

Noninvasive assessment of endothelial function and vascular parameters in patients with familial and nonfamilial hypercholesterolemia

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KEY WORDS

arterial stiffness,
atherosclerosis,
endothelial
dysfunction, familial
hypercholesterolemia

ABSTRACT

INTRODUCTION Endothelial dysfunction is one of the markers of atherosclerosis.

OBJECTIVES The aim of the study was to evaluate endothelial function by assessing flow-mediated dilation (FMD) and to measure the parameters of brachial arterial stiffness in patients with familial hypercholesterolemia (FH) and those with high low-density lipoprotein (LDL) cholesterol levels without FH mutations (nonfamilial hypercholesterolemia – non-FH).

PATIENTS AND METHODS The study involved 60 patients (mean age, 41.9 ± 7.7 y) without documented cardiovascular events and clinical symptoms of cardiovascular diseases: 21 patients with elevated plasma LDL cholesterol levels and genetically confirmed FH, 19 patients with elevated LDL cholesterol levels and without FH mutations, and 20 healthy controls. In each patient, ultrasound imaging was used to assess endothelium-dependent FMD and nitroglycerin-induced endothelium-independent dilation (EID) in the brachial artery. In addition, echo-tracking and photoplethysmography were used to assess the parameters of arterial stiffness.

RESULTS FMD was significantly lower in patients with FH ($11.0\% \pm 9.9\%$ vs. $21.0\% \pm 14.3\%$, $P < 0.01$) and non-FH ($14.2\% \pm 10.1\%$ vs. $21.0\% \pm 14.3\%$, $P < 0.05$) compared with controls. EID and arterial stiffness parameters were similar between the groups.

CONCLUSIONS Reduced FMD may suggest endothelial dysfunction. A lack of significant differences in arterial stiffness parameters may indicate that vascular remodeling is not advanced in patients with elevated LDL cholesterol levels. A lack of significant differences in FMD and arterial stiffness between patients with and without FH may indicate that FH mutation itself is not the main determinant of endothelial dysfunction and vascular remodeling in younger patients with hypercholesterolemia.

INTRODUCTION Atherosclerosis related to cardiovascular disease (CVD) is the most common cause of death worldwide. Moreover, hypercholesterolemia is considered as one of the main cardiovascular risk factors related to atherosclerosis.¹ It is known that lowering cholesterol levels significantly reduces total mortality and deaths from CVD in primary and secondary prevention.²

Familial hypercholesterolemia (FH) is defined as a heritable autosomal dominant disease, which in a vast majority of the patients is caused by a mutation in the low-density lipoprotein receptor (*LDLR*) gene.³ Efficient assembly and transport of low-density lipoprotein (LDL) cholesterol to hepatocytes is altered by a lack or dysfunction of the receptors.⁴ The FH phenotype may be

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TABLE 1 Characteristics of the study groups. Endothelial function and arterial stiffness parameters

