Endothelial and Autonomic Dysfunction at Early Stages of Glucose Intolerance and in Metabolic Syndrome

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ABSTRACT

This study evaluated sE-selectin, Endothelin-1, and cardiovascular autonomic neuropathy (CAN) at early stages of glucose intolerance and in metabolic syndrome (MetS). A total of 87 subjects – 39 males, of mean age 45.7 ± 11.6 years and mean BMI 31.4 ± 6.6 kg/m^2, divided according to glucose tolerance and the presence of MetS were enrolled. Glucose tolerance was studied during OGTT. Anthropometric indices, blood pressure, HbA1c, lipids, hsCRP, sE-selectin, Endothelin-1, and immunoreactive insulin were measured. Body composition was assessed by a bioimpedance method (InBody 720, BioSpace). Tissue AGEs accumulation was evaluated by skin autofluorescence (AGE-Reader, DiagnOpticsTM). CAN was assessed by ANX-3.0 technology. In the groups, according to glucose tolerance, the prevalence of CAN was 5.7 % in normal glucose tolerance (NGT), 8.6 % in prediabetes, and 23.5 % in newly diagnosed type 2 diabetes (NDD). In the groups, according to the presence of MetS, the prevalence of CAN was 12.3 % in those with MetS and 4.8 % in those without MetS. Parasympathetic activity was diminished at rest (p = 0.048, 0.015, respectively) in NDD as compared to prediabetes and NGT; and there was a numerically elevated heart rate at rest in NDD in comparison to NGT. There was a negative correlation between parasympathetic tone and waist circumference, BMI, and visceral and total fat. There was no difference in the measured endothelial function markers in the groups according to glucose tolerance and MetS. sE-selectin correlated with HOMA-IR (r = 0.275, p = 0.048). No association between Endothelin-1 levels and assessed metabolic parameters was observed. There is a high prevalence of CAN at early stages of glucose intolerance and in MetS, due to decreased parasympathetic activity. Slight elevation of glycemia and MetS probably do not affect endothelial function, since sE-selectin seems to be related to insulin resistance.

Introduction

Since prediabetes is a condition of high risk of developing diabetes and a category of increased cardiovascular risk [1, 2], prevention is the ultimate goal in this high-risk population.

As an independent risk factor for cardiovascular death in diabetes [3, 4], cardiovascular autonomic neuropathy (CAN) is a serious life-threatening complication of diabetes, affecting about 1/4 of subjects with type 1 diabetes and about 1/3 of those with type 2 diabetes (T2D) [5]. Since the first announcement of CAN preceding or accompanying the onset of diabetes has been published about 50 years ago [6], the exact nature of the association between early stages of glucose intolerance and autonomic nerve injury remains debatable. Meanwhile, the multifactorial determination of autonomic function has been implied [7] and a new concept of the so called “metabolic neuropathy” has been introduced [8].

Endothelin-1 is a vasoconstrictor and mitogenic peptide, described in a number of tissues and thought to modulate vascular tone, cell proliferation, and hormone production. The pivotal role

of Endothelin-1 system in endothelial dysfunction, insulin resistance, and atherosclerosis has been unraveled [9]. The mechanism of Endothelin-1 system activation in hyperglycemia-driven oxidative stress and insulin-related disorders are based on a specific impairment of the insulin-mediated PI3K pathway with sparing of the MAPK-dependent signaling cascade [10], thus promoting Endothelin-1 synthesis in the presence of blunted nitric oxide production [11]. Endothelin-1 modulates insulin signaling in vascular smooth muscle cells and therefore in the presence of elevated Endothelin-1 levels diminished insulin action in the vasculature may contribute to the development of cardiovascular disease in the presence of impaired glucose homeostasis [12].

