Arterial Stiffness and Endothelial Function in Patients With β-Thalassemia Major

Y.F. Cheung, MBBS; Godfrey C.F. Chan, MD; S.Y. Ha, MBBS

Background—Increased iron store has been linked to risk of cardiovascular disease. Structural alterations of arteries in β-thalassemia major patients and in vitro functional disturbance of vascular endothelial cells by thalassemic serum have been described. We sought to determine whether arterial stiffness and endothelial function are altered in vivo.

Methods and Results—Thirty thalassemia patients (16 male) aged 22.2±7.4 years were recruited. Left ventricular (LV) mass and function were assessed echocardiographically. Carotid and brachial artery stiffness was assessed by stiffness index and pulse-wave velocity (PWV), respectively. Brachial artery endothelial function was assessed by vascular response to reactive hyperemia (flow-mediated dilation [FMD]) and sublingual glyceryl trinitrate. These indexes were compared with those of 30 age- and sex-matched controls. None of the patients had LV systolic or diastolic dysfunction. When compared with controls, patients had greater absolute (113.8±38.0 versus 109.0±32.6 g, P=0.04) and indexed (82.4±17.5 versus 66.7±12.7 g/m², P<0.001) LV mass, carotid artery stiffness index (8.1±3.5 versus 5.5±1.6, P<0.001), and brachial PWV (8.9±2.4 versus 7.9±1.7 m/s, P=0.03). Their FMD was impaired (3.5±3.3% versus 8.8±3.9%, P<0.001), whereas glyceryl trinitrate–mediated dilation was preserved (17.9±7.6% versus 16.3±6.1%, P=0.40). Both stiffness index and PWV correlated inversely with magnitude of FMD (r=−0.40, P=0.03; r=−0.41, P=0.03) and positively with indexed LV mass (r=0.50, P=0.005; r=0.40, P=0.027). Nonetheless, no significant correlation existed between ferritin level and carotid stiffness, PWV, or FMD.

Conclusions—Increased arterial stiffness, endothelial dysfunction, and LV hypertrophy occur in patients with β-thalassemia major, which may result in reduction of mechanical efficiency of the heart. (Circulation. 2002;106:2561-2566.)

Key Words: endothelium • arteries • ventricles

Iron overload in patients with β-thalassemia major may result in systolic and diastolic dysfunction of the left ventricle.1,2 Although myocardial parenchymal damage occurs secondary to iron overload, atherogenic vascular complications have also been described in β-thalassemia patients, which has been attributed to an increase in lipid peroxidation products.3 Increased iron stores have been implicated in the association with increased risk of cardiovascular events.4,5 However, epidemiological evidence for such association is inconsistent.6 Furthermore, the role of iron in promotion of lipid peroxidation and development of atherogenesis-related pathologies remains controversial.7 Nonetheless, Duffy et al8 have recently shown that iron chelation with desferrioxamine in adults with coronary artery disease improves endothelium-dependent vasodilation. Their findings suggest that iron contributes to impaired nitric oxide function in atherosclerosis. In patients with β-thalassemia major, despite desferrioxamine therapy, their body iron load remains significantly higher than normal. Indeed, in vitro studies have shown disturbances of human vascular endothelial cell function when the cell culture is incubated with thalassemic serum.9,10 Vascular endothelial function in patients with β-thalassemia major in vivo hence may be similarly impaired but nonetheless has not been studied previously. Integrity of endothelial function is of particular relevance in these patients in light of the important role of endothelium in regulation of vascular tone11,12 and the propensity of these patients to develop cardiac failure.

Alterations of arterial structures with disruption of elastic tissue13 and calcification,14 on the other hand, have been demonstrated in patients with β-thalassemia major. These structural changes may translate functionally into alteration of arterial stiffness in vivo. Arterial stiffness is an important mechanical property, because it is related to vascular impedance and in turn to the afterload that is presented to the left ventricle.15 Its value in risk stratification has recently been shown in patients with hypertension.16

To date, however, no studies have yet been performed to examine the vascular functions in patients with β-thalassemia major. In the present study, we determined whether arterial...
endothelial function and stiffness are altered in patients with β-thalassemia major. We additionally assessed the relation between these indexes of vascular function and serum ferritin level, a widely used indicator of body iron load, and the interrelationship between endothelial function and arterial stiffness in this group of patients.

