Acta Ophthalmologica

Acta Ophthalmologica 2013

Impaired retinal vasodilator responses in prediabetes and type 2 diabetes

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ABSTRACT.

Purpose: **In diabetes, endothelial dysfunction and subsequent structural damage to blood vessels can lead to heart attacks, retinopathy and strokes. However, it is unclear whether prediabetic subjects exhibit microvascular dysfunction indicating early stages of arteriosclerosis and vascular risk. The purpose of this study was to examine whether retinal reactivity may be impaired early in the hyperglycaemic continuum and may be associated with markers of inflammation.**

Methods: **Individuals with prediabetes (n = 22), type 2 diabetes (n = 25) and healthy age and body composition matched controls (n = 19) were studied. We used the Dynamic Vessel Analyzer to assess retinal vasoreactivity (percentage change in vessel diameter) during a flickering light stimulation. Fasting highly sensitive c-reactive protein (hs-CRP), a marker of inflammation, was measured in blood plasma.**

Results: **Prediabetic and diabetic individuals had attenuated peak vasodilator and relative amplitude changes in retinal vein diameters to the flickering light stimulus compared with healthy controls (peak dilation: prediabetic subjects 3.3 ± 1.8%, diabetic subjects 3.3 ± 2.1% and controls 5.6 ± 2.6%, p = 0.001; relative amplitude: prediabetic subjects 4.3 ± 2.2%, diabetic subjects 5.0 ± 2.6% and control subjects 7.2 ± 3.2%, p = 0.003). Similar findings were observed in retinal arteries. Levels of hs-CRP were not associated with either retinal vessel response parameters.**

Conclusion: **Retinal reactivity was impaired in prediabetic and type 2 diabetic individuals in parallel with reduced insulin sensitivity but not associated with levels of hs-CRP. Retinal vasoreactivity measurements may be a sensitive tool to assess early vascular risk.**

Key words: flickering light stimulation – prediabetes – retinal reactivity – type 2 diabetes – vasodilation

Acta Ophthalmol. 2013: 91: e462–e469

doi: 10.1111/aos.12129

Introduction

More than 70 million Americans have prediabetes, an early stage in the hyperglycaemic continuum associated with an increased risk of developing future diabetes (Haffner 2003; Ford et al. 2010) and vascular complications (Milman & Crandall 2011). The retina is a unique site to study the human microcirculation. Retinal blood flow is controlled by autoregulatory metabolic and pressure mechanisms which are impaired in diabetes and may contribute to retinopathy and vision loss (Pournaras et al. 2008). Understanding the pathophysiologic basis for changed blood vessel responses across the hyperglycaemic continuum is important for the discovery of new treatments and preventive strategies during early disease stages.

A flickering light stimulus has been used to assess retinal vascular dysfunction. In healthy individuals, flickering light stimulus increases retinal blood flow and blood vessel diameter (Michelson et al. 2002; Dorner et al. 2003a; Nagel & Vilser 2004; Lott et al. 2012), whereas in diabetic individuals, retinal vasodilation is attenuated (Garhofer et al. 2004; Mandecka et al. 2007; Bek et al. 2008; Nguyen

et al. 2009; Pemp et al. 2009a,b; Lott et al. 2012), but it is unknown whether retinal vascular impairment exists in prediabetes.

Several studies indicate that inflammation plays an important role in the development of atherosclerosis and diabetes (Mazzone et al. 2008; D'Souza et al. 2009). While diabetes has been associated with elevated inflammatory biomarkers (Thomsen et al. 2010) as well as impaired macrovascular reactivity (Toda et al. 2010), whether inflammation is associated with retinal microvascular dysfunction across the hyperglycaemic continuum is not clear.

Thus, the purpose of this study was to examine whether retinal reactivity may be impaired early in the hyperglycaemic continuum and may be associated with markers of inflammation such as highly sensitive c-reactive protein (hs-CRP) levels. We hypothesized that individuals with prediabetes have an impaired retinal vasoreactivity and that attenuated retinal reactivity would be associated with higher levels of hs-CRP.

