Acute hyperinsulinemia increases the contraction of retinal arterioles induced by elevated blood pressure

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§ The authors deeply regret the untimely death of professor Ole Schmitz

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**Abstract:**

**Introduction:** Diabetic retinopathy is accompanied by disturbances in retinal blood flow which is assumed to be related to the diabetic metabolic dysregulation. It has previously been shown that normoinsulinemic hyperglycemia has no effect on the diameter of retinal arterioles at rest and during an increase in the arterial blood pressure induced by isometric exercise. However, the influence of hyperinsulinemia on this response has not been studied in detail.

**Methods:** In seven normal persons the diameter response of retinal arterioles to an increased blood pressure induced by isometric exercise, to stimulation with flickering light, and to the combination of these stimuli was studied during euglycemic normoinsulinemia (protocol N) on one examination day, and euglycemic hyperinsulinemia (protocol H) on another examination day.

**Results:** Isometric exercise induced significant contraction of retinal arterioles at all examinations, but during a repeated examination the diameter response was significantly reduced in the test persons following the N protocol and increased in the persons following the H protocol. Flicker stimulation induced a significant dilatation of retinal arterioles at all examinations, and the response was significantly higher during a repeated examination, irrespective of the insulin level.

**Conclusions:** Repeated exposure to isometric exercise reduces contraction whereas repeated exposure to flickering light increases dilatation of retinal arterioles in vivo. Hyperinsulinemia increases contraction of retinal arterioles induced by isometric exercise.

**Keywords:**

Hyperinsulinemia, pancreatic clamp, retinal autoregulation, isometric exercise
**Introduction:**

Diabetes mellitus is accompanied by disturbances in retinal blood flow (1-4) which may be secondary to changes in the baseline diameter of retinal arterioles (5), in vasomotion (6,7), pressure autoregulation (8,9), or secondary to changes in the metabolism (10-13). The coupling between metabolism and retinal blood flow have not been elucidated in detail, and especially the influence of the glucose metabolism on retinal blood flow is important since this may help understanding the development of diabetic retinopathy. It has been shown that hyperglycemia may cause retinal hyperperfusion (14,15), and in normal persons a high peroral intake of glucose has been shown to reduce retinal autoregulation (16). However, hyperglycemia may also induce other metabolic changes with an effect on retinal blood flow, such as increasing the release of insulin which may induce changes in retinal blood flow (17,18). Additionally, it has been shown that normoinsulinemic hyperglycemia has no effect on the diameter of retinal arterioles at rest and during an increase in the arterial blood pressure induced by isometric exercise in normal persons (19). However, the isolated effect of insulin on diameter regulation of retinal arterioles has not been studied in detail.

Therefore, the purpose of the present study was to investigate the effects of hyperinsulinemic normoglycemia on the baseline diameter, pressure autoregulation and metabolic autoregulation of retinal arterioles. In seven normal persons, the diameter response of retinal arterioles to an increased blood pressure induced by isometric exercise, to stimulation with flickering light, and to the combination of these stimuli was studied during euglycemic normoinsulinemia followed by euglycemic hyperinsulinemia.
**Materials and methods:**

**Study design:**

Seven men with no known ocular or systemic diseases (mean age 25 years, range 19-27; mean body weight 77.4 kg, range 61.3-87.6 kg) not taking any prescribed medicine were studied in a randomized, blinded, cross-over study performed on two study days separated by four to six weeks. On each day the experiment consisted of two periods. During the first period the test persons were kept normoglycemic and normoinsulinemic. After one hour on the first study day, the test person was randomized by the laboratory technician who drew an anonymous envelope containing information whether the patient should enter protocol H or N on the first examination day. In protocol H intravenous infusion of glucose, insulin and somatostatin were balanced to obtain normoglycemia and hyperinsulinemia, whereas in protocol N these variables were balanced to obtain normoglycemia and normoinsulinemia. The persons who entered protocol H on the first study day entered protocol N on the second day, and vice versa. The protocol was unknown to the test person and the ophthalmologist who made the eye examinations. A power analysis showed that with a test-retest variability of less than 1% (9,11,13), a significance level of 0.05 and a power of 0.8, the inclusion of seven individuals would be sufficient to detect induced diameter changes of at least 1%.

The study fulfilled the declaration of the Helsinki and was approved by the local ethical committee for medical sciences.

