UTILIZING DIGITAL THERMAL MONITORING (DTM) TO MEASURE REACTIVE HYPEREMIA OF TYPE 1 DIABETES MELLITUS ON CARDIOVASCULAR ENDOTHELIAL FUNCTION

by

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Abstract

The role of type 1 diabetes mellitus (T1DM) on the female cardiovascular system remains largely a mystery. However, it is widely accepted that insulin plays a role in cardiovascular protection. Hemoglobin A1C levels allow for further understanding of glucose impact upon endothelium and endothelial function in young females currently utilizing continuous glucose monitoring (CGM) along with insulin therapy. Flow-mediated dilation provides images of the dilation of the brachial artery under shear stress. The control group, those without a diagnosis of T1DM, are expected to have lower levels of hemoglobin A1C. The experimental group, women with T1DM, experienced significantly higher hemoglobin A1C levels and along with vascular reactivity index (VRI) scores than the control group. This finding has future influences in female cardiovascular health, protection of endothelium, and the wider debate over the influence T1DM and tighter controls of glucose regulation has on endothelial health.

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Introduction

According to the *World Journal of Diabetes*, cardiovascular disease (CVD) is the leading cause of death in those with type 1 diabetes mellitus (T1DM) (Bertoluci et al., 2015). Women with T1DM are more likely to have a CVD event than are non-diabetic women (Ferranti, 2014). Research has shown that individuals with T1DM are prone to accelerated atherosclerosis. Endothelial dysfunction plays a pivotal role in all stages of atherosclerosis. However, the pathophysiology involving T1DM is not well understood as research has only focused on certain CVD risk factors of type 2 diabetes mellitus (T2DM), such as chronic hyperglycemia. Chronic hyperglycemia is a weak predictor of macrovascular complications in both T1DM and T2DM while endothelial dysfunction (ED) can explain the residual unknowns of cardiovascular risk, specifically in T1DM (Bertoluci et al., 2015).

Clinical Question

This research project addresses the clinical question: How does endothelial dysfunction in females with type 1 diabetes change cardiovascular disease risk? The population of interest is female Texas Christian University (TCU) students with and without type I diabetes. Endothelial dysfunction will be measured using the FDA approved and non-invasive Vascular Endothelial Dysfunction (VENDYS-II) device to measure the function of vascular tone and blood flow in the upper extremity via digital thermal monitoring (DTM).

Background

Endothelial cells are a single layer of cells that form the innermost layer of blood vessel lining (tunica intima). Endothelial cells have multiple, vital functions in the body. They regulate fluid and molecule diffusion between blood and tissue; play a role in preventing clots; contribute to vascular homeostasis and repair; and play a central role in angiogenesis, tissue wound healing, vascular tone, and blood flow regulation (Deanfield, Halcox & Rebelink, 2017). The most important action of endothelium is the ability to alter blood supply by dilating or constricting the blood vessel as needed, also known as vasomotion. Vasomotion is controlled by the enzyme, specifically endothelial nitric oxide synthase (eNOS), nitric oxide (NO) diffuses to vascular smooth muscle to synthesize a short-acting gas. NO is made in response to change in homeostasis, peptides, hormones, proteins, platelets, immune cells, and more (Deanfield, Halcox & Rebelink, 2017). When activated in the vascular system, NO maintains the quiescent state of the vascular wall by controlling inflammation, cellular proliferation, and thrombosis. Therefore, reduced levels of NO lead to endothelial dysfunction, a systemic pathological state of an imbalance between vasodilating and vasoconstricting substances. Endothelial dysfunction precedes cardiovascular structural disorders, plaque buildup, and clinical events (Deanfield, Halcox & Rebelink, 2017).

