Ассоциации функциональных и биохимических параметров дисфункции эндотелия у женщин в постменопаузе с различным состоянием углеводного обмена

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Многие вопросы регуляции реологических и сосудистых свойств в норме и при нарушениях углеводного обмена остаются неясными, что важно в патогенезе ангиопатий. Так, отсутствуют сведения о функциях эндотелия по вазомоциям в условиях окклюзии. Особое внимание привлекают женщины в ранней постменопаузе, когда нередко клинически манифестируют метаболические и гемодинамические нарушения.

Цель*. Изучить ассоциации клинических и биохимических показателей с микроциркуляторными параметрами эндотелиального диапазона у постменопаузальных женщин в естественной постменопаузе при различном состоянии углеводного обмена.*

Материалы и методы*. Обследованных 94 постменопаузальных женщины разделили на 3 группы: 52 с сахарным диабетом 2 типа (СД2) составили группу 1; 16 с предиабетом — группу 2; 26 с нормогликемией — группу 3. Определяли показатели: антропометрические, гликемии натощак, гликированный гемоглобин, липидограмму; фактор роста эндотелия сосудов (VEGF) и эндотелин-1. Микроциркуляцию оценивали методом лазерной допплеровской флоуметрии (ЛДФ). Статистический анализ проводили с помощью программ SPSS (версия 17.0).*

Результаты*. Параметры ЛДФ в группе 3 достоверно различались с группой 1 во время окклюзионной пробы и при реперфузии; с группой 2 — в условиях базального кровотока. В группе 1 при окклюзии PMfEi (частота колебаний микрокровотока) была выше, а SO2aEi (амплитуда колебаний сатурации) ниже, чем в группе 3. Показатели VraEr и Vra/mEr в период восстановления кровотока в группе 1 и VrfEf при регистрации базального кровотока в группе 2 превышали таковые группы 3 (параметры Vr отражают вклад колебаний относительного объема фракции эритроцитов в зондируемом участке кожи в модуляцию сосудистого тонуса). В группе 1 окклюзионные показатели ЛДФ коррелировали с ОТ, VEGF и параметрами липидограммы, реперфузионные параметры VraEr — с показателями систолического АД, липидограммы. В группе 2 параметр VrfEf обратно коррелировал с показателем VEGF; в группе 3 показатель PMfEi — с показателями эндотелина-1.*

Заключение*. Выявлены ассоциации метаболических, антропометрических, гемодинамических факторов и биохимических маркеров дисфункции эндотелия с параметрами микроциркуляции в различных режимах (базальном, окклюзионном, реперфузионном) эндотелиального диапазона у постменопаузальных женщин в зависимости от состояния углеводного обмена. Детализировано влияние возраста на вазомоции микроциркуляторного русла.*

Ключевые слова*: дисфункция эндотелия; сахарный диабет 2 типа; предиабет; лазерная допплеровская флоуметрия; вазомоции*

Correlations between functional and biochemical parameters of endothelial dysfunction in postmenopausal women with normal and impaired carbohydrate metabolism

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Most of the questions regarding vascular and rheological regulation related to normal health and disorders of carbohydrate metabolism remain unclear, which is important in the pathogenesis of angiopathy. Consequently, in the literature, there is no information about the function of endothelial vasomotion during occlusion. The present study investigated early postmenopausal women, when clinical, metabolic, and hemodynamic disturbances often manifest.

Aim*. To study the association between clinical and biochemical indicators of endothelial microcirculation in naturally postmenopausal women with different carbohydrate metabolism statuses.*

Materials and methods*. We surveyed 94 postmenopausal women who were divided into three groups based on their carbohydrate metabolism status: group 1, type 2 diabetes mellitus (n = 52); group 2, prediabetes (n = 16); group 3, normoglycemia (n = 26). The following indicators were assessed: lipid profiles and anthropometric fasting plasma glucose, glycated hemoglobin, vascular endothelial growth factor (VEGF), and endothelin-1 levels. Microcirculation was evaluated by laser Doppler flowmetry (LDF). Statistical analysis was performed using SPSS software (version 17.0).*

