Endothelial Function, Adipokine Serum Levels, and White Matter Hyperintesities in Subjects With Diabetic Foot Syndrome

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Context: No study has analyzed the prevalence of white matter hyperintensities (WMHs) in subjects with diabetic foot syndrome (DFS) and their relationship to adipokine serum levels and indexes of endothelial and cognitive performance.

Objective: To evaluate omentin and vaspin serum levels and the prevalence of WMHs in subjects with DFS and to analyze their relationship with other endothelial, arterial stiffness, and cognitive functions.

Design: Case-control study enrolling 40 subjects with DFS, 40 diabetic subjects without foot complications, 40 controls with foot lesions without diabetes, and 40 patients without diabetes mellitus.

Main Outcome Measures: Pulse wave velocity (PWV), augmentation index, reactive hyperemia index (RHI), serum vaspin and omentin levels, Fazekas score, and Mini-Mental State Examination (MMSE).

Results: Subjects with DFS showed higher mean PWV values when compared with diabetic controls and lower RHI values when compared with controls. They also showed a lower mean MMSE score, significantly lower omentin serum levels, and a higher prevalence of grade 2 severity of periventricular hyperintensities (PVHs). We observed a significant positive correlation between PWV and PVH and between Fazekas score and PWV among diabetic subjects, whereas among subjects with diabetic foot we observed a significant negative correlation between PVH and RHI.

Conclusions: Diabetes seems to be more associated with endothelial function disturbance in comparison with patients with diabetic foot that exhibit a more strict association with microvascular brain damage as indicated by our significant finding of an association with PVHs. (***J Clin Endocrinol Metab*** **104: 3920–3930, 2019)**

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA

Received 20 November 2018. Accepted 8 April 2019.
First Published Online 12 April 2019

*A.T. and D.D. contributed equally to this study.

Abbreviations: BMI, body mass index; DFS, diabetic foot syndrome; DWMH, deep WMH; MMSE, Mini-Mental State Examination; OR, odds ratio; PVH, periventricular hyperintensity; PWV, pulse wave velocity; RH, reactive hyperemia; RHI, reactive hyperemia index; PAT, peripheral arterial tonometry; T2DM, type 2 diabetes mellitus; WMH, white matter hyperintensity.

Diabetic foot syndrome (DFS) is a severe microvascular and macrovascular disease complication of diabetes mellitus that leads to limb amputation, with concomitant disability and significant impairment of quality of life (1, 2).

Previous studies have shown an increase in mortality following amputation in both patients with diabetes and in nondiabetic patients (1–4). Several studies (5–10) have indicated that rates of mortality and morbidity of cardiovascular disease are twofold to fourfold higher among patients with type 2 diabetes mellitus (T2DM) than for nondiabetic subjects.

Despite the importance and complexity of this issue concerning diabetic foot ulcers and its consequences, little research has been conducted to investigate the epidemiological and prognostic pattern of subjects with T2DM complicated by diabetic foot, compared with diabetic patients without foot ulcers (9–11).

We recently reported (12) that subjects with diabetic foot in comparison with diabetic subjects without foot ulcers had higher mean values of pulse wave velocity (PWV), lower mean values of an endothelial function marker such as reactive hyperemia (RH) index (RHI), and lower mean Mini-Mental State Examination (MMSE) scores. These findings corroborate the concept of a higher degree of vascular damage in patients with DFS.

In the wake of these results published by our group, we have continued our investigations with the study presented herein to clarify the pathogenic reasons for a worse vascular damage profile in subjects with diabetic foot.

Indeed, only a few studies (13, 14) evaluated the brain small-vessel disease burden in subjects with diabetes, whereas no study evaluated differences in the prevalence of microvascular brain damage in patients with DFS.

Concerning the biohumoral mechanisms that can explain these aspects, cytokines released by adipose tissue (also called adipokines) are involved in initiating and promoting a proinflammatory status, contributing to insulin resistance and regulation of insulin sensitivity and secretion (15, 16). Moreover, adipokines can influence several steps in atheroma formation, from endothelial dysfunction to plaque rupture (17, 18).

