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The validity of point-of-care glucometers referenced against the YSI 2300 Stat Plus during aerobic activity and an oral glucose tolerance test

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ABSTRACT

THE VALIDITY OF POINT-OF-CARE GLUCOMETERS REFERENCED AGAINST THE YSI 2300 STAT PLUS DURING AEROBIC ACTIVITY AND AN ORAL GLUCOSE TOLERANCE TEST

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This thesis observes blood glucose and the dynamic changes that it undergoes within the human body. Blood glucose is carbohydrate located within the blood which is used for providing the body with energy in order to perform biological work. The level of carbohydrate in the blood will change dynamically depending on various factors such as exercising and fasting. Blood glucose is crucial to healthy metabolic function within the human body and it is important that it be monitored, especially in the case of diabetes. Various methods of measurement are used for reporting glucose levels ranging from the Yellow Spring Instruments 2300, the gold standard, to handheld glucose monitors.

This thesis examines the dynamic nature of blood glucose using two separate protocols in a laboratory study. The protocols being used allow for the examination of fasting, exercising and post-prandial blood glucose levels in humans as well as the validity of the Nova Max Plus handheld glucose monitor. Since glucose is required for the healthy functioning of the human body, it is imperative that monitoring systems be validated in order to determine their efficacy.

Keywords: Blood glucose, blood glucose monitor, glucose kinetics, glycolysis, metabolism

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BY

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DEDICATION

This work is dedicated to my parents

Mr. & Mrs. Timothy and Kari Granderson

and to my sibling

Timothy Granderson, Jr.

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INTRODUCTION

Blood glucose is an important physiological variable in clinical and human performance laboratories due to its relationship with various medical conditions and as an indicator of energy availability to metabolically active tissue. For example, the measurement of blood glucose following an overnight fast and oral glucose tolerance test are commonly used as screening and diagnostic techniques for diabetes mellitus (American Diabetes Association, 2018). Specifically, fasting blood glucose values of 100-125 $mg \cdot dL^{-1}$ are considered impaired or "prediabetic," whereas values >125 $mg \cdot dL^{-1}$ are classified as "diabetic" (American Diabetes Association, 2018). An oral glucose tolerance test (OGTT) involves the measurement of blood glucose following the ingestion of a drink typically containing 75 grams of sugar (American Diabetes Association, 2018). Blood glucose levels at the two-hour time point of 140-199 $mg \cdot dL^{-1}$ and $\geq$200 $mg \cdot dL^{-1}$ are classified as "prediabetic" and "diabetic," respectively (American Diabetes Association, 2018). In addition, blood glucose can be monitored during longer bouts of exercise to provide information related to carbohydrate metabolism and as a preventative measure against low blood sugar (i.e. hypoglycemia).

The maintenance of glucose in the blood is important for proper functioning of various metabolic processes of the body and reflects the balance of carbohydrate intake, cellular uptake, and glucose release by the liver (Angus et al., 2002; Coggan 1991; Coggan, 1997). Hypoglycemia, or low blood sugar, is a state in which the energy demands of the body may not be supplied, thereby leading to fatigue, decrements in mental acuity, and unconsciousness in extreme cases (American Diabetes Association, 2018; Cryer, 2007;). The risk of hypoglycemia

is of greatest concern to diabetics but can also play a factor in athletic performance during intense or extensive exercise and be indicative of glycogen depletion (Cermak & van Loon, 2013). Thus, the measurement of blood glucose is critical for the health and safety of both clinical and athletic individuals in home-, clinical-, or laboratory-based environments.

The reference techniques for measuring blood glucose typically involve using enzymatic assays or isotope-dilution gas chromatography-mass spectrometry as suggested by the Centers for Disease Control and Prevention (CDC; Hagvik, 2007). For example, the YSI 2300 STAT PLUS (Yellow Springs Instruments, Ohio) analyzes blood glucose using the glucose oxidase method and is considered the reference method by the U.S. Food and Drug Administration that most other glucose meters are calibrated against (Ekhlaspour et al., 2017). Although the YSI 2300 STAT PLUS offers a high level of precision (±2% of the reading or 2.5 $mg \cdot dL^{-1}$; User's Manual YSI 2300 STAT PLUS), it requires complicated, expensive, and time-consuming operation and maintenance procedures that are only realistic in laboratory and clinical settings. In addition to these complications, the YSI 2300 is designed to be stationary and requires an external energy source. Thus, for quick and easy blood glucose measurement in the field (e.g. health fairs, schools, exercise facilities) or as a point-of-care test at home, the portable handheld glucose monitors provide an attractive alternative over the more complex, laboratory-based procedures.

These portable handheld glucose monitors are considered highly user-friendly (e.g. simple to operate, small size, battery powered), cost-effective, and can produce results in approximately 5 seconds from less than a drop of blood (i.e. 0.3 µL). Although measures of quality control have been assessed in many handheld glucose monitors in the fasted state

(Robinson & Sharp, 2012; Tack et al., 2012; Thomas et al., 2008) or *in vitro* (Bedini et al., 2016; Ekhlaspour et al., 2017), there are limited data concerning these factors under dynamic conditions of blood glucose. Therefore, the purpose of the present study was to examine the validity and reliability of the Nova Max® Plus blood glucose monitoring system during an oral glucose tolerance test and 60-minute bout of exercise.

METHODS

Subjects

A total of 30 subjects (mean age ± SD = 22.3 ± 1.9 yrs; body mass = 77.6 ± 14.2 kg; height = 171.3 ± 9.6 cm; physical activity = 6.2 ± 4.3 $hr \cdot wk^{-1}$) were recruited to participate in a single visit to the laboratory for the OGTT (n = 15) or 60-minute treadmill exercise test (n = 15). In addition, the subjects did not report or exhibit any of the following that could significantly affect the outcome of the study: (i) history of medical or surgical events, including cardiovascular disease, heart disease, hypertension, diabetes mellitus, insulin resistance, hypoglycemia, or any other metabolic, renal, hepatic, or musculoskeletal disorder; (ii) phobia to needles or finger stick; (iii) fasting blood glucose level >100 $mg \cdot dL^{-1}$; (iv) any current physical injury due to the physical demands and requirements of the study. Subjects recruited for the OGTT (n = 15) were asked to avoid eating or drinking anything other than water for 8 hours prior to their visit, whereas the subjects recruited to participate in the 60-minute treadmill exercise (n = 15) were asked to avoid eating or drinking anything other than water for 2-3 hours prior to their visit. The study was approved by the Northern Illinois University Institutional Review Board, and all participants completed a health history questionnaire and signed a written informed consent document prior to testing.

Procedures

Oral Glucose Tolerance Test (OGTT)

Fifteen subjects (mean age ± SD = 22.4 ± 1.7 yrs; body mass = 77.0 ± 13.2 kg; height = 170.5 ± 10.6 cm; physical activity = 5.0 ± 2.5 $hr \cdot wk^{-1}$) were tested in the morning following an

8-hour overnight fast. Subjects had a baseline (0 min) blood glucose measurement taken immediately prior to the ingestion of the glucose drink to ensure the subjects were in a fasted state with normal levels of blood glucose (<100 $mg \cdot dL^{-1}$). If blood glucose levels were within normal range, the subjects were asked to ingest a drink (296 mL) that consisted of 75 grams of glucose (ThermoFisher Scientific, Waltham, MA). Subjects were then asked to sit quietly in the laboratory with their blood glucose being measured at the 10, 20, 30, 60, and 90 minute time points of the OGTT.

Treadmill Exercise

Fifteen subjects (mean age ± SD = 22.3 ± 2.2 yrs; body mass = 78.2 ± 15.6 kg; height = 172.2 ± 8.7 cm; physical activity = 7.3 ± 5.4 $hr \cdot wk^{-1}$) were tested for their blood glucose responses during 60 minutes of walking on a treadmill (Woodway Desmo HP, Waukesha, WI). Subjects had a baseline (0 min) blood glucose measurement taken immediately prior to the treadmill test in which the subjects were asked to walk at 3.5 mph for a total of 60 minutes. Blood glucose was measured at the 5, 10, 15, 30, and 60 minute time points of the test. At each of these time points, the treadmill belt was paused to safely allow for the measurement of blood glucose with the subject standing still. Following each measurement, the subject was instructed to continue the test at 3.5 mph until the 60 minute time point had been reached.

Measurement of Blood Glucose

Blood glucose concentrations were measured from the fingertip at six different time points during the oral glucose tolerance test (0, 10, 20, 30, 60, and 90 min) and treadmill test (0, 5, 10, 15, 30, and 60 min). The finger was cleaned with alcohol before the initial finger stick and

prior to each blood sample. In addition, the initial blood drop that formed on the finger was wiped away and the subsequent blood drops were sampled for analysis and used as the representative blood glucose concentrations.

During the oral glucose tolerance tests and treadmill exercise tests, one blood sample was analyzed four times at each time point, two by the YSI 2300 and two by the handheld device. Specifically, 50 µL of whole capillary blood was collected at each time point using two heparinized capillary tubes and transferred into a 1.5 mL graduated natural micro-centrifuge tube. This sample was analyzed twice using the YSI 2300 and twice using the Nova Max Plus using a micropipette to present the sample to the glucose monitor. The criterion reference method of blood glucose analysis used in the present study was the Yellow Springs Instruments (YSI) 2300 STAT PLUS Glucose and Lactate Analyzer (Yellow Springs, OH). In particular, the YSI 2300 measures blood glucose from whole blood using the glucose oxidase method. This technique required two 25 µL of whole blood collected into heparinized capillary tubes from a finger stick. Duplicate YSI blood glucose results were required to be within ±4 $mg \cdot dL^{-1}$ to be used for analysis and the average of these two measurements was used as the representative YSI 2300 value. The YSI 2300 whole blood glucose values were then converted to plasma equivalents (whole blood * 1.12 = plasma), and this value was compared with the test strip results of the handheld device (Nova Max® Plus; Nova Biomedical Corp., Waltham, MA). For quality control, the YSI 2300 was calibrated against 180 and 900 $mg \cdot dL^{-1}$ solutions according to the manufacturer's guidelines (User's Manual YSI 2300 STAT PLUS). The handheld device required 0.3 µL of whole blood for the measurement of blood glucose within 20-600 $mg \cdot dL^{-1}$ using an electrochemical glucose oxidase biosensor. In addition, the handheld device was

checked for accuracy against a known quality control solution prior to testing as recommended by the manufacturer (Nova Biomedical Corp.).