Parameters	FH (n = 21)	non-FH (n = 19)	FH and non-FH (n = 40)	Controls (n = 20)	P value				
					FH vs. NFH	FH vs. controls	controls	FH and non-FH vs. controls	
age, y	38.9 ± 7.4	45.4 ± 6.7	42.0 ± 7.7	41.6 ± 7.8	<0.01	NS	NS	NS	
men, n (%)	11 (52)	11 (53)	20 (50)	10 (50)	NS	NS	NS	NS	
LDLR mutation, n (%)	17 (81)	0 (0)	17 (42.5)	0 (0)	–	–	–	–	
APOB mutation, n (%)	4 (19)	0 (0)	4 (10)	0 (0)	–	–	–	–	
statins, n (%)	21 (100)	13 (68)	34 (85)	0 (0)	–	–	–	–	
ACEIs, n (%)	3 (14)	5 (26)	8 (20)	0 (0)	–	–	–	–	
TC _{max} , mmol/l	8.7 ± 1.9	7.7 ± 1.2	8.2 ± 1.7	4.6 ± 0.6	NS	<0.01	<0.01	<0.01	
LDL-C _{max} , mmol/l	6.4 ± 1.8	5.3 ± 1.3	5.9 ± 1.7	2.8 ± 0.5	<0.05	<0.01	<0.01	<0.01	
HDL-C _{max} , mmol/l	1.4 ± 0.4	1.7 ± 0.9	1.5 ± 0.7	1.4 ± 0.3	NS	NS	NS	NS	
TG _{max} , mmol/l	1.5 ± 0.7	1.6 ± 0.7	1.6 ± 0.6	1.1 ± 0.5	NS	<0.05	<0.01	<0.01	
TC, mmol/l	6.3 ± 1.9	5.9 ± 1.2	6.1 ± 1.6	4.6 ± 0.6	NS	<0.01	<0.01	<0.01	
LDL-C, mmol/l	4.3 ± 1.6	3.8 ± 1.1	4.0 ± 1.	2.8 ± 0.6	NS	<0.01	<0.01	<0.01	
HDL-C, mmol/l	1.4 ± 0.3	1.5 ± 0.4	1.4 ± 0.3	1.4 ± 0.3	NS	NS	NS	NS	
TG, mmol/l	1.4 ± 0.6	1.4 ± 0.6	1.4 ± 0.5	1.1 ± 0.5	NS	NS	<0.05	<0.05	
hypertension, n (%)	6 (29)	6 (32)	12 (30)	0 (0)	NS	<0.01	<0.01	<0.01	
current and passive smokers, n (%)	2 (10)	4 (21)	6 (15)	0 (0)	NS	NS	<0.05	NS	
diabetes, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	NS	NS	NS	NS	
SBP, mmHg	120.5 ± 12.0	120.6 ± 9.1	120.5 ± 10.6	114.8 ± 6.4	NS	<0.05	<0.05	<0.05	
DBP, mmHg	73.8 ± 8.0	75.8 ± 8.4	74.8 ± 8.2	70.0 ± 6.3	NS	<0.05	<0.05	<0.05	
height, m	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	NS	NS	NS	NS	
weight, kg	73.9 ± 14.8	75.2 ± 14.1	74.5 ± 14.3	75.1 ± 18.1	NS	NS	NS	NS	
BMI, kg/m ²	26.1 ± 4.1	26.0 ± 3.2	26.1 ± 3.7	24.6 ± 4.3	NS	NS	NS	NS	
baseline brachial artery diameter during systole, mm	4.4 ± 0.8	4.1 ± 0.9	4.3 ± 0.8	4.1 ± 0.8	NS	NS	NS	NS	
baseline brachial artery diameter during diastole, mm	4.2 ± 0.8	4.0 ± 0.9	4.1 ± 0.9	4.0 ± 0.8	NS	NS	NS	NS	
FMD, %	11.0 ± 9.9	14.2 ± 10.1	12.5 ± 10.0	21.0 ± 14.3	NS	<0.01	<0.05	<0.01	
EID, %	23.8 ± 11.4	24.7 ± 13.3	24.3 ± 12.2	26.0 ± 7.2	NS	NS	NS	NS	
e-Tracking	PWVβ, m/s	8.7 ± 3.3	9.5 ± 3.5	9.1 ± 3.4	10.2 ± 4.1	NS	NS	NS	NS
	ACI, mm ² /kPa	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	NS	NS	NS	NS
	Ep, kPa	233.6 ± 172.0	272.7 ± 182.4	252.2 ± 175.8	324 ± 231.0	NS	NS	NS	NS
	β	18.5 ± 13.0	21.2 ± 14.2	19.8 ± 13.4	26.2 ± 17.8	NS	NS	NS	NS
	AI, %	11.5 ± 14.9	23.9 ± 18.3	17.4 ± 17.6	17.1 ± 17.4	NS	NS	NS	NS
photoplethysmography	SI, m/s	6.8 ± 1.6	6.4 ± 1.1	6.7 ± 1.4	7.2 ± 1.8	NS	NS	NS	NS
	RI, %	54.2 ± 15.8	44.8 ± 15.3	49.8 ± 16.1	55.9 ± 15.1	NS	NS	NS	NS
	PPT, ms	257.1 ± 54.5	267.4 ± 38.9	262 ± 47.3	252.2 ± 52.2	NS	NS	NS	NS

Data are presented as mean ± standard deviation or number (percentage).