Endothelium is involved in a multitude of processes; it is important to know the degree of impact the T1DM has on endothelial function to intervene in a timely manner to prevent CVD. Assessing the endothelial role in vascular tone and blood flow regulation is the most effective way to measure endothelial function (Naghavi et al., 2016). VENDYS-II assesses reactive hyperemia, the transient increase in blood flow following a brief period of blood flow occlusion (ischemia). Ischemia results in tissue hypoxia or anaerobic metabolism which causes accumulation of lactate and other chemicals that cause the vessel to dilate. Once the cuff deflates, blood flow is restored, and the rate is increased to two to three times higher than previously. **The reactive hyperemia response relies on functional endothelial cells, therefore if arteries are functional, a greater hyperemic response will occur.** The machine uses smart temperature sensors at the fingertips and algorithms to measure vascular reactivity. It includes a

5-minute brachial vessel occlusion, where fingertip temperature falls due to a decrease in warm blood flow causing finger temperature to decrease. When the cuff is released blood flow rushes into forearm and hand temperature rebounds which is directly proportional to vascular reactivity. Since blood is warm, the more blood flow, the higher temperature at the fingertip which indicates better vascular function (Naghavi et al., 2016).

Literature Review

A literature review was conducted using EMBASE and PubMed. Keywords included *female*, *type 1 diabetes*, *diabetes*, *endothelial function*, *cardiovascular disease*, *FMD*, and *young adults*. Searches were limited to articles published within the past seven years and in English.

Bruzzi et al. (2014) conducted a long-term follow-up study to examine the differences in endothelial function in children and adolescents with type 1 diabetes and non-diabetic individuals. The researchers identified 84 children and adolescents between the ages of 5 and 19 and followed up with them over the period of a year. The subject group consisted of 39 individuals with insulin-dependent type 1 diabetes mellitus receiving care at the Pediatric Diabetes Clinic and 45 healthy individuals as the control population. The participants fasted overnight, and blood sample collections were obtained in the morning prior to insulin administration and the ultrasound test. A single vascular ultrasonographer conducted flow-mediated vasodilation (FMD) of the brachial artery and brachial artery diameters (BAD) at end-diastole for all participants. They inflated the sphygmomanometer to 200 mmHg for 5 minutes and monitored the brachial vessel with a Doppler echocardiogram during the resting scan, for 15 seconds after cuff deflation, and BAD measurements were taken 45 to 60 seconds after cuff deflation. FMD was calculated by the average maximal obtained diameter during ischemiainduced hyperemia over four cardiac cycles. Results showed that the subjects with T1DM had worsening FMD at follow-up with 74.3% showing endothelial function impairment than at baseline ($p=0.011$). These findings demonstrate that individuals with T1DM have impaired endothelial function even within a few years of diagnosis and FMD may be an effective prognostic tool for identifying development of cardiovascular risk (Bruzzi et al., 2014).

A meta-analysis published included 58 articles addressing vascular dysfunction and vascular smooth muscle attributable type 1 diabetes (Lespagnol et al., 2020). To be included in the analysis, studies had to have both a treatment and control group, assessed peripheral vasodilatory capacity, and be conducted in humans with T1DM without vascular complications. Researchers pooled data from all the included articles to come up with an overall standard mean difference (SMD) to statistically analyze data. This analysis revealed significant impairments in ED and small accompaniments of vascular smooth muscle (VSM) dysfunction. Additionally, macrovascular effects were more apparent in endothelial dysfunction than microvascular. (Lespagnol et al., 2020). However, the study did not account for individuals with uncomplicated type 1 diabetes. The correlations evident in numerous studies highlight the extensive, harmful effects T1DM has on early endothelial dysfunction, especially in larger vessels.

Wang et al. (2019) conducted a systematic review (SR) and meta-analysis of the relationship between atherosclerosis and T1DM. The review included 90 studies that met the strict inclusion and exclusion criteria set in advance of the review. Criteria included: cohort or case-control studies with data on both patients diagnosed as having T1DM and healthy controls, no clinical vascular disease, and pertaining to instruments and diagnostics tests. The results of the review showed greater cIMT and significantly lower FMD in T1DM persons. These findings suggest people with T1DM have a higher risk for cardiovascular disease, as evident in an increase in cIMT and arterial stiffness and an increase in FMD and endothelium-independent vascular dilation. T1DM people should have strict monitoring of their blood sugar, lipid profiles, blood pressure, and other atherosclerotic factors contributing to cardiovascular disease.

