Investigation of Muscle Bioenergetics in the Marfan Syndrome Indicates Reduced Metabolic Efficiency

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ABSTRACT

Background: The Marfan syndrome is an inherited multisystem disorder caused by mutations in fibrillin 1, with cardiovascular involvement being the most important feature of the phenotype. Affected individuals have impaired flow-mediated dilatation (FMD) of large arteries of a similar severity to patients with chronic heart failure (CHF). Aims: Skeletal muscle bioenergetics were studied in patients with the Marfan syndrome in order to evaluate the impact of impaired flow-mediated dilatation on skeletal muscle metabolism. Skeletal muscle metabolism is abnormal in CHF and the aetiology is unclear. Methods: Thirteen patients and 12 controls were studied by phosphorus Magnetic Resonance spectroscopy of the calf muscle using an incremental exercise protocol and by Magnetic Resonance imaging. Results: Metabolic variables measured at rest were normal in Marfan patients. For a similar total work output measured at end of the standardized incremental exercise, the total rate of energy consumption (EC) was significantly increased in patients (21.2 ± 2.3 mM ATP/min/W vs 13.6 ± 1.4 mM ATP/min/W in controls). Similarly, both PCr and pH time-dependent changes were significantly different between groups. The absolute contributions of aerobic and glycolytic pathways to energy production were significantly higher in patients whereas they were similar when expressed relative to EC. Conclusions: The higher EC measured in patients with Marfan syndrome was supported by both oxidative and anaerobic metabolic pathways, thereby suggesting a decrease in muscle efficiency and/or muscle mass, as previously described in other diseases affecting skeletal muscle function such as heart failure and peripheral vascular disease.

INTRODUCTION

The Marfan syndrome is an inherited connective tissue disorder (prevalence of 1/3000-5000) caused by mutations in the gene encoding fibrillin-1 (1). Fibrillin-1 is a key component of the elastic tissue associated microfibril, which, amongst other roles, is important for the attachment of the endothelium to the media of blood vessels (2).

The classical Marfan’s phenotype manifests in the skeletal, cardiovascular, and ophthalmic systems. The cardiovascular complications include ascending aortic dilatation and dissection, which may result from alterations in the elastic properties of the aortic wall. Skeletal involvement manifests as tall stature and lax joints while subluxed ocular lenses are part of the ophthalmic signs. Given the often fatal cardiovascular complications, the importance of vascular function cannot be underestimated and warrant further investigations.

Vascular dysfunction in the Marfan’s syndrome has been demonstrated by several different techniques. An increase in aortic stiffness and decrease in aortic distensibility have been shown by echocardiography and Magnetic Resonance (MR) imaging (3, 4). Impaired flow-mediated dilatation (FMD), a marker of endothelial dysfunction, has been found by ultrasound-guided brachial wall tracking (5), yet its functional impact on muscle energetics is still unsettled.

Received 18 September 2005; accepted 30 April 2006.
Keywords: Marfan syndrome, 31P-MR spectroscopy, skeletal muscle metabolism.
The study was supported by the British Heart Foundation and Medical Research Council.
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Interestingly, endothelial dysfunction has been also reported in chronic heart failure (CHF), a chronic condition for which muscle energetics alterations have definitively been reported (6). In CHF patients, \(^{31}\)P MRS studies have reported increased phosphocreatine (PCr) depletion in early exercise, larger exercise-induced acidosis, and decreased mitochondrial oxidative capacity (7, 8). These abnormalities could result from a defect in oxidative ATP synthesis, reduced metabolic efficiency and/or a primary over-activation of glycolgenolysis, and could be related to inadequate oxygen supply and/or reduced number of mitochondria (6–8). So far, it is still not known whether these energetic alterations are secondary events or if they occur independently of the endothelial dysfunction.

In that context, the investigation of muscle energetics in Marfan patients is of interest. On the one hand, it is an interesting way of analyzing the exact role of endothelial dysfunction on muscle energetics, and, on the other hand, it could contribute to delineating which of primary or secondary mechanisms are responsible for the metabolic anomalies reported so far in CHF patients.

In the present study, we aimed at investigating the possible contribution of impaired flow-mediated dilatation on skeletal muscle metabolism using a combination of \(^{31}\)P-MR spectroscopy and MR imaging.