Results. *LDF parameters in group 3 were significantly different from group 1 during occlusion and reperfusion and also from group 2 in basal blood flow. During occlusion, the frequency of microcirculation oscillation was higher in group 1, whereas the amplitude of oxygen saturation oscillation was lower in group 3 than those in group 2. VraEr and Vra/mEr indicators during reperfusion in group 1 and VrfEf during basal blood flow in group 2 were higher than those in group 3; Vr parameters reflect the contribution of fluctuations in the relative erythrocyte volume in the probed area of skin to the modulation of vascular tone. In group 1, occlusal LDF indicators correlated with waist circumference, whereas VEGF, lipid profile parameters, and reperfusion parameters, VraEr correlated with systolic blood pressure and lipid profile. In group 2, VrfEf inversely correlated with VEGF levels, and in group 3, the frequency of microcirculation oscillation inversely correlated with endothelin-1 levels.*

Conclusions. *We revealed the associations of metabolic, anthropometric, and hemodynamic factors as well as biochemical markers of endothelial dysfunction with microcirculation parameters in various modes of endothelial activity (basal, occlusion, and reperfusion) in postmenopausal women according to their carbohydrate metabolism status; we also describe the effect of age on microvasculature vasomotion.*

Key words: *endothelial dysfunction; type 2 diabetes mellitus; prediabetes; Laser Doppler Flowmetry; vasomotions*

Cardiovascular diseases (CVDs) are considered to be predominantly occurring in males; however, these are a leading cause of death in women associated with menopause [1]. The most important risk factor for cardiovascular mortality in women is type 2 diabetes mellitus (T2DM) because an improper management of this condition leads to the development of macro- and microvascular complications. According to the International Diabetes Foundation in 2013, the apogee of T2DM incidence lowered to 45−55 years of age, which coincides with the age range of natural menopause.

Microcirculation disorders and blood rheology play an important role in the pathogenesis of cardiovascular disease and complications related to T2DM [2]. In addition to nerve and local control mechanisms of skin microcirculation, changes in the lumen of blood vessels, called vasomotion, are also involved. Although its origin is not completely elucidated, vasomotion is the rhythmic oscillation of blood microvessels determined by endothelial, neurogenic, myogenic, and other factors. There is a speculation that changes in vasomotion result in the disruption of blood oxygen transport, which leads to an increase in tissue ischemia. This is the reason why microvascular blood flow in T2DM has been gaining more attention in recent decades.

Of the various microcirculation assessment techniques, the most common is laser Doppler flowmetry (LDF), a noninvasive method used to measure the overall level of perfusion that also shows particularities of microvascular blood flow. LDF enables the assessment of microhemodynamic indicators in vivo without direct contact with microvessels [3]. Results obtained with LDF can also be used for the evaluation of endothelial dysfunction (ED) [4].

To assess various risk groups of postmenopausal women, early discovery of subclinical organ damage with the help of modern noninvasive methods is very important. Despite certain advancements in the study of blood flow quality at the microvascular level, attention has been focused mostly on basal blood flow and vasomotion. Moreover, the data obtained in a number of those studies is inconsistent [5−10]. However, most authors believe that basal endothelial activity in patients with T2DM is lower than that in healthy individuals [5, 7, 8, and 11]. However, the influence of occlusion on endothelial vasomotor parameters of microcirculatory blood flow in various disorders of carbohydrate metabolism remains unexplored.

Aim

The purpose of the current research was to determine the connection(s) between clinical and biochemical parameters and endothelial parameters in naturally postmenopausal women with different carbohydrate metabolism statuses.

Materials and methods

We surveyed 94 women with a mean age ± standard deviation (SD) of 58.0 ± 5.9 years who underwent natural menopause duration 8,4 ± 6,9 years. The participants were divided into three groups according to their carbohydrate metabolism status as defined by the World Health Organization (1999−2013). Group 1 included 52 patients with T2DM (mean age, 58.6 ± 5.5 years); of these 52 patients, 5 were drug-naïve, 22 received metformin monotherapy, and 25 received metformin in combination with sulfonylurea or gliptin. Group 2 included 16 women with newly diagnosed prediabetes (mean age, 61.1 ± 5.5 years). Group 3 (control group) consisted of 26 women (mean age, 54.9 ± 4.9 years) without carbohydrate metabolism impairment (CMI; p1−3 = 0.005; p2−3 = 0.001). In groups 1−3, 46, 10, and 4 patients, respectively, had arterial hypertension; the mean duration of menopause was 8.8 ± 6.1, 12.7 ± 8.8, and 4.8 ± 5.0 years (p1−3 = 0.006; p2−3 = 0.004), respectively. None of the participants were alcohol- or tobacco-dependent.