It has been hypothesized that sE-selectin is one of the most important adhesion molecules for the evolution of atherosclerosis due to its expression only on activated endothelium [13]. Numerous lines of research suggest that high plasma sE-selectin concentrations predict the development of insulin resistance [14] and T2D [15, 16], especially the post-load glucometabolic status [17], and a 6-year risk for cardiovascular events [18] and a 5-year risk for peripheral neuropathy [19] in T2D. As improvement of glucose control in T2D [20] and lifestyle modification in prediabetes [21] have been demonstrated to diminish sE-selectin levels, measuring sE-selectin in early stages of glucose intolerance is important and makes it possible to detect initial endothelium activation, to intervene and to follow its reversal.

Since it is not clarified whether and to what extent different metabolic parameters affect endothelial and autonomic function at early stages of glucose intolerance, the aim of the present study was to evaluate plasma Endothelin-1 and sE-selectin levels, as markers of endothelial function, and cardiovascular autonomic function at different stages of glucose tolerance and in MetS, and their correlation with different cardio-metabolic parameters.

Subjects and Methods

A total of 87 subjects – 39 males (mean age 45.7 ± 11.6 years, mean BMI 31.4 ± 6.6 kg/m^2 – were enrolled in this cross-sectional study. They were divided into three groups according to glucose tolerance: 35 with normal glucose tolerance (NGT), 35 with prediabetes, and 17 with newly-diagnosed type 2 diabetes (NDD), and into two groups according to the presence of metabolic syndrome (MetS): 66 with MetS, of which 17 with NGT, 32 with prediabetes, and 17 with NDD; and 21 without MetS, of which 18 with NGT and 3 with prediabetes. The main characteristics of the groups are presented in ▶ **Tables 1** and ▶ **2**. Participants were recruited at the Department of Diabetology, Clinical Centre of Endocrinology, Medical University, Sofia within an ongoing diabetes screening program. All participants were interviewed and a questionnaire with a list of exclusion criteria was completed. Previously diagnosed diabetes or taking antidiabetic drug therapy, arrhythmias or taking anti-arrhythmic drug therapy, presence of macrovascular disease and any neurological conditions, which may affect autonomic nervous system function were adopted as exclusion criteria and these subjects were not eligible for the present study. All subjects declared their written informed consent in accordance with the Helsinki Declaration and rules of Good Clinical Practice and the study was approved by the Ethics Committee of the Medical University, Sofia.

Anthropometric indices – height, weight (BMI was calculated) and waist circumference – were measured. A standard oral glucose tolerance test was performed and glucose tolerance was defined in accordance with 2006 WHO criteria. Fasting and post-load plasma glucose were assessed by a hexokinase enzyme method (Roche Diagnostics). Fasting and post-load immunoreactive insulin were estimated by ECLIA method (Roche Diagnostics) and the homeostatic model assessment, indirectly quantifying insulin resistance – HOMA-IR – was calculated. Serum total cholesterol, HDL cholesterol, and triglycerides were assessed by an enzymatic colorimetric method (Roche Diagnostics). LDL cholesterol was calculated using Fridewald's formula. HbA1c (NGSP certified) in whole blood samples by immunoturbidimetric method (Roche Diagnostics), and high sensitive C-reactive protein (hsCRP) by a particle-enhanced turbidimetric method (CRP-Latex) (Roche Diagnostics) were assessed. These parameters were examined in all participants at fasting. Arterial blood pressure was measured with a manual sphygmomanometer under standard conditions – two times after 5 min rest. The IDF 2005 definition of metabolic syndrome was applied [22]. Body fat distribution was estimated by bio-impedance analysis (InBody 720). Tissue advanced glycation end products (AGEs) accumulation was assessed by skin autofluorescence (AGE-Reader, DiagnOpticsTM), which is a non-invasive method measuring the skin autofluorescence of ultraviolet light on the ventral side of the lower arm [23].

