of the insulin ratios and storage time, the functions with the base *e* were calculated with the insulin ratios as the Y axis and the storage time as the X axis as follows:  $Y = 0.991e^{-0.069x}$  ( $R^2 = 0.997$ ,  $P < .001$ ; 95% CI of Y: 0.974~1.009) for whole blood at 4°C,  $Y = 1.048e^{-0.126x}$  ( $R^2 = 0.998$ ,  $P < .001$ ; 95% CI of Y: 0.995~1.060) for whole blood at 25°C, and  $Y = -0.068\ln(x) + 0.9242$  ( $R^2 = 0.972$ ,  $P < .001$ ; 95% CI of Y: 0.885~1.126) for the centrifuged samples at 25°C (Figure 3), indicating that the insulin correction



**FIGURE 3** Regression equations were calculated with the insulin ratios as the Y axis and the storage time as the X axis. A, Whole blood samples were stored at 4°C, the exponential function with the base of the natural constant *e* was obtained:  $Y = 0.991e^{-0.069x}$  ( $R^2 = 0.997$ ,  $P < .001$ ; 95% CI of Y: 0.974~1.009); B, whole blood samples were stored at 25°C. The exponential function with the base of the natural constant *e* was obtained:  $Y = 1.048e^{-0.126x}$  ( $R^2 = 0.998$ ,  $P < .001$ ; 95% CI of Y: 0.995~1.060); C, centrifuged samples were stored at 25. The logarithmic function with the base of the natural constant *e* was obtained:  $Y = -0.068\ln(x) + 0.9242$  ( $R^2 = 0.972$ ,  $P < .001$ ; 95% CI of Y: 0.885~1.126)



**FIGURE 4** Comparison of insulin concentrations and the insulin ratios before and after correction at different times. A-C were the comparison of insulin ratio before and after correction; D-F were the comparison of insulin concentration before and after correction with the bar represent the mean. A and D, Whole blood samples were stored at 4°C; B and E, whole blood samples were stored at 25°C; C and F, centrifuged samples were stored at 25°C

formulas of the whole blood samples at 4°C or 25°C and the centrifuged samples at 25°C were  $C_{\text{correction}} = C_{\text{determination}} / 0.991e^{-0.069x}$ ,  $C_{\text{correction}} = C_{\text{determination}} / 1.048e^{-0.126x}$  and  $C_{\text{correction}} = C_{\text{determination}} / [-0.068\ln(x) + 0.9242]$ , respectively.

### 3.4 | Comparison of insulin levels between the corrected results and the original results

The correction formulas were used to correct the insulin levels at each time, and the corrected results were compared with the original values. There were no significant differences between the corrected insulin results and the original results at any time within 8 hours for the whole blood samples at 4°C or 25°C and the centrifuged samples at 25°C ( $P > .05$ ; Figure 4).

### 3.5 | Verification of correction formulas

The corrected insulin results at 3, 6, 12, and 24 hours were calculated by validation formulas and compared with the original results

