# Heart rate variability as a potential non-invasive marker of blood glucose level

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**Abstract**
Currently, monitoring of blood glucose level (BGL) is constrained by the invasive nature of BGL measures. We investigated heart rate variability (HRV) parameters as potential non-invasive markers of BGL. Healthy volunteers (n = 25; aged 27 ± 9 years) uninhibited by regular medications or chronic illness were recruited for this study. BGL and HRV were assessed during fasting (9:00am), postprandial (12:00pm), and postabsorptive (3:00pm) periods using self-monitoring of blood glucose techniques and ten-minute electrocardiogram, respectively. Frequency-domain HRV measures, which estimate contributions of sympathetic and parasympathetic systems to autonomic modulation of the heart, were correlated against BGL data with the following significant ($p<0.05$) findings. The change in BGL from fasting to postprandial levels was negatively correlated with fasting low frequency (LF) power and total power (TP). Postprandial BGL was negatively associated with fasting LF and TP, as well as with postprandial LF, high frequency (HF), and TP. The change in BGL from postprandial to postabsorptive levels was positively correlated with fasting LF power, as well as with postprandial LF, HF, and TP. Frequency-domain HRV parameters may be useful in predicting the magnitude and direction of acute fluctuations in BGL, and further research could develop them as non-invasive markers of BGL.

**Keywords**: Blood Glucose Self-Monitoring, Diabetes, Heart Rate Variability

## 1 Introduction

Diabetes is considered a leading epidemic of the 21st century, affecting an estimated 451 million adults worldwide (1). It is a group of metabolic disorders characterised by chronically-high blood glucose level (BGL), a condition which is also known as hyperglycaemia (2). Although it is well-known that indulging in high-sugar, high-fat diets and sedentary lifestyles is associated with diabetes and other metabolic disorders, adherence to these 'Westernised' lifestyle behaviours remains high (3, 4). Complications such as renal failure, blindness, and neuropathy can arise from uncontrolled hyperglycaemia (5) as certain cells in the renal nephrons, eyes, and neurons of the autonomic nervous system (ANS) are susceptible to hyperglycaemic damage (6). Consequently, the progression of diabetes is associated with a decline in autonomic modulation of the heart and a corresponding increase in the risk for cardiovascular disease (7-9). The literature agrees that the most effective means of reducing complications of diabetes is stringent control of BGL (10, 11).

Problematically, an estimated 45.8% of all people living with diabetes worldwide are undiagnosed or unaware of their condition, with higher prevalence in developing countries (12). Additionally, glycaemic control is not optimal even in populations of those who are aware of their condition; in one large-scale study, roughly half of a diabetes population did not maintain BGL at the recommended healthy level (13). Limitations such as the invasive nature of all commercially-successful measures of blood glucose may explain these alarming statistics (14). Invasive procedures, such as drawing a sample of blood using a lancet device and glucometer, are commonly used for their accuracy (15, 16). However, the associated pain presents a psychological barrier to self-monitoring of blood glucose (17). A non-invasive marker of BGL may improve patient compliance with self-monitoring of blood glucose and facilitate glycaemic control in diabetes (18, 19).

Heart rate variability (HRV) parameters reflect ANS output and can be recorded using a non-invasive electrocardiogram (ECG) (20). In a healthy human, the ANS modulates control over the heart via two counterregulatory branches – the sympathetic and parasympathetic systems – though this control is reduced in diabetes (21-24). HRV is one of the most commonly used measures of ANS activity (25), and is studied widely as a marker for sudden cardiac death after myocardial infarction and as an assessment of diabetic neuropathy (26-28). HRV can be extrapolated from short-term

ECG recordings using advanced computational methods. Spectral techniques are used to determine the total power of HRV, and this in turn can be divided into different parameters based on predefined frequency bands, such as low frequency and high frequency. These two parameters reflect the contributions of sympathetic and parasympathetic systems to autonomic modulation of the heart.

HRV measures are already appropriate for use in clinical settings as prognostic tools for cardiac autonomic neuropathy in diabetes (29). HRV may have further application in the evaluation of glycaemia as ANS regulation governs the cephalic phase of digestion, which primes the body for the inevitable rise in blood glucose after a meal. The long-term effects of abnormally-high BGL on HRV are well established in the literature, but the short-term relationship between BGL and HRV measures has only been investigated by a few cross-sectional studies (30-36). No study to date has successfully implemented autonomic markers alone as predictors of BGL.

