Effects of Insulin on Retinal and Pulsatile Choroidal Blood Flow in Humans

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Background: Insulin induces vasodilation in several tissues, including skeletal muscle and kidneys.

Objective: To investigate whether insulin may contribute to ocular blood flow regulation.

Methods: The study was performed in a balanced, randomized, placebo-controlled, single-masked, 3-way, crossover design in 9 healthy male subjects. Each subject received 2 doses of insulin (1.5 or 3 mU/kg per minute) or placebo on 3 different study days. Measurements of fundus pulsation amplitude with laser interferometry to assess pulsatile choroidal blood flow, of retinal blood flow with the blue-field entoptic technique, and of mean blood flow velocity in the ophthalmic artery with Doppler sonography were performed under euglycemic clamp conditions over 120 minutes.

Results: Hyperinsulinemia significantly increased fundus pulsation amplitude (1.5 mU/kg per minute: 8.7% ± 1.1% vs baseline; 3 mU/kg per minute: 13.2% ± 2.3% vs baseline; P < .001 vs placebo [analysis of variance]) and mean blood flow velocity (1.5 mU/kg per minute: 10.0% ± 4.3% vs baseline; 3 mU/kg per minute: 6.6% ± 3.5% vs baseline; P = .03 vs placebo). Retinal blood flow did not increase during administration of insulin (1.5 mU/kg per minute: 6.4% ± 8.0% vs baseline; 3 mU/kg per minute: 8.0% ± 5.1% vs baseline; P = .99 vs placebo). Neither the effect in the choroid nor that in the ophthalmic artery was dose-dependent.

Conclusion: Hyperinsulinemia significantly increases choroidal blood flow and mean blood flow velocity in the ophthalmic artery. By contrast, retinal blood flow was not influenced by hyperinsulinemia. The maximum effective dose of insulin for ocular hemodynamics is likely to be within the physiological range.


The role of insulin in glucose metabolism is well understood and has been described in detail. However, cumulative evidence indicates that insulin, independent of its endocrine effect, has potent action on vascular tone. Insulin is now considered an important vasoactive peptide involved in the hemodynamic regulation of skeletal muscle and kidney blood flow. Exogenous insulin has a dose-dependent effect that decreases vascular resistance and raises cardiac output with only small effects on blood pressure. Insulin exerts 2 antagonistic hemodynamic effects, inducing sympathetic vasoconstriction on the one hand and vasodilation via different pathways on the other hand. Direct effects on smooth muscle cells, endothelial nitric oxide, and adenosine seem to be involved in insulin-induced vasodilation.

Attempts have also been made to characterize the physiological role of insulin in the regulation of ocular blood flow. In vitro studies demonstrated that intraluminal application of insulin in potassium-contracted retinal artery segments causes a dose-dependent dilation via endothelial-derived nitric oxide. In a previous study, we have shown that mild hyperinsulinemia induces a small but significant effect on pulsatile choroidal blood flow. However, in vivo data on the retinal hemodynamic effects of insulin are still lacking.

Ocular vasodilator effects of insulin are of clinical importance, because retinal blood flow (RBF) is increased in patients with insulin-dependent diabetes mellitus even before the onset of diabetic retinopathy. Moreover, elevated ocular blood flow is assumed to play a role in the pathophysiological process of diabetic retinopathy. Whether this increase is solely related to hyperglycemia-induced biochemical alterations or is caused by other factors, such as hyperinsulinemia, remains unclear.
SUBJECTS AND METHODS

SUBJECTS

After approval from the Ethics Committee of Vienna University School of Medicine was obtained, 9 healthy, male, nonsmoking volunteers were studied (age range, 20-31 years; mean ± SD, 26.4 ± 3.8 years). The nature of the study was explained and all subjects gave written consent to participate. All volunteers passed a pre-study screening during the 4 weeks before the first day, which included a physical examination and medical history. 12-lead electrocardiogram, complete blood cell count, urine analysis, random urine drug screen, a standardized oral glucose tolerance test, and an ophthalmic examination. Inclusion criteria were normal findings in the screening examinations and ametropia of less than 3 dioptries.

EXPERIMENTAL DESIGN

The study was performed in a balanced, randomized, placebo-controlled, single-masked (analyst only), 3-way, crossover design, with a washout period of at least 4 days between the study days. Each subject received 2 doses of insulin (1.5 and 3 mU/kg per minute) or placebo (saline) on different study days in a randomized sequence. All subjects were asked to refrain from alcohol and caffeine for at least 12 hours before trial days and were studied after an overnight fast.

STUDY PROTOCOL

Two plastic cannulas (Venflon) were inserted into antecubital veins for administration of insulin, glucose, and potassium (cannula 1) and for the monitoring of plasma glucose concentrations (cannula 2). After a 20-minute resting period in a sitting position, baseline measurements of ocular hemodynamics, blood pressure, and pulse rate were performed.

