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A Preliminary Analysis On The Effects Of Acute Beetroot Juice Intake On Glycemic And Blood Pressure Control In Type 2 Diabetes

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A PRELIMINARY ANALYSIS ON THE EFFECTS OF ACUTE BEETROOT JUICE INTAKE ON GLYCEMIC AND BLOOD PRESSURE CONTROL IN TYPE 2 DIABETES

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ABSTRACT

Cardiometabolic diseases are becoming increasingly prevalent in modern society due to low activity lifestyles and poor nutrition. Inorganic dietary nitrate ($diNO_3$) has shown to potentially improve cardiometabolic health in a number of different populations but remains to be corroborated in humans with type 2 diabetes (T2D). This preliminary analysis aimed to determine the effects of consuming an acute, concentrated dose of $diNO_3$ containing beetroot juice (BRJ) on hemodynamic and glycemic responses in humans with T2D while controlling for pharmacological influences. Three participants with a clinical diagnosis of T2D were recruited into this study. Pulsewave Velocity (PWV) was used to assess hemodynamic responses and an Oral Glucose Tolerance Test (OGTT) was used to measure glycemic responses following the consumption of BRJ or a denitrolized BRJ placebo. Hemodynamic (systolic blood pressure, diastolic blood pressure, and systemic vascular resistance) and OGTT outcomes (Matsuda, HOMA, and QUICKI) were assessed between conditions. Blood plasma and saliva were collected at baseline, two hours post BRJ consumption, and immediately following the OGTT test to assess changes in nitrate and nitrite concentrations. The results from this preliminary study showed that acute ingestion of BRJ led to a decrease in systemic vascular resistance

(SVR). Calculated SVR change scores showed dramatic decreases in response to the consumed nitrate ($p = 0.036$). Using Pearson's R, SVR was shown to have a very strong correlation to plasma nitrate ($R = -0.986$) yet was not detected to be significant ($p = 0.107$). While all NOx responses were observed to peak at expected rates, the only variable in which we were able to detect a significant difference from baseline to the two-hour timepoint was plasma nitrate concentrations. No other hemodynamic or glycemic responses were observed following acute BRJ supplementation.

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AUC	area under the curve
BRJ	beetroot juice
$DiNO_3$	inorganic dietary nitrate
eNOS	endothelial nitric oxide synthase
NO	nitric oxide
NR	nitrate reductase
OGTT	oral glucose tolerance test
PWV	pulsewave velocity
T2D	type 2 diabetes

INTRODUCTION

Cardiometabolic and inflammatory diseases, such as type two diabetes (T2D) and hypertension have been, and remain, a growing concern for the health of individuals subject to typical high-fat western diets and sedentary lifestyle options [1]. Characteristics and conditions associated with T2D include hyperglycemia and insulin resistance [2]. Individuals diagnosed with T2D have an elevated chance of developing dyslipidemia, hypertension, and atherosclerosis due to the presence of excessive oxidative stress, inflammation, and impairments within the vascular endothelium often resultant from chronic hyperglycemia [2-5]. It has been demonstrated that the presence of nitric oxide (NO) does have restorative properties for these conditions [6, 7]. NO can be produced enzymatically in healthy vascular endothelium, using endothelial nitric oxide synthase (eNOS) which catalyzes the reaction of L-arginine in the presence of oxygen into NO and L-citrulline [8, 9]. Chronic oxidative stress, inflammation, and hyperglycemia have all been shown to reduce functionality and denature the enzymatic pathways responsible for endogenous NO production [5]. The reduced capability to synthesize NO, in turn, increases the pathogenesis of these conditions within the body [10].

Inorganic dietary nitrate ($diNO_3$) can be consumed and reduced to nitric oxide (NO), which has been shown to improve many cardiovascular health benefits, such as

vasodilation, endothelial cell proliferation, attenuation of atherosclerotic development and inhibition of platelet aggregation [6, 11-15]. It is now well known that consumption of foods rich in dietary nitrate, such as leafy greens, spinach, and beetroot can increase diNO_3 concentration independent of eNOS [16-19]. DiNO_3-rich foods begin digestion in the mouth, where it is masticated, swallowed, and passed through the stomach into the small intestine. Approximately 75% of the ingested nitrate remains unabsorbed and continues through the digestive tract to be excreted [11, 13, 18]. As the remaining nitrate moves through the small intestine it is absorbed into systemic circulation and collected in the salivary glands as the NO_3 -rich blood circulates through the oral cavity. Once concentrated within the salivary glands, the facultative bacteria use nitrate reductase (NR) to enzymatically mediate the reduction of nitrate to nitrite. When nitrite is swallowed and introduced to the acidic environment of the stomach (as nitrite) a small portion will be reduced to NO, and diffuse across the gastric wall into systemic circulation [11, 12, 20, 21]. Remaining nitrite continues to the small intestine where it is absorbed back into systemic circulation as the NO storage intermediary and can be reduced to NO when exposed to hypoxic environments within the vasculature [20].

Decreased NO production has been associated with a multitude of chronic cardiometabolic diseases; more recently, associations have been observed between vascular endothelial cell (a large source of systemically available NO) functionality and insulin sensitivity [7, 22]. While the mechanisms behind these pathways remain largely unknown, NO is thought to act as a macrovascular mediator of both vascular health and insulin delivery pathways [6, 7, 12, 13, 23]. Deficiencies in enzymatic activity within eNOS, in combination with excess oxidative stress within the vascular endothelium, is

thought to be a major contributor in the pathogenesis of cardiovascular and inflammatory diseases, such as hypertension and diabetes [15]. It has also been shown that arterial stiffening and atherosclerosis often result from chronic hyperglycemia in addition to low NO bioavailability [24].

Rodent studies, conducted by Khalifi et al. [2] and Gheibi et al. [25], have assessed the effects of nitrate supplementation on blood glucose regulation. Khalifi et al. [2], examined glucose tolerance in male Wistar rats separated by induced diabetes status and chronic nitrate supplementation. Their data suggests that $diNO_3$ may assist with decreasing blood pressure and could be beneficial for glucose disposal. Gheibi et al has also demonstrated that $diNO_3$ supplementation provides favorable effects in diabetic rats versus their nontreated diabetic cohorts [25]. Gheibi observed that the nitrate supplemented diabetic rats' levels of serum glucose and insulin levels remained lower than the levels assessed from non-treated cohort, however still elevated compared to the controls. Additionally, Gheibi demonstrated that nitrate supplementation enhanced insulin response to glucose injections in the diabetic cohorts. $DiNO_3$ supplementation has been confirmed to ameliorate many adverse cardiometabolic conditions in numerous human and animal trials [2, 9, 16, 24-29]. Positive associations have also been observed in healthy humans correlating systemic NO bioavailability and insulin sensitivity [30]. It has also been demonstrated that NO can mediate the delivery of insulin within the vascular system as a means of improving glycemic response [7]. However, it is only rodent studies that have shown consuming $diNO_3$ can improve glycemic responses and lipid profiles in populations with T2D [2, 25]. $DiNO_3$ has been shown to have the capability of mediating the adverse cardiometabolic conditions in populations with T2D,

however, $diNO_3$ has yet to be corroborated as a potential therapy in restoring insulin sensitivity in human diabetic trials [31, 32].

