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Female gender increases stiffness of elastic but not of muscular arteries in type I diabetic patients

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Summary
The reason for the particularly increased risk for cardiovascular complications in diabetic women is still unclear. We have previously found decreased distensibility of elastic arteries in type I diabetic women, indicating increased cardiac load, not seen in type I diabetic men, which might be one contributing factor. Whether the effect of gender is different in muscular arteries in type I diabetic patients has not been assessed. As estimates of arterial distensibility we measured stiffness ($\beta$) and pressure strain elastic modulus ($E_p$) in the muscular common femoral artery using echo-tracking sonography in 30 women (mean age 34 years, range 20–61) and 26 men (mean age 38 years, range 22–56) with type I diabetes. The results were compared with those of 89 healthy individuals of corresponding age and gender and with previously published results from elastic arteries in these patients obtained at the same occasion. The internal common femoral diameter was significantly decreased in both diabetic men and women. In sharp contrast to the highly significant decreased distensibility of the elastic abdominal aorta and common carotid artery in the type I diabetic women, the distensibility of the common femoral artery did not clearly differ between patients and controls, neither for women nor for men. Thus, the gender difference in changes of arterial distensibility found in elastic arteries was absent or far less obvious in the femoral artery. In conclusion, female gender seems to affect the mechanical properties of elastic, but not of large muscular arteries in type I diabetic patients. Thus, putative gender differences in arterial changes in type I diabetes are to be sought in elastic rather than muscular arteries.

Introduction
The risk for cardiovascular complications is particularly increased in women with diabetes (Kannel & McGee, 1979; Barret-Conner & Wingard, 1983; Lerner & Kannel, 1986). In addition, diabetic women have consistently been observed to have a worse prognosis than diabetic men after myocardial infarction (Garcia et al., 1974; Kannel, 1985; Tofler et al., 1987; Stone et al., 1989). Further, the relative risk for stroke is increased in diabetic women compared with diabetic men (Bell, 1994). The reason for this association between cardiovascular and cerebrovascular complications and female gender in diabetic patients is still unclear and inadequately explained by classical risk factors. Alterations of the mechanical properties of large arteries are increasingly recognized as a major factor for cardiovascular morbidity and mortality. Studies of the mechanical properties of arteries in type I diabetes have not been conclusive; both decreased (Pillsbury et al., 1962; Woolam et al., 1962; Christensen and Neubauer 1987; Oxlund et al., 1989; Airaksinen et al., 1993; Lambert et al., 1998, Giannattasio et al., 1999), increased (Lehmann et al., 1992) and unchanged (Kool et al., 1995; Zenere et al., 1995) distensibility have been reported. The discrepancy may partly be due to the fact that gender differences have rarely been considered. We have previously reported gender-related changes with decreased distensibility of central elastic arteries in type I diabetic women, indicating increased cardiac load, not seen in diabetic men (Ryden Ahlgren et al., 1995). In healthy subjects the mechanical properties of elastic arteries are age- (Kawasaki et al., 1987; Lanne et al., 1992a) and gender-dependent (Sonesson et al., 1993, 1994; Hansen et al., 1995). In contrast, muscular arteries, like the common femoral artery, are not clearly affected by either age (Kawasaki et al., 1987; Benetos et al., 1993; Rydén Ahlgren et al., 2001) or gender (Ryden Ahlgren et al., 2001). There is also a possibility that diabetes has different effects on muscular and elastic arteries. The aim of this study was to evaluate the mechanical properties of the common femoral artery in type I diabetic patients to clarify whether the gender-related
differences found in central elastic arteries are also present in this muscular artery.

