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Paper:

Bright, D., Dunseath, G., Peter, R. & Luzio, S. (2017). Stability of proinsulin in whole blood. _Clinical Biochemistry_
http://dx.doi.org/10.1016/j.clinbiochem.2017.10.005

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Accepted Manuscript

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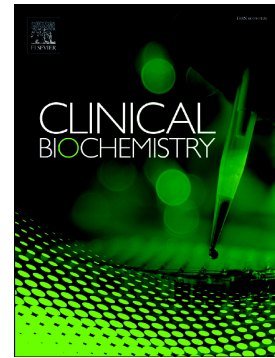
Please cite this article as: Dominic J. Bright, Gareth J. Dunseath, Rajesh Peter, Stephen Luzio , Stability of proinsulin in whole blood. The address for the corresponding author was captured as affiliation for all authors. Please check if appropriate. Clb(2017), doi:10.1016/j.clinbiochem.2017.10.005

Stability of Proinsulin in Whole Blood.

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Abstract

Proinsulin, the precursor for insulin, is secreted in higher concentrations when β-cells are under stress and previous studies have shown that elevated proinsulin could be used as a marker for individuals in a pre-diabetic state. The aim of this study was to assess the stability of proinsulin across a wide concentration range (3-882 and 2-187 pmol/L; total and intact proinsulin respectively) in whole blood to determine whether it could be used in routine clinical care. 51 subjects (26 normal glucose tolerance, 17 impaired glucose tolerance and 8 type 2 diabetes) had blood taken into EDTA tubes at 0, 60 & 120 minutes following a glucose load. The samples were kept at room temperature (~20°C) with aliquots taken, centrifuged and frozen at 0, 24, 48 and 72 hours. Comparison of the combined data (pre and post-glucose load) of baseline with 72 hour as a percentage of baseline gave an average of 123% (95% CI: 119-127) and 107% (95% CI: 105-109) for total and intact proinsulin respectively. A small change in the stability of total proinsulin was observed whilst there was no clinical difference over the 72 hour period for intact proinsulin.

1.

Introduction

Proinsulin is the precursor molecule to insulin, the biologically active agent that decreases blood glucose levels. Hyperproinsulinemia is a very common observation in patients with type 2 diabetes (T2DM) [1] and it has been postulated for over 20 years that an increased ratio of proinsulin to insulin in the blood could be a predictive marker of T2DM [2]. Use of fasting proinsulin concentration as a predictor of glucose intolerance was investigated by Wareham et al [3] who showed an association between elevated fasting proinsulin concentration and transitional progression to diabetes in a 4.5 year longitudinal study. Recently Pfützner et al [4] reported elevated levels of intact proinsulin indicated a progressive β-cell dysfunction and could be predictive of T2DM progression, these findings add to previous work by this author [5-7]. Other previous studies have confirmed this association along with progression of cardiovascular disease [8]. Measurement of proinsulin for early detection of beta-cell dysfunction, before deterioration of glycaemic control could allow introduction of lifestyle changes and/or pharmaceutical intervention at an early stage.

Proinsulin may potentially offer advantages over the measurement of insulin. Proinsulin is not as affected as insulin by hepatic metabolism, and furthermore proinsulin assays are now far more specific with little or no cross-reactivity with endogenous or exogenous insulin. As a consequence proinsulin concentrations may have the potential to be used to identify impaired beta cell function prior to the onset of glucose intolerance. In order to use proinsulin measurement in routine clinical practice, the stability of proinsulin needs to be established. Recent work has established the stability of proinsulin stability over a 24 hour period [9] however, the in this study the aim was to investigate the stability of both total and intact proinsulin in whole blood over a period of 3 days when stored at ambient temperature.

2.

Materials and Methods

2.1.

Subjects

Subjects were identified from a volunteer database or by using HbA1c values from a recent primary care visit. A total of 51 subjects were recruited across the glycaemic range. The study was approved by Wales Research Ethics Committee 6 and was sponsored by Abertawe Bro Morgannwg University Health Board. Subjects were grouped as normal glucose tolerant (NGT, n=26 (11 male)), impaired glucose tolerant (IGT, n=17 (10 male)) or type 2 diabetes mellitus (T2DM, N=8 (7 male)) according to a 75g oral glucose tolerance test (OGTT) performed during the study. Mean (±SD) HbA1c in the 3 groups was 37 (±3.76), 42 (±2.76) and 45 (±3.12) mmol/mol; NGT, IGT and T2DM respectively.

2.2.