Methods

Subjects

The study was approved by the Institutional Review Board at Penn State Hershey Medical Center and followed the Tenets of the Declaration of Helsinki. Middle aged to older non-smoking individuals with prediabetes ($n = 22$), type 2 diabetes ($n = 25$) and body mass index (BMI) and age similar matched healthy controls ($n = 19$) between 21 and 75 years of age participated in this study (Table 1). Diagnosis of diabetes and prediabetes was based on the American Diabetic Association's new classification standards for prediabetes (HbA1c ≥5.7% and <6.5%) and type 2 diabetes (HbA1c ≥6.5%) (2010). Subjects were recruited through physician letters and flyers in the general community. After signing informed consent, all subjects completed a medical history, physical examination and ocular screening which included a measurement of visual acuity and intraocular pressure. Subjects had a normal eye examination with corrected acuity of 20/30 or better and an intraocular pressure below 21 mmHg. Subjects were free from stroke, coronary, heart, lung and eye diseases (e.g. retinopathy, age-related macular degeneration and glaucoma) and were not morbidly obese (BMI > 45 kg/m^2) or currently pregnant. All subjects were non-smokers and controls had no history of hypertension. Diabetic subjects' blood pressures (BPs) were controlled by medications.

Table 1. Subject demographics.

	Healthy controls	Prediabetes	Type 2 diabetes	p-value
Number of subjects	19	22	25	
Number of women/men	12/7	12/10	16/9	
Ethnicity (%)				
Caucasian	100	95	92	
African/American	0	0	8	
Asian	0	5	0	
Age (years)	52 ± 9	60 ± 10*	56 ± 9	0.04
Weight (lbs.)	184 ± 45	183 ± 40	193 ± 41	0.69
BMI (kg/m^2)	28.4 ± 5.0	29.0 ± 5.5	30.5 ± 5.2	0.42
Fasting glucose (mg/dl)	85 ± 9	95 ± 11	123 ± 53*†	0.001
Fasting insulin (IU/ml)	4 ± 4	5 ± 3	18 ± 29*†	0.02
Insulin sensitivity (units)	0.41 ± 0.05	0.39 ± 0.05	0.34 ± 0.06*†	0.001
HbA1c (%)	5.3 ± 0.3	6.0 ± 0.3	7.5 ± 1.8*†	0.001
Total cholesterol (mg/dl)	200 ± 34	206 ± 34	180 ± 39†	0.04
Low-density lipoprotein (mg/l)	125 ± 28	128 ± 30	105 ± 36†	0.03
High-density lipoprotein (md/dl)	55 ± 19	54 ± 16	48 ± 11	0.21
High-/low-ratio lipoproteins	3.8 ± 1.4	4.1 ± 1.2	4.0 ± 1.2	0.75
Triglycerides (mg/dl)	91 ± 36	129 ± 71	137 ± 61*	0.03
Medications				
Oral diabetic (total % of individuals on)	0	0	84*†	0.001
Specifics – number of people on				
Sulfonylureas			12	
Glucophage			13	
Thiazolidinediones			3	
Dipeptidyl peptidase IV inhibitors			5	
Insulin (total % of individuals on)	0	0	28*†	0.001
Specifics – number of people on				
Glucagon-like peptide agonist			4	
Rapid acting insulin			2	
Long acting insulin			4	
Antihypertension (total % of individuals on)	0	32*	802*†	0.001
Specifics – number of people on				
Hydrochlorothiazide		2	3	
Ace inhibitors		4	9	
Beta-blockers		1	7	
Calcium channel blockers		3	7	
Alpha 2 adrenergic agonists		1	0	
Angiotensin II receptors blockers		1	3	
Fish oil (%)	26	36	16	0.28
Statins (%)	0	23*	68*†	0.001

BMI = body mass index; Hb = haemoglobin; Mean ± SD.
* Significantly different from controls.
† Significantly different from prediabetic individuals.

Experimental design

This comparative study examined retinal reactivity (i.e. changes in vessel diameter to flickering light) using the Dynamic Vessel Analyzer (DVA) in three groups of subjects (healthy controls and individuals with prediabetes and type 2 diabetes). Subjects refrained from alcohol, caffeine (Terai et al. 2012) and exercise for 24 hr prior to testing and fasted for approximately 10 hr prior to testing. Measurements were taken in a dimly lit room at room temperature. Individuals with type 2 diabetes held their diabetic medications on the morning of the study. None of the prediabetic subjects were on diabetic medications. Aspirin and non-steroidal anti-inflammatory medications were held for 24 hr prior to the study. After a 15-min rest period, venous blood sam-

Table 2. Resting haemodynamics.

