METHODS. Prospective study of 45 women with type 1 diabetes and 6 healthy pregnant women, from the first trimester of pregnancy to half a year after delivery, by using a fundus photographic method of determining retinal vessel diameters. Results. In diabetic women, the mean arterial blood pressure increased from 90.7 ± 10.9 mm Hg (mean ± SD) in the first trimester to 102.4 ± 16.4 mm Hg in the third trimester, accompanied by retinal arteriolar constriction, from a mean diameter of 95.5 ± 11.3 to 92.2 ± 12.9 μm (P = 0.007), arteriolar constriction being prominent in nonsmokers, from 96.6 ± 11.1 to 92.3 ± 13.0 μm (P < 0.001; n = 38), but absent in smokers, from 89.2 ± 11.5 to 92.0 ± 13.6 μm (P = 0.28). Healthy nonsmoking women demonstrated an increase in blood pressure during pregnancy comparable to that in diabetic women, but the change in mean retinal arteriolar diameter, from 88.5 ± 10.9 μm in the first to 91.6 ± 10.2 μm in the third trimester, did not reach statistical significance (P = 0.38). Diabetic retinopathy levels increased during pregnancy, but the change in arteriolar diameter from the first to the third trimester did not correlate with retinopathy, arterial blood pressure, HbA1c, or atrial natriuretic peptide. Plasma angiotensin II correlated positively with the change in arteriolar diameter in women who did not smoke (P < 0.05). After delivery, retinal vessel diameters returned to the first trimester range in all subgroups. Conclusions. From the first to the third trimester of pregnancy, blood pressure increased and retinal arteriolar diameter decreased in diabetic women, but the arteriolar constriction associated with pregnancy and systemic arterial blood pressure increase was remarkably absent in diabetic women who smoked tobacco before and during pregnancy. It is unknown whether smoking inhibits this vasoconstrictive response by deactivating mechanisms of physiological adaptation or by activating these mechanisms before the first trimester of pregnancy. (Invest Ophthalmol Vis Sci. 2005;46:709–713) DOI:10.1167/iovs.04-0604

Prospective studies of risk factors for the progression of diabetic retinopathy have documented a prominent role of hyperglycemia and arterial hypertension.1–3 The dynamics of vascular autoregulation have been implicated in the pathogenesis of diabetic retinopathy.4–6 Pregnancy is a condition in which the risk of progression of diabetic retinopathy is high,7–11 and it induces metabolic and hemodynamic changes that offer an opportunity to study adaptive circulatory mechanisms over a short time-span. Herein, we report the results of a fundus photographic study of the diameter and length of retinal arteries and veins in relation to blood glucose, arterial blood pressure, and retinopathy in pregnant women with or without diabetes.

METHODS

The protocol of the study was in keeping with the provisions of the Declaration of Helsinki for research involving human subjects and was approved by the local medical ethics committee. Informed consent was obtained from all who participated, after an explanation of the risks involved. The present report is based on a prospective study of pregnancy in diabetic women with type 1 diabetes or without diabetes enrolled after their first postconceptional ophthalmic examination and completed with fundus photographs of acceptable quality from the first and third trimester as well as three or more months after delivery.12–14 All patients had received insulin treatment from the onset of diabetes and had had type 1 diabetes diagnosed in routine clinical practice. In 45 women with diabetes aged 30.2 ± 4.7 years (mean ± SD) the duration of diabetes was 15.7 ± 7.9 years (Table 1). A control group was composed of six healthy nondiabetic women aged 30.5 ± 2.4 years with normal fasting blood glucose values.

The patients underwent a comprehensive examination including color fundus photography on film diapositives for documentation of the level of retinopathy. The analysis was based on optic-disc–centered 50° photographs of right eyes only, from one first trimester examination, one third trimester examination, and one examination at either 3 or 6 months after delivery. After exclusion of overexposed and underexposed photographs, acceptable imaging of the peripapillary trunk vessels was available for 45 subjects (Table 1). The image analysis was made fully masked with respect to the clinical study.

The best fundus photograph of three was chosen and the 35-mm diapositives were digitized at a nominal resolution of 4000 × 6000 pixels and a dynamic output range of 8 bits for either color channel, of which only the green one was analyzed. Absolute dimensions were calculated assuming a vertical optic nerve head diameter of 1500 μm.