Data Analysis

Data are provided as mean ±SD. Accuracy of the handheld glucose monitor compared to the reference YSI 2300 values involved multiple analyses. First, mean differences in blood glucose values between the YSI 2300 and handheld monitor across time during the OGTT and exercise tests were assessed using separate two-way (Method x Time) analyses of variance (ANOVAs) with repeated measures and follow-up paired-samples *t*- tests when appropriate. Accuracy was also assessed through: 1) calculation of the mean absolute relative deviation (MARD); 2) comparing test results of the handheld monitor against the ISO 15197:2013 performance standards (International Organization for Standardization); 3) simple linear regression from the calculation of constant error (CE = mean difference for YSI blood glucose – handheld blood glucose), Pearson correlation coefficient (r), and standard error of estimate ($\mathrm{SEE} = \mathrm{SD}\sqrt{1 - \mathrm{r}^2}$); and 4) Bland-Altman (1986, 2010) analyses. Intra-device reliability for the handheld glucose meter was assessed using coefficient of variation (CV), intra-class correlation coefficient (ICC), and paired-samples *t*- tests. An alpha of 0.05 was used for statistical significance.

Results

Accuracy. The results of the two-way repeated-measures ANOVA for blood glucose values during the OGTT indicated there was a significant ($p < 0.05$) Method x Time interaction [$F(5,70) = 2.806$, $p = 0.023$]. Follow-up paired-samples *t*- tests indicated that the handheld monitor resulted in significantly greater blood glucose values than the YSI 2300 at each time point (Table

Table 1

Mean (±SD) Blood Glucose Values from the YSI 2300 and Nova Max Plus During an Oral Glucose Tolerance Test and Treadmill Exercise.

	Oral glucose tolerance test time (minutes) (n = 15)					
Method	0	10	20	30	60	90
YSI 2300 ($mg \cdot dL^{-1}$)	87.3 (±4.7)	116.0 (±10.4)	143.4 (±13.5)	158.5 (±16.6)	132.7 (±21.8)	113.5 (±12.7)
Nova Max Plus ($mg \cdot dL^{-1}$)	97.3 (±8.0)*	130.9 (±12.6)*	157.7 (±17.4)*	170.9 (±19.7)*	141.5 (±22.3)*	120.2 (±15.2)*
	Treadmill exercise time (minutes) (n = 15)					
Method	0	5	10	15	30	60
YSI 2300 ($mg \cdot dL^{-1}$)	86.1 (±9.9)	84.6 (±9.5)	85.9 (±8.2)	85.3 (±7.2)	86.1 (±5.4)	87.6 (±6.0)
Nova Max Plus ($mg \cdot dL^{-1}$)†	91.8 (±9.2)	90.6 (±8.1)	89.9 (±8.0)	90.7 (±8.5)	92.4 (±8.0)	90.7 (±7.5)

*Significantly ($p < 0.05$) greater than YSI 2300 blood glucose value.

†Main effect ($p < 0.05$) for method (Nova Max Plus > YSI 2300) collapsed across time.

1). For blood glucose values during the treadmill exercise test, there was no significant Method x Time interaction [$F(5,70) = 0.868$, $p = 0.507$] or main effect for time [$F(5,70) = 0.533$, $p = 0.751$], but there was a main effect for method [$F(1,14) = 12.191$, $p = 0.004$]. A follow-up paired-samples *t*- test indicated that the handheld monitor (86.2 ± 18.8 mg·dL^{-1}) resulted in significantly greater blood glucose values than the YSI 2300 (81.3 ± 19.8 mg·dL^{-1}; collapsed across time; Table 1). The MARD (±SD) and accuracy values for the Nova Max Plus compared to the reference YSI 2300 method are provided in Table 2. Specifically, the overall MARD ±SD was 9.0 ±7.0% and the combined blood glucose values (OGTT and treadmill exercise tests, n = 180) that were within the ISO 15197 criteria was 87.2% (157/180; Table 2).

Table 2

Accuracy of Nova Max Plus in Different Reference Blood Glucose Ranges.

	Overall	<100 mg·dL^{-1}	>100 mg·dL^{-1}
MARD ± SD (%)	9.0 ± 7.0	9.0 ± 7.3	9.1 ± 6.6
95% CI	8.0 to 10.1	7.6 to 10.4	7.6 to 10.6
Values within			
±5%/5 mg·dL^{-1}	80/180 (44.4%)	53/105 (51.5%)	27/75 (36.0%)
±10%/10 mg·dL^{-1}	149/180 (82.8%)	76/105 (72.4%)	43/75 (57.3%)
±15%/15 mg·dL^{-1}	157/180 (87.2%)	92/105 (87.6%)	65/75 (86.7%)
ISO standards* met?	No	No	No

*ISO 15197:2013 standards require that 95% of values <100 mg·dL^{-1} be within ±15 mg·dL^{-1} of reference value and 95% of values >100 mg·dL^{-1} be within ±15%.

The regression analyses of the Nova Max Plus versus the YSI 2300 reference method during the OGTT, treadmill exercise, and overall combined (OGTT and treadmill exercise) resulted in significant correlations (r = 0.95, 0.57, and 0.96), SEE values (8.7, 6.3, and 7.8 $mg \cdot dL^{-1}$), and CE values (11.2, 5.1, and 8.2 $mg \cdot dL^{-1}$; Table 3).

Table 3

Regression Analyses of the Nova Max Plus Versus the YSI 2300 Reference Method During an Oral Glucose Tolerance Test and 60 Minutes of Treadmill Exercise.

			95% CIs				
	slope	intercept	slope	intercept	r	SEE	CE
OGTT (n = 15)	0.88*	5.65	0.81 to 0.94	-3.13 to 14.43	0.95	8.7	11.2
Exercise (n = 15)	0.55*	35.72	0.39 to 0.72	20.50 to 50.95	0.57	6.3	5.1
All (n = 30)	0.86*	7.81	0.82 to 0.90	3.47 to 12.15	0.96	7.8	8.2

*Significantly ($p < 0.05$) greater than zero.

OGTT = oral glucose tolerance test.

SEE = standard error of estimate.

CE = constant error.

The Bland-Altman plot indicated there was a significant ($p < 0.05$) negative relationship (r = -0.23) for CE (YSI 2300 – Nova Max Plus) versus the reference method (YSI 2300) as well as negative bias (CE = -8.15 $mg \cdot dL^{-1}$; Figure 1).

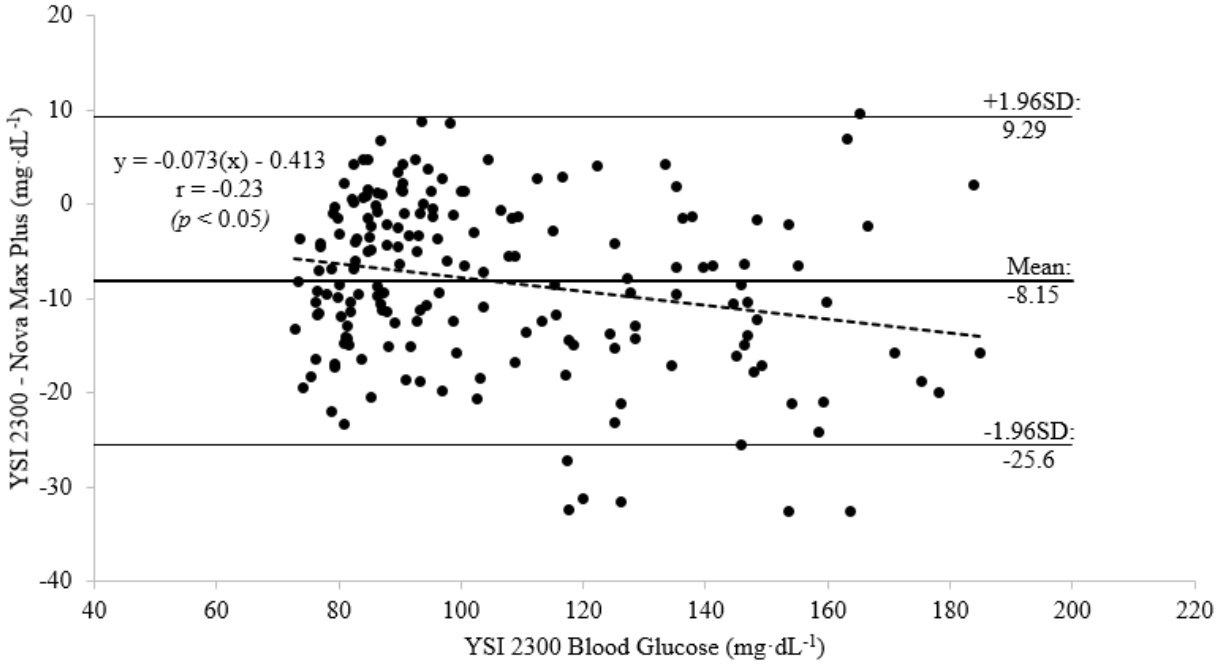

Figure 1. The relationship between constant error (YSI 2300 – Nova Max Plus) and the YSI 2300 blood glucose reference values (n = 180; 30 subjects x 6 time points = 180). Dashed line represents the regression line.

