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Laser Doppler flowmetry evaluation of skin microvascular endothelial function in patients with metabolic syndrome

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ABSTRACT

Background: Metabolic syndrome (MetS) is associated with high cardiovascular morbidity and mortality, and endothelial dysfunction is an early pathogenetic event in the MetS. Lifestyle changes and pharmacological intervention might partly restore endothelial function in MetS. Whereas an optimal non-invasive test for endothelial dysfunction is still being sought, the aim of this study was to assess the relationship between changes in skin microvascular endothelial function, detected by Laser Doppler flowmetry, and cardiovascular risk factors (CVRFs) of patients with MetS.

Design and methods: 3081 patients (1865 women and 1216 men, mean age 53 ± 6 years) with MetS were enrolled in the study, which was conducted during the period of 2010–2014 at Vilnius University Hospital Santaros Klinikos. Skin microvascular endothelial function was evaluated using the Laser Doppler flowmetry in combination with the post-occlusive reactive hyperaemia test. The percentage change of flow from peak to the rest flow (PF-RF) was calculated and used as the main measure of endothelial function.

Results: The study showed that decrease in flow-mediated dilatation reflected by PF-RF was associated with increased triglycerides ($p = 0.002$), male sex ($p < 0.001$), and diabetes ($p = 0.002$). Patients with quite a few CVRFs (body mass index $\geq$25 kg/m^2, smoking, diabetes, arterial hypertension, a positive history of dyslipidaemia) had significantly lower PF-RF score than patients only with one of these risk factors ($p < 0.001$).

Conclusions: Changes in skin microvascular endothelial function are significantly associated with most CVRFs and depend on the number of CVRFs.

1. Introduction

The 2021 European Guidelines on cardiovascular disease (CVD) prevention in clinical practice affirms that incidence and mortality rates of coronary artery disease are declining in many countries in Europe (Visseren et al., 2021). The World Health Organization states that more than three quarters of CVD deaths can be avoided by modifying cardiovascular risk factors (CVRFs) (Laucevičius et al., 2019). This encourages not only the identification of early CVRFs, but also the search for the most effective and convenient tests for easy detection of early signs of vascular aging. Timely identification of these signs could be used for the prevention of premature CVD and manifestation of possible its related complications.

Structural and functional changes, such as dysregulation of vascular tone, increased endothelium permeability, arterial stiffness, impairment of angiogenesis and vascular repair (Jia et al., 2019) are the hallmarks of senescence of vascular endothelial cells. Endothelial senescence is involved in age-related vascular dysfunction including atherosclerosis, arteriosclerosis and altered microcirculation (Honda et al., 2021). In fact, endothelial progenitor cells are responsible for balance between angiogenesis and microvascular rarefaction – an important pathophysiological sign of remodelling in vascular tree and early hypertension (Liang et al., 2019).

Since endothelial dysfunction is involved both in the early and late pathways of vascular remodelling, non-invasive tests of endothelial function are useful in identifying the patients at increased risk for CVD. Considering that various studies (Tziomalos et al., 2010; van der Heijden et al., 2017; Fuchs et al., 2017; Binggeli et al., 2003) confirmed that

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separate components of metabolic syndrome (MetS) (central obesity, impaired glucose metabolism, dyslipidaemia and arterial hypertension) have adverse effects on the endothelium, we suppose that endothelial dysfunction might be more prevalent at the presence of MetS and could play a significant role in the increased risk for CVD, and consequently indicate individuals with lower or higher cardiovascular (CV) risk.

LDF (Laser Doppler flowmetry) in combination with post-occlusive reactive hyperaemia (PORH) or other provocative tests is one of the methods for the evaluation of endothelial function, which allows to assess the endothelial function relatively quickly, completely independently on the investigator, and in a conveniently accessible location – the skin microvasculature (Lenasi, 2011). Endothelial dysfunction in the skin microvasculature is a significant indicator of early CV risk and could be considered as a prognostic marker for early vascular aging (Toya et al., 2021). LDF has been mainly used in cohorts with gestational diabetes, erectile dysfunction, chronic kidney disease, arterial hypertension, obesity, and diabetes mellitus (Sarnik et al., 2007; Gryglewska et al., 2020; Pontes et al., 2015; Fusco et al., 2020; Belcaro et al., 2000; Rossi et al., 2006; Levente Babos et al., 2013). These three latter conditions are the components of MetS. To our knowledge, there are a few studies that evaluate the association between CVRFs and endothelial function investigated by LDF in patients with MetS (van der Heijden et al., 2017; Genoud et al., 2008). The aim of this study was to evaluate the association between skin microvascular endothelial function – measured by LDF – and CVRFs in a high cardiovascular risk Lithuanian cohort with MetS. We hypothesize that endothelial function determined by LDF is significantly associated with risk factors for CVD and depends on the number of CVRFs.

