



Novel Detection and Restorative Levodopa Treatment for Preclinical Diabetic Retinopathy

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Diabetic retinopathy (DR) is diagnosed clinically by directly viewing retinal vascular changes during ophthalmoscopy or through fundus photographs. However, electroretinography (ERG) studies in humans and rodents have revealed that retinal dysfunction is demonstrable prior to the development of visible vascular defects. Specifically, delays in dark-adapted ERG oscillatory potential (OP) implicit times in response to dim-flash stimuli ($<-1.8 \log \text{cd} \cdot \text{s/m}^2$) occur prior to clinically recognized DR. Animal studies suggest that retinal dopamine deficiency underlies these early functional deficits. In this study, we randomized individuals with diabetes, without clinically detectable retinopathy, to treatment with either low- or high-dose Sinemet (levodopa plus carbidopa) for 2 weeks and compared their ERG findings with those of control subjects (no diabetes). We assessed dim-flash-stimulated OP delays using a novel handheld ERG system (RETeval) at baseline and 2 and 4 weeks. RETeval recordings identified significant OP implicit time delays in individuals with diabetes without retinopathy compared with age-matched control subjects ($P < 0.001$). After 2 weeks of Sinemet treatment, OP implicit times were restored to control values, and these improvements persisted even after a 2-week washout. We conclude that detection of dim-flash OP delays could provide early detection of DR and that Sinemet treatment may reverse retinal dysfunction.

Diabetes is a global health issue that affected ~451 million people in 2017 with incidence predicted to rise to 693 million by 2045 (1). Diabetic retinopathy (DR), one of the

most common complications of diabetes, is the leading cause of blindness in working-age adults in the U.S. (2). The incidence of DR is expected to double from 7.7 million to 14.6 million people by 2050 (National Institutes of Health National Eye Institute data: <https://nei.nih.gov/eyedata/diabetic#5>).

DR is currently identified in eye clinics by visually observing vascular lesions such as microaneurysms and dot blot hemorrhages on dilated ophthalmoscopy or through fundus photographs in teleretinal screening. DR in the early stages typically does not produce visual loss, but can progress and advance to late-stage disease, inducing neovascularization (proliferative retinopathy) with associated macular edema, vitreous hemorrhage, retinal detachment, and neovascular glaucoma, all conditions that lead to substantial visual impairment or blindness (3). Loss of visual function at both early and late stages of DR reduces quality of life (4). Modern therapies that reduce progression to blindness include pan-retinal laser photocoagulation, vitreoretinal surgery, or intravitreal injections of anti-vascular endothelial growth factor antibodies. However, these treatments are costly and come with risk of complications (5). Thus, earlier detection and treatment strategies are of great interest to detect retinal defects that occur prior to structural vascular changes and to investigate whether interventions can subsequently prevent progression of DR and vision loss.

The electroretinogram (ERG) is a standard ophthalmic test used to record retinal function. Although the ERG is not used clinically to detect DR, our laboratory and others

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have demonstrated retinal dysfunction as early as 3–4 weeks of diabetes in rodent models by measuring ERG oscillatory potential (OP) implicit time delays (6–9). OPs are small wavelets on the rising phase of the ERG b-wave that are generated by inner retinal neurons, specifically amacrine cells (10). OP implicit time delays can be detected in models of both type 1 and type 2 diabetes (6,11) and people with diabetes, without retinopathy (12), in response to dark-adapted dim-flash stimuli ($< -1.8 \log \text{cd} \cdot \text{s/m}^2$ value) that selectively activate rod pathways in the retina. These OP implicit time delays often occur prior to other ERG wave defects, such as a- and b-wave implicit time delays or amplitude reductions in diabetic animal models (6,12–14).

Although ERGs are performed in a clinical setting, the dim-flash stimuli that reveal deficits in diabetic retinal function are not currently used in standard clinical ERG protocols to record OPs. The International Society for Clinical Electrophysiology of Vision (ISCEV) standard recommends a dim- ($-2 \log \text{cd} \cdot \text{s/m}^2$) and bright-flash ($0.5 \log \text{cd} \cdot \text{s/m}^2$) stimuli with only the bright flash used for OP analysis (15). We have shown that the ISCEV standard dim flash is not strong enough to elicit measurable OPs in individuals with diabetes, and the ISCEV standard bright flash does not show OP delays in early-stage diabetes (12). Thus, a nonstandard flash stimulus that is brighter than the ISCEV standard dim but still dominated by rod function is required (12). Furthermore, standard ERGs require dilating drops for the pupils and numbing eye drops to permit placement of a corneal electrode for recording, which are cumbersome, prolong the procedure, are uncomfortable to the patient, and are impractical in the clinical setting. Trained personnel, typically only available at specialty centers, are also needed for administering and interpreting the ERG. To determine if dim-flash OP delays could be used as a screening test for early-stage DR, we tested a portable handheld ERG device (RETEval; LKC Technologies, Inc., Gaithersburg, MD) using only skin electrodes and no dilation. The RETEval has already been shown to have similar sensitivity to gold-standard fundus examination for detecting vascular defects in patients with DR (16,17). In this study, we determined if the RETEval with dim-flash stimuli could detect preclinical DR.