**Metabolic intervention:**

The test persons were examined after an overnight fast (10 h) to start the experiment
between 7.00 and 9.00 a.m. on the study day. The principles of the intervention are shown in Figure 1, and the time for the start of controlled insulinemia was set to t = 0. Two intravenous cannulae were inserted for blood sampling and for infusions. At t = -120 min, infusion of somatostatin 300 µg/h (Ferring AB, Malmö, Sweden) was initiated in order to control endogenous insulin secretion, and a replacement dose of insulin (0.10 mU/kg body weight/min; Insulin Actrapid, Novo Nordisk A/S, Copenhagen, Denmark) was commenced. During the entire examination, plasma glucose was maintained as close to 5 mmol/l as possible by adjustment of the infusions. At t = 0, plasma insulin infusion was either elevated acutely to 0.9 mU/kg/min (protocol H) or maintained at 0.1 mU/kg/min (protocol N) until t = 180 min. Additionally, in order to obtain euvoletic conditions during the two examination days, isotonic NaCl was infused at a constant rate (in protocol N 800 ml/h and in protocol H 300 ml/h) until t = 180 min. Plasma glucose was measured every 5-10 minutes, and blood samples for the measurement of insulin and C-peptide was collected every 30 minutes from t = -120 min, whereas serum electrolytes, osmolality, creatinine, urea, bicarbonate, albumin, growth hormone, and cortisol were measured at t = -60 min and at t = 180 min immediately before the eye examinations. Since no fluctuations were observed in the parameters measured on the blood samples before t = 0, the values at this time point were used as the pre-values. The level of insulin and glucose during the procedures of the two protocols are shown in Figure 2.

Biochemical analyses:

Plasma glucose was measured by a glucose oxidation method (Beckmann Instruments, Palo Alto, California, USA). Serum insulin was determined by a two-sided immunospecific ELISA method (Andersen et al. 1993). Plasma C-peptide was determined by a
commercially available two-sided ELISA kit (K6218, DAKO, Cambridgeshire, U.K.). Serum GH was analyzed with a double monoclonal immunofluorometric assay (DELFIA, Wallac Oy, Turku, Finland). Serum cortisol was measured with a solid-phase, time-resolved fluoroimmunoassay (DELFIA, Wallac Oy).

There was no significant difference between the baseline levels of plasma glucose, serum insulin, C peptide, and glucagon on the days of protocol N and H shown in Table 1 (Student's t-test, p > 0.48 for all comparisons).

**Ophthalmological examinations:**

The pupils were dilated on both eyes at t = -120 min using topical cyclopentolatehydrochloride (Cyclogyl 1%, Alcon, USA), and a sequence of eye examinations was started at t = -60 and was repeated starting at t = 180. Each sequence consisted of: Measurement of the intraocular pressure, diameter measurement of retinal arterioles before and during isometric exercise using a Dynamic Vessel Analyzer (Imedos, Jena, Germany), measurement of macular thickness by optical coherence tomography (OCT) scanning (Humphrey instruments, San Leandro, USA, Version A6.1) with six radial scans centered on the fovea. The mean thickness of the fovea was calculated from the center value provided by the instrument software.

**Diameter measurements of retinal arterioles:**

The measurement of the diameter of retinal arterioles was performed as described previously (13,20,21). The arterioles were studied in the left eye from all test persons. The study arteriole was chosen as the largest arteriole among first and second order arterioles located between two bifurcations within three disk diameters from the optic disk, resulting
in a segment length ranging between 500 and 1000 microns. (Figure 3)

The DVA examination had two sequences. The first sequence consisted of three minutes of rest (baseline), three minutes of exercise where the blood pressure was increased by isometric exercise induced by lifting a 2 kg hand weight, and three minutes of rest where the blood pressure could return to normal. The second sequence consisted of two minutes of rest (baseline) followed by two minutes where the retina was stimulated with flickering light, two minutes of rest, two minutes with combined isometric exercise and stimulation with flickering light, and two minutes of rest to ensure that the blood pressure returned to baseline. The blood pressure was measured on the upper left arm using oscillometric technique (Omron M4, Japan), at the beginning of the baseline period and 120 seconds after the beginning of the periods with exercise and/or flicker stimulation. The vascular diameters were calculated as the mean of the arteriolar diameters recorded during the baseline period and the diameters recorded between 120 and 150 seconds during the lifting part or the mean of the diameters recorded between 60 to 120 seconds during the periods with flicker stimulation.