Reliability. Test-retest intra-device reliability analyses for the Nova Max Plus during the OGTT and treadmill exercise test indicated there was no significant ($p > 0.05$) mean difference between measurement 1 (113.9 ± 31.2 mg·dL^{-1}) and measurement 2 (113.6 ± 31.4 mg·dL^{-1}) on the combined data (n = 180). The ICC and CV associated with measurement 1 versus measurement 2 were $R = 0.99$ and 3.0%, respectively. In addition, the relationship between measurement 1 and measurement 2 from the Nova Max Plus resulted in a significant correlation (r = 0.99; Figure 2).

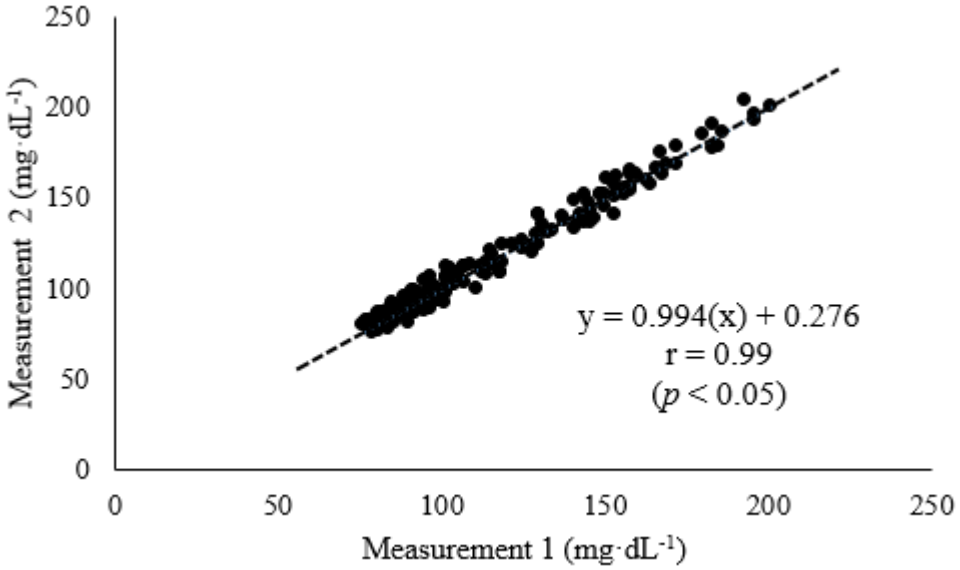

Figure 2. Test-retest reliability for the Nova Max Plus (n = 180; 30 subjects x 6 time points= 180).

Discussion

In the present investigation, the blood glucose responses during the OGTT and 60 minutes of treadmill exercise (Table 1) were comparable to those previously reported for healthy individuals (Lui et al. 2008; Zinker et al. 1990). Specifically, the blood glucose values (mean ±SD) during the 75-g OGTT at the 0-min (87.3 ± 4.7 mg·dL^{-1}) and 90-min (113.5 ± 12.7 mg·dL^{-1}) time points were below those associated with prediabetes (fasting: 100-125 mg·dL^{-1}; 120-min: 140-199 mg·dL^{-1}) and diabetes (fasting: >125 mg·dL^{-1}; 120-min: >200 mg·dL^{-1}; American Diabetes Association, 2018). In addition, the range in blood glucose values (84.6 ± 9.5 to 87.6 ± 6.0 mg·dL^{-1}) during the treadmill test were similar to those of Zinker et al. (1990) over 60 minutes of moderate-intensity exercise. Thus, the subjects in the present study exhibited blood glucose responses during the OGTT and 60-min exercise test that were consistent with healthy, non-diabetic individuals of comparable age (Lui et al., 2008; Zinker et al., 1990).

Accuracy. The accuracy of the Nova Max Plus handheld monitor was assessed during two dynamic conditions for blood glucose: an OGTT and 60 minutes of exercise. One of the main findings of the present study was that the Nova Max Plus provided significantly greater blood glucose values than the reference method (YSI 2300) under both conditions at all time points of the tests (Table 1). Therefore, the Nova Max Plus consistently overestimated blood glucose provided by the YSI 2300. In addition, the MARD and ISO 15197:2013 requirements were used to examine the accuracy of the handheld monitor within different reference ranges of blood glucose. Specifically, the calculation of MARD provides the mean absolute difference value between the Nova Max Plus and reference method (YSI 2300) expressed as a percent of the reference value (Obermaier et al., 2013). Thus, the MARD describes the magnitude of

percentage bias of each measurement (Tack et al., 2012) and a lower MARD value is associated with higher accuracy (i.e. closer to the reference value).

Although there are no set criteria for an "accurate" versus "inaccurate" MARD, values ranging from 4.2 to 39.2 have been reported for multiple handheld units (Ekhlaspour et al. 2017; Robinson & Sharp, 2012; Tack et al., 2012). Our results indicated that the Nova Max Plus exhibited an overall MARD (±SD) of 9.0 (±7.0; n = 180) and values of 9.0 (±7.3) and 9.1 (±6.6) at the low (<100 $mg \cdot dL^{-1}$, n = 105) and high (>100 $mg \cdot dL^{-1}$, n = 75) reference ranges of blood glucose, respectively (Table 2). The overall MARD value of 9.0 was consistent with previous findings for the Nova Max Plus under fasting conditions (MARD = 8.1; Robinson & Sharp, 2012) and *in vitro* preparations (MARD = 9.7; Ekhlaspour et al., 2017) that involved blood samples modified for glucose (20-440 $mg \cdot dL^{-1}$). In addition, Ekhlaspour et al. (2017) reported overall MARD (±SD) values ranging from 5.6 (±6.4) to 20.8 (±16.6) for 17 different handheld blood glucose monitors versus the YSI 2300, with the Nova Max Plus ranking 8th for the lowest MARD (9.7). Therefore, the findings of the present study and those of others (Ekhlaspour et al., 2017; Robinson & Sharp, 2012) indicated that Nova Max Plus provides consistent MARD values (9.0-9.7) within a wide range of blood glucose during fasting and dynamic conditions.

Although the present study was not conducted *in vitro* according to the strict compliance guidelines of ISO 15197:2013 that require the manipulation of blood glucose and hematocrit, their accuracy criteria was utilized in the evaluation of validity. The ISO 15197:2013 accuracy requirements for blood glucose monitoring systems specify that 95% of the measured values be within ±15 $mg \cdot dL^{-1}$ of the reference for values <100 $mg \cdot dL^{-1}$ and within ±15% for values >100 $mg \cdot dL^{-1}$ (ISO 15197:2013). Overall, the Nova Max Plus did not meet the 95% accuracy criteria,

yielding only 87.2% of readings within the required range (Table 2). To further examine the accuracy of the Nova Max Plus, we determined the percentage of readings that fell within the required range for values above and below 100 mg·dL^{-1}. In the reference range <100 mg·dL^{-1}, the Nova Max Plus was only within ±15 mg·dL^{-1} for 87.6% (92/105) of the values. For the reference range >100 mg·dL^{-1}, only 86.7% (65/75) of the values were within the ISO 15197:2013 requirements of ±15%. The results of these analyses indicated that the Nova Max Plus does not meet the ISO requirements in any of the three ranges (high, low, overall) of blood glucose that we examined. These findings were consistent with previous studies that have examined Nova Max Plus within fasting individuals and *in vitro* (Ekhlaspour et al., 2017; Robinson & Sharp, 2012) in which the Nova Max was found to have overall ISO 15197:2013 accuracy values of 89.0% and 88.2%, respectively.

Regression analyses were also completed to evaluate the prediction accuracy of the Nova Max Plus versus the actual values of the reference YSI 2300. Tack et al. (2012) used regression analyses in combination with MARD calculation to provide a comprehensive analysis of overall accuracy in handheld monitors. Specifically, Tack et al. (2012) reported slope coefficients and r values ranging from 0.98-1.04 and 0.97-0.99, respectively, for five different monitors. In the present study, the Nova Max Plus exhibited a significant slope coefficient (0.86), correlation coefficient ($r = 0.96$), and SEE value of ±7.8 mg·dL^{-1} on the overall combined data from the OGTT and exercise test (Table 3). The SEE value of ±7.8 mg·dL^{-1} provided the average error associated with predicting the reference YSI 2300 blood glucose value from the Nova Max Plus and represented 4.9% and 9.2% of the highest (158.5 mg·dL^{-1}) and lowest (84.6 mg·dL^{-1}) mean blood glucose values during OGTT and exercise test. More specifically, the Nova Max Plus and

YSI 2300 readings during the OGTT trial resulted in a strong correlation (r = 0.95), whereas the exercise trial resulted in a moderate correlation (r = 0.57). This discrepancy can be explained by the lower range of blood glucose values during the 60-minute exercise test (84.6-87.6 $mg \cdot dL^{-1}$) compared to the OGTT (87.3-158.5 $mg \cdot dL^{-1}$). In addition, the blood glucose values during exercise did not significantly ($p = 0.751$) change across the 60-minute test. Although exercise provides dynamic conditions in which glucose uptake by active skeletal muscle increases, this is balanced by glucose release from the liver, thereby maintaining nearly constant blood glucose values that change by less than 5 $mg \cdot dL^{-1}$.

The relationship between CE (YSI 2300 – Nova Max Plus) and the reference (YSI 2300) blood glucose values was analyzed using the method of Bland and Altman (1986; see Figure 1). For this relationship, the mean CE was -8.15 $mg \cdot dL^{-1}$ and correlation coefficient was r = -0.23. Constant error (CE) provides the mean difference between the actual (YSI 2300) and predicted (Nova Max Plus) blood glucose values. Specifically, the negative CE of -8.15 $mg \cdot dL^{-1}$ indicated that on average the Nova Max Plus overestimated the measurement of blood glucose by 8.15 $mg \cdot dL^{-1}$. In fact, 81% (145/180) of the blood glucose measurements from the Nova Max Plus resulted in overestimated values. The negative correlation (r = -0.23) suggested that the absolute CE values became greater at the high end of the reference (YSI 2300) blood glucose values. Thus, the Nova Max Plus overestimated blood glucose values by approximately 8.15 $mg \cdot dL^{-1}$ and this error tended to increase at higher blood glucose values.

