Peer reviewed article

Myocardial contrast echocardiography for the distinction of hypertrophic cardiomyopathy from athlete's heart and hypertensive heart disease

Andreas Indermühle, Rolf Vogel, Tobias Rutz, Pascal Meier, Christian Seiler

Department of Cardiology, University Hospital, Bern, Switzerland

Summary

Background: Myocardial contrast echocardiography (MCE) is able to measure *in vivo* relative blood volume (rBV, i.e., capillary density), and its exchange frequency β, the constituents of myocardial blood flow (MBF, ml min^{-1} g^{-1}). This study aimed to assess, by MCE, whether left ventricular hypertrophy (LVH) in hypertrophic cardiomyopathy (HCM) can be differentiated from LVH in triathletes (athlete's heart, AH) or from hypertensive heart disease patients (HHD).

Methods: Sixty individuals, matched for age (33 ± 10 years) and gender, and subdivided into four groups (n = 15) were examined: HCM, AH, HHD and a group of sedentary individuals without LVH (S). rBV (ml ml^{-1}), β (min^{-1}) and MBF, at rest and during adenosine-induced hyperaemia, were derived by MCE in mid septal, lateral and inferior regions. The ratio of MBF during hyperaemia and MBF at rest yielded myocardial blood flow reserve (MBFR).

Results: Septal wall rBV at rest was lower in HCM (0.084 ± 0.023 ml ml^{-1}) than in AH (0.151 ± 0.024 ml ml^{-1}, p <0.01) and in S (0.129 ± 0.026 ml ml^{-1}, p <0.01), but was similar to HHD (0.097 ± 0.016 ml ml^{-1}). Conversely, MBFR was lowest in HCM (1.67 ± 0.93), followed by HHD (2.8 ± 0.93, p <0.01), by S (3.36 ± 1.03, p <0.001) and by AH (4.74 ± 1.46, p <0.0001). At rest, rBV <0.11 ml ml^{-1} accurately distinguished between HCM and AH (sensitivity 99%, specificity 99%), similarly MBFR ≤1.8 helped to distinguish between HCM and HHD (sensitivity 100%, specificity 77%).

Conclusions: rBV at rest, most accurately distinguishes between pathological LVH due to HCM and physiological, endurance-exercise induced LVH.

Key words: hypertrophic cardiomyopathy; athlete's heart; capillaries; blood flow; vasodilation; ultrasound

Introduction

Hypertrophic cardiomyopathy (HCM) is diagnosed by the echocardiographic identification of a hypertrophied, non-dilated left ventricle (LV), in the absence of other cardiovascular diseases causing similar degrees of LV hypertrophy (LVH) [1, 2]. In adults, the most frequent disorder responsible for LVH is arterial hypertension; far less prevalent etiologies include myocardial storage diseases such as amyloidosis, hemochromatosis, or Anderson-Fabry disease, neuromuscular disorders, endocrinopathies, Noonan's or LEOPARD syndrome [2].

Conversely, physiological LVH in response to intense physical endurance exercise training may also closely resemble HCM. However, the distinction between HCM and physiological LVH (i.e., athlete's heart) is crucial, because the former accounts for approximately one third of exercise-induced sudden cardiac deaths in young, trained athletes [3]. In the attempt to determine whether LVH reflects a pathological process, such as in HCM, or a physiological, benign adaptation to training, no *single* test has, so far, been available [4,

Supported by a grant from the Swiss Heart Foundation.

List of abbreviations

HCM	hypertrophic cardiomyopathy
LV	left ventricle
LVH	left ventricular hypertrophy
MCE	myocardial contrast echocardiography
MBF	myocardial blood flow
MBFR	Myocardial blood flow reserve
rBV	relative blood volume
ROC	Receiver-operating-characteristics
ROI	regions of interest

5]. Approximately ten parameters have been proposed to distinguish between the two entities, such as gender, family history of HCM, the pattern of LVH, LV cavity and left atrial size, LV filling patterns, LV wall thickness change after physical deconditioning, and oxygen uptake.

Aside from the macroscopic structure of HCM as more or less asymmetric LVH, it is also a disease of the microcirculation. Histologically, HCM is characterized by myocyte disarray with an extended variation of their size and shape, as well as the presence of abnormal intercellular connections [6, 7]. Such a disordered histological architecture is related to expansion of the interstitial compartment and areas of replacement fibrosis [4], which is the basis for the macroscopic feature of LVH. Also, HCM is commonly associated with small vessel disease, in which intramural coronary arterioles are narrowed by medial hypertrophy [2, 7, 8]. Both of the mentioned microscopic aspects of HCM can be intuitively linked to structural as well as functional microvascular abnormalities detectable by contrast echocardiography [9]. Obviously, histological data for athlete's hearts, analogous to those described above for HCM, are not available, as they are only obtainable after the event of an athlete's sudden death (annual incidence of 1:200'000 participants of sport events) [3]. In a porcine model, cross-sectional vascular bed area increased by 37% after 36 weeks of exercise training and thus it may be reasoned that capillary density may increase in endurance-exercise induced LVH [10].