Recent basic and clinical studies have shed light on the role of two particular adipokines in vascular inflammation and atherosclerosis of diabetic subjects. These adipokines are omentin and vaspin. Omentin is an adipocytokine produced by adipose tissue able to modulate insulin resistance by improving insulin-stimulated glucose uptake and suppress vascular inflammation and oxidative stress by attenuating COX2 expression. A clinical study has shown that circulating omentin levels are independently associated with endothelial dysfunction even after matching for age and C-reactive protein in subjects with impaired glucose tolerance (19). Other studies have shown that circulating omentin are significantly lower in patients with carotid atherosclerosis and peripheral arterial disease compared with corresponding controls (20, 21).

One of the most recently discovered adipokines is vaspin, a visceral adipose tissue–derived serine protease inhibitor with insulin-sensitizing effects, belonging to the serpin superfamily, clade A (Serpina12) (22). It is thought that visceral adipose tissue–derived factors, including adipocytokines such as vaspin, play a local and endocrine role in the development of initial and advanced atherosclerosis in subjects with obesity by affecting the endothelium, vascular smooth muscle cells, and macrophages, thus disrupting vascular homeostasis (23, 24). Thus, vaspin and omentin serum levels may represent a possible candidate link between diabetic inflammatory-metabolic background and vascular damage in subjects with diabetic foot.

White matter hyperintensities (WMHs) are high-intensity lesions on both proton density and T2-weighted MRI scans and areas of lucency on head CT scans. The pathophysiological origins of WMHs are mainly due to advancing age and to the presence of cardiovascular risk factors, including hypertension (25–30).

A recent study reported that in subjects with nonalcoholic fatty liver disease the presence of WMHs is not associated with such disease (25).

No study, to the best of our knowledge, has analyzed the prevalence of WMHs in subjects with DFS and their relationship with adipokine serum levels and indexes of endothelial and cognitive performance.

Our study hypothesis was that higher serum values of some adipokines may explain the pathogenesis of vascular and microvascular damage in subjects with DFS.

Additionally, our study hypothesis was that serum values of vaspin and lower serum values of omentin, which are included in pathologic mechanisms of local inflammation on endothelium vessels, may be correlated with a higher degree of endothelial dysfunction, arterial stiffness, and microvascular brain damage in subjects with DFS.

Thus, we conducted a study in a new cohort of patients with diabetic foot and in controls with the aim of evaluating omentin and vaspin serum levels and the prevalence of WMHs in subjects with DFS and to analyze their relationship with other endothelial and cognitive indexes.

Methods

We recruited all subjects with T2DM and foot ulceration referred to the Diabetic Foot Intervention Clinical Group of the

Policlinico "P. Giaccone" Hospital of the University of Palermo (Palermo, Italy) from December 2016 to July 2017.

As controls, we recruited diabetic subjects without foot complications admitted from December 2016 to July 2017 for many conditions related to diabetic disease (decompensated diabetes, hypoglycemia, skin infections) at the Internal Medicine wards of the University Policlinico "P. Giaccone" of Palermo. We also enrolled as controls patients with foot lesions without diabetes (due to peripheral arterial disease or chronic venous disease), as well as consecutive patients without diabetes mellitus admitted to our wards for other causes than acute cardiovascular events from December 2016 to January 2019.

The study was accomplished in accordance with the principles of the Declaration of Helsinki as revised in 2001. All patients gave informed consent to take part in this research.

We have procured consent to publish from the participants (or legal parents) to communicate individual patient data.

DFS is defined, according to the World Health Organization, as "ulceration of the foot (distally from the ankle and including the ankle) related to neuropathy and different grades of ischemia and infection" (23). Foot ulcer was defined as a full-thickness skin lesion that required ≥14 days to heal (24).

Every subject with diabetic foot was matched for sex and age (±3 years) with one diabetic subject without diabetic foot and one healthy subject.

All subjects with cancer, autoimmune and inflammatory diseases or infectious and rheumatic diseases, hematological diseases, and severe liver or kidney failure, as well as those who were under treatment with anti-inflammatory drugs (not aspirin), were excluded. We also excluded subjects with recent deep vein thromboembolism and fever.

We also performed a physical examination of the lower limbs, which estimated the presence of the following characteristics: Charcot deformity, hammer toe/claw toe deformity, hallux limitus, hallux valgus, bony prominences, ankle and hallux mobility measured with goniometry, and prominent metatarsal heads.