Pareyn et al. (2013) engaged in a comparative study of reactive hyperemia peripheral artery tonometry (RH-PAT) in adolescents with T1DM and without diabetes. The study included 34 individuals with T1DM and 25 control subjects. All participants were Caucasian, aged 12 to 20 years old, and nonsmokers. The intervention group of adolescents with diabetes had to be officially diagnosed with T1D and treated with insulin for at least one year. There were no exclusion criteria for overweight or obese individuals. The study was conducted in a fasting state in the same noise and temperature-controlled room. Each participant's hands were placed at heart level with the fingers hanging freely. The RH-PAT device recorded a 5-minute baseline temperature with fingertip probes, then a blood pressure cuff on the non-dominant arm was inflated to occlude the arm for 5 minutes then the pulse wave amplitudes of reactive hyperemia in the nondominant fingertips were recorded for 5 minutes after cuff deflation. A software program calculated the ratio of the average blood volume before and after occlusion in both fingers to achieve a RH-PAT score. A lower RH-PAT score reflects more endothelial dysfunction. Results showed that adolescents with T1D had lower median RH-PAT scores than the control group ($p = 0.0154$), indicating that the experimental group had more ED present. Closer examination of differences within gender showed females with T1D had lower RH-PAT scores ($p = 0.0057$) compared to controls, while males had no significant difference in RH-PAT scores ($p = 0.7277$). This study indicates that females with T1DM have a higher risk of ED and subsequently CVD. However, findings cannot be generalized to individuals older than 20 years old or to ethnicities other than Caucasian.

Love et al. (2022) assessed arterial stiffness in individuals with type 1 diabetes via carotid-femoral pulse wave velocity (cfPWV) and FMD through a cross-sectional study. Participants included 41 adult and adolescents with an average age of 24 years and a diagnosis of type 1 diabetes. Participants fasted overnight and were instructed to avoid alcohol, exercise, and caffeine for 24 hours before admission to the Clinical Research Unit at the University of Virginia. Each individual had their hemodynamics, central aortic stiffness, left brachial artery FMD, and blood tested to be analyzed for arterial health. The results concluded that individuals with T1DM exhibited a high proportion of NO-dependent endothelial dysfunction, placing them at higher risk for cardiovascular events. The early impairment of NO-dependent endothelial function, HbA1c levels, and damaged vasodilation measured by FMD in people with T1DM demonstrate that endothelial function may be highly susceptible to glycemic control. Although, the cfPWV of the participants were comparable to their nondiabetic counterparts, FMD significantly predicted cfPWV. Therefore, FMD technology can be useful to determine multiple factors of endothelial dysfunction in adults and adolescents with T1DM early on.

Materials and Methods

Setting

The study will take place at Texas Christian University (TCU) in the second floor of the Bass Building in the Nursing Research Laboratory, room 2212.

Participants

The honors student will be completing the remainder of the VENDYS-II study for the principal investigator, Dr. Cheek. A convenience sample of 10 young women have already been recruited and an additional 10 young women will be recruited from a private university in Texas by Dr. Cheek. To be included, participants must be current Texas Christian University English speaking female students 18 years or older with either Type I diabetes mellitus (T1DM) or no history of T1DM or T2DM mellitus. The experimental group will consist of participants with T1DM who have been regularly taking various insulin replacement medications to control their blood sugar levels. The control group will include participants who neither had diabetes mellitus nor were taking any medication to control their glucose levels. Exclusion criteria will be limited to students with Raynaud's phenomenon. No exclusion will be made for individuals with hypertension, type of insulin used to control blood sugar, or other underlying health problems. Participation is completely voluntary, and participants can withdraw consent at any time without penalty. Students will not be offered course credit nor incentives to participate. All students will provide written informed consent before participation in the larger study. The study protocol was approved prior by the Institutional Review Board of Texas Christian University.

