Chapter 4

Carriers of The Hypertrophic Cardiomyopathy MYBPC3 Mutation are Characterized by Reduced Myocardial Efficiency in The Absence of Hypertrophy and Microvascular Dysfunction


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Abstract

Background: Next to left ventricular (LV) hypertrophy, hypertrophic cardiomyopathy (HCM) is characterized by coronary microvascular dysfunction and reduced myocardial external efficiency (MEE). Insights into the presence of these abnormalities as early markers of disease are of clinical importance in risk stratification, and development of therapeutic approaches. Therefore, the aim was to investigate myocardial perfusion and energetics in asymptomatic HCM mutation carriers.

Methods: Fifteen subjects with a MYBPC3 mutation underwent $[^{15}O]$water positron emission tomography (PET) to assess myocardial blood flow (MBF). $[^{11}C]$acetate PET was performed to obtain myocardial oxygen consumption ($MVO_2$). By use of cardiovascular magnetic resonance (CMR) imaging, LV volumes and mass were defined to calculate MEE, i.e. the ratio between external work and $MVO_2$. Eleven healthy family relatives, all non-carriers, underwent similar scanning protocols to serve as a control group.

Results: LV mass was comparable between HCM carriers and controls 93±25 vs. 99±21 g, $p=0.85$), as was MBF at rest (1.19±0.34 vs. 1.18±0.32 mL min$^{-1}$g$^{-1}$, $p=0.92$), and during hyperemia (3.87±0.75 vs. 3.96±0.86 mL min$^{-1}$g$^{-1}$, $p=0.77$). $MVO_2$ averaged 0.137±0.057 mL min$^{-1}$g$^{-1}$ in carriers and was not significantly different from controls (0.125± 0.043 mL min$^{-1}$g$^{-1}$, $p=0.29$). Cardiac work, however, was slightly reduced in carriers (7398±1384 vs. 9139±2484 mmHg mL in controls, $p=0.08$). As a consequence, MEE was significantly decreased in carriers (27±10 vs. 36±8% in controls, $p=0.02$).

Conclusion: Asymptomatic HCM mutation carriers display reduced myocardial work generation in relation to oxygen consumption, in the absence of hypertrophy and perfusion abnormalities. Hence, impaired myocardial energetics may constitute a primary component of HCM pathogenesis.
Introduction

Hypertrophic cardiomyopathy (HCM) is a genetic cardiac disease associated with mutations in various sarcomeric proteins of the myocardial contractile apparatus, including MYBPC3, βMHC, and troponin T.(1) Next to asymmetrical left ventricular hypertrophy, HCM is characterized by microvascular dysfunction indicated by a reduced coronary flow reserve (CFR) in the absence of obstructive coronary artery disease, the degree of which independently predicts clinical deterioration and death.(2) Furthermore, HCM patients exhibit a reduced energy efficiency of the myocardium, as reflected by a decreased generation of work in relation to oxygen consumption, i.e. myocardial external efficiency (MEE).(3-7) Although prognostic data related to an impaired energetic state in HCM are lacking, in analogy with dilated cardiomyopathy, it is believed to be of prognostic relevance as well.(8,9) Insight into the presence of microvascular dysfunction and reduced energy efficiency as early markers of disease and their relation to the onset of the HCM phenotype are of clinical importance in risk stratification, and the development of therapeutic approaches. Indeed, preliminary data have revealed a decreased phosphocreatin-in-to-adenosine triphosphate (PCr/ATP) ratio in HCM patients without LVH by use of $^{31}$P spectroscopy,(10) suggesting that a compromised energetic state may constitute an early component of the HCM phenotype. However, complementary studies regarding myocardial blood flow (MBF) are warranted as microvascular dysfunction is common in HCM, even in the absence of hypertrophy,(11) and repetitive stunning or myocardial hibernation due to ischemia are associated with reduced energy efficiency as well.(12,13) Therefore, the aim was to investigate myocardial energetics in asymptomatic HCM mutation carriers without hypertrophy, together with myocardial perfusion and contractile function by use positron emission tomography (PET) and cardiovascular magnetic resonance imaging (CMR).