Abbreviations: ACI – arterial compliance index, ACEIs – angiotensin-converting enzyme inhibitors, AI – augmentation index, APOB – apolipoprotein B receptor, BMI – body mass index, β – β-stiffness index, DBP – diastolic blood pressure, EID – endothelium-independent dilation, Ep – pressure-strain elasticity index (Young's modulus), FH – familial hypercholesterolemia, FMD – flow-mediated dilation, HDL-C – high-density lipoprotein cholesterol, LDL-C – low-density lipoprotein, LDLR – low-density lipoprotein receptor, max – maximum levels without pharmacotherapy, non-FH – nonfamilial hypercholesterolemia, NS – nonsignificant, PPT – peak-to-peak time, PWVβ – 1-point pulse-wave velocity, RI – reflexion index, SBP – systolic blood pressure, SI – stiffness index, TC – total cholesterol, TG – triglycerides

also caused by mutations in the apolipoprotein B (APOB) gene⁵ or proprotein convertase subtilisin/kexin type 9 (PCSK9) gene.⁶ All of these mutations result in highly increased plasma total cholesterol

(TC) levels, leading to premature atherosclerosis manifesting mainly as coronary artery disease. Patients with FH are particularly susceptible to atherosclerosis. Moreover, the response of these

TABLE 2 *LDLR* and *APOB* gene mutations

Sex	Age, y	Gene mutation
<i>LDLR</i>		
F	29	p.D221G
F	37	p.D221G
M	30	p.N564S
M	38	p.P526T
F	54	dup ex4-8
F	42	p.D221G
M	37	p.D221G
M	34	p.G592E
M	34	p.D221G
F	33	p.P608T
F	36	p.G592E
F	51	p.D227del
M	34	p.D221G
M	47	c.2140+5G>A
F	29	p.D221G
M	49	p.A431T
M	42	p.D90N
<i>APOB</i>		
M	37	p.R3527Q
F	40	p.R3527Q
M	35	p.R3527Q
F	50	p.R3527Q

Abbreviations: F – female, M – male

patients to standard hypolipemic treatment is often unsatisfactory.

A discovery that the endothelium is an endocrine and paracrine organ is thought to be crucial for the understanding of CVD pathogenesis. The presence of nitric oxide, a potent vasodilator, in the endothelium was reported.⁷ Physiologically, shear forces are the main activator of endothelial nitric oxide synthase (eNOS) and its activity is adjusting to changes in blood flow.⁸ Normal eNOS function is disturbed in endothelial dysfunction, which under favorable conditions may lead to atherosclerosis and CVD.⁹

Cardiovascular prevention is based mainly on the assessment of individual risk of CVD. Traditional cardiovascular risk factors such as sex, age, hypercholesterolemia, smoking, diabetes mellitus, and arterial hypertension have been identified.^{10,11} Because each individual risk factor is considered to have a different predictive value and often is not sufficient to assess the total risk, the search for new parameters that could help estimate the risk of cardiovascular events more precisely continues. New noninvasive imaging techniques may be helpful in the assessment of the atherosclerotic process, enabling early detection in the asymptomatic stage and evaluating progression at various stages of the disease.¹² Early endothelial dysfunction can be evaluated by brachial artery flow-mediated dilation (FMD) induced by ischemia.^{13,14} Progressive

atherosclerosis leads to structural and functional changes of the vessel wall resulting in increased arterial stiffness. In the early stage, these changes can be observed with ultrasonography and photoplethysmography.¹⁵⁻¹⁸ A number of independent clinical studies confirmed the association of endothelial dysfunction and increased arterial stiffness with elevated cardiovascular risk.^{19,20}

The aim of this study was to evaluate endothelial function assessed by FMD and arterial stiffness parameters measured by echo-tracking and photoplethysmography in the brachial artery in individuals with genetically confirmed FH and nonfamilial hypercholesterolemia (non-FH). The study included 3 groups of patients: with FH confirmed by molecular testing (*LDLR*, *APOB*, or *PCSK9* gene mutation), with elevated plasma levels of LDL cholesterol without a pathogenic mutation (non-FH), and healthy controls with plasma LDL cholesterol levels of less than 3.4 mmol/l.

PATIENTS AND METHODS **Participants** Endothelial function and arterial stiffness were analyzed in 60 individuals aged from 30 to 55 years (mean age, 41.9 ± 7.7 y). Forty subjects with elevated LDL cholesterol levels and tentative or definite diagnosis of FH (according to the Dutch Lipid Network Clinical Criteria of FH)²¹ were recruited from the consecutive patients of the Outpatient Clinic of the First Department of Cardiology, Medical University of Gdansk, Gdańsk, Poland. The FH group included 21 patients in whom mutations associated with FH (*LDLR* or *APOB*) were identified. The *PCSK9* mutation was not found in any of the patients. The non-FH group included 19 patients with elevated LDL cholesterol levels and without *LDLR*, *APOB*, or *PCSK9* mutation. Twenty healthy controls with normal LDL cholesterol levels (<3.4 mmol/l) were recruited from volunteers. The control group was matched for sex. None of the subjects had a history of cardiovascular events or cancer. Moreover, they did not have diabetes, infections within the previous 4 weeks, chronic diseases, or clinical symptoms of significant CVD. The study protocol was approved by the local ethics committee of the Medical University of Gdansk, and all individuals provided written informed consent to participate in the study. All subjects underwent a physical examination and had their weight and body mass index (BMI) measured. Moreover, a history of CVD, laboratory results of the maximum plasma LDL cholesterol levels, and lipid profile measured within the previous 6 months were recorded. Endothelial and vascular parameters were measured and evaluated by a single investigator. The characteristics of the study groups are presented in **TABLE 1**. The *LDLR* and *APOB* mutations identified in this study are listed in **TABLE 2**.