2. Design and methods

2.1. Study population

This observational cross-sectional study enrolled 3081 participants (1865 women and 1216 men) with established high CV risk (Laucevičius et al., 2019). Study sample was retrieved from the Vilnius University Hospital Santaros Klinikos registry according to the definite period – from year 2010 to year 2014, when study was conducted. All patients were referred by the primary health care institutions under Lithuanian High Cardiovascular Risk (LitHiR) primary prevention program. LitHiR program recruited women at the age of 50–65 years and men at the age of 40–55 years without overt CVD. All participants had the MetS according the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP-III) definition (Rezaianzadeh et al., 2012) if three or more of the following criteria were present:

- central obesity (waist circumference ≥102 cm in men and ≥88 cm in women),
- arterial hypertension (blood pressure ≥130/85 mmHg or treatment of previously diagnosed arterial hypertension),
- hypertriglyceridaemia (triglycerides (TGs) level ≥1.7 mmol/l),
- reduced high-density lipoprotein cholesterol (HDL-C) level (HDL-C level <1.03 mmol/l in men and <1.29 mmol/l in women or specific treatment for these lipid abnormalities),
- hyperglycaemia (fasting glucose level ≥5.6 mmol/l or previously diagnosed type 2 diabetes mellitus).

The exclusion criteria were as follows: clinically evident CVD, end-stage oncological disease, or any other end-stage somatic disease.

Blood sample collection.

Blood samples were collected from patients in the morning after overnight fasting. Blood samples for a high-sensitivity C-reactive protein (hsCRP), glucose concentration, a lipid panel (total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), HDL—C, TGs), and other makers were analyzed in the laboratory of Vilnius University Hospital Santaros Klinikos.

2.2. Assessment of endothelial function

The skin microvascular (arterioles, capillaries, venules and shunting vessels) endothelial function was evaluated using the LDF technique (PeriFlux System 5000, Perimed, Sweden). A laser doppler probe (Probe 457; Perimed) (Probes, 2020) was placed on the dorsal surface of the middle phalanx of the index finger of the non-dominant hand. Microvascular reactivity was evaluated using the LDF technology with a laser wavelength of 785 nm in combination with PORH test for non-invasive and continuous measurement of cutaneous microvascular perfusion changes. During the PORH test, arterial occlusion was performed with suprasystolic pressure (100 mmHg above the systolic arterial pressure), by applying a cuff of sphygmomanometer on a patient's upper arm longer than for 5 min. Following the release of the pressure, the maximum flux was measured. PORH was registered as perfusion change in a rest state and in a state after 5 min of arterial occlusion. Various microcirculation parameters were determined, the most important of which were as follows: rest flow (RF) – the flow determined in a rest state before arterial occlusion, biological zero (BZ) – the flow determined during arterial occlusion, peak flow (PF) – the flow determined after release of arterial occlusion. These parameters are presented in the Fig. 1 (Stupin et al., 2013). All these parameters were expressed in arbitrary perfusion units (APU). The percentage change of flow from peak to the rest flow (PF-RF) was calculated and expressed in percentage and used as the main measure of endothelial function.

The microcirculatory test was performed after a 10–15 min rest with the subjects in the supine position in a temperature-controlled room (22–25 °C) in the morning after overnight fasting. Patients were also asked to abstain from smoking or any tobacco consumption, exercising, drinking beverages that contain alcohol or caffeine at least 12 h before the study.

2.3. Ethical issues

The study conforms with the principles outlined in the Declaration of Helsinki. Permission No. 2019/3-1104-603 was issued by the Vilnius Regional Biomedical Research Ethics Committee. The written informed consent was obtained from all participants before their inclusion in the study.

