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Research Paper

Reduced global longitudinal strain at rest and inadequate blood pressure response during exercise treadmill testing in male heterozygous familial hypercholesterolemia patients

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ABSTRACT

Background: Heterozygous familial hypercholesterolemia (heFH) is a genetic disorder leading to premature coronary artery disease (CAD). We hypothesized that the subclinical pathophysiologic consequences of hypercholesterolemia may be detected before the occurrence of clinically overt CAD by stress testing and myocardial strain imaging.

Patients-methods: We evaluated the treadmill tests (ETTs) of 46 heFH men without known arterial hypertension/diabetes mellitus/vasculopathy like CAD and of 39 healthy men matched for age, baseline systolic/diastolic blood pressure (BP) and heart rate (HR), using Bruce protocol. Global longitudinal strain (GLS) of the left ventricle (LV) additionally to ejection fraction was obtained.

Results: heFH men reached a significantly higher peak systolic and diastolic BP compared to controls ($p = 0.002$ and $p < 0.001$, respectively). Mean rate pressure product was significantly higher in heFH patients ($p = 0.038$). Both duration of the ETT and workload in metabolic equivalents was lower in the heFH group ($p < 0.001$ and $p < 0.001$, respectively). Baseline to peak rise of systolic and diastolic BP in heFH men was higher ($p = 0.008$ and $p < 0.001$ for systolic and diastolic BP, respectively). Furthermore, heFH men had higher rise of HR from baseline to peak, compared to controls; ($p = 0.047$). GLS in heHF men was slightly decreased ($p = 0.014$), although the ejection fraction was similar in both groups.

Conclusion: heFH men have a higher rise in systolic/diastolic BP during ETT, which may reflect early, preclinical hypertension. Furthermore, slight impairment of LV GLS is present, despite the absence of apparent myocardial dysfunction in conventional 2D echocardiography.

Abbreviations: BP, blood pressure; CAD, coronary artery disease; DBP, diastolic blood pressure; EDV, end-diastolic volume; ESV, end-systolic volume; ETT, Exercise treadmill test; FH, Familial hypercholesterolemia; GLS, Global longitudinal strain; HDL, high density lipoprotein; heFH, heterozygous familial hypercholesterolemia; hoFH, homozygous familial hypercholesterolemia; HR, heart rate; LDL, low-density lipoprotein; LV, left ventricle; LVEF, LV ejection fraction; METs, metabolic equivalents; RPP, rate pressure product; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride.

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1. Introduction

Familial hypercholesterolemia (FH) is mainly caused by mutations of the gene encoding for the low-density lipoprotein (LDL)-receptor. FH is distinguished into homozygous familial hypercholesterolemia (hoFH) and heterozygous familial hypercholesterolemia (heFH). The prevalence of heFH is 1 in 200 and hoFH 1 in 160.000–300.000 people [1].

The most important consequence is the predisposition to early and accelerated atherosclerotic disease, especially coronary artery disease (CAD) and particularly in the homozygotes.

The serum lipid profile of these patients is characterized by elevated levels of both total (TC) and LDL cholesterol. Elevated triglyceride (TG) and low high density lipoprotein (HDL) levels might also coexist [9]. The most important consequence is the increased prevalence and the accelerated course of atherosclerotic disease particularly in the homozygotes that develop CAD before the age of 10. Moreover, patients with heFH are predisposed to CAD [2,3] Therefore, it is important to stratify asymptomatic heFH patients according to their cardiovascular risk for developing CAD. Exercise testing (ETT) is a well-established, easy, safe and low-cost procedure, used for many decades as a noninvasive test to diagnose CAD [4–6]. A limited number of studies have been published regarding ETT parameters in heFH patients [7,8].