Methods

Fifteen genotype-positive, phenotype-negative subjects (carriers) were enrolled whose LV wall thickness was less than 10 mm, as measured by 2D-echocardiography. Carriers were first-degree relatives of symptomatic HCM patients and recruited after genetic testing at a cardiogenetic outpatients’ clinic. All had a mutation in the cardiac myosin binding protein C (MYBPC3) gene. None of the carriers had systemic or cardiac disease, which might attribute to the development of LVH. Eleven family relatives of the carriers without the pathogenic mutation were included as a control group. All study subjects had normal physical examination, normal ECG, and were without relevant medical history. The imaging protocol consisted of PET and CMR. The study was
approved by the medical ethics committees and all patients gave written informed consent.

**Imaging Protocol**

*PET*

All scans were performed in 2D mode, using an ECAT EXACT HR+ (Siemens/CTI, Knoxville, Tennessee, USA). The protocol was performed as previously described,(14,15) In short, after overnight fasting, myocardial blood flow (MBF) was measured using 1100 MBq of oxygen-15-labeled water under resting conditions and during pharmacologically induced hyperemia with adenosine (140 μg·kg⁻¹·min⁻¹), and oxidative metabolism was assessed using 550 MBq [¹¹C]acetate.

Data were transferred to a SUN workstation (SUN Microsystems Inc.) and analyzed using Siemens/CTI software, together with additional tracer kinetic analysis software developed in MATLAB. Transaxial parametric MBF images were generated as described previously,(16) as well as maximum intensity [¹¹C]acetate uptake images. Subsequently, these images were reoriented according to the anatomic axis of the heart and slices were displayed as short-axis slices. The same reslicing parameters were applied to the dynamic [¹⁵O]water and [¹¹C]acetate images. Regions of interest (ROIs) were defined on these images corresponding to septal, anterior, lateral, and inferior walls of the left ventricle in the basal, mid, and apical planes.(14) Additional ROIs were defined in the left atrial and right ventricular chamber. This latter set of ROIs was projected onto the dynamic images in order to generate image derived input functions. Using a standard single tissue compartment model together with these input functions, MBF (mL·min⁻¹·g⁻¹ of perfusable tissue) was determined for all myocardial tissue time activity curves (TAC).(17) Average MBF was calculated by grouping all ROIs, whereas the regional blood flows were calculated by grouping specific ROIs. Corrections were made for left and right ventricular spillover effects by use of the method described by Hermansen et al.(18) Resting coronary microvascular resistance (CMVR) was calculated by dividing mean arterial pressure (MAP) with MBF, whilst minimal CMVR was derived in a similar fashion, only during administration of adenosine.(19)

Using automated analyses to determine the linear myocardial wash-out part of the [¹¹C]acetate TAC as previously described,(20) $k_{\text{mono}}$ was determined as an index of myocardial oxidative metabolism. To derive $\text{MVO}_2$ from average $K_{\text{mono}}$, a relationship between $K_{\text{mono}}$ and myocardial oxygen metabolism (mL·min⁻¹·g⁻¹),
previously established in humans, was used, where $K_{\text{mono}} = 0.0027(M\text{VO}_2) + 0.0197$.\(^{(21)}\)

**CMR**

CMR studies were performed on a 1.5-Tesla whole body scanner (Magnetom Sonata, Siemens, Erlangen, Germany), using a six-channel phased-array body coil. After survey scans, a retro-triggered, balanced steady-state free precession gradient-echo sequence was used for cine imaging. Image parameters were: slice thickness 5 mm, slice gap 5 mm, temporal resolution < 50 ms, repetition time 3.2 ms, echo time 1.54 ms, flip angle 60 degrees and a typical image resolution of 1.3*1.6 mm. The number of phases within the cardiac cycle was set at twenty.