Earlier detection of DR may reveal a treatment window in which neuroprotective agents could be administered to slow or halt the development of vision loss (18). One potential neuroprotective agent, dopamine (DA), is a key neuromodulator found throughout the body and within the retina, where it is released by dopaminergic amacrine cells. Diabetic animals have DA deficiencies in the retina (13), brain (19), and kidneys (20). When diabetic rodents are treated with levodopa (L-DOPA), a precursor to DA that crosses the blood-brain and blood-retina barriers, DA levels as well as early OP delays to dim-flash stimuli are restored (13). L-DOPA is already widely available as a U.S. Food and Drug Administration–approved drug to treat Parkinson disease, but it has not been evaluated for visual deficits in people with diabetes.

In this study, we proposed two goals: 1) to determine whether a handheld ERG device with a skin recording electrode had the sensitivity to measure OP delays in response to dim-flash stimuli in people with diabetes, without clinically detectable retinopathy; and 2) to evaluate whether L-DOPA treatments could restore OP implicit time delays in people with diabetes, without clinically detectable retinopathy.

RESEARCH DESIGN AND METHODS

Participant Inclusion/Exclusion Criteria

This clinical trial was registered with ClinicalTrials.gov (NCT02706977) and obtained Institutional Review Board approval (Emory University Institutional Review Board 83672). All participants were veterans, recruited from the Atlanta Veterans Affairs Health Care System. We recruited 15 control participants (male, $n = 12$; female, $n = 3$) between 37 and 69 years of age who had not been diagnosed with diabetes and without any confounding ocular diseases (i.e., retinitis, glaucoma, vitreous degeneration, high myopia, etc.) as verified by an eye examination within the last 6 months. To avoid difficulties when recording ERGs without dilation, patients with cataracts documented $>1+$ nuclear sclerosis were also not included (21). We recruited participants with diabetes between 29 and 71 years of age ($n = 44$, all male) (Table 1) who had been identified as not having signs of retinopathy based on diabetic teleretinal screening fundus photographs from the Atlanta Veterans Affairs Eye Clinic in the last 6 months. Individuals with diabetes were not included if they had any DA-dysregulating diseases, such as restless leg syndrome, Parkinson disease, or major depressive disorder. Additionally, to avoid confounding effects as well as prevent drug interactions once treated with Sinemet, participants were excluded if they were on any DA-enhancing drugs, such as DA agonists (i.e., bromocriptine, ropinirole, etc.) or monoamine oxidase inhibitors.

Participants were tested at baseline, 2 days (group with diabetes only), 2 weeks, and 4 weeks. Testing consisted of ERG recordings, uncorrected visual acuity testing, and drifting spatial contrast sensitivity thresholds. Fundus photographs of eyes from the group with diabetes included in the study were overread by a comprehensive ophthalmologist (A.Y.M.), masked to the treatment groups, who confirmed that no signs of retinopathy were present.

ERG Testing

The portable RETEval was preprogrammed with a protocol adapted from our previous animal and clinical work that reveals early DR deficits (6,12,14). Two dark-adapted flashes (“dim”: 1.13 Trolands [Tds] and “bright”: 85 Tds) were used to probe rod dominated and mixed rod-cone pathways, respectively. In addition, cone pathways were isolated using two light-adapted flicker steps (32 and 85 Tds at 30 Hz). To optimize the protocol as a screening procedure, we tested different dark-adaptation times (3, 10, or 20 min) and found 10 min to be sufficient to reveal the dim-flash OPs (Supplementary Fig. 1). Participants did

Table 1—Screening characteristics of study participants

	Healthy control subjects	Participants with diabetes with delayed OPs	Participants with diabetes with normal OPs (screen fails)
Patients, <i>n</i>	15	23	21
Sex, <i>n</i> of males	12	23	21
Age, years (mean ± SD)	55.8 ± 10.1	60.1 ± 7.3	55.8 ± 11.5
Disease duration, years (mean ± SD)	n/a	10.1 ± 7.6	9.3 ± 5.83
Type of diabetes (<i>n</i> of type 2)	n/a	23	19
HbA _{1c} , % (mmol/mol) (mean ± SD)	n/a	7.30 ± 1.06 (57.0 ± 11.3)	7.36 ± 1.01 (56.9 ± 12.0)
Race	11 AA, 4 W	19 AA, 4 W	9 AA, 12 W
Ethnicity, <i>n</i> of Hispanic or Latino	1	0	0

All individuals with diabetes were confirmed not to have retinopathy with fundus photography. AA, African American; n/a, not applicable; W, white.

not receive pupil dilation because pupil tracking within the ERG device adjusted the brightness of the flash stimuli automatically. Responses were recorded with skin electrodes (RETeval Sensor Strips; LKC Technologies, Inc.) that contained active, reference, and ground electrodes. Prior to electrode placement, the skin underneath the eye was scrubbed with gel (Nuprep Skin Prep Gel; Weaver and Company, Aurora, CO) to enhance signal conductivity and electrode sticking power. Any residue of the gel was wiped off with an alcohol preparation pad. Afterwards, the participant was asked to look straight ahead, and the nasal side of the electrode was aligned with the center of the pupil and positioned as close under the eye as possible without touching the eye or eyelashes.