Autonomic nerve system (ANS) function was assessed by ANX-3.0 method (ANSAR Medical Technologies, Inc., Philadelphia, PA, USA). This software is a monitoring technology that computes sympathetic and parasympathetic activity non-invasively, separately and simultaneously based on cardio-respiratory synchronization at rest and during standard cardiovascular autonomic reflex tests: deep breathing challenge, Valsalva challenge, and stand-up challenge. This methodology applies spectral analysis of heart rate variability with simultaneous spectral analysis of respiratory activity based on continuous wavelet transformation with Morlet wave. The spectral analysis is focused at low-frequency region of the spectrum between 0.04–0.15 Hz. The fundamental respiratory frequency in the spectrum of heart rate variability represents respiratory sinus arrhythmia and coincides with parasympathetic activity. It is termed respiratory frequency area (RFa), as a measurement of parasympathetic tone. The rest of the area under the curve from the heart rate variability spectrum reflects sympathetic activity and is termed low-frequency area (LFa), as a measurement of sympathetic tone. These parameters are measured in beats per square minutes (bpm^2).

The Ewing tests are performed as follows: ANS evaluation at baseline including a 5-minute interval in seated position at rest with normal breathing without any movements; ANS evaluation during deep breathing including 6 deep breathing cycles each 10 s for a total period of 1 min; ANS evaluation during 5 Valsalva maneuvers each 15 s; and ANS evaluation after standing from a seated position for a total period of 5 min. Normal ranges for each test, assessed by the ANSAR analysis, are individually based on the age group [24, 25].

The study was performed at least 24 h after the last dose of the following medications – antihypertensives, tricyclic antidepressants, and SSRIs – at least 12 h refraining from coffee and smoking, at least 30 min after the last meal, between 8–11 AM in the morning.

▶ **Table 1** Main characteristics of the groups according to glucose tolerance, normal glucose tolerance (NGT), prediabetes, and newly-diagnosed type 2 diabetes (NDD).