Vascular diameter was determined by prototype computerized image analysis software Retinalyze Danmark A/S (Hellerup, Denmark) enabling manual definition of the anchoring points for analysis of a given retinal vascular segment.15 For each eye, a single segment was examined from one retinal artery and one retinal vein on the basis of the following criteria: (1) proximity to the optic nerve head rim, (2) consistency of image quality throughout the study, (3) location (order of preference: upper temporal vascular arcade, lower temporal arcade, nasal), and (4) proximity of a vessel of the opposite class. Preferred
anchoring points were the center of dichotomous branchings, the intersection of the medians of crossing vessels of opposite class, and the crossing of the rim of the optic nerve head, if sufficiently well defined. For a given eye, the same anchoring points were used for the entire study. Only segments without bifurcations or major branchings were used. Of the chosen segments, 25 were found in the upper arcade and 20 in the lower arcade. For a given eye, all photographs were analyzed in a single session by the same observer.

Vascular contour tracing was avoided near vascular crossings and branchings, near hard exudate and cotton wool spots, and near strong reflexes from the posterior hyaloid. To allow precise tracking near the ends of the segments selected for analysis, a standard length of 40 pixels at either end of the tracing was subtracted before analysis. This corresponds to about twice the diameter of a large retinal vein at the rim of the optic disc.

After analysis, data from all photographs were scaled to the first of a series of photographs from the same eye. The scale was found to vary within ±3% for the same nominal fundus camera viewing-angle. Reproducibility was examined by repeat analysis of the same vascular segment using a hand-drawn sketch of the position of the anchoring points to assist communication between graders. Two observers tracking the same segment in each of 10 different eyes assessed interobserver and intraobserver reproducibility, the tracings being separated by no less than 3 hours. Intraobserver reproducibility (coefficient of variation) was 0.062% for vessel diameter and 0.31% for vessel length. Interobserver reproducibility was 0.060% for diameter and 0.59% for length.

Plasma concentrations of atrial natriuretic peptide and angiotensin II were assayed as previously described.16,17

Statistical analysis (SAS software, ver. 8e; SAS, Cary, NC) included an initial one-way analysis of variance (generalized linear model [GLM]) to test for differences between groups. For the major part of the analysis, a summary parameter of the change from first to third trimester was used, with a paired t-test. In case of small samples with sample size <10, the results were confirmed with nonparametric rank sum tests.

RESULTS

In diabetic women, mean arterial blood pressure increased from 90.7 ± 11.8 mm Hg (mean ± SD) in the first trimester to 102.4 ± 17.4 mm Hg in the third trimester (P < 0.001) whereas HbA1c decreased, from 7.17 ± 0.9 to 6.84 ± 0.3 (P = 0.004) and arterial diameter constricted, from a mean of 95.5 ± 11.3 to 92.2 ± 12.9 μm (P = 0.007). After delivery, retinal arteriolar diameter returned to baseline range.

between the latter two groups alone (P = 0.020). Among women with diabetes, arteriolar constriction was prominent in nonsmokers, from 96.6 ± 11.1 to 92.3 ± 13.0 μm (P = 0.001; n = 38, Fig. 2), but absent in smokers, who demonstrated a mean diameter of 89.2 ± 11.5 μm in the first trimester and 92.0 ± 13.6 μm in the third trimester (P = 0.28; n = 7, Fig. 3). The two groups demonstrated similar magnitudes of blood pressure increase (Table 2).

Retinal arteries were narrower in the first trimester in diabetic women who smoked than in diabetic women who did not smoke, but the difference did not reach statistical significance (P = 0.1). Other descriptive variables were comparable between smoking and nonsmoking women. Tobacco consumption, defined as daily consumption throughout pregnancy, amounted to less than 10 cigarettes per day in four women and to 10 or more cigarettes per day in three women. Tobacco consumption was only in the form of cigarettes. Four women with diabetes who had been smoking for more than 1

![Figure 1](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932931/ on 06/24/2017)

**Figure 1.** Retinal arteriolar diameter during pregnancy and after delivery in diabetic women (n = 45, values from first, second, and third trimesters, as well as from the 3rd and 6th months postpartum). From the first to the third trimester, retinal arteriolar diameter constricted, from 95.5 ± 11.3 to 92.2 ± 12.9 μm (P = 0.007). After delivery, retinal arteriolar diameter returned to baseline range.

![Figure 2](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932931/ on 06/24/2017)

**Figure 2.** From the first to the third trimester of pregnancy, diabetic women who did not smoke demonstrated retinal artery constriction (P = 0.001, △) and retinal vein constriction (P = 0.048, ✶) concomitant with an increase in mean systemic arterial blood pressure (P < 0.001, ♦).
The lengths of the retinal artery and vein segments selected for examination were stable throughout the study. Objective and subjective refraction was performed at each study time point. No systematic changes larger than 0.5 D occurred in refraction during the follow-up.