Human trials conducted by Cermak et al. and Gilchrist et al. indicate that improvements in glycemic responses from $diNO_3$ supplementation observed in diabetic rodents and healthy humans does not translate to humans with diabetes [31, 32]. Cermak et al. (2015) administered an acute dose of sodium nitrate ($NaNO_3$) or a sodium chloride placebo to obese, hypertensive, males with type 2 diabetes (T2D) 2.5 hours prior to a standard oral glucose tolerance test (OGTT) with a 75-g glucose load [32]. Basal plasma glucose did significantly decrease after nitrate supplementation, compared to the placebo, before the administration of the OGTT. No significant differences in glucose concentration or insulin sensitivity were found [32]. Gilchrist et al. (2013) administered a nitrate rich and nitrate depleted beetroot juice to obese, elderly individuals with T2D for two weeks before an assessment of glycemic response via hyperinsulinemic-euglycemic clamp [31]. An important factor that may have impacted these results in both studies is that participants were not able to halt utilization of antidiabetic medication [31, 32]. These medications could be confounding any measured results by pharmacologically mediating glucose disposal [18]. It is important to control for these medications when attempting to determine the effects of dietary nitrate on glycemic control due to their interference of the nitrate reduction cycle as well as competition over the molecular pathways affected by the consumption of $diNO_3$ [18, 33].

$DiNO_3$ consumption has been repeatedly shown to attenuate the development of atherosclerosis by improving vasodilatory response and decreasing blood pressure. And when combined with human clinical trials, $diNO_3$ has demonstrated itself to be an

effective regulator of hemodynamic responses. These findings suggest a role for $diNO_3$ in individuals at greatest risk of developing coronary artery disease [24, 27-29, 33]. In healthy humans, positive correlations have been observed between synthesized NO and insulin sensitivity [30]. The destructive effects of chronic hyperglycemia and an overwhelming presence of oxidative stress denaturing NOS enzymes is a proposed rationale for the positive correlation between NO bioavailability and carbohydrate metabolism [10]. Pre-clinical studies in rodents suggest that the exogenous $diNO_3$ pathway provides a supplemental increase in NO availability in individuals with incapacitated NOS, insulin resistance, or diabetes. Yet, only the glycemic responses remain to be corroborated in humans. Therefore, the purpose of this study is to assess the effects of $diNO_3$, in the form of a beetroot juice (BRJ) beverage, on vascular hemodynamics and glycemic responses in individuals with T2D, while controlling for blood pressure and diabetes medication.

METHODS

Study Design and Participants

This randomized crossover trial consisted of three participants (1 male, 2 female) aged between 40 and 65 years with a clinical diagnosis of T2D. Inclusion criteria for participants were HbA1c ≤ 8%, nonhypertensive ([SBP/DBP] < 140/90), a willingness to stop taking anti-diabetic medication for up to four weeks and blood pressure medication for up to three weeks. Participants were also required to halt mouthwash usage and planned physical activity for the duration of the trial. Exclusion criteria included a potential participant currently taking more than two anti-diabetic medications or more than two blood pressure medications. Additionally, if a patient was taking rosiglitazone or pioglitazone for their diabetes, or they were not able to qualify due to not meeting the inclusion criteria they were excluded from this trial. Participants were recruited from the University of Alabama at Birmingham's Diabetes and Endocrinology Unit.

Participants were scheduled for a screening visit where they were assessed to determine qualification for inclusion and two study visits. During the screening visit, participants Participants met with medical staff to determine eligibility and provide informed consent before beginning protocols to remove them from anti-diabetic and blood pressure medications. Participants were instructed to stop taking diabetic medications two-weeks prior to the second visit, and any blood pressure medications one

week prior to study visit one by clinicians from the UAB endocrinology and diabetes clinic.

Study visits were identical and started around the same time (between 0800 am and 0900 am). Participants arrived fasted and were assigned a condition in a randomized order. Anthropometric measures (height, weight, BMI) were taken as the participants arrived and then were given a 10-minute rest period to allow physiological measures to return to resting states. After the 10-minute rest period hemodynamic measures were collected using Pulsewave Velocity (HDI/Pulse Wave TM CR-2000, Hypertension Diagnostics. Eagan, MN) and an automated sphygmomanometer located in the UAB Clinical Research Unit (CRU). Upon obtaining all hemodynamic measures, a blood draw was taken to assess baseline serum nitrate and nitrite. Saliva was also collected to assess for baseline salivary nitrate and nitrite. Once all baseline measures had been obtained 140 mL of one of the two randomized conditions was administered; 1) NO_3^- rich beetroot juice (BRJ) approximately 6.4 mmol of NO_3^- per 70 mL; Beet it; James White Drinks, Ipswich, UK and 2) NO_3^- depleted beetroot juice (PLA) approximately 0.04 mmol of NO3- per 70 mL; Beet it; James White Drinks, Ipswich, UK). Participants remained seated in the CRU for two hours. Upon completion of the waiting period 2-hour follow up measures were taken with the pulsewave and a 2-hour post blood draw and saliva sample were collected. Data was collected in the same order as baseline testing. Participants then underwent a two-hour oral glucose tolerance test (OGTT). A flexible catheter was placed in the antecubital space of the right arm and blood was then drawn at 10- and 5- minutes before the administration of the dextrose load to provide fasting measures. At time 0 the participant was instructed to drink a 75g dextrose load within

five minutes. Blood was then collected at 10, 20, 30, 60, 90, and 120 minutes to assess changes in plasma glucose, insulin and c-peptide concentrations. At 120 minutes a third collection of blood and saliva was collected to assess nitrate and nitrite concentration for the duration of the visit. At the end of the visit a togue scrape was taken to assess activity levels of NR containing bacteria from the oral cavity. A disposable lab spatula was used to lightly scrape the back of the tongue five times to the right and five times to the left. The bacterial collection was submerged and mixed in 500 μL of BHI broth then set on ice until processing. Following processing the bacterial sample was stored at -80°C and remains to be analyzed. Each visit lasted approximately five to six hours and were separated by at least 72 hours as a washout period.