Methods

Patients with β-thalassemia major were recruited from the hematology clinic of Queen Mary Hospital. Smokers and patients with heart failure, systemic hypertension, diabetes mellitus, thyroid dysfunction, or parathyroid dysfunction were excluded. Healthy subjects matched for age and sex were recruited as controls. The institutional Ethics Committee approved the study, and all subjects gave informed consent.

The subjects attended for study after an overnight fast. For thalassemia patients, the study was performed at a median of 4 days (range, 1 to 7) after blood transfusion. An attempt was made to perform vascular studies within 1 week of transfusion so as to minimize potential confounding influence of anemia on the assessment results. Body weight and height were measured, and body surface area was calculated accordingly. All subjects rested for at least 15 minutes before blood pressure and cardiovascular assessments and remained supine throughout. Blood pressure in the right arm was measured twice using an automated oscillometric device (Dinamap, Critikon, Inc), and the average of 2 readings was taken. Assessments of left ventricular (LV) function, arterial stiffness, and endothelial function were performed sequentially, as described below. Venous blood was then withdrawn from all subjects for measurement of hemoglobin, fasting glucose, and total cholesterol levels. In control subjects, additional blood was taken for measuring serum ferritin level. In patients, the mean serum ferritin level was derived by averaging 6 to 8 values obtained approximately every 2 months over the past 12 months.

Echocardiographic Examination

Trans thoracic echocardiography was performed using a 2- to 4-MHz phased-array scanner, which was interfaced to a Hewlett-Packard Sonos 5500 ultrasound machine. Standard parasternal short-axis view at just below the tips of mitral valve leaflets was used to derive the M-mode measurements17 of LV systolic and end-diastolic dimensions and thickness of interventricular septum and posterior LV wall at diastole. Left ventricular fractional shortening and mass were calculated according to standard formula.18 Pulsed-wave Doppler examination was performed to obtain the following indexes of LV diastolic function:2 peak mitral inflow velocities at early (E) and late (A) diastole, early deceleration time, isovolumic relaxation time, forward pulmonary venous flow velocities and velocity time integral during ventricular systole and diastole, and pulmonary venous atrial reverse flow velocity. Average values of these indexes obtained from 5 consecutive cardiac cycles were used for analysis.

Measurement of Arterial Stiffness

Carotid artery stiffness was assessed by calculating the stiffness index.19 A 7- to 15-MHz linear-array transducer was used to image the right carotid artery at ~1 cm proximal to the carotid bifurcation. The maximum and minimum diameters at systole and end diastole, respectively, were measured. The stiffness index was calculated according to the following formula: (SBP/DBP)/(6ΔD/ΔD),19 where SBP and DBP are systolic and diastolic blood pressure respectively, ΔD is the difference between systolic and diastolic diameters, and D is the mean diameter. Stiffness of the brachioradial arterial segment was assessed by measuring pulse-wave velocity (PWV), which is related to square root of elastic modulus according to the Moens-Korteweg equation.13 Hence, the stiffer the artery, the faster the PWV. A previously validated photoplethysmographic technique20,21 was used to measure the transit time required for the pulse to travel from the brachial artery at the elbow to radial artery at the wrist. The average of 3 readings was taken. PWV was calculated by dividing distance between the 2 points by transit time. Intraobserver variability for measurement of PWV, as determined from the mean and SD of differences in 2 consecutive results from 20 studies, was 0.08±0.82 m/s.