**Materials and methods**

**Patients and control subjects**

Thirty women (mean age 34 years, range 20–61) and 26 men (mean age 38, range 22–56) with type I diabetes mellitus were investigated. Data regarding the mechanical properties of the abdominal aorta and the common carotid artery used for comparison have previously been published (Ryden Ahlgren et al., 1995, 1999). All subjects are followed-up regularly at the Department of Endocrinology at Malmö University Hospital, Sweden. The mean duration of diabetes at the time of this study was 19 years for both women and men (women: range 9–36 years, men: 9–38 years). None of the patients had a history of acute myocardial infarction, cerebrovascular events, or intermittent claudication. Moreover, none of the patients had angina pectoris or were being treated for coronary artery disease. Clinical details of the patients are given in Table 1; besides gender there were no significant differences in clinical features between women and men. One woman and four men showed clinical nephropathy (Table 1); two women were treated for hypertension with furosemide and metoprolol, respectively. Smoking was reported in 12 women and eight men. All patients gave informed consent according to the Helsinki declaration. To obtain age- and gender-matched reference values for common femoral artery diameter and distensibility, a control group of 158 healthy subjects (80 women: age range 7–81 years; 78 men: age range 8–78 years), was used (Ryden Ahlgren et al., 2001). Of these, 42 men and 47 women were in the age range 20–63 years, i.e. in the age range of the diabetic patients and these 89 subjects were used as controls in this study. None of the control subjects reported previous cardiopulmonary disease, intermittent claudication, diabetes or smoking and all were free from current medication. The ratio between ankle and brachial arterial pressure was >1 in all subjects indicating absence of obliterative atherosclerotic lesions in the arteries to the lower limb. The studies of normal subjects and diabetic patients were partly overlapping in time.

**Ultrasonic measurements of arterial diameter and distensibility**

The pulsatile changes in vessel diameter were registered with an ultrasound echo-tracking system (Diamove, Teltec AB, Lund, Sweden) (Lindström et al., 1987), interfaced with a 3-5 and a 5-MHz B-mode real time linear scanner (EUJ 240, Hitachi, Tokyo, Japan), capable of detecting vessel wall movements of less than 10 μm and with a time resolution of 1/2 ms (Lindström et al., 1987; Benthin et al., 1991). In combination with blood pressure measurements the pulsatile diameter changes form the basis for calculation of vessel wall distensibility. Details of the study technique have previously been described (Lanne et al., 1992b; Hansen et al., 1993; Sonesson et al., 1993).

All measurements were performed in a quiet room with the subject in the supine position after at least 15-min rest. An experienced ultrasound technician performed the examinations. The right common femoral artery was examined with the

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**Table 1** Demographical and clinical data in diabetic patients. All data, except frequencies, are presented as mean ± SD.

<table>
<thead>
<tr>
<th></th>
<th>Diabetic women</th>
<th>Diabetic men</th>
<th>Control women</th>
<th>Control men</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>30</td>
<td>26</td>
<td>47</td>
<td>42</td>
</tr>
<tr>
<td>Duration (years)</td>
<td>19 ± 7</td>
<td>19 ± 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165 ± 6</td>
<td>179 ± 7</td>
<td>166 ± 6</td>
<td>179 ± 5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>64 ± 9</td>
<td>76 ± 7</td>
<td>67 ± 10</td>
<td>81 ± 10</td>
</tr>
<tr>
<td>Body surface area (m²)</td>
<td>1:7 ± 0:1</td>
<td>1:9 ± 0:1</td>
<td>1:7 ± 0:1</td>
<td>2:0 ± 0:1</td>
</tr>
<tr>
<td>Body mass index (kg m⁻²)</td>
<td>23:4 ± 2:5</td>
<td>23:7 ± 2:2</td>
<td>24 ± 4:4</td>
<td>25:4 ± 3:0</td>
</tr>
<tr>
<td>Albuminurina &gt;0:5 g per 24 h</td>
<td>1</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microalbuminurina 30–300 mg per 24 h</td>
<td>5</td>
<td>3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Retinopathy

|                     |               |             |               |             |
| Background          | 11            | 8           |               |             |
| More severe than background | 3    | 4           |               |             |