Vascular Hemodynamics

Hemodynamic variables were measured using pulsewave velocity (HDI/Pulse Wave TM CR-2000, Hypertension Diagnostics. Eagan, MN). Data was collected for systolic (SBP), diastolic (DBP), and mean arterial pressure (MAP). The pulsewave also measured pulse rate (PR) and generated measures for large (LAE) and small artery elasticity (SAE), total vascular impedance (TVI), systemic vascular resistance (SVR), estimated cardiac output (Est. CO), and estimated cardiac index (Est. CI). Participants were placed in an upright, seated, position and a tonometer was set firmly over the right radial artery with an oscillometric cuff placed on the contralateral brachial artery. Upon acquisition of a stable pulse contour from the tonometer, the waverform was calibrated with the oscillometric cuff and a 30 second tracing of the waveform was analyzed. The waveform was analyzed three times at baseline and three times at the end of the two-hour

waiting period [34]. Blood pressure was also assessed via automated sphygmomanometer located in the UAB CRU three times prior to any PWV assessment. The cuff was attached to the same arm as the oscilometric cuff for the PWV.

Nitrate and Nitrite Analysis

Blood samples were collected from participants at every timepoint to assess changes in plasma nitrate and nitrite for the duration of the study visit. After obtaining all hemodynamic measures, blood was drawn from the antecubital vein of the right arm by phlebotomists in UAB's Clinical Research Unit (CRU). Blood was drawn into two vacutainers, one containing EDTA and the other containing citrate. The citrate tube was spun within two minutes at 5000 G's for three minutes to separate the blood sample. Supernatant was collected and placed into 500 µL aliquots, then flash frozen in liquid nitrogen and stored at -80°C until analysis. The EDTA tubes sat for at least ten minutes, then spun at 5000 G's for ten minutes to separate the blood. Supernatant was placed into 500 µL aliquots, flash frozen in liquid nitrogen, and stored at -80°C until analysis. Saliva samples were collected in tandem with serum samples. 1.5 mL of saliva was spun at 3000 G's for between three and five minutes, or until separation was visible. Two 500 µL aliquots of salivary supernatant were collected, flash frozen in liquid nitrogen and stored at -80°C until analysis. Serum and saliva samples were analyzed by the UAB Free Radical Biology Core.

Oral Glucose Tolerance Test

Insulin sensitivity and glycemic response were assessed using a two-hour oral glucose tolerance test (OGTT). The OGTT was initiated after the second round of hemodynamic measures were collected two hours after the ingestion of one of the BRJ supplements. Prior to the start of the OGTT an intravenous catheter was placed in the antecubital space of the right arm. Two blood samples were taken at 10- and 5- minutes before ingestion of a 75-g dextrose solution at time 0 to assess fasting glucose measures and provide baseline measures. After the ingestion of the dextrose load, blood samples were collected at 10, 20, 30, 60, 90, and 120 minutes. Blood was collected to measure serum glucose, insulin, and c-peptide concentrations. Serum was stored in a -80°C freezer until analysis. OGTT serum was analyzed in the Metabolism Core on the UAB campus.

Statistical Analysis

Preliminary data are expressed as group means ± SD and individually for the three participants. Means, standard deviations and 95% confidence intervals were calculated for glucose response variables (OGTT time points for serum glucose, insulin, and c-peptide and calculated area under the curve; and insulin sensitivity and resistance measures Matsuda, HOMA, QUICKI) and hemodynamic response variables (Cuff SBP and DBP, PWV SBP and DBP, MAP, PR, LAE, SAE, TVI, SVR, Est. CO, and Est. CI) and their respective change scores. Paired two-tailed t-tests were used to assess the differences between the absolute measures and change scores between experiment and placebo conditions. For all data analysis a p-value of less than 0.05 was determined

statistically significant. Statistical analysis was performed by using the Statistical Package for the Social Sciences (SPSS) version 26.0.0 (IBM, Armonk, NY). Area under the curve (AUC) was assessed by plotting time points on a curve (concentration by time) and calculated via trapezoidal summation.

RESULTS

Descriptive Statistics

In our attempt to investigate hemodynamic and glycemic responses to BRJ in people with type 2 diabetes we were able to recruit three participants between the ages of 55 and 66 years. We did not control for race or gender for this pilot study and had both African Americans and Caucasians represented in this analysis as well as males and females. Descriptive statistics have been displayed for our sample and each individual in **Table 1**.

Table 1

Descriptive Data

SUBJECT	AGE (years)	GENDER	RACE	HEIGHT (m)	WEIGHT (kg)	BMI (kg/m^2)
T2D-01	60	F	AA	1.55	71.10	29.62
T2D-02	66	F	AA	1.78	93.15	29.47
T2D-03	55	M	C	1.88	91.80	25.98
Sample means	60 ± 5.5			1.7 ± 0.2	85.4 ± 12.4	28.4 ± 2.1

Hemodynamic Responses

Hemodynamic response change scores for blood pressure variables and systemic vascular resistance (SVR) are reported in **Table 2**. Change scores were calculated by subtracting pre- scores from their respective post- scores for each hemodynamic variable. Paired two-tailed t-tests showed no significantly different blood pressure responses, but a significant difference between the change scores for SVR was detected ($p = 0.036$). Hemodynamic responses for the sample are demonstrated in **Figure 1** using absolute units. No significant changes were detected. Although the change scores for SVR were different, when analyzed as raw data SVR differences approached but was unable to show significance ($p = 0.07$). Individual hemodynamic responses affected by the BRJ supplement are demonstrated in **Figure 2**.