Flow-mediated dilation A high-resolution ultrasound scanner (Aloka SSD-Alpha 10-Miro, Hitachi Aloka Medical, Ltd. Tokyo Japan) was used to determine FMD and nitroglycerin-induced

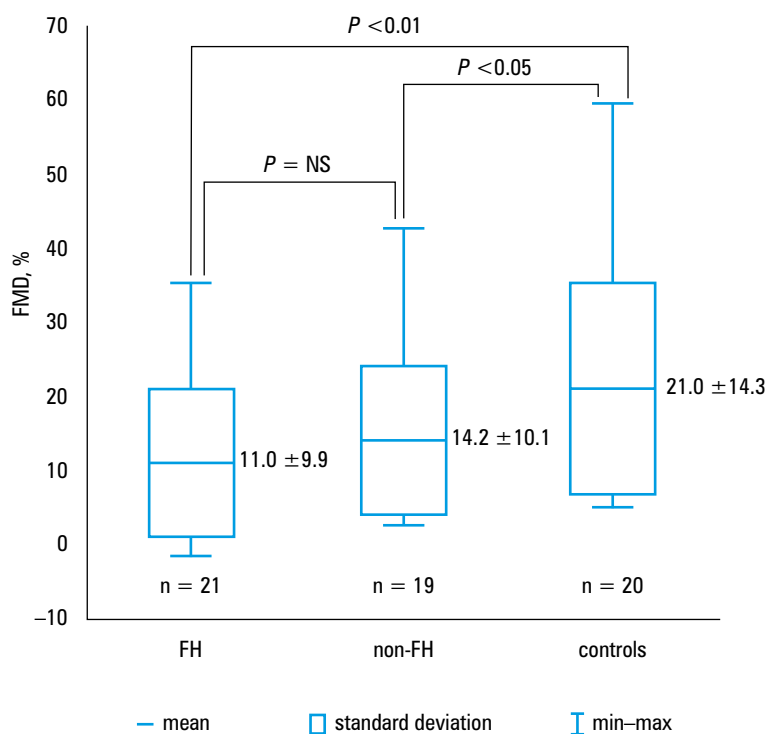


FIGURE Flow-mediated dilation in the study groups
Abbreviations: see
TABLE 1

endothelium-independent dilation (EID) in the brachial artery. The study was performed according to the protocol proposed by Celermajer et al.²² Participants were examined in the fasting state and were asked to abstain from smoking for at least 8 hours, using vasoactive medication, physical activity, and caffeine for 24 hours before the study. They remained in a quiet, temperature-controlled room (21–23°C), offset from the main roads, and rested in the supine position for 10 minutes before any measurement. A vascular ultrasound transducer was used to measure the diameter of the brachial artery in the dominant arm (the right arm in all subjects). Electrocardiography was recorded during each stage of the study. A 2-dimensional image of the brachial artery in the longitudinal plane was obtained 5 to 10 cm above the antecubital fossa. The artery diameter was measured at the peak of R wave (maximum diastole). In the first stage, the baseline diameter was measured between the anterior and posterior *m* line, which represents the boundary between the media and adventitia. Pulse-wave Doppler ultrasound was used to determine flow velocity. The brachial artery diameter was calculated by averaging the measurements automatically from at least 3 consecutive cardiac cycles. Next, a cuff was inflated to 200 mmHg for 5 minutes. Time was measured by a timer. Measurements were taken from 20 to 30 seconds after cuff's deflation and obtaining comparable baseline images. A maximal diameter of the artery was observed 1 minute after cuff deflation. The obtained values allowed to calculate FMD. In the second stage after reactive hyperemia, a patient rested for the next 10 minutes, and then the baseline diameter was measured again to assess EID. Measurements were repeated 3 to 4 minutes after sublingual administration of nitroglycerin spray (400 µg).