Previous research conducted by our research unit (33) has identified several key correlations between HRV parameters and BGL measured concurrently in a mixed sample of type 1 and type 2 diabetes, but found no correlations of significance within a healthy control group. This may be because healthy individuals experience only little glycaemic change over short-term periods. As such, the present study continues this research on healthy participants by assessing BGL and HRV across a longer time period and whilst participants were in different metabolic states, including fasting (at 9:00am), postprandial (12:00pm), and postabsorptive (3:00pm) periods. It is hypothesised that the ingestion of a meal will be marked by a significant rise in BGL across the sample, and that BGL will be correlated with measures of ANS activity. In this exploratory, proof-of-concept study, we highlight the potential of HRV as a non-invasive marker of BGL.

## 2 Experimental

### *2.1 Subjects*

A total of 25 healthy individuals were recruited from the local Sydney metropolitan area for this pilot study. Participants were excluded from the study if they suffered from a chronic health condition, were on regular medication, smoked > 10 cigarettes per day, consumed > 10 standard drinks per day, or were pregnant. To comply with the ethics protocol, participants were also screened for high blood pressure and were removed

from the study if systolic blood pressure exceeded 160 mmHg or if diastolic blood pressure exceeded 100 mmHg. All participants were required to sign a consent form, as well as abstain from food, drink (except water), nicotine, and alcohol for eight hours prior to study commencement (37, 38). The protocol was conducted in a controlled laboratory setting and was approved by the University of Technology Sydney Human Research Ethics Committee (HREC: 2014000110).

*2.2 Study procedure*

HRV and BGL were each assessed at fasting (9:00am), postprandial (12:00pm), and postabsorptive (3:00pm) periods using 10-minute ECG recordings and blood glucose 'fingerpicks', respectively. The timing of these assessments was based loosely on previous literature (39, 40). The fasting assessment provided baseline measurements as participants were required to undergo at least 8 hours of caloric restriction prior to study commencement. The postprandial or postbreakfast assessment was recorded at 12:00pm when glucose levels were expected to peak. Participants were encouraged to prepare and bring their own breakfast or to at least eat a meal they would consider 'regular' (41). Glucose peaks observed after a normal, daily meal are similar to glucose peaks after a two-hour glucose tolerance test (42). However, differences in meal composition between individuals can confound postprandial glucose profiles (43, 44) and, as such, participants were required to report all food and drink consumed during the study so that kilojoule intake could be used as a covariate in the correlation analysis. Finally, the assessment at 3:00pm recorded BGL when participants were in a postabsorptive state, when glucose levels were returning to baseline.

At each of the three assessments, BGL was assessed using an ACCU-CHEK® Performa II glucometer (43) (Roche Diagnostics GmbH, Mannheim, Germany) with a finger prick test applied to the ring finger on the dominant hand (45). Blood pressure (BP) was measured pre-study and post-study using an OMRON HEM-7000 automated BP monitor (OMRON, Kyoto, Japan) after each participant had been allowed to rest in a seated position for five minutes (37). Due to the dynamic nature of BP, the mean of the three BP recordings was used to provide better accuracy, with a two-minute rest interval between each recording (37, 46). HRV was recorded for 10 minutes using a three-lead FlexComp Infiniti (SA7550) encoder (Thought Technology Ltd., Montreal, Canada) at a sampling rate of 2048 Hz, using the Einthoven electrode placement (47).

Of the various short-term measures of HRV, 10-minute ECG recordings have excellent reproducibility and provide an accurate representation of ANS activity (48).