2.4. Statistical analysis

Statistical analysis was performed using the “IBM SPSS Statistics V23.0” and “Microsoft Excel 2010” programs.

The normality of the distribution across the variables was assessed using Kolmogorov–Smirnov test. Continuous and normally distributed variables were presented as mean with standard deviation, while continuous variables with asymmetric distribution were presented as median with interquartile range values. Categorical data were presented as frequencies and percentages. Continuous variables were compared using the *t*-test, analysis of variance ANOVA or the Mann–Whitney test. Categorical variables were compared using the *chi square* test. All *p*-values were two-tailed. A value of $p \leq 0.05$ considered to be statistically significant.

The linear dependence of sample characteristics was analyzed by calculating the Pearson's (for normally distributed variables) and Spearman's (for variables with asymmetric distribution) correlation coefficients.

A multiple linear regression model was used to assess the influence of risk factors on endothelial function. PF-RF was considered a dependent variable. The analysis provided a standardized regression coefficient beta (β) to access the significance of the influence of risk factors (independent variables) on PF-RF. Independent variables included in this model were as follows: age, body mass index (BMI), TGs, hsCRP level, sex, smoking, diabetes mellitus, arterial hypertension. TC level was not included in the model due to multicollinearity. 95% confidence interval

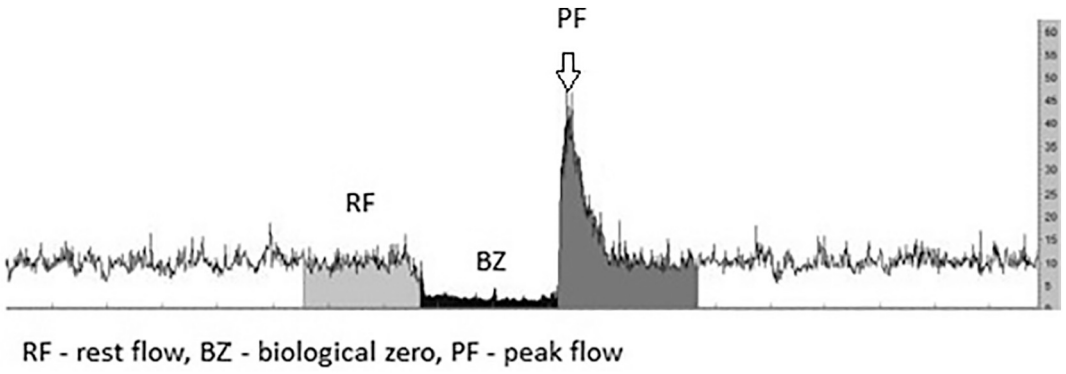

Fig. 1. LDF measurement of skin microcirculation.
(Modified from source (Stupin et al., 2013)).

(CI) were calculated.

The influence of different laboratory indicators: hsCRP ≥3.0 mg/l, HDL-C level <1 mmol/l, TGs level >1.7 mmol/l, low-density lipoprotein cholesterol (LDL-C) level >3 mmol/l, TC level ≥5 mmol/l, glucose ≥5.6 mmol/l, on endothelial function were analyzed.

In our study, we also analyzed the effect of different CVRFs such as BMI ≥25 kg/m^2, smoking, diabetes mellitus, arterial hypertension, dyslipidaemia (in our study, dyslipidaemia was defined as elevated TC ≥5 mmol/l or/and elevated LDL-C >3 mmol/l, or/and decreased HDL-C <1 mmol/l, or/and elevated TGs ≥1.7 mmol/l), and their combination on endothelial function.

3. Results

3.1. Sample characteristics

A total of 3081 patients were examined. The study sample consisted of 1865 (60%) women and 1216 (40%) men without overt CVD. The mean age of all subjects was 53 ± 6 years. These characteristics, anthropometric data, comorbidities, laboratory indicators and the measurements of endothelial function are presented in the Table 1.

BMI scores of 18.5 to 24.9 are considered normal, scores of 25 to 29.9 are overweight, scores of 30 and above are obese.

