Impact of age and hyperglycemia on the mechanical behavior of intact human coronary arteries: an ex vivo intravascular ultrasound study

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Tajaddini, Azita, Deborah L. Kilpatrick, Paul Schoenhagen, E. Murat Tuzcu, Michael Lieber, and D. Geoffrey Vince. Impact of age and hyperglycemia on the mechanical behavior of intact human coronary arteries: an ex vivo intravascular ultrasound study. Am J Physiol Heart Circ Physiol 288: H250–H255, 2005. First published August 26, 2004; doi:10.1152/ajpheart.00646.2004.—Despite their advantages, percutaneous coronary interventional procedures are less effective in diabetic patients. Changes in the mechanical properties of vascular walls secondary to long-term hyperglycemia as well as other factors such as age may influence coronary distensibility. This investigation is aimed at deciphering the extent of these effects on distensibility of postmortem human coronary arteries in a controlled manner. Excised human left anterior descending (LAD) coronary arteries were obtained within 24 h postmortem. With the use of intravascular ultrasound, vascular deformation was analyzed at midregions of 51 moderate lesions. Intraluminal pressure was systematically altered using a computerized pressure pump system and monitored by a pressure-sensing guidewire. Distensibility, a normalized compliance term, was defined as the change in lumen area normalized by the initial reference area over a given pressure interval. With the use of multivariate analysis and repeated-measures ANOVA, coronary distensibility was independently influenced by hyperglycemia and age (P < 0.05) through the entire pressure range. Within physiological pressure range, distensibility was significantly reduced with age in nonhyperglycemic coronary specimens (10.55 ± 4.41 vs. 6.99 ± 2.45, ×10⁻³ kPa⁻¹, P = 0.01), whereas the hyperglycemic vessels were stiff even in the younger group (7.90 ± 5.82 vs. 7.20 ± 3.36, ×10⁻³ kPa⁻¹, P = 0.79). Similar results were observed with stiffness index and elastic modulus of the arteries. Hyperglycemia and age independently influenced the distensibility of moderately atherosclerotic LAD coronary arteries. The stiffening with age was overshadowed in the hyperglycemic group by as-yet-undetermined factors.

arteriosclerosis; coronary disease; diabetes mellitus; elasticity

DIABETES MELLITUS is associated with a two- to threefold increase in the incidence of coronary artery disease (CAD). Micro- and macrovascular complications of diabetes result in increased cardiovascular morbidity and mortality (7). Diabetic patients have a higher tendency for thrombogenesis and neointimal proliferation after intervention (20) as well as multiple coexisting risk factors for CAD such as hypertension and age. Additionally, atherosclerosis in diabetic patients tends to be more diffuse with more complex lesions (28). Despite improvements in percutaneous coronary interventional technology, the outcome of these procedures is still less favorable for diabetic patients (7, 8, 20). The underlying pathophysiological mechanism is not completely understood, but changes in the elastic properties of coronary arterial walls could contribute to such increased morbidity.

The approach of this study was to quantify local coronary distensibility using diagnostic tools such as intravascular ultrasound (IVUS) catheters and pressure-sensing guidewires. The results could elucidate the factors affecting wall properties of moderately diseased vascular regions that would potentially impact the rate of disease progression and the complication rates of interventional procedures, particularly in patients with diabetes.

MATERIALS AND METHODS

Vessels. A total of 51 human left anterior descending coronary arteries (LAD) were examined within 24 h postmortem according to institutional regulations. Subjects with prior history of cardiac intervention and chemotherapy were excluded. An ~40-mm-thick portion of myocardium, which contained the LAD from its ostium to apex, was dissected from the heart. This extravascular tissue supported the LAD structurally by keeping its natural boundary conditions. Blood samples were also collected to analyze the glycated hemoglobin level (%HbA1c), where a level of 6.0 and above indicates hyperglycemia.

The age of subjects was between 40 and 75 yr old.

