Increased Hyaluronan and Hyaluronidase Production and Hyaluronan Degradation in Injured Aorta of Insulin-Resistant Rats

Abdesslam Chajara, Maha Raoudi, Bertrand Delpech, Marcelle Leroy, Jean Pierre Basuyau, Hervé Levesque

Abstract—Diabetic patients have a greater incidence of restenosis, which has been shown to be related to exaggerated intimal hyperplasia. Hyaluronan (HA) has been shown to be closely involved in arterial smooth muscle cell proliferation and migration, which provoke intimal hyperplasia after balloon catheter injury. Our aim was to determine the effect of fructose feeding, which produces certain characteristics of non–insulin-dependent diabetes (ie, insulin resistance, hyperinsulinemia, and hypertriglyceridemia), on production of HA and hyaluronidase and degradation of HA in rat aorta. Treated rats received fructose (25% in tap water) 12 weeks before balloon catheter injury and 14 days afterward. Fructose-fed rats had hyperinsulinemia and hypertriglyceridemia. Injury increased intima-media wet weight (7.5%) and DNA content (20%) in control rats. This increase was significantly greater in fructose-fed rats (22% for wet weight and 34% for DNA content) and was associated with greater HA and hyaluronidase production (123% and 41%, respectively) than in control rats (49% and 7%, respectively). Determination of HA molecular mass showed that balloon catheter injury increased the number of HA fragments in the aorta of control rats. Normal aorta of fructose-fed rats contained more HA fragments than that of control rats. Injury to the aorta of fructose-fed rats increased HA fragments and induced the appearance of a very-high-molecular-mass (≥2000 kDa) HA. In conclusion, fructose treatment, which induced hyperinsulinemia and hypertriglyceridemia, increased HA and hyaluronidase production and HA degradation in injured aorta. This finding suggests that HA, which has been shown to play a crucial role in proliferation and migration of arterial smooth muscle cells, may be involved in the promotional effect of long-term fructose feeding on arterial wall reaction to injury.

Key Words: diabetes mellitus ■ fructose feeding ■ hyperinsulinemia ■ hypertriglyceridemia ■ hyaluronan ■ hyaluronidase ■ aorta ■ injury
aorta. Moreover, others have demonstrated that staining of HA in injured rat artery is associated with proliferating and migrating ASMCs.\textsuperscript{13, 14} It also has been shown that HA stimulates ASMC migration through binding to its RHAMM receptor and stimulates DNA synthesis in ASMCs through binding to its CD44 receptor.\textsuperscript{15, 16} Evanko et al\textsuperscript{17} recently showed that 90% of ASMCs migrating in cultures of wounded tissue are surrounded by an HA-rich pericellular matrix that actively participates in the detachment of ASMCs and the mitotic process.

Hyaluronidase is the enzyme that degrades HA. Elevated activity of hyaluronidase in tissues is believed to be involved in the regeneration and restoration process in tissues, in increased vascularization, and in the uncontrolled cellular growth rate of tumors.\textsuperscript{18, 19} The role of hyaluronidase in ASMC activities, in either normal or abnormal conditions, remains unknown. However, the crucial role attributed to HA in ASMC proliferation and migration suggests that hyaluronidase may be implicated in the cellular events that occur, particularly those that occur in the injured vessel wall. It has been shown\textsuperscript{20} that ASMCs and endothelial cells from rat aorta produce hyaluronidase in culture, which requires strict acidic pH (3.7) for activity. Moreover, we previously found that hyaluronidase activity was increased in injured rat aorta.\textsuperscript{12}

The purpose of the present study was to determine the effect of fructose feeding, which has been shown to produce certain characteristics of NIDDM (ie, hypertriglyceridemia, insulin resistance, and hyperinsulinemia\textsuperscript{21, 22}), on HA production and degradation in injured rat aorta. To evaluate HA degradation, we quantified the activity of hyaluronidase and determined HA molecular mass in injured aorta.