HbA1c (%)           | 8·56 ± 1·76   | 7·85 ± 1·41 |               |             |
Systolic blood pressure (mmHg) | 121 ± 12    | 127 ± 14   | 117 ± 13      | 125 ± 11    |
Diastolic blood pressure (mmHg)  | 76 ± 6       | 77 ± 6     | 74 ± 8        | 79 ± 8      |
Mean arterial pressure (mmHg)     | 91 ± 7       | 94 ± 7     | 89 ± 9        | 94 ± 8      |
Mean common femoral diameter (mm) | 7·2 ± 0·65* | 8·8 ± 1·0** | 8·2 ± 0·8    | 9·7 ± 1·0   |
Common femoral artery strain (%) | 6·7 ± 3·5    | 6·0 ± 3·2  | 6·1 ± 2·4     | 5·6 ± 2·1   |
Common femoral artery stiffness | 9·2 ± 4·8    | 10·7 ± 5·8 | 8·6 ± 3·2    | 9·8 ± 4·7   |
Common femoral artery Ep (10⁵ N m⁻²) | 1·2 ± 0·7*** | 1·4 ± 0·8  | 1·1 ± 0·4  | 1·3 ± 0·6   |

*P < 0·00001 versus control women, **P = 0·002 versus control men, ***P = 0·04 versus control women.
5 MHz transducer and measurements were performed at the inguinal fossa with the hip joint as a landmark. The artery was visualized in the longitudinal section. Care was taken to minimize the pressure of the transducer to the skin. From each registration a sequence of at least five representative consecutive diameter cycles was manually chosen and the femoral artery diameters and the diameter changes calculated as mean of the selected diameter cycles. Blood pressure was measured by the auscultatory method using a sphygmomanometer on the upper arm immediately after measurement of the pulsatile common femoral artery diameter changes. Mean arterial blood pressure (MAP) was taken as the diastolic pressure plus one-third of the pulse pressure. Each vessel was examined three times with calculation of mean diameter, strain, stiffness (\(\beta\)), and the pressure strain elastic modulus (\(E_p\)) from the corresponding diameters, pulsatile diameter changes and blood pressures. The average of the values for each subject was then calculated.

The arterial strain, or the fractional diameter change, was defined as:
\[
\text{Strain} = \frac{D_{\text{systolic}} - D_{\text{diastolic}}}{D_{\text{diastolic}}}
\]
where \(D_{\text{systolic}}\) and \(D_{\text{diastolic}}\) are the end-systolic and end-diastolic diameters.

The mean vessel diameter was defined as \((D_{\text{max}} + D_{\text{min}})/2\).

Distensibility characteristics of large arteries depend on the extent to which they are expanded, the vessels being very distensible at low pressures and small diameters, but gradually stiffer with increasing pressure and diameter. Thus, there is a non-linear pressure–diameter relationship of the arterial wall. It was observed that the relation between the logarithm of relative pressure and strain (fractional diameter change) was linear in vitro. Based on this logarithmic relation, and thus independent of pressure in the physiological range, the index stiffness (\(\beta\)) was established as an estimate of vascular distensibility. It was later modified and used in vivo by Kawasaki et al. (1987). This index has been used in this study and is expressed as:
\[
\text{Stiffness (}\beta\text{)} = \frac{\ln\left(P_{\text{systolic}}/P_{\text{diastolic}}\right)}{(D_{\text{systolic}} - D_{\text{diastolic}})/D_{\text{diastolic}}}
\]
where \(P_{\text{systolic}}\) and \(P_{\text{diastolic}}\) are the systolic and diastolic blood pressure levels in mmHg.

We also calculated \(E_p\), defined according to Peterson et al. (1960) as:
\[
E_p = K \times \frac{P_{\text{systolic}} - P_{\text{diastolic}}}{(D_{\text{systolic}} - D_{\text{diastolic}})/D_{\text{diastolic}}}
\]
\(E_p\) is measured in N m\(^{-2}\). \(K = 133\,3\) is the factor for converting mmHg to N m\(^{-2}\).

Using this technique the intraobserver variability for repeated measurements have previously, at this laboratory, been shown to be 15% for the pulsatile diameter change, 5% for the mean diameter and 18% for stiffness (\(\beta\)) and \(E_p\) for the vessel investigated (Hansen et al., 1993).