Materials/Measurement

The VENDYS-II machine by Endothelix is a noninvasive, fifteen-minute procedure to measure reactive hyperemia via DTM after a blood pressure cuff on the right arm is inflated for 5 minutes. Hyperemia is the momentary increase in blood flow after brief ischemia which relies on endothelial cells. VENDYS-II is the only FDA cleared non-invasive, fully automated device for measurement of vascular reactivity and endothelial function. The control group includes non-diabetic females, and the experimental group is females with T1DM. Each participant will have the same VENDYS-II system used on them to collect data. The participants only must undergo the study once. The VENDYS-II system places a blood pressure cuff on the right arm and two temperature sensors to the first fingers of the left and right hands to monitor function of the arteries. The left arm and fingertips serve as a baseline for individual comparison during the procedure as the left arm's blood flow was not being restricted. A thermal insulation lap pad will be placed on each participant's lap to provide support for hands, ensure comfort, and reduce body heat interference during the procedure. If the patient is cold, a neck warmer will be placed on their neck.

Confidentiality Statement/Data Protection

After the participants provided written informed consent, a brief medical history, height and weight measurements, and medication list will be obtained. The participants will be consented and informed about HIPAA by the principal investigator. The participants' data and information will be deidentified by being coded with numbers to maintain confidentiality. All data and consents will be locked in a cabinet in the principal investigator's locked office after which they were be shredded. Electronic data will be stored without any identifying information in a password protected computer. Only the principal investigator will have access to stored data in the cabinet and electronically. The data will not have any identifying information when presented.

Procedures

Participants will verify that they did not drink caffeine or smoke prior to the VENDYS-II data collection. First, the participant's arms will be measured using a standardized ruler provided by VENDYS-II to ensure the right sized blood pressure cuff is used. Next, the thermal insulation lap pad will be placed on the patient's lap, the blood pressure cuff will be placed on the right arm, and fingertip probes will be placed on the first finger of each hand. The VENDYS-II device first takes the participant's blood pressure, temperature of each fingertip for baseline data, and room temperature. There is a 5-minute period of monitoring fingertip temperature in both arms for a baseline. Next, the hyperemia reaction procedure begins. The blood pressure cuff will inflate and stayed inflated for 5 minutes. The participant will be notified that their right arm may turn purple, and they may feel tingling in the hand and fingers. After 5 minutes, the blood pressure cuff will be released, and the fingertip probes measure the change in temperature which indicates hyperemia for another 5 minutes. The participant will be informed that their hand may turn red as the blood flows back through the right upper limb. The data will be graphed and organized by the VENDYS-II machine into a vascular reactivity index (VRI) report and saved to the hard-drive. The participant will be thanked for their participations and able to leave when comfortable. The participants and researchers will wear masks, dispose of finger probes, and sanitize surfaces with Super Sani-Cloth Surface Disinfectant Wipes in between each test to protect from COVID-19 infection.

Data Analysis

The endpoint for this study will be the vascular reactivity index (VRI) score produced by the VENDYS-II algorithm. Inferential statistics will be used to examine differences in changes of scores on the VRI between young adult females with T1DM and those without diabetes. A student t-test will show a statistically significant difference between the two groups, with a larger score indicating a higher level of difference. A p-value below 0.05 indicates statistical significance for the purposes of this test. The statistical analysis will be performed utilizing GraphPad Prism software ver 9.3.

Results

Demographics

The participants were all female: eight subjects without type 1 diabetes mellitus, the control group, and eight subjects diagnosed with type 1 diabetes using insulin therapy, as the experimental group. Age and body mass index (BMI) for the females with and without type 1 diabetes mellitus were not significant ($p < 0.05$) (see Table 1).

Hemoglobin A1C

The control group was found to have higher hemoglobin A1C levels than the experimental group. Overall, the females without type 1 diabetes did not have any individual data for their hemoglobin A1C levels since they do not need it regularly tested to assess how they are controlling their blood sugar externally. Therefore, the provided average in the control group was an AlC of 6% when calculating statistical significance, due to the average hemoglobin A1C for individuals without T1DM in the U.S. is 4% to 6% (ADA, 2022). Based on these calculations, the difference in hemoglobin A1C for a female with T1DM and without T1DM was statistically significant ($p < 0.0001$) (see Figure 1).