Thereafter, the euglycemic insulin clamp (or saline), was started for 120 minutes. Euglycemic clamps were performed according to the method of DeFronzo et al.1 Dosage 1: Each clamp was started with a loading dose of insulin of 3 mU/kg per minute for 2 minutes, followed by a slow decrease of the infusion rate by 0.3 mU/kg per minute every 2 minutes. After 6 minutes, the infusion rate remained constant at 1.5 mU/kg per minute. Dosage 2: Each clamp was started with a loading dose of insulin of 6 mU/kg per minute for 2 minutes, followed by a slow decrease of infusion rate by 1 mU/kg per minute every 2 minutes. After 6 minutes, the infusion rate remained constant at 3 mU/kg per minute. If indicated, potassium chloride was infused to prevent hypokalemia. Glucose was infused at a rate necessary to maintain the blood glucose level between 20 and 100 mg/dL. Arterialized venous blood samples were drawn every 5 minutes from the contralateral arm, which was heated in a 40°C blanket. Glucose concentrations were measured with a Beckman glucose analyzer (Beckman, Fullerton, Calif).

Hemodynamic measurements were taken every 15 minutes. The mean of 2 successive measurements was taken for data analysis. Blood pressure was measured in 5-minute intervals during the study period. Pulse rate and a real-time electrocardiogram were monitored continuously.

SYSTEMIC HEMODYNAMICS

Systolic, diastolic, and mean blood pressures were measured on the upper arm by an automated oscillometric device. Pulse rates were automatically recorded from a finger-pulse oxymeter (HP-CMS patient monitor; Hewlett-Packard, Palo Alto, Calif).

BLUE-FIELD ENTOPTIC TECHNIQUE

The retinal microcirculation was assessed with the blue-field simulation technique (BFS-2000; Oculix Sarl, Arbaz, Switzerland). This noninvasive method is described in detail by Riva and Petrig.13 The blue-field entoptic phenomenon is defined as the perception of leukocytes flowing through the subject’s perimacular retinal capillaries. If the fundus is illuminated with blue light, which, optimally, has a center wavelength of 430 nm and a narrow optical spectrum, many tiny corpuscles can be observed flying around swiftly in an area of 10 to 15 degrees of arc radius centered at the fovea. Most likely, this phenomenon is caused by the

Therefore, we set out to characterize the effects of insulin on RBF and pulsatile choroidal blood flow in healthy subjects, using laser interferometric measurement of fundus pulsation and the blue-field entoptic technique, respectively.

RESULTS

No significant differences in baseline ocular hemodynamic parameters between study days were observed (Table 1). Placebo had no relevant effect on systemic or ocular hemodynamic parameters.

Coefficients of variation for the ocular hemodynamic parameters assessed on the placebo day are presented in Table 2. The reproducibility was best for laser interferometric measurement of fundus pulsation. The coefficients of variation for measurements of RBF with the blue-field entoptic technique and the mean blood flow velocity in the ophthalmic artery were considerably larger. This is also evidenced by our calculations of intraclass correlation coefficients. The κ value of 0.97 for laser interferometric measurements of fundus pulsations represents excellent reproducibility. The intraclass correlation coefficients for RBF measurements of \( \kappa = 0.85 \) and for MFV determinations in the ophthalmic artery of \( \kappa = 0.70 \) indicate acceptable short-term reproducibility.

Mean blood pressure and pulse rate were not significantly changed during administration of insulin (Table 3). Effects of insulin on ocular hemodynamic parameters are illustrated in the Figure. Insulin increased fundus pulsation amplitude, with a maximum effect at 120 minutes (1.5 mU/kg per minute: 8.7% ± 1.1% vs baseline [95% CI: 6.0%-11.5%]; 3 mU/kg per minute: 13.2% ± 2.3% vs baseline [95% CI: 7.3%-19.1%]; P<.001). We observed no difference in fundus pulsation amplitude effects between the 2 dosages of insulin (P = .27).

Administration of insulin had no influence on RBF (1.5 mU/kg per minute: 6.4% ± 8.0% vs baseline [95% CI:
fact that short-wavelength light is almost totally absorbed by hemoglobin and therefore by red but not white blood cells. Thus, the passage of a white blood cell through a capillary loop close to the photoreceptors is perceived as a flying corpuscle.

For determination of retinal hemodynamic parameters, a simulated particle field was shown on a video monitor to the study subjects. By comparison, with their own entoptic observation, subjects adjusted the density and the mean flow velocity (MFV) of white blood cells at the monitor to match the simulated corpuscles to the perceived leukocytes of their fundus. Retinal blood flow was calculated as the product of MFV and density of white blood cells. Each measurement consisted of at least 5 matching tests, and the means of velocity and density were calculated. Only values with SDs lower than 15% were accepted as accurate. Since this depends on the skill of the volunteers, subjects who did not reach an SD lower than 15% were excluded from the trial.

