Endothelial Function in Marfan Syndrome
Selective Impairment of Flow-Mediated Vasodilation

Dirk G. Wilson, MB, MRCP (UK); Michael F. Bellamy, MB, MRCP (UK);
Mark W. Ramsey, BM, MRCP (UK); Jonathan Goodfellow, MB, MRCP (UK);
Moira Brownlee, RGN; Sally Davies, MA, MRCP (UK); John F. Wilson, BSc, PhD;
Malcolm J. Lewis, MB, DSc; A. Graham Stuart, MB, MRCP (UK)

Background—The cardiovascular complications of Marfan syndrome arise due to alterations in the structural and functional properties of fibrillin, a constituent of vascular connective tissues. Fibrillin-containing microfibrils are closely associated with arterial endothelial cells, indicating a possible functional role for fibrillin in the endothelium. Plasma concentrations of endothelial cell products are elevated in Marfan subjects, which indirectly indicates endothelial dysfunction. This study directly assessed flow- and agonist-mediated endothelium-dependent brachial artery reactivity in Marfan subjects.

Methods and Results—In 20 Marfan and 20 control subjects, brachial artery diameter, blood flow, and blood pressure were measured by ultrasonic wall tracking, Doppler ultrasound, and photoplethysmography, respectively. Measurements were taken during hand hyperemia (a stimulus for endothelium-derived nitric oxide [NO] release in the upstream brachial artery) and after sublingual administration of the endothelium-independent vasodilator nitroglycerin. In 9 Marfan and 6 control subjects, the above parameters were also assessed during intra-arterial infusions of acetylcholine and bradykinin (agonists that stimulate NO production) and NG-monomethyl-L-arginine (L-NMMA, an inhibitor of NO production). Flow-mediated responses differed markedly between Marfan and control subjects (−1.6±3.5% versus 6.50±4.1%, respectively; \( P<0.0001 \)), whereas nitroglycerin produced similar vasodilation (14.2±5.7% versus 15.2±7.8%; \( P=NS \)). Agonist-induced vasodilation to incremental intra-arterial infusions of acetylcholine and bradykinin were not significantly different between Marfan and control subjects, and intra-arterial L-NMMA produced similar reductions in brachial artery diameter in both groups.

Conclusions—These data demonstrate impaired flow-mediated but preserved agonist-mediated endothelium-dependent vasodilation in Marfan subjects and suggest preservation of basal NO release. Selective loss of flow-mediated dilation suggests a role for fibrillin in endothelial cell mechanotransduction. (Circulation. 1999;99:909-915.)

Key Words: Marfan syndrome ■ endothelium ■ arteries ■ endothelium-derived factors

Marfan syndrome is a hereditary connective tissue disorder caused by mutations of the gene encoding fibrillin, a complex glycoprotein.1–2 In addition to its important role in establishing and maintaining the integrity of elastic fibers within connective tissues,3,4 fibrillin also has an independent structural role.5,6 The cardiovascular complications of Marfan syndrome, including aortic root dilatation and dissection, are thought to result from alterations in the elastic properties of the fibrillin-rich aortic tunica media.7,8 Fibrillin-containing microfibrils have also been noted in abundance in the connective tissue matrix immediately subjacent to arterial endothelial cells.9–11 Although the role of subendothelial fibrillin is unclear, its close association with endothelial cells may indicate a functional as well as a structural role.

Endothelial cells continuously release nitric oxide (NO), which is synthesized by the endothelial isoform of NO synthase (NOS-III).12,13 NOS-III activity is stimulated by chemical agonists such as serotonin, acetylcholine, and bradykinin14–16 and by flow-induced shear forces on the endothelial cell wall.17–19 Endothelium-derived NO diffuses toward the underlying vascular smooth muscle, producing relaxation. The vascular endothelium, therefore, plays a central role in the modulation of arterial smooth muscle tone and thus influences large-artery distensibility and the mechanical performance of the cardiovascular system.20 Subjects with Marfan syndrome have elevated plasma levels of the endothelial cell products factor VIII (von Willebrand factor) antigen and thrombomodulin, providing
TABLE 1.  Subject Characteristics