Table 2

Hemodynamic Change Scores Comparisons

	Δ Cuff Sys (mmHg)		Δ Cuff Dia (mmHg)		Δ PWV Sys (mmHg)		Δ PWV Dia (mmHg)		Δ SVR	
	BRJ	PLA	BRJ	PLA	BRJ	PLA	BRJ	PLA	BRJ	PLA
T2D-01	-12.33	17.00	-3.00	-0.67	3.00	-8.67	-2.33	2.00	-104.00	86.67
T2D-02	3.67	-19.00	0.67	6.67	15.33	12.33	4.67	4.33	-47.33	96.33
T2D-03	-3.67	0.67	-1.33	-0.33	-4.67	13.00	-1.33	0.67	-10.33	86.33
Sample Means	-4.11 ± 8.01	-0.44 ± 18.03	-1.22 ± 1.84	1.89 ± 4.14	4.56 ± 10.09	5.56 ± 12.32	0.33 ± 3.79	2.33 ± 1.86	-53.89 ± 47.18 *	89.78 ± 5.68

Individual and population hemodynamic responses reported in change scores ($Variable_{POST}$ - $Variable_{PRE}$ = ΔVariable). Means reported as mean ± SD. (* = $p < 0.05$)

Glycemic Responses

Insulin sensitivity and resistance measures are reported in **Table 3**. No differences in insulin sensitivity or resistance measures were observed in response to the BRJ. Sample data for glycemic response measures have been shown in **Figure 3**. No differences were observed at any of the timepoints for serum glucose, insulin, or c-peptide concentration. Additionally, the inserted AUC measures indicate that there were no differences in the serum glycemic concentrations for the total duration of the OGTT and are reported in **Table 4**. **Figure 4** shows each individual's response for serum glucose, insulin, and c-peptide concentrations for the duration of the two-hour OGTT in response to both conditions. An area under the curve measure has been inserted into each curve to provide a more detailed individual response over time for each variable.

Table 3

Insulin Sensitivity/Resistance Comparisons

	Matsuda		HOMA		QUICKI	
	BRJ	PLA	BRJ	PLA	BRJ	PLA
T2D-01	1.761	3.288	8.404	2.844	0.283	0.327
T2D-02	2.668	2.325	2.326	2.465	0.336	0.333
T2D-03	7.013	6.265	1.213	1.306	0.372	0.367
Sample Means	3.81 ± 2.81	3.96 ± 2.05	3.98 ± 3.87	2.20 ± 0.80	0.33 ± 0.04	0.34 ± 0.02

Table 3. Individual and population insulin sensitivity and resistance measures. (Mean ± SD)

Table 4

OGTT AUC Data

	Glucose		Insulin		C- peptide	
	BRJ	PLA	BRJ	PLA	BRJ	PLA
T2D-01	35357.5	34672.5	4632.5	4254.8	929.3	788.8
T2D-02	15692.5	16405.0	15352.5	18162.8	1204.3	1348.5
T2D-03	28637.5	30932.5	2196.3	2610.5	652.0	646.6
Sample Means	26562.5 ± 9995.4	27336.7 ± 9650.0	7393.8 ± 6999.3	8342.7 ± 8544.1	928.5 ± 276.1	927.9 ± 371.1

Plasma and Salivary Nitrate and Nitrite Responses

Sample and individual nitrite and nitrate responses are displayed in **Figure 5** and **Figure 6**, respectively. A significant increase plasma nitrate was observed in our measured sample ($p = 0.023$; 0.019). No other variables were significant. There was over a twenty-fold increase in plasma nitrate by two hours after the consumption of the BRJ supplement and remained elevated for the entirety of the visit. No increases in concentrations of salivary nitrite or nitrate were detected between any timepoints. In **Table 5** correlations between the two-hour rise in plasma nitrate and study variables have been reported. No significant correlations were observed. SVR had the greatest correlation ($R^2 = -0.986$; $p = 0.106$) assessed in the study variables but was unable to reach significance.

Table 5.

Plasma Nitrate Correlations

Variable	Person's R	p- value
Hemodynamic Measures		
Δ Cuff Systolic	-0.501	.666
Δ Cuff Diastolic	-0.413	.729
Δ PWV Systolic	0.422	.722
Δ PWV Diastolic	-0.086	0.945
Δ Pulse Pressure	0.726	0.483
Δ Pulse Rate	-0.448	.704
Δ Est. Cardiac Output	-0.177	.887
Δ Est. Cardiac Index	-0.283	.817
Δ Systemic Vascular Resistance	-0.986	.106
Δ Total Vascular Impedance	0.605	.586
Δ Large Artery Elasticity	-0.486	.677
Δ Small Artery Elasticity	0.235	.849
Glycemic Responses		
Glucose AUC	0.292	.811
Insulin AUC	0.219	.859
C-Peptide AUC	0.541	.636