Subjects	Healthy controls	Prediabetes	Type 2 diabetes	p-value
HR (bpm)	62 ± 7	61 ± 9	68 ± 9†	0.01
MAP (mmHg)	93 ± 9	93 ± 8	98 ± 10	0.04
SBP (mmHg)	121 ± 11	128 ± 15	133 ± 15*	0.03
DBP (mmHg)	79 ± 8	76 ± 7	81 ± 8†	0.05

HR = heart rate; MAP = mean arterial pressure; SBP = systolic blood pressure; DBP = diastolic blood pressure; Mean ± SD.
* Significantly different from controls.
† Significantly different from prediabetic individuals.

ples of hs-CRP, glucose, insulin, lipid panel and HbA1c were drawn from the brachial antecubital location for later analysis. The eye with the best visual acuity was dilated with one or two drops of tropicamide (1%) and, if needed, phenylephrine (2.5%) was added to obtain optimal dilation.

Experimental protocol

After a rest period of 20 min to allow stabilization of baseline parameters, the subject's retinal vessels were imaged continuously for a total of 350 seconds. This protocol consisted of a 50-second baseline period, followed by three cycles consisting of a flickering light period (light flashes at a 12.5-Hz frequency for 20 seconds) followed by a 80-second rest period (Mandecka et al. 2007; Nguyen et al. 2009; Lott et al. 2012). BP and heart rate (HR) were measured continuously during the studies.

Measurements

Retinal vessel diameters
The DVA (Imedos Inc., Jena, Germany) uses a modified fundus camera (Zeiss FF450; Zeiss, Jena, Germany) and a video recording unit. The system visualizes retinal diameters in real time and vessel calibres can be analysed offline (Garhofer et al. 2010). To obtain images, the subject's fixation in the fundus camera was adjusted so that the optic nerve head was in the centre of the fundus monitor. The fundus cameras' focus and green background light were adjusted to provide crisp images on the fundus monitor. The region of interest (approximately 1.5 mm in length) was marked over a superior or inferior temporal retinal arteriole and venular between one and two optic disc diameters from the optic nerve disc. Selection criteria for the chosen segments included main vessels (segments >80 μm), a clear contrast to fundus background, no crossing or bifurcations and avoidance of nearby vessels within one vessel diameter of the chosen segment. This region of interest was scanned at a frequency of 25 times per second, and measurements were reported in arbitrary units (AU). Eye-tracking technology in the DVA compensated for small eye movements. Throughout the testing, the subject was verbally encouraged to maintain fixation and to blink frequently. All images were stored on a VHS videotape recorder for offline measurements. One observer analysed all measurements in a standardized fashion using the DVA software and corrected for any artefacts in the tracings due to spontaneous erroneous measurements.

Haemodynamics (HR and BP)
Heart rate, derived from the electrocardiogram, and BP were measured continuously by use of a Finameter device (model 2300; Ohmeda, Boulder, CO, USA, confirmed by an automated sphygmomanometer, Dinamap; Critikon, Tampa, FL, USA), and collected online at 200 Hz.

Plasma bloods
Plasma hs-CRP levels were assayed using radioimmunoassays (Diagnostic Products Corp., Los Angeles, CA, USA). Plasma glucose levels were assayed using a colorimetric method (Ortho Clinical Diagnostics, Auckland, New Zealand), and insulin levels were assayed using a chemiluminescent immunoassay (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA).