Correlations between changes in retinal vessel diameter, arterial blood pressure, HbA1c, and the level of diabetic retinopathy did not reach statistical significance for any group or subgroup, but a trend toward decreasing retinal artery diameters with increasing mean arterial blood pressure was seen in the largest group, nonsmoking diabetic women ($r = -0.172$, $P > 0.2$). Potential confounding factors that may obscure the relation between blood pressure and retinal artery diameter include refraction, baseline retinopathy, and intraocular pressure, as well as changes in these variables during pregnancy. Statistical analysis did not show any detectable effect of such confounders on the relation between blood pressure and retinal vascular caliber.

In nonsmoking diabetic women, increasing angiotensin II was associated with increasing retinal artery diameter ($r = 0.338$, $P < 0.05$, $n = 33$, Pearson's $r$). The diameter change did not correlate with atrial natriuretic peptide, nor did angiotensin II and atrial natriuretic peptide levels correlate with the changes in arterial blood pressure.

### Table 2. Blood Pressure, HbA1c, and Retinal Vessel Diameters during Pregnancy

<table>
<thead>
<tr>
<th></th>
<th>1st Trimester</th>
<th>3rd Trimester</th>
<th>1st Trimester</th>
<th>3rd Trimester</th>
<th>1st Trimester</th>
<th>3rd Trimester</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Arterial Blood Pressure (mmHg)</td>
<td></td>
<td></td>
<td>HbA1c (%)</td>
<td></td>
<td>Artery Diameter (µm)</td>
<td>Vein Diameter (µm)</td>
</tr>
<tr>
<td>Healthy ($n = 6$)</td>
<td></td>
<td></td>
<td>N/A</td>
<td></td>
<td>88.5 (10.9)</td>
<td>111 (19.6)</td>
</tr>
<tr>
<td>Diabetes, nonsmokers</td>
<td>85.3 (7.2)</td>
<td>93.6 (9.6)</td>
<td>N/A</td>
<td></td>
<td>89.2 (11.5)</td>
<td>110 (14.8)</td>
</tr>
<tr>
<td>Diabetes, smokers ($n = 38$)</td>
<td>89.9 (8.9)</td>
<td>101.7 (16.3)</td>
<td>7.09 (0.8)</td>
<td>6.75 (1.2)</td>
<td>96.6 (11.1)</td>
<td>122 (23.4)</td>
</tr>
<tr>
<td>($n = 7$)</td>
<td>94.8 (18.8)</td>
<td>106.1 (17.5)</td>
<td>7.61 (1.0)</td>
<td>7.53 (1.1)</td>
<td>92.5 (13.6)</td>
<td>118 (22.9)</td>
</tr>
</tbody>
</table>

Data are the mean ± SD.
* $P < 0.001$ for change compared to first trimester.
† $P = 0.004$ for change compared to first trimester.
‡ $P > 0.01$ in relation to first trimester of pregnancy.
§ $P = 0.048$ in relation to first trimester of pregnancy.
" $P > 0.05$ for all other comparisons between first and third trimester.
At the first trimester baseline examination, the median retinopathy grading level according to the Diabetes Control and Complications Trial (DCCT) implementation of the Early Treatment Diabetic Retinopathy Study (ETDRS) grading system was 20 (range, 10–45) in nonsmoking women with diabetes and 10 (10–45) in women with diabetes who smoked. In the third trimester, retinopathy levels in nonsmoking women with diabetes had decreased in 3 women, whereas it was unchanged in 22 women and had increased in 13 women (P = 0.02 for overall increase; sign test). Among the women with diabetes who smoked, the retinopathy level had increased in one patient, whereas it remained stable in 6 women (P > 0.05). Retinopathy levels were unchanged after delivery—that is, comparable to those found in the third trimester.

**DISCUSSION**

In the present study, mean arterial blood pressure increased during pregnancy, from the first to the third trimester, in both diabetic and nondiabetic women. Concomitant retinal arteriolar constriction was found in diabetic women, but subgroup analysis demonstrated that the arteriolar constriction was confined to the large subgroup of nonsmoking diabetic women (P = 0.001), whereas a tendency toward retinal arteriolar dilation was observed during the same period in the smaller subgroups of diabetic women who smoked and in healthy pregnant women. Thus, the nominal changes in retinal arteriolar diameter from the first to the third trimester were −4.5% in nonsmoking diabetic women, +3.5% in healthy women, and +2.8% in smoking diabetic women. After delivery, blood pressure and retinal vascular diameters returned to the baseline range, demonstrating that the effect of pregnancy was temporary.