Furthermore, the visual assessment of two-dimensional (2D) echocardiography provides a rapid evaluation of LV systolic function. A more thorough evaluation of LV systolic function requires calculation of LV ejection fraction (LVEF) using Simpson's biplane method [10]. Novel echocardiographic techniques allow the assessment of myocardial strain, which can measure myocardial deformation as an intrinsic mechanical property of the myocardium [11–13].

Our aim was to evaluate the change of systolic blood pressure (SBP) and diastolic blood pressure (DBP) during ETT and to combine it with probable early detection of myocardial systolic abnormality assessed measuring GLS in adult heFH men without known CAD/peripheral artery disease/arterial hypertension/diabetes mellitus and with normal LVEF.

2. Methods

2.1. Study population

This is a retrospective single-centre study investigating ETT parameters and LVEF- GLS from 2D echocardiography in asymptomatic heFH men. Forty-six ETTs of heFH men, aged 29.0–51.0 years, as well as 39 ETTs of healthy controls, aged 35.0–43.0 years from the database of the Department of Exercise Treadmill Testing in Onassis Cardiac Surgery Center were evaluated. Echocardiographic images were also studied from the digital archive of the Laboratory of Echocardiography. Two dimensional (2D) speckle tracking measurement of the GLS was evaluated from the 17 segments of the left ventricle (LV) using the four-, three- and two-chamber views.

The inclusion criteria for heFH men were clinical diagnosis of heFH [14] and the ability to walk on the treadmill at a moderate pace. Exclusion criteria were the presence of known CAD, the presence of symptoms suggestive of CAD/potentially related with CAD (angina pectoris, angina equivalents, exertional/resting dyspnoea), history of peripheral artery disease (stroke, carotid bruit, intermittent claudication, critical limb ischemia), the presence of ECG/ETT/echo findings suggestive of CAD and the presence of any known additional risk factor for CAD, apart from heFH and family history of CAD, as known arterial hypertension or diabetes mellitus. Study subjects did not receive any medication, since they visited our Lipid Clinic for the first time. On the other hand, healthy men matched for age, resting SBP and DBP and resting heart rate (HR) with a total cholesterol level <200 mg/dL and a blood glucose level <100 mg/dL were also assessed. No women were included either in the patient or in the control group, while obesity was not an exclusion criterion for both groups.

2.2. Lipid exams

The lipid profile was retrieved from the Laboratory Archive both for the heFH patients and the control group. The blood samples were collected after fasting for 9–12 h and analyzed using Hitachi Cobas 6000 analyzer in the biochemical laboratory of Onassis Cardiac Surgery Center.

2.3. Exercise treadmill test

All subjects were evaluated using standard ETT following the Bruce protocol [14]. Data were collected continuously, based on symptoms, rhythm, HR, BP, workload in metabolic equivalents (METs), rate pressure product (RPP) and HR at 3 min of exercise.

2.4. Echocardiography

A 2D echocardiographic evaluation for assessment of LV volumes, LVEF and GLS was performed in both groups. The studies were acquired with a Vivid 7 ultrasound cardiovascular system (GE Heathcare, Horten, Norway) using a 3-S phased array transducer. The transmission frequency was 1.7–3.4 MHz. All the digitally stored images were analyzed offline using EchoPAC, version 202 software (EchoPAC Dimension 08, GE Heathcare). End-diastolic and end-systolic LV volumes (EDV and ESV respectively) and LVEF were calculated from the 2D images, according to Simpson's biplane method from the apical four- and two- chamber views.

Myocardial strain measurements were performed using speckle tracking echocardiography [15,16]. GLS was obtained from the apical views at 55–80 frames/s and analyzed by experienced observers who had no information on the patients´ clinical status. The endocardial borders were traced in the end-systolic frame of the 2D images. The system automatically determined tracking quality for each analyzed segment. The user adjusted the border manually if the system could not track properly. Myocardial function by strain was evaluated on a frame-by-frame basis by automatic tracking of acoustic markers (speckles) throughout the cardiac cycle. The system then yield LV LS curve and the GLS was obtained by averaging the maximum systolic shortening in a 17-segment model of the LV (Fig. 1).