**Materials and Methods**

**Animals**

Male Wistar rats (300 to 320 g, 3 months old; Iffa Credo, l’Arbresle, France) were used. All animals were housed 3 per cage and fed, ad libitum, regular chow and tap water. All experiments were conducted in accordance with institutional guidelines and recommendations for the care and use of laboratory animals established by the French Ministry of Agriculture (authorization no. 002245, October 31, 1990). For all operative procedures, animals were anesthetized with ketamine hydrochloride (150 mg/kg IP Ketalar, Laboratoire Park Davis). To induce insulin resistance, rats were subjected to fructose treatment.\textsuperscript{21} The normal diet was supplemented with 25% fructose (Fluka), which was added to the drinking water of rats randomly assigned to treatment for 12 weeks before balloon catheter injury (BCI). Noninjured control rats were subjected to the same operation, but the catheter was not introduced into the carotid artery. All rats were killed and studied 14 days after BCI.

**Aortic Injury**

Injury to the thoracic aorta was induced by means of an inflated balloon catheter as previously described.\textsuperscript{23} In brief, a deflated embolomectomy catheter (Fogarty, size 2F, Edwards Laboratories) was introduced into the aorta through the left common carotid artery to the level of the renal arteries. The balloon was inflated with 0.05 mL of distilled water and withdrawn slowly. When the catheter reached the diaphragm, another 0.05 mL was added. This process was repeated twice, and the carotid artery was double ligated. Noninjured control rats were subjected to the same operation, but the catheter was not introduced into the carotid artery. All rats were killed and studied 14 days after BCI.

**Analysis of Intima-Media**

Animals were killed by exsanguination. The thoracic aorta, exactly delimited by the left subclavian and celiac arteries, was removed for study. The aorta was opened longitudinally, and residual blood was removed by flushing with Tris-EDTA (0.01 mol/L and 0.2 mmol/L, respectively), pH 7.4, and frozen in the same buffer solution until additional processing was performed. After thawing, the aortas were dissected into intima-media,\textsuperscript{24} which was further analyzed, and adventitia, which was discarded. Intima-media was weighed (wet weight) and homogenized in 2 mL of Tris-EDTA. The aorta was homogenized (2 minutes at 3000 rpm) in a glass tube using a glass pestle (size 22, Kontes). Four fractions were removed from the homogenate and used for DNA quantification (0.5 mL), HA quantification (0.1 mL), HA analysis by high-performance liquid chromatography (HPLC) (0.1 mL), and hyaluronidase quantification (0.1 mL). Sodium azide was added to fractions designated for HA and hyaluronidase analysis (final concentration 3 mmol/L). The remaining volume was stored at −20°C.

**Intima-Media DNA Content**

DNA content of intima-media was determined as described by Capron and Bruneval.\textsuperscript{25} After homogenization in Tris-EDTA, intima-media was digested by protease (from Streptomyces griseus, Sigma Chemical Co). DNA content of the lysate was then determined according to the method of Burton.\textsuperscript{26}

**Assay of HA in Intima-Media**

The HA assay was performed according to a previously described method using alkaline phosphatase–conjugated sheep brain hyaluronate (AP-sbH).\textsuperscript{27}

**Sheep Brain Hyaluronate**

sbH was obtained as previously described.\textsuperscript{11} In brief, sheep brains were homogenized in 0.2 mol/L glycine-HCl, pH 2.2 (1/2 wt/vol), and centrifuged at 40 000g (20 minutes at 4°C). The supernatant was neutralized, dialyzed in PBS, and incubated with a HA adsorbent overnight at room temperature. The HA adsorbent was prepared by coupling HA to aminohexyl-Sepharose (AH-Sepharose 4B, Pharmacia) using carbodiimide. The adsorbent was washed with PBS containing 1 mol/L NaCl, and HA was eluted with 0.2 mol/L glycine-HCl (pH 2.8), dialyzed in PBS, and concentrated to a final concentration of 1 mg/mL. The purity of HA was assessed immunologically by acrylamide gel electrophoresis and Western blotting and chemically by HPLC, which showed that the purified protein could bind to and was eluted with HA.