Data analysis:

The mean arterial pressure (MAP) was calculated from the diastolic (BP_{dia}) and the systolic (BP_{sys}) blood pressures according to the equation: MAP = \frac{2}{3}(BP_{dia}) + \frac{1}{3}(BP_{sys}). The systemic pulse pressure (PP) was calculated as PP = BP_{sys} - BP_{dia}. From the first examination sequence, the mean arteriolar diameter during the baseline period was compared to the mean diameter during the lifting period. From the second examination sequence, the mean arteriolar diameter in the baseline period was compared to the mean diameter during the two periods with flicker stimulation.
Statistics:
The change in the respective parameters during the H and the N study days, the change between baseline diameter, and the change in diameter from baseline to the exercise part, were tested using the paired sample t-test, and results are given as mean±SEM. For each of the three interventions two-way ANOVA was used to test for significant differences between the examination day and between the first and the second intervention on these days, followed by Bonferroni’s post test in order to identify the parameter(s) that differed significantly from the others.
**Results:**

The baseline systolic and diastolic blood pressure was (mean±SEM) 117.1±9.5 and 69.9±5.8 mmHg, respectively. The mean baseline blood pressure (MAP) was 85.6±6.6 mmHg, the baseline diameter of retinal arterioles was (124.5±23.4 AU), and the baseline central retinal thickness was (167.2±23.5 microns). There were no significant differences between these values at the beginning of (blood pressure and arteriolar diameter) or during (central retinal thickness) the two examination periods on each of the two examination days (p>0.67 for all comparisons).

Isometric exercise induced an overall increase in MAP of 16.5±9.5 mmHg which was significant (p<0.001 at all examinations, Student’s paired t-test), and there was no significant difference between the responses obtained at the different lifting periods in each patient on each examination day (p > 0.16 for all comparisons).

The results of the diameter measurements are shown in Table 2. During isometric exercise there was an overall decrease in the diameter of retinal arterioles of -4.57±0.69% (p<0.001, Students' paired t-test), and the responses at each of the four examinations are shown in the upper row. There was a significant difference between the diameter responses obtained during the first and the second examination using the two protocols (F(1,24) = 4.32, p = 0.049, η²=0.14), which was due to a significantly reduced diameter response in the patients following the N protocol and an increased response in the patients following the H protocol from the first to the second test (Bonferroni’s post test).

Examples of the diameter response during stimulation with flickering light during normoinsulinemia and hyperinsulinemia are shown in Figure 4. There was an overall significant dilatation of retinal arterioles of 5.98±0.99% (p<0.001 Students' paired t-test), and the responses at each of the four examinations are shown in the middle row. There
was a significant difference between the responses obtained during the first and the second examination using the two protocols ($F(1,24) = 8.80$, $p = 0.007$, $\eta^2=0.26$), which was due to a significant increase in the diameter response from the first to the second examination (Bonferroni's post test).

Stimulation with both isometric exercise and flickering light induced an overall significant dilatation of retinal arterioles ($5.66\pm0.66\%$, $p<0.001$ Students' paired t-test), and the responses at each of the four examinations are shown in the lower row. There was no significant difference between the responses obtained at the four examinations ($F(1,24) = 0.33$, $p = 0.88$).
Discussion:

The present study demonstrates the effect of hyperinsulinemia on diameter regulation of retinal arterioles in vivo. The study was designed as a double-blinded cross-over study which allowed a separation of the influence of insulin on vessel diameters from the influence of other derived consequences of changes in plasma insulin, as well as the influence of habituation when the experiments were repeated. Additionally, the study was performed on younger persons where the interventions on blood pressure could be expected to induce a sufficient diameter response in the retinal arterioles (20) to allow the detection of changes after interventions on this response.

The study showed contraction of retinal arterioles during isometric exercise which was comparable to previous results obtained in the same age group (19). However, it appeared that the diameter response was reduced when isometric exercise was repeated which may indicate habituation to this stimulus. Thus, in another study reduced arteriolar contraction after isometric exercise was attributed to ingestion of glucose (16), but the response may have been due to habituation since no control group not ingesting glucose was included.