Recently, it has been demonstrated that myocardial contrast echocardiography (MCE) is able to quantify myocardial blood flow (MBF) via determination of its microvascular constituents, relative blood volume (rBV) and its exchange frequency β (MBF = rBV x β / tissue density [1.05g ml^{-1}]) [11]. rBV corresponds to the intravascular volume fraction and reflects capillary density, whereas β is the turnover of rBV and a measure of the circulatory conductance to MBF. In this context, the hypothesis of the present study was that MBF or its constituents are able to accurately differentiate between HCM and athlete's heart as well as hypertensive heart disease.

Materials and methods

Study population

Sixty individuals (age 33 ± 9 years, 56 men, 4 women) with LVH (n = 45) or with entirely normal hearts (control group; n = 15) were included in the study. Aside from the group with normal hearts, the three groups with LVH comprised of 15 individuals each, with hypertrophic cardiomyopathy, with physiological LVH in the context of physical endurance exercise training (athlete's heart), and with hypertensive heart disease. HCM and athlete's heart were defined as the presence of echocardiographically determined LVH, without identifiable cause and in the presence of endurance exercise, respectively. The group of patients with HCM was recruited from our HCM database (n~70) and matched for age and gender to the following previously examined groups [12]: semi-professional triathletes (athlete's heart group) selected from a respective sports team (n = 29); patients with arterial hypertension and LVH (hypertensive heart disease group); and sedentary individuals (control group) with normal hearts recruited from the hospital staff, not engaged in regular exercise training and without arterial hypertension or LVH. The study participants had no cardiovascular risk factors except for arterial hypertension in the subjects with hypertensive heart disease. Valvulopathies or shunts were excluded by baseline echocardiography.

The investigation conforms with the principles outlined in the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the Canton of Bern, Switzerland, and all the participants gave written informed consent to participate in the study.

Study protocol

All subjects abstained from methyl-xanthines, including caffeine, 24 hours before the start of the protocol and were studied after an overnight fasting period. Baseline echocardiography was performed in all participants to exclude relevant valvulopathies or shunts. Left ventricular diastolic dimensions were measured from standard two-dimensional guided M-mode registrations. LVH was defined as follows: according to the Teichholz formula as LV mass index ≥134g m^{-2} in men and ≥109 g m^{-2} in women; as a ratio of end-diastolic septal plus posterior wall thickness to LV cavity dimension at end-diastole >0.45; or as an end-diastolic septal or posterior wall thickness >12 mm. The study individuals subsequently underwent MCE at rest and during adenosine-induced hyperemia. MCE was obtained from apical two and four chamber views.

Myocardial contrast echocardiography

A Sequoia C512 ultrasound scanner (Siemens Medical Solutions, Mountain View, California, USA) equipped with a 4V1c transducer and the contrast echo software Coherent Pulse Sequences® was used. The machine settings were as follows: mechanical index (MI) for microsphere detection 0.13, mechanical index for microsphere destruction 1.3, dynamic range 50 dB, linear post-processing, clip length 300 and 200 frames with intervals of 75 ms for rest and stress imaging, respectively. Refill sequences were generated using the manual bubble destruction feature of the scanner and captured digitally for offline quantification.

The ultrasound contrast agent V08DA (SonoVue, Bracco SA, Mendrisio, Switzerland) was infused into the right cubital vein at a constant rate of 0.5–1 ml min^{-1}. At rest, saline was infused at a rate of 2.8 ml $kg^{-1} \cdot h^{-1}$ as a substitute for the adenosine infusion, in order to have the same steady state concentration of the contrast agent in the intravascular compartment as during hyperemia. When stable myocardial enhancement was reached, the contrast infusion rate was kept constant, and baseline image acquisition was performed. Steady state and refill sequences of contrast agent were derived before and after microsphere destruction, respectively. After completion of resting perfusion sequences, contrast and saline infusion were stopped, and hyperemia was induced by intra-

venous adenosine 140 μg · kg^{-1} · min^{-1} (adenosine 3 mg ml^{-1} diluted in NaCl 0.9%) in a parallel port resulting in an infusion rate of 2.8 ml · kg^{-1} · h^{-1}. After 4 minutes of adenosine infusion, contrast infusion was started at the same rate as during resting conditions. After 6 minutes of adenosine infusion, hyperemia perfusion sequences were obtained.