Vital Signs

The systolic, diastolic, and mean blood pressure were taken at the beginning of every individual's procedure to formulate baseline data. The systolic blood pressure, diastolic blood pressure, and mean blood pressure were similar amongst both groups of females. The difference was not significant (see Figures 2, 3, 4).

Furthermore, the baseline heart rates of the sixteen participants were determined before the procedure as well. The variance in heart rate for females with T1DM and without T1DM was not statistically significant (see Figure 5).

Vascular Reactivity Index (VRI)

Vascular reactivity is the responsiveness of a blood vessel tone and diameter to a specific stimulus. The vascular reactivity index (VRI) is used to shows the endothelial function health since the endothelial cells play critical roles in the vascular beds. The VRI scores range from 0.0 to 3.5 with a goal range of a "good" VRI being greater than 2.0. In this particular study, the difference in VRI between the females with and without T1DM were not statistically significant

($p = 0.543$) (see Figure 6). Consequently, based on this study we cannot correlate a relationship between females with T1DM and poor endothelial function health.

Discussion

The control group, those females that do not have T1DM, had insignificantly lower VRI scores despite having significantly lower hemoglobin A1C percentages. Therefore, based on having better long-term control of blood sugars naturally, it cannot be concluded that the endothelial function is better in the nondiabetic individuals compared to the experimental group. The insulin therapy used by the diabetic participants may provide enough protective effects to regulate blood flow. There is no significant difference in VRI because of a diagnosis with T1DM in the experimental group.

While diabetes mellitus has known long-term negative effects on the cardiovascular system if not managed properly, it is important to recognize that proper adherence to an insulin treatment regimen even with a significantly higher hemoglobin A1C, can help preserve healthy endothelium and regulation of blood flow. With a growing number of young females becoming diagnosed with type I diabetes mellitus, the continuation of this study can impact millions of women.

Limitations

The small sample size and homogenous subject group may limit these results to a certain population of young females. The subjects somewhat varied in age, BMI, and ethnicity leading to differing results in vascular reactivity. Vascular reactivity may have been influenced by the participant’s medication regimen and diet the day of utilizing the VENDYS-II system. The inability to directly measure the hemoglobin A1C levels of the control group forced the authors to rely on the average score for nondiabetic individuals to conclude results.

Conclusions

With over 585,000 women in the United States having type I diabetes mellitus, more research is necessary to discover the vast effects of the disease and insulin treatment on endothelial health (CDC, 2018). The risk for poor endothelial health remains even though there are more technological advances in diabetes management today such as continuous glucose monitoring (CGM) systems. However, based on our findings insulin therapy seems to have a beneficial impact on vascular response and dilation. The exogenous insulin provided by insulin treatments and CGMs to the diabetic population helps to provide tighter blood glucose management which increases blood vessel dilation in response to ischemia. The increased vasodilation can prevent risk factors for cardiovascular disease. Well-maintained insulin therapy may be a protective factor to preserve functional endothelium in females with T1DM. This discovery adds beneficial points to the debate of diabetes and endothelial health (Nathan et al, 2014, King, Peacock & Donnelly, 1999).

Future Research

In the future, these results should be replicated, but with revisions to the methodology. The participants should participate in the study at the same time of day while fasting and use VENDYS-II on each arm with adequate time between experiments. For the nondiabetic group, it would be beneficial to determine an exact hemoglobin A1C. This experiment can be altered to include only diabetic patients utilizing CGMs to study time in range of their target blood glucose levels for better accuracy in determining endothelial impact. The influence of these factors being more evenly compared can yield more usable and accurate data. The vascular reactivity index would continue to be measured with each of these individuals to determine if there is a correlation between T1DM and endothelial function. Lastly, although a homogenous group allows for easier comparison, a larger, more diverse sample would provide more information.

Appendix

Table 1.