<table>
<thead>
<tr>
<th></th>
<th>MFS Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (male)</td>
<td>20 (13)</td>
<td>20 (13)</td>
</tr>
<tr>
<td>β-Blocked: non-β-blocked</td>
<td>10:10</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>22 ± 10</td>
<td>22 ± 10</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.82 ± 0.15</td>
<td>1.73 ± 0.12</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>70.1 ± 12.4</td>
<td>67.3 ± 17.1</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>21.2 ± 2.3</td>
<td>22.5 ± 3.7</td>
</tr>
<tr>
<td>Blood samples (fasting)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, † mg/dL</td>
<td>142 ± 33</td>
<td>147 ± 32</td>
</tr>
<tr>
<td>Triglyceride, ‡ mg/dL</td>
<td>122 ± 57</td>
<td>89 ± 53</td>
</tr>
<tr>
<td>Glucose, § mg/dL</td>
<td>83 ± 13</td>
<td>82 ± 10</td>
</tr>
<tr>
<td>Total homocysteine, mmol/L</td>
<td>8.3 ± 2.6</td>
<td>8.6 ± 3.1</td>
</tr>
</tbody>
</table>

MFS indicates Marfan syndrome.

Data are mean ± SD. There were no significant differences between the Marfan and control groups (n=19 in both groups; 1 Marfan subject aged 11 and 1 control subject aged 14 declined to give blood samples). To convert values for cholesterol, triglyceride, and glucose to mmol/L, multiply by 0.0298, 0.0144, and 0.0556, respectively.

indirect evidence of endothelial dysfunction in this condition. More direct quantification of endothelial function in Marfan syndrome is lacking. The aim of the present study was to assess flow-mediated and agonist-mediated endothelium-dependent vasodilation in otherwise healthy subjects with Marfan syndrome.

Methods

Marfan Subjects

Twenty subjects aged 11 to 44 years (22 ± 10 years) were recruited from patients under regular follow-up at the institutions participating in the study. All satisfied clinical criteria for Marfan syndrome. Seven of the subjects came from 3 families with established Marfan syndrome pedigrees, and the remaining 13 had no known affected first-degree relatives. Echocardiographic data obtained within 6 months of the study were available on each subject, and all were known to have aortic root dilatation as described by Roman et al. No subjects had ventricular dysfunction or significant valvar regurgitation. All Marfan subjects were clinically well, but because of aortic root dilatation, 9 were taking β-blockers and 1 was taking a β-blocker/enalapril combination. Three Marfan subjects were taking 1 of the following: combined oral contraceptive pill, carbamazepine, and fluoxetine. Some, but not all, subjects had obvious phenotypic features of Marfan syndrome, and therefore, complete blinding was not possible. However, the observers in the present study were not informed whether subjects were cases or controls.

Control Subjects

Twenty age- and sex-matched healthy control subjects were recruited from 3 sources: healthy blood donors, employees known to our institutions or their family members, and patients with primary cardiac arrhythmias who were off therapy in preparation for electrophysiological investigation.

Exclusions

Subjects were not recruited if there was a history of smoking within the previous 4 years or if there was a history of hypertension (blood pressure >160/90 mm Hg in adults or >95th percentile in children), diabetes, hypercholesterolemia (cholesterol >240 mg/dL), hyperhomocysteinemia (serum homocysteine >16 μmol/L), or ischemic heart disease. Potential Marfan subjects were not recruited if there was a previous history of aortic valve or root surgery or if orthopedic problems prevented the adoption of a comfortable supine position.

All adult participants gave informed written consent. In the case of minors, written consent was obtained from 1 parent. The study met with the approval of the appropriate ethics committee.

Study Design

The study was divided into 2 phases: noninvasive and invasive. In the noninvasive phase, changes in end-diastolic brachial artery diameter were measured in response to (1) endothelium-dependent flow-mediated dilution induced by hand hyperemia and (2) endothelium-independent vasodilation induced by administration of sublingual nitroglycerin. In the invasive phase, changes in end-diastolic brachial artery diameter were measured in response to intra-arterial infusions of (1) the endothelium-dependent vasodilators acetylcholine and bradykinin and (2) the NOS-III antagonist Nω-monomethyl-L-arginine (L-NMMA). The ethics committee permitted subjects aged ≥16 years to participate in the invasive phase. Twelve subjects were eligible; of these, 9 (aged 29 ± 10 years, 6 males) agreed to take part, 4 of whom were taking β-blockers. Six subjects (aged 29 ± 9 years, 4 males) from the control group agreed to participate in the invasive phase.