Data analysis

Offline image analysis and quantification were done with DataPro® 2.11 software (Noesis S.A., Courtaboeuf, France). Logarithmic signal compression was removed, and linearized signal intensity data were expressed in arbitrary units. MBF was calculated as previously described and validated by our group [11]. Corresponding to the territories of the main coronary arteries, regions of interest (ROI) were placed in mid-septal, mid-lateral and mid-inferior segments. The ROIs were tracked manually within the myocardium and in the adjacent LV cavity. Myocardial intensity data were corrected for non-contrast signals arising from the tissue, by subtracting the signal intensity of the first frame after manual bubble destruction. Myocardial plateau signal intensity A was calculated by averaging the myocardial signal intensity data from end-systolic frames before manual bubble destruction. Averaging adjacent LV signal intensities of all end-systolic frames, except those *during* and the first *after* manual bubble destruction, yielded the signal intensity in the nearby LV A_{LV}; rBV in ml ml^{-1} was calculated according to the following equation:

$$rBV = A / A_{LV}.$$

The exchange frequency β (min^{-1}) was derived from the fitting of myocardial intensity data after MBD to the following refill equation:

$$y(t) = A\,(1\text{-}e^{-\beta t}).$$

The product of rBV and β divided by tissue density yielded MBF. Myocardial blood flow reserve (MBFR) was calculated as the ratio of hyperemic and resting MBF.

Statistics

Data are expressed as mean value ± standard deviation. All statistical tests were two-sided. As groups were matched, repeated-measures analysis of variance was performed for continuous participant characteristics, echocardiographic, and MCE data. In case of significance, paired student *t*-test was used for *post-hoc* analysis. For analyses of categorical data, the Friedman test for several paired groups was used. Uncorrected *p* values are given for inter-group comparisons. Receiver-operating-characteristics (ROC) analysis was performed to assess discriminatory accuracy of predictors for the differentiation between the groups. Statistical significance was defined at $p < 0.05$. Statistical analyses were performed using the software package SAS® (version 9).

Results

Participant characteristics and clinical data

The characteristics of the study population are shown in table 1. Body mass index was higher in the group of hypertensive patients than in the other groups. The following parameters were different between the groups at rest, as well as during hyperemia: heart rate (lowest in the athlete's group), blood pressure (highest in the hypertensive group). Heart rate increased significantly in all groups ($p = 0.001$; $p = 0.022$; $p = 0.025$; $p = 0.013$) but was not different between the groups. Systolic blood pressure decreased in all but the control group ($p = 0.027$; $p = 0.001$; $p = 0.03$; $p = 0.219$), and there was no significant difference between the groups, apart from a more significant decrease in the athletes compared to the group with HCM ($p = 0.01$). By definition, the occurrence of systemic hypertension was present in the group with hypertensive heart disease but apart from this none of the study participants had any cardiovascular risk factor.

Individuals in the two patient groups were treated significantly more often with cardiovascular drugs than subjects in the two groups without cardiovascular disease (athlete's heart and normal heart), the latter of whom took none. Patients

Table 1

Participant characteristics and clinical data.

	Hypertrophic cardiomyopathy	Athlete's heart	Hypertensive heart disease	Normal heart	P
Number of individuals	15	15	15	15	
Age (years)	35 ± 11	33 ± 9	33 ± 9	32 ± 8	.87
Male gender	14	14	14	14	1.0
Body mass index (kg m^{-2})	25 ± 6 ^{A*}	22 ± 1	27 ± 4 ^{A**,N**}	23 ± 3	.0027
Rest					
Heart rate (beats min^{-1})	69 ± 11^{A**}	56 ± 5^{N**}	65 ± 10^{A*}	67 ± 10	.0024
Systolic blood pressure (mm Hg)	117 ± 11^{N*}	122 ± 9	142 ± 17^{A**,H**,N**}	111 ± 10	<.0001
Diastolic blood pressure (mm Hg)	70 ± 11	73 ± 9^{N*}	82 ± 16^{H*,N*}	64 ± 11	.0017
Hyperemia (adenosine)					
Heart rate (beats min^{-1})	81 ± 17^{A*}	68 ± 13^{N**}	90 ± 13^{A**}	82 ± 13	.0009
Systolic blood pressure (mm Hg)	115 ± 12	114 ± 11	134 ± 9^{A**,H**,N**}	109 ± 10	<.0001
Diastolic blood pressure (mm Hg)	71 ± 7^{N*}	66 ± 12^{N*}	76 ± 9^{A**,N**}	62 ± 11	.0013

Abbreviations: A: comparison with "athlete's heart"; H: comparison with "HCM" respectively "HHD";
HCM: hypertrophic cardiomyopathy; HHD: hypertensive heart disease; N: comparison with "normal heart".
Values are numbers or means ± SD; *: $p < .05$; **: $p < .01$

Table 2

Doppler echocardiographic data.

