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Adolescents with Type 2 Diabetes: Early Indications of Focal Retinal Neuropathy, Retinal Thinning and Venular Dilation

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Abstract

Purpose—The eye provides a unique window into the neural and vascular health of a patient with diabetes. The present study is the first of its kind to examine the neural retinal function, structure and retinal vascular health in adolescents with type 2 diabetes.

Methods—Focal neural responses from 103 discrete retinal regions of the eye were tested using multifocal electroretinography. Optical coherence tomography was utilized to measure retinal thickness. Digital fundus photographs were examined for the presence of retinopathy and to measure vascular caliber using retinal vessel analysis. Fifteen adolescents diagnosed with type 2 diabetes, aged 13 to 21 years with a mean diabetes duration of 2.1 ± 1.3 yrs, were tested. Twenty-six age-matched control subjects were also tested.

Results—mfERGs of the type 2 diabetic group were significantly ($p = 0.03$) delayed by 0.49 ms. The diabetic group also showed significant (both; $p \leq 0.03$) retinal thinning ($10.3 \mu\text{m}$) and significant venular dilation ($16.2 \mu\text{m}$).

Conclusions—The present study shows early indications of focal retinal neuropathy, retinal thinning and venular dilation in adolescents with type 2 diabetes. Early detection of functional and structural changes will hopefully aid in the prevention of permanent damage or further functional loss.

Keywords

Adolescent; Children; Type 2 Diabetes Mellitus; Diabetic Neuropathy; Diabetic Retinopathy; Retina; Vascular Health; multifocal electroretinogram; optical coherence tomography; retinal vessel analysis

Introduction

The prevalence of type 2 diabetes in adolescents is an ever-increasing issue throughout the world.¹ According to the Center for Disease Control and Prevention, the incidence of obesity and diabetes in the United States has reached epidemic proportions.² On a regional basis, the

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Summary Statement: Through the use of multifocal electroretinography, optical coherence tomography and vascular analysis we have been able to detect subtle but significant retinal changes in adolescents with type 2 diabetes. The present study shows early indications of focal retinal neuropathy, retinal thinning and venular enlargement in adolescents with type 2 diabetes.

rates of type 2 diabetes in adolescents ranges from 8% to 45% of all newly diagnosed cases of diabetes in adolescents.³ As these rates increase we expect to see an associated increase in the rates of cardiovascular disease, neuropathy, nephropathy, retinopathy and mortality in earlier stages of life. The trend necessitates greater efforts to understand the early pathogenesis of this disease in young people in order to ultimately prevent or minimize future damage from these complications.

Adolescents with type 2 diabetes often present with comorbidities such as obesity, urine microalbumin, hypertension and dyslipidemia.⁴ All of these conditions have considerable impacts on both the vascular and neural health of the patient and can present daunting challenges in terms of treatment and prevention of future vision and life-threatening complications. Unfortunately, research into type 2 diabetic vasculopathy and neuropathy in the eye has been largely limited to adult studies, where clear and early vascular and neural changes have recently been shown to occur well before there is any documented vision change.^{5, 6} These changes in neural function have been revealed from recordings of light evoked neural responses generated in the posterior pole of the retina.⁷ The multifocal electroretinogram (mfERG) is a non-invasive method for testing over a hundred retinal locations simultaneously. From our mfERG studies in adults with diabetes, we know that focal retinal neuropathy is present in the absence of any clinically visible vascular damage (retinopathy).^{5, 6} Perhaps more critical is the finding that the measures are predictive of future location-specific retinopathy development, and that as the neuropathy progresses in severity, there is an accompanying increase in the retinal vascular damage.^{5, 6, 8, 9}

Using optical coherence tomography (OCT) imaging of the retina in-vivo, structural changes have been reported at every stage in the progression of diabetic eye disease. Retinal thickening from edema, as well as retinal nerve fiber loss, has been described in adults with type 2 diabetes, even in the absence of retinopathy.¹⁰⁻¹³ Furthermore, there is evidence for both arteriole narrowing and venular enlargement in the retinal vasculature of children with elevated blood pressure (BP) and body mass index (BMI).^{14, 15} In adults these vascular characteristics have been associated with increased risk of vascular related pathology throughout the body.¹⁶⁻²⁰

Taken together, these research results reveal very early vascular and neural functional changes in adult diabetes and that some measures are predictive of subsequent vasculopathy and complications. Clearly there is a need for such early indicators of ocular and neural health. The issues are no less critical for adolescents and one could argue that they are even more critical given the number of years that they are likely to live with diabetes. Yet such studies in adolescents with type 2 diabetes have yet to be performed.