**METHODS AND SUBJECTS**

**Subjects**

Patients included in the present study (n = 13; 9 males) were recruited from an integrated hospital-based outpatient clinic for families with Marfan syndrome. They were between 15 and 47 years of age (mean age = 35 years). The diagnosis of Marfan syndrome was made according to the Ghent criteria (9), and 8 patients had a mutation identified in the FBN1 gene. Nine patients were taking prophylactic atenolol or verapamil, and none of them had previously undergone aortic root surgery. The control group was composed of twelve age and sex-matched subjects (8 males; mean age = 33 years; range: 14–46 years). These volunteers were free of symptoms or signs of cardiovascular or neuromuscular disease and were not taking any treatment. All procedures were conducted on the same day. Subjects had MR imaging of the right calf followed by a \(^{31}\)P-MR spectroscopic investigation of skeletal muscle during a standardized exercise protocol (10). The study conformed with the principles outlined in the Declaration of Helsinki. All patients gave their written informed consent, and the study was approved by the Central Oxford Research Ethics Committee.

**\(^{31}\)P-MRS exercise study**

\(^{31}\)P-MRS of the right gastrocnemius muscle was performed using a 2T super-conducting magnet (Oxford Magnet Technology, Eynsham, Oxford, United Kingdom) interfaced to a Bruker spectrometer (Bruker, Coventry, United Kingdom). The protocol has been described in detail previously (7). Subjects lay supine with a 6 cm surface coil centered beneath the maximal circumference of the right calf muscle. The position of the surface coil centered was marked on the subject’s leg, and the mark was used to guide localization for the MR images. Spectra were acquired using a 2 s inter-pulse delay at rest (64 scans), during exercise (16 scans), and recovery. As soon as the last 16 scan exercise spectrum was collected, 1 additional 8-scan spectrum was also recorded while the subject was still exercising, and this data point was considered as “zero time” of the recovery period. The exercise was stopped immediately afterwards. Data were collected for 10 minutes in recovery (four 8-scan spectra followed by 4 of 16 scans, 3 of 32 scans, and 2 of 64 scans). The muscle was exercised by plantar flexion at 0.5 Hz, lifting a weight of 10% lean body mass (LBM) (calculated from body weight and skin fold thickness) (11) through a distance of 7 cm. After the first 4 minutes of exercise (8 spectra), the weight was incremented by 2% of LBM for each alternate spectral acquisition. Subjects exercised until they had consumed approximately 50% of their resting PCr. This was checked through a continuous visualisation of \(^{31}\)P MR spectra. This standardization procedure has been already used in a large number of studies (10, 12–17).

**MR imaging**

MR imaging of the right calf was carried out using an 18 cm diameter quadrature birdcage coil. Three sagittal gradient-echo images (repetition time (TR) = 130 ms; echo time (TE) = 10 ms; matrix size (MS) = 128 × 128; field of view (FOV) = 30 cm) were obtained to identify the location of a small phantom, which had been placed over the mark made on the patient’s calf during the \(^{31}\)P-MRS study, indicating the point of maximal circumference. T1-weighted images were obtained in the transaxial orientation using a spin-echo pulse sequence (TR = 600 ms; TE = 20 ms, MS = 256 × 256; FOV = 25 cm, and eight slices of 10 mm section thickness separated by 10 mm gaps.

**Data analysis**

**Metabolite concentrations and pH**

\(^{31}\)P MR spectra were processed using a time-domain fitting routine (VARPRO, R de Beer, Delft, The Netherlands) with the appropriate prior knowledge (18). Relative concentrations of inorganic phosphate (P\(_i\)), phosphocreatine (PCr), and ATP were obtained after correction for magnetic saturation. This correction was performed from the comparative measurements of spectra recorded at rest under fully relaxed conditions (i.e., with a repetition time of 20 s) and spectra recorded under partially relaxed conditions (i.e., with a 2 s inter pulse delay). Absolute concentrations were obtained by assuming that the concentration of cytosolic ATP was 8.2 mM (i.e., mmol/L of intracellular water). Intracellular pH was calculated from the chemical shift of the P\(_i\) peak relative to PCr (\(\delta P_i\), measured in parts per million), as

\[
\text{pH} = 6.75 + \log_{10}[(\delta P_i - 3.27)/(5.69 - \delta P_i)].
\]

Free cytosolic (ADP) was calculated from pH and (PCr) using a creatine kinase equilibrium constant of 1.66 × 10\(^{9}\) L/mol and
assuming a normal total creatine content of 42.5 mM (19).

\[ [\text{ADP}] = [\text{total creatine}][\text{ATP}]/[\text{PCr}][\text{H}^+](K_{eq}) \]

The total creatine concentration has been shown to be normal in a variety of neuromuscular disorders even when the PCr was reduced, so it is not unreasonable to hypothesize a normal total creatine concentration in patients.