	Hypertrophic cardiomyopathy	Athlete's heart	Hypertensive heart disease	Normal heart	P
LV ejection fraction (%)	64 ± 11	65 ± 3	64 ± 4	65 ± 3	.82
LV end-diastolic (ED) diameter (mm)	41 ± 10^{A**,N*,N**}	50 ± 4	46 ± 6	49 ± 4	.0022
Interventricular septum (ED, mm)	19 ± 4^{A**,H**,N**}	13 ± 2^{N**}	16 ± 3^{A*,N**}	10 ± 1	<.0001
Posterior wall (ED, mm)	12 ± 3^{H*,N**}	12 ± 2^{N**}	14 ± 2^{A*,N**}	10 ± 1	<.0001
Septal-to-posterior wall ratio (ED)	1.7 ± 0.5^{A**,H**,N**}	1.1 ± 0.2	1.2 ± 0.2	1.1 ± 0.1	<.0001
LV mass index (g m^{-2})		134 ± 15^{N**}	140 ± 30^{N**}	94 ± 13	<.0001
Left atrial diameter index (mm m^{-2})	22.5 ± 3.9 ^{H*,N*}	21.4 ± 2.7^{H*}	19.5 ± 2.2	18.9 ± 2.4	.004
Early (E') mitral annular velocity (cm s^{-1})	6.6 ± 2.0^{A**,N**}	12.1 ± 2.4	7.9 ± 1.6^{A**,N**}	10.5 ± 1.6	<.0001

Abbreviations: A: comparison with "athlete's heart"; H: comparison with "HCM" respectively "HHD"; HCM: hypertrophic cardiomyopathy; HHD: hypertensive heart disease; LV: left ventricular; N: comparison with "normal heart"; eValues are means ± SD.*: p <.05; **: p <.01

Table 3

Myocardial contrast echocardiography data.

	Hypertrophic cardiomyopathy	Athlete's heart	Hypertensive heart disease	Normal heart	P
Septal wall region					
rBV, rest (ml ml^{-1})	0.084 ± 0.023^{A**,N**}	0.151 ± 0.024^{N*}	0.097 ± 0.016^{A**,N*}	0.129 ± 0.026	<.0001
β, rest (min^{-1})	10.9 ± 5.4^{A**}	6.9 ± 2.1	11.4 ± 4.5^{A**}	9.2 ± 2.9	.0124
MBF, rest (ml min^{-1} g^{-1})	0.89 ± 0.51	0.95 ± 0.26	0.93 ± 0.37	1.11 ± 0.36	.44
rBV, hyperemia (ml ml^{-1})	0.092 ± 0.030^{A*,N**}	0.162 ± 0.045	0.106 ± 0.024^{A*,N*}	0.169 ± 0.036	<.0001
β, hyperemia (min^{-1})	15.0 ± 7.8^{A**,H**,N*}	30.3 ± 11.8^{N*}	25.7 ± 8.5	23.0 ± 8.2	.0007
MBF, hyperemia (ml min^{-1} g^{-1})	1.33 ± 1.03^{A*,H*,N**}	4.39 ± 1.36^{N*}	2.50 ± 0.79^{A**,N*}	3.52 ± 0.96	<.0001

Abbreviations: A: comparison with "athlete's heart"; β: exchange frequency; H: comparison with "HCM" respectively "HHD"; HCM: hypertrophic cardiomyopathy; HHD: hypertensive heart disease; MBF: myocardial blood flow; N: comparison with "normal heart"; rBV: relative myocardial blood volume. Values are means ± SD; *: p <.05; **: p <.01

