Hyperoxia improves contrast sensitivity in early diabetic retinopathy

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Abstract

***Aim*—The cause of vascular and visual pathology in diabetic retinopathy remains unknown. If retinal hypoxia plays a role, then early in the course of diabetes 100% oxygen breathing should normalise both contrast sensitivity and retinal blood flow.**

***Methods*—This hypothesis was tested in 12 diabetic patients with minimal retinopathy who, none the less, exhibited reduced contrast sensitivity ($p=0.003$ versus 12 age and sex-matched controls) and prolonged retinal arteriovenous dye transit ($p=0.0001$ versus controls).**

***Results*—Isocapnic hyperoxia failed to alter contrast sensitivity in controls, while it significantly improved contrast sensitivity in patients (at 12 cpd; $p=0.042$) to levels indistinguishable from normal. Individual improvement in contrast sensitivity correlated positively with the severity of the initial defect ($r=+0.84$, $p=0.0008$). Hyperoxia also had haemodynamic effects: it slowed retinal arteriovenous passage of fluorescein dye in controls, but did not further slow this transit time in patients.**

***Conclusions*—These results demonstrate the reversibility of early contrast sensitivity deficits in diabetes mellitus, and support the hypothesis that factors linked to tissue hypoxia initiate both visual and vascular dysfunction in diabetic retinopathy.**

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Diabetes mellitus patients, both with and without visible retinopathy, demonstrate reductions in contrast sensitivity and colour perception, and alterations in the pattern electroretinogram.[1–4] In patients with advanced disease, visual function losses correlate well with both the level of retinopathy and the extent of retinal oedema.[1 5] Further, patients whose retinal oedema regresses after panretinal photocoagulation also show improved contrast sensitivity.[6] None the less, in patients with early stage diabetes, little is known about how the disease provokes visual loss. In these patients, no visible evidence of damage in the retina is available to explain reductions in visual function.

Among the earliest vascular changes associated with diabetes are vasodilatation and increased retinal blood flow.[7] These changes have been hypothesised to result from either retinal tissue hypoxia[8 9] or from hyperglycaemia pseudohypoxia.[7] This latter theory holds that hyperglycaemia induced increases in cytosolic $NADH/NAD^+$ ratio provoke hyperaemia, endothelial damage, and vascular wall sclerosis.[7] If either of these theories is correct, then in early diabetic retinopathy, before major changes in vessel architecture have occurred, reducing tissue hypoxia (or $NADH/NAD^+$ ratios) with hyperoxia should normalise both blood flow and visual function. To test this possibility, in this study we examined in early stage diabetes the simultaneous influence of 100% oxygen breathing on both visual function (contrast sensitivity) and retinal perfusion (retinal arteriovenous passage time).

Patients and methods

Twelve insulin dependent diabetic patients and 12 healthy subjects were tested. All participated after signing informed consent to procedures reviewed and approved by a committee for protection of human subjects. The research conformed to the tenets of the Declaration of Helsinki.

DIABETIC SUBJECTS

Patients were recruited from the clinical practice of the Retina Service at the Indiana University Medical Center. All subjects presented for routine ophthalmic screening for diabetic retinopathy. Subjects had had insulin dependent diabetes for at least 5 years and no concurrent systemic illness (for example, hypertension or renal failure). An ophthalmic examination consisting of history, refraction, visual acuity measurement, slit-lamp, and detailed fundus examination was performed on each subject. Fundus examination was performed with a 90 dioptre lens at the slit-lamp and indirect ophthalmoscopy was carried out with a 20 dioptre lens. Visual acuity was 20/25 or better (corrected) in all subjects. The level of retinopathy was estimated in each eye. Only eyes with no detectable retinopathy or with haemorrhages and/or microaneurysms grade Ia or less (by the DRS modified Airlie House classification[10]) and no other signs of retinopathy were included.

NORMAL SUBJECTS

Healthy volunteers were recruited from the subject pool at the Indiana University Medical Center; they were chosen so that their mean age and distribution by sex would match that of the diabetic subjects. All normal subjects had 20/20 or better best corrected visual acuity.