The following anthropometric parameters were assessed: body mass index (BMI), waist circumference, and blood pressure (BP). Parameters of carbohydrate metabolism evaluated were fasting plasma glucose using the glucose oxidase test and glycated hemoglobin (HbA1c) on a DCA Vantage analyzer (Siemens, Germany). Lipid parameters evaluated were triglycerides, high-density (HDL), and low-density lipoproteins (LDL) using a

colorimetric method, and plasma atherogenic index [AIP; calculated using the equation (total cholesterol - HDL)/HDL ratio]. Levels of vascular endothelial growth factor (VEGF) and endothelin-1 were determined using enzyme-linked immunosorbent assay.

Microcirculation was evaluated using a computerized laser capillary flow analyzer (LAKK-01; R&D Enterprises, "Lazma," Russia) that combines two diagnostic techniques, LDF and optical tissue oximetry [3]. The regulation of active (endothelial, neurogenic, and myogenic) and passive (cardiac and respiratory) microcirculation factors was evaluated using wavelet analysis; microcirculation reserve capacity was analyzed by an occlusion test. The following indicators were calculated: basal peak oscillation frequency relative to the volume of the erythrocyte fraction in the probed area of skin in the endothelial range (VrfEf); occlusive peak oscillation frequency of blood flow in the endothelial range (PMfEi), peak oscillation amplitude of oxygen saturation in the endothelial range (SO2aEi), postocclusive peak oscillation frequency relative to the volume of the erythrocyte fraction in the probed area of skin in the endothelial range (VraEr), and the ratio of amplitude fluctuation input relative to the volume of the erythrocyte fraction in the probed area of skin divided by the modulation of vascular tone relative to the mean value of a microcirculation indicator in the endothelial range (Vra/mEr).

SPSS (version 17.0) software was used for statistical analysis. For parameters with normal distribution, we calculated the mean ± SD. For nonGaussian distribution, median and interquartile range was used. For correlation analysis, the Spearman method and partial correlation analysis were used. A Bonferroni correction with the Mann−Whitney criterion was applied for comparison of the three groups. Statistical significance was set at $p \leq 0.05$. All study participants signed informed consent. This study was approved by the Ethics Committee of Novosibirsk State Medical University, Ministry of Health, Russian Federation, Minutes No. 19, December 18, 2009.

Results

In patients with CMI (groups 1 and 2), the mean BMI was significantly higher than that in group 3, indicating the prevalence of obesity (Table 1) and excess abdominal adipose tissue in patients with T2DM. The mean systolic and diastolic BP in patients with CMI corresponded to high−normal levels (World Health Organization, 1999), which indicates controlled hypertension. Fasting plasma glucose and HbA1c in groups 1 and 2 were significantly higher than those in the control group. Systolic BP measurements were lower in group 3 than those in patients with T2DM (group 1). Group 3 patients had higher LDL levels, without an increase in AIP than group 1 patients, despite the fact that patients with T2DM had the metabolic lipid triad (Table 1). It is possible that LDL levels in women without CMI were because of the absence of dietary restrictions; the majority of patients in group 3

either did not have conditions frequently associated with an increase in LDL (including CVDs, hypothyroidism) or such conditions were only marginally expressed (e.g., extra body mass).

A number of significant differences in ED (Table 2) and endothelial range LDF (Table 3) parameters were found between the three groups. Although VEGF levels did not differ between groups 2 and 3, its levels in these groups were higher than those in group 1; endothelin-1 levels were comparable in all groups (Table 2).

LDF demonstrated significant differences in PMfEi and SO2aEi during occlusion testing, and VraEr andVra/mEr during reperfusion testing between groups 1 and 3, as well as in VrfEf between groups 2 and 3 during basal recording. Since there were age differences between groups, to confirm the validity of these findings, we conducted partial correlation analysis in the sample of patients with T2DM but without CMI (Remark: It is unclear whether this refers to groups 1 and 3, respectively, or a subgroup of group 1. Please clarify by rephrasing.) using age as control variable. The results showed that the inverse correlation between absence of CMI and PMfEi was not statistically significant (r = -0.145, p = 0.130), but the direct correlation between absence of CMI and SO2aEi, VraEr, and Vra/mEr were (r = 0.232, p = 0.035; r = 0.259, p = 0.017; and r = 0.375, p = 0.001; respectively). Hence, the absence of a significant connection between CMI and SO2aEi (Remark: This statement is in direct contrast the previous sentence which states that there was a significant correlation between these two parameters. Please clarify this issue by rephrasing.) supports the definitive influence of age on this link. Partial correlation analysis between groups 2 and 3, using age as control variable, demonstrated a significant correlation between absence of CMI and VrfEf (r = -0.402, p = 0.008).