The present study is the first to examine these structural and neural measures (mfERG, OCT and retinal vascular analysis) in eyes of adolescents with Type 2 diabetes. The results we report here show that functional and structural change can be documented in many of their retinas.

Methods

Subjects

15 adolescent patients diagnosed with type 2 diabetes, aged 13 to 21 (16.0 ± 1.9 , Mean $\pm$ SD) with a mean duration of 2.1 ± 1.3 yrs, were tested. All of these adolescents were patients of the endocrinology department at Children's Hospital and Research Center, Oakland (CHRCO). The group consisted of 6 (40%) males and 9 (60%) females, which is representative of the type 2 population at CHRCO. Type 2 diabetes was diagnosed based on fasting blood glucose (BG) level, HbA1c, oral glucose tolerance, fasting C-peptide/insulin levels and the absence of autoantibodies upon presentation. 26 control subjects without diabetes, aged 13 to 21 with a mean age of 17.6 ± 3.0 yrs, were also included in the study. Control group inclusion criterion

was based on blood glucose (random, non-fasting) as well as the subject/guardian reports of the subject's health. None of our adolescent control subjects reported having been diagnosed with diabetes or pre-diabetic symptoms. All of our control subjects fell within normal blood glucose levels. This criterion is somewhat crude and could include an insulin insensitive patient with enough residual beta-cell function to present within the normal BG range. The inclusion of a pre-diabetic subject in the control group would only push the results toward the Null. The group was comprised of 10 (38%) males and 16 (62%) females. The ages of the diabetes and control groups did not differ ($p = 0.13$).

The purposes and potential consequences of the study were explained and informed consent was obtained from all subjects and parents (in the case of minors) before testing. Procedures adhered to the tenets of the Declaration of Helsinki, and the University of California, Committee for the Protection of Human Subjects, approved this research.

BMI, BP and blood glucose (BG) levels were also measured at the time of recording in both groups. Hemoglobin A1c (HbA1c) was also measured at the time of recording in the diabetic group using A1C At-Home testing kits (FlexSite Diagnostics, Inc., Palm City, FL). HbA1c measurements made at CHRCHO were measured with the DCA 2000 (Bayer Diagnostic, Milan, Italy). In order to compare HbA1c values across testing locations, 12 subjects with diabetes were tested at both sites within a one-week period. There was a linear relationship ($y = 1.0x - 1.4$, ($R^2 = 0.97$, $P < 0.001$)) between the two measurements, with the A1C At-Home being 1.4% per unit higher than the DCA. Based on this data, the A1C At-Home measurements were adjusted by -1.4%. Clinical covariates are summarized in Table 1.

All subjects had best-corrected visual acuity of 20/20 or better, no spherical equivalent refractive errors outside the range of -6.0 to +4.0 D, no history of disease unrelated to diabetes, and clear ocular media. Both eyes were examined but only the right eye tested in all but one of the type 2 adolescents was analyzed in this study. Data from the one left eye was reflected in order to match the others. Digital fundus photos subtending approximately 50° , were taken at the time of testing and were graded for the presence of diabetic retinopathy by a retinal specialist who was masked to all other data on the individual. All of the adolescents were determined to be free of retinopathy in both eyes. Retinal blood vessels within $\frac{1}{2}$ to 1 disc diameter around the optic nerve were analyzed with IVAN (University of Wisconsin-Madison, WI), an automated but supervised program that measures and summarizes the diameters of retinal arterioles and venules. These methods followed a standard protocol described in detail in previous reports.²¹⁻²³

Multifocal Electroretinogram

The mfERG methods have been described in detail previously.⁵ Briefly, mfERGs were recorded using VERIS Science 4.3 (EDI, San Mateo, CA). The stimulus consisted of a 103-element hexagonal array (Figure 1a). Each hexagon alternated between white (200 cd/m^2) and black ($< 2 \text{ cd/m}^2$). Pupils were dilated using 2.5% phenylephrine and 1.0% tropicamide, and the cornea was anesthetized with 0.5% proparacaine. Retinal signals were acquired with a standard bipolar contact lens electrode placed on the cornea. Total recording time was approximately 8 minutes per eye. Response amplitude is the voltage difference between the N1 trough and P1 peak (Figure 1b, "Amplitude") and the implicit time is measured from the onset of the local flash to the P1 peak (Figure 1b, "Implicit Time"). The waveforms in Figure 1c were obtained from a normal control subject and a subject with type 2 diabetes.