Frequency-domain HRV measures are a set of highly-reputable variables which are favoured in certain studies as they reflect specific types of autonomic modulation (32, 33). These HRV measures were extrapolated from each of the ECG recordings using Kubios HRV Premium, a program which was used to automatically detect R-waves, which represent each contraction or main electrical depolarisation of the heart (49), as well as remove artefacts (50). A time-series graph was produced for each ECG trace by plotting the R-R intervals, which denote the distance in milliseconds between each R-wave. The time-series graph was converted to a power frequency spectrogram using Welch's method, which is a periodogram based method to solve the discrete Fourier transform (38, 49, 51). Within the spectrogram, three predefined frequency bands were identified: low frequency (LF) power (0.04-0.15 Hz), high frequency (HF) power (0.15-0.4 Hz), and total power (TP), which was represented by the entire spectrogram (0.00-0.4 Hz) (51, 52). LF power and HF power estimate the contributions of sympathetic and parasympathetic activity to autonomic modulation of the heart. LF power reflects sympathetic activity, the branch of the ANS dedicated to the fight or flight response; HF power reflects parasympathetic activity, the opposing branch associated with the rest and digest phase; and TP reflects overall ANS activity (52, 53). LF, HF, and TP parameters were all natural logarithm transformed as their distribution was revealed to be highly skewed, though this is typical of HRV studies (37, 54, 55).

*2.3 Statistical analysis*

A paired-sample t-test (two-tailed) was used to determine whether BGL or HRV parameters changed significantly in the sample across each of the three assessments. A Partial Pearson's correlation identified associations between HRV parameters and BGL, adjusting for kilojoule intake as a covariate. As no kilojoule information was available for the fasting assessment, Pearson's correlation was used instead. Significance level was set at $p<0.05$. The HRV parameters used in the analysis included LF power, HF power, and TP. The BGL variables were defined as fasting (BGL1), postprandial (BGL2), postabsorptive (BGL3), the change from BGL1 to BGL2 ($\Delta$BGL 1-2), and the change from BGL2 to BGL3 ($\Delta$BGL 2-3). Finally, multiple regression analysis was used in cases where three or more HRV measures were

found to be significantly correlated with BGL to determine the strongest individual predictor of BGL.

## 3 Results

Data was collected from 25 participants at three different time points across the day, though ECG data from two participants was excluded from the statistical analysis due to noisy ECG data. The mean age of the sample was 27 ± 9 years, with a sex breakdown of ~48% males, and the mean body mass index was 24 ± 3 kg/m$^2$. The results of the t-tests are presented in Table 1. BGL increased significantly from Test 1 (9:00am) to Test 2 (12:00pm) ($p<0.01$) after all participants consumed a regular meal.

**Table 1.** Changes in mean HRV parameters and BGL between fasting, postprandial, and postabsorptive assessments

| | | | | p | | |
|---|---|---|---|---|---|---|
| **Variable** | **Test 1 (9:00am)** | **Test 2 (12:00pm)** | **Test 3 (3:00pm)** | **Test 1 vs 2** | **Test 2 vs 3** | **Test 1 vs 3** |
| **BGL (mmol/L)** | 4.9 ± 0.4 | 5.5 ± 0.7 | 5.3 ± 0.9 | <0.01* | 0.46 | 0.06 |
| **LF (ms$^2$)** | 7.0 ± 0.9 | 6.9 ± 1.1 | 7.0 ± 0.8 | 0.26 | 0.69 | 0.67 |
| **HF (ms$^2$)** | 5.9 ± 1.3 | 5.8 ± 1.2 | 5.9 ± 1.0 | 0.62 | 0.64 | 0.89 |
| **TP (ms$^2$)** | 7.5 ± 1.0 | 7.3 ± 1.0 | 7.4 ± 0.7 | 0.16 | 0.59 | 0.45 |

**Key**: * = Statistically significant ($p<0.05$); BGL = Blood glucose level; HF = High frequency; LF = Low frequency; mmol/L = Millimoles per liter; ms$^2$ = Millisecond squared; TP = Total power. Data presented as mean ± standard deviation. LF, HF, and TP values are presented as the natural logarithm.

### *3.1. Correlation analysis*

The Partial Pearson's correlation was used to determine associations between HRV parameters and BGL variables, in which kilojoule intake was applied as a covariate (Table 2). The mean kiljoule intake following the fasting assessment was 3072 kJ ± 1517. Several correlations were observed between postabsorptive HRV parameters and fasting BGL, however these findings were omitted from Table 2 as they were not relevant to the aims of the present study, which is interested in the capacity for HRV to predict future or concurrent glucose levels or changes.