Participant Demographic Data

	Nondiabetics (n = 8)	Diabetics (n = 8)	p value	Significance
Age (years) (Mean$\pm$SEM)	21.13 ± 0.48	21.13 ± 0.51	0.85	Not Significant
BMI (kg/m^2) (Mean$\pm$SEM)	21.13 ± 0.87	24.33 ± 1.17	0.45	Not Significant

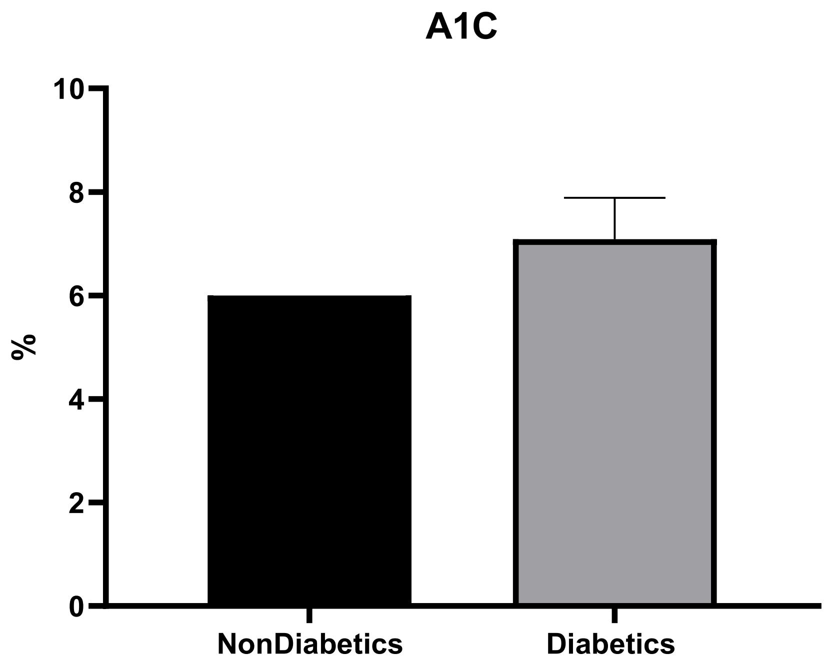

Figure 1. The hemoglobin A1C levels were compared between the nondiabetic and diabetic female participants. The females with diabetes had a higher hemoglobin A1C than the control group without diabetes ($p < 0.0001$). It is important to note that only the diabetic participants knew their exact hemoglobin A1C level, every individual in the nondiabetic group was given an A1C of 6% as it is at the high range of the average A1C.

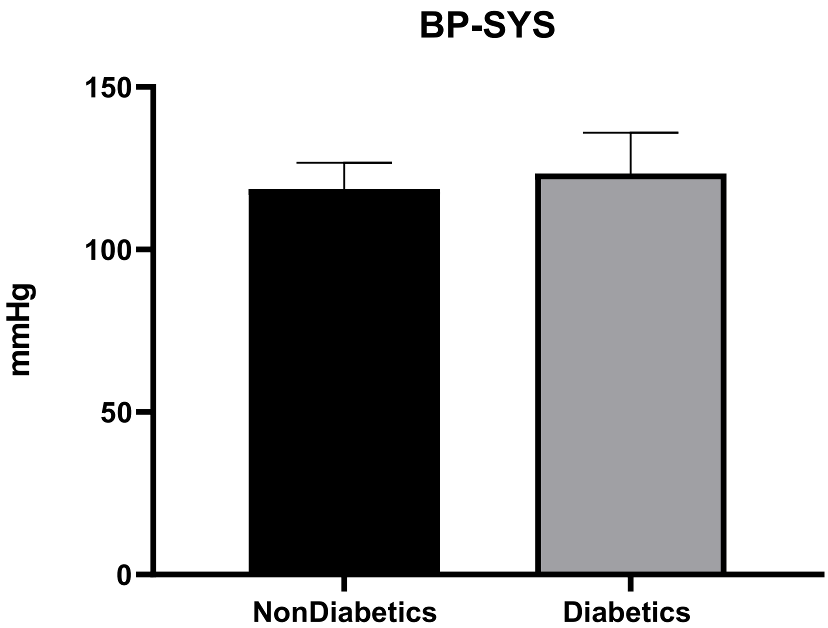

Figure 2. The systolic blood pressure between the control and experimental groups were compared. The experimental group had insignificantly higher systolic blood pressure compared to the control group ($p = 0.26$).