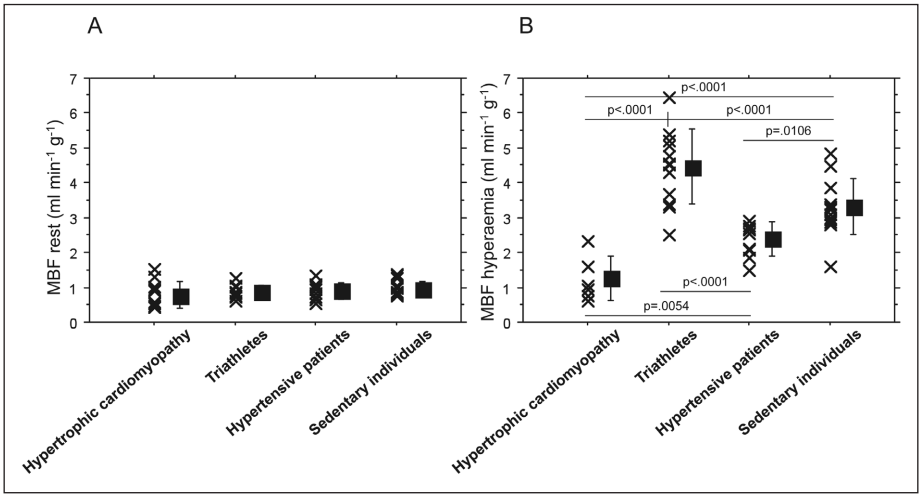

Figure 1

Individual data of septal myocardial blood flow (MBF, ml min^{-1} g^{-1}) at rest (A) and during adenosine-induced hyperaemia (B) in patients with hypertrophic cardiomyopathy (HCM; n = 15) with left ventricular hypertrophy (LVH), in semiprofessional triathletes with LVH (n = 15), in hypertensive patients with LVH (n = 15), and in healthy, sedentary individuals without LVH (n = 15). The squared symbols with error lines indicate mean values ± standard deviation.

with HCM and with hypertensive heart disease received the following medication: betablockers in 6 cases each (p <0.01 among all groups); calcium anatagonists in 7 cases of HCM and in 3 cases of hypertensive heart disease (p <0.01); angiotensin converting enzyme inhibitors in exclusively 9 cases of hypertensive heart disease (p <0.01); angiotensin-2 antagonists in exclusively 5 cases of hypertensive heart disease (p <0.01); diuretics in 4 cases with HCM and in 7 cases of hypertensive heart disease (p <0.01); acetylsalicylic acid in 1 case of hypertensive heart disease (p = 0.38); nitroglycerine in 1 case of hypertensive heart disease (p = 0.38).

Echocardiographic data

Doppler echocardiographic data are summarized in table 2. LV ejection fraction was not different between the groups. By definition, echocardiographic parameters indicative of LVH were present in all but the group of sedentary individuals with normal hearts. The patients with HCM had predominantly asymmetric septal LVH (14 patients) and only one had symmetrical LVH.

MCE data

Analysis was feasible in 162 out of 180 investigated myocardial segments, and the success rate of mid-septal, mid-lateral and mid-inferior re-

gions was 100, 72 and 98%, respectively. Regional MCE data was not significantly different within the groups although the success rate was higher in the septal wall. Therefore, only values obtained in the septal wall are presented. MBF at rest (fig. 1A and table 3) was not different between the groups. Conversely, MBF during hyperemia (fig. 1B and table 3) was lower in patients with HCM than in individuals with either hypertensive heart disease, athlete's heart or normal hearts without LVH. Furthermore, hyperemic MBF in hypertensive patients was lower than in subjects with athlete's heart or normal heart. Consequently, MBFR (fig. 2) was lowest in patients with HCM (1.61 ± 0.48), followed by patients with hypertensive heart disease (2.68 ± 0.31), by individuals with normal hearts (3.37 ± 0.77), and by those with athlete's heart (4.94 ± 1.08).

rBV at rest (table 3 and fig. 3) differed significantly among the groups. During hyperemia, rBV showed similar differences between the study groups: it was lowest in the group with HCM followed by the group with hypertensive heart disease, the group with normal hearts and finally the group with athlete's heart.

β at rest (table 3) was different between the groups with LVH, that is it was significantly higher in the groups with pathological rather than with physiological LVH (athlete's heart). During hyperemia, it was lowest in the group with HCM and highest in the group with athlete's heart with the other two groups in-between (table 3).

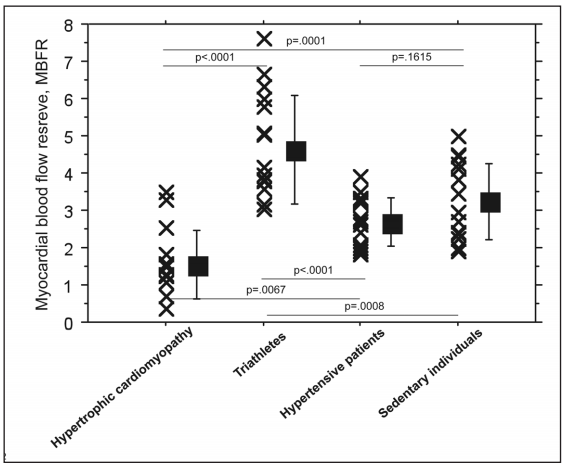

Figure 2

Individual data of myocardial blood flow reserve (MBFR) obtained at the septal wall in patients with hypertrophic cardiomyopathy (HCM; n = 15) with left ventricular hypertrophy (LVH), in semi-professional triathletes with LVH (n = 15), in hypertensive patients with LVH (n = 15), and in healthy, sedentary individuals without LVH (n = 15). The squared symbols with error lines indicate mean values ± standard deviation.