LASER INTERFEROMETRY

Pulse synchronous pulsations of the ocular fundus were assessed by a laser interferometric method, described in detail by Schmetterer et al.\textsuperscript{14,15} The ocular fundus of the subject is illuminated by a high-coherence laser beam (\( \lambda = 783 \text{ nm} \)) along the optical axis. The laser power of approximately 80 \( \mu \text{W} \) at a diameter of 1 mm is much lower than the limit set by the American National Standards Institute.\textsuperscript{16} The light is reflected at both the retina and the outer surface of the cornea, the latter serving as the reference wave. The relative distance changes between cornea and retina during a cardiac cycle can be evaluated by analysis of the interference fringes, which are produced by the 2 reemit- ted waves. The distance between cornea and retina decreases during systole and increases during diastole in the order of several micrometers. The fundus pulsation amplitude, which is the maximum distance change between cornea and retina during the cardiac cycle, has been shown to estimate pulsatile blood flow in the selected fundus location.\textsuperscript{17} The interferometer is coupled to a fundus camera (FK-30; Zeiss, Oberkochen, Germany), which allows real-time inspection of the measurement point on the retina.

DATA ANALYSIS

Data are presented as mean ± SEM. The effect of insulin or placebo on outcome parameters was assessed with repeated-measures analysis of variance. Post hoc comparison was done with a paired \( t \) test using the Bonferroni correction for multiple comparisons. A value of \( P < .05 \) was considered significant.

To estimate the short-term reproducibility of each method, the coefficient of variation was determined from each subject’s SD using the 5 measurements obtained on the placebo day. The mean of these individual coefficients of variation is presented as a measure of short-term variability of the measurements. The 95% confidence intervals (CIs) for drug effects at 120 minutes were calculated from individuals’ percentage of change from baseline.

In addition, the intraclass correlation coefficient, was calculated (according to the method described by Kramer and Feinstein\textsuperscript{19}) from the variance among subjects (\( \upsilon_s \)), the variance among levels (\( \upsilon_m \)), and the residual error variance (\( \upsilon_r \)): \( \kappa = (\upsilon_s - \upsilon_r)/(\upsilon_s + \upsilon_m + 2\upsilon_r) \). The \( \kappa \) statistic is a generally accepted measure of reliability of repeated measurements.\textsuperscript{20} A \( \kappa \) value of 1 represents perfect reproducibility.

intravenous administration of insulin at 1.5 mU/kg per minute produces plasma levels of around 100 \( \mu \text{U/mL} \). This corresponds with insulin’s action on leg blood flow, where the maximum effective dose is reached at plasma levels of around 80 \( \mu \text{U/mL} \).\textsuperscript{1}

In humans, the hemodynamic effects of insulin have been investigated in the forearm,\textsuperscript{2} leg,\textsuperscript{21} kidney,\textsuperscript{8,22} and choroid.\textsuperscript{5} In the forearm and leg, insulin increased blood flow in a dose-dependent fashion and decreased regional vascular resistance. The latter effect was more pronounced than the effect on systemic vascular resistance, indicating substantial differences in insulin’s vasoactive properties in different vascular beds. This is in agreement with renal studies in which mild hyperinsulinemia increased renal plasma flow by approximately 10%\textsuperscript{22} but did not affect systemic hemodynamics.

In the eye, studies of hemodynamic actions of insulin in vivo were only available on pulsatile choroidal blood flow, which was increased by approximately 10%
The present study confirms and extends these findings, in that we found that the maximum effective dose of insulin’s action on ocular hemodynamics is likely below plasma levels of around 100 µU/mL and therefore within postprandial physiological ranges.

With regard to retinal circulation, data are limited to in vitro studies. In potassium-precontracted retinal artery segments, intraluminal application of insulin caused a dose-dependent vasodilation. However, there is evidence from studies of patients with non–insulin-dependent diabetes mellitus that the vasodilator effect of insulin is less pronounced than that of glucose. A decrease in plasma glucose levels via exogenous administration of insulin causes a marked decrease of RBF. However, the vasodilator properties of glucose are potent, and have been studied by several investigators in animal and human studies. Thus, the present experiments were conducted under carefully controlled euglycemic conditions.

We did not observe a significant effect of insulin on RBF. The coefficient of variation of measurement for RBF with the blue-field entoptic technique was 8.8%. Hence, our data indicate that the effect of insulin on RBF is small. The reproducibility obtained in the present trial is in keeping with the findings of Grunwald and Zinn, who showed that the minimum percentage change of the mean leukocyte velocity that could be detected with the blue-field entoptic technique was 9%. Our calculation of the intraclass correlation coefficient also indicates that the short-term variability of the measurements with the blue-field entoptic technique is acceptable in skilled healthy young subjects. It should be noted, however, that subjects who were not able to match their own perception consistently were excluded from the present trial. The subjects under study therefore represent a positive selection of volunteers with regard to blue-field entoptic technique.