Materials

Total proinsulin was measured using a total proinsulin ELISA kit (IV2-003, Invitron Ltd, Monmouth, UK). The cross-reactivities in this assay were: intact proinsulin 100%; insulin 2.2%; C-peptide 0%; 32-33 split proinsulin 97%; des 31-32 proinsulin 100%. Intra-assay CVs at 3.0, 51, and 257 pmol/L were 9.9%, 6.3% and 2.4%, respectively. Intact proinsulin was measured using an intact proinsulin luminescence assay kit (IV2-002, Invitron Ltd, Monmouth, UK), which has stated cross-reactivity of: intact proinsulin 100%; insulin 0%; C-peptide 0%, 32-33 split proinsulin 5.6%; des 31-32 proinsulin 1.4%. Intra-assay CVs at 3.5 and 54 pmol/L were 4.9% and 5.3%, respectively. Both proinsulin assays were calibrated against the WHO 1st International Standard for proinsulin (IRP 84/611). Results were processed on MikroWin curve fitting software (Mikrotek Laborsysteme GmbH, Germany). Glucose was determined using a glucose oxidase method (YSI 2300, Yellow Springs Instruments, UK).

2.3.

Methods

Following an overnight fast, all subjects underwent a 75g OGTT. 4ml of blood was drawn from all subjects into K2EDTA blood tubes (Becton Dickinson, Oxford, UK) at 0, 60 and 120 minutes during an OGTT. Samples were transported immediately to the lab as whole blood and the samples inverted and mixed thoroughly before a 1ml aliquot was taken from each. Within 5 minutes of sample collection, the aliquot was centrifuged at 4000g for 5 minutes and the plasma separated and frozen at -20°C immediately. The remaining blood sample was left at ambient temperature (approximately 20°C) until further 1ml aliquots were taken after thorough mixing at 24, 48 and 72 hours and plasma separated using the same procedure. Each aliquot was stored at -20°C until analysis was performed on all time points in a single assay. Results are displayed as a percentage of the baseline value, as scatterplots and as a Bland-Altman plot of the measured concentration at 72 hour minus baseline concentration. Paired t-tests were performed to measure for differences between baseline and 72 hour values.

3.

Results

A range of total and intact proinsulin concentrations were achieved by taking both fasting and post-glucose load samples (total proinsulin range 3-882 pmol/L; intact proinsulin range 2-187 pmol/L).

3.1.

Stability of total proinsulin

Stability of total proinsulin in whole blood over 72 hours is shown in Figure 1. There was a small increase in total proinsulin over the 72 hour period (mean concentration difference of 20.3pmol/L) (figure 1A). The mean percentage value over the 72 hour time period versus baseline was 126.7% (95% CI: 118.7-134.5) for fasting samples, 123.5% (95% CI: 115.9-