Phase 1: Noninvasive Assessment of Flow-Mediated Vasodilation

Subjects lay supine in a temperature-controlled room (21°C to 23°C) with their nondominant arm outstretched on a pneumatic cushion. Using well-described and validated techniques, we measured brachial artery end-diastolic diameter using high-resolution vessel wall tracking (Vadirec; Medical Systems; resolution ≥3 μm), blood pressure by finger photoplethysmography (Finapres; Ohmeda), and blood flow by continuous-wave Doppler ultrasound, derived from the mean velocity-time integral corrected for Doppler angle and internal brachial artery diameter (Dopstation; SciMed). Measurements were made (1) at baseline (after 15 minutes of supine rest), (2) after 60 seconds of hand hyperemia (produced by releasing a pneumatic wrist cuff inflated for 5 minutes 50 mm Hg above systolic blood pressure), and (3) 3 minutes after 400 μg of sublingual nitroglycerin (following the return of hemodynamic variables to the resting state, after ≥10 minutes). At the end of the study, blood was drawn for analysis from all but 2 subjects (1 young subject each from the Marfan and control groups declined venipuncture).

Phase 2: Intra-Arterial Infusion of Acetylcholine, Bradykinin, and L-NMMA

At a separate visit, 9 Marfan and 6 control subjects lay supine with their nondominant arm outstretched on a pneumatic cushion. With the use of 1% lidocaine local anesthesia, a 24-gauge cannula (Venisystems Abbocath-T; Abbott) was inserted percutaneously into the brachial arterial 2 to 5 cm proximal to the antecubital fossa. Brachial artery end-diastolic diameter, blood flow, and blood pressure were measured noninvasively distal to the puncture site in the manner described in phase 1, during the following sequential infusions: (1) 0.9% saline (with added heparin, 1 U/mL; Baxter Healthcare) for ≥15 minutes to allow vessel stabilization; (2) acetylcholine (Miochol; Ciba Vision Ophthalmics) given in incremental doses (7.5, 15, 30, and 60 μg/min), with brachial artery parameters measured 5 minutes after each increment (end points for cessation of infusion: visible limb hyperemia or completion of the infusion regimen); (3) 0.9% saline (with added heparin, 1 U/mL) until resting hemodynamic state returned (15 to 30 minutes); (4) bradykinin (Clinalfa AG) given in incremental doses (0.1, 0.2, 0.4, and 0.8 μg/min), with measurements recorded 5 minutes after each increment (end points for cessation of infusion: visible limb hyperemia or completion of the infusion regimen); (5) 0.9% saline (with added heparin, 1 U/mL) until resting hemodynamic state returned (15 to 30 minutes); and (6) L-NMMA (Clinalfa AG; 3 mg/min) with measurements recorded after 20 minutes of infusion.
The drug solutions were freshly prepared by use of an aseptic technique in a sterile environment and were administered with a P3000 infusion pump (Wellmed). The rate of intra-arterial infusion was maintained at 0.5 mL/min throughout the study. After the infusion regimen was completed, the cannula was removed and gentle pressure was applied over the brachial artery for 15 minutes to ensure hemostasis.

**Blood Samples and Assays**

Serum cholesterol and glucose were analyzed by use of a Hitachi 747 AutoAnalyzer. Cotinine concentrations were determined by a competitive microplate immunoassay (STC Diagnostics). Total plasma homocysteine was measured by high-performance liquid chromatography with SBD-F (ammonium 7-fluoro-oxa-1,3-diazole-4-sulfonate) derivatization.25,26

**Statistical Analysis**

In phase 1, between-group results were compared by use of unpaired t tests. ANOVA analyses were used to compare hemodynamic parameters in Marfan subgroups treated and not treated with β-blockers.

