Coronary Microvascular Dysfunction in Male Patients
With Anderson-Fabry Disease and the Effect of Treatment
With Alpha Galactosidase A

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ABSTRACT

Background: Anderson-Fabry disease (AFD) is a cause of unexplained left ventricular hypertrophy and premature stroke. Recent studies suggest that patients develop progressive systolic dysfunction and myocardial fibrosis. We hypothesised that this is secondary to coronary microvascular dysfunction. The aim of this study was to measure coronary flow reserve (CFR), an index of microvascular function, in AFD at baseline and after enzyme replacement therapy (ERT).

Methods and results: Myocardial blood flow (MBF) at rest and during hyperaemia (adenosine 140 µg/kg/min) was measured in 10 male, non-smoking patients (53.8±10.9 years, cholesterol 5.5±1.3 mmol/l) and 24 age matched male, non-smoking controls (52.0±7.6 years, cholesterol 4.5±0.6 mmol/l) using positron emission tomography (PET). Resting and hyperaemic MBF and CFR (hyperaemic/resting MBF) were reduced in patients compared to controls (0.99±0.17 vs. 1.17±0.25 ml/g/min, p<0.05; 1.37±0.32 vs. 3.44±0.78 ml/g/min, p<0.0001 and 1.41±0.39 vs. 3.03±0.85, p<0.0001, respectively). This coronary microvascular dysfunction was independent of cholesterol levels. A repeat PET scan was carried out in 5 patients after 10.1±2.3 months of ERT; resting and hyperaemic MBF and CFR were unchanged after ERT (0.993±0.16 vs. 0.991±0.16 ml/g/min, p=ns; 1.56±0.29 vs. 1.71±0.3 ml/g/min, p=ns and 1.6±0.37 vs. 1.74±0.28, p=ns respectively).

Conclusions: The results of the present study demonstrate that patients with AFD have markedly abnormal coronary microvascular function. Our preliminary data suggest that ERT has no effect on coronary microvascular dysfunction. Further work is necessary to determine whether treatment at an earlier stage in the course of the disease may improve coronary microvascular function in AFD patients.

Key Words: Cardiomyopathy; coronary circulation; myocardial blood flow; myocardial ischaemia; cardiac imaging.

Word Count: 246
**INTRODUCTION**

Anderson-Fabry disease (AFD) is a multisystem disorder caused by an X-linked deficiency of lysosomal α-galactosidase A (A-Gal) that results in renal, cardiac, cerebrovascular disease and premature death. Patients with AFD complain of angina despite angiographically normal coronary arteries. Recent studies have also shown that there is progressive deterioration in left ventricular systolic function and myocardial scarring in patients with AFD cardiomyopathy. We hypothesised that these clinical abnormalities may be caused by coronary microvascular dysfunction.

The aims of this study were to measure coronary flow reserve (CFR), an index of coronary microvascular function, in a consecutive cohort of patients with AFD using positron emission tomography (PET), and to determine the effect of ERT on microvascular abnormalities.

**METHODS**

**Patient and Controls**

Ten non-smoking male patients with AFD (53.8 ± 10.9 years, range 43-82, cholesterol 5.5±1.3 mmol/l) referred to the Heart Hospital, London, UK were studied. The diagnosis of AFD was based on the identification of an α-Gal gene mutation and a low plasma α-Gal (mean 0.45 ± 0.35 nmol/hr/ml, range 0.04-0.99 nmol/hr/ml). Nine patients were identified through screening of patients presenting with unexplained left ventricular hypertrophy (LVH); six of these patients have been reported previously. One patient was referred with an established diagnosis of AFD (# 6), and 1 was identified through family screening; (# 10). At the time of diagnosis, none of these patients were receiving ERT.

All patients had ECG and 2-D/Doppler echocardiography performed using previously described methods. Left ventricular mass (LVM) was calculated by M-mode echocardiography using the Devereux formula and indexed to body surface area. Nine patients had symptoms and/or signs suggestive of myocardial ischaemia and underwent coronary angiography to exclude coronary artery disease.