Reliability. Intra-device test-retest reliability analyses of the Nova Max Plus were performed on the two blood glucose measurements taken at each time point during the OGTT and treadmill test (30 subjects x 6 time points = 180 comparisons). As recommended by Atkinson and Nevill

(1998), a number of statistical methods should be utilized for assessing reliability in variables relevant to sports medicine to provide a comprehensive analysis. Our findings indicated there were no significant mean differences between the first (113.9 ± 31.2 mg·dL^{-1}) and second (113.6 ± 31.4 mg·dL^{-1}) measurements. Although suitable, the *t*-statistic does not provide an indication of random variation between tests and should be interpreted with caution (Atkinson & Nevill 1998). Thus, we also utilized the ICC and CV as two additional statistical procedures that are commonly used to assess relative and absolute reliability, respectively (McLain et al., 2015). The ICC is used to describe the strength of the similarity of the values within a group, and categories suggested by Vincent (1994) include "excellent" (*R* close to 1), "high" (*R* > 0.90), "good" (*R* = 0.80-0.89), and "questionable" (*R* = 0.70-0.79). For CV, an acceptable boundary of <10% has been previously proposed (Atkinson & Nevill 1998; McLain et al., 2015). Therefore, the Nova Max Plus exhibited excellent relative reliability (ICC, *R* = 0.99) and acceptable absolute reliability (CV = 3.0%). Collectively, these analyses indicated that the Nova Max Plus provided highly reliability blood glucose values on two consecutive measurements taken at the same time points during the OGTT and treadmill test.

In summary, the blood glucose values provided by the handheld Nova Max Plus were significantly higher than those of the YSI2300 reference method at all time points of the OGTT and 60-minute treadmill test. In addition, the Nova Max Plus exhibited an overall MARD (±SD) of 9.0 (±7.0) and failed to meet the 95% accuracy requirements of ISO 15197:2013 (only 87.2% of all values met the criteria). The YSI 2300 versus Nova Max Plus blood glucose relationships resulted in a significant correlation (r = 0.96) and an SEE of ±7.8 mg·dL^{-1}. The Bland-Altman plot for CE (YSI 2300 – Nova Max Plus) versus the reference method (YSI 2300) indicated an

average negative bias (CE = -8.2 $mg \cdot dL^{-1}$) that tended to increase (r = -0.23) at higher blood glucose values. The intra-device reliability analyses of the Nova Max Plus, however, demonstrated that the ICC was R = 0.99 and CV = 3.0%, with no significant mean differences between test and retest values. These findings suggest that the Nova Max Plus provided highly reliable, yet inaccurate blood glucose values compared to the YSI 2300 during the dynamic conditions associated with an OGTT and exercise.

LITERATURE REVIEW

Carbohydrates are the preferred substrate from which the body yields adenosine triphosphate (ATP) in order to fuel various processes. Glucose is a six-carbon sugar that may be found within the blood readily available for the utilization of energy. Blood glucose comes from two sources: foodstuffs that are consumed and from stored glucose in the form of glycogen in the liver. The main source of blood glucose is food, namely foodstuffs with considerable amounts of starch. The starches and sugars located within food are entered directly into the bloodstream as glucose. The carbohydrates are first broken down into glucose by salivary and pancreatic amylase and then absorbed through active transport (Wong & Jenkins, 2007). Other carbohydrates, such as fructose and sucrose, are converted into glucose in either the small intestine or liver. Excess glucose is synthesized into glycogen through the process of glucogenesis and stored within the liver and muscles for later use.

Glucogenesis is a four-step process: (1) an ATP must provide a phosphate to the glucose in order to form a glucose-6-phosphate through hexokinase, (2) the enzyme glucose-6-phosphate isomerase isomerizes glucose-6-phosphate into glucose-1-phosphate, (3) the enzyme uridyl transferase reacts uridine triphosphate (UTP) with the glucose-1-phosphate to create uridine diphosphate-glucose and (4) the UDP-glucose attaches itself to a pre-existing glycogen polymer chain (Bollen et al., 1998; Geddes, 1986; McGarry et al., 1987; Wilson, 2003). UTP is uridine and three phosphates linked to the carbohydrate ribose (Leloir & Cardini, 1957). UTP acts as a source of energy during metabolic reactions, and when UTP activates a substrate, a phosphate is released and the remaining UDP binds with the substrate (Leloir & Cardini, 1957; Leloir &

Goldemberg, 1960; Luck, 1961). This results in UDP-glucose in glucogenesis (Leloir & Cardini, 1957; Leloir & Goldemberg, 1960; Luck, 1961). In the case that there is a deficit of glucose in the bloodstream, the liver will break down glycogen to glucose through the process of glycogenolysis (König et al., 2012). Glycogen is made up of linkages of glucose; therefore, in order to revert it back into glucose, the links must be broken (Wolfrom et al., 1951). Most linkages are end-to-end while a few are cross, or side, linkages. The end-to-end linkages are hydrolyzed by the enzyme phosphorylase while the cross-linkages are hydrolyzed by various debranching enzymes (Wolfrom et al., 1951). These two processes work in tandem in order to mobilize reserved glucose from the glycogen deposits. Glucose synthesis is not solely dependent on glycogen stores because the body also possesses the ability to create glucose from non-carbohydrate substances such as amino acids, glycerol, lactate and pyruvate (Cori, 1931). This process is referred to as gluconeogenesis and takes place in the liver just as glucogenesis and glycogenolysis.

Where Does Glucose Go?

The body relies on glucose for the utilization of energy in most cases. Glucose goes to the muscles that are in need of ATP. Glucose then goes through the process of glycolysis in which ATP is formed from the sugar. Once ATP is synthesized from glucose it may be utilized to perform biological work such as muscle contraction. Glycolysis is fundamentally the break down of glucose into a usable form of energy (i.e. ATP) for the muscles and organs. Glycolysis may be aerobic or anaerobic due to its ability to take place both in the presence and absence of oxygen. Aerobic glycolysis yields larger amounts of ATP but takes considerably longer than anaerobic glycolysis, which yields less ATP but at a greater rate. Both anaerobic and aerobic glycolysis

produce pyruvate, which has the opportunity to be oxidized in the presence of ample oxygen or reduced to lactate. Both anaerobic and aerobic glycolysis begin following the same general pathway of events, starting with glucose. Throughout the cascade of events, enzymes react with the six-carbon sugar in order to alter its composition, resulting in the phosphorylation of adenosine diphosphate (ADP) into ATP that may be used by the body for energy. Energy is later available as the ATP catabolizes into ADP and free energy.

At lower metabolic rates, resting muscle may oxidize lactate (Gladden, 2008). At higher intensities requiring larger metabolic rates, however, the body fails to oxidize lactate due to the oversaturation of CO_2, or hypercapnia (Gladden, 2008; Jones, 1980). In anaerobic environments, the final product of lactic acid will be without the oxygen needed to oxidize the hydrogen ions associated with it due to the state of hypercapnia induced by high-intensity exercise (Gladden, 2008; Jones, 1980). This leads to lactic acidosis by way of accumulated hydrogen ions (H^+) which have dissociated from the lactate (Gladden, 2008; Jones, 1980). Increased glucose utilization, such as during anaerobic exercise, is responsible for increasing H^+ when oxygen demand cannot be met (Jones, 1980). In the case of ample oxygen consumption to meet energy requirements, the pyruvate created as a product of glucose catabolism in glycolysis will be able to convert to acetyl-CoA (Jones, 1980; Krebs & Lowenstein, 1954) and continue to create ATP by way of the citric acid cycle and electron transport chain (Krebs & Lowenstein, 1954). To summarize, glucose is capable of producing ATP for the energy requirements of the body in both highly oxidative and anaerobic environments (Gladden, 2008; Jones, 1980; Krebs & Lowenstein, 1954). If given the oxygen, the byproducts of glycolysis may continue to create increased

amounts of ATP without the negative effects associated with the dissociation of H^+ ions from lactic acid accumulation (Krebs & Lowenstein, 1954).

Changes in Blood Glucose

The levels of glucose in any given body are not constant and the liver is constantly working to maintain a homeostatic figure. Blood glucose levels change in relation to quite a few factors. Factors such as fasting, consumption of food and exercise all maintain a specific blood glucose level reaction within the body. Fasting blood glucose is defined as the level of glucose in the blood of an individual who has not consumed food over an extended period of time (i.e. ≥8 hrs), such as when waking from sleep. Fasting blood glucose levels in healthy individuals typically range from 70 to 100 $mg \cdot dL^{-1}$ (American Diabetes Association, 2018). When the body enters a fasted state, it will continue to use blood glucose for energy. As the amount of glucose in the blood is not supplemented through eating, it will continue to drop. When blood glucose drops below a specific value, insulin levels begin to fall as a protective measure, preventing further uptake of glucose into the muscles and other tissues (Horwitz et al., 1975). When insulin is low enough, the body will begin to create more glucose in order prevent hypoglycemia. Glycogenolysis will become an active process in order to replenish blood glucose and return the individual to homeostatic levels. In the case that glycogen stores are entirely depleted, gluconeogenesis will allow for the conversion of amino acids and glycerol from triglycerides in order to meet energy needs (Majumder et al., 2016).

Digestion and glucose. Postprandial blood glucose is defined as the blood glucose concentration following the consumption of food. It is important that individuals consume glucose throughout the day because falling below 55-60 $mg \cdot dL^{-1}$ is indicative of hypoglycemia (Cryer et al., 2009). Glucose levels typically experience a change following consumption and during digestion due to the carbohydrate within the food. Approximately 10 minutes after a meal, the blood glucose levels will begin to rise due to the absorption of carbohydrates and continue to rise for approximately an hour before peaking (American Diabetes Association, 2001). Postprandial blood glucose in healthy individuals will rarely exceed 140 $mg \cdot dL^{-1}$ prior to returning to pre-prandial blood glucose levels approximately two to three hours following their meal. Glucose will increase after a meal and then continue to decrease to previous levels. This pattern is typical and expected in healthy individuals due to postprandial insulin release.