In phase 2, the infusion sequence was terminated when limb hyperemia was visible. With acetylcholine, this frequently occurred at doses >15 μg/min and for bradykinin at doses >0.2 μg/min. Therefore, only responses below these doses were analyzed. Because some subjects exhibited hyperemia after the lowest dose of both agonists, these subjects could not be included in the analysis for the 15 and 0.2 μg/min doses for acetylcholine and bradykinin, respectively. Nine Marfan and 6 control subjects received the lowest dose of each agonist. Eight Marfan and 6 controls received 15 μg/min acetylcholine and 6 Marfan and 5 controls the 0.2 μg/min dose of bradykinin. Analyses of these data were performed by univariate repeated-measures ANOVA. An unpaired t test was used to compare between-group responses of the nonincremental infusion of L-NMMA.

Descriptive data are expressed in the text and tables as group mean (±SD) and shown in the figures as group mean (±SE). A value of P<0.05 was considered statistically significant.

**Results**

There were no significant differences in age, sex, height, weight, body mass index, or biochemical variables between the Marfan and control groups (Table 1). Although all subjects denied a history of active or heavy passive smoking, 1 subject in the Marfan group (male, aged 14, taking β-blocker treatment) and 1 subject in the control group (male, aged 11) had cotinine levels of 147 and 224 ng/mL, respectively, suggesting that they were active smokers. Cotinine aged 11) had cotinine levels of 147 and 224 ng/mL, respectively, suggesting that they were active smokers. Cotinine levels were <25 ng/mL in all other Marfan and control subjects. In phase 1 of the study, basal brachial artery end-diastolic diameters and basal hemodynamic variables were similar in Marfan and control groups, and hemodynamic parameters did not change significantly during interventions (Table 2). In phase 2 of the study, small differences were observed in basal heart rate and blood pressure measurements between the Marfan and control subjects. However, only the heart rate differences reached statistical significance, and this could probably be accounted for by β-blocker treatment in 4 of the Marfan subjects. Basal brachial artery end-diastolic diameters were similar between interventions, and hemodynamic variables did not change significantly within these groups during interventions (Table 3). In phase 1, flow-
mediated endothelium-dependent vasodilation was significantly impaired in Marfan subjects compared with control subjects ($P<0.0001$; Figure 1), whereas the endothelium-independent response to nitroglycerin was similar. In phase 2, both endothelium-dependent agonists produced significant increases in brachial artery end-diastolic diameter from baseline in the Marfan and control groups ($P<0.05$; Figure 2). There was no significant difference in diameter for either acetylcholine ($P>0.6$) or bradykinin ($P>0.7$) between the 2 groups (Table 3). Analyses indicated that there was no significant interaction between either drug dose and the study groups ($P>0.3$). L-NMMA produced a 3% reduction in mean end-diastolic diameter in both Marfan and control subjects ($P=NS$; Figure 2).

**Adverse Effects**

In phase 1, no adverse effects were reported. In phase 2, 1 control subject experienced an urticarial reaction confined to the arm during the infusion of bradykinin, which resolved on cessation of the infusion. In addition, 1 Marfan subject complained of mild elbow discomfort 4 days after the study, which resolved spontaneously.

**Discussion**

The major finding of this study is that flow-mediated endothelium-dependent vasodilation is impaired in Marfan syndrome, whereas agonist-mediated endothelium-dependent vasodilation is preserved. These results provide support for in vitro evidence of different NO release mechanisms from endothelial cells in response to different stimuli. L-NMMA produced arterial vasoconstriction in Marfan subjects equivalent to control subjects, suggesting preservation of basal NO activity.

$\beta$-Blockers prevent cardiovascular complications in Marfan syndrome. All 20 Marfan subjects in this study had aortic root dilatation; 10 of these subjects were taking atenolol. Withdrawal of therapy, even for a short period, was considered unwise, and therefore subjects taking atenolol were included. $\beta$-Blockers are known to produce changes in the composition of the arterial wall and arterial compliance, and their use by some Marfan subjects may have influenced the results. However, flow-mediated endothelium-dependent brachial artery responses were similarly impaired in $\beta$-blocker– and non–$\beta$-blocker–treated groups and there was no change in hemodynamic variables in either group during any intervention.