Parameters	Groups			
	NGT	Prediabetes	NDD	p-Value
Number	35	35	17	
Sex (male/female)	16/19	16/19	7/10	0.945
Age (years)	45.5 ± 14.1	44.8 ± 10.2	48.0 ± 8.5	0.649
BMI (kg/m^2)	28.7 ± 6.5	33.3 ± 5.9 * *	33.2 ± 6.8 *	**0.009 vs. NGT * ***
				0.027 vs. NGT§
				0.049 vs. NGT *
				0.147 vs. NGT§
Waist circumference (cm)	99.8 ± 16.8	108.7 ± 11.9	109.9 ± 18.3 *	0.039 vs. NGT *
				0.117 vs. NGT§
Visceral fat area (cm^2)	131.8 ± 48.0	164.5 ± 50.7	165.0 ± 50.3 *	0.021 vs. NGT *
				0.063 vs. NGT§
Total body fat (%)	31.7 ± 10.6	36.8 ± 10.8	37.4 ± 7.9	0.068
Fasting plasma glucose (mmol/l)	5.5 ± 0.4	6.4 ± 0.5 *	9.3 ± 2.6 * #	**< 0.001 vs. NGT ***
				< 0.001 vs. NGT§
				0.001 vs. prediabetes#
				0.003 vs. prediabetes§
120-Min plasma glucose (mmol/l)	5.2 ± 1.2	7.6 ± 1.9 *	14.6 ± 4.9 * #	**< 0.001 vs. NGT ***
				< 0.001 vs. NGT§
				< 0.001 vs. prediabetes#
				< 0.001 vs. prediabetes§
HbA1c (mmol/mol IFCC)	38 ± 3	39 ± 6	61 ± 15 * #	**< 0.001 vs. NGT ***
				< 0.001 vs. NGT§
				< 0.001 vs. prediabetes#
				< 0.001 vs. prediabetes§
Fasting immunoreactive insulin (mIU/l)	12.4 (6.6–16.1)	13.1 (9.5–22.7)	12.2 (8.6–21.2)	0.150
120-min immunoreactive insulin (mIU/l)	28.5 (20.4–57.3)	49.7 (17.8–84.7)	44.3 (21.3–91.9)	0.136
HOMA-IR	3.2 (1.6–4.2)	3.6 (3.0–6.9) *	5.1 (3.9–7.5) * *	**< 0.001 vs. NGT * ***
				< 0.001 vs. NGT§
				0.021 vs. NGT *
				0.063 vs. NGT§
Systolic blood pressure (mmHg)	122 ± 14	125 ± 12	127 ± 19	0.500
Diastolic blood pressure (mmHg)	78 ± 11	79 ± 9	81 ± 13	0.606
Total cholesterol (mmol/l)	5.6 ± 1.3	5.3 ± 1.0	5.6 ± 0.9	0.451
HDL-cholesterol (mmol/l)	1.3 ± 0.4	1.2 ± 0.3	1.1 ± 0.2	0.305
LDL-cholesterol (mmol/l)	3.8 ± 1.6	3.6 ± 1.8	4.0 ± 1.8	0.775
Triglycerides (mmol/l)	1.5 (1.0–2.2)	1.7 (0.9–2.4)	1.9 (1.6–2.7)	0.192
hsCRP (mg/l)	1.8 (1.5–3.5)	3.1 (1.2–6.1)	4.4 (3.1–6.5)	0.081
AGEs accumulation	1.8 ± 0.4	1.8 ± 0.4	1.9 ± 0.2	0.820
sE-selectin (ng/ml)	17.3 (12.7–23.4)	18.9 (12.3–32.0)	24.2 (16.1–42.2)	0.157
Endothelin-1 (pg/ml)	50.0 (20.8–79.0)	54.0 (20.5–83.0)	48.0 (19.3–93.3)	0.976
Heart rate (bpm)	77 ± 9	79 ± 11	86 ± 11 *	0.024 vs. NGT *
				0.073 vs. NGT§
LFa baseline (bpm^2)	2.1 (1.2–3.7)	2.5 (1.4–4.0)	1.5 (0.7–2.6)	0.355
RFa baseline (bpm^2)	1.4 (0.6–2.6)	1.3 (0.5–2.6)	0.5 (0.2–1.0) * #	**0.005 vs. NGT ***
				0.015 vs. NGT§
				0.016 vs. prediabetes#
				0.048 vs. prediabetes§
LFa deep breathing (bpm^2)	1.7 (0.8–2.6)	1.8 (1.0–3.4)	1.2 (0.7–4.1)	0.985

▶ **Table 1** Continued

Parameters	Groups			
	NGT	Prediabetes	NDD	p-Value
RFa deep breathing (bpm²)	22.5 (9.2–57.4)	23.6 (6.9–44.1)	14.7 (6.7–34.4) * #	0.046 vs. NGT *
				0.138 vs. NGT§
				0.043 vs. prediabetes#
				0.129 vs. prediabetes§
LFa Valsalva maneuver (bpm²)	27.6 (7.2–45.2)	34.9 (17.6–52.0)	30.7 (10.1–53.5)	0.385
RFa Valsalva maneuver (bpm²)	4.5 (1.4–8.2)	3.8 (2.3–10.0)	2.4 (1.0–4.2)	0.167
LFa standing (bpm²)	3.2 (1.1–8.1)	2.9 (1.1–5.8)	2.2 (0.5–4.2)	0.581
RFa standing (bpm²)	0.8 (0.3–2.0)	0.5 (0.3–2.1)	0.3 (0.2–1.0)	0.426

Data are mean ± standard deviation; and median and interquartile range. LFa: Sympathetic activity; RFa: Parasympathetic activity. § Corrected p-value after Bonferroni correction; * p-value after comparing NDD and prediabetes versus NGT; * * p-value after comparing NDD and prediabetes versus NGT; §corrected p-value after Bonferroni correction ; #p-value after comparing NDD versus prediabetes.