Consequently, we observed no significant correlations between any HRV parameter and fasting BGL, and no correlations between any HRV parameter and postabsorptive BGL. There were, however, multiple significant correlations between the various HRV parameters and postprandial BGLs, as well as the change in BGL from fasting to postprandial and from postprandial to postabsorptive. Postprandial BGL was found to be negatively associated with fasting LF power ($r = -0.62$, $p<0.01$) and fasting TP ($r = -0.57$, $p<0.01$), as well as postprandial LF power ($r = -0.60$, $p<0.01$), postprandial HF power ($r = -0.49$, $p=0.02$), and postprandial TP ($r = -0.56$, $p=0.01$).The change in BGL from fasting to postprandial was negatively correlated with fasting LF power ($r = -0.52$, $p=0.02$) and fasting TP ($r = -0.50$, $p=0.02$). Finally, the change in BGL from postprandial to postabsorptive was positively correlated with fasting LF power ($r = 0.46$, $p=0.04$), postprandial LF power ($r = 0.51$, $p=0.02$), postprandial HF power ($r = 0.45$, $p=0.04$), and postprandial TP ($r = 0.51$, $p=0.02$).

**Table 2.** Associations between heart rate variability parameters and blood glucose levels

| Parameter | | BGL1 (9:00am) | ∆ BGL 1-2 | BGL2 (12:00pm) | ∆ BGL 2-3 | BGL3 (3:00pm) |
|---|---|---|---|---|---|---|
| Test 1 (9:00am) | LF | | | | | |
| | r | -0.23 | -0.52 | -0.62 | 0.46 | 0.09 |
| | p | 0.31 | 0.02* | <0.01* | 0.04* | 0.69 |
| | HF | | | | | |
| | r | -0.08 | -0.41 | -0.43 | 0.24 | -0.03 |
| | p | 0.74 | 0.07 | 0.05 | 0.29 | 0.90 |
| | TP | | | | | |
| | r | -0.18 | -0.50 | -0.57 | 0.42 | 0.07 |
| | p | 0.44 | 0.02* | <0.01* | 0.06 | 0.75 |
| Test 2 (12:00pm) | LF | | | | | |
| | r | - | - | -0.60 | 0.51 | 0.17 |
| | p | - | - | <0.01* | 0.02* | 0.47 |
| | HF | | | | | |
| | r | - | - | -0.49 | 0.45 | 0.18 |
| | p | - | - | 0.02* | 0.04* | 0.42 |
| | TP | | | | | |
| | r | - | - | -0.56 | 0.51 | 0.19 |
| | p | - | - | 0.01* | 0.02* | 0.40 |
| Test 3 (3:00pm) | LF | | | | | |
| | r | - | - | - | - | 0.29 |
| | p | - | - | - | - | 0.20 |
| | HF | | | | | |
| | r | - | - | - | - | -0.05 |
| | p | - | - | - | - | 0.82 |
| | TP | | | | | |
| | r | - | - | - | - | 0.24 |
| | p | - | - | - | - | 0.29 |

**Key**: * = Statistically significant ($p<0.05$). BGL = Blood glucose level; HF = High frequency; LF = Low frequency; TP = Total power; Δ = Change in. Retroactive findings were not presented in this table as this was a prospective analysis. The intention was to determine whether HRV parameters were related to concurrent or future BGL or BGL changes. Thus, it is not relevant to this study to observe correlations between HRV parameters and BGL that were recorded in earlier time points.

As there were multiple significant correlations between postprandial BGL (BGL2) and HRV variables, a multiple regression analysis was performed to determine which HRV parameter was the strongest predictor of BGL (Table 3). The regression retained all five of the originally entered variables (fasting LF power, fasting TP, postprandial LF power, postprandial HF power, postprandial TP), and had an overall significance of $p<0.019$ ($p<0.075$). Together, these five variables explained 52% of the variance in postprandial BGL ($F = 3.708$; $DF = 5, 17$; $p<0.019$; $R = 0.722$; $R^2 = 0.522$; $AR^2 = 0.381$). Furthermore, the retained variables did not present as independently

significant predictors, although fasting LF power approached statistical significance (p=0.084).

Similarly, multiple HRV variables were significantly correlated to the change in BGL between postprandial and postabsorptive (Δ BGL 2-3); and, as such, a multiple regression analysis was performed to determine which HRV parameter was the strongest predictor (Table 4). The regression retained all four of the originally entered variables (fasting LF power, postprandial LF power, postprandial HF power, postprandial TP), but was found to be non-significant overall.