Following a meal, peptides responsible for glucose homeostasis within the gastrointestinal system are released. Glucose-dependent insulinotropic polypeptide and glucagonlike peptide-1 stimulate the activation of insulin after the consumption of glucose (Tseng et al., 1996). Insulin is the hormone responsible for allowing the uptake of glucose into the various tissues of the body (Baron et al., 1988; Morgan et al., 1961). If blood glucose is rising above normal level, insulin will be released in order to upregulate the absorption of glucose into bodily tissue. The opposite happens in cases of fasting in which glucose levels drop below normal levels as described above. In this case, insulin levels drop in order to allow what little glucose that is present in the blood to remain. There are, however, cases in which postprandial glucose concentrations do not follow the expected rise above and return to pre-prandial levels as described previously (Dunstan et al., 2012). Factors such as diet or insulin

resistance may have an exaggerated effect on postprandial blood glucose. The repeated consumption of meals high in calories and processed carbohydrates may lead to uncharacteristic spikes in postprandial blood glucose concentrations (Dunstan et al., 2012). Due to the wavering nature of blood glucose, it is important to monitor and report uncharacteristically high or low levels in diabetics.

Exercise and glucose. As with fasting and postprandial measures, blood glucose levels vary in response to differing exercise modalities and intensities (Ahlborg et al., 1974; Coggan et al., 1995; Fujimoto et al., 2003; Zinker et al., 1990). Blood glucose levels are critical for exercise because the body requires an increased amount of fuel in order to maintain the muscular activity. Low levels of blood glucose and muscle glycogen are factors that contribute to fatigue in exercising individuals (Hultman et al., 1991). Blood glucose levels increase at the onset of exercise due to the increased requirement of energy, then begin to decline as the body continues to use the glucose that is available (Foskett et al., 2008; Rose & Richter, 2005; Zinker et al., 1990). After a peak in blood glucose measures, blood glucose will continue to drop until exercise ceases (Foskett et al., 2008; Zinker et al., 1990). Though a decline in blood glucose is apparent, it will typically remain within 10% of normal glucose values until exercise stops (Zinker et al., 1990). The body maintains these blood glucose levels during exercise by way of hepatic glucose release. One way that the body does so is by the increased release of glucose within the gut, liver and kidneys into the blood during onset of exercise to compensate for the decreased levels (Meyer, Dostou, et al., 2002; Meyer, Stumvoll, et al., 2002). The liver is also capable of releasing glucosyl units that were stored in glycogen to stimulate gluconeogenesis in the liver and kidneys using precursor molecules such as lactate (Meyer, Stumvoll, et al., 2002). During

exercise, uptake of glucose within the muscles is largely increased in order to provide energy for the activity being performed and this is why a decline in glucose values is observable (Rose & Richter, 2005; Zinker et al., 1990).

The more muscles recruited for an activity, the larger the peripheral glucose uptake (Rose & Richter, 2005). At rest, glucose uptake in peripheral muscles is minimal. At the onset of exercise, glucose uptake within the involved muscles increases, which ultimately increases whole-body glucose uptake (Kjaer et al., 1991). This explains the phenomena of blood glucose levels dropping as exercise is maintained. Due to the nature of increased blood glucose uptake within the body during exercise, it is important that the levels are monitored in order to prevent hypoglycemia.

Importance of Measurement

The measurement of blood glucose in various conditions has both clinical and performance implications. For example, diabetes mellitus is a disease characterized by abnormally high blood glucose levels due to either insulin resistance or insulin dependence (American Diabetes Association, 2018). Type II diabetes mellitus is an insulin-resistant condition in which uptake of glucose into tissues is largely suppressed due to the inability to efficiently use insulin (American Diabetes Association, 2018). Type I diabetes mellitus is an insulin-dependent condition in which the pancreas creates little or no insulin, resulting in decreased glucose uptake within the tissues (American Diabetes Association, 2018). Both of these conditions result in a state of hyperglycemia, which is when blood glucose levels are well above the normal healthy levels (American Diabetes Association, 2001).

As stated previously, insulin is the hormone responsible for glucose uptake within the muscles and other tissues of the body (Baron et al., 1988; Morgan et al., 1961). When insulin availability is miniscule, or if the insulin available is not effective enough, the amount of glucose allowed into bodily tissue is minimal, which leads to increased amounts of glucose in the blood, or hyperglycemia. Chronically high levels of blood glucose associated with type II diabetes are also related to an increased risk of various forms of heart disease (Cinar et al., 2001; Haffner & Cassels, 2003; Kannel & McGee, 1979; Qazi & Malik, 2013). The Framingham Heart Study (Mahmood, Levy, Vasan & Wang, 2014; Qazi & Malik, 2013) is an ongoing cardiovascular cohort study in which various risks of cardiovascular disease are assessed and reported. One of the first associations between cardiovascular disease and diabetes was made in 1979 with the aid of this study (Kannel & McGee, 1979; Qazi & Malik, 2013). The mortality of cardiovascular disease had been reported to be three times higher in individuals with diabetes due to increased levels of heart failure and hypertensive heart disease (Kannel & McGee, 1979; Mahmood et al, 2014).

Hyperglycemia. Both pre-prandial and postprandial hyperglycemia have also been linked to increased rates of atherosclerosis and cardiovascular disease (Bonora & Muggeo, 2001). Hyperglycemia may lead to these conditions due to an increase in blood viscosity. Viscosity of the blood increases by 25% when blood glucose is increased to 400 $mg \cdot dL^{-1}$ from 100 $mg \cdot dL^{-1}$ (Cinar, Senyol & Duman, 2001). When the viscosity of the blood is increased, the heart is required to work harder in order to maintain cardiac output. In most cases, heart rate will increase due to a decreased left ventricular end diastolic volume as the result of decreased blood flow. Blood flow decreases by 20% when blood glucose is raised from 100 $mg \cdot dL^{-1}$ to 400

$mg \cdot dL^{-1}$ (Cinar, Senyol & Duman, 2001). This decrease in left ventricular end diastolic volume is responsible for the decrease in stroke volume that follows. Blood pressure increases as a result of increased resistance. Blood pressure increases by 25% to compensate for the increase in blood viscosity in the aforementioned instance of a 300 $mg \cdot dL^{-1}$ increase in blood sugar (Cinar, Senyol & Duman, 2001). All of these effects increase the stress experienced by the heart and collectively exacerbate symptoms of various cardiovascular diseases (Haffner & Cassells, 2003). In short, the clinical data supports a connection between hyperglycemia and cardiovascular disease (Haffner & Cassells, 2003; Haffner et al., 1998; Levitan et al., 2004). Though hyperglycemia occurs in individuals with diabetes, the cardiovascular implications of abnormally high blood glucose are not limited to those diabetics.

Hypoglycemia. At rest, the liver is constantly undergoing glycogenolysis in order to maintain normal glucose levels. This process is accelerated at the onset of exercise as the muscles continue to draw energy through catabolizing the available glucose in the bloodstream. Hypoglycemia occurs when liver glycogen stores are depleted and there is no longer a source from which to produce glycogen. The body continues to absorb glucose into tissue as long as the requirement of energy exists and exercise is a condition that increases the metabolic demands of the body. These demands must be met with the energy yielded from glucose through the process of increased peripheral glucose uptake.

When an individual exercises, the blood sugar will experience an increase subsequently followed by a decrease in the glucose availability for the duration of exercise. Hypoglycemia is when blood glucose levels are well below values for healthy functioning and, just as hyperglycemia, this possesses health implications (American Diabetes Association, 2018; Cryer,

2007). Hypoglycemia is characterized as a blood glucose level that has fallen to ≤55-60 $mg \cdot dL^{-1}$ (Cryer et al., 2009) and is often accompanied by hunger, confusion, dizziness, weakness, seizures, coma and even hospitalization (Bonds et al., 2012). The brain possesses its own glycogen stores within astrocytes in order to combat hypoglycemia (Brown & Ransom, 2007; Suh et al., 2007). In severe cases an individual may turn unconscious (Musen et al., 2008) and sustain decrements to mental processes (Cryer, 2007; Gonder-Frederick et al., 2009). The control center of the body requires glucose as an energy source just as other tissue, and an inadequate supply results in hypoglycemia of the brain leading to the aforementioned symptoms. This condition may occur as the result of many preventable behaviors and states such as decreased carbohydrate consumption, failure to eat and hyperinsulinemia due to taking an excess amount of insulin as a diabetic. Hypoglycemia is especially dangerous in exercise conditions as well as in individuals with diabetes mellitus as the hazard of seizing or fainting and entering a coma driven by a lack of energy required for brain functioning is increased (Bohn et al., 2015). There are quite a few antecedents to hypoglycemia that may warn an individual of impending complications, such as hunger, irritability, blurry vision, sweating, and weakness and fatigue (Bonds et al., 2012). The normoglycemic range is 70-99 $mg \cdot dL^{-1}$ in a fasted state and below 140 $mg \cdot dL^{-1}$ two hours postprandial (American Diabetes Association, 2001; Hill et al., 2011). It is important that individuals with glucose management difficulties monitor blood glucose levels and are take actions to prevent falling outside of the recommended normative blood glucose levels in order to avoid the aforementioned symptoms and complications.