After the 4-, 3-, and 2-chamber view cines were obtained, a stack of 6-10 transversely oriented slices was planned on an end-diastolic (ED) 2-chamber view at the level of the lower leading edge of the mitral valve annulus to cover the left atrium (LA).\(^{(22)}\) Subsequently, a stack of 10-12 short axis slices were acquired for full coverage of the LV.\(^{(23)}\) Cine images were acquired during one breath-hold in mild expiration. Left ventricular volume analysis was performed by manually drawing epicardial and endocardial contours on all ED and ES LV short-axis images. Global LV function parameters, including ED volume (LVEDV), ES volume (LVESV), and LV mass (LVM), were then derived from the cine images with use of the MASS software package (MEDIS, Leiden, The Netherlands). The forward stroke volume (fSV) was obtained from the velocity-encoded phase-contrast aortic flow maps by dividing the forward cardiac output by HR.

Cine imaging with myocardial tagging was applied to create noninvasive markers (tags) within the myocardium for the calculation of strain.\(^{(24)}\) Five short-axis tagged images with complementary spatial modulation of magnetization tagging for improved strain calculations were acquired as previously described.\(^{(25)}\) The tagging images were used to generate circumferential strain curves for each myocardial segment. Subsequently, $E_{cc}$, which indicates maximum contractile function, was derived for each segment from the strain curves.\(^{(25)}\) Similar segmentation for average and regional analyses of $E_{cc}$ was used as described for the PET data. Since circumferential shortening is determined by the shortening of myofibers, $E_{cc}$ is expressed as a negative value.

**Myocardial external efficiency**

MEE was calculated according to the equation depicted below.\(^{(4)}\) External work (EW) was defined as the product of fSV derived by MRI and mean arterial
pressure (MAP). The caloric equivalent of 1 mmHg•mL is $1.33\cdot10^{-4}$ J, whereas 1 mL O$_2$ is $\approx$ 20 J.(26)

$$\text{MEE} = \frac{\text{EW} \cdot \text{HR} \cdot 1.33 \cdot 10^{-4}}{\text{MV}_2 \cdot \text{LVM} \cdot 20}$$

Statistics

Data were expressed as mean ± SD. For comparison of two data sets, a paired or unpaired Student’s t test was performed where appropriate. For the comparison of multiple data sets, one-way analysis of variance (ANOVA) was applied with post hoc Bonferroni adjustment for inequality. All analyses were performed using SPSS 14 (SPSS Inc., Chicago, Illinois, USA). A $p$ value < 0.05 was considered statistically significant.

Results

Baseline characteristics

Study group characteristics are shown in table 1. The mean age of subjects in the carrier group was significantly lower compared to the control group, as was the percentage of men (both $p < 0.001$). LV mass, volumes and ejection fraction were not significantly different between groups.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Carriers</th>
<th>Controls</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>4 men (27%)</td>
<td>7 men (64%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>35 ± 11</td>
<td>53 ± 3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>LVM (g)</td>
<td>93 ± 25</td>
<td>99 ± 21</td>
<td>0.85</td>
</tr>
<tr>
<td>LVEDV (mL)</td>
<td>172 ± 31</td>
<td>186 ± 32</td>
<td>0.53</td>
</tr>
<tr>
<td>LVESV (mL)</td>
<td>68 ± 16</td>
<td>73 ± 21</td>
<td>0.80</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>52 ± 7</td>
<td>54 ± 10</td>
<td>0.58</td>
</tr>
</tbody>
</table>

Table 1. Baseline characteristics. LVM, left ventricular mass; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; LVEF, left ventricular ejection fraction.
Hemodynamics

The hemodynamics of both study groups are depicted in table 2. Heart rate, blood pressure, and the RPP were comparable between carriers and controls, both at rest and during hyperemia. Heart rate and the RPP were higher during the adenosine studies as compared to rest (both \( p < 0.001 \), for both groups).