e-Tracking Pulse-wave analysis of the right brachial artery was performed in all patients using the echo-tracking method (e-Tracking) using Aloka SSD-Alpha 10-Miro and the automatic system of ultrasound vascular evaluation. Blood pressure was measured on the right upper limb using a sphygmomanometer and uploaded to ultrasound before the test. First, stable images of the anterior and posterior vascular walls were obtained. For continuous evaluation of artery diameter, the e-Tracking gate was placed between the near and far *m* line of the vessel. The vascular wall motion and changes in the wall dimension were followed during diastole and systole. The measurements were performed automatically while obtaining the baseline diameter for FMD evaluation. The following parameters of arterial stiffness were assessed: β -stiffness index (β), pressure-strain elasticity index (Young's modulus, E_p), arterial compliance index (ACI), 1-point pulse-wave velocity (PWV β), and augmentation index (AI).

Photoplethysmographic pulse waveform analysis

Photoplethysmographic analysis was performed with Pulse Trace 2 (Micro Medical, CareFusion Corporation, San Diego, California, United States) simultaneously with the brachial dilation test. Pulse volume was measured with a sensor placed on the index finger of the dominant upper limb. The following indices were assessed: peak-to-peak time (PPT), stiffness index, and reflexion index.

Reproducibility of flow-mediated dilation and e-Tracking

To assess the reproducibility of FMD and e-Tracking parameters, the baseline values of the brachial artery diameter prior to reactive hyperemia and nitroglycerin administration were compared.

Statistical analysis Data were expressed as mean \pm standard deviation. The Saphiro-Wilk test was used to assess the normal distribution of data. Data were analyzed by the analysis of variance with post hoc analysis. Correlations between variables were assessed by the Spearman rank test. The χ^2 test was used to evaluate correlations between categorical variables. A *P* value of less than 0.05 was considered statistically significant. Data were analyzed using the Statistica 9.1 StatSoft software (StatSoft, Inc, Tulsa, Oklahoma, United States).

RESULTS The non-FH group was older compared with patients with FH (45.4 \pm 6.7 years vs. 38.9 \pm 7.4 years; *P* < 0.01). The study groups differed in the maximum levels of LDL cholesterol with the highest values observed in patients with FH. Six FH patients and 6 non-FH patients suffered from arterial hypertension but, during the study, all subjects had well-controlled blood pressure, confirmed by home monitoring. Two patients with FH and 4 patients with non-FH were current smokers. The subjects did not differ

TABLE 3 Correlations between flow-mediated dilation and arterial stiffness parameters assessed by e-Tracking and photoplethysmography

Parameters		FMD, %									
		all patients (n = 60)		FH (n = 21)		non-FH (n = 19)		FH and non-FH (n = 40)		controls (n = 20)	
		r	P	r	P	r	P	r	P	r	P
e-Tracking	β	-0.4	<0.01	-0.6	<0.05	-0.4	<0.05	-0.5	<0.01	-0.5	<0.05
	Ep, kPa	-0.4	<0.01	-0.6	<0.01	-0.4	<0.05	-0.5	<0.01	-0.5	<0.05
	PWV β , m/s	-0.5	<0.01	-0.7	<0.01	-0.6	<0.01	-0.6	<0.01	-0.6	<0.01
	AC, mm ² /kPa	0.2	<0.05	0.5	<0.05	0.4	<0.05	-0.4	<0.01	-0.4	<0.05
	AI, %	0.4	NS	0.3	NS	-0.2	NS	-0.2	NS	-0.2	NS
photoplethysmography	SI, m/s	-0.1	NS	0.0	NS	-0.2	NS	-0.1	NS	-0.1	NS
	RI, %	-0.0	NS	-0.1	NS	0.3	NS	0.1	NS	0.1	NS
	PPT, ms	0.0	NS	0.0	NS	0.0	NS	-0.0	NS	-0.0	NS

Abbreviations: see TABLE 1

TABLE 4 Correlations between arterial stiffness parameters assessed by e-Tracking and photoplethysmography

Parameters		e-Tracking									
		β		Ep, kPa		PWV β , m/s		AC, mm ² /kPa		AI, %	
		r	P	r	P	r	P	r	P	r	P
photoplethysmography	SI, m/s	0.0	NS	0.0	NS	0.1	NS	-0.0	NS	0.0	NS
	RI, %	0.0	NS	0.0	NS	0.0	NS	0.2	NS	-0.1	NS
	PPT, ms	0.0	NS	0.0	NS	-0.0	NS	0.0	NS	-0.1	NS