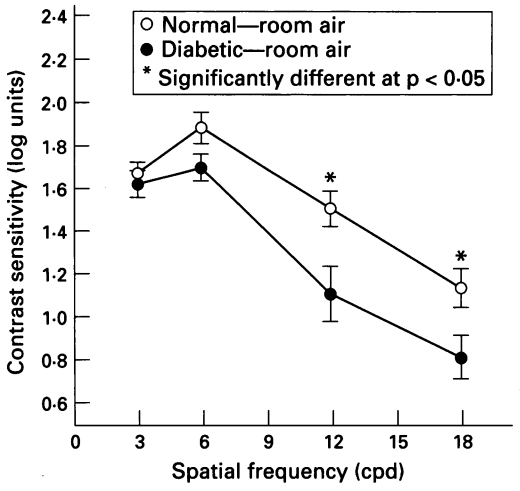

Figure 1 Contrast sensitivity at four spatial frequencies in normals and in diabetics with minimal retinopathy. Diabetics displayed depressed sensitivity at 12 and 18 cycles per degree (asterisks indicate a statistically significant difference (p=0·003 at 12 cpd; p=0·006 at 18 cpd) between normal and diabetic groups).

SCANNING LASER OPHTHALMOSCOPY

To assess retinal arteriovenous passage time nine diabetic subjects and 11 control subjects underwent scanning laser video fluorescein angiography during normal conditions and then during isocapnic hyperoxia created by breathing 100% oxygen. The two conditions were randomised and the subjects and technical staff masked to this order. Carbon dioxide was added during hyperoxic exposure to render control and high oxygen conditions isocapnic.[11] Subjects breathed from a mouthpiece connected to a low resistance, two way breathing valve.[11] On the inspired side, 100% oxygen was added to a mixing chamber, to which small amounts of carbon dioxide were added for isocapnia.[9] This protocol raises end tidal partial pressure of oxygen (P_{O_2}) to 600–700 mm Hg while leaving end tidal partial pressure of carbon dioxide (P_{CO_2}) constant at 35–40 mm Hg.[11] Both of the end tidal gases were monitored by rapid response specific gas analysers placed in the initial portion of the expired air stream.[11] Hyperoxia (or normoxia) was maintained for 15 minutes before fluorescein angiography and contrast sensitivity measurements were carried out. Arteriovenous passage time was measured by computer from digitised angiograms by calculating the time between the dye appearance (defined as attainment of 10% maximum dye intensity) on the temporal superior artery and the corresponding temporal vein.[12]

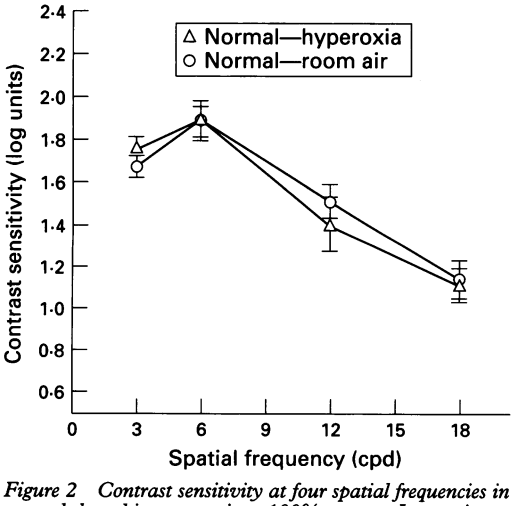

Figure 2 Contrast sensitivity at four spatial frequencies in normals breathing room air or 100% oxygen. Isocapnic hyperoxia failed to alter contrast sensitivity at any frequency.

CONTRAST SENSITIVITY

Contrast sensitivity was assessed in all 20 subjects during normal conditions and during isocapnic hyperoxia created by breathing 100% oxygen. The two conditions were randomised and the subjects were masked to their order.

Contrast sensitivity was assessed with the CSV-1000 (VectorVision; Dayton, OH, USA) contrast testing instrument. This instrument has recently been demonstrated to provide the necessary test/retest stability for assessing disease related changes in contrast sensitivity.[13] The instrument provides a retroilluminated translucent chart at a standardised light level of 85 cd/m^2. Four spatial frequencies (3, 6, 12, and 18 cpd) are tested using an orientation free two-alternative quasi-forced choice procedure. Testing time required is approximately 1 minute per eye.