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Hyperemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carriers</td>
<td>Controls</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>119 ± 16</td>
<td>123 ± 11</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>68 ± 8</td>
<td>72 ± 8</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>85 ± 10</td>
<td>89 ± 9</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>64 ± 9</td>
<td>68 ± 11</td>
</tr>
<tr>
<td>RPP</td>
<td>7639 ± 1620</td>
<td>8350 ± 1759</td>
</tr>
</tbody>
</table>

Table 2. Hemodynamic parameters obtained during PET studies. *BP, blood pressure; MAP, mean arterial pressure; RPP, rate-pressure product.*

Transmural MBF

Table 3 represents the mean MBF at rest and during adenosine for both study groups. In the carrier group, resting MBF averaged 1.19 ± 0.34 mL min\(^{-1}\) g\(^{-1}\) and was comparable to the control group (1.18 ± 0.32 mL min\(^{-1}\) g\(^{-1}\), \( p = 0.94 \)). Hyperemic MBF in the carrier group averaged 3.87 ± 0.75 mL min\(^{-1}\) g\(^{-1}\), which not significantly different from the control group (3.96 ± 0.86 mL min\(^{-1}\) g\(^{-1}\), \( p = 0.77 \)). Consequently, the CFR was comparable between the carriers and controls (3.37 ± 0.71 vs. 3.64 ± 0.92, \( p = 0.40 \), respectively). In both groups, resting and hyperemic MBF were distributed homogeneously across all regions. In addition, no significant differences were found between groups on a regional level.
<table>
<thead>
<tr>
<th>Region</th>
<th>Carriers</th>
<th>Controls</th>
<th>Carriers</th>
<th>Controls</th>
<th>Carriers</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior</td>
<td>1.21 ± 0.32</td>
<td>1.44 ± 0.55</td>
<td>3.42 ± 0.84</td>
<td>3.46 ± 1.03</td>
<td>-16.2 ± 3.0</td>
<td>-16.7 ± 2.7</td>
</tr>
<tr>
<td>Lateral</td>
<td>1.23 ± 0.35</td>
<td>1.26 ± 0.33</td>
<td>4.00 ± 0.91</td>
<td>4.21 ± 0.97</td>
<td>-17.9 ± 2.8*</td>
<td>-18.7 ± 3.0*</td>
</tr>
<tr>
<td>Inferior</td>
<td>1.06 ± 0.34</td>
<td>0.96 ± 0.25</td>
<td>3.52 ± 0.93</td>
<td>3.65 ± 1.11</td>
<td>-16.5 ± 3.5</td>
<td>-17.2 ± 3.4</td>
</tr>
<tr>
<td>Septal</td>
<td>1.13 ± 0.33</td>
<td>1.08 ± 0.32</td>
<td>3.98 ± 0.94</td>
<td>3.98 ± 1.19</td>
<td>-16.7 ± 3.2</td>
<td>-16.8 ± 3.2</td>
</tr>
</tbody>
</table>

**Table 3.** Regional MBF and contractile function data. In the carrier group, the \( p \) values (as determined by ANOVA) for MBF, hMBF, \( E_{cc} \) were: \( p = 0.49, p = 0.18, \) and \( p = 0.04, \) respectively. In the control group, the \( p \) values (as determined by ANOVA) for MBF, hMBF, \( E_{cc} \) were: \( p = 0.09, p = 0.45, \) and \( p = 0.02. \)

*\( p < 0.05 \) versus anterior
Coronary microvascular resistance

CMVR at rest was not significantly different between carriers and controls (77 ± 24 mmHg•ml⁻¹•min⁻¹ vs. 80 ± 20 mmHg•ml⁻¹•min⁻¹, p = 0.74), whereas minimal CMVR was also comparable between groups (22 ± 5 mmHg•ml⁻¹•min⁻¹ vs. 23 ± 5 mmHg•ml⁻¹•min⁻¹, p = 0.69, respectively).