Methods of Measurement

The Yellow Springs Instruments (YSI) Biochemistry Analyzer is capable of measuring molecules that may be found in the blood such as lactate, ketones and glucose (Yellow Springs Instruments, 2009). Validation studies (Lovrenčić et al., 2013; Robinson & Sharp, 2012; Salacinski et al., 2014; Tack et al., 2012) often use the YSI Biochemistry Analyzer as the reference method in order to determine the effectiveness of various blood analyzers. This analyzer is quite effective due to its ability to report accurate and reliable values for a variety of blood measures. The YSI has had its accuracy validated by glucose standards traceable to the National Institute of Standards and Technology Standard Reference Material (Robinson & Sharp, 2012). One of the benefits of the YSI is that it does not require blood samples to be centrifuged prior to being presented to the sipper. The YSI is capable of measuring values through both whole and parted blood. Though the YSI is considered to be the gold standard, it suffers from a few shortcomings. There are a myriad of technical weaknesses that are the direct result of human error. The YSI uses a buffer solution that is used after a sample is processed in order to flush the chamber in preparation for a new sample (Yellow Springs Instruments, 2009). This solution flushes into a waste container that must be emptied once full. The buffer and the waste should have a volume equal to a full container. For example, if the buffer is 75% full, then it should follow that the waste is 25% full. This is because the volume from within the buffer container is flushed into the waste container after each sample. Issues will ensue if the buffer is prepared incorrectly or if the sum of the volume of buffer and waste exceeds a full container. The YSI will continue to draw from the buffer solution as it is available. There are stainless steel shafts in each container that terminate testing if either the calibration or buffer containers are empty or if the

waste container is full (Yellow Springs Instruments, 2009). If the waste and buffer solution container volumes are not equal to a full container, then there may be an extra termination than necessary. This will halt testing until the respective containers are dealt with accordingly.

The YSI also requires the use of enzyme membranes as well as reagent and calibration solutions. Improper installation of membranes or preparation of solutions will result in less than accurate measures. One of the most prominent human-error complications that may halt data collection is forcing a calibration to be made. When the YSI is ready to receive a sample, the tester must press “Sample” and wait for the sipper to finish descending from the machine. Once the sipper is completely descended, the tester may present the sample. It is imperative that the tester keeps a steady hand for the entirety of the process until the sipper returns into the YSI. If the sipper is bumped or deviated by the tester, then the YSI will retract the sipper and run a calibration without testing the sample it has just received. This will halt data collection and will require another blood draw from the participant if ample blood was not gathered for two samples. This calibration will also start if the sipper descends for a sample yet no sample is presented when it is ready. Calibration is required in order to uphold the accurate and reliable results of the YSI Biochemistry Analyzer. The YSI may be configured to calibrate when desired by the tester (Yellow Springs Instruments, 2009). This, however, poses an issue of data collection time versus accuracy and reliability. For example, if the YSI is set to calibrate after every five samples, then data collection for a study that requires multiple samples at multiple data points will come to a halt numerous times. This results in the tester possibly limiting data collection to one subject per session, which will ultimately extend the period of time required to

complete data collection. Though the amount of calibrations taking place during a testing session is a limiting factor, it is necessary that the calibrations are completed.

Aside from mechanical and user errors, the YSI also falls short as a practical method of measuring blood glucose. Due to the price of the YSI Biochemistry Analyzers, they typically will not be used in exercise physiology labs. The Biochemistry Analyzer is not an efficient field testing method for the various blood variables that a tester may measure in combination with exercise. The YSI is by no means intended to be a mobile device due to its need of electricity for use, and the downtime needed to gather the sample for use with this machine also interferes with exercise testing. It is also important to review the YSI Biochemistry Analyzer in terms of practicality for diabetics. Glucose management and tracking are vital for the survival of individuals with either type I or type II diabetes so is important that these individuals have a cost-effective, reliable substitute for blood glucose measurement.

Glucometers. Handheld glucose monitors are small, mobile devices used for the measurement of blood sugar. There are various brands and types of handheld glucose monitors but the function of each is the same. These monitors report blood glucose through the use of specialized strips that measure glucose through analyzing less than a drop of capillary blood. As opposed to the YSI Biochemistry Analyzer, these monitors are portable, cost-effective, fast to produce results and easy to operate. This technology is an exceptionally accessible option for diabetic individuals who must manage their glucose levels manually throughout the day. The efficacy of these monitors as reliable glucose observers has been determined by various studies (Lovrenčić et al., 2013; Robinson & Sharp, 2012; Salacinski et al., 2014; Tack et al., 2012) in which subject pools range from healthy to diabetic. These studies (Lovrenčić et al., 2013;

Robinson & Sharp, 2012; Salacinski et al., 2014; Tack et al., 2012) examine the relationship between blood glucose and various situations, such as exercise, and the effectiveness of the handheld monitor in regards to reflecting those expected responses. Due to the importance of accurate glucose measures for the diagnosis and management of diabetes, it is imperative that these handhelds be both reliable and accurate regardless of the circumstance in which the subject may be. Validation studies have used OGTTs, exercise, and fasting glucose levels in order to examine monitor reliability and accuracy referenced against either the YSI Biochemistry Analyzer or results gathered from accredited laboratory practices.

Validation studies are used as a means to determine whether the glucometers possess criterion validity. Criterion validity is when the accuracy and reliability of the tested technology are compared to a criterion method referred to as the reference. In the case of glucose monitors, the monitor is compared against either the YSI or conventional laboratory methods as the criterion.

Accuracy and reliability of glucometers. The results of the aforementioned studies were varied. Many studies have made claims supporting the accuracy and reliability of handheld glucose monitors while others deemed the monitors unfit for diagnosis and research purposes. The StatStrip glucose meter produced by Nova Biomedical has been determined a reliable tool for diabetes diagnosis. This particular monitor was produced for hospital use with technology resistant to both hematocrit and drug-interference. The study conducted in order to determine the efficacy of this product as a reliable diabetes diagnosis technology consisted of subjects diagnosed with fasting or intermediate hyperglycemia (Lovrenčić et al., 2013). The subjects arrived after an overnight fast and performed a two-hour OGTT in which measures were taken

using venipuncture for the traditional laboratory glucose measurement procedure and capillary blood for the Nova StatStrip monitors. Two StatStrip monitors using two separate batches of StatStrip reagent strips were used at each time point immediately following the venous collection.

The reference laboratory procedure and StatStrip monitor fasting plasma glucose measures were highly correlated ($r = 0.97$) without a significant difference between the two methods (Lovrenčić et al., 2013). The two-hour plasma glucose values upheld an excellent correlation ($r = 0.98$); however, a significant difference was found between the two methods.

With the main purpose being to assess the diagnostic accuracy of modern handheld glucose monitors for type II diabetes, the results of this study provide the first evidence that point-of-care glucose meters, specifically the Nova StatStrip monitor, were comparable to the validity of the reference laboratory procedures. Though most validation studies reference blood glucose values of the monitors to the YSI Biochemistry Analyzer, it is important that these monitors were also validated against other methods such as the laboratory procedures used in the previous study. Due to the time consumption of remotely testing blood for glucose levels by way of laboratory procedures, hospitals typically opt for the use of point-of-care handheld glucose monitors for bedside treatment. Due to the need for effective bedside diabetes management it is important that these monitors are not only fast but also valid. There are specific, international standards for the accuracy of blood glucose monitors published by the International Organization for Standardization (ISO) that hospitals must abide by. A study conducted in 2012 examined the accuracy of six different handheld blood glucose monitors using the accuracy criteria listed by the ISO 15197 published in 2003 (Robinson & Sharp, 2012). For this study, the YSI 2300 STAT

PLUS was used as the reference for the comparison of the blood glucose monitoring handheld test strips results. The methodology for the data collection was not specified, but it can be assumed that there was no control over subject behavior due to the blood glucose concentrations gathered over the course of the study ranging from 49 $mg \cdot dL^{-1}$ to 444 $mg \cdot dL^{-1}$.

Each of the 108 diabetic subjects received a finger prick from which all six handhelds gathered capillary blood samples followed by YSI sample taken from the same finger stick within 15 minutes of the first handheld sample being taken (Robinson & Sharp, 2012). According to the criteria of the ISO 15197, five of the six handhelds used and referenced against the YSI were up to accuracy standards. These results demonstrated the efficacy of handheld glucose monitors as an effective method for bedside diabetes management (Robinson & Sharp, 2012). With these outcomes it can be inferred that handheld glucose monitors are effective for diabetes management outside of the hospital environment, as well.

Another study using the ISO 15197:2003 standards as a means of accuracy determination examined five commercially available monitors not used in the previous study. In this study 453 subjects with type I or type II diabetes arrived to the facility in a fasted state and performed the sample collection just as they would at home. This was repeated for three of the five monitors, each of which was randomly assigned to each subject. A healthcare professional gathered capillary blood for the YSI 2300 STAT PLUS before and after each self-test performed by the subject (Tack et al., 2012). Regression analyses were performed for each blood glucose monitoring system against the YSI, resulting very high correlations for each system ($r = 0.97$ to 0.99). Three of the five handheld glucose monitors were found to be highly accurate in comparison to the YSI 2300 STAT PLUS (Tack et al., 2013). The results of this study provide

data demonstrating that the commercially available monitors meant for use by type I and II diabetics are valid for self-monitoring when used by the diabetics themselves. Though there are multiple studies showing the accuracy and validity of handheld glucose monitors for both hospital and home use, there are conflicting data. In particular, some studies have found that these monitors should only be used for patient management rather than for hospital and research purposes. A study using 15 moderately to highly active men and women attempted to precisely measure the validity and reliability of handheld glucose meters for biomedical research (Salacinski et al., 2014). This validation study referenced the values of the handheld glucose monitor against the YSI 2300 STAT PLUS, using a fasted OGTT as the protocol. Measurements were taken prior to glucose administration and then at 10, 20, 30, 60 and 90 minutes postprandial. The ISO necessitates that handheld glucose monitors be within 20% of the reference value if glucose concentrations are above 76 $mg \cdot dL^{-1}$; however, the results of this study found that only 82% of the total number of glucometer readings fell within this criteria (Salacinski et al., 2014). Glucometer technology is progressing in order to improve accuracy, and due to the conflicting results among the currently available validity and reliability studies, it is important that more research is conducted in order to properly determine the efficacy of glucometer usage.