Optical Coherence Tomography

Retinal thickness was measured using OCT (Stratus OCT 3, Carl Zeiss Meditec, INC., Dublin, CA). Subjects fixated on a central target while 12 radial cross-sectional images of the retina

were acquired sequentially. Each 6-mm scan was composed of 512 axial samples (9-10 μ m axial resolution by 11 μ m transverse resolution) centered on the fovea. Retinal thickness is defined as the difference between the vitreoretinal interface and the pigment epithelial/photoreceptor outer-segment interface (Figures 1d).²⁴ These measurements formed the basis of the topographic thickness map of the central 20 degrees of the retina (Figure 1e). This thickness map was then divided into 37 hexagonal regions that corresponded to the central 37 elements of the mfERG stimulus (Figure 1f). An average retinal thickness for each hexagon was then computed from all points falling within the given region, which permitted local analysis of retinal thickness.

Data Analysis: mfERG and OCT

Figure 1a shows the spatial relationships between the mfERG and OCT regions tested. In order to correct for the normal retinotopic variation in the mfERG and OCT measurements, the mean and standard deviation at each retinal location of the control group was used to generate Z-score values. To compare control and diabetic groups, we averaged Z-scores across subjects at each tested retinal location, creating an average eye for each subject group. This allowed for an equal N (mfERG = 103, OCT = 37) for each group. T-tests were used to compare the mean differences between the groups' average eyes. To examine local abnormalities within subjects, retinal measurements with Z-scores ≥ 2 were considered abnormal ($p \leq 0.023$). An eye with 6 or more abnormal mfERG responses and 3 or more abnormal OCT measurements ($p < 0.03$) was considered abnormal. To compare the number of abnormal eyes between groups, χ^2 analyses with Fisher's Exact (FE) corrections were applied. Univariate ANOVA and multiple linear regression analysis were used to examine relationships between the mfERG, OCT, vascular measurements and clinical covariates (i.e., HbA1c, BG or BMI). Clinical covariates were entered into the regression model and removed systematically until the most parsimonious and statistically significant model remained. Results are presented as means $\pm$ SE unless otherwise stated, and critical p-values were adjusted for multiple comparisons.

Results

Clinical Covariates

Mean BG at the time of testing (without fasting) was 197.0 ± 133.8 mg/dL (Mean $\pm$ SD) in subjects with diabetes and was significantly higher ($P = 0.01$) than that of the control group (97.0 ± 10.9 mg/dL). The mean HbA1c of the diabetic group was $7.6 \pm 3.0\%$, which is consistent with the yearly average for type 2 adolescents at CHRCHO ($7.6 \pm 3.0\%$). The BMI of the diabetic group (34.6 ± 6.4) was significantly higher ($P < 0.001$) than that of the control group (22.3 ± 3.6). Whereas the mean BPs of the diabetic group (systolic = 115.7 ± 7.4 ; diastolic = 72.7 ± 5.6 mm/Hg) were not outside of clinical norms, the systolic pressure was significantly higher ($p < 0.05$) than the control group (107.0 ± 10.3 / 68.2 ± 9.2 mm/Hg). In agreement with the type 2 diabetes BMI archetype, 84% of the adolescents with type 2 diabetes in the present study were at or above the 95th percentile for their age and gender.⁴ Twenty-five percent of the diabetic subjects had either systolic or diastolic pressure outside of the 90th percentile for their age, gender and height.