We estimated diabetic peripheral neuropathy through careful patient history review and physical examination of the feet with the combination of a patient's neuropathic symptoms, diagnostic tests, and clinical signs. To evaluate the neuropathic symptoms, we used the neuropathy symptom score (25), which is generally used in clinical practice and showed high validity and sensitivity. We used the Semmes-Weinstein monofilament to estimate diabetic peripheral neuropathy (26).

We used the revised criteria of the American Diabetes Association to diagnose T2DM, using a value of fasting blood glucose ≥126 mg/dL, or it was determined using a clinically based algorithm that considered presenting weight and symptoms, age at onset, onset of insulin treatment, family history, and history of ketoacidosis (31).

Hypertension was diagnosed according to the 2013 European Society of Cardiology/European Society of Hypertension criteria (32).

Dyslipidemia was defined as triglyceride level ≥150 mg/dL and high-density lipoprotein cholesterol level <40 mg/dL, regardless of the patient's sex (33).

The study protocol was approved by the Ethics Committee of the Policlinico "P. Giaccone" University Hospital of Palermo.

Clinical and laboratory assessment

We accumulated clinical and anthropometric data at the time of enrollment. Patients were classified as obese [body mass index (BMI) ≥30], overweight (BMI 25–29.9), and normal weight (BMI 18.5–24.9 kg/m^2).

At the time of enrollment, a 12-hour overnight fasting blood sample was drawn to define the serum levels of alanine aminotransferase, triglycerides, plasma glucose, total cholesterol, and high-density lipoprotein cholesterol.

Assessment of cognitive function (MMSE)

We administered the MMSE, an instrument that can be used to assess mental status systematically and thoroughly. It is an 11-question measure that assesses five areas of cognitive function (language, attention, orientation, registration, recall, and calculation). The maximum score is 30, whereas an MMSE value <24 is suggestive of cognitive impairment (33).

PWV measurement

Carotid-femoral PWV was assessed in the supine position using an automatic device (SphygmoCor version 7.1) that evaluated the time delay between the rapid upstroke of the carotid and femoral artery pulse waves. The distance between the two arterial points was measured using a tape measure on the surface of the body. PWV was evaluated as the distance traveled by the arterial pulse wave (meters) divided by the time delay between the two arterial points (seconds), thus expressed as meters per second.

Pulse wave analysis

We used applanation tonometry to record radial artery pressure waveform continuously, and mean values of two or more screens of pulse waves of good quality were used for analysis. In consideration of the collected data, an averaged radial pressure waveform was created, as well as a corresponding aortic pressure waveform and blood pressure calculated by the validated transfer function (SphygmoCor version 7.1). The aortic pressure waveform was used to calculate the augmentation index (difference in height between the first and second systolic peaks expressed as a percentage of pulsatory pressure).

Reactive hyperemia–peripheral arterial tonometry

The principle of reactive hyperemia (RH)–peripheral arterial tonometry (PAT) has been described previously by some researchers (19). In sum, a blood pressure cuff was positioned on one upper arm, while the contralateral arm served as a control. PAT probes were positioned on one finger of each hand. After a 5-minute equilibration period, the cuff was inflated to 60 mm Hg above the systolic pressure or 200 mm Hg for 5 minutes and then deflated to induce RH. RH is a temporary increase of blood flow in an area as result of induced ischemia and expresses "the health state" of endothelium.

The RH-PAT data were digitally analyzed online by Endo-PAT 2000 software version 3.0.4. The RH-PAT index (RHI) reproduces the range of RH and was measured as the ratio of the mean amplitude of the PAT signal over 1 minute starting 1.5 minutes after cuff deflation (control arm, A; occluded arm, C) divided by the mean amplitude of the PAT signal of a 2.5-minute period before cuff inflation (baseline) (control arm, B; occluded arm, D). Thus, RHI = (C/D)/(A/B) × baseline correction. A value of RHI <1.67 indicated endothelial dysfunction.

Brain MRI evaluation

For the assessment of white matter alterations to brain MRI, the presence of periventricular hyperintensity (PVH) and deep white matter hyperintensity (DWMH) graded through a score between 0 and 3 was evaluated.