STATISTICAL ANALYSIS

Unpaired *t* tests were used to compare baseline or hyperoxic measurements in normal versus diabetic people. Paired *t* tests were used to assess changes in each group as induced by hyperoxia; Bonferroni's correction was applied when multiple *t* tests were performed using a single data set.[14] A p value of $<0·05$ was regarded as statistically significant; all *t* tests were two tailed.

Results

The insulin dependent diabetic patients and the normal controls were similar in mean age (23 (SD 8) years for patients; 26 (7) years for controls; p=NS), and in sex distribution (seven male, five female controls; six male, six female patients). Two hours before the study, blood glucose in the diabetic patients (n=9) averaged 202 (34) mg/dl.

ROOM AIR

While breathing room air, diabetics displayed reduced contrast sensitivity at 12 and 18 cpd compared with controls (Fig 1; p=0·003 at 12 cpd, p=0·006 at 18 cpd). Diabetic patients also demonstrated slowed arteriovenous passage time through the retina while breathing room air (Fig 6; p=0·0001).

HYPEROXIA

During the imposition of isocapnic hyperoxia, normal subjects showed no alterations in contrast sensitivity (Fig 2) while arteriovenous passage time was substantially slowed in these subjects (Fig 6; p=0·0001). In contrast,

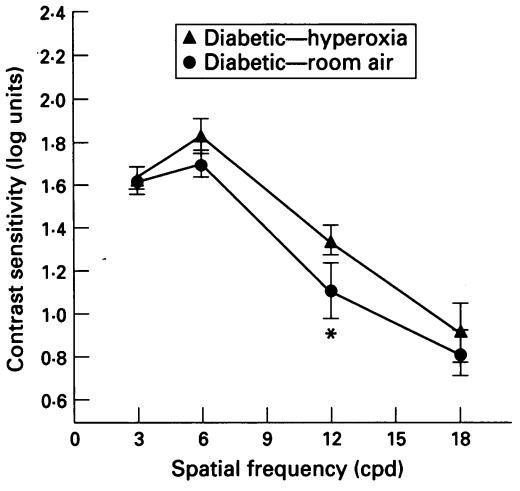

Figure 3 Contrast sensitivity at four spatial frequencies in diabetic patients with minimal retinopathy breathing room air or 100% oxygen. Isocapnic hyperoxia improved contrast sensitivity at 12 cycles per degree (asterisk indicates p=0·042).

hyperoxia significantly improved contrast sensitivity in diabetic patients at 12 cpd (Fig 3; p=0·042), without substantially altering arteriovenous passage time (Fig 6). Further, the improvement in contrast sensitivity at 12 cpd in diabetic patients during hyperoxia eliminated the differences in contrast sensitivity between the two groups; no significant differences in contrast sensitivity were found between normals and diabetic patients in hyperoxia (Fig 4). Similarly, hyperoxia eliminated the differences between normals and diabetic patients in arteriovenous passage time seen during room air breathing (Fig 6).

The improvement in contrast sensitivity at 12 cpd with hyperoxia in diabetic patients was proportional to the initial decrement in contrast sensitivity (Fig 5; $r=+0{\cdot}84$; p=0·0008). Impairment was determined relative to the mean sensitivity at 12 cpd as measured in the present control group.

Discussion

In this study we confirmed that insulin dependent diabetic patients with minimal retinopathy exhibit both visual (reduced contrast sensitivity) and retinovascular (slowed arteriovenous dye passage) abnormalities. When hyperoxia

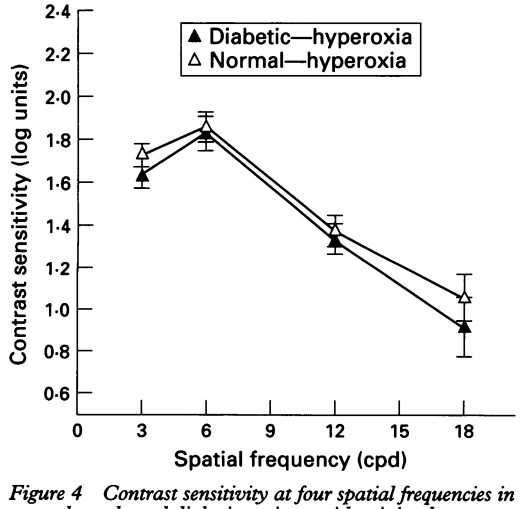

Figure 4 Contrast sensitivity at four spatial frequencies in normal people and diabetic patients with minimal retinopathy during isocapnic hyperoxia in both groups. No statistically significant differences in contrast sensitivity existed between the groups during hyperoxia.