All echocardiographic measurements were performed twice by 2 independent observers.

It should be mentioned that the lipid sampling, the ETT and the echocardiographic study were performed in a time period of 1–3 months.

2.5. Statistical analysis

Statistical analysis was performed with Stata v.15 SE. Normality of continuous variables was determined based on a visual evaluation of Q-Q plots and histograms. Normally distributed continuous variables are presented as mean ± standard deviation, not-normally distributed continuous variables are presented as median (interquartile range) and categorical variables are presented as n (%). Normally distributed variables were compared between groups with independent-sample *t*-test, not-normally distributed variables were compared with the Mann-Whitney *U* test and categorical variables with the Chi-square test or Fisher's exact test when appropriate. Correlation between normally distributed continuous variables was determined with Pearson's r coefficient calculation. For data deviating from normal distribution, Spearman's rho was applied. For echocardiography measurements, intra- and inter-observer variability were assessed with Pearson's r and Cronbach's alpha coefficient calculation. A p-level <0.050 was considered statistically significant.

3. Results

3.1. Patient characteristics

As already mentioned, all subjects were male with no known comorbidity or other risk factors for CAD. Median age was 35.5 years

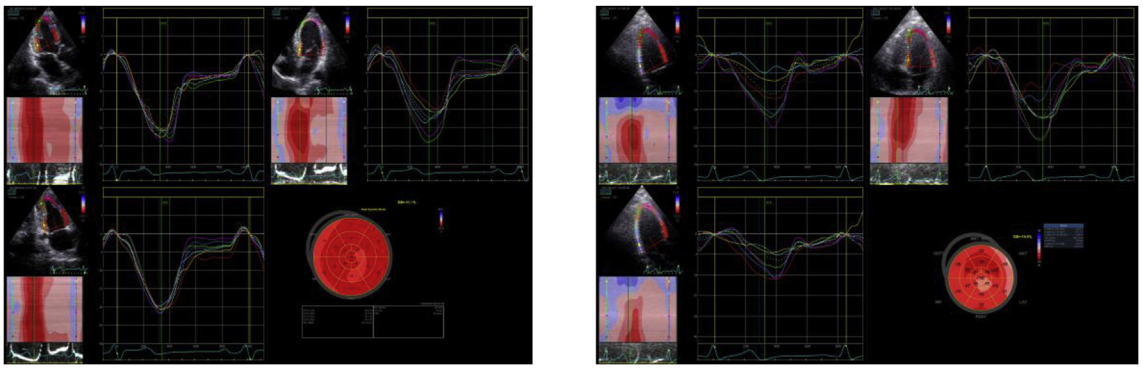

Fig. 1. Measurement of peak systolic longitudinal strain of 17 segments of the left ventricle as depicted by the respective "Bull's eye" of a healthy control (left) and a heFH patient (right).

(29.0–51.0). Both patients and controls had normal mean body mass index (BMI), namely 25.8 ± 22.4. As expected, patients featured elevated TC an LDL values (TC: 326 ± 63 mg/dL, LDL: 250 ± 63 mg/dL) and acceptable TG and HDL (TG: 148 ± 78 mg/dL, HDL: 44 ± 11 mg/dL), while the above parameters were normal in the control subjects.

