Carotid Artery Stenosis is Exacerbated in Spontaneously Obese Model Rats with Diabetes

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Aim: Population studies have shown obesity and diabetes to be risk factors for atherosclerosis. We assessed changes in the common carotid arteries in rat models of obesity and diabetes without hypertension.

Methods: Twenty 30-week-old male spontaneously diabetic and obese model Otsuka Long-Evans Tokushima Fatty (OLETF) and 20 control Long-Evans Tokushima Otsuka (LETO) rats were used in the experiments. The animals were considered diabetic if the plasma glucose level peaked at >300 mg/dL and remained at >200 mg/dL for 120 minutes. Blood gas physiological parameters were continuously monitored under anesthesia, and the flow of the carotid artery was assessed with ultrasoundography. All animals were sacrificed with an overdose of anesthesia at the end of the experiment. Sections of the middle portion of the internal carotid artery were cut and stained with hematoxylin and eosin to assess the overall morphology.

Results: All OLETF rats were diabetic, and all LETO rats were non-diabetic. The physiological parameters did not differ significantly between the control and model rats, whereas the carotid artery wall thickness (19.3 ± 3.2 vs. 6.1 ± 4.5 μm) was significantly different between the two groups. The blood flow velocity in the common carotid artery determined using ultrasonography and color Doppler sonography was significantly increased during systole in the model rats compared with that observed in the control rats (203 ± 20.3 vs. 55.3 ± 21.4 cm/sec).

Conclusions: The OLETF rats were obese, and diabetes worsened the degree of carotid artery stenosis. These results indicate the possibility of new therapies for carotid artery stenosis in obese and diabetic patients.


Key words: Obesity, Diabetes, Carotid artery, Wall thickness, OLETF rat
Blood pressure was determined in the morning and calculated as the average value of four to five successive measurements.

**Anesthesia**

After determining the rats' diabetic status, the animals were sedated with atropine (1 mg) and initially anesthetized via the intraperitoneal injection of chloral hydrate (36 mg/100 g of body weight). The rats were tracheally intubated after relaxation, and ventilation was controlled using a Harvard Model 683 small animal respirator (Harvard Apparatus, Holliston, MA, USA). Anesthesia was induced and maintained with 2% and 1% halothane, respectively, in a mixture of 70% nitrous oxide and 30% oxygen. The rectal temperature was maintained at 37°C using a CMA 150 feedback heating pad (Carnegie Medicine AB, Stockholm, Sweden).

Polyethylene catheters were inserted into the tail artery and left femoral vein. Blood gas parameters, including the partial pressure of oxygen (PaO₂) and partial pressure of carbon dioxide (PaCO₂), as well as the arterial pH, blood pressure and glucose concentration, were continuously monitored through the intraarterial catheter connected to an ABL 550 pressure transducer (Radiometer, Copenhagen, Denmark).

**Ultrasound Assessment of the Carotid Arterial Flow**

The neck was shaved to reduce artifacts and then slightly hyperextended, after which each supine rat was assessed via color duplex sonography using a ProSound alpha 7 ultrasound system (Hitachi-ALOKA Medical, Tokyo, Japan). The system was equipped with a phased array transducer containing 128 elements and a length of 13 mm. The lateral and axial resolution was 0.45 and 0.5 mm, respectively, according to the manufacturer. The optimal settings for detecting a fast- and low-volume depth of 2 cm with this array were preliminarily determined. A standoff pad was not required to achieve good near-field resolution owing to the density of the array elements. No contrast agent was administered. A transmitting and receiving frequency of 7.6 MHz was used for all color Doppler, pulsed Doppler and B-mode examinations, and the pulse repetition frequency was set to maximum velocity detection, with no aliasing. Angle correction was maintained below 60° degrees in order to minimize errors from false angle correction during the flow measurements. The Doppler and color Doppler gains were adjusted to minimize background noise and overamplification of the signal with the smallest gate possible. The blood velocity was measured in
areas of maximum stenosis.

**Histopathology**

All animals were sacrificed via an overdose of anesthesia at the end of the experiment. The chest was opened, a cannula was placed into the left ventricle and the cardiovascular system was perfused at a constant pressure of 120 mm Hg for 10 minutes with a fixative solution (300 mM glutaraldehyde in 100 mM phosphate buffer). Five sections (5 mm thick) of the common carotid artery obtained 1 cm proximal from the bifurcation were cut and stained with hematoxylin and eosin (HE) to assess their overall morphology.

**Geometry of the Carotid Artery**

Vessel cross-sections with a long-axis to short-axis ratio of <1:3 were used for the evaluation and quantitative scoring, based on our previous study of the vessels of OLETF and LETO rats. Vascular cross-sections of the left common carotid artery (CCA; near the bifurcation) were quantified on HE-stained slides, and the ratio of the tunica media to the lumen (wall area/luminal area) was calculated as an index of wall thickening, in which the total vessel area was defined as the sum of the tunica media and vessel lumen. The wall thickness (tunica intima + tunica media) and the inner circumference were measured using light microscopy. The ratios of the luminal area/total vessel area were calculated from these data. Images were obtained with the Scion image software program (National Institutes of Health).