MfERG Implicit Time

Mean mfERG implicit time for the diabetic subjects (27.6 ± 0.1 ms) was significantly delayed ($P = 0.01$) compared to the control group (27.1 ± 0.1 ms). Figure 2 (Implicit Time) shows the Z-score distributions for the average eyes. The distribution of Z-scores for the diabetic group (median = 0.7) was significantly delayed (Student T-test; $N = 103$, $p < 0.001$) compared to the control group (median = 0.00). As shown in Figure 3, 6/15 (40%) eyes of the diabetic group had 6 or more abnormally delayed implicit times (abnormal eye) compared to 2/26 (8%) in the control group. Figure 4A shows the frequency of implicit time abnormalities at each retinal

location for the control and diabetic groups. This was a significant difference in frequency of abnormality between the two groups (FE: $\chi^2 = 6.32$, $p = 0.035$).

MfERG Amplitude

The mean amplitude across retinal locations for the diabetic subjects (219.6 ± 15.1 nV) did not differ ($P = 0.2$) from controls (241.4 ± 10.3 nV). For the groups' average eye data (Figure 2, Amplitude) the diabetic group had significantly ($N = 103$, $p < 0.0001$) smaller amplitudes (median Z-score = -0.4) than the control group, (Z-score = 0.00). Four of the 15 eyes (27%) of the diabetic group were abnormal based on amplitude compared to 1 out of 26 (4%) in the control group (Figure 3). Figure 4B shows the frequency of amplitude abnormality at each retinal location in both the control and diabetic groups. The frequency of abnormal eyes, based on amplitude, was not significantly different between the two groups (FE: $\chi^2 = 4.63$, $p = 0.06$).

Retinal Thickness

The mean retinal thickness of the diabetic subjects, 243.4 ± 3.7 μm , was significantly less than the 253.7 ± 2.5 μm of the control group ($p = 0.02$). Examination of the distribution (median = -0.6) of Z-scores of the diabetic average eye showed that retinal thickness measures (Figure 2) were significantly thinner ($N = 37$, $p < 0.0001$) than measures obtained from the control group (median = 0.00). As shown in Figure 3, 4/15 eyes (27%) in the diabetic group were abnormally thin compared to 2/26 eyes (8%) in the control group. Figure 4C shows the frequency of abnormal retinal thicknesses at each retinal location in both the control and diabetic group. There was not a significant difference between the groups (FE: $\chi^2 = 2.74$, $p = 0.17$).

Vessel Caliber

The venular caliber of the diabetic group (235.8 ± 5.9 μm) was significantly larger ($p = 0.03$) than the control group's (219.6 ± 4.0 μm). Arteriolar caliber did not differ ($p = 0.22$) between the diabetic (165.5 ± 4.0 μm) and control groups (172.1 ± 3.5 μm).

Association between function, structure and clinical measures

There were no significant spatial associations between mean retinal thickness and either mfERG implicit time or amplitude ($p > 0.05$). There were also no significant relationships between any of the clinical covariates (Age, Duration, BG, HbA1c, BMI and BP) and either implicit time or vascular caliber in the present sample. After systematically removing clinical covariates from the multiple linear regression models, univariate regression was the most parsimonious model for describing the relationships between: (1) mfERG amplitude and duration (Figure 5), indicating that as diabetes duration lengthened there was a significant trend towards larger amplitudes ($R^2 = 0.41$, $N = 15$, $p = 0.01$); and (2) retinal thickness and BG at the time of recording, suggesting that as BG levels increased there was a marginal trend towards decreasing retinal thickness ($R^2 = 0.28$, $N = 15$, $p = 0.04$).

Discussion

Using the mfERG, OCT and retinal vessel analysis, the present study reveals early indications of focal retinal neuropathy, retinal thinning and venular enlargement in adolescents with type 2 diabetes and no retinopathy.

Retinal Function

Forty percent of the subjects in the diabetes group presented with 6 or more abnormal mfERG implicit times compared to 8% of the control subjects, and are thus considered to have functionally abnormal retinas. When looking at the mean differences between the average eyes for each group, adolescents with type 2 diabetes had significantly delayed implicit times

compared to the control subjects ($p < 0.0001$). As seen in Figure 4a, the peripheral retina and areas around the optic disk are affected more than that of the central macular region. This pattern of abnormalities is similar to that seen in adult diabetics without retinopathy albeit to a lesser degree.²⁵ In adults with diabetes, a delayed mfERG implicit time is predictive of future development of retinopathy in a given retinal location.^{5, 6, 8, 9, 26}