Data analysis

For flickering light trials, the resting baseline was averaged from the last 15 seconds prior to each flicker stimulation. Peak vasodilation period was an average of the highest diameters achieved during flicker and within approximately the first 3 seconds after the stimulus ended to capture maximal vasodilation. Percentage change in vessel diameter was calculated comparing baseline diameter with the peak diameter using the following equation: percentage change in diameter = $((\text{diameter}_{\text{peak}} - \text{diameter}_{\text{baseline}})/\text{diameter}_{\text{baseline}}) \times 100$). In addition, we measured maximal vasoconstriction after peak vasodilation (i.e. lowest diameter period was calculated by averaging the three consecutive lowest diameters after peak dilation). Lastly, we calculated the range of diameter change, known as relative amplitude (percentage change in relative amplitude = percentage change in peak vasodilation + |percentage change in vasoconstriction|; Lott et al. 2012). Vessels chosen for analysis were localized in the superior or inferior temporal quadrants. Quantitative insulin sensitivity check index (QUICKI) was calculated to assess insulin sensitivity (IR = 1/[(log insulin) + (log glucose)] (Muniyappa et al. 2008) in all subjects.

Statistical analysis

From previous published retinal studies on healthy and diabetic populations (Garhofer et al. 2004; Mandecka et al. 2007), power was calculated for a two-group comparison (control versus prediabetic or diabetic groups) for the main outcome measure, a change in retinal diameter. Using a one-way analysis of variance for group comparisons, 18 subjects per group were calculated to yield a power of 98% to detect a difference in retinal diameters. Analyses of covariance were used to examine the effects of potential confounding variables [i.e. age, mean arterial blood pressure (MAP), and BMI] (Mandecka et al. 2007; Kneser et al. 2009). *Post hoc* testing was done with Bonferroni. Repeated measures were performed for examining changes in vital signs during flicker between groups. With the groups' data merged, the correlation between two continuous variables (i.e. changes in diameter to flickering light stimuli and hs-CRP) was assessed using Pearson's correlation coefficients. Statistical significance was accepted at $p < 0.05$, 95% confidence interval. All data were reported as mean ± SD.

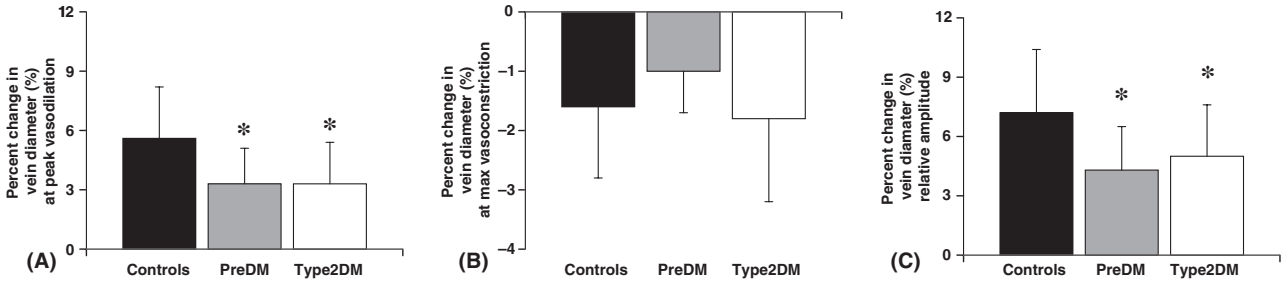

Fig. 1. Prediabetes and type 2 diabetes reduces retinal vein vasodilation to flicker stimulus. Prediabetic and diabetic subjects compared with healthy controls had attenuated retinal vein vasodilation responses to flicker-induced stimuli ($p = 0.001$; A). There was no significant difference in vein maximal vasoconstriction after peak vasodilation comparing the different groups ($p = 0.07$; B); however, the percentage change in relative amplitude in dilation showed attenuated responses in prediabetic and diabetic subjects, compared with controls for the vein ($p = 0.003$; C). There was no difference between groups on time to maximal vein vasoconstrictor responses (data not shown; *Significantly different from controls; $p < 0.05$; prediabetics = PreDM; type 2 diabetics = Type 2 DM).