**Table 1. Animal Characteristics 8 and 14 wk After the Fructose-Enriched Diet Was Started**

<table>
<thead>
<tr>
<th></th>
<th>Control Rats (n=16)</th>
<th>Fructose-Fed Rats (n=14)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 wk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight, g</td>
<td>486±6.52</td>
<td>493±10</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.62±0.25</td>
<td>6.47±0.30</td>
<td>0.037</td>
</tr>
<tr>
<td>Insulin, pmol/L</td>
<td>128±8.95</td>
<td>210±11.7</td>
<td>0.0008</td>
</tr>
<tr>
<td>Triglycerides, g/L</td>
<td>2.17±0.12</td>
<td>3.1±0.31</td>
<td>0.0087</td>
</tr>
<tr>
<td>14 wk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight, g</td>
<td>528±8.2</td>
<td>529±11.3</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>7.59±0.27</td>
<td>7.89±0.32</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin, pmol/L</td>
<td>288±22</td>
<td>340±17</td>
<td>0.046</td>
</tr>
<tr>
<td>Triglycerides, g/L</td>
<td>1.49±0.12</td>
<td>2.43±0.25</td>
<td>0.0009</td>
</tr>
</tbody>
</table>

Values are mean±SE. The Mann-Whitney U test was used for comparisons.
### Preparation of AP-sbHN

AP-sbHN was prepared as previously described.27 AP-sbHN was used to detect tissue HA and to assay HA in intima-media extract.

### HA Assay

Samples were digested with protease (1 mg/mL from *S. griseus*) overnight at 37°C. Protease was destroyed by treating samples at 100°C for 20 minutes. After centrifugation (30 000 g overnight at 37°C, Protease was destroyed by treating samples at 100°C for 20 minutes. After centrifugation (30 000 g), the supernatant was injected into the column. Assay of HA in fractions of HPLC was performed with the ELSA technique described previously (see HA Assay).

### Assays of Plasma Glucose, Insulin, Total Cholesterol, and Triglycerides

Blood was collected from the tail 8 weeks after fructose feeding was started and from the abdominal aorta at the time of killing. Plasma glucose, total cholesterol, and triglycerides were determined using the automated methods detailed in the manual for the Kodak Ektachem 750 RC analyzer (Jansson & Jansson). Plasma immuno-reactive insulin was measured using a radioimmunoassay kit (Kabi Pharmacia Diagnostics AB).

### Statistics

Multiple comparisons of the 4 groups (C0, C14, F0, and F14) were performed by using the Kruskal-Wallis test. If the resulting *H* value was significant (*P*<0.05), the Mann-Whitney *U* test was used for simple comparisons.

### Results

#### Animal Characteristics

Table 1 describes the experimental groups and summarizes the main animal characteristics (body weight, nonfasting plasma glucose, insulin, and triglyceride concentrations). Fructose treatment induced hyperinsulinemia and hypertriglyceridemia and increased to a lesser extent glycemia in rats. These modifications were not associated with a change in body weight gain.

### Intima-Media Wet Weight and DNA Content

Intima-media wet weight and DNA content were slightly lower in the noninjured aorta of rats treated with fructose than in noninjured aorta of control rats (*P*=0.47, Table 2). BCI content 2 weeks later in control rats (*P*=0.058 for wet weight

### Table 2. Wet Weight, DNA Content, HA Content, and Hyaluronidase Activity of Aortic Intima-Media in Sham-Operated Rats (Groups C0 and F0) and 14 d After BCI (Groups C14 and F14)

<table>
<thead>
<tr>
<th></th>
<th>Control Rats</th>
<th>Fructose-Fed Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C0 (n=8)</td>
<td>C14 (n=8)</td>
</tr>
<tr>
<td>Wet weight, mg</td>
<td>33.6±0.86</td>
<td>36.1±0.91*</td>
</tr>
<tr>
<td>DNA content, μg Aorta</td>
<td>54.6±0.84</td>
<td>65.9±1.68§</td>
</tr>
<tr>
<td>HA content</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total, ng</td>
<td>591±71.6</td>
<td>881±37.7‡</td>
</tr>
<tr>
<td>Aorta extract, ng/mg aorta</td>
<td>17.5±1.94</td>
<td>24.4±0.88†</td>
</tr>
<tr>
<td>HA/DNA ratio, ng/μg DNA</td>
<td>10.8±1.3</td>
<td>13.4±0.42</td>
</tr>
<tr>
<td>Hyaluronidase activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total, nU</td>
<td>19.8±0.07</td>
<td>21.3±1.1</td>
</tr>
<tr>
<td>Aorta extract, nU/mg aorta</td>
<td>0.59±0.01</td>
<td>0.59±0.03</td>
</tr>
<tr>
<td>Activity/DNA ratio, nU/μg DNA</td>
<td>0.36±0.01</td>
<td>0.32±0.02</td>
</tr>
</tbody>
</table>