The definition for CAN was based on the number of abnormal autonomic tests. Confirmed CAN was defined as the presence of at least two out of three abnormal autonomic tests based on Toronto Diabetic Neuropathy Expert Group classification [26]. There is no universal reference value for parasympathetic and sympathetic activity as ANSAR system uses individual age-based low "cut-off" values above which sympathetic or parasympathetic response during a particular test is normal for the particular examined patient.

Statistical analysis

Statistical analysis of the data was performed by SPSS 21.0 (SPSS, Chicago, USA). The data are expressed as mean ± standard deviation (SD) and median and interquartile range. Logarithmic transformation was used for skewed data distribution. Principal component analysis was performed to define a principal component variable for sympathetic and parasympathetic power. For continuous variables with a normal distribution and for log-normal variables, one-way analysis of variance (one-way ANOVA) was used for comparison of the groups with post-hoc analysis with Tamhane correction for multiple comparisons. Partial correlation test was used to compare variables with normal and log-normal distribution. A p-value (two tailed) of less than 0.05 after Bonferroni correction was considered statistically significant.

Results

The prevalence of CAN was 5.7 % in NGT, 8.6 % in prediabetes, 23.5 % in NDD; and 12.3 % in the presence of MetS as compared to 4.8 % in subjects without MetS. No significant difference was observed in plasma sE-selectin and Endothelin-1 levels between the groups according to glucose tolerance and in endothelial markers and autonomic parameters according to the presence of MetS (▶ **Tables 1**, and ▶ **2**). Our results showed significantly diminished parasympathetic activity at rest (p = 0.048, 0.015, respectively) in NDD as compared to prediabetes and NGT; and a numerically elevated heart rate at rest in NDD in comparison to NGT (▶ **Table 1**). Plasma sE-selectin levels correlated with HOMA-IR (r = 0.275, p = 0.048) (▶ **Table 3**). There was a negative correlation, controlling for age and the presence of hypertension, between parasympathetic power and waist circumference, BMI, visceral fat area, and total body fat (▶ **Table 3**).

Discussion

The results of the present study confirm the data from our previous study in a different cohort, showing a high prevalence of CAN at early stages of glucose intolerance and in the presence of MetS [27, 28] and largely overlap with literature data [29–31]. According to Vinik classification of CAN, based on the high-sensitive ANX-3.0 method, applied in the current study, our data meet the criteria for early CAN with parasympathetic tone weakness and relative SNS hyperactivity [32], manifested by increased heart rate, which we recorded. Regarding the presence of MetS, the group with MetS encompasses 17 subjects with NGT (26 %), 32 with prediabetes (48 %), and 17 with NDD (26 %); and the group without MetS includes 18 subjects with NGT (86 %) and 3 with prediabetes (14 %), respectively. This study found no difference in frequency-domain autonomic tone parameters between the aforementioned groups. Our previous work has shown no difference in autonomic function between subjects with prediabetes with or without MetS, in contrast to NGT, where MetS has been found to be strongly associated with autonomic dysfunction [33]. Therefore, probably glucose tolerance is the most powerful metabolic factor, which drives the autonomic function deterioration and blunts the relationship between autonomic tone and other metabolic parameters.

Despite the prevailing notion for a strong correlation just between abdominal obesity, as a hallmark of insulin resistance, and autonomic imbalance [34–36], including in prediabetes [37] and in NGT [38, 39], our findings demonstrate a significant negative correlation between parasympathetic tone and markers of both

▶ **Table 2** Main characteristics of the groups according to the presence of metabolic syndrome – with metabolic syndrome (MetS+) and without metabolic syndrome (MetS–).