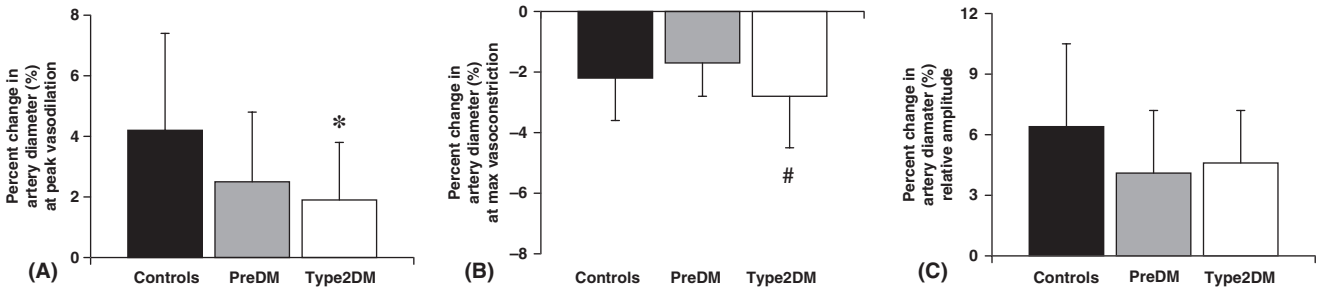

Fig. 2. Type 2 diabetes reduces retinal artery vasodilation to flicker stimulus. Type 2 diabetic subjects compared with controls had attenuated retinal peak artery vasodilator response to the flickering light stimulus ($p = 0.015$; A) with a greater maximal artery vasoconstrictor responses following the peak vasodilation compared with prediabetic subjects ($p = 0.034$; B). Prediabetic and type 2 diabetic individuals had a trend towards reduced retinal artery amplitude compared with controls ($p = 0.084$; C). No difference between groups in time to maximal vasoconstrictor responses was observed (data not shown); *Significantly different from controls, $p < 0.05$; #Significantly different from prediabetic group, $p < 0.05$; prediabetics = PreDM; type 2 diabetics = Type 2 DM).

Results

Subject characteristics

Groups were comparable in weight and BMI (Table 1), but those with prediabetes were slightly older than the controls. The majority of the subjects were Caucasian and women. Individuals had been diagnosed with prediabetes for an average of 1 year or type 2 diabetes for 6 years. Several prediabetic individuals were using statin (23%) and antihypertensive therapy (32%), but none used diabetic medications. Most of the subjects with diabetes were treated with oral diabetic medications (84%), and several were on combination therapy with long and/or short acting insulin (28%; Table 1). Most of the diabetic subjects were using single or multiple antihypertensive (80%) and statin (68%) therapy. All medications except beta-blockers were held the morning of the study. Diabetic subjects had higher fasting glucose and HbA1c levels and lower insulin sensitivity compared with controls and prediabetic subjects. Diabetic compared with prediabetic subjects had lower total cholesterols and LDL levels. Type 2 diabetic subjects had higher resting HR compared with controls and prediabetic subjects. Lastly, type 2 diabetic subjects had slightly higher resting systolic and diastolic BPs (Table 2).

Flicker light–induced vasodilation

Resting retinal artery and vein diameters were similar at baseline between all groups (artery: 116 ± 9 AU, 123 ± 20 AU, 118 ± 17 AU and vein: 139 ± 22 AU, 149 ± 19 AU, 148 ± 24 AU in controls, prediabetic and type 2 diabetic subjects, respectively; differences between groups $p > 0.05$). Flickering light evoked vasodilation in all groups; however, peak vein dilator responses were attenuated in the prediabetic and diabetic subjects compared with controls (Fig. 1A). Despite no difference in maximal vein vasoconstriction after peak dilation, prediabetic and diabetic subjects compared with controls also had attenuated relative amplitude changes in venular diameter (Fig. 1B,C). Similar findings were observed for retinal arterioles (Fig. 2A–C). Controlling for the covariates (i.e. age, MAP and BMI) did not alter the findings. There were no significant gender differences in arteriole or venular vasodilator or vasoconstrictor responses to the flickering light stimulus across all groups. Flickering light stimuli did not significantly change resting HR or BP in any of the groups (data not shown).