**Statistics**

Unpaired t-test or analyses of covariance were used to compare the vessel diameters, strain, stiffness and \(E_p\) in diabetic men and women with control subjects in corresponding age ranges. The effects of age, duration and HbA1c on the studied parameters were tested by correlation analysis (Pearson or Spearman). Differences between smoking and non-smoking patients and differences in clinical characteristics (HbA1c, blood pressure) were statistically tested with Mann–Whitney U-test. Multiple regression analysis, correcting for age, were used to sought correlations between the arterial indices and blood pressure. Statistical significance was defined as \(P<0.05\). Data are presented as the mean value ± SD, unless otherwise stated.

**Results**

Blood pressures in the diabetic patients did not differ significantly from those in the control group of corresponding age, either for women or for men (Table 1). There was no significant difference in HbA1c levels between diabetic men and women (Table 1). Body mass index (BMI) was slightly less in diabetic patients than in controls (Table 1).

**The common femoral artery diameter**

The internal common femoral artery mean diameter (Table 1) was significantly decreased in both diabetic men and diabetic women compared with the corresponding control subjects (men: \(P = 0.002\), women: \(P<0.00001\)). The difference in diameter between diabetic patients and controls could not be explained by differences in body surface area. No increase in diameter with ageing could be detected in either diabetic men or diabetic women. No significant correlation between the common femoral artery diameter and the duration of diabetes or the HbA1c levels, respectively, could be detected.

**The common femoral artery strain**

There were no significant differences in strain in the common femoral artery between men with type I diabetes and control men (\(P = 0.5\)) or between women with type I diabetes and control women (\(P = 0.3\)). Further, no significant correlation between strain and age was seen (diabetic men \(r = -0.21\), \(P = 0.3\); control men \(r = -0.14\), \(P = 0.4\); diabetic women \(r = -0.32\), \(P = 0.09\); control women, \(r = 0.18\), \(P = 0.2\)).

**The common femoral artery stiffness (\(\beta\))**

Figure 1a shows stiffness (\(\beta\)) of the common femoral artery in men with type I diabetes and in control men. There was no significant difference in stiffness between diabetic men and control men (\(P = 0.5\)) and no correlation between stiffness and age was seen (diabetics \(r = 0.08\), \(P = 0.7\); controls \(r = 0.15\), \(P = 0.4\)).
Figure 1b shows stiffness (β) of the common femoral artery in women with type I diabetes and in control women. In diabetic women a significant positive correlation between age and stiffness was seen (r = 0.45, P = 0.01), not detected in control women (r = 0.20, P = 0.2). In agreement, the difference in slope between diabetic women and control women reached significance (P = 0.046) and, in fact, the level of Ep tended to be increased in diabetic women, although the difference was low (P = 0.04).

The arterial indices and clinical characteristics

The strain, stiffness and Ep values in the few patients with nephropathy and/or proliferative retinopathy, did not differ from those without complications and our results were the same if these patients were excluded. No significant correlation between common femoral artery strain, stiffness (β) or Ep and the duration of diabetes could be detected in either men or women. Further, no correlation between strain, stiffness or Ep and HbA1c could be detected. Using multiple regression analyses, correcting for age, no correlation between the arterial indices and blood pressure, analysed as MAP, systolic and diastolic pressure, was seen. No significant differences in strain, stiffness or Ep between smoking and non-smoking patients for either women or men could be detected.

The arterial pressure strain elastic modulus (Ep)

As for stiffness (β), there was no significant difference in Ep between men with type I diabetes and control men (P = 0.4) and similarly there was no correlation between Ep and age (diabetic men r = 0.11, P = 0.6; control men r = 0.17, P = 0.3).

As for stiffness (β), in diabetic women there was a correlation between age and Ep (r = 0.48, P = 0.007), not seen in control women (r = 0.20, P = 0.2). In agreement, the difference in slope between diabetic women and control women reached significance (P = 0.046) and, in fact, the level of Ep tended to be increased in diabetic women, although the difference was low (P = 0.04).