The study’s most important contribution is the absence of arteriolar constriction associated with blood pressure increase in the subgroup of diabetic women who smoked. This may reflect an influence of smoking on the normal vasostroictive response to hypertension. The small sample size of the study, particularly the subgroups of diabetic women who smoked and healthy, nonsmoking, nondiabetic women, makes it difficult to determine conclusively whether there is a significant effect modification of cigarette smoking on the association of blood pressure increase and arteriolar narrowing. These limitations should be appreciated and the findings tested in an independent and larger study.

The study was not powered to examine differences in characteristics at baseline, but smoking diabetic women did have lower arteriolar baseline diameters than nonsmokers. Hence, we cannot exclude the possibility that the retinal arteries in smokers were in a state of relative constriction at the initial examination in the first trimester. If so, then the arteries would appear to have been nonresponsive to the arterial hypertensive that caused constriction in nonsmoking women. This may have potential implications for the progression of retinopathy. Indeed, we found a higher frequency of retinopathy progression in diabetic women who did not smoke than in those who did smoke. No clinical recommendations should be made from the present study, in view of its exploratory character and relatively crude quantitation of duration and magnitude of tobacco consumption, but our results suggest that smoking has hemodynamic actions in patients with diabetes that may influence the course of diabetic retinopathy.

Under stable metabolic conditions, the volumetric blood flow rate and capillary flow in the retina should also be stable. In the present study population, capillary blood flow in diabetic subjects was increased compared with that in healthy subjects, but did not change during pregnancy. This was despite an increase in arterial blood pressure from the first to the third trimester in all subgroups. These observations indicate that autoregulation was active in diabetic women in the present study except that it may have been anomalous in those who smoked tobacco.

The overall change from the first to the third trimester was one of arteriolar constriction in nonsmoking diabetic women, concomitant with an increase in blood pressure, and this was significantly different from nonsmoking diabetic women and nondiabetic women. When analyzing correlation per patient, a trend toward decreasing arteriolar diameter with increasing blood pressure was present, although it failed to reach statistical significance. Thus, the results of the two analyses are in agreement, but they may be influenced by confounding variables that we did not map in full detail—for example, IOP, the degree of retinal ischemia, and autoregulatory dysfunction. We found no indication of an effect of HbA1c, retinopathy level, or other descriptive parameters tested, suggesting that important pathophysiological variables remain to be identified.

Angiotensin II has vasostroictive activity, with both fast and slow pressor effects in nonpregnant subjects. In normal pregnancy, the reduced peripheral vascular resistance is associated with a reduced arteriolar vasostroictive response to angiotensin II. In the largest subgroup in the present study, nonsmoking diabetic women, increasing angiotensin II during pregnancy was associated with retinal artery dilation. This would be a paradox under stable nonpregnant conditions, but when observed during pregnancy, it may be explained by a decreasing vasostroictive response to angiotensin II during pregnancy.

Smoking during pregnancy has been shown to decrease the risk of preeclampsia. In addition, smoking is associated with biochemical changes in umbilical cord vessels compatible with placental vasostroiction. This is likely to contribute to the intrauterine growth retardation effect of smoking. Our results support that these effects are caused by a direct influence of smoking on important hemodynamic functions, but it should be noted that our subjects were smoking before conception.

In conclusion, we have found indications that autoregulation is active in a group of pregnant diabetic women with mild to moderate diabetic retinopathy, but we also found that tobacco smoking appears to induce a state of altered vascular function in pregnant diabetic women, where little or no change in retinal arteriolar diameters occurs in relation to pregnancy-induced arterial hypertension. Diabetic women who smoked before and during pregnancy demonstrated no arteriolar constriction during pregnancy and had less progression of diabetic retinopathy, perhaps because the habit of smoking was established before conception and had induced a state of chronic arteriolar constriction that protected against the detrimental effect of hyperglycemia-induced hyperperfusion.

The angiotensin II receptor antagonist losartan has vasodialatory and blood-pressure-lowering activity. There are both theoretical arguments and clinical observations supporting that retinal vasodilation may be harmful to the edematous retina. In a controlled trial, the short-term effect of losartan treatment on fovea-involving diabetic macular edema was to increase foveal thickness. Although retinal vessel diameter was not examined, this finding indicates that retinal vasodilation may have adverse effects on the progression of diabetic macular edema. Indeed, despite the beneficial preventive effect of antihypertensive treatment in relation to diabetic retinopathy, such treatment has never been documented to be of therapeutic value in established macular edema. Smoking is not to be encouraged, because of its many adverse effects, but the implication of the present study and a previous demonstration of

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a retinal blood flow-reducing and vasoreactivity-eliminating effect of smoking is that vasoactive pharmaceuticals that induce retinal artery contraction without increasing systemic blood pressure should be examined for their ability to influence the course of diabetic retinopathy.

References