ERG Analysis

Custom software was developed to both extract and analyze the ERG data (MATLAB, Version R2018a; MathWorks, Inc., Natick, MA). The a-wave amplitude was measured from baseline to bottom of the leading edge of the first large negative trough and implicit time from flash onset (Fig. 1A and C). The b-wave amplitude was measured from a-wave trough to the peak of the large positive wave of the recorded signal, and implicit time was measured from flash onset to peak (Fig. 1A and C). OPs filtered by the ERG software (band pass: 85–190 Hz) (RETeval) were superimposed on the raw ERG waveform in the custom analysis program. OPs were marked such that the first peak following the a-wave nadir was identified as OP1. OPs 2–4 were then marked in sequential order (Fig. 1B and D). OP amplitude was measured from trough to peak, and OP implicit times were measured from flash onset to peak. ERGs of all control subjects were collected and analyzed first to establish 95% CIs for normal values. These values were then used to determine inclusion of people with diabetes at baseline. For all participants (control and those with diabetes), recordings from both eyes were taken. For statistical analyses, each participant's most delayed eye was selected.

Screening of Individuals With Diabetes and Drug Dosage

People with diabetes and without retinopathy were tested at baseline using the ERG protocol described above. If the OP implicit times fell outside of the 95% CI of the control values collected at baseline (OP2: mean ± SD: 34.13 ± 4.34 ms; 95% confidence limit: 36.53 ms), the participant was randomized to either low-dose (25 mg carbidopa/100 mg L-DOPA) or high-dose (50 mg carbidopa/200 mg L-DOPA) of Sinemet Controlled Release Generic (McKesson Medical-Surgical, Las Colinas, TX). Prior to dispensing the drug, participants were screened for Parkinson disease by a physician (D.E.O. and P.M.T.) using the Unified Parkinson Disease Rating Scale test (all participants passed). Participants were instructed to take the oral Sinemet 12 h apart twice a day, preferably with a meal. The “2 day” visit was scheduled after the participant had taken three pills. Participants continued taking the medication for a total of 2 weeks (± 1 day), at which point they were retested and then underwent a 2-week washout period without the drug followed by a final testing session. Medication compliance was determined through verbal interview and tallying the remaining pills at the end of the study. In this study, two persons with diabetes withdrew from the study after experiencing known side effects of Sinemet: one for headaches and the other for frequent urination. Additionally, one participant was excluded at baseline, after a second, more recent fundus photo revealed retinal vascular abnormalities.

Drifting Spatial Contrast Sensitivity

A drifting spatial contrast sensitivity test was performed to assess visual function (Metropsis; Cambridge Research Systems, Ltd., Kent, U.K.). Participants were seated 1.5 m away from the monitor and used a button box to respond to a four-alternative forced-choice stimuli presented monocularly (contralateral eye was patched), with the room lights off. Prior to the stimulus, a gray fixation X in the middle of the screen was presented briefly, followed by the stimulus

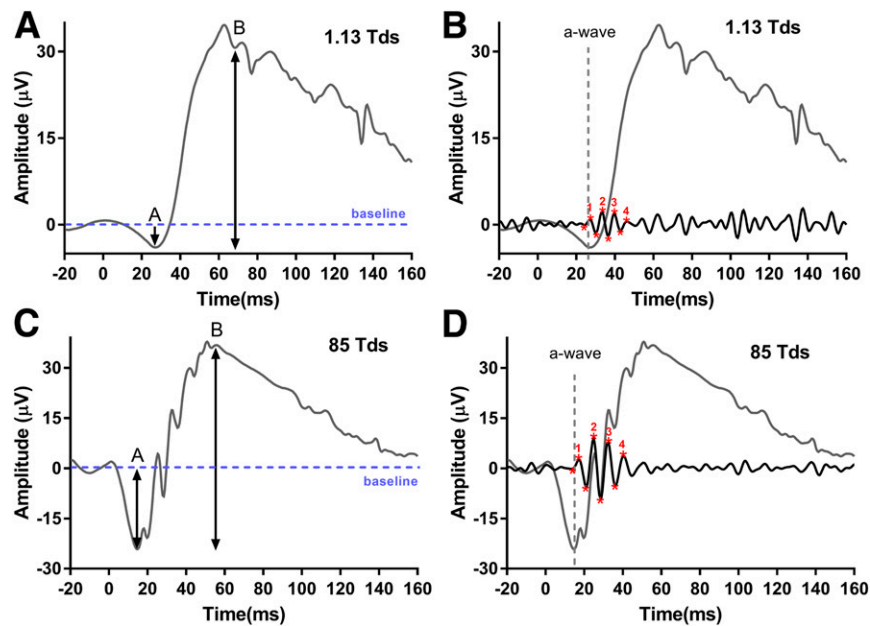


Figure 1—This waveform is an averaged representation of the ERG recordings and measurements to dim (A and B) and bright (C and D) flash stimuli from the control group. The a- and b-waves were marked first. OPs were filtered offline and then overlaid onto the full ERG waveform. OP1 was identified as the first peak after the a-wave trough. Red asterisks indicate the trough and peak of each OP wave.

presented simultaneously with a sound. The stimulus was a Gabor patch sized $\sigma 2$ with a sinusoidal grating that varied in spatial frequency (0.5–8 cycles/degree [c/d]) and contrast (starting at 50%) presented in one out of four orientations. A contrast sensitivity curve was generated for each eye at each visit. The contrast sensitivity was corrected for based on calculations from the screen's luminance (i.e., $[\text{maximum} + \text{minimum}]/[\text{maximum} - \text{minimum}]$) as a reciprocal of the Michelson contrast, as previously described (6). All participants had normal distance vision (no prescription for distance), and testing was done uncorrected.