3.2. Influence of CVRFs on endothelial function

Difference in PF-RF score with regards to CVRFs is presented in Supplemental Table 1. PR-RF score was similar by presence of arterial hypertension, hypertriglyceridaemia, increased LDL-C, and hyperglycaemia.

After adjustment for age and sex, TGs level was an independent variable significantly associate with PF-RF (Table 2).

3.3. Correlation between endothelial function and CVRFs

The multiple linear regression model with PF-RF score as dependent variable (Table 3) identified CVRFs that are associated with endothelial function: male sex (β = 56.69, $p < 0.001$), diabetes mellitus (β = 40.07, $p = 0.002$) and TGs level (β = −6.29, p = 0.002).

3.4. Influence of the number of CVRFs on endothelial function

In our study, we analyzed the effect of different numbers of CVRFs (from 1 to 5 CRFs) on endothelial function. We found that the PF-RF score decreases as the number of CRFs increases. The results are presented in the Table 4 and the Fig. 2.

Hence, patients with maximum number of CRFs (BMI ≥25 kg/m^2, smoking, diabetes mellitus, arterial hypertension, a positive history of dyslipidaemia) had significantly lower PF-RF score than patients with one of these risk factors ($n = 85, 235 \pm 169$ vs. $n = 15, 477 \pm 453$, $p < 0.001$).

4. Discussion

In our study we analyzed the influence of components of the MetS and other CVRFs on endothelial function measured by LDF in high CV risk cohort with MetS. The study showed that TGs, male sex and diabetes were independent factors significantly associated with endothelial function. Whereas even two components of the MetS significantly differed from the other CVRFs by revealing the essential association with endothelial function. The role of MetS in the increased risk for CVD is indisputable as much as male sex remains one of the most important non-modifiable CVRFs. Moreover, the study revealed that decrease in flow-mediated dilatation directly correlates with the number of CVRFs.

MetS is related to 50–60% higher CV risk compared to subjects without MetS (Qiao et al., 2007). Therefore, MetS is one of the main challenges we need to tackle to prevent the incidence of CVD. It is known that MetS and its components, e.g. obesity and diabetes mellitus (Hong et al., 2020), are considered as pro-inflammatory conditions, and thorough inflammatory pathways affect endothelial function (Tziomalos et al., 2010). Inflammatory state of microvascular endothelial cells is a probable factor for the microvascular rarefaction (Goligorsky, 2010), which plays a significant role in the development of hypertension which increases peripheral vascular resistance, and eventually contributes to the development of CVD and its complications (Liang et al., 2019). Microvascular rarefaction is associated with microvascular endothelial dysfunction (Mohammed et al., 2015) and both processes are thought to contribute to chronic ischemia in CVD by limiting coronary blood flow during reactive hyperaemia (Kaul and Jayaweera, 2008). We used hsCRP as a marker of inflammation and could confirm that elevated hsCRP was associated with impaired endothelial function as reported in previous studies (Tziomalos et al., 2010; Title et al., 2008).

Perivascular adipose tissue plays an important role in vasomotor regulation by excreting vasoactive adipocytokines, reduces smooth muscle tone and enhances insulin-dependent vasodilatation. In obesity this tissue switches its function to attenuation of adipose tissue-dependent vascular relaxation as a consequence of reduced nitric oxide (NO) bioavailability (van der Heijden et al., 2017). Here we show that higher BMI was associated with a significant lower microvascular hyperaemic response to PORH compared to normal BMI. Heijden DJ et al. used the same endothelial function evaluation method as we did and confirmed interrelationship between BMI and endothelial function (van der Heijden et al., 2017).

The state of low-grade inflammation, especially in obese patients, can lead to insulin resistance and type 2 diabetes mellitus, an important CVRF (van der Heijden et al., 2017). Inflammatory and oxidative

Table 1
Main characteristics of the subjects.