Previous studies have shown that insulin has a vasodilating effect on retinal arterioles in vitro when applied intraluminally, but not extraluminally (22) and on the systemic circulation in general (23,24), which is mediated by nitric oxide (25,26). This suggests a physiological effect of insulin transported in the blood stream on diameter regulation of arterioles in the retinal circulation and elsewhere in the body. However, in the present study the contraction of retinal arterioles to isometric exercise was found to be increased during hyperinsulinemia. The finding is in accordance with a recent study that reported improved vascular function after infusion of insulin in children with type 1 diabetes mellitus (27), and suggests a more complex interaction between pressure autoregulation and
metabolic autoregulation. The finding may indicate the presence of insulin-induced activation of the sympathetic nervous system with contraction of the larger arterioles supplying the eye (28,29) and may be involved in modulating the response of intermittent pulsatile insulin secretion on diabetic complications in the eye and elsewhere (30).

The study also showed flicker induced vasodilatation which is comparable to previous reports (11,31). This flicker induced dilatation was significantly increased during the second examination which may be due to the different time of the day, but may also be a carry over effect from the first examination because the initial flicker stimulation had increased the sensitivity to the subsequent flicker stimulation. A similar response has been found in a study where patients with primary open angle glaucoma were exposed to increasing flicker frequencies (32), whereas this response has not been found in normal persons and in diabetic patients (10). The background for this finding therefore remains to be elucidated.

Finally, the study showed that a simultaneous increase in the arterial blood pressure by isometric exercise and stimulation with flickering light induced vasodilation similar to dilatation induced by flicker stimulation alone (11,13). Additionally, it was shown that this diameter response was unaffected by repeating the examination and by hyperinsulinemia, which is in accordance with studies showing that reduced diameter responses to isometric exercise and flicker stimulation in patients with diabetes mellitus are neutralized when the two stimulus paradigms are presented simultaneously (11,33,34). This evidence suggests that the mechanisms leading to vasodilatation during activation of the retinal metabolism can overrule the mechanisms leading to vasoconstriction during increased arterial blood pressure.

The results of several studies suggest that insulin may have trophic effects in the retina
other than those directly related to uptake of glucose in peripheral tissues. Thus, in animal models insulin can stimulate the development of axial myopia (35) and delay the death of photoreceptors in tapetoretinal degeneration (36), whereas silencing of insulin receptors has been shown to increase the loss of retinal Müller cells (37). These effects on cellular growth and survival may have implications for retinal metabolism, and thereby for the regulation of retinal blood flow. However, these effects of insulin are probably initiated more gradually and have a more sustained action than the effects of acute changes in plasma insulin observed in the present study.

Altogether, the study has shown that hyperinsulinemia can increase the contraction of retinal arterioles in normal persons during isometric exercise. This evidence was obtained during an acute increase in the insulin level, but may potentially reflect the changes in type 2 diabetic patients where insulin levels are increased several years before disturbances in autoregulation can be registered. This hypothesis might be tested in a clamp study in type 2 diabetic patients designed to elucidate whether changes in the diameter response of retinal arterioles during hyperinsulinemia correlate with the severity of diabetic retinopathy or other diabetic late complications. Assuming that the perfusion pressure is constant, changes in the diameter of retinal arterioles will reflect changes in retinal blood flow. Therefore, the findings indicate that changes in plasma insulin may contribute to the changes in retinal blood flow involved in the development of diabetic retinopathy.
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Disclosures

None
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Legends to Figures and Tables

Legend to Table 1:
Baseline levels of plasma glucose, serum insulin, C peptide and glucagon before the beginning of the experiments on the two protocol days. p-values result from comparisons using Student's paired t-test.

Legend to Table 2:
Percentage change in the diameter of larger retinal arterioles. Arrows indicate significant contraction (arrow down) or dilatation (arrow up) resulting from the Bonferroni post-test.

Legend to Figure 1:
The experimental protocol followed

Legend to Figure 2:
The increase in insulin concentration during protocol H (solid squares) as compared to protocol N (solid triangles) depicted together with the stable glucose levels during protocol H (open squares) and protocol N (open triangles). Error bars indicate standard deviation.

Legend to Figure 3:
A frame from a video recording of the ocular fundus during an examination with the Dynamic Vessel Analyzer. The fixation bar is seen to enter the image at the lower edge with a direction upward to the right to end corresponding to the fovea. The white line indicates the arterial segment subjected to continuous diameter measurements.
Legend to Figure 4:

Figure 4: Diameter traces before and during flicker stimulation from the arteriolar segment shown in the previous figure during A: Normoinsulinemia and B: Hyperinsulinemia.
### Table 1

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<th>H</th>
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<td>mean</td>
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<td>[mU/l]</td>
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<tr>
<td><strong>C peptide</strong></td>
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### Table 2

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