Combined time point data (0, 60 & 120 minutes) of baseline versus 72 hour values showed R^2= 0.980 & y = 1.0447 + 13.703, p <0.001 (figure 1B). Mean bias at 72 hours was 18.8% (figure 1C).
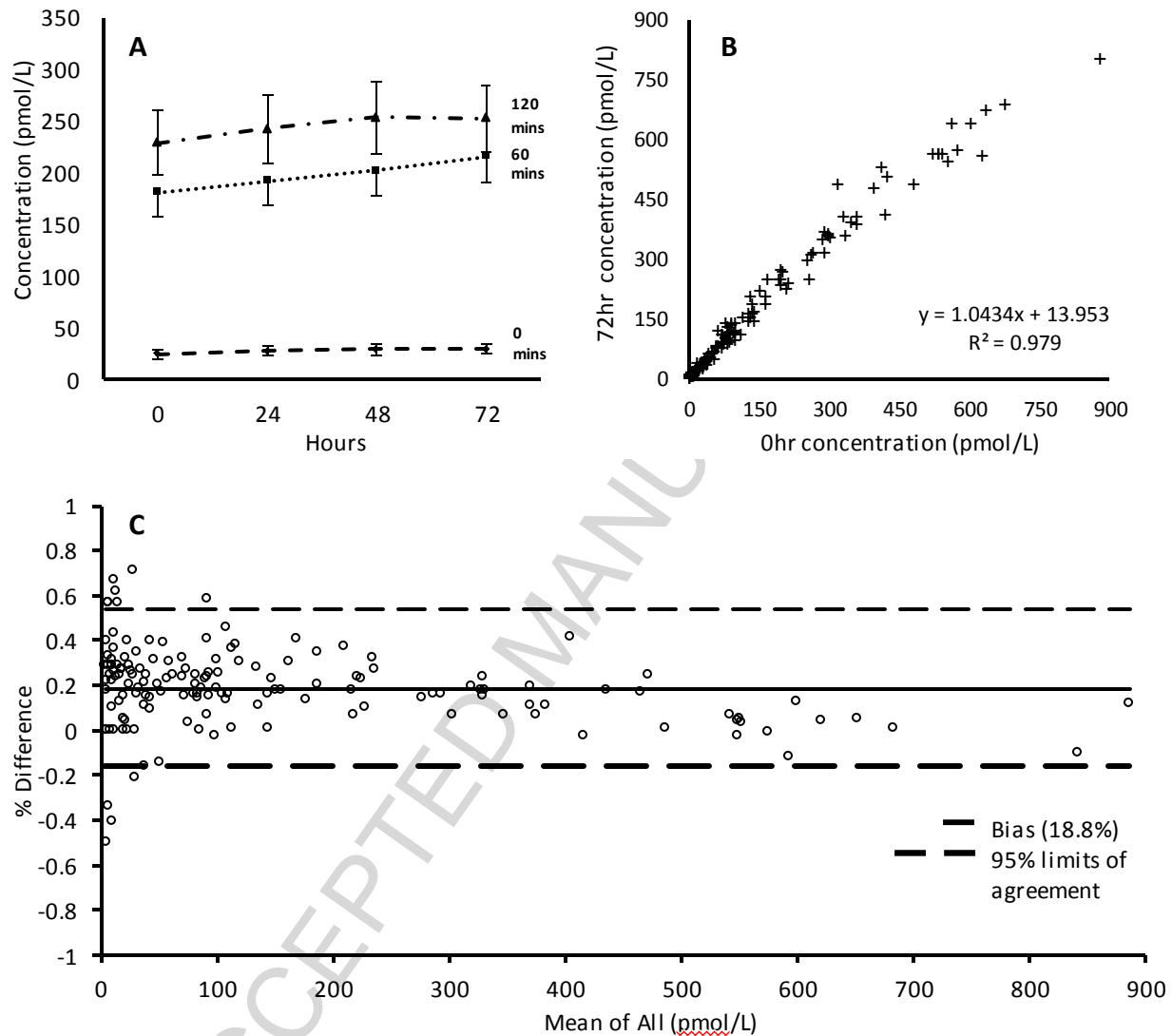
Fig. 1. Stability of total proinsulin over 72 hours in whole blood EDTA at room temperature. Figure A: Mean±SEM total proinsulin concentrations at 0, 60 & 120 minutes over the 72 hour period. Figure B: Day 0 (combined 0, 60 & 120 minutes) total proinsulin concentration versus 72 hour concentrations with y intercept and R^2 value. Figure C: Bland-Altman plot of the mean % concentration difference (72 hour minus baseline)

3.2.

Stability of intact proinsulin

Stability data for intact proinsulin in whole blood over 72 hours is displayed in Figure 2. There was negligible degradation of intact proinsulin over 72 hours compared to baseline (0 hour) concentrations (mean concentration difference of 1.6pmol/L) (figure 2A). Mean percentage values over the 72 hour time period versus baseline of 106.4 (95% CI: 101.9-111.0) for fasting samples, 103% (95% CI: 109.0-113.0) for 60 minute and 102% (95% CI: 106.4-109.4) for 120 minute samples. Combined time point data (0, 60 & 120 minutes) of baseline versus 72 hour values, showed R^2= 0.982 & y = 1.036x + 0.5101, p <0.001 (Figure 2B). Mean bias at 72 hours was 6.2% (Figure 2C).
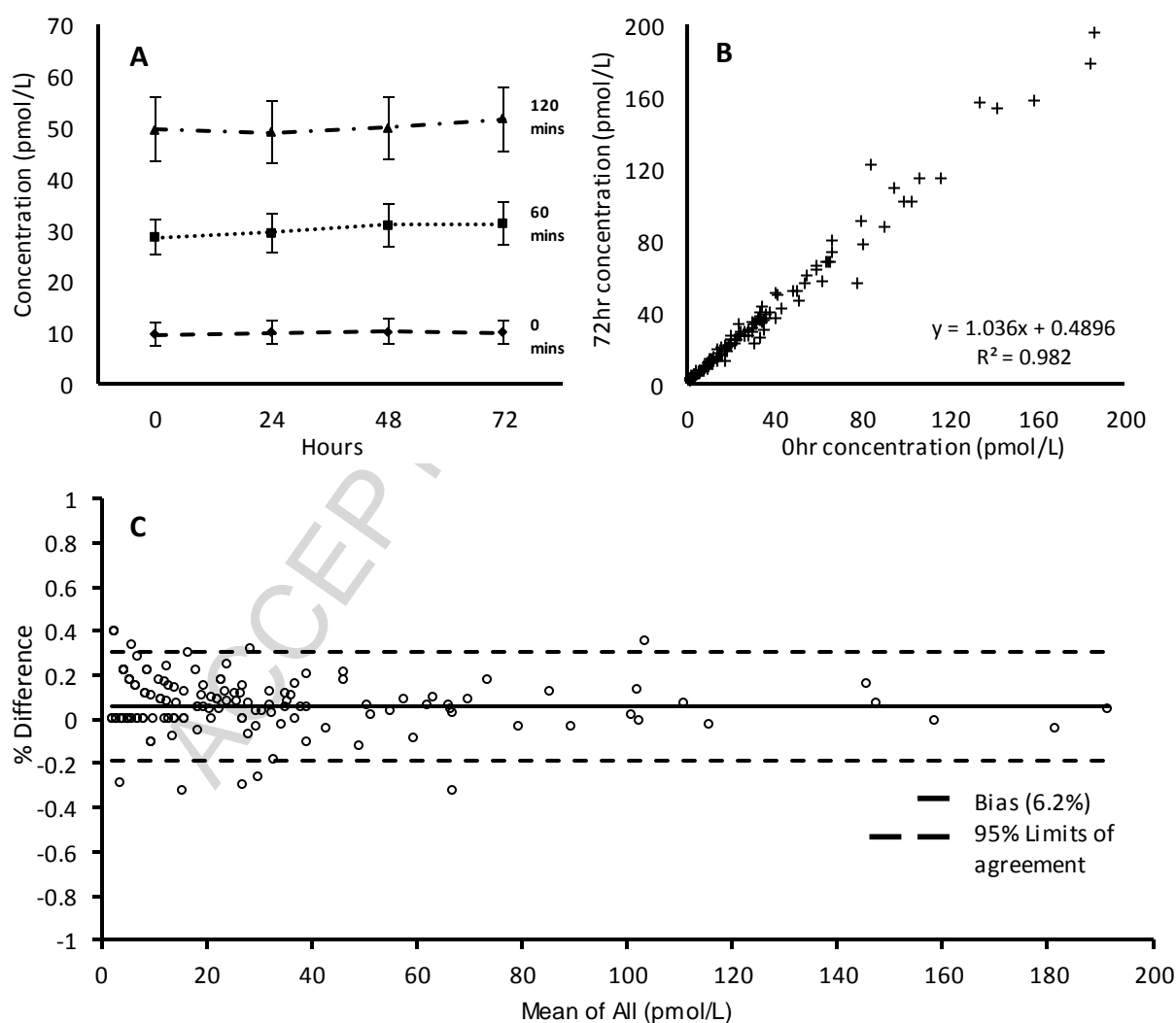