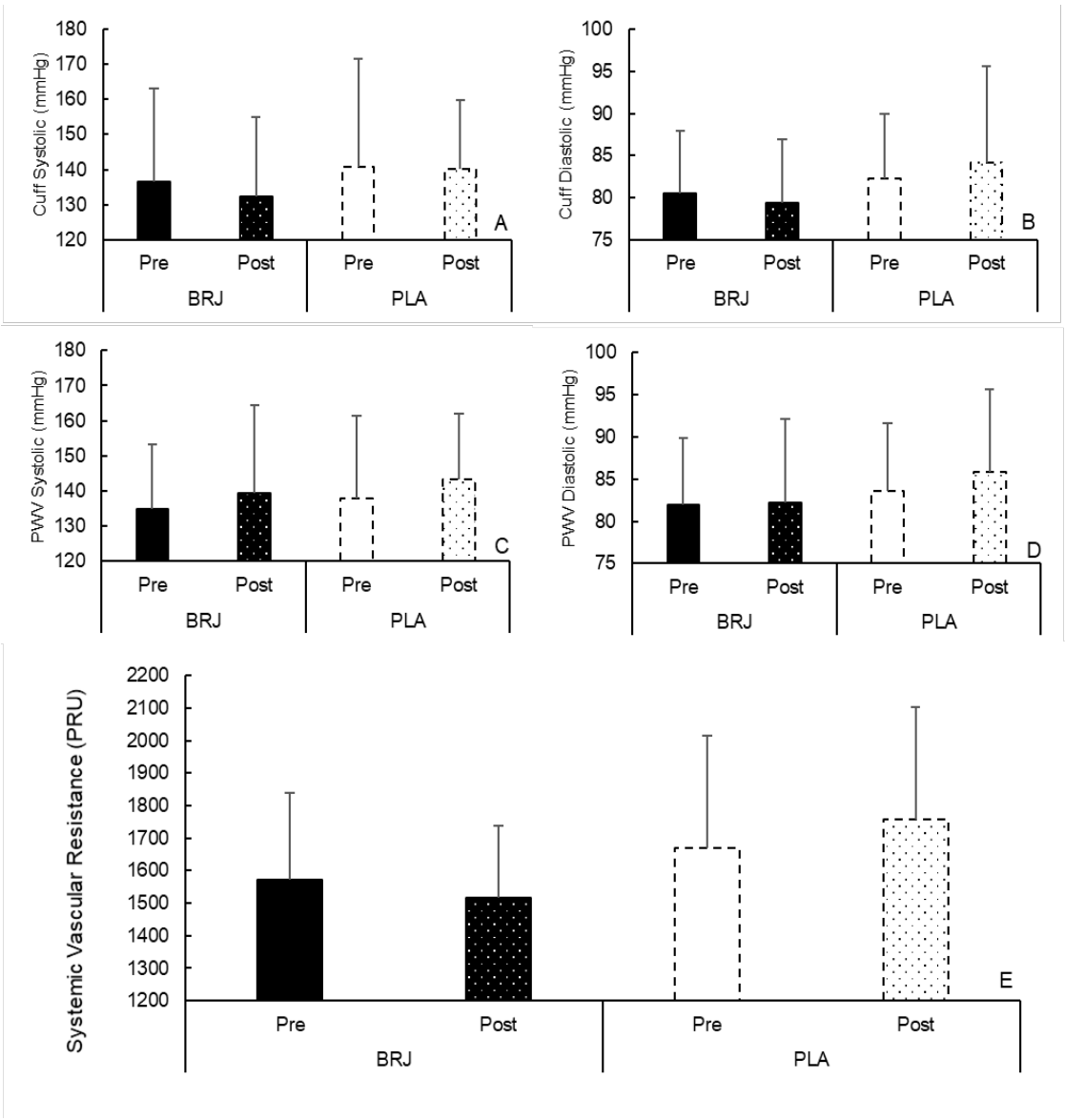

Figure 1. Sample Hemodynamic Responses
(Mean ± SD)

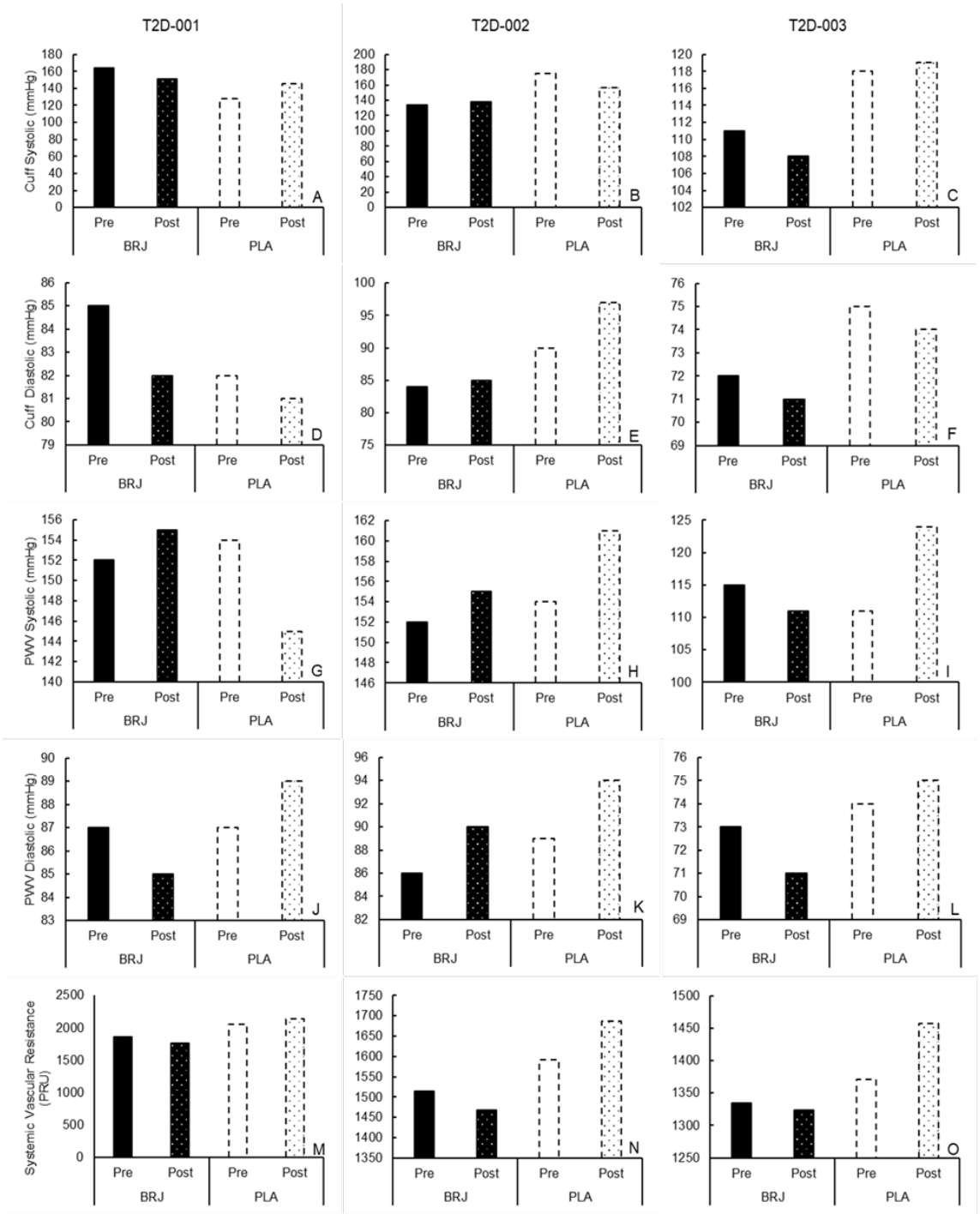

Figure 2. Individual Hemodynamic Responses
(Raw scores)