The difference in the number of subjects presenting with a significant number of abnormal mfERG amplitudes (27% vs 4%) approached but did not reach significance. However, the amplitude Z-scores for the diabetic group average eye were significantly smaller than that of the control group. Greco et al.²⁷ found amplitude loss in children (age 5-14yrs) with type 1 diabetes, using the steady-state focal electroretinogram within the central 9 degrees of the retina. Their results are in agreement with ours: amplitude differences can be detected early in children with diabetes. However, in our study, mfERG response amplitudes did not appear to be affected to the same degree as response implicit time in the diabetic group. The topography of amplitude abnormalities differs from that of abnormalities in implicit time; implicit time delays are more frequent in the periphery, whereas amplitude abnormalities tend to be seen in the central retina. In adult onset type 2 diabetes, implicit time abnormalities appear to precede amplitude abnormalities.^{5, 6, 8, 9, 26} While there were few significant associations between the functional measures and the clinical covariates in the current sample, we cannot definitively say that associations do not exist given the present sample size. Further examination with a larger sample will give a better understanding of the relationship between function and clinical indicators

Retinal Thickness

This is the first study to examine retinal thickness in adolescents with type 2 diabetes. On average, the overall retinal thickness of the diabetic group was about 10 μ m thinner than that of the control group. Our results are in agreement with an abstract reported by Van Dijk et al.²⁸ who found an average 15 μ m thinning in the pericentral retina of adults with type 2 diabetes with little or no retinopathy. However, there are reports of retinal *thickening* in patients without retinopathy.^{10, 11} Nerve fiber layer thinning in the adult type 2 diabetic retina has also been reported.^{10, 12, 13} Some have theorized that the changes in retinal thickness and nerve fiber in the adult type 2 population are due to neural tissue loss.^{10, 12, 13, 28} This could be true in the adult type 2 diabetic retina but this explanation is less likely in our adolescent sample given the relatively short duration of diabetes. The retinal thinning in the adolescent diabetic group was unexpected and its basis is currently unknown. Further investigation with a larger population is needed.

Vascular diameter

Altered microvascular function has been found at every stage in the development of adult type 2 diabetes. Many of the changes in vascular regulation have even been shown prior to and predictive of future onset of diabetes in adults.^{17, 29-31} In our study we found that venular caliber of adolescents with type 2 diabetes was significantly larger than that of our control group. This is in agreement with Cheung et al.¹⁴ who found that BMI (86% of our diabetic group was at or above the 95th percentile) was significantly associated with retinal venular dilation in a large cross-sectional study with children aged 6-8 yrs old. In adults, venular dilation has been associated with carotid artery disease, cerebrovascular disease, measures of atherosclerosis and diabetic nephropathy.³²⁻³⁵ The fact that we found no relationship between venular diameter and BMI within the present sample of adolescents with diabetes is likely due to our relatively small sample size. Future advances in image analysis should allow us to measure vessel diameter within more localized regions of the retina away from the optic disc

Potential Mechanisms

The pathophysiological mechanisms underlying the functional, structural and vascular changes presented here are not well understood. Adolescents with type 2 diabetes often present with comorbidities such as obesity, hyperinsulinemia, hypertension and hyperlipidemia. All of these conditions can impact both the vascular and neurological health of the patient.

The retinal responses measured here are largely generated by the bipolar cells within the middle retinal layers.^{36, 37} These cells are non-myelinated and respond to stimulation with a graded potential. The Müller cells are major glial support mechanisms in the retina that help regulate bipolar cell function and contribute to the maintenance of the blood retinal barrier, vascular regulation and retinal homeostasis.³⁸⁻⁴² We know from experimental diabetes studies that Müller cells are affected early and prior to the onset of visible retinopathy, and that they play a critical role in the development and progression of diabetic eye disease.⁴³⁻⁴⁵ The functional and vascular structural changes presented here could be due to damage to or altered function of these cells. It has also been suggested that retinal venular dilation could be due to systemic inflammation associated with elevated BMI.^{14, 46-48}

The relationship between duration and amplitude in Figure 5 is one that is not easily explained. Our search through the literature revealed a lack of both data and an appropriate animal model. We can speculate that the mechanisms underlying the amplitude response could be affected prior to the onset of the hyperglycemic state and diagnosis of diabetes. The presumed pre-diabetic hyperinsulinemia and presence of inflammatory factors could well be toxic to the neural retina and thus be responsible for the abnormally low amplitudes seen very early in diagnosis.^{49, 50} The slow recovery toward normal of the amplitude response as duration lengthens could be the result of diabetes treatment or reflective of the pathological stage of pancreatic insulin production. These are important factors that should be examined in a longitudinal study that includes those adolescents at risk for the development of diabetes in order to fully understand pathological progression of events.