Abnormalities of endothelium-dependent arterial reactivity are widely believed to be early markers of arteriosclerosis; impaired flow-mediated vasodilation has been described in hypercholesterolemia, hypertension, diabetes mellitus, and hyperhomocysteinemia, all of which were excluded in the subjects participating in the present study. Active and passive exposure to tobacco smoke are also known to impair flow-mediated vasodilation and subjects were excluded from the present study on the basis of their smoking history. However, 1 subject from each study group had elevated serum cotinine levels. The results from these subjects were not excluded from analysis because this did not alter the overall findings, and neither participated in phase 2 of the study.

Loss of endothelium-dependent vasodilation has previously been reported to follow a hierarchical course, with loss of agonist-mediated responses preceding flow-mediated responses. The observation reported in the present study of selective loss of flow-mediated endothelium-dependent vasodilation with preservation of agonist-mediated vasodilation is unique in human subjects. However, similar findings have been reported in hypercholesterolemic rabbits. Although only 9 Marfan and 6 control subjects participated in phase 2 of the study, the Marfan subjects showed similar responses to the control subjects after acetylcholine and bradykinin infusion. It is unlikely, given the low level of statistical signifi-
cance that these findings would differ if a larger subgroup were studied. Moreover, any relaxant response to agonists in Marfan subjects is remarkable given the degree of abnormality in the flow-mediated responses.

Stimulation of normal endothelial cells by receptor-dependent agonists or by shear stress elevates intracellular calcium ion concentrations, which leads to increased NOS-III activity and NO release. Agonist-stimulated release is mediated by phosphatidylinositol hydrolysis via membrane-bound phospholipase C, but the mechanisms underlying flow-mediated NO release (mechanotransduction) are less well understood. The preserved vasodilatory responses to agonist-stimulated NO release and the preserved vasoconstrictive response to NOS-III antagonism in Marfan syndrome suggest that the abnormal response to increased flow lies at the level of mechanotransduction rather than resulting from an abnormality of NO synthesis or diffusion.

Endothelial cell signal transduction may be mediated in part by the luminal glycocalyx, but mechanotransduction may also be dependent on the conduction of physical forces through the cell to the abluminal surface. The mechanical forces to which endothelial cells are subjected must be balanced by equal and opposite resistant forces. The latter are produced by the intracellular cytoskeletal network and are distributed as mechanical stresses to the anchoring subendothelial extracellular matrix via highly specialized focal adhesion sites. Membrane-bound integrins at these sites attach intracellular cytoskeletal elements to structural components in the extracellular matrix. If endothelial cells lacked inherent tension or had inadequate external attachments, their perception of externally applied forces might be distorted. In the isolated rabbit aorta, chemical disruption of the endothelial cytoskeleton results in impaired flow-mediated endothelium-dependent vasodilation, with preservation of agonist-mediated endothelium-dependent NO release. Fibrillin-containing microfibrils in the subendothelial matrix are produced by and are closely associated with arterial endothelial cells. Furthermore, there is preliminary evidence that fibrillin binds with integrin and with other structural components in the subendothelial extracellular matrix.
unpublished data, 1997). We hypothesize that disordered fibrillin disrupts the normal association between the endothelial cytoskeletal network and the structural components of the extracellular matrix. This may interfere with normal cell attachment and the ability of endothelial cells to adequately sense and resist external forces, thereby preventing normal mechanotransduction.

The potential clinical consequences of impaired flow-mediated endothelium-dependent vasodilation deserve consideration. The elastic components of large arteries (collagen, elastin, and smooth muscle) play an important role in converting pulsatile cardiac ejection into virtually constant tissue perfusion. Loss of flow-mediated endothelium-dependent vasodilation may reduce large-artery distensibility, increase cardiac workload, and increase pulse wave velocity, thereby subjecting the proximal aorta to larger and earlier reflected wave pressures.20,32 In addition, because fibrillin abnormalities alter the structural integrity of elastin,3,4 the ability of the aorta to withstand the increased wall stress may be impaired, predisposing it to aortic root dilatation.

Conclusions
Selective loss of flow-mediated endothelium-dependent vasodilation is described in subjects with Marfan syndrome and aortic root dilatation. The possibility that fibrillin may play a role in endothelial cell attachment and mechanotransduction of flow-induced NO release has implications for large-artery distensibility and the pathogenesis of arterial disease in Marfan syndrome.

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