Parameters	Groups		
	MetS+	MetS–	p-Value
Number	66	21	
Sex (male/female)	32/34	7/14	0.277
Age (years)	46.5 ± 11.0	43.1 ± 13.2	0.445
BMI (kg/m^2)	33.5 ± 5.7	25.0 ± 5.2	**<0.001**
			<0.001§
Waist circumference (cm)	110.3 ± 13.5	89.6 ± 12.2	**<0.001**
			<0.001§
Visceral fat area (cm^2)	167.1 ± 45.4	102.1 ± 38.3	**<0.001**
			<0.001§
Total body fat (%)	37.3 ± 9.1	27.6 ± 11.1	**0.001**
			0.003§
Fasting plasma glucose (mmol/l)	6.9 ± 2.0	5.6 ± 0.4	**0.015**
			0.045§
120-Min plasma glucose (mmol/l)	8.6 ± 4.6	5.8 ± 1.6	0.029
			0.087§
HbA1c (mmol/mol IFCC)	45 ± 13	38 ± 4	0.037
			0.111§
Fasting immunoreactive insulin (mIU/l)	17.6 ± 11.9	10.5 ± 6.4	0.034
			0.102§
120-Min immunoreactive insulin (mIU/l)	64.8 ± 8.4	36.7 ± 28.0	0.168
HOMA-IR	5.3 ± 3.6	2.6 ± 1.8	**0.007**
			0.021§
Systolic blood pressure (mmHg)	127 ± 14	116 ± 10	**0.004**
			0.012§
Diastolic blood pressure (mmHg)	81 ± 11	72 ± 9	**0.005**
			0.015§
Total cholesterol (mmol/l)	5.5 ± 1.0	5.3 ± 1.5	0.625
HDL-cholesterol (mmol/l)	1.1 ± 0.3	1.5 ± 0.3	**<0.001**
			<0.001§
LDL-cholesterol (mmol/l)	3.9 ± 1.8	3.3 ± 1.4	0.395
Triglycerides (mmol/l)	2.2 ± 1.9	1.2 ± 0.7	0.044
			0.132§
hsCRP (mg/l)	3.2 (1.6–6.1)	1.6 (0.9–3.8)	0.754
AGEs accumulation	1.9 ± 0.3	1.7 ± 0.4	0.101
sE-selectin (ng/ml)	18.3 (13.6–30.7)	15.3 (12.2–23.0)	0.311
Endothelin-1 (pg/ml)	50.0 (23.8–76.8)	61.0 (18.5–101.0)	0.846
Heart rate (bpm)	81 ± 11	77 ± 10	0.152
LFa baseline (bpm^2)	2.0 (1.1–3.0)	2.8 (1.6–4.8)	0.087
RFa baseline (bpm^2)	1.0 (0.4–2.2)	1.1 (0.5–4.7)	0.281
LFa deep breathing (bpm^2)	1.5 (0.9–3.4)	1.7 (0.8–2.6)	0.560
RFa deep breathing (bpm^2)	17.4 (7.1–38.6)	29.9 (9.1–69.1)	0.351
LFa Valsalva maneuver (bpm^2)	32.2 (14.8–53.5)	25.1 (8.3–43.7)	0.659
RFa Valsalva maneuver (bpm^2)	3.5 (1.8–6.4)	4.6 (1.5–9.6)	0.328
LFa standing (bpm^2)	2.7 (0.9–5.2)	4.4 (1.6–9.1)	0.234
RFa standing (bpm^2)	0.5 (0.2–1.5)	0.9 (0.3–2.6)	0.362

Data are mean ± standard deviation; and median and interquartile range. LFa: Sympathetic activity; RFa: Parasympathetic activity. § Corrected p-value after Bonferroni correction.

▶ **Table 3** Correlations between sE-selectin, Endothelin-1 levels, sympathetic and parasympathetic tone components and metabolic indices in the studied cohort.