Fig. 2. Stability of intact proinsulin over 72 hours in whole blood EDTA at room temperature. Figure A: Mean ±SEM intact proinsulin concentrations at 0, 60 & 120 minutes over the 72 hour period. Figure B: baseline

(combined 0, 60 & 120 minutes) intact proinsulin concentration versus 72 hour concentrations with y intercept and R^2 value. Figure C: Bland-Altman plot of the mean % concentration difference (72 hour minus baseline)

4.

Discussion

Elevated circulating proinsulin levels can result from a stressed pancreas releasing insulin precursors, in an attempt to counteract insulin resistance and increased blood glucose concentrations. Measurement of proinsulin may therefore be of use as a measure of β-cell dysfunction for screening individuals who, without intervention, could progress to glucose intolerance. However, for this to be possible particularly within a primary care setting, proinsulin would need to be sufficiently stable in whole blood to account for the transfer time to the local laboratory. Our study was designed to mimic the transportation setting that such samples would be exposed to; alongside this, the study also followed the recommended validation and stability protocols of blood collection tubes [10, 11]. A recent study found no degradation over a 24 hour period, however, transportation time in routine use may exceed this [9]. We observed stability based on the 72 hour values as a percentage of baseline concentration for total proinsulin of 123% (95% CI 119-127%) and 107% (95% CI 105-109%) for intact proinsulin. This related to a small increase in a mean difference of 20.3pmol/L for total proinsulin and a negligible change of 1.6pmol/L for intact proinsulin which would not be considered clinically significant. The larger change in total proinsulin over 72 hours was not due to assay variation, but may be explained by changes in proinsulin split products after several days of standing, which have different cross-reactivities in the total proinsulin assay. Variations in cross-reactivities for the different split products in alternative total proinsulin assays should therefore be a consideration.

5.

Conclusion

In conclusion proinsulin, especially intact proinsulin, is very stable in whole blood. When collected into EDTA tubes and left unseparated at ambient room temperature for at least 72 hours, there was little degradation; this enables transport and handling from a primary care setting to a laboratory testing facility, enabling its potential use as a biomarker for diagnosis of glucose intolerance. Due to showing better agreement with baseline levels after 72 hours, intact proinsulin may be the more appropriate choice.

6. References

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Highlight

- Proinsulin could offer early indication of β cell dysfunction
- This study aimed to establish the stability of proinsulin in whole blood
- Intact proinsulin is stable over a 72 hour period at room temperature

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