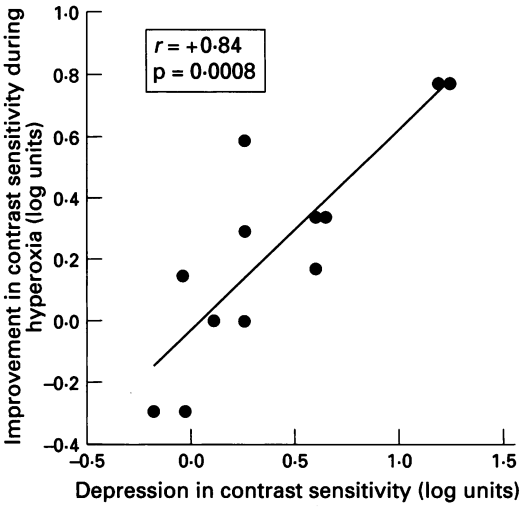

Figure 5 Significant positive correlation in diabetic patients of contrast sensitivity improvement during hyperoxia (y axis) with initial contrast sensitivity depression (x axis). All sensitivities were measured at 12 cycles per degree. Initial sensitivity depression was measured relative to the normal mean (n=12; r=+0·84; p=0·0008).

was imposed upon these patients, contrast sensitivity significantly improved at 12 cpd to levels indistinguishable from normal, even as arteriovenous passage time was unchanged. This result in patients contrasts with that found in healthy people, in whom hyperoxia did not alter contrast sensitivity even as it substantially slowed arteriovenous passage time.

The retinal vascular and visual defects caused by diabetes have been hypothesised to result from either localised tissue hypoxia[8 9] or from hyperglycaemic pseudohypoxia.[7] According to the latter hypothesis, cytosolic NADH/NAD^+ ratios, elevated in true hypoxia, are also elevated when hyperglycaemia results in excess reduction of glucose to sorbitol (via aldose reductase) and the subsequent excessive oxidation of sorbitol to fructose.[7] The resulting redox imbalance mimics that caused directly by low Po_2.[7] If either theory is correct, then delivering more oxygen to a hyperglycaemic tissue should correct either low tissue Po_2 or the redox imbalance and improve tissue function.[7]

We found both that hyperoxia improved mean contrast sensitivity in a group of diabetic patients, and that the improvement in sensitivity was proportional to the initial impairment. This result suggests that, at least in diabetic patients with minimal retinopathy, some aspects of diminished visual function remain acutely reversible. That these changes were seen at 12 cpd, and not so clearly, at 18 cpd, probably results from the smaller variance of repeated measures obtained at 12 cpd.[13] The result also suggests that some factor linked to retinal tissue hypoxia (or pseudohypoxia) causes the contrast sensitivity decline seen early in the course of insulin dependent diabetes. Earlier studies using the pattern electroretinogram hypothesised that diabetes mellitus may affect the larger retinal ganglion cells most severely,[15] although more generalised effects at every retinal neurosensory cell could not be ruled out.[15] The mechanism linking diabetes to retinal neurosensory cell dysfunction is

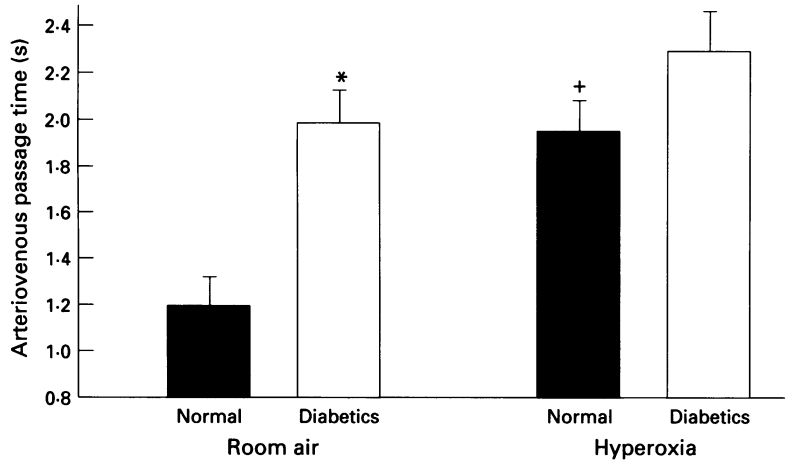

Figure 6 Retinal arteriovenous passage time in normal people and diabetic patients with minimal retinopathy under room air and hyperoxic conditions. While breathing room air, diabetics displayed slower arteriovenous passage of fluorescein dye (asterisk indicates p=0·0001 versus normals). Imposition of isocapnic hyperoxia failed to substantially change passage time in diabetics, while it did significantly slow transit in normals relative to the room air condition (plus indicates p=0·0001 versus room air for normals).