In group 1, PMfEi correlated with waist circumference (r = 0.34, p = 0.034) and VEGF levels (r = -0.44, p = 0.012), whereas SO2aEi correlated with levels of triglycerides (r = 0.36, p = 0.03), LDL (r = 0.37, p = 0.027), and AIP (r = 0.36, p = 0.033). Following restoration of blood flow, there were significant correlations between the following LDF parameters: VraEr and systolic BP (r = -0.33, p = 0.027), Vra/mEr and systolic BP (r = -0.34, p = 0.023), and Vra/mEr and AIP (r = 0.34, p = 0.032). In group 2, there was an inverse correlation between VrfEf and VEGF (r = -0.54, p = 0.045). In group 3, there was an inverse correlation between PMfEi and endothelin-1 (r = -0.51, p = 0.016).

Discussion

Multilevel control of microcirculation is achieved through a feedback system. In the process of microcirculation self-organization, endothelial activity, neurogenic and myogenic mechanisms, and pulse and breathing rhythms form positive and negative feedback connections [12]. Oscillations near 0.01 Hz, which are slower than neurogenic and myogenic oscillation frequencies, are the result of endothelial functioning and periodic changes in the concentration of the vasodilator

Table 1

Comparison of clinical and biochemical parameters between groups 1–3			
Parameter	Group 1 n = 52	Group 2 n = 16	Group 3 n = 26	*p1–3 **p1–2 ***p2–3
WC, cm	102.0 ± 14.4	93.7 ± 18.0	84.8 ± 10.5	0.000*
BMI, kg/m2	33.4 ± 6.1	31.0 ± 7.5	25.8 ± 4.1	0.001* 0.017***
SBP, mmHg	137.1 ± 10.8	133.1 ± 7.3	129.9 ± 10.1	0.006*
DBP, mmHg	85 (76.3; 90)	84 (80; 89.8)	80 (78.8; 85)	p > 0.05
FPG, mmol/l	6.6 ± 1.2	5.4 ± 0.8	5.1 ± 0.6	0.000* 0.000**
HbA1c, %	7.3 ± 1.1	6.2 ± 0.2	5.5 ± 0.4	0.000* 0.001** 0.000***
HDL, mmol/l	1.1 ± 0.3	1.5 ± 0.4	1.6 ± 0.3	0.000* 0.009**
LDL, mmol/l	3.4 ± 1.4	4.1 ± 1.2	4.1 ± 0.9	0.033*
TG, mmol/l	2.1 (1.6; 2.8)	1.2 (1.2; 1.5)	1.3 (1.1; 1.6)	0.001* 0.000**
AIP	4.4 ± 1.6	3.6 ± 1.6	2.9 ± 0.7	0.000*

Data are presented as means ± standard deviation and median values at 25% and 75%. p1–3, p1–2, and p2–3, represent the p value calculated between groups 1 and 3, 1 and 2, and 2 and 3, respectively.
WC, waist circumference; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglycerides; AIP, plasma atherogenic index

Table 2

Biochemical parameters of endothelial dysfunction			
Parameter	Group 1, n = 52	Group 2, n = 16	Group 3, n = 26	*–p1–3 **–p1–2 ***–p2–3
VEGF, pg/ml	66.3 (51.3; 90.3)	163.7 (78.4; 211.9)	149.3 (71.2; 232.3)	0.000* 0.018** >0.05***
Endothelin-1, fmol/ml	0.188 (0.175; 0.199)	0.194 (0.170; 0.200)	0.186 (0.170; 0.196)	>0.05

Data presented as median values at 25% and 75%.
p1–3, p1–2, and p2–3, represent the p value calculated between groups 1 and 3, 1 and 2, and 2 and 3, respectively.
VEGF, vascular endothelial growth factor