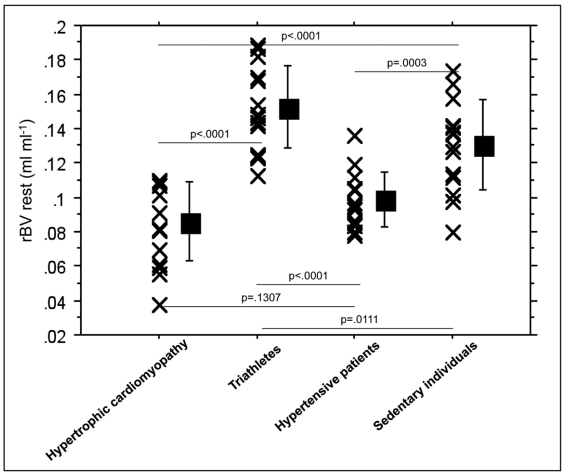

Figure 3

Individual data of relative myocardial blood volume (rBV) obtained during resting conditions and at the septal wall in patients with hypertrophic cardiomyopathy (HCM; n = 15) with left ventricular hypertrophy (LVH), in semi-professional triathletes with LVH (n = 15), in hypertensive patients with LVH (n = 15), and in healthy, sedentary individuals without LVH (n = 15). The squared symbols with error lines indicate mean values ± standard deviation.

Receiver-operating-characteristics (ROC) analysis

Table 4 provides accuracy data of MCE-related and -unrelated parameters obtained at rest and during hyperemia for the distinction of HCM from athlete's heart and hypertensive heart disease. At rest, rBV at a threshold of 0.11 very accurately differentiated between HCM and athlete's heart. During hyperemia, MBFR of <2.8 distinguished better between HCM and athlete's heart, than rBV. MBFR at a lower threshold of 1.8 also accurately differentiated between HCM and hy-

Table 4

Receiver operating characteristic (ROC) analysis.

	Threshold	AUC	Sensitivity	Specificity	P
HCM versus Athlete's heart					
Rest					
Septal wall rBV (ml ml^{-1})	<0.11	1.0	1.0	1.0	<.0001
Early (E') mitral annular velocity (cm s^{-1})	<8.8	0.94	0.8	1.0	<.0001
Interventricular septum (ED, mm)	≥15	0.842	0.8	0.86	<.002
Septal-to-posterior wall ratio (ED)	≥1.3	0.83	0.8	0.71	0.031
Left atrial diameter index (mm/m^{-2})	>22.5	0.58	0.60	0.80	0.1295
Hyperemia (adenosine)					
Septal wall rBV (ml ml^{-1})	≤0.124	0.949	0.8	1.0	<.0001
Septal myocardial blood flow reserve	<2.8	0.97	1.0	085	<.0001
HCM versus HHD					
Rest					
Early (E') mitral annular velocity (cm s^{-1})	≤0.093	0.90	0.87	0.87	.0001
Septal-to-posterior wall ratio (ED)	≥1.3	0.87	0.87	0.73	.0009
Hyperemia (adenosine)					
Septal myocardial blood flow reserve	≤1.8	0.85	1.0	0.77	0.002

Abbreviations: AUC: area under the ROC curve; HCM: hypertrophic cardiomyopathy; HHD: hypertensive heart disease; LV: left ventricular; rBV: relative blood volume

pertensive heart disease (table 4). An MCE-unrelated differentiator at rest between HCM and athlete's heart, and hypertensive heart disease was the ratio between septal and posterior wall thickness at a value of ≥1.3.

Discussion

The present study, for the first time, demonstrates that MCE-derived rBV at rest most accurately differentiates between pathological LVH due to HCM and physiological, endurance-exercise induced LVH. As a further novel result, MBFR at thresholds of 2.8 and 1.8 is able to distinguish LVH in the context of HCM from physiological and pathological forms of LVH, respectively.

Myocardial contrast echocardiography

Over the last two decades, co-evolution of contrast agents and contrast-sensitive imaging methods rendered MCE applicable for clinical practice. In their landmark paper, Wei et al. were the first to propose an algorithm for the quantification of MBF [13]. They found excellent correlations between MBF and the MCE-derived product of plateau signal intensity A and exchange frequency β. However, they conceded that absolute values for rBV and thus MBF were not obtainable due to their use of intermittent imaging. Thus, expression of myocardial acoustic intensities A_{myo} as a percentage of that obtained from the LV cavity does not provide an accurate estimation of rBV using intermittent imaging [13]. Nonetheless, Le et al. calculated rBV as the ratio of steady-state acoustic intensities in the myocardium A_{myo} and the left ventricular cavity A_{LV}, derived by intermittent imaging [14, 15]. Wu et al. applied the indicator-dilution theory and calculated rBV in dogs, as the ratio of MBF (derived by radiolabeled microspheres) and exchange frequency (derived by MCE) [16]. However, both groups only provided percent changes of rBV from rest to stress and under varying driving pressures, respectively.