Comparison between the common femoral artery and the elastic arteries

Figure 2 shows schematically common femoral artery stiffness (β) data in diabetic women and men in comparison with previously published results from the abdominal aorta and the common carotid artery in the same patients examined at the same occasion (Ryden Ahlgren et al., 1995). The clear increase in stiffness (β) in the abdominal aorta (mean 64%) and the common carotid artery (mean 22%) in diabetic women is in sharp contrast to the lack of increase in stiffness (β) in the common femoral artery in the same diabetic women.
Discussion

Alterations of the mechanical properties of large arteries are increasingly recognized as an important factor for cardiovascular morbidity and mortality. In a previous study of patients with type I diabetes (Ryden Ahlgren et al., 1995) we reported gender-related changes with decreased distensibility of the elastic abdominal aorta and the elastic common carotid artery in diabetic women compared with control women, whereas in diabetic men no difference compared with control men was seen, thus indicating increased cardiac load in diabetic women. We have now evaluated the muscular common femoral artery in the same patients, examined at the same occasion as the elastic arteries, i.e. with the same duration and metabolic control, to clarify whether the gender-related differences in changes of arterial distensibility are also present in the muscular common femoral artery.

In agreement with our findings from the abdominal aorta in the same patients (Ryden Ahlgren et al., 1995) we found the common femoral artery mean internal diameter to be decreased in both diabetic men and women. This is in agreement with a previous study (Christensen & Neubauer, 1988), but in contrast to Kool et al. (1995), who did not find a decreased common femoral artery diameter in type I diabetic patients. The patient material in the latter study was, however, small and the duration of diabetes was shorter. Our finding of a decreased inner vessel diameter suggests a thicker vessel wall in the diabetic patients than in the control subjects. Intima-media thickness has been shown to be increased in several studies of arteries in diabetic patients (Yamasaki et al., 1994; Frost & Beischer, 1998; Giannattasio et al., 1999). Another possible explanation for found decreased diameter might be an effect of reduced vasodilatory function in type I diabetic patients, which has been described for the common femoral artery (Zenere et al., 1995).

In sharp contrast to the findings from the abdominal aorta, where a 60–70% increase in stiffness (β) was noted in diabetic women, no clear-cut differences in distensibility of the common femoral artery were seen in either diabetic women or men compared with control subjects. Accordingly, the pronounced gender-related differences in changes of arterial distensibility, found in the central elastic arteries in these patients, are far less obvious or absent in the common femoral artery, indicating that the diabetic state has a different influence on elastic and muscular arteries in type I diabetic women, probably because of differences in histological and histochemical structure. The common femoral artery is a predominantly muscular artery with large content of smooth muscles, while the abdominal aorta and the common carotid are predominantly elastic arteries with a large content of elastin and collagen and only sparse smooth muscle cells.

Studies of the mechanical properties of the common femoral artery in type I diabetic patients are sparse and putative gender differences have not been evaluated. Kool et al. (1995), in a study of 30 type I diabetic patients, found the distensibility of the femoral artery to be decreased. Zenere et al. (1995), however, in a smaller number of patients, including those with microalbuminuria, did not find changes in the mechanical properties of the femoral artery, thus supporting our findings.

Decreased distensibility of central arteries increases central pulse pressure and left ventricular load (Nichols & O’Rourke, 1998). Thus, if further confirmed, our finding of a gender difference with more decreased distensibility in central arteries in diabetic women than in diabetic men, is likely to be an important factor for the particularly increased risk for cardiovascular complications in diabetic women, including their poor prognosis after myocardial infarction with increased incidence of congestive heart failure, reinfarction and death (Garcia et al., 1974; Kannel, 1985; Tofler et al., 1987). Studies regarding putative gender-related differences in arterial changes in diabetic patients are few. Our findings of gender-related differences in changes of arterial distensibility in elastic arteries in type I diabetic patients have, however, support from Hu et al. (1997) in a study of adolescents with type I diabetes. Further, recently, Lambert et al. (1998) reported female gender to be a determinant of decreased distensibility of the common carotid artery in type I diabetic patients. Indeed, gender-related differences have also been reported in type II diabetic patients with findings of increased aorto-femoral pulse wave velocity in women, but not in men (Lehmann, 1996). These studies are all based on findings from predominantly elastic arteries, thus supporting our initial finding. The present study with no clear cut gender-related changes in the common femoral artery, despite clear gender-related changes in the central elastic arteries, thus shows that gender-related changes in type I diabetic patients can be present in one type of arteries, but not in others. Further, our study shows that putative major gender-related differences in arterial changes in type I diabetes are to be sought in central arteries rather than in large muscular arteries. As a factor for cardiac load the elastic arteries are also those of the most importance.