Table 3

Parameters of endothelial range basal, occlusion, and reperfusion laser Doppler flowmetry (LDF)			
LDF parameter	Group 1, n = 52	Group 2, n = 16	Group 3, n = 26	*–p 1–3 **–p 1–2 ***–p 2–3
VrfEf, Hz	0.0190 (0.0150; 0.0200)	0,0195 (0.0183; 0.0200)	0.0180 (0.0120; 0.0190)	0.013***
PMfEi, Hz	0.0190 (0.0180; 0.0200)	0.0180 (0.0180; 0.0185)	0.0180 (0.0180; 0.0190)	0.042*
SO2aEi, perfusion units	0.460 (0.245; 0.583)	0.550 (0.395; 0.665)	0.530 (0.410; 0,800)	0.043*
VraEr, perfusion units	0.250 (0.135; 0.393)	0.260 (0.143; 0.365)	0.325 (0.258; 0.428)	0.043*
Vra/mEr, perfusion units	2.43 (1.43; 3.19)	2.72 (1.89; 4.17)	3.27 (2.41; 4.24)	0.007*

Data presented as median values at 25% and 75%.
p1–3, p1–2, and p2–3, represent the p value calculated between groups 1 and 3, 1 and 2, and 2 and 3, respectively.
VrfEf, basal peak oscillation frequency relative to the volume of the erythrocyte fraction in the probed area of skin in the endothelial range
PMfEi, occlusive peak oscillation frequency of blood flow in the endothelial range
SO2aEi, peak oscillation amplitude of oxygen saturation in the endothelial range
VraEr, postocclusive peak oscillation frequency relative to the volume of the erythrocyte fraction in the probed area of skin in the endothelial range
Vra/mEr, ratio of amplitude fluctuation input relative to the volume of the erythrocyte fraction in the probed area of skin divided by modulation of vascular tone relative to the mean value of an indicator of microcirculation in the endothelial range

nitric oxide [13]. Kvandel et al. (2003) showed that of the two vasodilators produced by the endothelium, which were nitric oxide and prostaglandins, only nitric oxide causes myogenic contractions with a frequency of about 0.01 Hz [12]. The release of nitric oxide by the endothelium occurs during the physiological regulation of muscle tone and plays an important role in regulation of the pressure and distribution of blood flow [13]. Although the role of the endothelium as a local regulator of vascular tone was confirmed previously [3], LDF parameters allow us to evaluate the degree of ED and adequately demonstrate the state of microcirculation in tissues [4.12.13].

The current LDF results demonstrated a number of differences in basal and occlusal parameters as well as during the reperfusion period between groups 1 and 3. Partial correlation analysis of patients with T2DM and without CMI using age as a control variable allowed us to elucidate the effect of this parameters on microcirculation. Results showed that the basal VrfEf in healthy women correlated significantly with absence of CMI (r = -0.40, p = 0.008). At the same time, the absence of a significant correlation between CMI and occlusal PMfEi supports the idea that age has a crucial effect on this correlation. However, correlation of SO2aEi, another occlusion parameter thatreflects oxygen saturation in the endothelial range, with the absence of CMI (r = 0.23, p = 0.035) did not depend on the age. Furthermore, correlations between reperfusion VraEr and Vra/mEr and absence of CMI HMD did not depend on the age (r = 0.26, p = 0.017 and r = 0.38, p = 0.001, respectively).

In the available literature, we have not found any information on the function of epithelial vasomotion under conditions of occlusion, whereas basal blood flow has been evaluated in a number of studies. In one of the first studies in patients with diabetes, a significant decrease of basal vasomotion was demonstrated in the low frequency range (0.1 Hz) [5]. However, their sample of patients differed significantly from ours with respect to the mean duration of T2DM (17.1 ± 2.3 years) and mean HbA1c (9.1±0.4%). Moreover, another previous study reported that a sample of patients with T2DM having similar mean age as that of ours (58 ± 8.2 years) showed an increase in vasodilation compared with healthy individuals [6]. However, Muris et al. (2014) reported that healthy individuals and patients with T2DM who were comparable in age and waist circumference, but had lower HbA1c levels, did not show differences in the endothelial range [7]. In all of these studies, gender differences were not significant. It should be noted that basal endothelial activity in patients with T2DM are even lower than that in healthy elderly individuals [8].