	All subjects (n = 3081)	Men (n = 1216)	Women (n = 1865)
Demographic data			
Age (years)	53 ± 6	48 ± 4	57 ± 4
Anthropometric data			
BMI (kg/m^2)	32 ± 5	31 ± 4	32 ± 5
Normal BMI	150 (5%)	43 (4%)	107 (6%)
Overweight	1086 (35%)	436 (36%)	650 (35%)
Obese	1834 (60%)	733 (60%)	1101 (59%)
Waist circumference (cm)	106 ± 11	109 ± 10	103 ± 11
Waist circumference ≥88 cm in women, and ≥102 cm in men	–	966 (79%)	1784 (96%)
Comorbidities			
Smoking	683 (22%)	431 (63%)	252 (37%)
Diabetes mellitus	547 (18%)	188 (34%)	359 (66%)
Arterial hypertension	2823 (92%)	1120 (40%)	1703 (60%)
Dyslipidaemia	3007 (98%)	1193 (40%)	1814 (60%)
Laboratory indicators			
TC (mmol/l)	7 ± 2	6 ± 2	7 ± 1
TC ≥5 mmol/l	2672 (87%)	1003 (82%)	1669 (89%)
LDL-C (mmol/l)	4 ± 1	4 ± 1	5 ± 1
LDL-C >3 mmol/l	2563 (83%)	930 (76%)	1669 (89%)
HDL-C (mmol/l)	1 ± 0.3	1 ± 0.3	1 ± 0.3
HDL-C <1 mmol/l	614 (20%)	448 (73%)	166 (27%)
TGs (mmol/l)	2 [1]	2 [2]	2 [1]
TGs ≥1.7 mmol/l	1781 (58%)	798 (45%)	983 (55%)
hsCRP (mg/l)	2 [3]	2 [3]	2 [3]
hsCRP ≥3.0 mg/l	945 (31%)	366 (39%)	579 (61%)
Glucose (mmol/l)	6 ± 1	6 ± 1	6 ± 1
Glucose ≥5.6 mmol/l	1918 (62%)	799 (42%)	1119 (58%)
Measurements of endothelial function			
PF-RF (%)	354 ± 271	324 ± 235	374 ± 291
RF (APU)	48 ± 33	49 ± 34	48 ± 33
BZ (APU)	6 ± 4	6 ± 4	6 ± 5
PF (APU)	171 ± 76	166 ± 75	174 ± 76
Number of CVRFs			
Subjects with 1 CVRF	15 (1%)	1 (0%)	14 (1%)
Subjects with 2 CVRFs	207 (7%)	59 (5%)	148 (8%)
Subjects with 3 CVRFs	1725 (58%)	616 (51%)	1109 (63%)
Subjects with >3 CVRFs	1006 (34%)	524 (44%)	482 (28%)

Data are presented as number (percentage), as mean ± SD, and as median [interquartile range].
BMI – body mass index, TC – total cholesterol, LDL-C – low-density lipoprotein cholesterol, HDL-C – high-density lipoprotein cholesterol, TGs – triglycerides, hsCRP – high-sensitivity C-reactive protein, PF-RF – percentage change of flow from peak to the rest flow, RF – rest flow, BZ – biological zero, PF – peak flow, APU – arbitrary perfusion units, CVRFs – cardiovascular risk factors.

reactions in endothelial cells are basically influenced by advanced glycation end-products (AGEs), which concentrations are elevated in diabetes. AGEs significantly decrease NO synthase expression in endothelial cells and NO production, and cause endothelial dysfunction and vascular inflammation (Ren et al., 2017). The meta-analysis on diabetes mellitus (Fuchs et al., 2017) described inferior values of skin microvascular function, assessed by LDF and laser speckle contrast imaging in combination with local thermal hyperaemia, in comparison to healthy subjects. And our results are in line with the latter report and could be an

Table 2
Assocciation of Lased Doppler flowmetry derived endothelial function with cardiovascular risk factors.

	β	SE	p value
BMI (kg/m^2)	−6.97	1.04	0.19
Smoking	51.76	12.45	0.33
Arterial hypertension	15.70	20.18	0.43
Diabetes mellitus	57.57	13.05	0.11
hsCRP (mg/l)	−1.84	1.18	0.11
TC (mmol/l)	6.04	3.32	0.06
LDL-C (mmol/l)	7.26	3.98	0.06
HDL-C (mmol/l)	73.97	16.62	0.89
TGs (mmol/l)	−6.97	2.11	**<0.001**
Glucose (mmol/l)	−25.12	3.93	0.19

Linear regression analysis with PF-RF as dependent variable adjusted for age and sex. Variables with significance level <0.05 indicated as bold font.
β – standardized coefficient, SE – standard error, CI – confidence interval for standardized coefficient.
BMI – body mass index, TC – total cholesterol, LDL-C – low-density lipoprotein cholesterol, HDL-C – high-density lipoprotein cholesterol, TGs – triglycerides, hsCRP – high-sensitivity C-reactive protein.