The oral glucose tolerance test. Testing glycemia is an essential step in the diagnosis of diabetes in patients. Tests used for diagnosis have been established over the years, such as random and fasting blood glucose, HbA1c and the OGTT. Fasting and random glucose measures are simply when glucose is measured either in a fasted state or at a random moment during the

day. HbA1c is the amount of hemoglobin in the blood that has become glycated (i.e. joined with glucose). This value allows for the estimation of average glucose over the previous months (Rohlfing et al., 2002). The OGTT has become the gold standard for diabetes diagnosis due to its use in a multitude of studies (Barr et al., 2002). The OGTT is a dynamic test due to its ability to allow the examiner to observe both fasting blood glucose and the way a patient's body processes glucose following ingestion. Unlike fasting blood glucose in which the subject refrains from eating and blood glucose is measured, the OGTT expresses postprandial blood glucose levels as well. Postprandial blood glucose is important because it allows for a look at how efficient the patient's systems are at storing and utilizing the glucose that has been consumed.

Glucose intolerance, or an elevated glucose concentration, is a sign of insulin resistance which may be used to diagnose diabetes or a pre-diabetic state in a patient. The normoglycemic range should be below 140 $mg \cdot dL^{-1}$ two hours after glucose consumption, which means that a healthy body is storing and using the glucose effectively after a meal (American Diabetes Association, 2018; Hill et al., 2011). Glucose will increase following a meal due to the carbohydrate content of the food and then continue to decrease to homeostatic values. In healthy individuals, peptides responsible for glucose homeostasis within the gastrointestinal system are released and these peptides facilitate the release of insulin. Diabetics tend to be insulin resistant, which leads to postprandial blood glucose concentrations above healthy levels well after consumption.

As fasting glucose tests determine an individual's insulin resistance by observing where blood glucose is after an extended period of not eating, OGTTs aid in the detection of insulin resistance in the form of blood glucose concentrations following meal consumption. OGTTs

consist of the subject consuming carbohydrate, typically a 75 g glucose solution, after fasting. Blood glucose is measured multiple times over a period of one to three hours, with two hours being the norm due to the ADAs classification of normoglycemia being >140 $mg \cdot dL^{-1}$ two hours postprandial. The subject's fasting glucose is to be taken prior to ingestion of the glucose and is used as the baseline measure for the test. The results of this test have implications for exercise. Hyperglycemia affects the viscosity of the blood, which leads to an increased stroke volume in order to combat the decrease of blood returned to the heart. Individuals with hyperglycemia typically have a higher blood pressure than normal, which poses an issue in the case of resistance training and exercises that are responsible for increasing blood pressure in healthy individuals. Many validation studies opt for the use of OGTTs because they allow for the examination of handheld glucose monitor values against the reference method values over the course of a couple hours. This gives the examiner a chance to observe the accuracy of the handheld glucose meter throughout the course of the insulin response.

REFERENCES

Ahlborg, G., Felig, P., Hagenfeldt, L., Hendler, R. & Wahren, J. (1974). Substrate turnover during prolonged exercise in man: Splanchnic and leg metabolism of glucose, free fatty acids, and amino acids. *Journal of Clinical Investigation, 53*(4), 1080.

American Diabetes Association. (2001). Postprandial blood glucose. *Clinical Diabetes*, *19*(3), 127-130.

American Diabetes Association. (2018). 2. Classification and diagnosis of diabetes: Standards of medical care in diabetes-2018. *Diabetes Care, 41*(Supplement 1), S13-S27.

Angus, D.J., Febbraio, M.A. & Hargreaves, M. (2002). Plasma glucose kinetics during prolonged exercise in trained humans when fed carbohydrate. *American Journal of Physiology-Endocrinology and Metabolism, 283*(3), E573-E577.

Atkinson, G., & Nevill, A.M. (1998). Statistical methods for assessing measurement error (reliability) in variables relevant to sports medicine. *Sports Medicine, 26*(4), 217-238.

Baron, A.D., Brechtel, G., Wallace, P. & Edelman, S.V. (1988). Rates and tissue sites of non-insulin-and insulin-mediated glucose uptake in humans. *American Journal of Physiology-Endocrinology and Metabolism*, *255*(6), E769-E774.

Barr, G.R., Nathan, D.M., Meigs, J.B. & Singer, D.E. (2002). Tests of glycemia for the diagnosis of type 2 diabetes mellitus. *Annals of Internal Medicine, 137*(4), 263-272.

Bedini, J.L., Wallace, J.F., Pardo, S. & Petruschke, T. (2016). Performance evaluation of three blood glucose monitoring systems using ISO 15197: 2013 accuracy criteria, consensus and surveillance error grid analyses, and insulin dosing error modeling in a hospital setting. *Journal of Diabetes Science and Technology*, *10*(1), 85-92.

Bland, J.M. & Altman, D.G. (1986). Statistical methods for assessing agreement between two methods of clinical measurement. *The Lancet, 327*(8476), 307-310.

Bland, J.M. & Altman, D.G. (2010). Statistical methods for assessing agreement between two methods of clinical measurement. *International Journal of Nursing Studies, 47*(8), 931-936.

Bohn, B., Herbst, A., Pfeifer, M., Krakow, D., Zimny, S., Kopp, F. ... & Holl, R. W. (2015). Impact of physical activity on glycemic control and prevalence of cardiovascular risk factors in adults with type 1 diabetes: A cross-sectional multicenter study of 18,028 patients. *Diabetes Care, 38*(8), 1536-1543.

Bollen, M., Keppens, S. & Stalmans, W. (1998). Specific features of glycogen metabolism in the liver. *Biochemistry Journal, 336*(1), 19-31.

Bonds, D.E., Miller, M.E., Dudl, J., Feinglos, M., Ismail-Beigi, F., Malozowski, S. … & Sood, A. (2012). Severe hypoglycemia symptoms, antecedent behaviors, immediate consequences and association with glycemia medication usage: Secondary analysis of the ACCORD clinical trial data. *BMC Endocrine Disorders, 12*(1), 5.

Bonora, E. & Muggeo, M. (2001). Postprandial blood glucose as a risk factor for cardiovascular disease in type II diabetes: The epidemiological evidence. *Diabetologia, 44*(12), 2107-2114.

Brown, A.M. & Ransom, B.R. (2007). Astrocyte glycogen and brain energy metabolism. *Glia, 55*(12), 1263-1271.

Cermak, N.M. & van Loon, L.J. (2013). The use of carbohydrates during exercise as an ergogenic aid. *Sports Medicine, 43*(11), 1139-1155.

Cinar, Y., Senyol, A.M. & Duman, K. (2001). Blood viscosity and blood pressure: Role of temperature and hyperglycemia. *American Journal of Hypertension, 14*(5), 433-438.

Coggan, A.R. (1991). Plasma glucose metabolism during exercise in humans. *Sports Medicine, 11*(2), 102-124.

Coggan, A.R. (1997). Plasma glucose metabolism during exercise: Effect of endurance training in humans. *Medicine and Science in Sports and Exercise, 29*(5), 620-627.

Coggan, A.R., Raguso, C.A., Williams, B.D., Sidossis, L.S. & Gastaldelli, A. (1995). Glucose kinetics during high-intensity exercise in endurance-trained and untrained humans. *Journal of Applied Physiology*, *78*(3), 1203-1207.

Cori, C.F. (1931). Mammalian carbohydrate metabolism. *Physiological Reviews, 11,* 143-275.

Cryer, P.E. (2007). Hypoglycemia, functional brain failure, and brain death. *The Journal of Clinical Investigation, 117*(4), 868-870.

Cryer, P.E., Axelrod, L., Grossman, A.B., Heller, S.R., Montori, V.M., Seaquist, E.R. & Service, J.F. (2009). Evaluation and management of adult hypoglycemic disorders: An endocrine society clinical practice guideline. *Journal of Clinical Endocrinology and Metabolism, 94*(3), 709-728.

Dunstan, D.W., Kingwell, B.A., Larsen, R., Healy, G.N., Cerin, E., Hamilton, M. T. … Owen, N. (2012). Breaking up prolonged sitting reduces postprandial glucose and insulin responses. *Diabetes Care, 35,* 976-983.

Ekhlaspour, L., Mondesir, D., Lautsch, N., Balliro, C., Hillard, M., Magyar, K. …& Russel, S.J. (2017). Comparative accuracy of 17 point-of-care glucose meters. *Journal of Diabetes and Technology, 11*(3), 558-566.

Foskett, A., Williams, C., Boobis, L., & Tsintzas, K. (2008). Carbohydrate availability and muscle energy metabolism during intermittent running. *Medicine and Science in Sports and Exercise, 40*(1), 96-103.

Fujimoto, T., Kemppainen, J., Kalliokoski, K.K., Nuutila, P., Ito, M., & Knuuti, J. (2003). Skeletal muscle glucose uptake response to exercise in trained and untrained men. *Medicine and Science in Sports and Exercise*, *35*(5), 777-783.

Geddes, R. (1986). Glycogen: A metabolic viewpoint. *Bioscience Reports, 6*(5), 415-428.

Gladden, L.B. (2008). A "lactatic" perspective on metabolism. *Medicine & Science in Sports & Exercise, 40*(3), 477-485.

Gonder-Frederick, L.A., Zrebiec, J.F., Bauchowitz, A.U., Ritterband, L., Magee, J.C., Cox, D.J., & Clarke, W.L. (2009). Cognitive function is disrupted by both hypo-and hyperglycemia in school-aged children with type 1 diabetes: A field study. *Diabetes Care, 32*(6), 1001-1006.

Haffner, S.J. & Cassells, H. (2003). Hyperglycemia as a cardiovascular risk factor. *The American Journal of Medicine, 115*(8), 6-11.