Experimental setup. The harvested tissue was mounted in a tissue bath, cannulated, and secured at the ostium, maintaining its in vivo orientation. The tissue bath containing PBS was kept at 37 ± 1°C to reproduce in vivo conditions. Luminal pressure was altered with a computer-controlled pump system, as described previously (30). To measure intraluminal variations accurately, a pressure-sensing guide-wire (Pressurewire, RADI Medical Systems) was calibrated and inserted proximally into the mounted vessel. The Pressurewire, with a diameter of 360 μm, was positioned to avoid contact with the vascular wall. The input pressure, vascular pressure, and temperature of the PBS entering the LAD were continuously monitored and displayed.

To measure the arterial wall dimensions, a 2.9-Fr, 30-MHz IVUS catheter (Scimed) was inserted into the vessel over the Pressurewire. While a low pressure (~100 mmHg) was applied, side branches were ligated until the measured lumen pressure approached the input. When the pressure stabilized, usually in 30–45 min, the catheter was manually pulled back for an overview of the vessel geometry and extent of atherosclerosis. Vessels were excluded if they failed to reach a stable pressure due to excessive leakage at branch sites.

Site selection. A site with concentric intimal thickening with plaque burden <50% (by area) was selected from each vessel (Fig. 1) to examine the properties of a homogenous section of the arterial wall. This single site was chosen from the middle section of the lesion to represent the behavior of that particular lesion.

Distensibility tests. After mechanical preconditioning of the vessel, the IVUS catheter was placed at the selected site and secured exter-
Distensibility at physiological coronary pressure ($D_P$) was defined for each subject at $\Delta P$ between 60 and 140 mmHg (24). A stiffness index ($\beta$) was also calculated based on the same pressure range as $\beta = [\ln (P_2/P_1)]/[r_2 - r_1/r_1]$, where $r$ is the radius of the vessel at the corresponding pressure $P$ (9, 11). Similar to distensibility, $\beta$ provides a normalized compliance term including a logarithmic pressure term. Elasticity of a material can also be defined using structural-independent terms of stress and strain commonly used in the engineering discipline. Circumferential Cauchy stress ($\sigma$) was calculated in the middle of the arterial wall using the equation for thick-walled cylinders as $\sigma = P \times \left[ \frac{r_2^2(r_2^2 - r_1^2)}{2} \right] [1 + (r_2/r_1)^2]$, where $r$, $r_n$, and $r_o$ indicate midwall, inner, and outer radii, respectively. Circumferential strain ($\varepsilon$) was defined as the change in midwall radius within the same pressure range as above with respect to the reference radius $\bar{r} = (r_2/r_1) - 1$.

The elastic modulus ($E$) was calculated as the slope of the stress-strain curve in the physiological range of pressure. $E$ is a material property independent of the structure of the specimen. However, calculation of stress depends on the structure of the specimen, which in this case was assumed to be a thick-walled cylinder.

**Statistical analysis.** For each case, the subject’s age, plaque burden, and %HbA1c levels were included. A statistical mixed model of repeated-measures ANOVA (SAS, release 6.12, SAS Institute) was used to extract the influence of fixed variables on distensibility. Continuous variables such as age, plaque burden, and $D_P$ are described as means $\pm$ SD. They were compared between the subject groups using a t-test assuming an equal variance. A significance level of 0.05 was used for all tests.