### Table 1. Baseline Ocular Hemodynamic Measurements on the 3 Study Days (n = 9)*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo</th>
<th>Insulin, 1.5 mU/kg per minute</th>
<th>Insulin, 3 mU/kg per minute</th>
</tr>
</thead>
<tbody>
<tr>
<td>FPA, µm</td>
<td>3.4 ± 0.3</td>
<td>3.4 ± 0.3</td>
<td>3.5 ± 0.4</td>
</tr>
<tr>
<td>RBF, arbitrary units</td>
<td>93.3 ± 10.9</td>
<td>90.7 ± 10.9</td>
<td>82.3 ± 10.2</td>
</tr>
<tr>
<td>MFV, cm·s⁻¹</td>
<td>20.2 ± 1.5</td>
<td>19.8 ± 1.4</td>
<td>20.4 ± 1.6</td>
</tr>
</tbody>
</table>

*Results are presented as mean ± SEM. FPA indicates fundus pulsation amplitude; RBF, retinal blood flow; and MFV, mean flow velocity in the ophthalmic artery.

### Table 2. Intraclass Correlation Coefficient and Coefficient of Variation for Measurements of Ocular Hemodynamic Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>k</th>
<th>Coefficient of Variation, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>FPA</td>
<td>0.97</td>
<td>4.2</td>
</tr>
<tr>
<td>RBF</td>
<td>0.85</td>
<td>8.8</td>
</tr>
<tr>
<td>MFV</td>
<td>0.70</td>
<td>9.8</td>
</tr>
</tbody>
</table>

*FPA indicates fundus pulsation amplitude; RBF, retinal blood flow; and MFV, mean flow velocity in the ophthalmic artery.

### Table 3. Systemic Hemodynamic Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Testing Time, min</th>
<th>0</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>79.3 ± 1.3</td>
<td>79.1 ± 2.1</td>
<td></td>
</tr>
<tr>
<td>Insulin, 1.5 mU·kg⁻¹·min⁻¹</td>
<td>77.3 ± 2.2</td>
<td>76.2 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>Insulin, 3 mU·kg⁻¹·min⁻¹</td>
<td>78.1 ± 3.6</td>
<td>78.1 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>Pulse rate, bpm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>73.4 ± 3.4</td>
<td>64.8 ± 3.2</td>
<td></td>
</tr>
<tr>
<td>Insulin, 1.5 mU·kg⁻¹·min⁻¹</td>
<td>73.6 ± 4.2</td>
<td>70.9 ± 4.4</td>
<td></td>
</tr>
<tr>
<td>Insulin, 3 mU·kg⁻¹·min⁻¹</td>
<td>76.7 ± 3.7</td>
<td>70.1 ± 4.0</td>
<td></td>
</tr>
</tbody>
</table>

*Results are presented as mean ± SEM (n = 9).
oxide formation between the choroid and the retina may well contribute to differences in the vascular responsiveness to insulin in the 2 vascular beds. Whether adenosine also contributes to insulin-induced vasodilation in the eye, as it presumably does in the forearm, is unclear. Another issue may arise from differences in the adrenergic innervation of retinal and choroidal vessels. The vasodilatory effect of insulin is partially blunted by stimulation of the sympathetic nerve system innervating the vessels in the forearm. However, there is evidence that retinal vessels, in contrast to choroidal vessels, are not adrenergically innervated. Finally, local counter-regulatory mechanisms induced by high levels of insulin may well account for different hemodynamic effects in the retina and the choroid.

Mean flow velocity in the ophthalmic artery also significantly increased during administration of insulin, but not in a dose-dependent fashion. An increase in flow velocity in the ophthalmic artery during insulin administration does not necessarily reflect increased blood flow. However, insulin-induced vasoconstriction of the ophthalmic artery is very unlikely, and our data therefore indicate an increase in ophthalmic artery blood flow. Since only a small amount of blood flow in the ophthalmic artery contributes to the blood supply of the eye, increase of blood flow in the ophthalmic artery does not necessarily reflect an increase in choroidal blood flow or RBF.

CONCLUSION

We observed a non–dose-dependent effect of insulin on pulsatile choroidal blood flow and on MFV in the ophthalmic artery. Retinal blood flow was not influenced by hyperinsulinemia in the present study, but we cannot exclude effects on the order of 10% because of the reproducibility of the blue-field entoptic technique. The maximum effective dose of insulin on ocular hemodynamics is likely to be within the physiological range.

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