3.2. ETT parameters

The study population consisted of 85 participants, 46 (54%) with heFH and 39 (46%) healthy controls. The most important ETT parameters are presented in Table 1. Median test duration was significantly longer in the control group compared to heFH patients [780.0 (750.0–840.0) sec vs. 660.0 (600.0–720.0)sec, $p < 0.001$] and controls achieved a higher median number of METs than heFH patients [17.1 (15.1–17.3) vs. 13.0 (11.0–13.7), $p < 0.001$]. HeFH patients presented a significantly higher peak SBP and DBP compared to controls [180.0 (165.0–200.0) mmHg vs. 165.0 (160.0–180.0) mmHg, $p = 0.002$ and 90.0 (85.0–100.0) vs. 80.0 (75.0–90.0), $p < 0.001$, respectively]. Median changes of SBP and DBP from rest to peak exercise, namely delta SBP and delta DBP were also higher in heFH patients [55.0 (50.0–70.0) vs. 45.0 (35.0–60.0), $p = 0.008$ and 10.0 (10.0–20.0) vs. 0.0 (0.0–5.0), $p < 0.001$, respectively]. The mean rate pressure product was significantly higher in heFH patients compared to controls [30475.3 ± 5492.5 vs. 28089.2 ± 4797.5, $p = 0.038$]. HR at 3 min was lower in heFH patients compared to controls, which tended towards but did not reach statistical significance [61.2 ± 20.9 vs. 69.2 ± 15.0, $p = 0.051$]. Delta HR at 3 min was significantly lower in heFH patients compared to controls [36.2 ± 10.9 vs. 40.6 ± 8.4, $p = 0.043$]. However, no significant differences were identified between groups with regard to peak and delta HR as well as maximum HR adjusted for age or the percentage of maximum HR achieved. No significant statistical difference was identified between groups regarding age and resting HR, resting SBP and resting DBP. Nonspecific ST-T changes occurred both in heFH patients and controls during ETT, but no subjects with ischemic ST/T changes were included, as this was an exclusion criterion (Fig. 2).

3.3. Echocardiography

Intra-observer and inter-observer variability were acceptable for all echocardiographic measurements, including LVEF and GLS (Supplementary Table). Both groups (heFH males and control group) had similar EDV, ESV and LVEF. However, GLS was found −16.7 ± 2.3% in heFH males and −19.2 ± 3.8% in control group ($p = 0.014$), as shown in Table 2. No correlation between LVEF/GLS and exercise BP response was found. However, a non-significant correlation between GLS and resting DBP was obtained (rho = −0.625, $p = 0.053$).

4. Discussion

In our study, we combined parameters of ETT and GLS to evaluate the presence of preclinical cardiovascular impairment in a population of heFH males. We found that heFH men showed a higher peak SBP and DBP at ETT and higher corresponding delta values, compared with healthy men. A slight but statistically significant reduction of GLS was also documented, although no difference in LVEF or LV volumes was identified. No correlation between resting GLS and exercise BP parameters was observed.

ETT parameters are of prognostic significance in heFH subjects without clinically overt vascular disease (CAD, peripheral artery disease). A recent study of 451 asymptomatic heFH subjects without any evidence of ischemia has shown that parameters of ETT may predict cardiovascular disease after three decades of observation [7]. A previous study of the same research team including 639 heFH patients had shown that decreased exercise capacity, a delayed decrease in HR during the first minute of graded exercise and increased peak pulse pressure are strong predictors of coronary events [8]. In particular, the authors report that increased pulse pressure is an indirect measure of arterial stiffness. Furthermore, arterial stiffness has been shown to be a major determinant of myocardial ischemic threshold in another study [17].

Few studies have questioned BP response during exercise in heFH. Kolovou et al. showed that heFH women had an inadequate rise in their SBP and DBP compared with healthy women [18]. Another group showed that heFH men with known CAD had a significantly higher DBP rise during ETT compared with patients without hypercholesterolemia. However, there were no differences in the peak exercise SBP in these patients, probably because they were under relevant medication [19]. Increased SBP and DBP response during exercise has also been described in adolescents with increased LDL [20]. The present study included asymptomatic treatment-naive heFH males without known CAD/peripheral arterial disease, with normal LVEF and no other risk factors as formal arterial hypertension or diabetes mellitus. A higher increase of both SBP and DBP during ETT was detected. Taken together, these findings strongly suggest that patients with heFH are at a higher risk of developing hypertensive state.