### RESULTS

A summary of subject and vessel characteristics is shown in Table 1. Age, %HbA1c, and plaque burden ranged between 40 and 75 yr, 4.5% and 14.1%, and 20% and 50%, respectively. The majority of the subjects were males ($n = 39$), some of whom were hyperglycemic ($n = 12$). Most of the subjects were Caucasian ($n = 32$), mostly nonhyperglycemic. Hyperglycemic subjects were not significantly older ($P = 0.06$). Also, there was no difference in age between male and female subjects ($51.3 \pm 8.0$ and $53.3 \pm 0.3$ yr, respectively, $P = 0.48$). The LAD sections had a similar plaque burden compared with the nonhyperglycemic group ($P = 0.38$). The morphometric measurements of the two groups were similar at an intracoronary pressure of 100 mmHg (Table 1). The LAD sections had similar histological profiles, including fibrous or fibrolipidic intima without calcification.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Hyperglycemic</th>
<th>Nonhyperglycemic</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>19</td>
<td>32</td>
<td>0.08</td>
</tr>
<tr>
<td>Male/female</td>
<td>12/7</td>
<td>27/5</td>
<td></td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>7.33\pm2.1</td>
<td>5.29\pm0.3</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>Age, yr</td>
<td>56.2\pm9.0</td>
<td>50.8\pm9.8</td>
<td>0.06</td>
</tr>
<tr>
<td>Lumen CSA, mm$^2$</td>
<td>8.7\pm3.3</td>
<td>8.1\pm3.4</td>
<td>NA†</td>
</tr>
<tr>
<td>Plaque CSA, mm$^2$</td>
<td>3.9\pm1.9</td>
<td>3.9\pm1.8</td>
<td>0.93</td>
</tr>
<tr>
<td>Plaque burden, %</td>
<td>30.1\pm6.0</td>
<td>31.9\pm7.3</td>
<td>0.38</td>
</tr>
<tr>
<td>Stiffness index</td>
<td>52.6\pm8.0</td>
<td>21.6\pm10.8</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Values are means $\pm$ SD; $n$, no. of subjects. The cross-sectional area (CSA) and plaque burden (100 $\times$ lesion CSA/external elastic membrane CSA) were calculated at an intraluminal pressure of 100 mmHg. The stiffness index was calculated within the physiological range of pressure. NA, not applicable.

*Statistical significance, $P$ value $< 0.05$. †See discussion.
On the basis of multifactorial analysis, hyperglycemia influenced distensibility both independently and significantly ($P = 0.02$) for the entire CSA-P curve of each subject. The CSA-P curves (Fig. 2) for subject groups based on median age and hyperglycemia reflect their differences. Vessels from older individuals without hyperglycemia were significantly stiffer than those from young individuals ($10.55 \pm 4.41$ vs. $6.99 \pm 2.45$, $P = 0.01$) within physiological coronary pressure. Such a difference was not observed in the hyperglycemic group ($7.90 \pm 5.82$ vs. $7.20 \pm 3.36$, $P = 0.76$; Fig. 3). Distensibility decreased with age by about 12% per year with a higher decline in the nonhyperglycemic subjects (16% vs. 10%). The age distribution within each group is depicted in Table 2 as well as the indexes of mechanical behavior, namely, distensibility, $\beta$, and $E$. $\beta$ was higher but not significantly with hyperglycemia with or without outliers, which were taken as one order of magnitude away from the group mean ($52.6 \pm 88.0$ vs. $21.6 \pm 10.8$, $P = 0.14$, and $24.4 \pm 20.3$ vs. $21.6 \pm 10.8$, $P = 0.6$, respectively). However, when age and hyperglycemia were considered, the influence of age on $\beta$ was significant in nonhyperglycemic subjects and lacking in the hyperglycemic group (Fig. 4), as seen with distensibility. Similarly, $E$ significantly increased with age only in the nonhyperglycemic group (Fig. 5).