Glucose and insulin and BP effects on vasodilation

When the control, prediabetic and diabetic groups were combined, fasting insulin and HbA1c levels were associated with larger resting retinal vein diameters ($r = 0.25$, $p = 0.043$ and $r = 0.25$, $p = 0.046$). There was a trend towards an inverse relationship

between fasting insulin and venous peak flicker dilation ($r = -0.23$, p = 0.062) and relative amplitude change in venular diameter ($r = -0.21$, p = 0.088) and HbA1c and arterial peak flicker dilation ($r = -0.22$, p = 0.082). Fasting insulin was also significantly correlated to resting HR and MAP ($r = 0.37$ p = 0.002; $r = 0.25$, p = 0.040, respectively). Insulin sensitivity showed an inverse relationship to BMI ($r = -0.43$, p = 0.0001), HR ($r = -0.36$, p = 0.003), MAP ($r = -0.43$, p = 0.0001), systolic BP ($r = -0.40$, p = 0.001) and diastolic BP ($r = -0.37$, p = 0.003). Lastly, systolic BP was correlated to resting vein diameters ($r = 0.25$, p = 0.069) and inversely correlated to venous diameter peak flicker dilation ($r = -0.30$, p = 0.014) and relative amplitude change in venular diameter ($r = -0.32$, p = 0.008, respectively). No correlations in retinal reactivity and the subjects' lipid profiles were detected.

Hs-CRP levels and vasodilation

There was no significant difference in plasma hs-CRP levels between groups (Fig. 3). When the control, prediabetic and diabetic groups were combined, higher hs-CRP levels were associated with smaller resting retinal artery diameters ($r = -0.30$, p = 0.022). We observed no significant relationship between hs-CRP levels and the magnitude of the artery or vein dilator responses to flicker (i.e. peak vasodilation or relative amplitude; Fig. 4). However, hs-CRP was significantly associated with BMI ($r = 0.37$, p = 0.004).

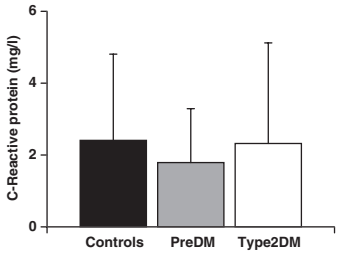

Fig. 3. Highly sensitive c-reactive protein (hs-CRP) levels similar across groups. There was no significant difference in hs-CRP levels between healthy controls, prediabetic and type 2 diabetic subjects (p = 0.71).

Discussion

In this study, we examined retinal vascular dilation responses to flicker in prediabetic and type 2 diabetic individuals and the relationship between retinal vasodilator responses and an inflammation biomarker (hs-CRP). We found similar attenuated vasodilator responses in individuals with prediabetes and diabetes compared with healthy controls. There was no relationship between flicker-induced vasodilation and hs-CRP levels. These data suggest that prediabetic subjects already have an impaired endothelial function early in the disease which is equivalent in magnitude to those found in diabetic subjects and mechanisms other than inflammatory hs-CRP responses may be involved in this early impairment.

Prediabetes, an early stage in the hyperglycaemic continuum, is associated with a greater risk of later developing type 2 diabetes (Haffner 2003; Ford et al. 2010; Milman & Crandall 2011) endothelial dysfunction and cardiovascular risk (Ford et al. 2010). In prediabetes and diabetes, both elevated glucose and insulin levels can lead to vasodilation of resting blood vessels potentially through an increase in vasodilators such as nitric oxide (Muniyappa & Quon 2007). Our data support prior retinal studies (Kifley et al. 2008; Sun et al. 2009) in which higher HbA1c and insulin levels were associated with larger retinal venular diameters.

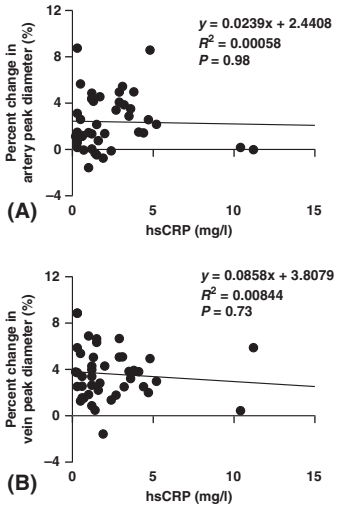

Fig. 4. No correlation between retinal dilation to flicker response and hs-CRP. When all groups were combined, there were no significant correlations between hs-CRP and percentage change in peak arterial or venular diameter to flicker dilation (p = 0.98 and p = 0.73, respectively).