Values are mean±SE. For each variable, the Kruskal-Wallis test was used to compare the 4 experimental groups. If the resulting *H* value was significant (*P*<0.05), the Mann-Whitney *U* test was used for simple comparisons.

*P*<0.05, †*P*<0.01, ‡*P*<0.005, signed differences between injured and noninjured rats (C14 vs C0 and F14 vs F0). | *P*=0.059, ¶*P*<0.05, and #*P*<0.01, signed differences between control and fructose-fed rats (F0 vs C0 and F14 vs C14).

**TABLE 2. Wet Weight, DNA Content, HA Content, and Hyaluronidase Activity of Aortic Intima-Media in Sham-Operated Rats (Groups C0 and F0) and 14 d After BCI (Groups C14 and F14)**
and $P<0.005$ for DNA) and rats receiving fructose ($P<0.01$ for wet weight and $P<0.005$ for DNA). Wet weight of the intima-media and its DNA content in injured aorta of fructose-fed rats were higher than in injured aorta of control rats; the differences did not, however, reach significance ($P=0.11$ and $P=0.13$, respectively). When we focused on stimulation of wet weight and DNA content of the intima-media after BCI (Figure 1), we found that wet weight stimulation was 3 times higher in fructose-fed rats (22.7±3.31%) than in control rats (7.5±2.72%, $P=0.0045$). Stimulation of DNA content was also more pronounced (34.3±3.8% in fructose-fed rats and 20.7±3.31% in control rats, $P=0.03$).

**Intima-Media HA Content**

The amount of HA in noninjured aorta was slightly higher in control rats than in fructose-fed rats (Table 2). BCI significantly increased HA content in both groups of rats ($P<0.005$ for both). On the other hand, HA content was higher in injured aorta of fructose-fed rats than in control rats ($P<0.05$). HA concentration (HA per milligram of aorta) in the intima-media increased significantly after BCI in both control ($P<0.01$) and fructose-fed rats ($P<0.005$) with no significant difference between the 2 groups at day 14. The amount of HA related to cell number in the intima-media (reflected by DNA content) was determined as the HA/DNA ratio (Table 2), which reflects the capacity of arterial cells to produce HA. HA/DNA was only slightly enhanced by BCI on day 14 in control rats but was significantly increased in fructose-fed rats ($P<0.005$).

The proportion of HA content increase in the intima-media after BCI was about 2.5 times higher in fructose-fed rats than in control rats (Figure 2a, $P=0.0008$). HA concentration and HA/DNA augmentation were also more pronounced after BCI in fructose-fed rats: HA concentration and HA/DNA were 2-fold ($P=0.001$) and 3-fold ($P=0.001$) higher, respectively, than in control rats (Figure 2b).

**Intima-Media Hyaluronidase Activity**

To determine whether the difference in production of HA by injured aorta observed between fructose-fed and control rats was associated with a change in the activity of the enzyme that degrades HA, we determined the activity of hyaluronidase in the aorta extract (Table 2). Total hyaluronidase activity in sham-operated aorta was similar in the 2 groups of rats. However, the concentration of hyaluronidase activity (nU/mg aorta) was higher in uninjured aorta of fructose-fed rats than in uninjured aorta of control rats ($P=0.058$). At day 14, hyaluronidase activity of the intima-media was not affected by BCI in control rats; however, it increased significantly in fructose-fed rats ($P<0.005$). The concentration of hyaluronidase activity in the aorta extract also remained unchanged in control rats but was significantly enhanced in fructose-fed rats after BCI ($P<0.01$). The ratio of hyaluronidase activity to DNA was not significantly influenced by BCI in either group of rats. Both hyaluronidase concentration and the hyaluronidase activity/DNA ratio were significantly greater in injured aorta of fructose-fed rats than in injured aorta of control rats 14 days after BCI ($P<0.005$ and $P<0.01$, respectively).