Twenty four healthy non-smoking age matched males (52.0±8 years, cholesterol 4.5±0.6 mmol/l) with normal electrocardiograms (ECG), and no evidence of cardiac disease served as controls for the MBF and CFR data.

**Enzyme Replacement Therapy**

Five patients (Table 1) received Fabrazyme (Genzyme Corporation, Cambridge MA, USA) at a dose of 1 or 2 mg/kg every two weeks as part of a separate randomised study. Follow up PET scans were obtained 17.1±1.9 months after the baseline scan while patients were on treatment. Plasma globotriaosylceramide (neutral glycosphingolipid) levels were measured using tandem mass spectrometry pre- and post ERT.

The study was approved by the Local Research Ethics Committees and all participants gave written informed consent. Radiation exposure was approved by the UK Administration of Radioactive Substances Advisory Committee (ARSAC).
**Positron Emission Tomography (PET)**

All patients and controls underwent PET scanning to measure MBF at rest and during adenosine-induced hyperaemia (140 µg/kg/min i.v.). Scanning was performed with an ECAT 931-08/12 15-slice tomograph with a 10.5-cm axial field of view (CTI/Siemens, Knoxville, TN). Resting and hyperemic MBF were measured using oxygen-15 labeled water ($H_2^{15}O$) as previously reported\(^8\). Arterial blood pressure was recorded by automatic cuff sphygmomanometer at one-minute intervals and the ECG was monitored continuously throughout the procedure.

**PET Data Analysis**

All emission and transmission data were reconstructed using a Hanning filter with a cut-off frequency of 0.5 units of the reciprocal of the sampling interval of the projection data resulting in an image resolution of 8.4x8.3x6.6 mm full width at half maximum at the centre of the field of view. Myocardial and blood pool images were then generated directly from the dynamic $H_2^{15}O$ study as previously reported\(^9\). Regions of interest were drawn within the left atrium and ventricular myocardium on consecutive image planes. These were projected onto the dynamic $H_2^{15}O$ images to generate blood and tissue time activity curves. Arterial and tissue activity curves were fitted to a single tissue compartment tracer kinetic model to give values of MBF (ml/g/min)\(^9\). Coronary resistance (mmHg/ml/min/g) was calculated as the ratio of mean arterial pressure to MBF and CFR as the ratio of hyperemic MBF to resting MBF.

**Statistical Analysis**

Comparisons between continuous variables were performed using the paired Student t-test (Statview V 5.0, SAS institute Inc.; Cary, NC, USA). Linear regression was performed to assess the relationship between cholesterol levels, LVM index, MBF and CFR. Data are reported as mean ± SD values. A p<0.05 was considered significant.

**RESULTS**

**Patient Characteristics**

The clinical characteristics of the patients are shown in Table 1. Six (60%) patients complained of exertional chest pain. None of the nine patients had angiographically significant coronary artery disease. Three patients had rate responsive dual chamber permanent pacemakers: one for left ventricular outflow tract gradient reduction and two for heart block. None of the patients had a PR interval less than 120ms. Two patients had non-sustained ventricular tachycardia on Holter (# 2 and 3), and 2 were in atrial fibrillation (# 5 and 11). Seven years prior to the diagnosis of AFD, patient #1 had undergone an assessment of coronary sinus pH during dipyridamole infusion\(^10\). The peak change in coronary sinus pH during hyperaemia was -0.086 units, indicating severe myocardial ischaemia.