unknown, but could include the following sequence: (1) hyperglycaemia increases NADH/NAD$^+$ ratios via a series of oxidation/reduction reactions on glucose; (2) the consequent overactivation of aldose reductase reduces (Na,K) ATPase and (Ca, Mg) ATPase activities; and (3) reduced ATPase activities cause defects in neural conduction or sensitivity.[7 15] This line of speculation is strengthened by the finding that these ATPase activities are reduced in retinal endothelial cells in experimental diabetes,[16] and that aldose reductase inhibition in diabetic tissues restores ATPase activities to normal.[17] Unfortunately, treatment of diabetic humans with aldose reductase inhibitors has thus far been somewhat disappointing.[18] Certainly any comprehensive theory of the biochemical mechanism linking diabetes to defective visual function must also potentially include many other factors, such as changes in lipid metabolism, superoxide anion products, and the role of nitric oxide.[7 19 20] Along this same line, it will be important to determine how fluctuations in blood glucose (in this study only measured 2 hours before testing) affect contrast sensitivity and blood flow.

Biochemical changes consequent to diabetes also result in vascular dysfunction.[21] Retinal tissue oedema, capillary leakage, vascular sclerosis, and frank under or overperfusion are all well known consequences of chronic insulin dependent diabetes.[12 22 23] It remains debatable, however, to what extent vascular changes occur early in disease, before the emergence of frank retinopathy. For example, some authors find unchanged retinal blood flow in diabetics with only mild retinopathy[24]; others find early evidence of hyperaemia[22] or of altered capillary perfusion.[25] Our finding of slowed arteriovenous passage time in diabetic patients with minimal retinopathy, but with visual function impairment, confirms the presence of simultaneous vascular and visual dysfunction. Whether such vascular changes are essential for depression of contrast sensitivity remains unknown. It is also important to note that arteriovenous passage time is only partly dependent on bulk retinal blood flow: many factors, both blood flow and non-blood flow dependent, affect its value.[7 21] Consequently, the slower arteriovenous passage time exhibited by diabetic patients could be created entirely by variables independent of flow, such as tissue blood volume or vascular permeability.[7 21 23]

When healthy tissue is perfused with blood with increased oxygen content, blood flow decreases to allow total oxygen delivery to remain constant.[11] This response, seen in the healthy retina,[11] probably slowed arteriovenous dye passage in hyperoxia in this study in healthy individuals.[11] These hyperoxia induced changes in retinal haemodynamics in healthy eyes left contrast sensitivity unchanged. On the other hand, earlier work suggests that diabetes blunts or abolishes hyperoxia induced vasoconstriction in affected tissues: retinal blood flow diminishes less than normal, or not at all, in diabetic patients breathing 100% oxygen.[8] Our finding that there was no change in retinal arteriovenous passage time in diabetic patients given 100% oxygen to breathe may indicate a blunted vasoconstrictor or autoregulatory capacity in that tissue. However, as before, the indirect nature of the measurement makes it impossible to determine whether blood flow, or tissue oedema or permeability factors, are altered by hyperoxia in diabetes. What our data do indicate is that the haemodynamic response to an autoregulatory challenge is abnormal in diabetic patients with minimal retinopathy, providing further evidence that vascular abnormalities are present early in disease.

In conclusion, we have demonstrated reversibility (via 100% oxygen breathing) of the contrast sensitivity defects seen early in the course of diabetic retinopathy, and the association of these defects with retinal haemodynamic abnormalities. That an increase in arterial oxygen content can restore visual function in these people testifies both to the potential reversibility of early visual deficits in diabetes, and to the role of hypoxia (or hyperglycaemic pseudohypoxia) in their initiation.

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