In conclusion, the eye provides a unique window into the vascular as well as the neural health of a patient with diabetes. As a result of recent advances in the assessment of both neural retinal functioning and vascular health we have been able to detect subtle but significant changes early in the pathogenesis of type 2 diabetes in adolescents. The present study shows early indications of focal retinal neuropathy, venular enlargement and retinal thinning in adolescents with type 2 diabetes. Given the associated risks of retinopathy, nephropathy and cardiovascular disease seen in the adult type 2 diabetic populations, the present data suggest that these children are likely to face greater health care challenges early in life. It is hoped that early detection of functional and structural changes will aid in the prevention of permanent damage or further loss. These outcomes might be averted through improved glycemic control or behavioral changes. The associated risks in this population will need to be explored longitudinally with larger study groups in the future.

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Figure 1.

Multifocal Electroretinogram (mfERG) and Optical Coherence Tomography (OCT). **(A)** Five field fundus mosaic depicting the retinal regions measured with the 103 mfERG stimulus pattern and 12 OCT scans. **(B)** Amplitude is the voltage difference between the N1 trough and P1 peak; implicit time is measured from the onset of the local flash to the P1 peak. **(C)** Example waveforms obtained from a normal control subject (black trace) and a subject with diabetes (red trace). **(D)** Black and white depiction of OCT scan indicating retinal layers. Retinal thickness is measured from the vitreoretinal interface to the pigment epithelial/photoreceptor outer-segment interface. **(E)** Interpolated thickness map of the central 20 degrees of the retina of a control subject. **(F)** 12 radial scans used to generate retinal thickness maps. The thickness

map was then scaled and divided into 37 hexagonal regions. An average retinal thickness for each hexagon was then computed from all points falling within it.

Figure 2.

Response Distribution for Average Eye. Box plots represent the 5th, 25th, 50th, 75th and 95th percentiles. Control distributions for each measure are centered on a Z-score of zero. Median mfERG implicit time (0.7), mfERG amplitude (-0.4) and retinal thickness (-0.6) all differed from the control group (Student T-test; $N_{\text{mfERG}} = 103$, $N_{\text{OCT}} = 37$, $p < 0.001$).

Figure 3. Frequency of Abnormality. The percentages of subjects with an eye with 6 or more abnormal mfERG response Z-scores and 3 or more abnormal OCT Z-scores (probability of less than 0.03) are shown. White bars = Control Group; Black bars = Type 2 Diabetes.

Figure 4.

Retinal distributions of abnormalities. Percentages of subjects with a response or measurement equal to or beyond ± 2.0 Z-scores at each retinal location are coded gray to black from 0% to 42%. (A) Frequency of implicit time abnormalities at each retinal location for the control and diabetic groups. (B) Frequency of amplitude abnormality at each retinal location. (C) Frequency of abnormal retinal thicknesses at each retinal location.

Figure 5.

Diabetes duration vs mfERG amplitude. As diabetes duration lengthens there is a significant increase in amplitude towards normal ($R^2 = 0.41$, $N = 15$, $p = 0.01$).

Table 1

Characteristics of subject groups

Characteristics	Control (n = 26)	Type 2 Diabetes (n = 15)
Age, mean $\pm$ SD, yrs	17.6 $\pm$ 3.0	16.0 $\pm$ 1.9
Duration, yrs	N/A	2.1 $\pm$ 1.3
Blood Glucose, mg/dL	97.0 $\pm$ 10.9	197.0 $\pm$ 133.8 *
HbA1c _{adjusted} , %	N/A	7.6 $\pm$ 3.0
Body Mass Index, kg/m ²	22.6 $\pm$ 3.6	34.6 $\pm$ 6.4 *
Systolic BP, mm Hg	107.0 $\pm$ 10.3	115.7 $\pm$ 7.4 *
Diastolic BP, mm Hg	68.2 $\pm$ 9.2	72.7 $\pm$ 5.6

All values are mean $\pm$ SD.* P $\leq$ 0.05