Serum cholesterol was higher in the patients compared to controls (mean difference 0.97 mmol/l, 95% CI = 0.3-1.6, p = 0.006). Patients also had higher HDL cholesterol levels compared to controls (1.7±0.5 mmol/l vs. 1.1±0.4 mmol/l, respectively, p = 0.002). There were no differences in LDL cholesterol (3.1±0.8 mmol/l vs. 3.7±1.3 mmol/l, patients vs. controls respectively, p = 0.2) and triglyceride levels (1.4±0.7 mmol/l vs. 1.8±1.6 mmol/l, patients vs. controls respectively, p = 0.5).
Two patients had acroparaesthesia, 1 had hypohidrosis and 1 had renal dysfunction (creatinine clearance 52 ml/min). None of the patients were hypertensive and one was a type 2 diabetic. No angiokeratoma were noted in any of the patients. All patients had a maximal left ventricular wall thickness $\geq 13$ mm ($17 \pm 4.0$ mm, range 14-26 mm). Nine patients had concentric left ventricular hypertrophy (LVH) and 1 had asymmetric septal hypertrophy. At the time of study no patient had left ventricular outflow tract obstruction; the mean left ventricular end-systolic, end-diastolic and left atrial diameters were $33 \pm 0.9$, $5.2 \pm 0.8$ and $4.7 \pm 1.1$ mm respectively. The mean LVM index was $234 \pm 81.3$ g/m$^2$ (range 141-407 g/m$^2$).

**Myocardial Blood Flow and Coronary Flow Reserve**

Heart rate and mean arterial blood pressure were similar in controls and AFD patients at rest and during adenosine infusion. The rate pressure product (RPP= systolic blood pressure x heart rate) was similar in the controls and patients during all study conditions.

Resting and hyperaemic MBF and CFR were significantly reduced in AFD patients compared to controls ($0.99 \pm 0.17$ vs. $1.17 \pm 0.25$ ml/g/min, $p<0.05$, $1.37 \pm 0.32$ vs. $3.44 \pm 0.78$ ml/g/min, $p<0.0001$ and $1.41 \pm 0.39$ vs. $3.03 \pm 0.85$, $p<0.0001$), respectively (Figure 1 and table). This reduction in coronary microvascular function was independent of cholesterol and HDL cholesterol levels. Resting coronary resistance was comparable in patients and controls ($82.2 \pm 27.8$ vs. $89.3 \pm 31.2$ mmHg/ml/min/g $p=\text{ns}$ respectively). The minimal resistance during hyperaemia was higher in AFD patients compared to controls ($65.1 \pm 16.9$ vs. $26.5 \pm 6.0$ mmHg/ml/min/g $p<0.0001$). No correlation was found between LVM index and resting or hyperaemic MBF or CFR.

**Response to ERT**

In those patients that underwent a repeat PET scan there was a decrease in plasma globotriaosylceramide with ERT (mean change=5.3 µg/ml, 95% CI = 0.5, 10.1, $p=0.04$). Resting and hyperaemic MBF and CFR, before and after 10.1±2.3 months of ERT were similar ($0.99 \pm 0.16$ vs. $0.99 \pm 0.16$ ml/g/min, $p=\text{ns}$, $1.56 \pm 0.29$ vs. $1.71 \pm 0.3$ ml/g/min, $p=\text{ns}$ and $1.6 \pm 0.37$ vs. $1.74 \pm 0.28$, $p=\text{ns}$ respectively, Figure 2). Resting coronary resistance was similar pre- and post- ERT ($83.9 \pm 18.9$ vs. $90.2 \pm 26.5$ mmHg/ml/min/g, $p=\text{ns}$) respectively. The minimal resistance during hyperaemia was unchanged following ERT ($56.9 \pm 14.7$ vs. $52.8 \pm 18.7$ mmHg/ml/min/g, $p=\text{ns}$). There was no change in LVM index (mean change=0.4±37.8 g/m$^2$, ranging from -66.3 to +26.4, $p=\text{ns}$, Figure 3). There were no changes in the left ventricular cavity dimensions (mean change in end systolic cavity dimension=0.1±0.3mm, $p=\text{ns}$; mean change in end diastolic cavity dimension=0.1±0.2mm, $p=\text{ns}$). There was a trend to a reduction in fractional shortening (mean fractional shortening pre-ERT=34.8±6.7%, mean post-ERT=31.4±6.3%, $p=0.08$). There was no change in the maximal left ventricular wall thickness (mean change=0.02±0.3mm, $p=\text{ns}$).