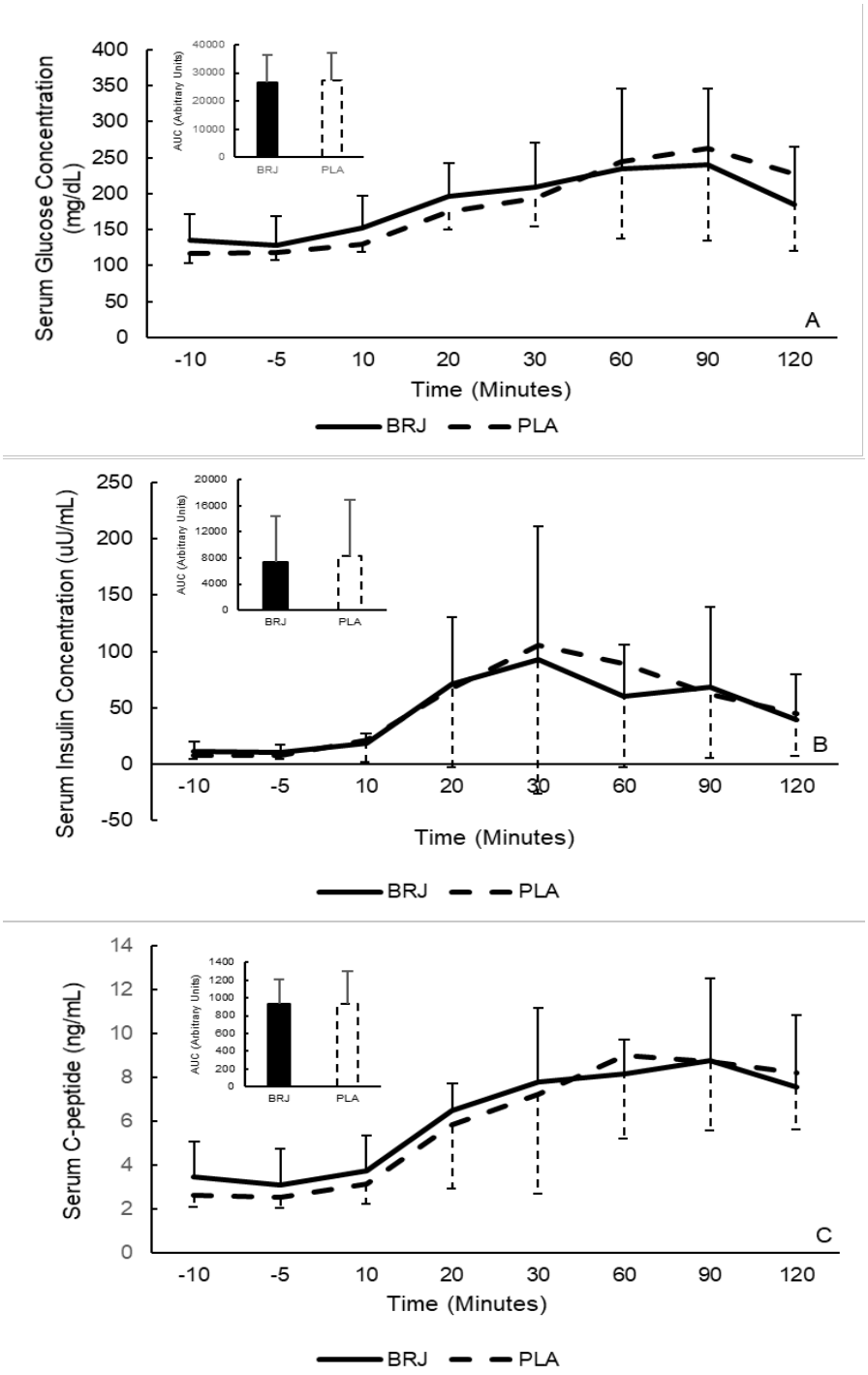

Figure 3. Sample Glycemic Responses
(Mean ± SD); AUC inserted and reported in arbitrary units.

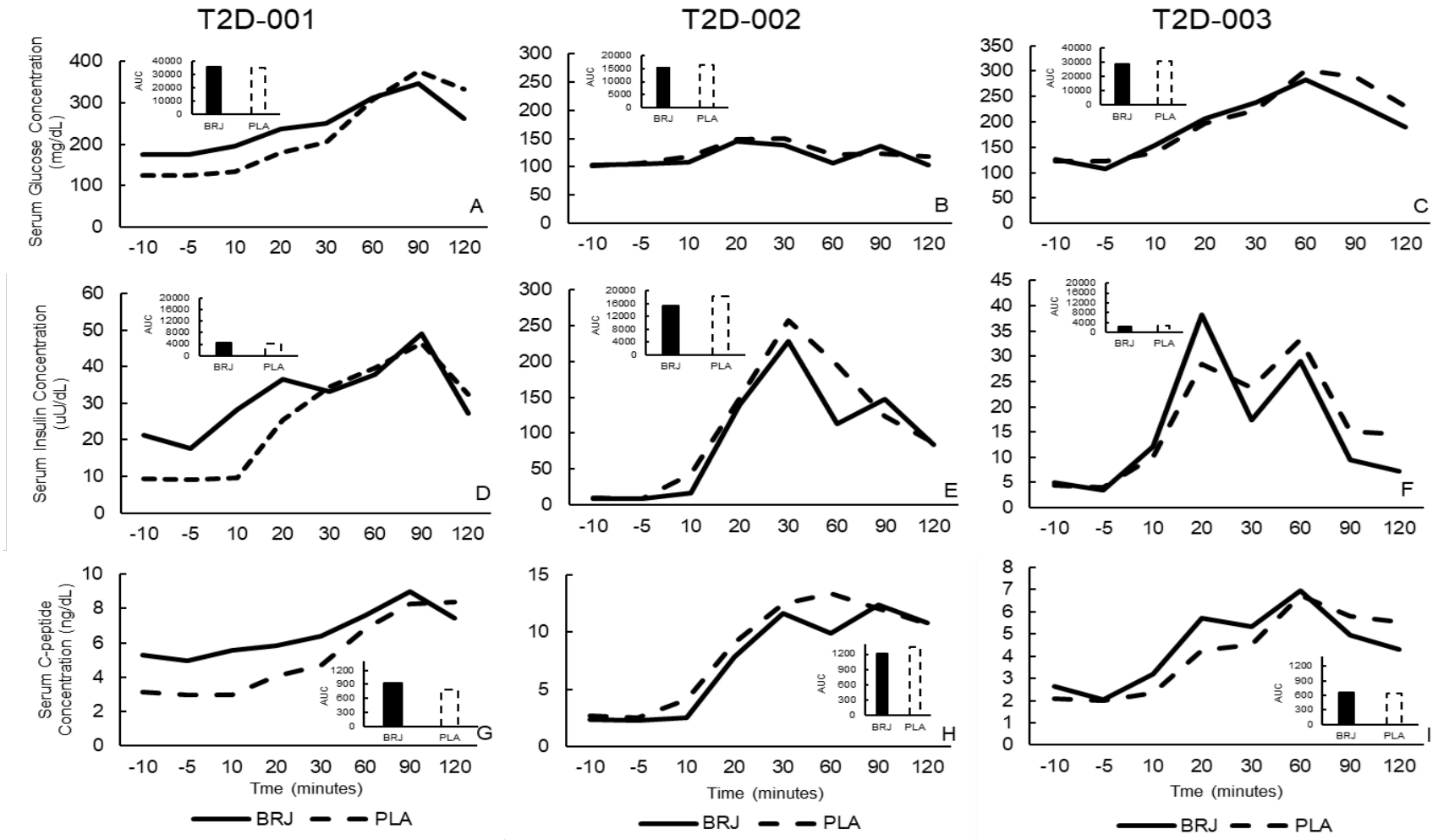

Figure 4. Individual Glycemic Responses
(Raw scores); AUC inserted and reported in arbitrary units.