**Table 3.** Regression analysis for postprandial glucose level (BGL2), and the significantly correlated HRV parameters

| **Regression summary for dependant variable: BGL2**<br>$R = 0.722$; $R^2 = 0.522$; $AR^2 = 0.381$; $F(5,17) = 3.708$<br>$p<0.019$, SE of Estimate = 0.517 | | | | | |
|---|---|---|---|---|---|
| **Variable** | **β** | **B** | **SE of B** | **t** | **p** |
| Intercept | | 6.717 | 1.580 | 4.254 | 0.001 |
| Fasting LF | -1.023 | -0.719 | 0.392 | -1.834 | 0.084 |
| Fasting TP | 0.715 | 0.493 | 0.310 | 1.594 | 0.129 |
| Postprandial LF | -1.234 | -0.768 | 0.875 | -0.878 | 0.392 |
| Postprandial HF | -1.089 | -0.608 | 0.365 | -1.705 | 0.106 |
| Postprandial TP | 1.804 | 1.214 | 1.159 | 1.048 | 0.309 |

**Key**: BGL = Blood glucose level; HF = High frequency; LF = Low frequency; SE = Standard Error; TP = Total power

**Table 4.** Regression analysis for change in BGL from postprandial to postabsorptive (Δ BGL 2-3), and significantly correlated HRV parameters

| **Regression summary for dependant variable: Δ BGL 2-3**<br>$R = 0.442$; $R^2 = 0.195$; $AR^2 = 0.017$; $F(4,18) = 1.093$<br>$p=0.390$, SE of Estimate = 1.114 | | | | | |
|---|---|---|---|---|---|
| **Variable** | **β** | **B** | **SE of B** | **t** | **p** |
| Intercept | | -2.335 | 3.395 | -0.688 | 0.500 |
| Fasting LF | 0.106 | 0.127 | 0.488 | 0.261 | 0.797 |
| Postprandial LF | 1.003 | 1.066 | 1.765 | 0.604 | 0.553 |
| Postprandial HF | 0.391 | 0.373 | 0.766 | 0.487 | 0.632 |
| Postprandial TP | -0.979 | -1.125 | 2.412 | -0.466 | 0.646 |

**Key**: BGL = Blood glucose level; HF= High frequency; LF = Low frequency; SE = Standard Error; TP = Total power; Δ = Change in

## 4 Discussion

Our findings that HRV measures are inversely related to BGL are congruent with the literature (30, 35). Many studies in this area base this on pooled data from both healthy participants and participants with diabetes and impaired fasting glucose. Our study is one of few that have identified significant correlations in healthy participants alone and is the first to determine that HRV measures obtained during fasting and postprandial periods are associated with healthy ranges of BGL. In general, the literature has focused on applying HRV measures as the gold standard for assessing cardiac autonomic neuropathy in diabetes (56, 57), and so our findings may be considered novel. It is well-known that duration of diabetes is a strong predictor for autonomic neuropathy (58). Long-term hyperglycaemic damage in diabetes targets neurons of the vagus nerve and reduces autonomic tone (59), and this mechanism contributes to the consensus that higher BGL is associated with lower HRV measures. It is interesting to note, however, that HRV measures are inversely related to BGL even in the absence of this mechanism, or the absence of complications of diabetes. Our findings concur with the few studies that have focused on healthy people in that higher levels of blood glucose are related to lower levels of autonomic modulation of the heart, even in groups with normal glycaemia and normal autonomic function (35, 60).

The values for LF power, HF power, and TP observed in this study were similar to those reported by other studies on healthy subjects (32-34), and all glucose levels were verified to be in the healthy range. The difference between fasting and postprandial values of LF power are natural and correlate with changes in blood glucose. Correlation analysis between HRV measures and BGL recorded at three different periods revealed multiple relationships of significance, particularly in regard to postprandial BGL. Of the six HRV measures that were correlated against postprandial BGL, five were statistically significant with moderate effect sizes, and the sixth (fasting HF power) was approaching significance ($p<0.1$). These findings indicate a promising relationship between autonomic activity and fluctuations in BGL, but do not infer a causal relationship. Instead, we conclude that HRV measures, which explained 52% of the variance in postprandial BGL (Table 3), may be useful in predicting the magnitude and direction of changes in BGL. This is also reinforced by our novel findings that HRV measures were correlated with the change in BGL from fasting to postprandial, and from postprandial to postabsorptive periods.