The proportion of hyaluronidase increase in the intima-media after injury in comparison to that in sham-operated
aorta was about 4.5 times higher in fructose-fed rats than in control rats ($P=0.0003$, Figure 3a). The concentration of hyaluronidase in the aorta did not change and the hyaluronidase activity/DNA ratio decreased in control rats after BCI (Figure 3b). These 2 parameters, however, increased in fructose-fed rats, and differences between the 2 groups were significant ($P=0.027$ and $P=0.03$, respectively; Figure 3b).

HA Molecular Mass in Intima-Media

Because the ability of injured aorta of rats treated with fructose to produce hyaluronidase was greater than that of control rats, we attempted to verify (1) whether it contained more HA fragments than control injured aorta and (2) whether the profile of HA fragments, regardless of their molecular mass, in injured aorta of fructose-fed rats is somewhat different from that in injured aorta of control rats. HPLC of protease-digested extract of noninjured aorta of control rats showed that HA was present mainly in a high-molecular-mass form, which was observed at a retention time of 20 kDa (Figure 4a). Two other forms, each with a lower molecular mass, were also observed in a smaller amounts, one at a retention time of 41 minutes and the other at a retention time of 56 minutes. The molecular mass of these 2 HA fragments was $<20$ kDa. This HA profile was compared with that of the intima-media of noninjured aorta of fructose-fed rats. In group F0, the peak corresponding to the 2000-kDa form was also observed and was slightly greater than that observed in group C0 (Figure 4b). However, more HA fragments (8 forms) were present in the intima-media extract of group F0. Three forms had a molecular mass $>20$ kDa (669, 200, and 29 kDa); the other 5 forms had a molecular mass $<20$ kDa. In the injured aorta of control rats (C14), the 2000-kDa form of HA was still present; the peak of this high molecular mass was only slightly enhanced in comparison to that of the same molecular mass detected in group C0 (Figure 4c). On the other hand, injury elicited the formation of new HA fragments that were not present in the uninjured aorta (C0). Five HA fragments were present in group C14, 1 with a molecular mass of 66 kDa and 4 with a molecular mass of $<20$ kDa. In the injured aorta of fructose-fed rats (F14), the 2000-kDa form of HA was also observed; however, the extent of the peak of this HA form was 2.3-fold higher than that observed in the corresponding noninjured aorta (F0). Moreover, the 2000-kDa form of HA was not the highest form present in the intima-media extract of F14 (Figure 4d). A HA form with a molecular mass $>2000$ kDa was observed in group F14 but was not present in the 3 others groups studied (C0, C14, and F0). In addition to the 2 high-molecular-mass forms mentioned, intima-media of group F14 contained 10 HA forms with a lower molecular mass (4 forms with a molecular mass ranging from 1200 to 34 kDa and 5 forms with a molecular mass $<20$ kDa).

Discussion

The purpose of the present study was to analyze production of HA and hyaluronidase and degradation of HA in injured aorta under conditions that reproduce in the animal model certain characteristics of NIDDM. To obtain conditions similar to those occurring in NIDDM, we subjected rats to fructose treatment (25% in tap water). Fructose feeding is known to provide a model of hypertriglyceridemia, insulin resistance, and hyperinsulinemia, which are common in NIDDM. In the present study, we observed that long-term fructose feeding induced hyperinsulinemia, hypertriglyceridemia, and a transient increase of glycemia. The mechanism by which fructose provokes this metabolic response is not completely understood. It was found, however, that insulin resistance resulting from long-term fructose feeding is due to the diminished ability of insulin to suppress hepatic glucose output and to a decrement in peripheral glucose disposal.$^{30,31}$ Hypertriglyceridemia, which appears rapidly (a few days after starting a fructose diet), is believed to play an active role in induction of insulin resistance and hyperinsulinemia. Fructose likely provokes hypertriglyceridemia by increasing the formation of glycerol-3-phosphate, a precursor of lipid synthesis.$^{22}$ Increased circulating levels of triglycerides are known to reduce the number of insulin receptors, thereby reducing insulin sensitivity. On the other hand, enhanced production of triglycerides may lead to stimulation of lipoprotein lipase, an important regulatory enzyme of triglyceride uptake.