**Quantitative analysis**

Rates of aerobic and anaerobic ATP production were calculated in exercising muscle as originally described by Kemp et al. (20). Briefly, the energy cost (EC) of contraction was calculated at the onset of exercise: the oxidative contribution being regarded as negligible at that time. Rates of glycolytic ATP production were calculated taking into account changes in PCr and pH during exercise in addition to buffering capacity (21) and proton efflux (22). Considering that EC retains a constant proportionality to power output throughout the entire exercise period, rates of aerobic ATP production can be calculated at any time of exercise as the difference between EC and anaerobic ATP production scaled to power output (23).

**Muscle bioenergetics during recovery from exercise**

Recovery half-times \( (\tau_{1/2}) \) of PCr, P, and ADP were calculated from semi-logarithmic plots. Initial rates of PCr resynthesis after exercise \( (V, \text{in mM/min}) \) were calculated from the exponential rate constant of PCr recovery \( (k = 0.693/\tau_{1/2}) \) and the total fall in PCr during exercise \( (\Delta \text{[PCr]}) \) as \( V = k \Delta \text{[PCr]} \). Apparent maximal mitochondrial capacity, \( V_{\text{max}} \) (mM/min), was calculated using the hyperbolic relationship between the initial rate of PCr resynthesis and ADP, assuming a normal \( K_m \) for ADP of 30 µM as

\[ V_{\text{max}} = \frac{V(1+(K_m/[\text{ADP}])(24))}{1} \]

**1H MRI**

From MR images, we calculated the cross-sectional area (CSA) of the skeletal muscle in the slice corresponding to the position of the phantom. The boundaries of the skeletal muscle area, tibia, fibula, subcutaneous fat, and whole leg circumference were traced by hand using the Xtip software package (Bruker, Ettlingen, Germany), and the respective areas were calculated. Respective contributions of muscle and fat have been separated on the basis of a thresholding method as previously described (25).

**Statistics**

Data are presented as means ± SE except otherwise stated. Time-dependent changes in high-energy phosphate compounds and pH were compared using one way ANOVA with repeated measurements (repeated factor being time) using the General Linear Models procedure of the SAS software (26), options REPEATED, LSMEANS and CONTRAST. Wilk’s lambda tests were performed to analyse the effects of time (either during exercise or in recovery), and in the case of a significant difference, post-hoc repeated comparisons (Scheffe’s contrasts) were performed to test specific hypotheses. A value of \( p < 0.05 \) was considered significant.

**RESULTS**

The baseline characteristics of the patients and controls are summarized in Table 1. Although there were small differences in height, weight, LBM, and calf muscle CSA between patients and controls, none of them reached the significant threshold. In addition, muscle and fat relative contributions were identical in both patient and control groups (Table 1). Similarly, metabolic variables such as PCr and ADP concentrations and pH values measured at rest were similar between the two groups (Table 2, Fig. 1).

**Metabolic changes in exercising muscle**

Typical \( ^{31}P \) spectra recorded at rest, at the end of the first minute of exercise, and at the end of exercise are shown in Fig. 1. Patients exercised for an average of 8 minutes, whereas the exercise duration in controls was significantly longer, i.e., 11.6 minutes (Table 2). However, it is noteworthy that this significant result was mainly due to a single patient who abnormally exercised for less than 3 minutes. Given that the other results were not significantly affected by this subject’s data, he was included in the final results. As previously described, exercise intensity was standardised on the basis of LBM measurements (15). Work-rate measured during incremental exercise (Fig. 2A), and the total work output measured at end of exercise (Table 2) were similar in both groups. In addition, the difference between the groups was even smaller when total work output was scaled to CSA measurements (23 ± 2 vs 21 ± 3 J/cm² in patients). The metabolic changes associated with exercise are displayed in Figs. 2B and 2C. As expected, exercise was accompanied in the initial phase by a rapid PCr breakdown and intracellular

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<th>Table 1. Demographic characteristics of Marfan subjects and controls</th>
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BMI = body mass index; CSA = cross-sectional area; LBM = lean body mass.

*Expressed as % of the whole leg CSA.
The amount of PCr consumed was significantly larger in patients. The rates were not significantly different between groups (Table 2). Exercise (Figs. 2B and C). At end of exercise, PCr and pH values were not significantly modified in patients. Similarly, proton efflux was not significantly impaired in patients as compared to controls (Table 2).