A possible explanation is that subjects with high cholesterol level may have endothelial dysfunction, which has been shown to correlate with the extent of atherosclerotic process [28]. Atherosclerosis is associated with increased arterial stiffness and decreased elasticity in conduit vessels. In previous studies, effects of hypercholesterolemia on vascular reactivity have been demonstrated in small subject groups. Moreover, there is evidence that endothelial dysfunction in hypercholesterolemic subjects is generalized and extends beyond the coronary circulation [21,22,24]. These findings show the potential of forthcoming hypertension in hyperlipidemic patients [21,25,26]. Furthermore, studies in humans with hypercholesterolemia, revealed that there is a decreased effect of

Table 1
Exercise treadmill test results of the entire cohort. Statistical significance is considered for $p \leq 0.05$.

Variable	Entire Cohort	HeFH	Controls	p-value
Group size	85	46	39	N/A
Resting HR (bpm)	80.1 ± 14.0	79.7 ± 13.6	80.6 ± 14.6	0.760
Peak HR (bpm)	173.0 (162.0–181.0)	173.0 (162.0–181.0)	173.0 (162.0–181.0)	0.750
ΔHR (bpm)	89.6 ± 15.7	88.7 ± 16.9	90.6 ± 14.3	0.570
ΔHR (%)	52.7 ± 7.3	52.5 ± 7.0	53.0 ± 7.6	0.770
HR after 3 min (bpm)	103.0 (93.0–115.0)	107.0 (96.0–118.0)	97.0 (88.0–109.0)	0.088
Resting SBP (mmHg)	120.0 (110.0–130.0)	120.0 (115.0–130.0)	120.0 (110.0–125.0)	0.170
Peak SBP (mmHg)	170.0 (160.0–190.0)	180.0 (165.0–200.0)	165.0 (160.0–180.0)	**0.002**
ΔSBP (mmHg)	50.0 (40.0–65.0)	55.0 (50.0–70.0)	45.0 (35.0–60.0)	**0.008**
ΔSBP (%)	30.1 ± 8.3	31.8 ± 8.4	28.1 ± 7.7	**0.036**
Resting DBP (mmHg)	80.0 (75.0–80.0)	80.0 (75.0–80.0)	80.0 (75.0–80.0)	0.660
Peak DBP (mmHg)	85.0 (80.0–90.0)	90.0 (85.0–100.0)	80.0 (80.0–80.0)	<**0.001**
ΔDBP (mmHg)	5.0 (5.0–10.0)	10.0 (10.0–20.0)	0.0 (0.0–5.0)	<**0.001**
ΔDBP (%)	6.3 (5.6–12.5)	12.1 (10.0–20.0)	0.0 (0.0–6.3)	<**0.001**
RPP (bpm × mmHg)	29380.5 ± 5291.9	30475.3 ± 5492.5	28089.2 ± 4797.5	**0.038**
Test duration (sec)	720.0 (660.0–781.0)	660.0 (600.0–720.0)	780.0 (750.0–840.0)	<**0.001**
Maximum achieved HR adjusted for age (bpm)	182.0 (177.0–187.0)	184.5 (169.0–191.0)	182.0 (177.0–185.0)	0.480
% of maximum HR achieved by patient	94.2 (91.0–99.0)	94.5 (91.4–97.7)	93.8 (90.5–100.0)	0.74
Workload (METs)	13.6 (13.0–17.1)	13.0 (11.0–13.7)	17.1 (15.1–17.3)	<**0.001**
Peak HR- 3 min HR (bpm)	64.9 ± 18.8	61.2 ± 20.9	69.2 ± 15.0	0.051
Δ(Peak HR- 3 min HR) (%)	38.2 ± 10.1	36.2 ± 10.9	40.6 ± 8.4	**0.043**

bpm, beats per minute; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; RPP, Rate-Pressure Product (Peak HR* Peak SBP); MET, metabolic equivalents. $p < 0.05$ is highlighted with bold.