Under these conditions of hyperinsulinemia and hypertriglyceridemia, we observed that stimulation of wet weight and DNA content of the intima-media (parameters reflecting the
Figure 4. HPLC determination of HA molecular mass in normal aorta of control (a) and fructose-fed rats (b) and in injured aorta of control (c) and fructose-fed rats (d). In all cases, the HA assay, performed after elution, showed the presence of a high-molecular-mass (2000-kDa) HA. Injury increased the number of HA fragments in the aorta of control rats. Normal aorta of fructose-fed rats contained more HA fragments than that of control rats. Injury to the aorta of fructose-fed rats increased HA fragments and induced the appearance of a very-high-molecular-mass (>2000kDa) HA.
proliferative response of ASMCs) after injury was greater in animals receiving a fructose-rich diet than in control rats. This finding is concordant with findings of previous studies using another animal model, the obese Zucker rat, which has characteristics similar to those of the fructose-fed rat (ie, hyperinsulinemia, hyperlipidemia, and a mild increase of plasma glucose levels). These previous studies demonstrated that the extent of neointima formation in vessel wall injured by means of a catheter balloon was increased in Zucker rats.6,7

Injury increased the amount and concentration of HA in the intima-media of injured aorta. This finding is concordant with our previous observations11,12 and those of several other studies, showing that production of HA increased in the catheterized aorta. Rats receiving fructose produced more HA than control rats after injury. HA, a highly charged, high-molecular-mass glycosaminoglycan, has been shown to be actively involved in ASMC migration and proliferation in vitro and in vivo.11–17 ASMCs, which undergo migration across the internal elastic lamina in injured artery, strongly express both HA and RHAMM.14 This observation has been substantiated by the demonstration in culture that HA stimulates ASMC migration through binding to its receptor RHAMM.15 HA has also been found to be involved in ASMC proliferation. Jain et al16 demonstrated that the binding of HA to the HA receptor CD44 stimulated DNA synthesis in human and rat ASMCs in vitro. Investigation of the potential contribution of HA to the pathogenesis of restenosis in humans produced concordant results: HA was found to be strongly and mainly expressed around stellate ASMCs in fibroproliferative tissue, which characterizes restenotic lesions.13 According to all of these previous investigations, our results suggest that the increased production of HA in injured aorta of fructose-fed rats may be involved in the enhanced ASMC reaction to injury observed in these animals.

However, the growth-promoting effect of HA on ASMCs in injured aorta may not be the only consequence of HA deposition in the aorta of fructose-fed rats. Hyaluronan is an important component of the healing wound.9,10 In addition, evidence supports the participation of wound healing in arterial remodeling that leads to lumen narrowing after BCI. Moreover, Geary et al15 provided data indicating that HA, which is strongly expressed early in the media of injured arteries and later in the neointima, may be involved in wound contraction, which is actively involved in lumen narrowing. Although participation of HA in arterial remodeling remains to be clearly demonstrated, it is probable that this glycosaminoglycan plays an important role in extracellular matrix modulation in injured aorta of fructose-fed rats. The factor responsible for increased HA production in fructose-fed rats is unknown. However, some factors, like hyperinsulinemia and hypertriglyceridemia, likely play a role. We recently found that production of HA in insulin-dependent diabetic rats was similar to that in nondiabetic rats. Interestingly, when diabetic rats were treated with insulin, both neointima formation and HA production were stimulated in comparison to nondiabetic rats.33 This suggests that insulin may promote HA synthesis in injured rat aorta.