Parameters	ln sE-selectin		ln Endothelin-1	
	Corr. Coeff (r)	p-Value	Corr. Coeff (r)	p-Value
BMI	0.03	0.797	−0.08	0.495
Waist circumferernce	0.07	0.573	−0.11	0.342
Visceral fat area	0.06	0.59	−0.09	0.454
Total body fat	0.04	0.736	0.1	0.393
Fasting plasma glucose	0.17	0.131	−0.04	0.743
120-Min plasma glucose	0.15	0.202	−0.08	0.501
HbA1c	0.15	0.189	−0.09	0.463
ln (fasting immunoreactive insulin)	0.24	0.037	0.2	0.075
		0.111§		
ln (120-min immunoreactive insulin)	0.07	0.564	−0.14	0.240
ln (HOMA-IR)	**0.28**	**0.016**	0.2	0.082
		0.048§		
Systolic blood pressure	0.18	0.112	0.18	0.122
Diastolic blood pressure	0.2	0.080	0.09	0.419
Total cholesterol	−0.04	0.752	−0.04	0.713
HDL-cholesterol	−0.12	0.296	−0.01	0.974
LDL-cholesterol	−0.01	0.932	−0.17	0.131
ln (triglycerides)	0.12	0.315	−0.21	0.062
ln (hsCRP)	0.5	0.664	0.1	0.406
AGEs accumulation	0.06	0.600	0.13	0.250
	Sympathetic tone component		**Parasympathetic tone component**	
	Corr. Coeff (r)	**p-Value**	**Corr. Coeff (r)**	**p-Value**
BMI	−0.17	0.126	−**0.39**	**<0.001**
				<0.001§
Waist circumferernce	−0.17	0.140	−**0.34**	**0.002**
				0.006§
Visceral fat area	−0.04	0.712	−**0.3**	**0.007**
				0.021§
Total body fat	−0.11	0.320	−**0.31**	**0.004**
				0.012§
Fasting plasma glucose	−0.06	0.610	−0.15	0.193
120-min plasma glucose	−0.12	0.296	−0.25	0.028
				0.084§
HbA1c	−0.13	0.246	−0.22	0.042
				0.126§
ln (fasting immunoreactive insulin)	0.09	0.439	0.01	0.958
ln (120-min immunoreactive insulin)	0.03	0.814	−0.07	0.545
ln (HOMA-IR)	0.05	0.637	−0.04	0.726
Systolic blood pressure	0.01	0.960	0.02	0.863
Diastolic blood pressure	−0.04	0.709	0.01	0.908
Total cholesterol	0.03	0.775	0.04	0.741
HDL-cholesterol	0.1	0.398	0.2	0.073
LDL-cholesterol	−0.1	0.375	−0.07	0.520
ln (triglycerides)	0.05	0.692	0.02	0.892
ln (hsCRP)	−0.06	0.607	−0.11	0.333
AGEs accumulation	−0.07	0.530	−0.09	0.429

§ Corrected p-value after Bonferroni correction.

generalized and visceral obesity after controlling for age and presence of hypertension. There are some data quite similar to ours, demonstrating a significant correlation between BMI [39, 40] and total body fat accumulation [41–43], and CAN. There are some data for parasympathetic dysfunction in the absence of insulin resistance [44], and thus, as a mortality predictor even in subjects without cardiovascular disease [45], a reduced parasympathetic tone carries a serious risk in individuals with mild changes in blood glucose levels and obesity.

Glycemia has been proposed as the most vigorous marker for the presence of CAN in most huge studies – the Hoorn Study [46] and the ARIC Study [47] – even in subjects with NGT [48, 49]. On the other hand, CAN has been considered as a strong marker of mortality [50]. The present study fails to show significant relation between any of the glucose parameter and ANS function indexes probably due to the small sample size of the examined cohort, since the results for post-load glucose and HbA1c has been statistically significant before Bonferroni correction has been performed. Available data from continuous glucose monitoring have revealed a significant relationship between heart rate variability and glucose excursions, the underlying mechanism being oxidative stress and inflammation [51, 52]. HbA1c has been shown to be an independent risk factor for all types of nerve deficits in diabetes [53], in particular CAN in NDD [54], and in prediabetes [55].