Visual Acuity

Uncorrected visual acuity was tested in each eye using a logMAR chart starting at -0.3 c/d (Metropsis; Cambridge Research Systems, Ltd.). With room lights off and opposite eye patched, participants were seated 4.0 m away from the screen. All acuity measures were converted to Snellen decimal values due to technical difficulties, which only captured some participants best line read data.

Statistical Analyses

All data analyses were performed in Prism 7.02 (GraphPad Software, San Diego, CA) and SigmaPlot 13.0 (Systat Software, San Jose, CA). Data analyses for baseline ERGs were performed using an unpaired Student *t* test. Outcome measures recorded longitudinally across time were analyzed using two-way repeated-measures (RM) or mixed-effects model ANOVA with Tukey multiple comparisons. For all analyses, significance was set at an α of 0.05. Data shown in this study are means \pm SEM, unless otherwise stated.

Data and Resource Availability

The data sets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request. No applicable resources were generated or analyzed during the current study.

RESULTS

OP Delays Were Detected With Dim Flash in Eyes in the Group With Diabetes Without Clinical Retinopathy

OP delays in response to dark-adapted dim-flash (1.13 Tds) stimuli were detectable in 52% of all participants screened. OP2 implicit time test-retest variability for healthy control subjects was 2.54 ± 1.81 ms within a single session and 3.08 ± 1.45 ms for control participants across testing sessions. Analysis of clinical characteristics (age, disease duration, type of diabetes, HbA_{1c}, race, and ethnicity) did not reveal associations with early retinal dysfunction (Table 1) in this population study.

In response to a dark-adapted dim flash, OP implicit times were more delayed in eyes in the group with diabetes without retinopathy compared with control subjects (Student *t* test: $t = 3.47$, $P = 0.001$) (Fig. 2A). Control eyes had OP2 implicit times of 34.13 ± 1.12 ms compared with 38.67 ± 0.76 ms in eyes in the group with diabetes. OP2 was plotted for consistency, but other OPs showed a similar trend. In response to bright flash (Fig. 2B), OP implicit times were not different between eyes from the group with diabetes and the control group.

In addition to being able to detect OP delays in eyes in the group with diabetes without retinopathy, the dark-adapted dim flash also revealed a selective delay in a-wave (Student *t* test: $t = 2.49$, $P = 0.018$) and b-wave implicit

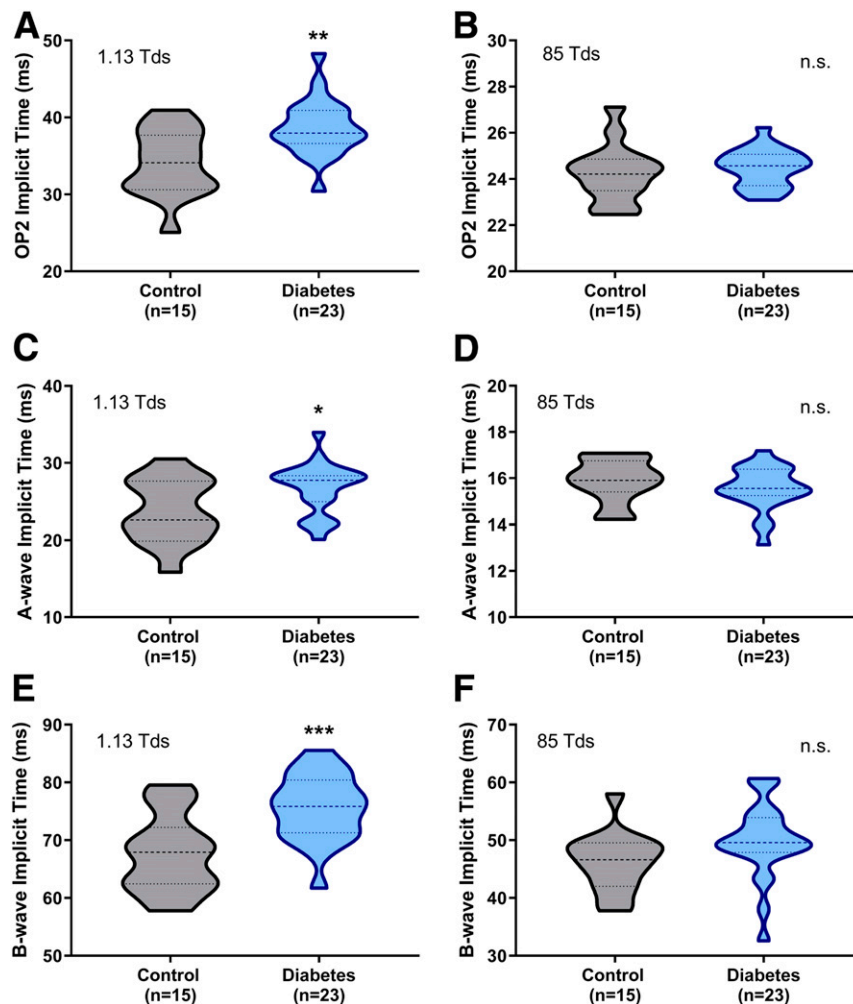


Figure 2—Dim-flash stimuli revealed ERG delays in diabetic eyes without retinopathy. *A*: OP2 delays in participants with diabetes were significantly delayed compared with control subjects in response to dim-flash stimuli (1.13 Tds; $P < 0.01$). *B*: In contrast, bright-flash stimuli (85 Tds) did not reveal any differences in OP implicit times in the same subject groups. Dim-flash stimuli also revealed delays in a-waves ($P < 0.05$) and b-waves ($P < 0.001$) in eyes from the group with diabetes compared with control subjects (*C* and *E*), while a- and b-wave implicit times to bright-flash stimuli (*D* and *F*) were similar. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