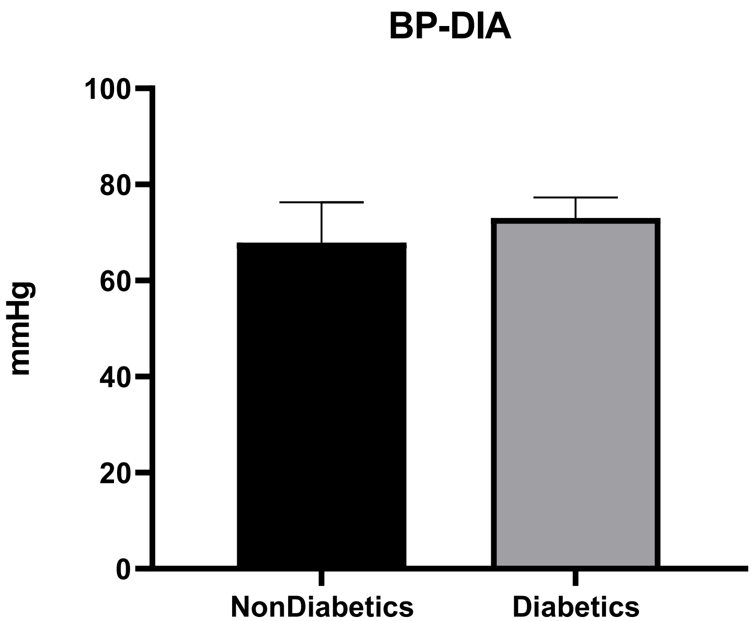

Figure 3. The diastolic blood pressure between the control and experimental groups were compared. The experimental group had insignificantly higher diastolic blood pressure compared to the control group ($p = 0.09$).

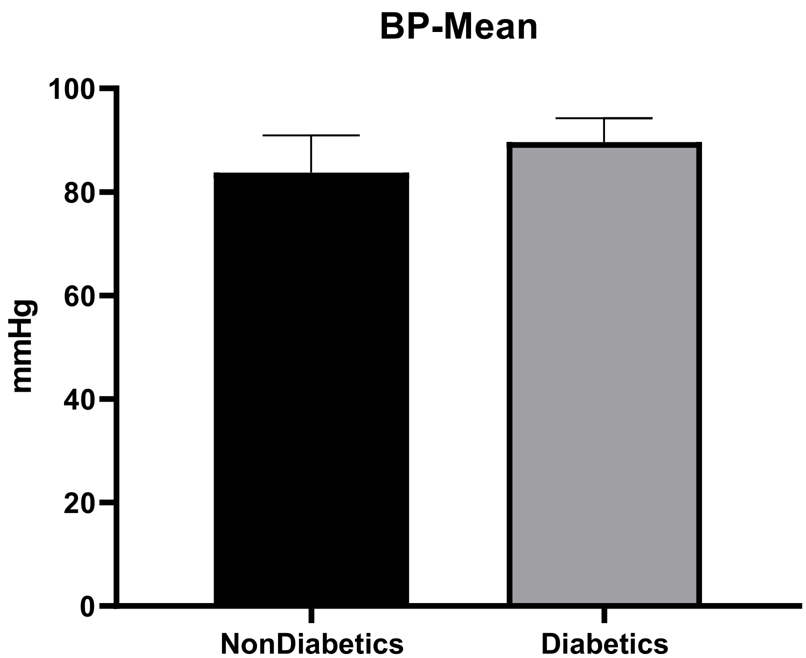

Figure 4. The mean blood pressure between the nondiabetic and diabetic female participants were compared. The diabetic participants had insignificantly higher mean blood pressure ($p = 0.26$).