Although there is some evidence that AGEs accumulation in the skin [56] and hsCRP [57–59] correlate with the severity of CAN even in subclinical stages, our results show no relationship between AGEs and autonomic tone in the studied cohort. Our data also demonstrate no association between lipid profile parameters and autonomic tone, supported by the findings of Gerritsen et al. [60] and Meyer et al. [61].

There is some evidence that plasma Endothelin-1 levels predict the development of prediabetes and diabetes 10 years later [62]. Numerous studies have revealed elevated plasma Endothelin-1 levels in the presence of insulin resistance [63, 64] and MetS [65], in prediabetes, and in first-degree relatives of T2D with NGT [66]; as well as increased basal Endothelin-1 vasoconstrictor tone in obesity [67], in the presence of MetS [68, 69], and in prediabetes [70]. Contrary to the above, we found no significant difference in plasma Endothelin-1 levels between the groups according to glucose tolerance and the presence of MetS, reaffirming some available data showing similar plasma Endothelin-1 levels in subjects with T2D and NGT [71–73]. Plasma Endothelin-1 levels probably do not fully reflect its activity because its secretion is largely polarized, as Endothelin-1 is a local paracrine regulator of vasotone [74]. The discrepancy between plasma Endothelin-1 levels and its activity might be also due to changes in the clearance of the peptide not reflecting its production and biological effects in diabetes [75].

Our results demonstrate no correlation between plasma Endothelin-1 concentration and the studied cardio-metabolic parameters, in line with the previous studies, which have failed to establish correlations between plasma Endothelin-1 levels and insulin concentrations [67, 76], glycemia, lipid profile and obesity [73, 77].

Most studies have shown higher serum concentrations of plasma sE-selectin in prediabetes [78, 79], in subjects with obesity and NGT [80–83] and in nonobese T2D subjects with insulin-resistance, defined as HOMA-IR > 2.5 [14]. Our study failed to establish increased plasma levels of sE-selectin in prediabetes and NDD and in the presence of MetS, which is in support of the data from some previously conducted studies, reporting no difference in sE-selectin concentrations between subjects with T2D [84–86] and prediabetes [87], and NGT.

Insulin sensitivity, measured directly [88] and indirectly [80], has been found to be an independent factor correlating with plasma sE-selectin levels [88], which is in accordance with our data. It is widely suggested that reduced nitric oxide release probably induces high expression of sE-selectin in the state of insulin resistance [89, 90]. Our results established no association between glycemia and plasma sE-selectin levels. In contrast, it has been reported that plasma sE-selectin concentration correlates with fasting glucose, post-load glucose and post-load insulin, suggesting that hyperglycemia increases plasma sE-selectin, which reflects excessive formation of atherosclerotic plaques in subjects with impaired glucose metabolism [91].

Plasma sE-selectin concentrations are not related to serum lipids which is supported by the findings of Cominacini et al. [92]. Contrary to the prevailing observations [81–84], plasma sE-selectin levels showed no correlation with obesity parameters.

Limitations

An inherent limitation of cross-sectional design studies is the inability to establish causality. As prediabetes is a heterogeneous condition, a large sample size will allow subdivision of this group with a more detailed analysis. The present study reports on circulating plasma concentrations of Endothelin-1. Since Endothelin-1 is predominantly released abluminally [20], circulating levels provide little information on the vascular effects of the peptide.

Conclusion

Our results demonstrate a high prevalence of CAN in early stages of glucose intolerance and in the presence of MetS based on parasympathetic dysfunction with main determinants being hyperglycemia and obesity. A slight increase in plasma glucose and the presence of MetS do not influence plasma Endothelin-1 and sE-selectin levels, and sE-selectin concentrations seem to be related to fasting insulin concentration and sensitivity in this population.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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