Contractile parameters

Regional $E_c$ values are depicted in table 3. $E_c$ in the carrier group averaged -17.0 ± 3.2%, which was not significantly different from controls (-17.5 ± 3.2%, p = 0.69). Interestingly, $E_c$ was distributed heterogeneously across the myocardium in both groups, with increased contractile function in the lateral region as compared to the anterior region (p < 0.05 in both). Between groups, however, no significant differences were found on a regional level.
Myocardial oxygen consumption, external work and myocardial efficiency

Estimated MVO$_2$ averaged $0.137 \pm 0.057$ mL$\cdot$min$^{-1}\cdot$g$^{-1}$ in the carriers, which was not significantly different from the controls ($0.125 \pm 0.043$ mL$\cdot$min$^{-1}\cdot$g$^{-1}$, $p = 0.29$, Fig. 2A). EW was $7398 \pm 1384$ mmHg$\cdot$mL in the carriers as opposed to $9139 \pm 2484$ mmHg$\cdot$mL in the controls, reaching a borderline significance of $p = 0.08$ (Fig. 2B). As a consequence, MEE was significantly lower in the carriers ($27 \pm 10\%$), as compared to the controls ($36 \pm 8\%$, $p = 0.02$, Fig. 2C).

Figure 2. Scatter plots depicting myocardial oxygen consumption (A), external work (B), and myocardial external efficiency (C) values for carriers and controls.
Discussion

It was found that myocardial external efficiency, as defined by the ratio between cardiac work and oxygen consumption, is reduced in HCM mutation carriers without LVH. An important supplementary finding of this study is that myocardial perfusion was comparable to healthy subjects, both on a global and regional level. Furthermore, the tissue tagging studies showed normal global and regional systolic function. Hence, these results provide evidence that impaired myocardial energetics constitute an early component of the HCM phenotype, preceding the onset of ventricular hypertrophy, microvascular dysfunction and contractile dysfunction.

Myocardial blood flow

In HCM patients, the presence of coronary microvascular dysfunction results in a reduced hyperemic MBF, and has been associated with a relative decrease in capillary density due to extensive hypertrophy, i.e. vascular rarefaction,(26) in combination with a blunted maximal vasodilatory capacity of intramural arterioles.(27) Impaired hyperemic MBF values, however, are also seen in myocardial segments with normal wall thickness, suggesting that microvascular dysfunction is an early and widespread phenomenon in HCM, also affecting non-hypertrophied areas.(11,28) Reductions in CFR have even been reported in carriers, and has therefore been suggested as an early marker of disease expression in HCM pathogenesis.(29) This study, however, revealed that MBF at rest and during pharmacologically induced vasodilation was similar between carriers of the MYBPC3 mutation and controls. In addition, CMVR was also comparable between study subjects, thereby excluding autoregulation of the microvascular bed and coronary driving pressure as confounders of myocardial perfusion.(19) Hence, these results strongly suggest a normal micro- and macrocirculatory function of the heart in carriers.

Contractile function

Next to alterations in myocardial perfusion, HCM patients are also characterized by regional contractile dysfunction, especially of the hypertrophied septum.(28,30,31) In contrast, the tissue tagging studies in the carriers showed normal contractile function, both on a global and regional level. Although some regional heterogeneity in contractile function was present, i.e. circumferential shortening was highest in the lateral wall, a similar pattern was observed in the control group, and has been noted previously in healthy volunteers.(32)
Myocardial efficiency

To date, noninvasive in vivo studies of myocardial energetics in HCM have predominantly focused on subjects with established hypertrophy. (3-7,33) In general, these studies have shown that myocardial efficiency is reduced due to an increased cost-to-force ratio in terms of energy, presumably the result of inefficient substrate metabolism and ATP wastage by the cardiomyocyte. (34,35) Previously, Crilley and coworkers investigated myocardial energetics in HCM patients with various degrees of LVH by use of phosphorus-31 MR spectroscopy. (10) It was shown that HCM subjects without LVH also exhibit reduced PCr/ATP ratio’s, suggesting that abnormal cardiac energetics may be an early feature of HCM manifestation. In contrast to this study however, measurements of myocardial perfusion and contractile function were not performed. This seems warranted, inasmuch as repetitive stunning due to myocardial ischemia has also been associated with alterations in myocardial metabolism, and longterm microvascular dysfunction in HCM may therefore hamper metabolic, and thus mechanical efficiency as well, even in asymptomatic subjects without hypertrophy. Correspondingly, contractile dysfunction due to ischemia or cardiomyocyte disarray also render the heart mechanically less efficient. (36,37) Although the presence of coronary arteriole remodeling and disarray in preclinical HCM patients remains unknown due to ethical objections regarding cardiac biopsies, normal in vivo myocardial perfusion and contractile function strongly suggest that the observed reduction in myocardial efficiency cannot be attributed to these morphological aberrations. Consequently, these results corroborate the hypothesis that compromised myocardial energetics constitute a central role in HCM pathogenesis. (38)