**Statistical Analysis**

All data are expressed as the mean ± S.E.M. Statistical significance (p < 0.05) was assessed using the Student-Newman-Keuls test with a repeated-measures analysis of variance for the flow velocity and physiological data. Vessel wall histopathology was assessed according to a two-way analysis of variance. All data were statistically analyzed using the Sigma-Stat software package (Jandel Scientific, San Rafael, CA, USA).

**Results**

**Determination of the Diabetic Status**

All OLETF rats were significantly heavier than the LETO rats at 30 weeks of age (566 ± 11 vs. 342 ± 12 g, p < 0.01; Table 1). The fasting glucose levels and peak glucose responses were significantly elevated in the OLETF animals compared with that observed in the LETO animals (112.3 ± 2.1 vs. 72.6 ± 3.1 mg/dL, p < 0.05 and 313.4 ± 10.3 vs. 130.6 ± 4.2 mg/dL, p < 0.01, respectively). Moreover, the glucose levels remained significantly higher in the OLETF group than in the LETO group at 120 minutes after administration (243.2 ± 2.1 vs. 117.2 ± 3.7 mg/dL, p < 0.05). Taken together with previous findings, these results indicate that OLETF rats become diabetic by 30 weeks of age, whereas the glucose levels remain within the normal range in LETO rats.

**Physiological Parameters**

The mean arterial blood pressure, pH, PaO₂, PaCO₂ and hematocrit (Ht) values did not differ significantly between the two groups either before or after the US examinations. However, the blood glucose concentrations were significantly higher in the OLETF rats than in the LETO rats (Table 1). The physiological parameters (other than the glucose concentration) did not differ significantly between the strains (Table 1).

**Histology of the Carotid Artery**

As shown in Fig. 1, the ratio of wall thickness to the luminal area was significantly higher in the OLETF rats (Fig. 1A) than in the LETO rats (Fig. 1B). Both intimal and media hyperplasia was observed in the OLETF rats (Fig. 1A).

The wall thickness (tunica intima + tunica media) was increased (19.3 ± 3.2 vs. 6.1 ± 4.5 μm; Fig. 2A) and the luminal area/total vessel area ratio was significantly decreased (51.4 ± 10.2% vs. 83.3 ± 10.1%; Fig. 2B) in the 30-week-old OLETF rats compared with that observed in the LETO rats.

### Table 1. Physiological Data

<table>
<thead>
<tr>
<th>Rats</th>
<th>LETO</th>
<th>OLETF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weights (g)</td>
<td>342 ± 12</td>
<td>566 ± 11*</td>
</tr>
<tr>
<td>MABP (mmHg)</td>
<td>110.5 ± 10.3</td>
<td>120.4 ± 9.8</td>
</tr>
<tr>
<td>PaO₂ (mmHg)</td>
<td>120.4 ± 6.5</td>
<td>123.5 ± 5.3</td>
</tr>
<tr>
<td>PaCO₂ (mmHg)</td>
<td>43.5 ± 6.4</td>
<td>44.6 ± 5.8</td>
</tr>
<tr>
<td>pH</td>
<td>7.37 ± 0.11</td>
<td>7.39 ± 0.22</td>
</tr>
<tr>
<td>Ht (%)</td>
<td>45.4 ± 1.3</td>
<td>44.3 ± 1.1</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>80.3 ± 4.3</td>
<td>332 ± 7.3*</td>
</tr>
</tbody>
</table>

Ht, hematocrit; LETO, Long-Evans Tokushima Otsuka; MABP, mean arterial blood pressure; OLETF, Otsuka Long-Evans Tokushima Fatty; PaO₂, partial pressure of oxygen; PaCO₂, partial pressure of carbon dioxide.

All data are expressed as the mean ± SEM. *Significantly higher in the OLETF rats (p < 0.01). No other measurements differed significantly between the two strains.
Ultrasound Assessments of the Carotid Arterial Flow and Plaque

The CCA and carotid bifurcation were visualized in all rats using color duplex sonography in the color Doppler mode without the use of a contrast agent. The procedure was well tolerated by all animals and completed within 15 minutes. The blood flow was antegrade in all animals. The blood flow in the CCA during systole was faster in the OLETF rats (Fig. 3A) than in the LETO rats (Fig. 3B) (203 ± 20.3 vs. 55.3 ± 21.4 cm/sec), whereas that during diastole was not significantly increased in the OLETF rats compared with that observed in the LETO rats (77.3 ± 27.4 vs. 45.7 ± 30.4 cm/sec) (Fig. 3C). Carotid arterial plaque was more prominent in the OLETF rats (Fig. 4A) than the LETO rats (Fig. 4B) on ultrasound sonography.
Carotid Artery Stenosis is Worsened in Spontaneously Diabetic Model Rats