times (Student t test: $t = 3.94$, $P < 0.001$) (Fig. 2*C* and *E*) that were not seen with bright-flash stimuli (Fig. 2*D* and *F*). ERG flicker responses were not different between the subject groups (Supplementary Fig. 2).

Sinemet Treatment Restored OP Delays in Eyes in the Group With Diabetes Without Retinopathy

Of the 44 participants recruited, 23 had delayed OPs and were randomized to low-dose ($n = 13$) or high-dose ($n = 10$) Sinemet (see participant characteristics in Supplementary Table 1). Of these participants, four were lost to follow-up after the first visit ($n = 2$ in each dosing group) due to known side effects of Sinemet (one for headaches and one for frequent urination) or absences at scheduled appointments.

After only 2 days of Sinemet treatment, OP implicit times were significantly faster (Fig. 3*A*). After low-dose treatments, 100% of eyes in the group with diabetes had faster OP2 implicit times (Student paired t test: $t = 4.54$,

$P = 0.001$). Furthermore, with high-dose treatments, 87.5% of eyes in the group with diabetes had faster OP2 implicit times, although this difference did not reach significance. After 2 weeks of Sinemet treatment and a 2-week washout period, OP2 implicit times were significantly faster in eyes in the group with diabetes compared with baseline, approaching the control values (two-way RM ANOVA, main effect of time: $F[1.86, 53] = 11.39$, $P < 0.001$) (Fig. 3*B*). The high- and low-dose groups were not statistically different from each other; however, when analyzed separately, the low-dose group appeared more effective. While the control eyes had consistent OP values across time, the low-dose group was significantly faster compared with baseline at 2 weeks ($P < 0.001$) and 4 weeks ($P = 0.005$; two-way RM ANOVA: $F[2, 44] = 4.42$, $P = 0.02$). The high-dose group also had trends for faster OP2 values at 2 weeks but did not reach statistical significance. By 4 weeks, the OP2 values for the high-dose group were increasing

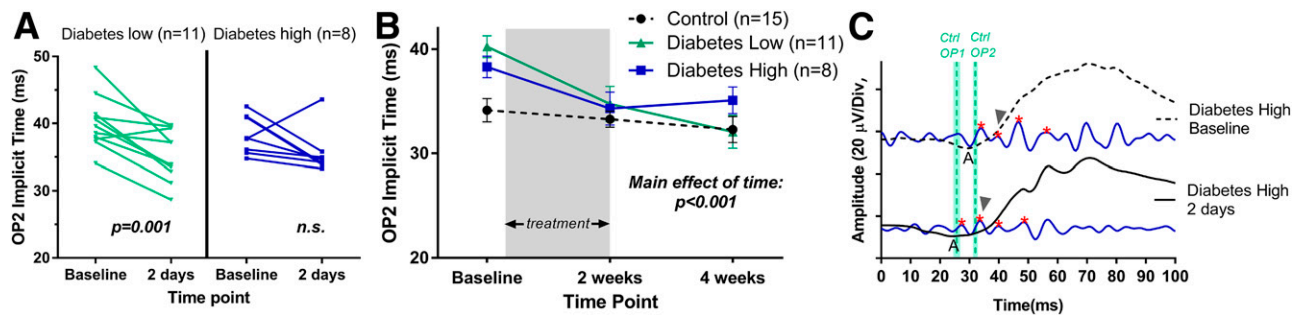


Figure 3—Sinemet treatments improve OP implicit times in eyes in the group with diabetes without retinopathy. **A:** After 2 days of low-dose treatment, inner retinal function, as measured by the OP implicit time, was significantly improved (Student paired t test: $t = 4.54$, $P = 0.001$). High-dose treatment produced faster OP2 implicit times in the majority of eyes in the group with diabetes but did not reach statistical significance. **B:** After 2 weeks of Sinemet treatments, both high- and low-dose groups had OP2 implicit times that were indistinguishable from control subjects. This effect was maintained at 4 weeks, following a 2-week washout period of the drug. **C:** Representative OP waveforms from a Diabetes High participant at baseline and 4 weeks (2 weeks of Sinemet treatment plus 2-week washout period). The OP waveforms are overlaid with the full ERG. Red asterisks indicate the OP peaks and show the improvement in implicit time across all OPs. Arrowheads indicate OP2 peaks.

toward baseline values (Fig. 3B). These differences in OP timing were easily observable in the waveforms, as shown in Fig. 3C. Even after the 2-week washout period, the Sinemet low dose–treated group retained OP implicit time improvements over baseline. OP amplitudes did not show any statistically significant differences between the group with diabetes and control participants (data not shown).