Yano et al. were the first to denote absolute values of rBV using the above-mentioned normalization and estimated rBV to be 4.0 ± 2.2 ml 100 g^{-1} in normal human myocardium [17]. However, their low value compared to the current study (12.9 ± 2.6 ml 100 g^{-1}) and to animal data (5 to 20 ml 100 g^{-1}) most likely arose from inappropriate conversion of signal intensities [18]. Moreover, intermittent imaging using harmonic imaging with progressively longer pulsing intervals may be too short to allow complete filling of the microvasculature, especially when perfusion is very low (e.g., in hypertrophic cardiomyopathy).

Other investigators simply equated rBV with A_{myo}, which depends on variations in contrast agent concentration and acoustic properties of patients, thus A_{myo} may only loosely correlate with rBV. Moreover, the unit of A_{myo} is acoustic intensity, whereas rBV has the unit ml ml^{-1} or ml g^{-1}. Probably therefore, Di Bello et al. were not able to detect reduced capillary density in patients with hypertensive heart disease when compared with controls using A_{myo} [19].

In conjunction with real-time imaging using pulse inversion and a volumetric model based on contrast agent kinetics, an algorithm for the quantification of absolute MBF was developed on physical grounds of the continuity equation of flow within a circulation, and it was tested *in vitro* and *in vivo* using several approaches [11]. During coronary angioplasty, MCE accurately measured collateral blood flow as compared with the reference method using coronary pressure measurements [20]. In this context, the absolute perfusion threshold preventing ECG signs of myocardial ischemia could be defined using MCE [21]. Thus, the algorithm employed in the present study can be reasonably regarded as precise and also robust over the entire normal and pathological range of MBF, and in measurement settings of variable technical difficulty.

Comparison with other perfusion imaging methods

Apart from myocardial biopsy, only MCE allows for the estimation of capillary density, that is rBV, whereas this parameter may not be obtained by other perfusion imaging methods. In humans, absolute myocardial perfusion can be measured by positron emission tomography (PET) and recently by MCE [11]. The current findings are in line with previous studies using PET: Radvan et al. found decreased MBFR in patients with HCM as compared to a group of elite rowing athletes [22]; Choudhury et al. found lower values of MBFR in patients with HCM and hypertensive heart disease, respectively, compared to controls [23]. Reduced MBFR was a strong, independent predictor of clinical detoriation and death in a prospective study [24].

Conversely, single-photon emission computed tomography (SPECT) only allows for qualitative assessment of myocardial perfusion. In patients with HCM, subendocardial perfusion defects in patients with HCM have been found using SPECT [25]. An abnormal perfusion scan was associated with increased risk of cardiocascular death [26]. Neither PET nor SPECT reported diagnostic accuracies for the above mentioned distinctions and both use ionizing radiation which precludes their use for screening purposes. Similar to myocardial biopsy, MCE allows for the measurement of the underlying structural alteration, that is reduced or increased capillary density, in individuals with HCM and athlete's heart, respectively. This may explain the superior accuracy of rBV compared to MBFR.

Cardiovascular magnetic resonance (CMR) is

an emerging imaging modality which provides structural as well as functional information during the same session. Left ventricular dimensions and mass may be more accurately determined than with echocardiography, especially, in asymmetric LVH. Moreover, late-gadolinium enhancement allows for the detection of myocardial scarring which is an important predictor of ventricular arrhythmia [27]. So far, CMR has not been validated for perfusion imaging, mainly due to the fact that no purely intravascular contrast agent is currently available for clinical use.

LVH in HCM and in athlete's heart

Pathoanatomic, morphometric data on capillary density in humans are generally scarce, but they are consistent with the findings of the present study documenting an rBV in the sedentary group of individuals with normal hearts of 13% at rest and 17% during hyperaemia [28]. Indirect morphometric data on rBV in HCM are accessible by the frequently obtained parameter of interstitial collagen content volume fraction, which has been reported to be 14% in patients with HCM but only 2% in individuals with normal hearts [29]. Inversely, rBV at rest in HCM in the present study was reduced to 9%, as compared to 14% in the presence of physiological LVH in endurance athletes.

Sharma and co-workers documented, in a recent study using over 700 elite junior athletes aged approximately 16 years, that rowers and triathletes had the highest LV wall thickness (on average 11 and 10 mm), and that an LV wall thickness exceeding 12 mm occurred only very rarely [30]. Interestingly, the rate of triathletes with a septal wall thickness above 12 mm in the present study was exceedingly higher (12/15; posterior wall thickness above 12 mm in 5/15 cases), the fact of which may be explained by one of the selection criteria used for the definition of LVH and study inclusion (septal or posterior wall thickness >12 mm), and by the investigation of adults in the present study compared to adolescents in Sharma's study.