Measures of arterial distensibility or stiffness have been proposed as surrogate markers for atherosclerosis (Arnett et al., 1994; Lehmann et al., 1996), at least in central arteries. At present the putative relation between early atherosclerosis and changes in the mechanical properties of the arterial wall are, however, far from clear (Nichols & O’Rourke, 1998). In this study we found the common femoral inner diameter to be decreased in both diabetic men and women, which might be due to early atherosclerosis. Thus, our finding that the mechanical properties of the common femoral artery, a vessel prone to atherosclerotic disease, are not clearly affected in patients with long-term type I diabetes, may seem surprising. It is well known that the structure of arterial walls change with ageing with an increase in collagen and thickening of the vessel wall and diabetic patients have in several studies been shown to have increased intima-media thickness (Yamasaki et al., 1994; Frost & Beischer, 1998; Giannattasio et al., 1999), a factor likely to increase vessel wall stiffness. This leads to the hypothesis that the smooth muscle within the common femoral artery has a
larger impact on vessel wall mechanics than age- and putative diabetes-related histological changes per se. Further, it gives support to the opinion that there is no clear relation between atherosclerosis and vessel wall motion. One must, however, keep in mind that in this study all patients investigated were without clinical signs of vascular disease and the vast majority of the patients were free of diabetic late complications. Further, although the relation between femoral artery distensibility and atherosclerosis is not clear, it might still be possible that our finding of a decrease in femoral artery distensibility with age in diabetic women, but not in men also indicates an increased vulnerability related to gender in the common femoral artery.

In several studies the arterial wall properties of central elastic arteries in healthy, non-diabetic subjects have been shown to differ between men and women. This suggests that these differences may result from hormonal influence. In contrast, in the common femoral artery, a large muscular artery, gender- and age-related changes, seem to be sparse or lacking (Ryden Ahlgren et al., 2001). Thus, our finding that the normal gender-related differences in arterial distensibility of the common carotid artery and the abdominal aorta are absent in patients with type I diabetes, whereas no clear cut differences in changes of the mechanical properties of the common femoral artery are seen, favours the assumption that the sex hormonal balance is affected in type I diabetic women. Indeed, women with type I diabetes have recently been shown to have a high prevalence of hyperandrogenic disorders (Escobar-Morreale et al., 2000). Future studies will show whether an altered sex hormone balance contributes to the alterations in arterial distensibility seen in type I diabetic women.

In attempting to measure indices of arterial distensibility the arterial diameter change and the intra-arterial blood pressure ideally should be measured simultaneously at the same location, as it is well known that the arterial pressure waves undergo transformation in the arterial tree. Although widely used, the method of using the auscultatory blood pressure in the upper arm, when calculating distensibility indices for, e.g. the femoral artery, may be questioned. Comparison between intra-arterial pressure in the common femoral artery and the brachial blood pressure obtained by the auscultatory method in healthy subjects has, however, shown that the pulse pressure is systematically underestimated when the auscultatory method is used without obvious differences between gender or between young and elderly (Ryden Ahlgren et al., 2001). Hence, our use of auscultatory blood pressure cannot explain our findings.

In conclusion, this study shows that the internal common femoral diameter significantly decreases in both type I diabetic men and women without clinical signs of vascular disease. Further, it shows that female gender has a major effect on the mechanical properties of elastic, but not on muscular arteries in type I diabetic patients. Accordingly, the obvious gender-related differences found in elastic arteries with decreased arterial distensibility in women, but not in men, are far less evident or absent in the muscular common femoral artery. This indicates that the metabolic state has different effects on elastic and muscular arteries in diabetic women. Further, it suggests that putative gender-related differences in arterial changes in type I diabetes are to be sought in central elastic arteries rather than in muscular arteries, which are also of most importance for cardiac load.

Acknowledgements

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