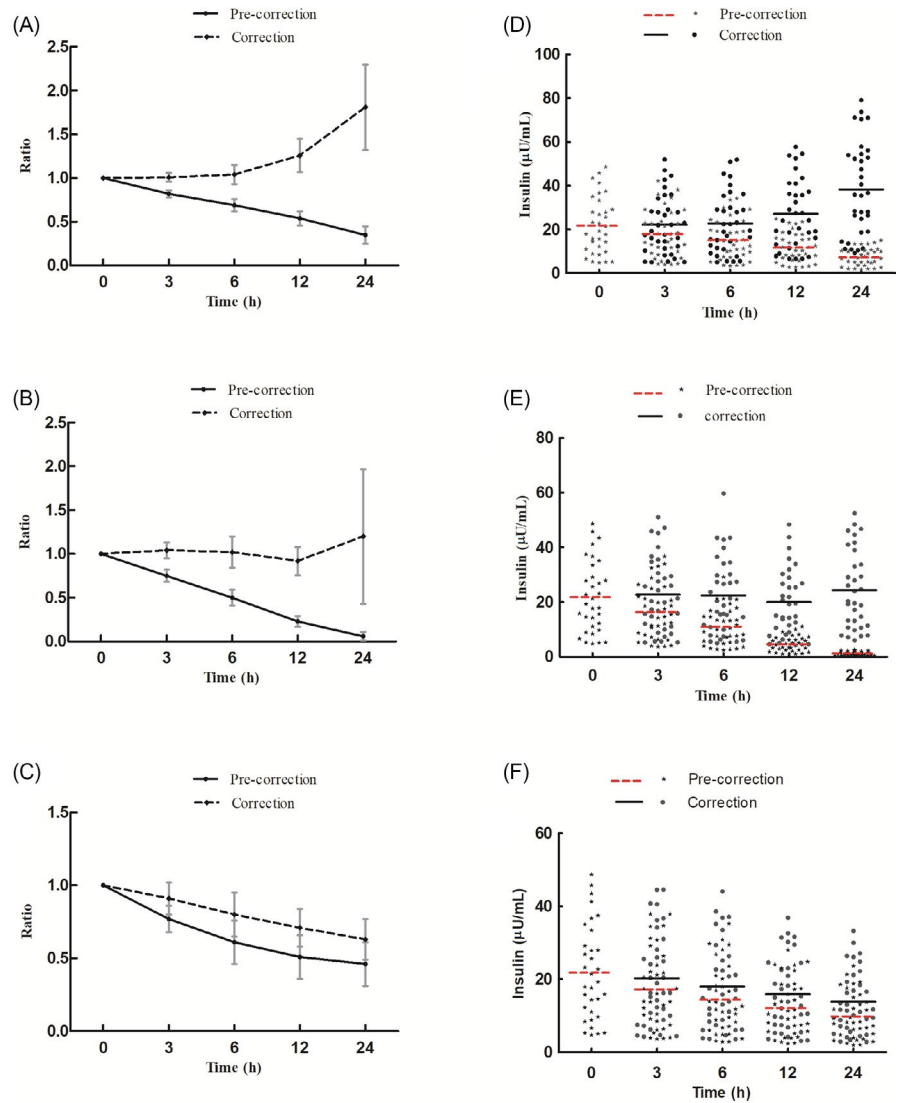
at each time. The results showed that there was no significant difference between the corrected insulin results and the original results at 3, 6 and 12 hours ( $P > .05$ ), but there were significant differences at 24 hours for all of the correction formulas ( $P < .05$ ; Figure 5).

## 4 | DISCUSSION

Insulin is a protein hormone consisting of three peptide chains, A and B. Its molecular weight is 5734 D, and its half-life is approximately 5 minutes. Additionally, due to the existence of insulin-degrading enzymes in the blood, insulin levels decrease rapidly in the blood.<sup>20</sup>The insulin secretion curve is generated according the levels of insulin at 0, 1, 2 and 3 hours. The inaccurate determination of insulin at each point time should affect the distribution of the curve. Although many studies have reported that the stability of insulin is mainly affected by the hemolysis degree, storage time, and temperature of the specimens, these studies have also proposed the best way to preserve the specimen. Due to improvements in blood collection technology, hemolysis of specimens has decreased considerably and has even become rare; however, a protracted time



**FIGURE 5** Comparison of insulin concentrations and the insulin ratios before and after correction at different verified times. A-C were the comparison of insulin ratio before and after correction; D-F were the comparison of insulin concentration before and after correction with the bar represent the mean. A and D, Whole blood samples were stored at 4°C; B and E, whole blood samples were stored at 25°C; C and F, centrifuged samples were stored at 25°C



between collection and analysis and room-temperature transportation of insulin specimens are common in the clinic, which inevitably affect the accuracy of the insulin results and insulin secretion curve. Incorrect insulin results can mislead clinicians regarding the diagnosis of diabetes, such as the classification and staging of the disease; the choice of treatment for diabetes; and the etiological analysis of various other diseases, such as the various forms of mature diabetes, calcified fibroid pancreatitis, and mitochondrial genome defects.<sup>26-29</sup> Therefore, to improve the accuracy of insulin detection and obtain a more realistic insulin value, it is important to establish a correction formula for clinical use.