Nevertheless, it should be noted that myocardial energy efficiency in carriers appears to be less impaired compared to symptomatic HCM patients with LVH, where MEE ≈ 21%. (4) This suggests that the onset of hypertrophy, microvascular dysfunction and myofiber disarray during the natural course of disease may further exacerbate myocardial energy deficiency.

Clinical implications

The results suggest a therapeutic potential for myocardial energy modulators in ameliorating myocardial energetics and preventing the pathophysiological cascade in HCM. Indeed, a recent study performed by Abozguia and coworkers showed that enhancement of myocardial carbohydrate usage, by inhibition of fatty acid metabolism with perhexilin, resulted in improvement of myocardial energetics and exercise capacity in symptomatic HCM patients. (39) Despite these promising results, however, future interventional studies with pro-
energetic agents in asymptomatic carriers are warranted, in addition to the long-
term effect on delaying or preventing the onset of manifest HCM.

Technical considerations

Although LVM is related to indices of body size, no such corrections were
performed during the calculation of MEE. It should be noted, however, that LVM
is also related to SV, assuming that the LV is not hypertrophied or dilated. For
instance, a larger, yet structurally normal LV will deliver a greater amount of
work per cardiac cycle, defined as EW in the numerator of the formula. Correspondingly, however, this will also be accompanied by a proportional
increase in total LV oxygen consumption, as defined in the denominator. Hence,
interindividual differences in LV size theoretically do not affect the ratio of
energy expenditure and consumption.

Limitations

Due to the relatively small sample sizes of both study groups, the statistical
results should be interpreted with certain care. However, this is the first study to
simultaneously assess myocardial energetics, perfusion and function in a cohort
of carriers, and the results clearly demonstrate a significant reduction in energy
efficiency in these subjects. In addition, the carriers were significantly younger
hence partially limiting the inferences that can be drawn from the comparison of
myocardial perfusion values with the control subjects. A previous study by Uren
and coworkers, however, showed that hyperemic myocardial blood flow (hMBF)
values are fairly consistent among healthy subjects up to 60 years of age. Only
above 70 years, apparent declination of hyperemic flow was observed.(40)
Nonetheless, the current results need validation in a larger study cohort,
preferably in comparison with age and sex matched control subjects. Finally,
non-invasive calculation of oxygen consumption and work by PET and CMR,
respectively, are hampered by several factors. In the present study a relationship
obtained in healthy subjects was used to calculate MVO₂ from [¹¹C]acetate
clearance rates. Whether this relationship is valid in HCM carriers is unkown,
since $K_{\text{mono}}$ is, among multiple factors, dependent on metabolic conditions of the
myocardium. Furthermore, EW is determined non-invasively in the present
study and represented as a rectangle (i.e. SV • MAP), also including the area
under the end-diastolic pressure volume relationship (EDPVR) curve. This
results in overestimation of EW and consequently MEE values, especially in
patients with decreased LV diastolic elastance, which has been previously
observed in HCM carriers.(41)
Conclusion

Phenotype-negative MYBPC3 mutation carriers show reduced myocardial work generation in relation to oxygen consumption, in the absence of left ventricular hypertrophy and myocardial flow or functional abnormalities. Hence, impairment of myocardial energetics precedes the onset of the HCM phenotype, and may constitute a primary component of HCM pathogenesis.
Reference list