Assessment of Brachial Artery Endothelial Function

Arterial endothelial function of the brachial artery was assessed noninvasively by ultrasound examination of the vasodilation response to endothelium-dependent and -independent stimuli, as previously reported.22 The right brachial artery, proximal to the antecubital fossa, was imaged longitudinally using the linear-array transducer. Flow-mediated endothelium-dependent vasodilation was assessed by measuring the brachial artery diameter at baseline and during reactive hyperemia. Reactive hyperemia was induced by deflating a cuff previously inflated to 300 mm Hg for 4.5 minutes in the forearm. Arterial flow velocity was measured at baseline and during reactive hyperemia using pulse-waved Doppler. After 10 to 15 minutes, the endothelium-independent response was assessed by the change in artery diameter at 3 to 4 minutes after a 400-μg dose of sublingual glyceryl trinitrate (GTN). All scans were recorded on super-VHS videotapes for analysis. The parameters were measured for 3 consecutive cardiac cycles, and the average was taken. The percentages of diameter and flow changes during reactive hyperemia and after sublingual GTN were calculated. The intraobserver and interobserver coefficients of variation for flow-mediated dilation were 1.8% and 2.1%, respectively.

Statistical Analysis

Data are presented as mean ± SD unless otherwise stated. Data on LV function, arterial stiffness, and endothelial function were analyzed offline, blinded to whether the subject had β-thalassemia. Differences in demographic data, echocardiographic parameters, and vascular indexes between patients and controls were compared using paired Student’s t test. Within the patient group, Pearson correlation analysis was used to assess for possible relationships between serum ferritin level and vascular indexes, indexed LV mass and vascular indexes, and flow-mediated dilation and arterial stiffness. Stepwise multiple linear regression was used to identify significant determinants of arterial stiffness and flow-mediated dilation. Statistical significance was defined as P<0.05. All statistical analyses were performed using SPSS Version 10.0 (SPSS, Inc).

Results

Thirty patients (16 male) were studied at age 22.2±7.4 years. Monthly blood transfusion was started at a median age of 0.5 years (range, 0.1 to 4.0), whereas desferrioxamine was started at a median age of 5.1 years (range, 2.6 to 16). Five patients had undergone splenectomy. The demographic data, clinical parameters, and hematologic profile of patients and controls are summarized in Table 1. Thalassemia patients had significantly smaller body size (P<0.001) and, expectedly, a higher serum ferritin level (P<0.001) and lower hemoglobin level (P<0.001).

Echocardiographic Findings

The M-mode measurements and Doppler indexes are summarized in Table 2. When compared with controls, patients had significantly greater absolute (P=0.04) and indexed (P<0.001) LV mass, E wave velocity (P=0.01), ratio of E to A wave velocities (P=0.049), forward systolic pulmonary venous flow velocity (P=0.001), and ratio of systolic to diastolic pulmonary venous flow velocity (P=0.04). These Doppler diastolic index abnormalities were similar to those found in conditions with increased preload, which is probably related to chronic anemia.2 None of our patients had Doppler...
Patients and Controls

TABLE 2. Comparison of Echocardiographic Indexes Between Patients and Controls

<table>
<thead>
<tr>
<th></th>
<th>Patients (n=30)</th>
<th>Controls (n=30)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>22.2±7.4</td>
<td>22.7±7.6</td>
<td>0.13</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>44.0±11.7</td>
<td>60.8±16.2</td>
<td>&lt;0.001*</td>
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<tr>
<td>Height, cm</td>
<td>152.2±14.8</td>
<td>166.6±12.0</td>
<td>&lt;0.001*</td>
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<tr>
<td>Body mass index, kg/m²</td>
<td>18.6±2.8</td>
<td>21.6±4.3</td>
<td>0.002*</td>
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<tr>
<td>Heart rate, min⁻¹</td>
<td>72±13</td>
<td>68±13</td>
<td>0.14</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>113±15</td>
<td>119±10</td>
<td>0.07</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>60±12</td>
<td>66±13</td>
<td>0.07</td>
</tr>
<tr>
<td>Haemoglobin, g/dL</td>
<td>13.8±0.6</td>
<td>14.8±1.2</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Serum ferritin, pmol/L</td>
<td>5422±3366</td>
<td>392±243</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.2±0.7</td>
<td>5.0±0.3</td>
<td>0.15</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>3.4±0.8</td>
<td>3.8±0.9</td>
<td>0.08</td>
</tr>
</tbody>
</table>

BP indicates blood pressure.