The literature proposes that a key link between autonomic activity and BGL fluctuations is the involvement of the ANS in the cephalic, or preabsorptive, phase of digestion. Research has shown that activation of the ANS during both the preabsorptive and absorptive phases of digestion is important in determining postprandial insulin activity (61, 62), which is a determinant of postprandial BGL. As such, changes in HRV, a measure of autonomic activity, precede the release of insulin (62). The cephalic phase is important in preparing the gastrointestinal tract for a meal, such as increasing gastric secretion, and involves parasympathetic input to the stomach via the vagus nerve before food even arrives in the stomach (61). This anticipatory response is required because food digested in the gastrointestinal tract is absorbed into the blood quickly, and there is a rapid demand for insulin to move glucose molecules into the cells of the body to maintain blood glucose homeostasis. Thus, to prevent glucose levels from accumulating rapidly in the blood, the ANS coordinates a release of insulin in anticipation of a meal, proportional to the quantity of ingested carbohydrate (63). This may explain why changes in HRV precede changes in BGL. This was evident in the present study as HRV measures were associated with postprandial BGL as well as the change in BGL before and after a meal.

### *4.1 Postprandial state*

Although the literature agrees that HRV measures are inversely related to BGL, there are discrepancies concerning the association between specific HRV measures and BGL during certain periods. There are few studies which have investigated HRV measures in both fasting and postprandial states (31, 36), and even fewer that have assessed these in healthy individuals (33). Klimontov and colleagues determined that when BGL increases within the healthy range, both LF power and HF power are reduced in the postprandial state compared to fasting (31). They concluded that daytime LF power and HF power are inversely related to glucose concentration, though, in a similar study, Weissman and colleagues found that HF power, but not LF power, is lower in postprandial periods compared to fasting periods and that HF power is inversely related to BGL (36). Both studies were conducted on pregnant women between 24-28 weeks gestation, so discrepancies between their findings and the findings of the present study may be due to the number of confounders present during pregnancy. For one, an increase in insulin resistance normally occurs in pregnancy

(64), so it may not be relevant to compare their findings with a cohort of healthy people. Furthermore, our findings that postprandial BGL are inversely related to fasting LF power and TP, as well as postprandial LF power, HF power, and TP conflict with the previous study conducted by Rothberg and colleagues which identified no significant correlations between HRV measures and BGL in healthy participants (33). This may be because the previous study design did not incorporate kilojoule intake as a covariate in the statistical analysis and did not standardize the food content that participants consumed. We do agree with the previous study in that HRV measures are not significantly related to fasting BGL. However, in a similar study on 42 healthy participants, Lutfi and Elhakeem identified that fasting BGL is positively correlated with HF power ($r = 0.33$, $p=0.03$) (32). Inconsistencies in the findings of these studies may be due to the small effect size between fasting BGL and HRV measures.

### *4.2 Fasting state*

Studies with larger sample sizes have consistently identified that fasting BGL is inversely correlated with both LF power and HF power in individuals with normal autonomic function (30, 34, 35). The findings from these studies were similar when they pooled data from their sample groups of different glycaemic statuses, including normal, impaired fasting glucose, and diabetes. A study of 2441 participants from a range of normal and abnormal glycaemic statuses found that fasting BGL was inversely correlated with LF power ($r = -0.27$, $p<0.001$) and HF power ($r = -0.27$, $p<0.001$)(30). The findings from the Framingham Heart Study were congruent with this as fasting BGL was inversely correlated with LF power ($r = -0.26$, $p<0.0001$) and HF power ($r = -0.21$, $p<0.0001$) in a sample of 1919 participants (34). The effect sizes between HRV measures and fasting BGL reported by these studies are similar, as well as small. Small effect sizes are more difficult to correctly detect with a small sample size such as the present study. The reason for these smaller effect sizes may be because levels of fasting blood glucose exist in a narrower range compared to postprandial glucose profiles, which demonstrate greater variability. This is supported by several studies in which fasting BGL had a smaller standard deviation compared to postprandial BGL (33, 40), and is also supported by this study (Table 1). It is possible that the greater range of physiological data seen in postprandial glucose profiles may correspond with a larger effect size when correlated against HRV measures. As such, future studies should also aim to assess BGL during postprandial periods, and not just

during fasting periods. Postprandial BGL also contributes more to overall glycaemia than fasting BGL, at least at lower levels of $HbA_1c$ or overall glycaemia (40), and thus may be more relevant to studies investigating glycaemia in the healthy range.