**DISCUSSION**

Both age and long-term glycemic state reflected by $\%$HbA1c (29) were found to significantly influence the distensibility-pressure relationship of moderately diseased regions of human LAD. A significant reduction in distensibility was observed with age within the physiological pressure range for nonhyperglycemic subjects. However, the LAD of younger hyperglycemic subjects was as stiff as the older nonhyperglycemic subjects, illustrating the impact of hyperglycemia on coronary

![Fig. 2. Relationship between lumen cross-sectional area (CSA) and pressure (P) based on hyperglycemia and median age.](http://ajpheart.physiology.org/)

![Fig. 3. Coronary distensibility based on hyperglycemia and median age. The distensibility ($D$) was calculated within physiological pressures as changes in lumen CSA ($\Delta\text{CSA}$) normalized by the initial lumen CSA (CSAi) for each pressure interval ($\Delta P$) as $D = \Delta\text{CSA}/(\text{CSAi} \times \Delta P)$.](http://ajpheart.physiology.org/)

![Table 2. Mechanical behavior of arteries from hyperglycemic and nonhyperglycemic subjects in terms of distensibility, elastic modulus, and stiffness index](http://ajpheart.physiology.org/)
distensibility. In all biomechanical indexes used, distensibility, \( \beta \), and \( E \), hyperglycemia masked the effect of aging clearly seen in the nonhyperglycemic group.

Fundamental biomechanical vascular changes with early metabolic dysfunction are likely to impact the clinical complication rates in diabetic patients as suggested by these results. Although the components of diabetic lesions do not differ greatly from those in nondiabetic patients (29), hyperglycemia could affect the inherent properties of components in the vascular walls. Several molecular and cellular changes in diabetic vessels occur in their basement membrane (5), connective tissue matrix (6), and platelet function (4). Impairment of endothelial function in nitric oxide production (31) as well as decreased smooth muscle responsiveness influences the effective arterial capability for vasodilation (4) and distension (1, 23, 27). Long-term hyperglycemia leads to nonenzymatic glycation of proteins such as collagen fibers and accumulation of the byproducts, advanced glycation end products (AGEs), which may contribute to vascular stiffness in both diabetes and aging. Recent studies have verified an increase in mean arterial compliance of older diabetic patients after using a cross-link breaker to reduce glycation (14). As a result of such findings, vascular stiffness has been attracting more attention in the study of diabetic cardiovascular disease (CVD).

Because of the difficulty of testing small coronary vessels, most detailed vascular studies have examined noncoronary/peripheral arteries as surrogates of the extent and impact of disease in the coronary tree. Arterial compliance has been reported to change in large arteries even before any evidence of complications from Type 1 diabetes (26). Direct ex vivo studies of aortic tissue in mechanical testing systems (25) and various noncoronary vessels in animals have shown a reduction of compliance with diabetes (19). Noncoronary, indirect methods, such as measuring carotid intima-media thickness with Doppler ultrasound techniques, and pulse wave velocity methods have been used to investigate vasculopathy in diabetic patients. With the use of these methods, loss of compliance (up to 20%) of various noncoronary arteries is reported with both Type 1 and 2 diabetes (2, 10, 13, 18, 21, 26). Such techniques apply to large and relatively superficial vessels with inherent limitations in studying smaller vessels, such as the coronaries. Although all evidence points to the stiffening of vascular tissue with diabetes, the data collected in a controlled manner from coronary arteries are scarce, underscoring the need for a direct and accurate investigation as attempted in this study.

The unique capability of IVUS in showing the arterial wall layers with a resolution of 125 \( \mu \)m axially and 250 \( \mu \)m laterally (22) has been utilized in vascular elasticity testing, with few studies including diabetes vasculopathy (1, 23, 27, 31–33). While showing the feasibility of coronary distensibility studies in a clinical setting, selection criteria for both subjects and lesions are varied in these studies as well as including a few diabetic patients (1, 23, 27, 31, 33). Coronary compliance has been shown to correlate negatively with lesion area. Specifically, the content, severity, and distribution of a lesion alter the local mechanical behavior of the artery (3, 12). To remove the effect of lesion distribution on the arterial wall, this study focused on the relatively homogenous concentric sections with moderate plaque burden (<50% by area). Calcified regions were specifically avoided to ensure a uniform distribution of material properties as evidenced by histology.