In patients with T2DM (group 1), we detected a correlation between PMfEi and waist circumference (r = 0.34, p = 0.034), but there were no evidence of a correlation between epithelial range vasomotion and anthropometric parameters during ischemia. However, in a number of previous studies of basal blood flow, a decrease in endothelial function was demonstrated in patients with abdominal obesity. For example, in the

skin of T2DM patients without CVD, there is an inverse correlation between waist circumference and vasomotion in all frequency ranges, including the endothelial that is independent of gender [7]. The subjects in this study were similar to ours in age and waist circumference but had lower HbA1c levels. According to Walther et al. (2015), fluctuations in the endothelial range in response to acetylcholine and sodium nitroprusside iontophoresis are predominantly inversely related to the amount of abdominal adipose tissue and independent of presence or absence of metabolic syndrome and T2DM [14]. Even in children, the presence of obesity in metabolic syndrome results in decreased endothelial activity at the arteriolar level of basal blood flow regulation, increased vascular tone, and worsening of hemostatic disorders [11].

At the same time, Sanip et al. (2012) found no connection between microvascular basal endothelial fluctuations and obesity indices in a sample of younger women (mean age, 34.9 ± 7.9 years) who had a mean BMI (32.9 ± 4.8 kg/m2) comparable to group 1 in our study [9] but did not have diabetes. Seywert et al. (2004) also did not find a difference in the intensity of reactive hyperemia between healthy obese pre- and postmenopausal women after hand occlusion testing [10]. Hence, we have reason to believe that the direct correlation between occlusal PMfEi and waist circumference in patients with T2DM in our study is a result of microcirculatory compensation mechanisms in the early stages of diabetes (mean T2DM duration herein, 5.8 ± 5.0 years). This is consistent with findings which show that upon manifestation of type 1 diabetes mellitus, there is an acceleration of blood flow in microcirculatory vessels due to the increased production of nitric oxide in response to hyperglycemia [15]; whereas chronic hyperglycemia results in a decrease of nitric oxide concentration and predominance of microcirculatory vasospasms [15].

We also demonstrated statistically significant correlations between lipid levels and microcirculation parameters in endothelial range frequencies in patients with T2DM. The SO2aEi, which indicates the peak amplitude of fluctuations in the endothelial oxygen saturation range during the 3-min ischemia occlusion test, directly correlated with triglyceride and LDL levels. This is probably due to the higher viscosity of blood in the presence of dyslipidemia [6, 16] and to a large erythrocyte fraction in the probed area of skin. It is possible the same mechanism explains the link between Vra/mEr and AIP during reperfusion after ischemia in patients with T2DM. At the same time, both LDF parameters in women with T2DM were significantly lower than those without CMI (Table 3). A previous study observed that lipid profiles influence microcirculation during occlusion testing, which the authors associated with the decrease in postischemic vasodilation in obese patients, or more precisely, the concomitant dyslipidemia (decreased HDL and increased triglyceride levels) [17]. We believe that the observed changes are consistent with the glucolipotoxicity hypothesis, followed by activation of oxidative stress in

patients with T2DM, and the role of these factors in the genesis of micro- and macroangiopathy [18].

Kraemer et al. (2012) demonstrated that in middle-aged and older patients, oxygen saturation of the basal microcirculation of the skin on the dorsum of the hands is higher in healthy individuals than in patients with T2DM [19]. Herein, we found similar changes in women during occlusion testing: the SO2aEi in healthy individuals was significantly higher than in patients with T2DM (Table 3). It has also been shown that the microcirculatory mean relative oxygen saturation and oxygen saturation perfusion index, which describe the relationship between microcirculatory blood flow and oxygen not consumed by tissues, are higher in patients with T2DM ($p \leq 0.05$) compared with controls [6]. This means that increased basal blood oxygen saturation may indicate a high risk for development of adverse changes in tissues.

In the current study, we identified a number of significant differences between groups using parameters of ED (Table 2). It has been suggested that ED with impaired physiological vasodilation is a link between development and progression of diabetes and cardiovascular disorders [20]. ED may be defined as inadequate or excess production of biologically active substances in the endothelium, the level of which correlates with the severity of ED. In our study, there was no difference in the level of VEGF in groups 2 and 3, but both were higher than those in group 1; the level of endothelin-1 was comparable in all studied groups (Table 2).