Distinction between HCM and athlete's heart

The structural myocardial parameter, rBV, conveniently obtained during resting conditions at the thickened interventricular septum, detected HCM very dependably, if it was equal or less than 11%. The reliability of the test using rBV was not better during hyperaemic myocardial conditions (see table 4), the fact of which renders the detection procedure more simple. Currently, 9 different clinical criteria are used to distinguish whether LVH is pathological and due to HCM or physiological in the context of endurance exercise [4]. Using receiver operating characteristics analysis (not all data shown), some of these predictors for or against HCM could be confirmed in the present investigation (asymmetrical pattern of LVH, the presence of diastolic dysfunction, family history of HCM, peak VO_2), others not (left atrial size, LV cavity size, female gender, wall thickness behaviour in response to deconditioning). In fact, female gender and wall thickness behaviour in response to deconditioning were not tested in the current study.

The MCE-derived parameter β obtained at rest was not discriminatory between HCM and athlete's heart. In contrast, the product of rBV and β during hyperaemia divided by the constant of tissue density, yielding MB (or MBF), discerned HCM from athlete's heart very reliably. Since rBV during hyperaemia remained statistically unchanged compared to resting conditions, in HCM patients and in the group of athletes, the main contribution to the highly distinctive parameter of MBF must have come from β. Indeed, it revealed a small change in response to adenosine in HCM patients (+3.83 min^{-1}, $p = 0.0508$), but a large and highly significant one in athletes (+23.34 min^{-1}, $p < 0.0001$). Thus, it can be reasoned that β is a *functional* microvascular parameter representing directly vascular conductance or the inverse of resistance. With regard to LVH in HCM, its blunted hyperaemic response draws attention to the second of two pathophysiological aspects of developing ischemia aside from a reduced content of vessels (or reduced rBV), that is their impaired functional capacity for dilatation. It is partially consumed already at rest (increased β) in order to maintain normal perfusion close to 1 ml min^{-1} g^{-1} of myocardium.

Distinction between HCM and hypertensive heart disease

A similar pathophysiological pattern of the microcirculation, with invariably reduced vascularity and compensatory up-regulation of vascular conductance at rest, can be observed in pathological LVH due to arterial hypertension [12]. In this context, the question evoked is whether MCE, consequently, is unable to distinguish between the two forms of pathological LVH. At rest they could not be discerned using MCE in this study (see table 4). In this situation, a practical solution to the problem is to check for elevated blood pressure and/or a history of hypertension and whether the septal-to-posterior wall ratio is below 1.3, in which case the presence of hypertensive heart disease is very likely. During hyperaemia, MBFR at a threshold of ≤1.8 (see table 4) distinguished patients with HCM very dependably from those with hypertensive heart disease. Impaired coronary vasodilator reserve in secondary LVH despite normal epicardial coronary arteries is a well-known phenomenon [31]. To the authors' knowledge, no investigations have been performed in patients with HCM or hypertensive heart disease focusing on the contribution of vascular resistance changes to the hyperaemic MBF.

Study limitations

Ideally, patients with HCM should have been recruited who were involved in endurance exercise training similar to that of the triathletes. However, considering the HCM prevalence of 1:500 [3], it is unrealistic to locate enough of such individuals.

The influences of vasoactive drugs taken by the

patients with pathologic LVH on MBF and vasodilator reserve have only been examined in a small number of studies, which mainly found that beta-blockers and angiotensin-converting enzyme inhibitors improved coronary flow reserve [32, 33]. However, the respective effects on rBV and β are not known.

Coronary angiography was not performed in the patients with pathological LVH (nor in the healthy individuals), and thus, an influence of early-stage atherosclerosis not detected during bicycle ergometry (data not shown) on the study findings cannot be excluded.

So far, only MCE with its purely intravascular tracer allows for the *in vivo* measurement of rBV in humans. Therefore, a comparison to another technique is lacking, and assessment of rBV based on myocardial biopsies (which were not taken in this study) should be interpreted cautiously because filling of the vasculature is affected by the fixation process, and may not correspond to rBV obtained *in vivo*.

In conclusion, MCE-derived measurement of the microvascular alterations of pathological or physiological LVH, such as capillary density, yields superior diagnostic accuracy when compared to the previously suggested conventional echocardiographic parameters.

Correspondence:
Christian Seiler, MD, FACC, FESC
Professor and Co-Chairman of Cardiology
University Hospital
CH-3010 Bern
E-Mail: christian.seiler @insel.ch

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