Because of the nonlinear viscoelasticity of arteries, using isobaric settings is essential for vascular studies (1, 12, 21, 23, 31, 33), emphasizing the benefits of local intracoronary pressure measurement. The aforementioned studies were based on the pulse pressure measured in a vessel other than the one imaged. The pressure-sensing guidewires used in this study are...
small enough to allow measurement very close to the imaged site, with a design easily allowing for future in vivo studies in both animals and humans.

Employing the IVUS imaging capability in arteries with isobaric conditions measured at the lesion site, the arterial distensibility was significantly reduced in uniform lesions from excised coronary LADs by both aging and hyperglycemia. Similar to studies of larger, noncoronary arteries, the present data showed the extent of hyperglycemia to be a significant independent factor in arterial distensibility. Within the physiological range of coronary pressure, the influence of age was even more distinct than that of hyperglycemia. Other indexes of the arterial behavior, β and E, calculated over the physiological range of pressure, also showed that hyperglycemia overshadowed the effect of age in stiffening of the arterial walls. Aging has been associated with reduced large artery (2) and coronary compliance (1, 27) as well as linked to a higher prevalence of both diabetes and CVD. Cross-linking of collagen fibers, common in both aging and diabetes, may contribute to arterial stiffness and be physically influenced by the extent of hyperglycemia, its duration, and treatment approaches.

Beyond the biomechanical changes reported in this study, higher systolic pressure has been reported in some diabetic patients (26), which would further compromise the overall distensibility range of their vessels. Therefore, the combined effects of aging, hyperglycemia, and accompanying problems such as high blood pressure impact the predisposition for CVD and treatment challenges and complications in diabetic patients. Further combined biochemical and distensibility studies would elucidate the relationship between local physiological factors such as coronary pressure, vascular wall composition, and the state of the connective tissue fibers and matrix components in arterial tissue layers, with the pathological arterial mechanisms in diabetic patients. The main limitation from a clinical standpoint is the lack of data from individual cases, which may affect the development and progression of CVD (20) including the duration, extent, and treatment of diabetes.

From the experimental perspective, the maximum pressure obtained was variable among the specimens, due to unpredictable remaining leakage from branches of the postmortem vessels. However, in this study, unlike previous ones (16, 18, 21, 25–27, 33), local pressure was measured within millimeters of the target lesion for accurate assessment of the true arterial pressure. The catheter was centrally placed within the vessel and externally secured to avoid increased local pressure due to its contact with the wall. Minor inaccuracy in plaque burden resulted because of the well-known IVUS challenge in discerning the media-intima border in images. Therefore, the reported plaque area includes media, leading to a consistent overestimation of plaque burden throughout the study.

Other studies have found that diabetic patients have smaller lumen CSA, larger plaque CSA, and similar EEM CSA compared with nondiabetic patients (17, 27). But, due to the focus on homogenous moderately diseased lesions, arterial sections in this study were not selected based on the same anatomical reference point but rather on size and distribution. This led to the variation in mean CSA shown in Table 1, which is mostly attributable to the site selection within the LAD. Therefore, no conclusions can be drawn regarding the arterial size and hyperglycemic status.

In conclusion, with a systematic study, long-term hyperglycemia as indicated by %HbA1c was found to influence distensibility of moderately occluded (<50%) atherosclerotic coronary arteries. This relationship persisted even when controlling for potential confounding factors such as age and plaque burden. Within physiological pressures, distensibility decreased significantly with age for nonhyperglycemic subjects. All measures of arterial stiffness in this study indicated a significant change with age in nonhyperglycemic subjects only. The results imply that the changes in coronary artery mechanics due to hyperglycemia occur even before the processes associated with aging and with progression of atherosclerosis in terms of increasing vascular wall thickness. These findings could ultimately impact the use of interventional techniques and devices as applied to the diabetic population through using clinically available diagnostic tools. Further detailed biochemical and distensibility studies would improve our understanding of the changes in behavior of lesions with various extents of atherosclerosis in hyperglycemic patients.

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GRANTS

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