The present study made certain improvements on the previous study design (33). Recording kilojoule intake in a food diary and applying it as a covariate in the analysis was an important method of controlling for the different diets of the participants, which can impact glucose profiles. Another strength was standardising the time points in which participants were assessed to reduce the effect of circadian rhythms on HRV, which can be substantial (65, 66). Future studies could attempt to expand on this by sampling a broader range of ages. As a requirement of the study, participants could not be living with any chronic health condition or be taking regular medication, and these increase in prevalence with age. As a result, the present sample was relatively young, which may limit the applications of our findings (67, 68). One of the concerns raised in this study was the limited ability of glucose meters to track changes in glucose profiles over time. If HRV measures were developed as a suitable replacement or supplement for current invasive self-monitoring of blood glucose techniques, it could also provide continuous glucose if recorded using a wearable ECG, such as a Holter monitor.

## 5 Conclusions

Current standards in BGL monitoring are inadequate considering the scope of diabetes and hyperglycaemia. As a leading epidemic of the 21$^{st}$ century, more rigorous technologies need to be developed to assist glycaemic control for people living with diabetes. There is a growing interest in the development of non-invasive, continuous markers of BGL that may aid in diabetes management. Generally, the aim of non-invasive glucose markers is to combine data derived from biosensors with continuous glucose monitoring data to increase the precision of the glucose level prediction (69).

For example, a novel algorithm presented by Cichosz and colleagues has shown promising results by combining information from a Holter monitor with concurrent values from a continuous glucose monitoring system. The algorithm detected 16/16 hypoglycaemic events in a sample of type 1 diabetes patients with a sensitivity of 79% and a specificity of 99% (70). This study represents an appealing line of research, as

it aims to overcome some of the current problems people with diabetes face. Current self-monitoring of blood glucose is costly (17), though a portable ECG, such as a Holter monitor, may present a reduced financial burden and, at the very least, would provide a non-invasive option. The R-waves from an ECG, used in the determination of HRV parameters, have distinct profiles that make them suitable for detection by computer algorithms (71). Consequently, the commercialisation of an inexpensive portable ECG may be expanded.

We observed numerous significant correlations between components of HRV and BGL. We conclude that HRV parameters derived from an ECG have the potential to be used in predicting the magnitude and direction of changes in BGL, though this needs to be confirmed in future studies. The scope of this area of study is not limited to diabetes – management of other metabolic diseases may be improved by the introduction of a non-invasive marker of BGL. Additionally, such a technology may interest professional athletes, cyclists, and marathon runners, as well as any group who might benefit from continuous, non-invasive glucose monitoring. The development of HRV parameters as non-invasive markers of BGL represents an optimistic line of research and could be critical in managing the diabetes epidemic. Such research could lead to the development of an algorithm capable of predicting BGL in real-time using purely non-invasive recordings from an ECG.

**Author Contributions**: Conceptualization, Roderick Clifton-Bligh, Ann M Simpson, Najah Nassif and Sara Lal; Data curation, Ty Lees; Formal analysis, Jaymen L Elliott; Investigation, Luke R Jarman; Methodology, Luke R Jarman, Jaymen L Elliott and Sara Lal; Resources, Ty Lees; Supervision, Roderick Clifton-Bligh, Ann M Simpson, Najah Nassif and Sara Lal; Writing – original draft, Luke R Jarman; Writing – review & editing, Ty Lees, Jaymen Elliott, Roderick Clifton-Bligh, Ann M Simpson, Najah Nassif and Sara Lal.

**Funding**: This research received no external funding.

**Acknowledgments**: The authors would like to acknowledge the Neuroscience Research Unit of the University of Technology Sydney for providing the equipment and facilities necessary to complete this study.

**Conflicts of Interest**: The authors declare no conflict of interest.

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