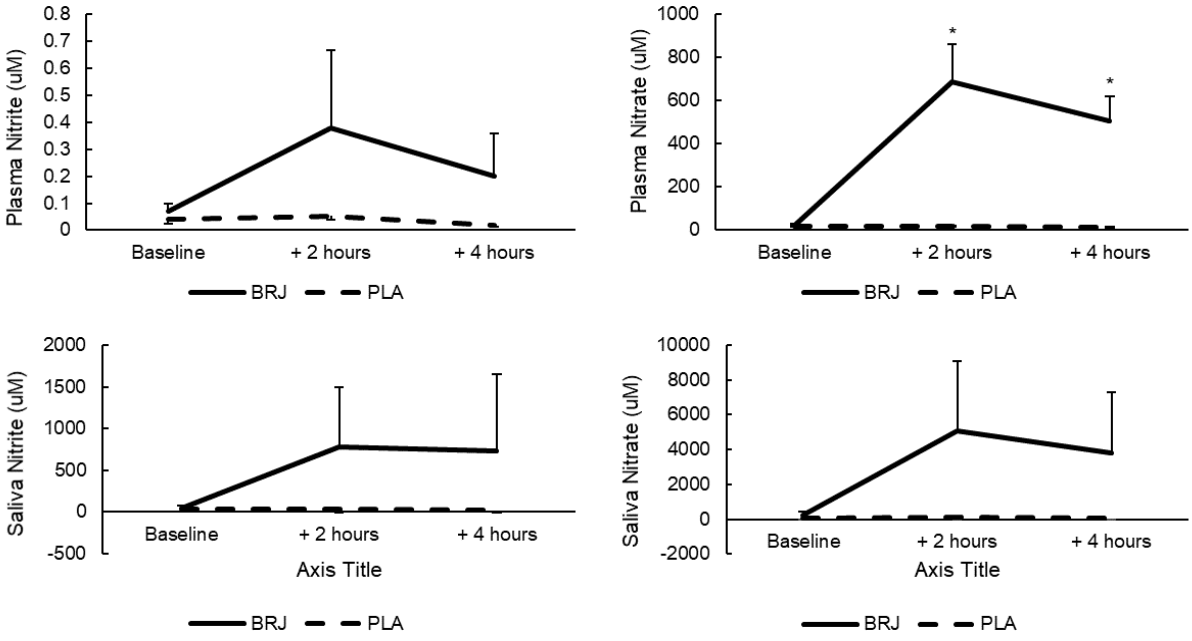

Figure 5. Sample Plasma and Salivary Nitrite and Nitrate Responses
(Mean ± SD)

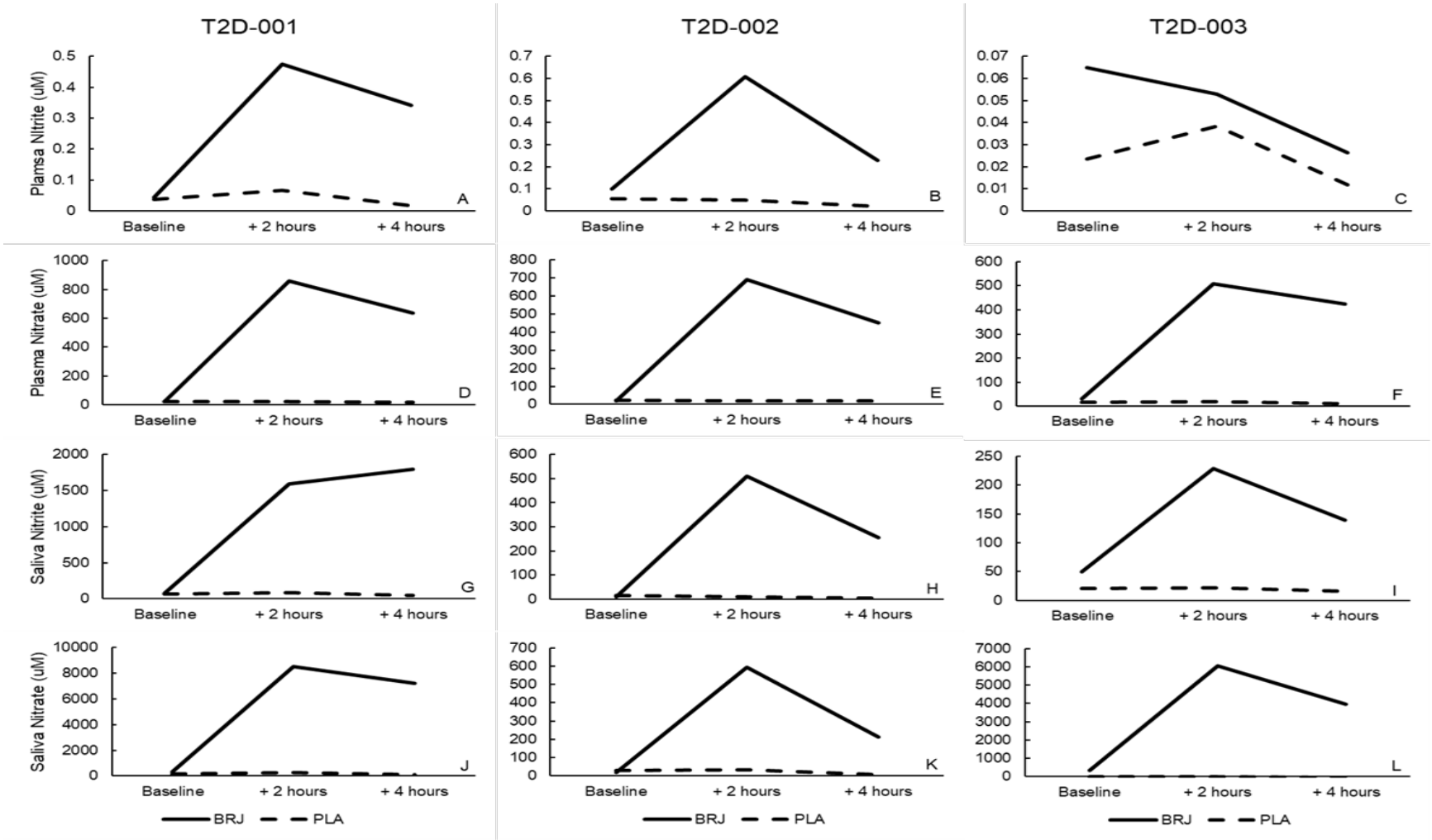

Figure 6. Individual Plasma and Salivary Nitrite and Nitrate Responses (Raw scores)