Abbreviations: see TABLE 1

TABLE 5 Reproducibility of flow-mediated dilation and e-Tracking

Parameters		Before FMD	Before EID	P value	Correlation	
					r	P value
FMD	diameter in systole, mm	4.2 ± 0.8	4.3 ± 0.8	NS	0.9	<0.01
	diameter in diastole, mm	4.1 ± 0.8	4.2 ± 0.8	NS	0.9	<0.01
e-Tracking	β	21.9 ± 15.2	23.0 ± 14.7	NS	0.3	<0.05
	AC, mm ² /kPa	0.2 ± 0.1	0.1 ± 0.1	NS	0.8	<0.01
	PWV β , m/s	9.5 ± 3.6	10.1 ± 3.7	NS	0.5	<0.01
	Ep, kPa	276.0 ± 197.0	290.0 ± 190.5	NS	0.3	<0.05
	AI, %	17.3 ± 17.3	9.7 ± 28.9	NS	0.2	NS

Data are presented as mean ± standard deviation.

Abbreviations: see TABLE 1

significantly in height, weight, and body mass index (TABLE 1).

FMD was lower in patients with FH compared with controls (11.0% ± 9.9% vs. 21.0% ± 14.3%, $P < 0.01$) and in those with non-FH compared with controls (14.2% ± 10.1% vs. 21.0% ± 14.3%, $P < 0.05$), as shown in the FIGURE. FMD did not differ between FH and non-FH subjects. Moreover, it did not correlate with lipid profile parameters in patients with FH and non-FH. EID did not differ between the groups. In addition, there were no differences in arterial stiffness parameters assessed by e-Tracking and photoplethysmography between any of the groups (TABLE 1).

A significant positive correlation between FMD and the ACI and significant negative correlations between FMD and β , Ep, and PWV β were noted. The results of correlation analysis between endothelial function assessed by FMD and arterial

stiffness parameters obtained by e-Tracking and photoplethysmography are presented in TABLE 3. There were no significant correlations between arterial stiffness parameters measured by e-Tracking and those measured by photoplethysmography (TABLE 4).

There were no differences in the baseline diameter of the brachial artery measured during systole and diastole before FMD and 10 minutes after the test, just prior to the EID test. A strong correlation was observed in the mean vessel diameter before FMD and before EID (correlation index during diastole: $r = 0.90$; $P < 0.01$). Likewise, the reproducibility of arterial stiffness parameters assessed by e-Tracking, prior to FMD and EID measurement, was analyzed. No statistically significant differences were found between β , ACI, Ep, PWV β , EP, and AI obtained prior to FMD and EID tests. Significant correlations were noted

between β , AC, Ep, PWV β , and EP obtained prior to FMD and EID tests. The reproducibility of FMD and e-Tracking assessments is presented in [TABLE 5](#).

DISCUSSION Atherosclerosis, often determined by high plasma cholesterol levels, is an underlying cause of CVD and cardiovascular events. Early diagnosis of vascular dysfunction may allow early prevention of acute cardiovascular events.²³ New noninvasive methods assessing endothelial function and arterial stiffness may be helpful in early detection of atherosclerosis.²⁴ Our FMD results are consistent with the findings of the previous studies that assessed this parameter in patients with hypercholesterolemia. In a meta-analysis of 8 studies conducted in patients with FH, Masoura et al.²⁵ showed that subjects with FH have significantly lower FMD values compared with normolipidemic controls (pooled mean difference: -5.31%; 95% confidence interval, -7.09 to -3.53%; $P < 0.001$). Of note, in all our study groups, mean FMD values were within the normal range. However, all subjects were relatively young and had no history of cardiovascular events. Despite a difference in age between FH and non-FH patients, we noted lower FMD values in a significantly younger group of FH patients. It suggests that the effect of genetically determined hypercholesterolemia on FMD values is more significant than that of age itself. The importance of EID in vascular function assessment has also been emphasized by researchers. Reduced FMD values in hypercholesterolemic patients in our study may indicate that endothelial dysfunction had already occurred in this group but had not yet been advanced enough to substantially affect EID values. Therefore, we suggest that FMD can be used for early detection of endothelial dysfunction preceding the atherosclerotic process in patients with FH and non-FH without significant CVD.