<table>
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<th>Table 2. Metabolic variables in Marfan subjects and controls</th>
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<td>Initial rate of PCr resynthesis (mM/min)</td>
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<td>Maximum rate of aerobic ATP production (mM/min)</td>
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<td>Rate of proton efflux (mM/min)</td>
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TWO = total work output. Δ refers to the difference between the value measured at rest and the corresponding value measured at end of the incremental exercise. ² units are mM ATP/min/W.

**DISCUSSION**

In this study, we have shown that the EC of contraction calculated early in exercise was larger in subjects with the Marfan syndrome as compared to controls. Likely as a consequence, the PCr time-dependent changes indicated greater PCr consumption in patients throughout the incremental standardized exercise. Also, the end-of-exercise PCr consumption, when scaled to the total work output, was significantly higher in patients. Similarly to data previously reported in both humans and rats, the rates of both PCr degradation and glycogenolysis declined with time throughout exercise whereas oxidative contribution to ATP production increased so that ATP turnover became virtually all oxidative by five minutes of exercise (13, 27).

The higher energy consumption measured during exercise was supported by both oxidative and anaerobic metabolic pathways thereby suggesting a decrease in muscle efficiency and/or muscle mass as previously described, among other diseases affecting skeletal muscle function, heart failure and peripheral vascular diseases (7, 14). Several abnormalities including fiber type changes and muscle atrophy have been proposed as factors accounting for the increased EC (28, 29). On the basis of MRI measurements of CSA, muscle and fat relative contents, it seems unlikely that reduced muscle mass would account for the higher EC. This result is similar to what has been previously concluded from studies in claudicant patients (13) or patients with heart failure (7) and suggest that a significant part of the abnormality reported in the present study is due to a loss of intrinsic metabolic efficiency. Before going any further, one has to keep in mind that metabolic changes in exercising muscle are conditioned by the balance between energy demand, i.e., work output and energy production, i.e., ATP synthesis. Thus, any increase in energy demand would result in increased energy production. Given that exercise intensity was standardized to lean body mass as previously described (15, 25), it is unlikely that poor standardization could account for the increased EC. However, one could argue that this standardization procedure would not be accurate for subjects with the Marfan phenotype. In that regard, we have used MRI in order to accurately estimate muscle mass using CSA measurements. Considering that this index was similar in both groups, we can clearly conclude, on the one hand, that energy demand was similar in both groups and, on the other hand, that metabolic abnormalities would be more likely related to energy demand rather than energy production. Deconditioning has also been proposed as a possible factor accounting for the increased EC (30). Again, the similarity between MRI-estimated...
Figure 1. Examples of $^3$P spectra from a control (A–C) and a Marfan subject (D–F); A and D: spectra at rest (64 scans); B and E: after 1 minute of exercise (16 scans); C and F: at end exercise (8 scans). $P_i =$ inorganic phosphate; $PCr =$ phosphocreatine; $\delta P_i =$ chemical shift in ppm of $P_i$ with respect to $PCr$ (required for calculation of $\text{pH}^{10}$); ppm = parts per million.

muscle mass clearly rules out this factor as accounting for the increased EC of exercise.

In the present study, the increased EC and the corresponding reduction in metabolic efficiency is supported by an overall increased contribution of ATP synthesis from both aerobic and non oxidative pathways. This overall increase indicates that neither oxidative nor glycolytic ATP production was impaired. A reduced oxidative capacity can be further discarded on the basis of the normality of recovery measurements. $PCr$ repletion during recovery is regarded as a purely oxidative process and, therefore, the initial rate of $PCr$ recovery and $V_{\text{max}}$ (the maximal rate of oxidative ATP synthesis) are both considered to be non invasive indices of mitochondrial function (31–33). As these were not significantly different between patients and controls, this is consistent with the conclusion of intrinsic muscle mitochondrial function being normal in Marfan subjects.

On the basis of measurements of endothelium-dependent and independent changes in blood flow, it has been clearly shown that flow-mediated vasodilation was selectively impaired in Marfan subjects (5). Considering that vasodilation is effective for the lowering of resistance and the redistribution mechanism of blood flow, it is considered as an important factor controlling blood flow in exercising muscle (34). Consequently, one may wonder how far the metabolic abnormalities reported in the present
Figure 2. Power (A), PCr (B) and pH (C) time-dependent changes during the standardised incremental exercise in controls (white circles) and patients (black circles). Results are displayed as means ± SE. * indicates significant difference by 2 factor ANOVA. Given that exercise duration was different for each subject, for the sake of clarity results are presented for the first 8 minutes of exercise. At this time, 75% of the patients and 91% of the controls were still exercising.