Table 3
Influence of multiple cardiovascular risk factors on endothelial function.

	β	SE	p value
Age (years)	−1.48	1.20	0.22
Male sex	56.69	15.11	**<0.001**
Diabetes mellitus (yes)	40.07	13.25	**0.002**
Arterial hypertension (yes)	−4.4	20.22	0.82
Smoking	57.22	12.38	0.39
BMI (kg/m^2)	−6.90	1.10	0.75
TGs (mmol/l)	−6.29	2.09	**0.002**
hsCRP (mg/l)	0.71	1.21	0.55

Variables with significance level <0.05 indicated as bold font.
β – standardized coefficient, SE – standard error, CI – confidence interval for standardized coefficient.
BMI – body mass index, TGs – triglycerides, hsCRP – high-sensitivity C-reactive protein, PF-RF – percentage change of flow from peak to the rest flow. PF-RF (%) as dependent variable.

Table 4
Endothelial function depending on the number of risk factors.

Number of CVRFs	1	2	3	>3
PF-RF (%)	477.48 ± 453.36	445.37 ± 337.19	370.33 ± 270.15	306.64 ± 242.31

Data are presented as mean ± SD.
CVRFs – cardiovascular risk factors, PF-RF – percentage change of flow from peak to the rest flow.

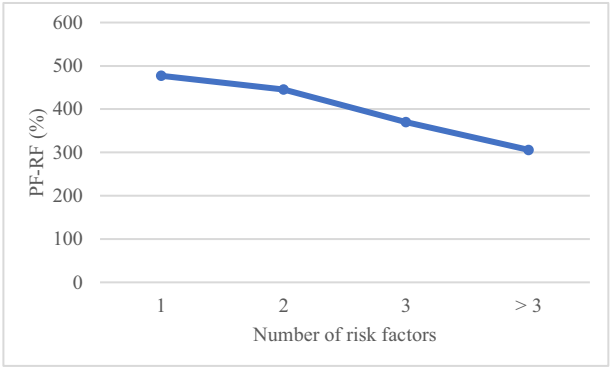

Fig. 2. Influence of the number of risk factors on endothelial function.

indirect evidence of microvascular rarefaction (Horton and Barrett, 2021).

TGs might impair endothelial function both through direct and indirect mechanisms (Kajikawa et al., 2016; Kajikawa and Higashi, 2019). However, most studies support the indirect pathway since triglyceride-rich lipoproteins (TRLs) are sufficiently small to enter the arterial wall and promote inflammation and alter endothelial function. Basically, TRLs trigger proatherogenic responses by enhancing monocyte recruitment and attachment to endothelial cells (Kajikawa et al., 2016). TGs level reflects on the balance between secretion and removal of TRLs. Binggeli C et al. reported altered skin blood flow in subjects with hypercholesterolaemia and especially with increased LDL-C levels (Binggeli et al., 2003). We found an additional relationship with TGs, additionally to male sex and diabetes. Based on previous studies, increased TGs levels are stronger correlated to increase in CV risk in women as compared to men (Palmisano et al., 2018). So that our results should be interpreted carefully and could depend on lower adherence to drug treatment in men and is important uncertainty to answer.

The negative effect of smoking on endothelium function that we report are in line with previous studies (Rossi et al., 2014). It could be explained by increased production of reactive oxygen species (ROS) and dysfunctional uncoupled NO synthase, that contribute to decrease of vascular NO bioavailability (Münzel et al., 2020).