Importantly, Sinemet treatment did not alter the OP timing in response to dark-adapted bright flashes ($P = NS$) (Fig. 4A). Furthermore, participants who had normal OPs and received treatment (one undelayed eye or excluded later when data reanalyzed) did not show any changes in their dim-flash OP2 implicit times with either high- or low-dose drug treatment ($P = NS$) (Fig. 4B).

ERG a- and b-Waves of Eyes in the Group With Diabetes Have Faster Implicit Times With Sinemet Treatment

Low- and high-dose treatment significantly improved implicit times in a-waves elicited from the dark-adapted dim flash (Fig. 5A) (two-way RM ANOVA, main effect of time: $F[1.97, 56.1] = 9.07$, $P < 0.001$), showing consistent improvements even after the 2-week washout period (Fig. 3B). The b-wave implicit times from eyes in the group with diabetes did not significantly benefit from Sinemet treatment (two-way RM ANOVA, main effect of group: $F[2, 31] = 5.51$, $P = 0.010$) (Fig. 3C). The a- and b-wave amplitudes did not show any difference with time point or treatment (data not shown).

Visual Acuity and Contrast Sensitivity Not Altered by Diabetes or Sinemet

Visual acuity did not show a deficit at baseline in people with diabetes compared with control subjects (control, 0.76 ± 0.02 decimal; diabetes low, 1.05 ± 0.04 decimal; diabetes high, 1.06 ± 0.02 decimal). Furthermore, Sinemet treatment did not alter visual acuity over the course of the study (Fig. 6A). Contrast sensitivity function curves of control subjects and people with diabetes were

indistinguishable (Fig. 6B) with the peak at 2.0 c/d . Evaluation of peak contrast sensitivity threshold with Sinemet treatment did not show any significant changes between groups (Fig. 6C).

DISCUSSION

Our results show that early retinal dysfunction in individuals with diabetes is detectable prior to clinically recognized vascular changes using a handheld ERG device with a novel dim stimulus, a skin electrode, and no dilating drops. These data indicate that recording dim-flash OP delays could be used as a screening method to detect preclinical diabetic retinal dysfunction. Other RETeval studies have demonstrated ERG defects have equal to greater sensitivity than fundus photos in detecting DR at later stages (16,17). Additionally, using this early marker of preclinical diabetic retinal dysfunction, we demonstrated that Sinemet treatment could restore inner retinal function to normal levels very rapidly (within 2 weeks) and continue to provide benefit for at least 2 weeks after the drug treatments were stopped. These preliminary data suggest that dim-flash OP delays are very sensitive to diabetic changes and that reduced DA may be partially responsible for the early retinal dysfunction.

While current screening methods for DR have focused on vascular change detectable on direct visual inspection of the retina or through fundus photography, functional deficits measured by ERG have been reported in the context of DR for many years (22–25) despite variation in methodology (scotopic vs. photopic, flash intensity, waveform components analyzed, etc.) and disease stage (17,26). The ERG reflects activity in multiple layers of the retina, with the a-wave generated by photoreceptors (27), the b-wave by ON bipolar cells (28), and the OPs by amacrine cells (10). Abnormalities in these waves suggest underlying retinal defects in the respective cell type. Patients with diabetes with and without clinically detectable DR have been shown to have abnormal a-wave (29,30), b-wave (25,31), and

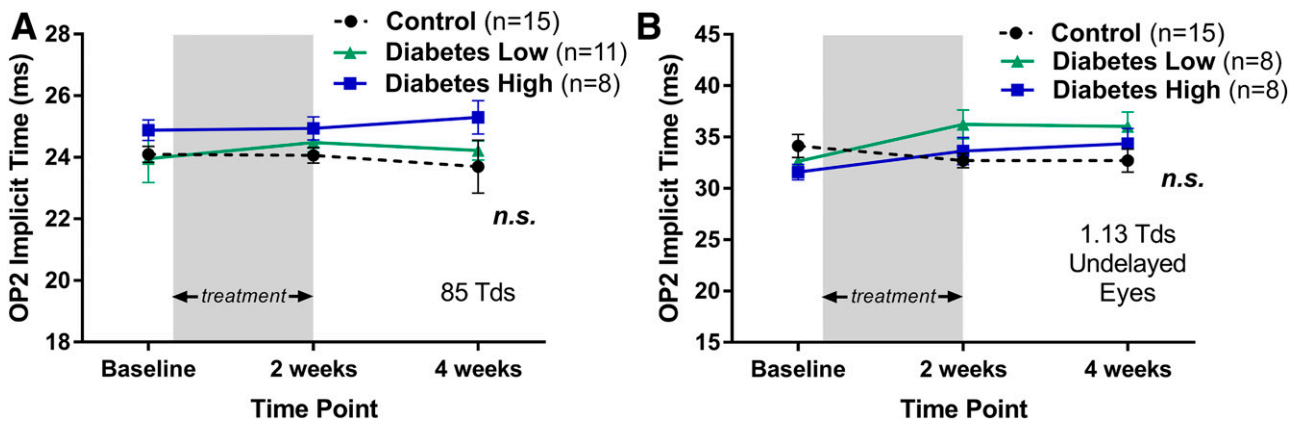


Figure 4—Sinemet treatment did not change OP timing in response to bright-flash stimuli or in eyes that did not have a delay. *A*: In response to bright flash, Sinemet treatment did not alter the OP implicit time. *B*: Eyes from participants with diabetes who had normal OP implicit times were not affected by the treatment. These results indicate that Sinemet only benefited eyes in which rod-driven inner retinal function was abnormal.

flicker (17,32) amplitudes and/or implicit times. However, OPs are thought to be the most sensitive measure of dysfunction prior to retinal neovascularization (6,12,33,34). While some studies report OP amplitude changes (26), we find the delay in OP implicit time in response to dim-flash stimuli to be the most sensitive OP parameter in preclinical studies of diabetic rodents in which progression of DR can be closely monitored, with OP implicit time delays occurring prior to a- and b-wave delays (6,11–14).