In this study, the six point times were tested, based mainly on the following three considerations: First, Insulin release test time points. Second, the time between clinical laboratory specimen collection and analysis cannot be more than 8 hours.<sup>30</sup> Third, with the earlier time for analysis and establishing the corrected formulas, and then verification at the other times, the applicability of the calibration formulas could be effectively evaluated, and the cost of the test could be reduced to a certain extent. Before establishing the correction formula, besides the effects of storage conditions, time, and temperature on

insulin concentration, the experiment also considered whether the level of insulin concentration and the concentration of Hb affected the degradation of insulin. According to the statistical analysis of the experimental results (Figures 1 and 2), there was no correlation between them.

Consistent with the time saving advantage of measuring insulin concentrations early, the results of the study showed that the insulin concentration in the whole blood samples declined continuously, decreased more quickly at 25°C than at 4°C, and was significantly decreased in 4 hours (Table 1, Figure 4). This decline may be related to the activity of insulin-degrading enzymes; the higher the temperature is, the stronger the activity of the enzymes will be, and the longer the enzymatic action is, the more obvious decrease of the insulin concentration will be. Centrifuged blood samples, and the changes at 25°C were more obvious than those at 4°C (Figure 4). The plasma samples were separated and stored at 4°C and 25°C, and the insulin concentrations were either not obviously changed or unchanged. The results of this study are consistent with those reporting that insulin levels in whole blood samples decreased significantly after 24 hours and that insulin levels in isolated plasma samples could be

stabilized for 24 hours or several days in some studies.<sup>17-19</sup> However, the conclusion that insulin can be stabilized for 4–5 hours is inconsistent with the data reported in other studies.<sup>21</sup> This inconsistency may be related to anticoagulants or differences in analysis or the shorter design time.

Therefore, according to the experimental results, the insulin concentration is significantly affected by time and temperature, and the degree of the insulin decline is independent of the insulin concentration and Hb concentration. This suggests that the delayed analysis of specimens is most likely to result in patients with normal insulin secretion being diagnosed as having delayed or insufficient insulin secretion and in the disease conditions of diabetic patients being considered more serious. Therefore, the establishment of the correction formula for insulin level has very important clinical significance for clinicians to evaluate the insulin secretion level and to stage the conditions of diabetic patients correctly. The correction formulas for the insulin concentration of whole blood samples at 4°C and 25°C and centrifuged samples at 25°C were established as follows:  $C_{\text{correction}} = C_{\text{determination}} / 0.991e^{-0.069x}$ ,  $C_{\text{correction}} = C_{\text{determination}} / 1.048e^{-0.126x}$ , and  $C_{\text{correction}} = C_{\text{determination}} / [-0.068\ln(x) + 0.9242]$ . Figures 4 and 5 showed that the insulin concentration can basically be restored to the initial concentration after correction. The corrected differences of upon 95% whole blood samples which were stored at 4°C and 25°C could basically reach the acceptable difference of  $\pm 20\%$  within 8 hours; and within 4 hours for centrifuged blood samples stored at 25°C. This is particularly important for the evaluation of clinical applicability of the correction formulas, for the detection of clinical insulin samples is basically completed within 8 hours.

In conclusion, to improve the accuracy of plasma insulin concentration measurements, samples should be sent to the laboratory as soon as possible for testing or separation of plasma for cryopreservation. If the samples cannot be sent to or measured in time, according to the state of the sample, the clinical laboratory can correct the insulin concentration through the corresponding correction formula, and then directly feed it back to the clinic. Therefore, the correction formula can correct the insulin results to reduce the effects of time and temperature on insulin levels and provide more accurate insulin results for clinical diagnosis and treatment.

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**How to cite this article:** Zeng X, Chen Q, Gong G, et al. A simple formula to correct for the effects of storage time and temperature on the insulin concentration. *J Clin Lab Anal*. 2020;34:e23255. <https://doi.org/10.1002/jcla.23255>