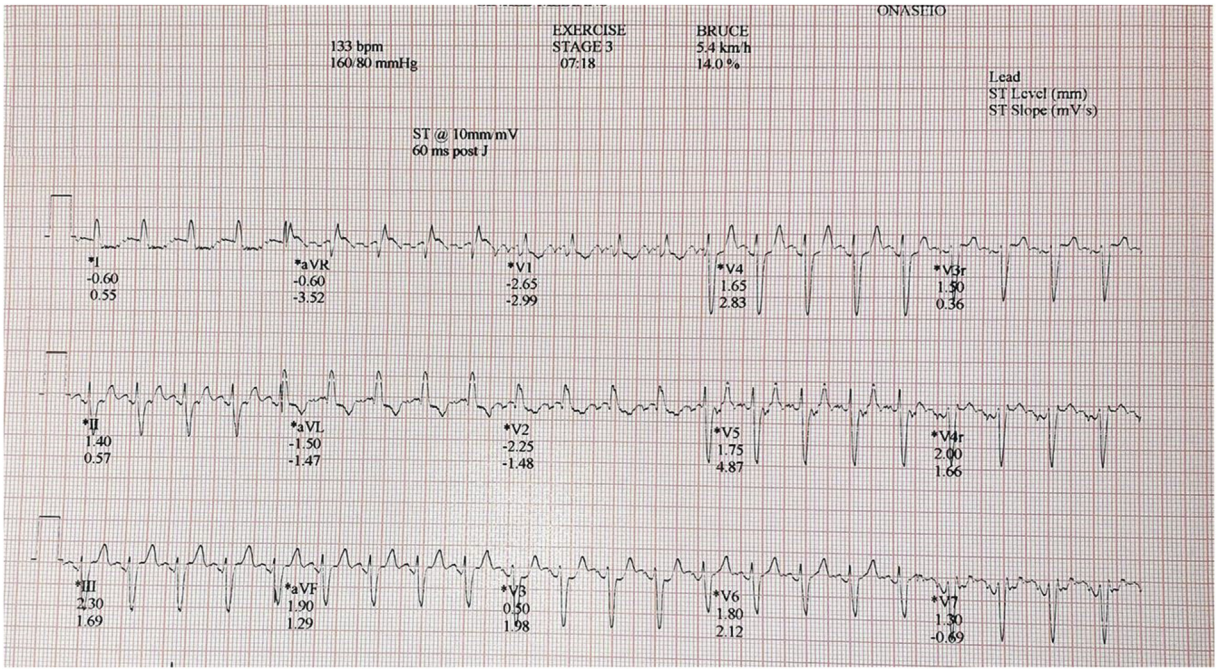

Fig. 2. Electrocardiogram from the peak of a heFH patient's exercise treadmill test.

Table 2
Echocardiography data in heFH men and Control group.

	heFH men	Control group	p
LV EDV (mL)	87 ± 19	103 ± 28	0.058
LV ESV (mL)	39 ± 10	47 ± 14	0.072
Stroke Volume (mL)	47 ± 11	56 ± 18	0.105
LVEF (%)	54.5 ± 5.2	54.8 ± 8.2	0.915
GLS (%)	−16.7 ± 2.3	−19.2 ± 3.8	**0.014**

$p < 0.05$ is highlighted with bold.

vasodilators like endothelium-derived relaxing factor (nitric oxide, NO) on the vascular smooth muscle of resistance vessels [23,27].

Reduced endothelium-dependent relaxation can be expressed as a higher SBP and DBP with exercise, as was the case in our study. Brett et al. demonstrated a significant positive correlation between the increase in DBP during exercise (delta DBP) and serum concentrations of TC, which further supports a causative relationship between hypercholesterolemia and BP deregulation explained by endothelial dysfunction [29]. Hypercholesterolemia may inhibit the vasodilator mechanisms resulting in elevated BP during exercise in hypercholesterolemic subjects. Subclinical atherosclerosis of renal vasculature may also contribute.