While mechanisms leading to retinal dysfunction in diabetes remain elusive, these data provide important insights into retinal cell types and pathways that are affected. The abnormality in OP implicit time with dim flash implicates inner retinal neurons in the rod photoreceptor pathway as being susceptible to the diabetes insult. Studies show that OPs are generated by several inner retinal neurons, including AII (10) and dopaminergic amacrine cells (35), and thus, diabetes likely creates an insult on multiple inner retinal cell types due to oxidative stress (36,37), metabolic changes (38), and other factors.

Our data indicate that photoreceptor and ON bipolar cell function, reflected by delayed a- and b-wave implicit times, respectively, is also abnormal prior to visible vascular structural defects in the diabetic retina. The delay in both a-wave and OP implicit times may suggest that photoreceptors are contributing to the downstream OP implicit time delays. However, analysis of OP implicit times relative to a-wave trough in each waveform still retained a significant delay in eyes in the group with diabetes (one-way ANOVA: $F[1, 31] = 5.69, P = 0.02$), suggesting that inner retinal dysfunction may be independent of photoreceptor dysfunction. Importantly, only the dim-flash OP and a-wave implicit times were significantly improved by Sinemet treatment, suggesting that reduced DA may underlie these abnormalities and/or that increased DA bioavailability may selectively enhance function of these cell types. L-DOPA treatments in diabetic rodents benefit multiple aspects of the visual system by slowing the progression of retinal dysfunction (ERG delays), as well as visual loss (spatial frequency and contrast sensitivity declines) (13,14). The absence of bright

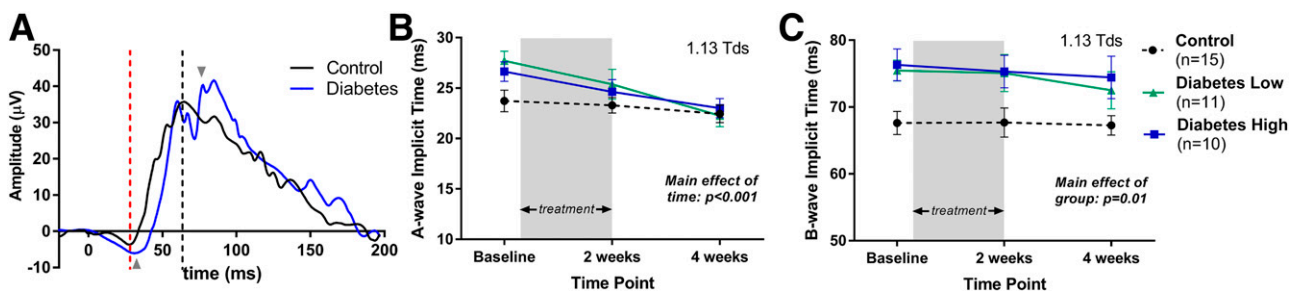


Figure 5—High- and low-dose Sinemet treatment resulted in faster a-wave implicit times in eyes from the group with diabetes. *A*: Representative control and diabetic waveforms that illustrate the delay in a- and b-wave at baseline. The red and gray dashed vertical lines indicate the a- and b-wave peaks in the control waveform, respectively. The gray arrowheads indicate the delayed peak for each wave. *B*: The a-wave implicit times were significantly improved at 2 weeks with values that became similar to the control group by 4 weeks. *C*: The b-wave implicit times were slightly improved by 4 weeks but did not reach control values.

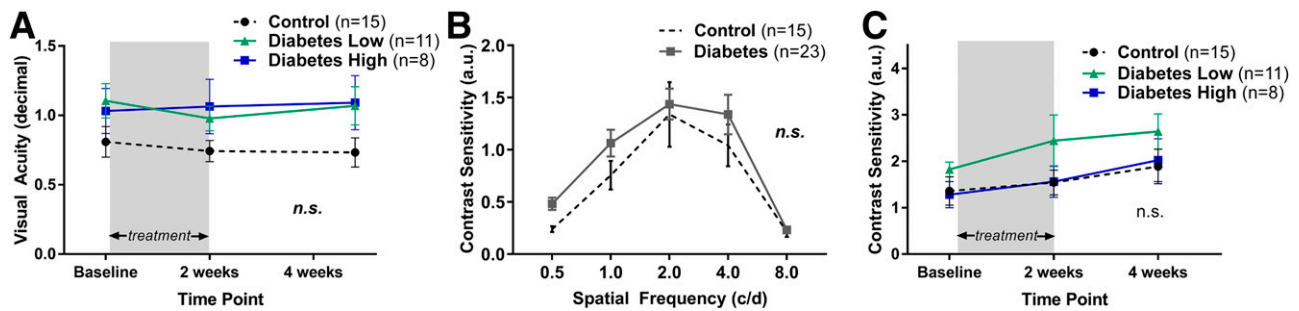


Figure 6—Visual function did not change in participants with diabetes and control participants with Sinemet treatment. *A*: Visual acuity thresholds were not different between the diabetic and control eyes at baseline. Sinemet did not alter thresholds. *B*: Contrast sensitivity function for diabetic and control eyes was identical with a peak at 2 c/d. *C*: Plot of contrast sensitivity at 2.0 c/d across time shows that treatment did not alter contrast sensitivity thresholds. a.u., arbitrary units.

flash or flicker ERG abnormalities in our data suggest that cone pathway function is not affected by diabetes at this early stage of the disease.