*Statistically significant.

indexes that suggest LV restrictive or relaxation abnormalities.

Arterial Stiffness

The carotid artery stiffness index was significantly higher in patients than controls (8.1±3.5 versus 5.5±1.6, P<0.001).

TABLE 2. Comparison of Echocardiographic Indexes Between Patients and Controls

<table>
<thead>
<tr>
<th></th>
<th>Patients (n=30)</th>
<th>Controls (n=30)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVDD, cm</td>
<td>4.48±0.56</td>
<td>4.69±0.41</td>
<td>0.09</td>
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<tr>
<td>LVDS, cm</td>
<td>2.99±0.47</td>
<td>3.09±0.36</td>
<td>0.36</td>
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<tr>
<td>FS, %</td>
<td>33.6±4.1</td>
<td>34.0±4.9</td>
<td>0.74</td>
</tr>
<tr>
<td>LV mass, g</td>
<td>113.8±38.0</td>
<td>109.0±32.6</td>
<td>0.04*</td>
</tr>
<tr>
<td>Indexed LV mass, g/m²</td>
<td>82.4±17.5</td>
<td>66.7±12.7</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Mitral inflow Doppler indexes</td>
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</tr>
<tr>
<td>E, cm/s</td>
<td>90.2±14.2</td>
<td>78.2±20.1</td>
<td>0.01*</td>
</tr>
<tr>
<td>A, cm/s</td>
<td>40.5±10.6</td>
<td>41.4±8.3</td>
<td>0.68</td>
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<tr>
<td>E/A ratio</td>
<td>2.4±0.7</td>
<td>2.1±0.5</td>
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<tr>
<td>E deceleration, ms</td>
<td>134±26</td>
<td>124±30</td>
<td>0.10</td>
</tr>
<tr>
<td>IVRT, ms</td>
<td>67±9</td>
<td>71±10</td>
<td>0.12</td>
</tr>
<tr>
<td>Pulmonary venous Doppler indexes</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>S, cm/s</td>
<td>51.7±13.0</td>
<td>42.1±7.2</td>
<td>0.001*</td>
</tr>
<tr>
<td>D, cm/s</td>
<td>51.7±10.8</td>
<td>51.6±12.9</td>
<td>0.99</td>
</tr>
<tr>
<td>S/D ratio</td>
<td>1.0±0.3</td>
<td>0.9±0.3</td>
<td>0.04*</td>
</tr>
<tr>
<td>VTIS, cm</td>
<td>11.9±3.7</td>
<td>11.1±2.5</td>
<td>0.36</td>
</tr>
<tr>
<td>VTID, cm</td>
<td>11.5±3.3</td>
<td>12.5±3.5</td>
<td>0.22</td>
</tr>
<tr>
<td>AR, cm/s</td>
<td>19.2±6.6</td>
<td>19.1±4.0</td>
<td>0.91</td>
</tr>
</tbody>
</table>

LVDD indicates left ventricular dimension at diastole; LVDS, left ventricular dimension at systole; FS, fractional shortening; LV, left ventricular; E, peak mitral inflow velocity at early diastole; A indicates peak mitral inflow velocity at late diastole; IVRT, isovolumic relaxation time; S, forward systolic pulmonary venous flow velocity; D, forward diastolic pulmonary venous flow velocity; VTIS, pulmonary venous systolic velocity-time integral; VTID, pulmonary venous diastolic velocity-time integral; and AR, atrial reverse flow velocity.

*Statistically significant.

Figure 1. Scatter plots showing a positive correlation between carotid artery stiffness index and indexed left ventricular mass (A) and a similar correlation between brachioradial PWV and indexed LV mass (B).

Likewise, brachioradial PWV, and hence stiffness, was significantly higher in patients (8.9±2.4 versus 7.9±1.7 m/s, P=0.03). The correlation between carotid artery stiffness index and brachioradial PWV was significant (r=0.37, P=0.004).