DISCUSSION AND CONCLUSIONS

In our study of men and women with type 2 diabetes, we found that ingestion of beetroot juice decreased systemic vascular resistance but had no effects on any other hemodynamic or glycemic responses. To our knowledge, this is the first investigation that has allowed participants with diabetes to be removed from both hypertension and antidiabetic medications to assess the effects of increased $diNO_3$ ingestion without pharmacological influences. $DiNO_3$ has been repeatedly shown to increase serum NOx and mediate specific hemodynamic responses in humans and has demonstrated utility in increasing rates of glucose shuttling in rodents [2, 7, 24, 25, 30]. While the NOx and hemodynamic responses have been corroborated by human trials, glycemic responses remain to be observed in humans with type 2 diabetes [31, 32].

Hemodynamic Responses

These preliminary data suggest that beetroot juice may decrease systemic vascular resistance but had no effects on other hemodynamic measures or glycemic responses. Within our sample no significant differences were detected, however SVR was the only hemodynamic variable that had a lowering effect in all three participants, demonstrated in **Figure 2**. It is also worth noting that within participant 1 and participant 3 that every hemodynamic variable (excluding T2D-01 PWV Systolic blood pressure) had a lowering

response to the nitrate-rich BRJ. Participant 2 seemed to have either no or a raised response in their hemodynamic variables (excluding SVR) to the nitrate-rich BRJ. With such a small sample size, this one dissimilar response carries much weight when running statistical analysis.

Glycemic Responses

No affect was observed in glycemic response from the nitrate-rich BRJ. AUC remained slightly lower across all three variables in the sample, but no significance was detected. While observing individual responses, participant 2 appeared to have the greatest insulin concentrations by a factor of approximately 2.5 to 4 times the other two participants, and the highest c-peptide concentrations, regardless of the condition. It does appear that the BRJ conditions lowered serum glucose concentration between 20 and 100 mg/dL by the end of the OGTT compared to the placebo. However, there were no statistically significant changes between conditions or major differences between timepoints on the glycemic response variables for any participant. No differences were observed in any insulin resistance or sensitivity measures.

Plasma and Salivary Nitrate and Nitrite Responses

A significant increase in plasma nitrate was observed in our sample. No changes in salivary nitrate or nitrite or plasma nitrite concentrations were detected. Plasma nitrate was significantly higher two hours after the consumption of the nitrate-rich BRJ supplement and remained elevated for the duration of the study. There appeared to be a

25-fold increase in plasma nitrate which is comparable to other studies assessing the rate of plasma nitrate concentration in response to a high $diNO_3$ load [27, 35, 36]. Jonvik et al were also able to detect significant increases in plasma nitrite in addition to the plasma nitrate peaking. It is difficult to determine the effects these compounds have within the vasculature when we are unable to detect a significant increase in their prevalence within the body's environment. Due to only finding a significant increase in plasma nitrate correlations were calculated to ASSESS if the peak in plasma nitrate affected any of our measured variables. While no correlations may be deemed significant, there was a very strong correlation between a change in plasma nitrate from baseline to hour 2 and a change in SVR in the same time period ($R = -0.986$; $p = 0.106$). While we cannot say that dietary nitrate does beneficially effect SVR in people with Type 2 diabetes, this does show potential that nitrate consumption may promote total vascular health in this population.

Drawbacks and Implications

The primary weakness of this analysis was a lack of participants, and consequently, statistical power to indicate any other potential physiological responses. In the study conducted by Gilchrist et al, researchers were able to observe significant increases in plasma nitrate and nitrite even without evidence of our hypothesized physiological responses [31]. In various other publications $diNO_3$ consumption has still been shown to significantly upregulate the concentration of plasma NOx in subjects with diabetes and healthy humans [2, 25, 27]. While we were able to observe a significant increase in plasma nitrate, we did not see the expected rise in serum nitrite, like

mentioned in the previous studies, and subsequently lacked the NO bioavailability necessary to observe known physiological responses.

Moving forward, more participants will be needed to observe any hemodynamic or glycemic responses to the $diNO_3$ present in BRJ. While no conclusions have yet to be drawn from this study it would also be worth looking into the effects of chronic dosing with $diNO_3$, similar to the protocol used by Gilchrist et al under careful medical supervision. Additionally, experimenting with BRJ to alleviate some of the stress that developing chronic hyperglycemia may put on the vascular endothelium could prove beneficial as a preventative therapy in those who are prediabetic. This study remains ongoing and plans to continue recruitment and testing to provide a more complete dataset. With an increased sample size and statistical power, dietary nitrate remains being investigated as a potential therapy to increase NO bioavailability in those with type 2 diabetes to improve insulin sensitivity and glucose shuttling.

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APPENDIX

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM
Office of the Institutional Review Board for Human Use

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Birmingham, AL 35294-0104
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APPROVAL LETTER

TO: Fisher, Gordon

FROM: University of Alabama at Birmingham Institutional Review Board
Federalwide Assurance # FWA00005960
IORG Registration on # IRB00000196 (IRB 01)
IORG Registration on # IRB00000726 (IRB 02)

DATE: 20-Sep-2019

RE: IRB-300002656
A Pilot Study on the Effects of Acute Beetroot Juice Intake on Glycemic and Blood Pressure Control in Type 2 Diabetics

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The IRB reviewed and approved the Initial Application on submitted on 20-Sep-2019 for the above referenced project. The review was conducted in accordance with UAB's Assurance of Compliance approved by the Department of Health and Human Services.

Type of Review: Full (Institutional Review Board 01 (UAB))
Determination: Approved
Approval Date: 20-Sep-2019
Approval Period: One Year
Expiration Date: 09-Jul-2020

Documents Included in Review:

- partialwaiver.181206
- hsp.190920.docx
- Patient Diary.19.0816.docx
- consent.190920.docx
- Assessment Checklist.19.0813.docx