It has been proved that increased arterial stiffness in atherosclerosis may result in higher risk of cardiovascular events.²⁶ There are a few studies investigating the use of e-Tracking for the assessment of arterial stiffness. Jaroch et al.²⁷ examined 58 individuals with normal intima-media thickness using e-Tracking, including 27 healthy subjects and 31 patients with cardiovascular risk factors. They showed higher values of β , Ep, and PWV β and significantly lower values of the carotid artery ACI in patients with cardiovascular risk factors. In another study, Jaroch et al.²⁸ showed higher values of β , Ep, and PWV β and lower values of the carotid artery ACI in patients older than 50 years of age compared with patients younger than 50 years of age. In the current study, no significant difference in arterial stiffness parameters between FH and non-FH patients without a history of CVD was found. A lack of significant differences in arterial stiffness parameters most likely shows that the process of vascular remodeling may not yet be advanced in relatively young patients with elevated LDL cholesterol levels.

There is a significant correlation between novel arterial stiffness parameters assessed by ultrasonography and endothelial dysfunction, which can be used for early detection of functional arterial remodeling in patients with hypercholesterolemia. A relationship between e-Tracking parameters and FMD reflects interactions between endothelial dysfunction and arterial stiffness. A lack of a significant correlation between FMD values and arterial stiffness parameters measured by photoplethysmography may result from the fact that these methods evaluate different aspects of the atherosclerotic process. Photoplethysmography may also have lower sensitivity compared with FMD. A lack of significant correlations between arterial stiffness parameters assessed by e-Tracking and photoplethysmography may suggest that these tests are used to evaluate different aspects of early atherosclerosis development. The PulseTrace 2 estimates large artery stiffness from the pulse waveform obtained at the finger with an infrared sensor. The speed at which the pulse travels along the arterial tree is related to arterial stiffness. The measurement of the time it takes for the pulse waves to travel through the arterial system provides a way of measuring arterial stiffness. The shape of the volume waveform in the finger is directly related to the time it takes for the pulse waves to travel through the arterial tree. It is expected to be independent of local changes of the vasculature but determined mostly by large artery stiffness (estimated by the stiffness index) and vascular tone (estimated by the reflexion index).

The strongest correlation between FMD values and arterial stiffness parameters measured by e-Tracking was noted in patients with FH. Therefore, a new ultrasound method for the assessment of arterial stiffness parameters and endothelial dysfunction may be useful in early detection of vascular remodeling in patients with FH. A lack of significant differences in FMD and arterial stiffness parameters in patients with FH and non-FH may indicate that the *LDLR* or *APOB* mutation is not the main determinant of endothelial dysfunction and vascular remodeling in relatively younger patients.

In previous studies, arterial stiffness parameters measured by e-Tracking were usually assessed in the carotid arteries.²⁷ Our study is the first to have successfully used this method for the brachial arteries. However, a limited number of published data regarding e-Tracking and photoplethysmography makes it difficult to verify our results.

Our study has several limitations. It was an observational study evaluating only 40 patients with elevated LDL cholesterol levels (with FH and non-FH). FH is a rare condition. Moreover, the subgroup of FH patients included only those with genetically confirmed FH and without a history of CVD. Unfortunately, the occurrence of cardiovascular events (mainly acute coronary syndrome) in this group of patients usually results in

establishing a diagnosis and introducing specialist care.²⁹ This was a major limitation of the study since the sample size could be too small to show significant differences between FH and non-FH groups regarding FMD and arterial stiffness parameters. The study groups differed significantly in age. There were also a few participants with arterial hypertension. Therefore, any conclusions should be drawn with caution. It should be emphasized that this was an experimental study and we did not perform an epidemiological evaluation of endothelial function and arterial stiffness parameters in the studied groups.

We did not evaluate the mechanisms by which FMD was impaired in patients with FH and non-FH. The measurement of plasma or urinary sympathetic nervous activity markers, inflammatory markers, and oxidative stress markers would have allowed to draw more specific conclusions.

There is evidence that statins exert pleiotropic effects and modify vascular dysfunction,³⁰ and, in our study, numerous patients were treated with statins. We cannot exclude that the lack of arterial structural remodeling in the study group despite high LDL cholesterol levels may be a result of effective statin treatment. Several studies have also shown a protective effect of endogenous estrogens on endothelial function in women.³¹ As there were only 12 postmenopausal women (3 in the FH, 6 in non-FH, and 3 in control groups) in our study, we could not perform additional statistical analysis.