The paradoxically low level of VEGF in patients with T2DM may be attributed to the inhibitory activity of nitric oxide. It is known that VEGF stimulates release of nitric oxide as an endothelial trophic factor and is a potent mitogen for endothelial cells. This response is feedback-regulated [21]; an increased level of nitric oxide inhibits the activity of VEGF. With diabetes, this process is disrupted over time, and the nitric oxide–VEGF link becomes dissociated; then unrestrained high levels of VEGF lead to the development and progression of complications [22]. However, in the early stages of ED, levels of nitric oxide may be multiplied due to "nitric oxide stress" and its enhanced inactivation [23]. With a shorter T2DM duration (as in our study), a temporary decrease in blood VEGF concentration may result. Analogous to the "diabetes honeymoon," we suggest an "ED honeymoon." As diabetes and ED progresses, compensatory synthesis of nitric oxide decreases and VEGF concentration increases which in turn leads to microvascular complications.

Interestingly, PMfEi inversely correlated with VEGF levels in group 1 under ischemic conditions ($r = -0.44$, $p = 0.012$) and endothelin-1 levels in group 3 ($r = -0.51$, $p = 0.016$). We have not seen similar data in the literature. VEGF is known to enhance vasodilation [24], and endothelin-1 is one of the most potent vasoconstrictors. In patients without CMI, the perfusion index logically decreased under conditions of ischemia, when endothelin-1 levels and corresponding vasoconstriction increased. In T2DM, a decrease in the level of VEGF (Table 2) did not result in the

reduction of perfusion but enhanced it instead. We believe that our data indicate a shift in the balance of endothelial vasodilation mechanisms in patients with T2DM.

In addition, in women with prediabetes, we obtained higher VrfEf values for basal blood flow compared with the control group ($p = 0.013$). Notably, Vr indicates the percentage of hemoglobin content in the total volume of the tested biological tissue [5]. If diabetes is not controlled, hemoglobin glycation determines development of tissue hypoxia. In this regard, a higher VrfEf in women with prediabetes compared to controls probably reflects a compensatory response to local hypoxia, resulting from an increase in the glycated fraction, by increasing hemoglobin content. Hence, an increase in hemoglobin content determines endothelial vasomotion of basal blood flow in women with prediabetes.

During evaluation of basal blood microcirculation in the prediabetic group, we observed a significant inverse correlation between VrfEf and VEGF ($r = -0.54$, $p = 0.045$). When the relative hemoglobin content decreases, local hypoxia develops, resulting in production of vasodilators, including VEGF. Our data demonstrates reciprocal relationships between endothelial vasomotion of microcirculation and VEGF, a biochemical indicator of endothelial function.

Occlusion testing demonstrated that during restoration of blood flow (reperfusion) in patients with T2DM, there were inverse correlations between systolic BP and VraEr and Vra/mEr ($r = -0.33$, $p = 0.027$ and $r = -0.34$, $p = 0.023$, respectively). While the VraEr in women with T2DM was significantly lower than that in the control group (Table 3), systolic BP, on the other hand, was higher (137.1 ± 10.8 versus 133.1 ± 7.3 mmHg, $p = 0.006$). In addition, not only was the VraEr, which shows the effect of the hemoglobin amount on the endothelial spectrum vasomotion, lower in T2DM versus healthy individuals (Table 3), it decreased further with increasing BP, exacerbating local hypoxia. We believe that our findings demonstrate the influence of BP on the disruption of postocclusive microcirculation restoration in patients with diabetes.

Conclusions

There are direct links between microcirculatory endothelial range vasomotion under conditions of occlusion and lipid profile indicators, such as triglycerides and LDL (SO2aEi) and waist circumference (PMfEi), in postmenopausal women with T2DM. Furthermore, we found inverse correlations between endothelial range LDF parameters (VraEr and Vra/mEr) and systolic BP during the postocclusive period in female patients with T2DM. In patients with T2DM, age had a crucial impact on the occlusal PMfEi but did not influence other microcirculation parameters, including basal and occlusal SO2aEi and reperfusion. We also established that occlusal parameters of vasodilation inversely correlated with endothelin-1 in postmenopausal women without CMI and VEGF in patients with T2DM. In prediabetic women, basal

microcirculation measurement of VEGF levels inversely correlated with the relative hemoglobin content (Vr).

ADDITIONAL INFORMATION

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