The LV GLS, currently useful clinical tool [30], assessed from the apical views in heFH males, was slightly decreased, compared to that of the control group. A previous study reported that obese dyslipidemic children and adolescents (6–18 years of age) present impaired LV strain parameters. More specifically, LV GLS was lower in dyslipidemic children compared with normal controls. Reduced GLS was documented in obese

subjects compared with normal-weight dyslipidemic children. It should be noticed that the study population was free of other cardiovascular risk factors or structural cardiac abnormalities [31]. Further, in a study of childhood obesity with dyslipidemia 2D speckle tracking-derived longitudinal LV strain was reduced in obese dyslipidemic children ($-18.2 \pm 2.0\%$ vs $-20.5 \pm 2.3\%$, $p < 0.001$) compared with nonobese ones [32]. In addition, a recent study including both hoFH and heFH patients revealed that there is an inverse correlation between LDL levels and GLS [33]. To the best of our knowledge, the current study is the first to assess both ETT parameters and 2D echocardiography-derived parameters, especially strain analysis, as potential markers of clinically silent impaired vascular function with prognostic significance. In our study, lower GLS values among heFH patients could be explained either by a relative increase in afterload or by indigenously reduced myocardial contractility. The first explanation is supported by the greater increase of SBP and DBP during exercise in patients; however resting values did not differ significantly between patients and controls. Moreover, no correlation between GLS and exercise BP parameters was shown in this study despite the known inverse relationship between LV longitudinal strain and afterload, namely BP. Thus, reduced endogenous myocardial contractility seems to be the most adequate explanation of this finding and may be caused by diffusely impaired vascular function, the latter being also responsible for the inadequate BP response of heFH subjects during exercise. Another explanation could be the direct toxicity of excess fat deposits in the myocardial cells, as it was shown in a previous experimental study [34].

The current study has the following limitations: a) the sample included was relatively small, b) follow-up data regarding the potential occurrence of any cardiovascular event were not available, c) no imaging data of the coronary anatomy were available and no perfusion data were dispensable. Moreover, echocardiography was performed only at rest, while exercise echocardiography, for example with supine bicycle ergometry, may provide further information regarding LV adaptation and BP response. The results of correlation analysis between GLS and exercise BP parameters should be interpreted with caution, as ETT and echocardiography were not performed simultaneously. Last, GLS calculation was based on 2D echocardiography, while 3D echocardiography may overcome some limitations of the 2D method.

5. Conclusion

Asymptomatic heFH men with no known CAD/peripheral arterial disease/arterial hypertension/diabetes mellitus present a higher rise in both systolic and diastolic BP during ETT compared with healthy age- and sex-matched controls. Furthermore, GLS of the LV is slightly reduced, although normal LVEF is maintained. Impaired GLS and altered exercise BP response do not correlate with each other. These findings highlight the presence of subclinical vasculopathy in male heFH subjects and provide the clinical tools for its early identification. Long-term prospective studies are needed to confirm their prognostic importance on top of the mere presence of heFH.

Author contributions

V. Vartela and **G. Kolovou** designed the study, **V. Vartela** and **D. Leivadarou** collected the data, **I. Armenis** performed statistical analysis, **V. Vartela** and **I. Armenis** wrote the manuscript, **G. Athanassopoulos** and **G. Karatasakis** performed and analyzed the echocardiograms, **G. Kolovou** was responsible for patient management, **S. Mavrogeni**, **K. Toutouzas** and **K. Makrilakis** critically reviewed and corrected the manuscript, **D. Perrea** coordinated the whole process, proposed explanations of the findings and reviewed the manuscript.

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Declaration of competing interest

All the authors declare that they have no competing interests regarding the present work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijchy.2021.100083.

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