The LV mass of patients, indexed to body surface area, increased with an increase in carotid artery stiffness index (r=0.50, P=0.005) (Figure 1A) and brachioradial PWV (r=0.40, P=0.027) (Figure 1B). Nonetheless, there was no significant correlation between serum ferritin level and either carotid artery stiffness index (P=0.77) or brachioradial PWV (P=0.77).

Brachial Artery Endothelial Function

The brachial artery baseline flow (117±88 versus 141±90 mL/min, P=0.32) and flow increase during reactive hyperemia (321±204% versus 381±222%, P=0.25) were similar between patients and controls. However, baseline brachial artery diameter (3.13±0.06 versus 3.94±0.07 mm, P<0.001) was significantly smaller, and flow-mediated dilation was markedly less in patients (3.5±3.3% versus 8.8±3.9%,
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In contrast, the vasodilation response to sublingual GTN was similar between the 2 groups (17.9 ± 7.6% versus 16.3 ± 6.1%, P = 0.40) (Figure 2B), suggesting that reduced flow-mediated dilation in thalassemia patients is related to endothelial dysfunction.

In patients, magnitude of flow-mediated dilation correlated inversely with carotid artery stiffness index (r = -0.40, P = 0.03) (Figure 3A) and brachioradial PWV (r = -0.41, P = 0.03) (Figure 3C). However, there was no correlation between flow-mediated dilation and serum ferritin level (P = 0.23) or indexed LV mass (P = 0.30).

Determinants of Arterial Stiffness and Flow-Mediated Dilation

Multiple linear regression analysis of the entire cohort of 60 subjects was used to identify significant determinants of arterial stiffness and flow-mediated dilation. To identify determinants of arterial stiffness, the following dependent variables were included: age, body mass index, systemic blood pressure, hemoglobin level, fasting glucose and total cholesterol levels, magnitude of flow- and GTN-mediated dilation, ferritin level, and patient status. For identifying determinants of flow-mediated dilation, additional variables included were baseline brachial artery diameter, degree of reactive hyperemia, carotid artery stiffness index, and PWV.

For carotid artery stiffness index, significant determinants were age (β = 0.29, P = 0.03), being a thalassemia patient (β = 0.3, P = 0.002), and magnitude of flow-mediated dilation (β = -0.46, P = 0.003). The magnitude of flow-mediated dilation was also the main determinant of brachioradial PWV (β = -0.47, P < 0.001). When flow-mediated dilation was entered as the independent variable, significant determinants were baseline brachial artery diameter (β = -0.36, P = 0.005), as reported previously, pulse wave velocity PWV (β = -0.26, P = 0.02), and being a thalassemia patient (β = -0.77, P < 0.001).

Discussion

This study demonstrates systemic arterial endothelial dysfunction and increased arterial stiffness in patients with β-thalassemia major. Importantly, these phenomena occur in the absence of cardiac dysfunction that is known to alter arterial endothelial function and vascular tone. Endothelial dysfunction probably contributes in part to the increase in arterial stiffness, given the important role of endothelium-
derived nitric oxide as an inhibitor of smooth muscle contraction.

Functional disturbances of human vascular endothelial cells, when incubated with thalassemic serum, have been demonstrated in vitro. These include an increase in levels of soluble adhesion molecules in the supernatant of cell culture, a reduction in mitosis of endothelial cells, and morphological changes characteristic of apoptosis. Recently, soluble adhesion molecules including intercellular adhesion molecule 1, vascular adhesion molecule 1, and E-selectin have also been shown to be significantly elevated in the plasma of thalassemia patients. The present study provides evidence that arterial endothelial dysfunction occurs in vivo in patients with β-thalassemia major.

Despite the controversy of epidemiological evidence, experimental studies have demonstrated that iron may reduce endothelium-derived nitric oxide bioactivity either directly, by decreasing endothelial and inducible nitric oxide synthase activity, or indirectly, by stimulating membrane lipid peroxidation to generate lipid peroxyl radicals. Our findings also suggest that endothelial dysfunction might be the link in this association and corroborate with findings recently reported by Duffy et al.