Although the cause of FH is monogenic, it is characterized by a substantial variation in the onset and severity of atherosclerotic disease symptoms. Additional atherogenic risk factors of the metabolic and genetic origin and the effect of environment, in conjunction with the LDL receptor defect, are presumed to affect the clinical phenotype of FH. However, it is not clear whether these are synergistic interactions or simply additive effects. The mechanism of action of these factors is thought at least in part to be mediated by the differences in the plasma levels of atherogenic lipids and lipoproteins. The specific genes involved in these processes and their variants in the general population are the subject of numerous ongoing studies.³² Endothelial dysfunction is caused by a wide range of factors including inflammatory processes, smoking, or elevated levels of lipoproteins such as LDL. We did not find any results indicating that particular gene mutations determine endothelial dysfunction. In addition, our study group was too small to analyze those associations.

In conclusion, preserved EID in all subjects may suggest that reduced FMD is due to endothelial dysfunction. A lack of significant differences in arterial stiffness parameters may indicate that the process of vascular remodeling may not be advanced in younger patients with elevated LDL cholesterol levels. No significant differences in FMD and arterial stiffness parameters between patients with FH and those with non-FH

may indicate that the gene mutation itself is not the main determinant of endothelial dysfunction and vascular remodeling in younger patients with hypercholesterolemia.

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Contribution statement MG conceived the idea for the study. PL, MG, and AR contributed to the design of the research. All authors were involved in data collection. PL analyzed the data. AW coordinated funding for the project. All authors edited and approved the final version of the manuscript.

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Nieinwazyjna ocena parametrów naczyniowych i funkcji śródbłonna u pacjentów z hipercholesterolemią rodzinną

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SŁOWA KLUCZOWE

dysfunkcja
śródbłonna,
hipercholesterolemia
rodzinną, miażdżycę,
sztywność naczyń

STRESZCZENIE

WPROWADZENIE Dysfunkcja śródbłonna jest jednym z markerów procesu miażdżycowego.

CELE Celem pracy była ocena funkcji śródbłonna oszacowanej za pomocą badania rozszerzalności tętnicy ramiennej pod wpływem niedokrwienia (*flow-mediated dilatation* – FMD) oraz ocena parametrów sztywności tętnicy ramiennej u pacjentów z hipercholesterolemią rodzinną i pacjentów z wysokim stężeniem cholesterolu frakcji lipoprotein o małej gęstości (*low-density lipoprotein* – LDL), bez mutacji powodującej hipercholesterolemię rodzinną.

PACJENCI I METODY Do badania włączono 60 osób (średnia wieku $41,9 \pm 7,7$ roku) bez udokumentowanych zdarzeń sercowo-naczyniowych i objawów klinicznych chorób sercowo-naczyniowych: 21 pacjentów z podwyższonym poziomem cholesterolu LDL w osoczu i genetycznie potwierdzoną hipercholesterolemią rodzinną, 19 pacjentów z podwyższonym poziomem cholesterolu LDL w osoczu bez mutacji genów powodujących hipercholesterolemię rodzinną oraz 20 zdrowych ochotników. Za pomocą aparatu ultrasonograficznego każdemu pacjentowi wykonano zależne od funkcji śródbłonna badanie FMD i niezależne od funkcji śródbłonna badanie rozszerzalności tętnicy ramiennej pod wpływem nitrogliceryny (*endothelium-independent dilatation* – EID). Metodę e-Tracking i metodę fotopletyzmoграфiczną wykorzystano do oceny parametrów sztywności naczyń.

WYNIKI Wartości FMD były istotnie mniejsze u pacjentów z hipercholesterolemią rodzinną ($11,0 \pm 9,9\%$ vs $21,0 \pm 14,3\%$; $p < 0,01$) oraz pacjentów z wysokim poziomem cholesterolu LDL, bez mutacji powodującej hipercholesterolemię rodzinną ($14,2 \pm 10,1\%$ vs $21,0 \pm 14,3\%$; $p < 0,05$) w porównaniu z grupą kontrolną. Wartości EID oraz parametry sztywności naczyniowej były porównywalne we wszystkich grupach.

WNIOSKI Obniżone wartości FMD mogą świadczyć o dysfunkcji śródbłonna. Brak istotnych różnic w parametrach sztywności tętnic może sugerować, że proces przebudowy naczyń nie jest zaawansowany u chorych ze zwiększonym stężeniem cholesterolu LDL w surowicy. Brak istotnych różnic wartości FMD oraz parametrów sztywności naczyń między pacjentami z hipercholesterolemią rodzinną i bez niej może wskazywać, że obecność mutacji nie jest głównym czynnikiem determinującym wystąpienie dysfunkcji śródbłonna i przebudowy strukturalnej naczyń tętniczych w grupie młodszych pacjentów z hipercholesterolemią.

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