Sinemet treatment started prior to retinal vascular changes in diabetes could have long-term effects on the disease. Decreased DA levels have been reported in diabetic rodent retinas (13,14), with eventual loss of dopaminergic amacrine cells in late-stage disease (39). Treating the diabetic retina with L-DOPA at early stages of DR may restore DA levels to enhance retinal function and perhaps even promote survival of dopaminergic amacrine cells (18). Additionally, DA has antiangiogenic effects (40,41), and thus, L-DOPA may also help prevent the vascular defects that characterize clinically recognized DR, some of which are driven by ischemia to the retina and resultant angiogenesis (e.g., neovascularization of the disk). Further studies are needed to determine the long-term benefits of L-DOPA to the diabetic retina and whether it can slow the progression of DR.

A limitation of using Sinemet to treat DR is that people with diabetes would potentially need to take the drug for several years or even decades. Chronic use of Sinemet could have side effects, as observed in Parkinson disease in which dyskinesias develop (42). However, L-DOPA has been used to treat a variety of other diseases, such as restless leg syndrome (43), cardiovascular disease (44), and most recently as a therapy for age-related macular degeneration (ClinicalTrials.gov NCT03022318). Importantly, patients with Parkinson disease seem to be susceptible to the side effects of L-DOPA due to the loss of dopaminergic neurons in the substantia nigra, as well as a reduction in DA transporter (42) and dysfunctional *N*-methyl-D-aspartate receptors, which are important for normal DA metabolism (45). Similar changes have not been reported in individuals with diabetes, and thus, similar side effects may not be expected in this patient population.

In the current study, low-dose Sinemet was more effective than high-dose Sinemet in restoring OP implicit time delays. These data suggest that lower dose or even intermittent dosing of L-DOPA may be effective in reversing measurable early-stage DR that could minimize or prevent

potential side effects. The low-dose Sinemet used in this study contained 100-mg L-DOPA, a human equivalent dose (46) to the one showing efficacy in diabetic rodent studies (13,14). Initial studies suggest that lower doses may also be effective in diabetic rodents (47). The eye is also a unique organ for localized drug delivery. Future studies are needed to determine if eye drops or other ocular delivery methods would be as effective. However, it may be that systemic delivery is more efficacious for people with diabetes because L-DOPA may also benefit the diabetes-related DA reduction in the brain and kidney (48,49).

Although contrast sensitivity as well as visual acuity deficits have been shown to be decreased in diabetic rodent models as well as humans with diabetes (50,51), we did not observe any changes in our study for either test. We adopted a moving grating test for contrast sensitivity because robust declines in optomotor response are found in diabetic rodents (6,13), and we hypothesized that this would have more sensitivity to detect visual dysfunction compared with a static test. The lack of visual acuity and contrast sensitivity deficits in this study may be due to the fact that the study population had no clinically detectable DR, and visual acuity changes are often not seen until after clinical DR onset (50,52,53). Furthermore, although contrast sensitivity changes have been reported prior to vasculopathy in rodent models (6,11), as well as humans (50), the range of spatial frequency used in this study may not have been optimized for this detection, as the only notable contrast sensitivity difference reported for people with diabetes and without retinopathy was for a spatial frequency of 22.8 c/d. Most studies have also reported decreased contrast sensitivity in type 1 diabetes (54), and this study included mostly type 2 diabetes.

There are several limitations to the current study. It was a small study with limited sample size, which prevented sex and racial balance. A larger study is needed to determine potential sex or racial contributions to early OP delays detected in this study. Future studies are needed to determine if dim-flash OP delays are correlated with progression of DR and to determine if dim-flash OP delays may be a sensitive marker for monitoring HbA_{1c} levels,

blood glucose levels, etc. Furthermore, a longer study with L-DOPA treatment is needed to determine if the retinal vascular defects in DR benefit from L-DOPA. However, Sinemet is already approved by the U.S. Food and Drug Administration, with decades of clinical assessment, and is available as a generic, which would greatly facilitate testing.

In summary, these results show that early retinal dysfunction is detectable in the diabetic retina prior to clinically recognized retinopathy using a handheld ERG system with skin electrodes. This ERG testing approach could be used to screen individuals with diabetes in primary care clinics and other nonspecialty eye clinics. These findings also show that early retinal dysfunction is reversible using L-DOPA treatments, suggesting reduced DA levels underlie early retinal function deficits. The recovery of dim OP delays within 2 weeks of treatment demonstrate that OP delays may be sensitive to early-stage retinal dysfunction and provide a means to monitor both systemic and retinal-specific treatments for diabetes.

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