**DISCUSSION**

This study shows that patients with AFD have a substantial reduction in hyperaemic MBF and CFR compared to normal controls. The significance of these findings for individual patients remains to be determined, but the severity of the microvascular
dysfunction suggests that these abnormalities may have a substantial influence on the natural history of AFD.

**Mechanisms of Microvascular Dysfunction**

Symptoms and signs suggestive of myocardial ischaemia in the absence of coronary disease are frequent in patients with cardiomyopathies and in individuals with left ventricular hypertrophy secondary to pressure overload. Reductions in CFR similar to those seen in this study have been demonstrated in all these conditions, although different mechanisms are involved. In patients with hypertrophic cardiomyopathy, remodelling of the intramural arterioles is important\textsuperscript{11,12}, whereas in aortic stenosis, increased perivascular fibrosis and intramyocardial pressure and reduced diastolic filling are the dominant mechanisms for microvascular dysfunction\textsuperscript{13,14}.

In AFD, a number of mechanisms may contribute to microvascular dysfunction. AFD cardiomyopathy is characterised by GB3 deposition in myocytes, conduction tissue, vascular endothelium and valvular tissue. This is accompanied by secondary changes such as myocyte hypertrophy and fibrosis\textsuperscript{15} which cause raised coronary vascular resistance and increased myocardial oxygen demand. Although endothelial GB3 deposits may lead to microvascular dysfunction, a recent publication has shown that patients with AFD have enhanced nitric oxide independent endothelial function measured using forearm venous plethysmography\textsuperscript{16}. It remains to be seen whether similar abnormalities are seen in coronary microvasculature.

**Response to ERT**

In this study there was a significant reduction in the plasma levels of globotriaosylceramide with ERT, but no improvements in coronary microvascular function or LVM were seen. However, interpretation of this observation is limited by the small size of the cohort and by the fact that the cohort was considerably older, and had more severe cardiac disease compared to patients included in previous trials of ERT\textsuperscript{17,18,19}. Along with an improvement in AFD symptoms these trials have demonstrated a reduction in LVM and an improvement in systolic function with ERT. In order to understand if the impact of ERT on coronary microvascular function a larger study conducted over a longer period of time is required.

**CONCLUSION**

This study shows that microvascular dysfunction is important in the pathophysiology of AFD cardiomyopathy. Further studies are required to assess the impact of enzyme replacement therapy on microvascular function.

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REFERENCES


LEGEND TO FIGURES

**Figure 1**
Myocardial Blood Flow at Rest and During Adenosine-Induced Hyperaemia in Patients with Anderson-Fabry Disease and Controls.

MBF = Myocardial blood flow, REST = MBF at rest, ADO = MBF during adenosine.

**Figure 2**
Myocardial Blood Flow at Rest and During Adenosine-Induced Hyperaemia Pre- and Post Enzyme Replacement Therapy (Fabrazyme™).

MBF = Myocardial blood flow, REST = MBF at rest, ADO = MBF during adenosine.

**Figure 3**
Left Ventricular Mass Index Pre- and Post Enzyme Replacement Therapy (Fabrazyme™).

Left Ventricular Mass Index expressed as g/m², cumulative data represented as mean ± standard deviation (pre-treatment = 226.3±54.1; post treatment = 226.7±39.0, p = ns).
**Table: Patient Characteristics**

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**Legend:** NYHA = New York Heart Association, Max LVWT = maximal left ventricular wall thickness, FS = fractional shortening, %=percentage, CFR = coronary flow reserve, * = 5 patients with follow-up PET scans following Enzyme Replacement Therapy.
Coronary microvascular dysfunction in male patients with Anderson-Fabry disease and the effect of treatment with alpha galactosidase A

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