Increased HA production in injured aorta of rats receiving fructose was associated with a greater ability to produce hyaluronidase activity than observed in control rats. Because hyaluronidase degrades HA, this finding suggests that the increased amount of HA in injured aorta of fructose-fed rats reflects increased production rather than decreased degradation. The elevated production of hyaluronidase activity in the injured aorta of fructose-fed rats prompted us to verify whether this is associated with (1) the presence of more HA fragments in injured aorta of fructose-fed rats than in injured aorta of control rats or (2) the presence of new HA fragments in fructose-fed rats not observed in control rats. To do this, we analyzed the molecular mass of HA in the intima-media extract. In the normal aorta, HA was mainly present in a form of 2000 kDa. Two other forms >29 kDa were also detected, suggesting that HA is degraded under normal conditions. It has been effectively demonstrated that HA is actively degraded in normal connective tissue, because one third of HA present in the whole body is degraded each day.14 Injury increased the number of low-molecular-mass forms of HA in the intima-media of control rats, suggesting that BCI enhanced HA degradation in the intima-media. Hyaluronidase activity was not found, however, to be significantly increased in injured aorta of control rats. This may suggest that the moderate increase of hyaluronidase activity in these rats is associated with a visible degradation of HA in the catheterized aorta. The weak increase of hyaluronidase activity in injured aorta of control rats contrasts with our previous data demonstrating a significant increase of this enzyme after injury in normal rats.12 This discrepancy may be related to the difference in age of the rats studied. In the previous study,12 we analyzed hyaluronidase activity in young rats (12 weeks old, mean body weight of 368 g), whereas control rats in the present study were, because of the protocol used, 24 weeks old with a mean body weight of 562 g. This hypothesis is supported by our previous finding that hyaluronidase activity is not significantly increased in injured aorta of old rats.12 The uninjured intima-media of fructose-fed rats contained more HA fragments than that of control rats, suggesting enhanced degradation of HA in fructose-fed rats. This supports the finding that the concentration of hyaluronidase activity in normal aorta of fructose-fed rats was significantly higher than that of controls. Injured aorta of fructose-fed rats contained a greater number of low-molecular-mass forms of HA, supporting the observation that hyaluronidase activity was increased more in injured aorta of fructose-fed rats than in injured aorta of control rats. In addition, we found that the injured aorta of fructose-fed rats contained an HA form with a molecular mass >2000 kDa, which was not observed in the other groups studied. This finding suggests that HA of a very high molecular mass can be synthesized by arterial cells. Why this high-molecular-mass form of HA is not present in the aorta of the others groups analyzed remains to be shown. Two explanations are plausible. The first is that this high-molecular-mass form of HA is produced in the aorta of the others groups but is not detected because it is rapidly digested by hyaluronidase in smaller forms. This form may be visible in injured aorta of fructose-fed rats because HA production is so strong that hyaluronidase, despite being increased, did not arrive to degrade all of the HA produced. The second explanation is that the HA form >2000 kDa may be specifically synthesized by arterial cells of rats treated with fructose in response to injury. This explanation supposes that this HA form is involved in the promotional effect of a high-fructose diet on the aortic response to injury. It will be interesting to
explore the effect of the >2000-kDa HA form on ASMC proliferation and migration in vitro.

Nevertheless, the increased production of both high- and low-molecular-mass forms of HA in injured aorta of fructose-fed rats indicates that activated arterial cells actively produce and degrade HA. One explanation is that HA may be used and recycled continuously by ASMCs, which are activated by balloon injury. Another explanation is that HA is produced and then degraded to obtain HA oligosaccharides, which have been shown to have a specific effect on cell activities. HA oligosaccharides comprising 3 to 16 disaccharides, obtained by hyaluronidase treatment of HA, have been found to stimulate angiogenesis in vivo and proliferation of endothelial cells in culture.33,35 Recently, Evanko et al17 showed that HA fragments were more likely to bind to the surface of ASMCs than a high-molecular-mass HA. Those observations, along with our finding in injured aorta of fructose-fed rats, suggest that hyaluronidase probably plays an active role in the process that leads arterial cells to produce HA and to use it during the process that leads them to proliferate in the media and then to migrate to the intima to form a thickened neointima.

In conclusion, long-term fructose feeding, which provokes hypertriglyceridemia and hyperinsulinemia, increased HA and hyaluronidase production and HA degradation in the intima-media of injured rat aorta. Considering the crucial role that HA plays in ASMC migration and proliferation,11–17 our results suggest that hypertriglyceridemia and hyperinsulinemia, which are observed in patients with NIDDM, promote an arterial response to BCI through a mechanism that may involve HA production and degradation in the arterial wall.

Acknowledgment

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References

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