Previous studies examining retinal vessel responses to flickering light stimuli in normal subjects demonstrated robust increases in retinal diameter and blood flow responses (Michelson et al. 2002; Dorner et al. 2003a,b; Nagel & Vilser 2004; Lott et al. 2012), whereas diabetic individuals had an attenuated retinal dilator responses (Garhofer et al. 2004; Mandecka et al. 2007; Bek et al. 2008; Nguyen et al. 2009; Pemp et al. 2009a,b; Lott et al. 2012). In addition, retinal attenuation further increased with the progression of diabetic retinopathy (Mandecka et al. 2007; Tilma & Bek 2012). Retinal vascular dysfunction is proposed to contribute to the pathogenesis of diabetic retinopathy (Schmetterer & Wolzt 1999), but the exact microvascular changes that precede diabetes are not clearly understood (Caballero 2005).

In our study, we confirmed an attenuated retinal vein vasodilator response in type 2 diabetics and showed that similar changes are already present in prediabetic individuals (diabetic individuals 3.3 ± 1.8% and prediabetic individuals 3.3 ± 2.1%) compared with healthy controls (5.6 ± 2.6%). Similar findings were found in the retinal arterioles in response to the flickering light stimuli. As aging, high BP and obesity are associated with attenuated flickering light responses (Mandecka et al. 2007; Kneser et al. 2009; Kotliar et al. 2011), we adjusted our analysis for age, BP, and BMI as covariates but the findings did not change with these adjustments. Differences in flicker magnitude of our subject groups compared with prior studies may reflect the use of different flicker light protocols (duration and Hz) as well as the recent change in classification of prediabetes and diabetes (2010). Similar to our results, previous retinal studies have shown that the flicker response was not associated with HbA1c, in type 1 and type 2 diabetic individuals (Garhofer et al. 2004; Mandecka et al. 2007). We also demonstrated that lower insulin sensitivity was associated with an attenuated venous vasodilator response. This is in agreement with previous studies which showed

that a lower QUICKI index was associated with type 2 diabetes (Katz et al. 2000) and prediabetes (Festa et al. 2003).

As it appears that prediabetic subjects may have an increased cardiovascular risk and may develop retinopathy, we now see that individuals with prediabetes also have altered retinal microvascular function. Thus, monitoring retinal reactivity may be an early marker of microvascular disease or endothelial dysfunction that clinicians can follow non-invasively.

Reduced retinal vasodilation in response to flickering light in prediabetes and diabetes may indicate several underlying pathological processes. These include impaired autoregulation and endothelial dysfunction. Vascular abnormalities may cause retinal damage such as pericycte loss which may change the release of local metabolites. Animal and human studies suggest that part of the flickering light vasodilation can be explained by an increase in the production of nitric oxide (Kondo et al. 1997; Dorner et al. 2003b). The attenuated flicker response in diabetes has been suggested to be partly due to reduced nitric oxide (Schmetterer et al. 1997). In a recent study, it was shown that retinal vessels in persons with type 1 diabetes have similar responses to exogenous NO as healthy controls (Pemp et al. 2009a), implying that the diabetic retinal endothelium is not less sensitive to NO. Thus, other factors may play a role in the altered vasoreactivity observed in prediabetes and diabetes. The attenuated responses in those with prediabetes and diabetes could also be due to the arteries already being in a dilated state to meet metabolic demand. However, as we found no significant difference in resting arteriolar and venular diameters between groups, we do not think that these vessels were predilated in the diabetic or prediabetic subjects. Lastly, impaired vasomotor responses observed in those with prediabetes and diabetes could result from impaired signalling between the neurosensory retina and the vessels. These impaired neurosensory coupling mechanisms may include glial cell or retinal barrier dysfunction and altered vascular endothelium growth factor signalling pathways (Pournaras et al. 2008; Antonetti et al. 2012).