Figure 3. A: Individual and mean values of energy cost calculated in controls (white circles) and patients (black circles). Error bars represent SE. Time-dependent evolution of the absolute rates of aerobic (B) and anaerobic (C) ATP production during the standardised incremental exercise in controls (white circles) and patients (black circles). Results are displayed as means ± SE. * indicates significant difference by 2 factor ANOVA. Given that exercise duration was different for each subject, for the sake of clarity results are presented for the first 8 minutes of exercise. At this time 75% of the patients and 91% of the controls were still exercising.

Study are related to the endothelial dysfunction already reported in Marfan patients. In line with this hypothesis, one could expect to find in Marfan subjects, typical abnormalities observed in patients with impaired blood flow, such as patients with peripheral vascular disease (PVD) and/or chronic heart failure (CHF). In both conditions, some of the metabolic abnormalities measured in the present study have already been reported such as increased PCr consumption and higher EC (5, 13, 35). However, while increased PCr consumption has been linked to abnormal blood flow, the increased EC is more compatible with reduced muscle efficiency and/or muscle mass rather than impaired muscle perfusion (13).

Previous studies have shown that whilst cardiac output is an important determinant of exercise capacity in healthy subjects, peripheral factors are predominant in CHF (36, 37). 31P-MRS and muscle biopsy investigations have shown, in agreement with
the present results, abnormal muscle energetics with increased phosphocreatine (PCr) depletion in early exercise, lower pH during exercise and notably decreased mitochondrial oxidative capacity (6, 35, 38, 39). However, impaired blood flow at a macrovascular level has been thought not to be an important determinant of the metabolic findings on exercise (40). Whether skeletal muscle metabolism is adversely affected by endothelial dysfunction is unclear. The occurrence of abnormalities in human heart failure during ischemic exercise would indicate a loss of metabolic efficiency rather than an impaired blood flow as a causative factor of altered muscle energetics (39–41). It is noteworthy that inadequate blood flow has been proposed as a causative factor of exercise intolerance in CHF patients (6, 42, 43), but normal leg blood flow has been measured (44). Overall, higher EC and larger PCr consumption during exercise would not be linked in Marfan patients to either abnormal perfusion or abnormal endothelium-dependent vasodilation but rather to a reduced muscle efficiency. In that respect, it has been suggested previously (7, 21) that in the face of increased EC, one has to determine whether both aerobic and anaerobic ATP production pathways are involved or not. Regardless of its cause, a reduction in muscle efficiency would increase the required rate of ATP synthesis by all pathways. On the contrary, a reduction in oxidative capacity would only affect the contribution of oxidative synthesis with a likely compensatory increase in the non oxidative contribution. In the present study, the overall increased relative contributions of both aerobic and non oxidative pathways point towards a reduction in muscle efficiency rather than impaired muscle energy provision. The reduction in muscle efficiency could be due to gross muscle atrophy and/or a loss of intrinsic metabolic efficiency, which could be related, among other things, to changes in fiber types as previously suggested on the basis of a comparative analysis between ischemic and aerobic exercises (40).

One factor that could have influenced the data is the advice to most Marfan patients to avoid strenuous physical exertion. There was no evidence on direct questioning of the patients or controls that there was any difference in the level of regular aerobic exercise undertaken. We did not perform treadmill exercise tests to determine VO\textsubscript{2max} due to ethical considerations, but we excluded patients who had undergone any type of surgery likely to
have impact on myocardial function. In addition, the metabolic response during the type of incremental exercise protocol used in this study (plantar-flexion whilst semi-recumbent) has been previously thought to be poorly correlated with intrinsic cardiac output (40).

The data from this study suggest that impaired endothelial function which could affect blood flow would not be a causative factor of the metabolic abnormalities recorded in Marfan patients. Rather, impaired muscle efficiency as previously reported in both PVD and CHF (7, 13) would explain the overall higher activation of both oxidative and glycogenolytic contributions to ATP synthesis. As discussed earlier, a reduction in the muscle mass could be excluded on the basis of accurate MRI measurements.

ACKNOWLEDGMENTS

We are grateful to Anne Roberts, Clinical Nurse Specialist, Churchill Hospital, Oxford, UK who helped with patient recruitment.

REFERENCES