In our study, microvascular perfusion and endothelial function of the subjects were assessed not only by LDF with PORH, but also by peripheral arterial tonometry (PAT) with PORH test. Whereas PAT methodology included placing the probe on the finger, we placed the probe on the finger instead of the forearm during the LDF test in order to compare the results of both tests. It is likely that we would have obtained slightly lower hyperemic response results by choosing the forearm skin for the study. Freccero C et al. study showed the higher microvascular flow response to heating, as a provocative test, in the fingertip compared with the forearm skin. These differences are likely to be due to the different amount of arteriolar venular anastomoses – the finger contains more arteriolar venular anastomoses than the forearm skin (Freccero et al., 2003).

To our knowledge, there is a lack of studies in the literature that examine in detail the multiple effect of many CVRFs rather than a single factor on endothelial function determined by LDF with PORH in patients without any CVD. Based on our finding it can be assumed that the presence of multiple risk factors indicates individuals with progressive vascular damage. In fact, the more risk factors a person has, the more likely he or she is to develop a CVD (Elfi et al., 2021). It is important to control as many risk factors as possible through lifestyle changes, treatment, or both, in order to preserve endothelial function and to prevent early vascular aging.

5. Conclusions

In conclusion, changes in skin microvascular endothelial function determined by LDF in combination with PORH are significantly associated with most risk factors for CVD and depend on the number of CVRFs. The more risk factors a person has, the more likely he or she is to develop a CVD, because of the altered endothelial function. Thus, it is important to control as many risk factors as possible.

6. Limitations

The main limitation of the study was the non-application of the pharmacological stimuli directly to the skin to test endothelial function. Whereas endothelium is one of many other local factors regulating microcirculation in the skin, it is important to try to distinguish the true cause of the decreased hyperaemic response.

Moreover, in our study we applied one more microvascular perfusion and endothelial function assessment method – flow mediated dilatation (FMD) in brachial artery with PORH test. Whereas in the FMD in brachial artery with PORH methodology suprasystolic pressure was considered 100 mmHg above the systolic arterial pressure, the same suprasystolic pressure was used during the LDF test on the possibility of more reliable comparison of these tests.

One more limitation of our study was that we had no data on antihypertensive and lipid lowering treatment that may have affected endothelium. Furthermore, there were relatively few comparable studies that are related to our topic in the literature. Seeing that we analyzed a high CV risk cohort, which already had MetS, we were not able to assess normal endothelial function hypertension and endothelial function was mainly due to the lack of statistical power as 92% of participants had arterial hypertension.

The publication of our study was delayed until the year of 2022 because of the intention to select a larger group of subjects and to compare the results obtained by all three tests: LDF, PAT and FMD in brachial artery in combination with PORH. Evaluation of endothelial function by LDF with PORH was stopped performing earlier than other tests due to certain financial and technical aspects, which made an influence on the delay of publication.

Abbreviations

AGEs	advanced glycation end-products
BMI	body mass index
BZ	biological zero
CV	cardiovascular
CVD	cardiovascular disease
CVRFs	cardiovascular risk factors
FMD	flow mediated dilatation
HDL-C	high-density lipoprotein cholesterol
hsCRP	high-sensitivity C-reactive protein
LDF	laser Doppler flowmetry
LDL-C	low-density lipoprotein cholesterol
LitHiR	Lithuanian High Cardiovascular Risk
MetS	metabolic syndrome
NO	nitric oxide
PAT	peripheral arterial tonometry
PORH	post-occlusive reactive hyperaemia
PF	peak flow
PF-RF	percentage change of flow from peak to the rest flow
APU	arbitrary perfusion units
RF	rest flow
ROS	reactive oxygen species
TC	total cholesterol
TGs	triglycerides
TRLs	triglyceride-rich lipoproteins

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Data availability

Dataset generated during and analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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CRediT authorship contribution statement

Zivile Girkantaite: Conceptualization, Methodology, Software, Formal analysis, Data Curation, Writing – Original draft, Writing – Review & Editing, Visualization. **Agne Laucyte-Cibulskiene**: Conceptualization, Methodology, Formal analysis, Writing – Original draft,

Writing – Review & Editing, Visualization. **Ligita Ryliskyte**: Conceptualization, Methodology, Investigation, Writing – Review & Editing. **Agne Juceviciene**: Conceptualization, Investigation, Writing – Review & Editing. **Jolita Badariene**: Conceptualization, Methodology, Validation, Investigation, Resources, Data Curation, Writing – Review & Editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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