Highly sensitive c-reactive protein, a general index of inflammation, has been primarily used in large epidemiological studies. Hs-CRP is produced by the liver and rises during inflammatory processes, at least in part due to increased interleukin-6 produced by macrophages and adipocytes. Elevations of hs-CRP have been associated with increased risk of developing diabetes (Doi et al. 2005; Dehghan et al. 2007; Hu et al. 2009) and heart disease (Wilson et al. 2008; Buckley et al. 2009) and risk of a having a myocardial infarction (Ridker 2004). Elevated hs-CRP has also been associated with elevated fasting glucose (Wu et al. 2002; Aronson et al. 2004; Nakanishi et al. 2005) and HbA1c levels (Wu et al. 2002). However, there are mixed findings of elevated hs-CRP and prediabetes (Doi et al. 2005; Sabanayagam & Shankar 2011). In our study, there were no significant differences between groups on hs-CRP. Factors that may have affected these results included the use of statins (Ridker et al. 1999; Tan et al. 2002) and good glycaemic control (King et al. 2003) of our diabetic individuals and/or obesity (Meng et al. 2007) in matching our controls to the other groups. In our study, we did see an association between higher hs-CRP levels and smaller resting artery diameters. However, in a large population study of individuals with a range of risk factors for coronary artery disease, elevated hs-CRP levels were associated with wider venules (Klein et al. 2006; Wong et al. 2006). Our study did not find a significant correlation between hs-CRP and vasodilator or constrictor responses to the flicker stimulus across the hyperglycaemic continuum. Other studies measuring macrovascular function such as brachial flow–mediated vasodilation have also not seen significant associations with hs-CRP (Vita et al. 2004; Kullo et al. 2007; Lippincott et al. 2008). As hs-CRP is only one of several indices of inflammation, we cannot totally exclude the effects of inflammation on the changes that we observed in the retinal vascular beds of prediabetic and diabetic subjects. Thus, altered retinal vasoreactivity may prove to be a significantly more sensitive indicator of atherosclerosis and vascular risk than plasma hs-CRP.

Limitations of the study

First, it is possible that there were differences in ocular perfusion pressures between groups in which higher ocular perfusion pressures would lead to an attenuated dilator response. Although intraocular pressures were not directly measured at the study visit, all subjects had recent eye examinations in which intraocular pressures were in the normal range (<21 mmHg). In addition, our findings did not change when we used BP as a covariate. Second, DVA requires optimal pupil dilation. Some of our prediabetic (27%) and diabetic (52%) subjects required additional dilation with the use of phenylephrine, which theoretically could elevate MAP through increasing sympathetic stimulation; however, our findings were still observed when these individuals were excluded from the analysis. Thus, we do not feel that the use of phenylephrine negatively impacted our study results. Third, we were only able to measure diameters, not blood flow, so it is possible that velocity may be altered differently by the flicker stimulus; however, we could not measure retinal blood velocity. Lastly, our hs-CRP levels were based on only a single measurement. In addition, the lack of group differences in hs-CRP may have been due to our diabetic subjects current statin use (Tan et al. 2002). As our study was powered for the percentage change in retinal diameter (observed power was 81–95%), it may be that a larger sample size may be needed to see differences in hs-CRP between groups. This may also help to explain why our study showed only trends for inverse relationships between fasting insulin and vessel diameter responses to flicker.

In summary, we have demonstrated that retinal reactivity is impaired in both prediabetic and diabetic individuals, compared with healthy controls. The inflammatory biomarker, hs-CRP, was not associated with changes in retinal vasoreactivity. Retinal vasoreactivity measurements may therefore be a more sensitive non-invasive indicator of early stages of atherosclerosis than traditional markers of cardiovascular risk such as hs-CRP. Prospective studies may determine whether this change in individuals with prediabetes is a harbinger of

future cardiovascular disease or retinopathy.

Acknowledgements

We would like to thank Surju Patel, Cheryl Blaha and Jessica Mast for their help with the study. We would like to thank the individuals who participated in the studies, as well as the nursing staff of the General Clinical Research Center for technical assistance. Support was provided by a grant from Pennsylvania Tobacco Settlement Funds, UL1 RR033184, and UL TR000127 as well as C06 RR016499. The DVA was partially funded by the Penn State Diabetes and Obesity Institute Equipment Grant and the Pennsylvania Lions Sight Conservation and Eye Research Foundation and the Jack and Nancy Turner Professorship at Penn State supported Dr Tom W Gardner. Dr Gardner is currently supported by the A. Alfred Taubman Medical Research Institute at the University of Michigan and a Research to Prevent Blindness Physician-Scientist Award. Lastly, the studies were performed in Penn State's Clinical Research Center which was supported by the National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health, through Grant UL1TR000127.

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Received on September 17th, 2012.
Accepted on February 14th, 2013.

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