Haffner, S.M., Lehto, S., Rönnemaa, T., Pyörälä, K. & Laakso, M. (1998). Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *New England Journal of Medicine, 339*(4), 229-234.

Hagvik, J. (2007). Glucose measurement: Time for a gold standard. *Journal of Diabetes Science and Technology, 1*(2), 169-172.

Hill, N.R., Oliver, N.S., Choudhary, P., Levy, J.C., Hindmarsh, P. & Matthews, D.R. (2011). Normal reference range for mean tissue glucose and glycemic variability derived from continuous glucose monitoring for subjects without diabetes in different ethnic groups. *Diabetes Technology & Therapeutics, 13*(9), 921-928.

Horwitz, D.L., Rubenstein, A.H., Reynolds, C., Molnar, G.D. & Yanaihara, N. (1975). Prolonged suppression of insulin release by insulin-induced hypoglycemia: Demonstration by C-peptide assay. *Hormone and Metabolic Research, 7*(06), 449-452.

Hultman, E., Greenhaff, P.L., Ren, J.M. & Söderlund, K. (1991). Energy metabolism and fatigue during intense muscle contraction. *Biochemistry of Exercise, 19*, 347-353.

International Organization for Standardization. (2013). *ISO 15197:2013: In vitro diagnostic test systems - Requirements for blood-glucose monitoring systems for self-testing in managing diabetes mellitus.* Retrieved from https://www.iso.org/standard/54976.html

Jones, N.L. (1980). Hydrogen ion balance during exercise. *Clinical Science, 59*(2), 85-91.

Kannel, W.B. & McGee, D.L. (1979). Diabetes and cardiovascular risk factors: The Framingham study. *Circulation, 59*(1), 8-13.

Kjaer, M., Kiens, B., Hargreaves, M. & Richter, E.A. (1991). Influence of active muscle mass on glucose homeostasis during exercise in humans. *Journal of Applied Physiology, 71*(2), 552-557.

König, M., Bulik, S. & Holzhütter, H.G. (2012). Quantifying the contribution of the liver to glucose homeostasis: A detailed kinetic model of human hepatic glucose metabolism. *PLoS Computational Biology, 8*(6), e1002577.

Krebs, H.A. & Lowenstein, J.M. (1954). The tricarboxylic acid cycle. In *Chemical pathways of metabolism* (p. 129-203).

Leloir, L.F. & Cardini, C.E. (1957). Biosynthesis of glycogen from uridine diphosphate glucose[1]. *Journal of the American Chemical Society, 79*(23), 6340-6341.

Leloir, L.F. & Goldemberg, S.H. (1960). Synthesis of glycogen from uridine diphosphate glucose in liver. *Journal of Biological Chemistry, 235*(4), 919-923.

Levitan, E.B., Song, Y., Ford, E.S. & Liu, S. (2004). Is nondiabetic hyperglycemia a risk factor for cardiovascular disease? A meta-analysis of prospective studies. *Archives of Internal Medicine, 164*(19), 2147-2155.

Liu, T., Liu, Y., Lee, S., Huang, C., Chien, K., Cheng, I. ... & Kuo, C. (2008). Effects of short-term detraining on measures of obesity and glucose tolerance in elite athletes. *Journal of Sports Sciences, 26*(9), 919-925.

Lovrenčić, M.V., Biljak, V.R., Božičević, S., Medvidović, E. & Ljubić, S. (2013). Validation of point-of-care glucose testing for diagnosis of type 2 diabetes. *International Journal of Endocrinology, 2013.*

Luck, D.J. (1961). Glycogen synthesis from uridine diphosphate glucose. *The Journal of Cell Biology, 10*(2), 195-209.

Mahmood, S.S., Levy, D., Vasan, R.S. & Wang, T.J. (2014). The Framingham heart study and the epidemiology of cardiovascular disease: A historical perspective. *The Lancet, 383*(9921), 999-1008.

Majumder, P., Mazumdar, A., Kar, A., Halder, S. & Choudhury, P. (2016). Integration and regulation of fuel metabolism in maintaining homeostasis in human body. *International Journal of Applied Research, 2*(3), 481-487.

McGarry, J.D., Kuwajima, M., Newgard, C.B., Foster, D.W. & Katz, J. (1987). From dietary glucose to liver glycogen: The full circle round. *Annual Review of Nutrition, 7*(1), 51-73.

McLain, T.A., Wright, G.A., Camic, C.L., Kovacs, A.J., Hegge, J.M. & Brice, G.A. (2015). Development of an anaerobic sprint running test using a nonmotorized treadmill. *Journal of Strength and Conditioning Research, 29*(8), 2197-2204.

Meyer, C., Dostou, J.M., Welle, S.L. & Gerich, J.E. (2002). Role of human liver, kidney, and skeletal muscle in postprandial glucose homeostasis. *American Journal of Physiology, Endocrinology & Metabolism, 282,* 419-427.

Meyer, C., Stumvoll, M., Dostou, J., Welle, S., Haymond, M. & Gerich, J. (2002). Renal substrate exchange and gluconeogenesis in normal postabsorptive humans. *American Journal of Physiology, Endocrinology & Metabolism, 282,* 428-434.

Morgan, H.E., Henderson, M.J., Regen, D.M. & Park, C. R. (1961). Regulation of glucose uptake in muscle I. The effects of insulin and anoxia on glucose transport and phosphorylation in the isolated, perfused heart of normal rats. *Journal of Biological Chemistry, 236*(2), 253-261.

Musen, G., Jacobson, A.M., Ryan, C.M., Cleary, P.A., Waberski, B.H., Weinger, K. … & White, N. (2008). Impact of diabetes and its treatment on cognitive function among adolescents who participated in the Diabetes Control and Complications Trial. *Diabetes Care, 31*(10), 1933-1938.

Obermaier, K., Schmelzeisen-Redeker, G., Schoemaker, M., Klötzer, H. M., Kirchsteiger, H., Eikmeier, H., & del Re, L. (2013). Performance evaluations of continuous glucose monitoring systems: precision absolute relative deviation is part of the assessment. *Journal of Diabetes Science and Technology, 7*(4), 824-832.

Qazi, M.U. & Malik, S. (2013). Diabetes and Cardiovascular Disease: Insights from the Framingham Heart Study. *Global Heart, 8*(1), 43-48.

Robinson, C.S. & Sharp, P. (2012). Tighter accuracy standards within point-of-care blood glucose monitoring: How six commonly used systems compare. *Journal of Diabetes Science and Technology, 6*(3), 547-554.

Rohlfing, C.L., Wiedmeyer, H.M., Little, R.R., England, J.D., Tennill, A. & Goldstein, D.E. (2002). Defining the relationship between plasma glucose and HbA1c. *Diabetes Care, 25*(2), 275-278.

Rose, A.J. & Richter, E.A. (2005). Skeletal muscle glucose uptake during exercise: how is it regulated? *Physiology*, *20*(4), 260-270.

Salacinski, A.J., Alford, M., Drevets, K., Hart, S. & Hunt, B.E. (2014). Validity and reliability of a glucometer against industry reference standards. *Journal of Diabetes Science and Technology, 8*(1), 95-99.

Suh, S.W., Bergher, J.P., Anderson, C.M., Treadway, J.L., Fosgerau, K. & Swanson, R.A. (2007). Astrocyte glycogen sustains neuronal activity during hypoglycemia: Studies with the glycogen phosphorylase inhibitor CP-316,819 ([R-R*S*]-5-Chloro-N-[2-hydroxy-3-(methoxymethylamino)-3-oxo-1-(phenylmethyl) propyl]-1H-indole-2-cardboxamide). *Journal of Pharmacology and Experimental Therapeutics, 321*(1), 45-50.

Tack, C., Pohlmeier, H., Behnke, T., Schmid, V., Grenningloh, M., Forst, T. & Pfützner, A. (2012). Accuracy evaluation of five blood glucose monitoring systems obtained from the pharmacy: A European multicenter study with 453 subjects. *Diabetes Technology & Therapeutics, 14*(4), 330-337.

Thomas, L.E., Kane, M.P., Bakst, G., Busch, R.S., Hamilton, R.A. & Abelseth, J.M. (2008). A glucose meter accuracy and precision comparison: The FreeStyle Flash versus the Accu-Check Advantage, Accu-Check Compact Plus, Ascenia Contour, and the BD Logic. *Diabetes Technology & Therapeutics, 10*(2), 102-110.

Tseng, C., Kieffer, T.J., Jarboe, L.A., Usdin, T.B. & Wolfe, M.M. (1996). Postprandial stimulation of insulin release by glucose-dependent insulinotropic polypeptide (GIP). *The American Society for Clinical Investigations, 98*(11), 2440-2445.

Vincent, J. (1994). *Statistics in kinesiology.* Champaign, IL: Human Kinetics Books.

Wilson, J.E. (2003). Isozymes of mammalian hexokinase: Structure, subcellular localization and metabolic function. *Journal of Experimental Biology, 206*(12), 2049-2057.

Wolfrom, M.L., Lassettre, E.N. & O'Neill, A.N. (1951). Degradation of glycogen to isomaltose[1]. *Journal of the American Chemical Society, 73*(2), 595-599.

Wong, J.M.W. & Jenkins, D.J.A. (2007). Carbohydrate digestibility and metabolic effects. *The Journal of Nutrition, 137*(11), 2539-2546.

Yellow Springs Instruments. (2009). *YSI 2700 Select Biochemistry Analyzer user's manual*. Retrieved from https://www.ysi.com/File%20Library/Documents/Manuals%20for%20Discontinued%20Products/YSI-2700-Select-Biochemistry-Analyzer-Manual-n.pdf.

Zinker, B.A., Britz, K. & Brooks, G.A. (1990). Effects of a 36-hour fast on human endurance and substrate utilization. *Journal of Applied Physiology, 69*(5), 1849-1855.