We have additionally demonstrated that arterial stiffness is increased in both central elastic arteries and peripheral conduit arteries in patients with β-thalassemia major. Diffuse arterial elastorrhexis, as characterized by fragmentation and defects of the internal elastic lamina, has been observed in the surgically removed spleens and liver biopsy specimens of patients. Additionally, radiological studies have demonstrated calcifications in their posterior tibial artery. Furthermore, alteration of glycosaminoglycan composition with increased fibrosis has been documented histologically in the aorta, iliac, and pulmonary arteries in postmortem examination. Undoubtedly, these structural changes may explain the increase in arterial stiffness in vivo. Nonetheless, the fact that vasodilation response to GTN is preserved suggests that alteration of arterial wall composition may not be the sole predominant mechanism to account for the increased stiffness.

Stiffness of both carotid and brachial-radial arteries is inversely related to the magnitude of flow-meditated dilation in our patients. This corroborates with findings of previous studies that demonstrated a critical role of endothelium in the control of vascular tone. Decrease in arterial compliance and increase in PWV during vascular smooth muscle contraction have been demonstrated previously by Bank et al. Thus, it is likely that functional alteration of arterial tone, coupled with structural alteration of arterial wall, contributes to the overall increase in systemic arterial stiffness.

The relevance of our findings in the pathogenesis of cardiac failure in thalassemia becomes obvious when the heart and arterial system are considered from a mechanical perspective. Although congestive heart failure is the main cause of death in patients with β-thalassemia major, thromboembolic episodes, stroke, and myocardial infarction are uncommon. Their extremely low total and low-density lipoprotein cholesterol plasma levels might have conferred protection against atherogenic risk and explain the rather uncommon atherosclerotic complications despite iron deposition in arteries. On the other hand, increased arterial stiffness and increased wave reflection as a result of a faster PWV increase the input impedance that is presented to the left ventricle. Interaction between LV ejection and systemic arterial impedance may thus become less favorable, because the impedance modulus may no longer be the least at where the flow harmonics are highest. Our findings additionally support the view of a multifactorial etiology of LV failure in patients with β-thalassemia major. Thus, apart from myocardial iron deposition, myocarditis, and immunogenetic profile, arterial dysfunction may also be contributory.

Serum ferritin level does not correlate with the degree of arterial stiffness or impairment of flow-mediated dilation. A poor correlation between liver iron stores and serum ferritin level has been shown in patients receiving iron chelator. The correlation is especially poor when serum ferritin exceeds 5500 pmol/L, a level that is exceeded in 30% (9 of 30) of our patients. It remains to be determined whether by using a more robust indicator of tissue iron load, such as liver iron content, a positive correlation between iron load and indexes of vascular function would emerge. Alternatively, these parameters may not be related in a dose-dependent fashion. These speculations, however, require additional studies for clarification.

A potential limitation to this study is the possible confounding influence of anemia on assessment of PWV and flow-mediated dilation. According to the Moens-Korteweg equation, a lower blood density increases PWV. Nonethe- less, the small difference in hemoglobin levels between patients who were studied shortly after transfusion and control subjects is unlikely to be associated with a significant difference in blood density and viscosity. Furthermore, the fact that stiffness index, assessment of which is independent of hemoglobin level, correlates significantly with PWV strengthens the evidence of a genuine increase in systemic arterial stiffness. Likewise, because the brachial artery flow is similar between patients and controls, the wall shear stress induced during reactive hyperemia to cause flow-mediated vasodilation is unlikely to be significantly different between the 2 groups.

In conclusion, this study provides the first evidence that function of the arterial system in patients with β-thalassemia major is jeopardized by endothelial dysfunction and increased stiffness, which may result in reduction of mechanical efficiency of the heart. Apart from rigorous iron chelation, interventions targeted at improving arterial dysfunction may perhaps delay deterioration of cardiac function in the long-term.

Acknowledgments

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