**Fig. 3.** Color Doppler ultrasound assessments of the carotid arterial flow in the left common carotid artery (CCA) and carotid bifurcation. The systolic and diastolic blood flow was significantly increased in the obese OLETF rats (A) compared with that observed in the control LETO rats (B). Systolic and diastolic blood flow in the common carotid artery (CCA). The blood flow velocity in the common carotid artery (CCA) was significantly higher in the OLETF rats than in the LETO rats during systole (203 ± 20.3 vs. 55.3 ± 21.4 cm/sec; *p < 0.05), but not diastole (77.3 ± 27.4 vs. 45.7 ± 30.4 cm/sec) (C).
Discussion

In the present study, the basic physiological parameters and geometry of the carotid artery conduit differed significantly between the 30-week-old spontaneously obese OLETF and control LETO rats. Hypertriglyceridemia and hyperinsulinemia positively correlate with hyperglycemia in OLETF rats. Previous reports have shown stimulation of the sympathetic nervous system to be a potential mechanism underlying the development of vasoendothelial dysfunction. In the present report, blood pressure was slightly elevated in the 30-week-old OLETF rats, although the values did not differ significantly from those observed in the age-matched LETO rats, in agreement with our previous findings. In contrast, the inner diameter of the carotid artery was significantly decreased in the OLETF rats. Arterial wall hypertrophy is generally accompanied by a decrease in the inner diameter of the artery under conditions of chronic hypertension. However, blood pressure was not increased significantly in the OLETF rats compared with the LETO rats, indicating that factors other than hypertension influence the degree of carotid artery stenosis.

One layer of endothelial cells covers the intimal layer of the carotid artery, with smooth muscle cells covering the medial layer. Dysfunction of endothelial cells is observed in animals in a diabetic state. Diabetes causes hypertrophic remodeling of the peripheral vasculature characterized by a decreased lumen diameter, enlarged tunica media and abnormal expression and localization of extracellular matrix components, including the deposition of collagen and laminin in vessel walls and the accelerated formation of atherosclerotic plaque and intimal proliferation.

We previously observed typical cerebral microvascular atherosclerotic changes in thickened small vessel walls with perivascular fibrosis in OLETF rats. In the present report, we demonstrated increased carotid arterial wall thickening and luminal narrowing in diabetic and obese rats. Typical metabolic syndrome leading to vessel dysfunction has been documented in OLETF rats. We did not observe any vessel dysfunction in the present experiments; however, such dysfunction may worsen atherosclerotic changes.

In humans, type 2 diabetes tends to be associated with hyperinsulinemia due to the presence of insulin resistance resulting in hyperglycemia. Hyperinsulinemia has been reported in OLETF rats in previous studies. In the present analysis, metabolic correction with insulin notably reversed the enhanced endothelial function in the diabetic model rats, which may be due to the downregulation of endothelial nitric oxide synthase (eNOS)/heme oxygenase (HO)-1. Insulin administration also resulted in intimal hyperplasia, particularly subendothelial thickening with the potential to impede the diffusion of nitric oxide into smooth muscle required to induce relaxation. Intimal hyperplasia is also induced by insulin in rats with type 1 diabetes after balloon injury and may be responsible for accelerated carotid artery stenosis in humans. Therefore, metabolic correction with insulin may lead to unintentional hyperinsulinemia and intimal hyperplasia that may be detrimental to the endothelial function and promote vascular atherosclerosis. In this study, intimal thickening was more pronounced in the OLETF rats than the LETO rats.
In this report, we assessed the effects of obesity and diabetes on arteriosclerosis of the carotid artery, as we previously found that arteriosclerotic changes in the cerebral small vessels progress as advanced glycation end-products (AGEs) accumulate in OLETF rats. However, antiplatelet therapy does not improve these changes. Our findings indicate marked arteriosclerotic changes and stenosis in the carotid arteries of obese OLETF rats. Yang et al. compared the prevalence and manifestations of extracranial and intracranial artery stenosis using digital subtraction angiography in patients with and without type 2 diabetes and found a tendency towards a higher incidence of multiple stenosis in the diabetic patients. Non-obstructive stenosis arises more often in diabetic subjects than in non-diabetic individuals. Other risk factors, such as dyslipidemia and diabetes, may also contribute to endothelial dysfunction in OLETF rats. Furthermore, in humans, obesity may be associated with diabetes, leading to arterial wall dysfunction and stenosis.

Based on the present findings, hyperglycemia contributes to stenosis of the common carotid arteries in obese model OLETF rats. Although we did not examine the serum insulin or adiponectin levels in this study, a previous study reported high HbA1c, blood serum insulin, cholesterol and adiponectin values in OLETF rats. The lack of assessment of these parameters is a limitation of this study.

Conclusion

In the present report, we demonstrated that hypertension and diabetes contribute to the development of stenosis of the carotid arteries in a model of obese rats. Our findings suggest the possibility of developing new therapies for carotid artery stenosis in obese patients.

Conflicts of Interest

The authors received no financial support or grants for this research.

References