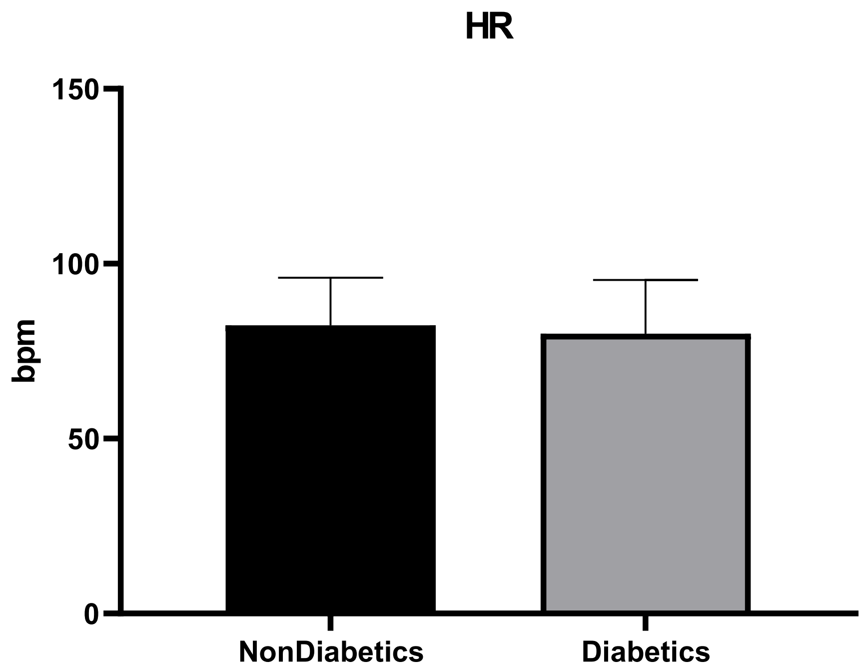

Figure 5. The VENDYS-II system compared the average heart rates (HR) of the diabetic and nondiabetic participants. There was not a significant difference between the heart rates of the nondiabetic and diabetic females ($p = 0.76$).

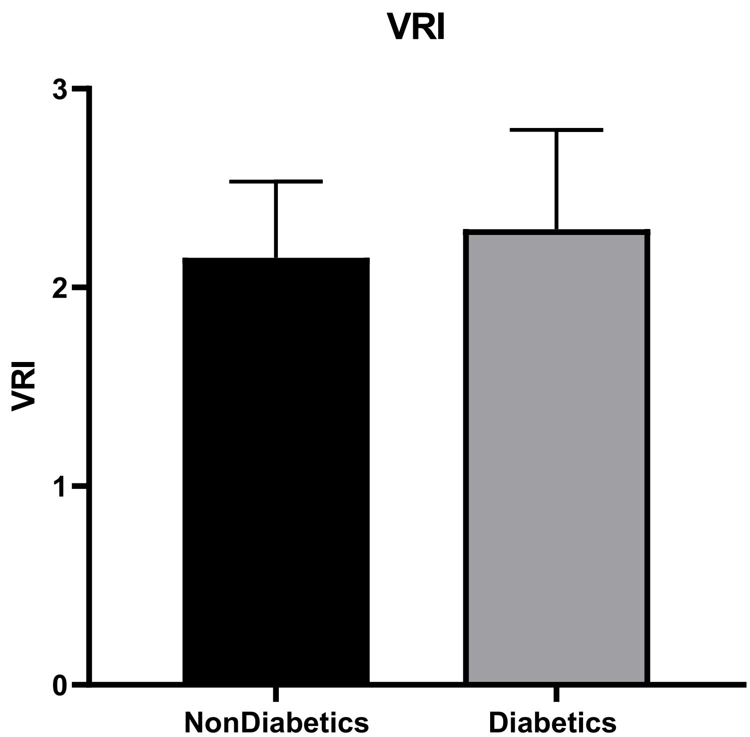

Figure 6. The vascular reactivity index (VRI) for the control and experimental group were tested. The experimental group consisting of the diabetics had insignificantly better VRI scores compared to the control group (p = 0.543). .

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