Fazekas score

We also calculated the Fazekas score to estimate the overall extent of changes in the cerebral white matter.

The Fazekas scale is used to quantify the amount of T2 hyperintense lesions of white matter and is usually attributed to chronic ischemia of small vessels, although not all of these lesions are attributable to this.

This classification was proposed by Fazekas *et al.* (34) in 1987, and it represents the most widespread system to describe the severity of white matter disease in publications. This scale divides the cerebral white matter between periventricular matter (PVH) and deep white matter (DWMH). At each location a score is assigned in relationship to the size and degree of confluence of the periventricular white matter lesions: 0, absent ; 1, pencil-tip lesions or caps; 2, smooth "halo"; 3, irregular periventricular signal extending to deep white matter; 4, deep white matter (DVMH) .

Biochemical analysis

Serum vaspin and omentin levels were evaluated on blood samples collected on fasting. Serum was separated by centrifugation for 10 minutes at 3000 rpm at room temperature and stored at −80°C until testing. All samples were analyzed in the same analytic session.

Serum vaspin concentration was measured by a commercial ELISA kit (BioVendor; BioVendor Laboratory Medicine, Brno, Czech Republic) (detection range, 0.1 to 1000 ng/mL; sensitivity, <1 ng/mL).

Serum omentin was measured by a commercial ELISA kit (RayBio human omentin enzyme immunoassay; RayBiotech, Atlanta, GA) (detection range, 0.031 to 2 ng/mL; sensitivity, 0.01 ng/mL).

Intraassay and interassay coefficients of variation were <10%, as declared by the manufacturers of both kits.

Statistical analysis

The sample size was estimated to ensure sufficient power in the analysis of variance, considering as the main outcome omentin and vaspin serum level differences of 30% in diabetic subjects with and without diabetic foot vs healthy subjects. To achieve 80% power with a significance level of 0.05, 40 subjects in each group were needed.

Data were tested for normal distribution using the Kolmogorov–Smirnov test. Continuous data are expressed as mean ± SD, unless otherwise specified. Baseline differences between groups were assessed by the χ^2 test or Fisher exact test, as needed for categorical variables, and by the univariate ANOVA for parametric variables. Pearson correlation analysis was conducted to examine the association between vascular and cognitive health indexes and other clinical and laboratory variables in patient groups. Multivariable logistic regression analysis examined the correlation between patient characteristics (independent variables such as clinical variables, endothelial function markers, and brain WMH markers that resulted in significance at univariate analysis) and patient groups (dependent variables, including diabetic subjects and subjects with diabetic foot vs healthy subjects) in the multiple regression model. Odds ratios (ORs) and their 95% CIs were also calculated and adjusted for confounding factors such as other cardiovascular risk factors (hypertension, diabetes, dyslipidemia, smoking habit) and drug therapy as covariates. Data were analyzed by the SPSS software version 22.0 (IBM Corporation, Armonk, NY). All P values were two-sided, and a P value <0.05 was considered statistically significant.

Results

General, demographic, and laboratory variables of subjects, with and without diabetic foot and of healthy controls, are listed in Table 1.

We recruited 40 subjects with diabetic foot, 40 diabetic subjects without diabetic foot, 40 with foot lesions without diabetes, and 40 healthy controls.

Patients with diabetic foot showed in comparison with control subjects higher mean systolic blood pressure, higher mean diastolic blood pressure, higher mean BMI, and higher mean HbA1c percentage (see Table 1) .

Patients with diabetic foot in comparison with diabetic controls, foot ulcer controls, and healthy controls were also more likely to have hypertension, whereas no significant difference was observed between subjects with DFS and patients with diabetes without DFS with regard of prevalence of previous cardiovascular and cerebrovascular events, nephropathy and retinopathy, and duration of diabetes (see Table 1) .

Subjects with diabetic foot compared with control subjects showed higher mean PWV values (11.4 ± 4.8 m/s vs 9.0 ± 3.9 m/s vs 8.1 ± 2.4 m/s vs 6.8 ±1.9 m/s; $P <$ 0.0005), higher mean augmentation index (137.4% ± 21.3% vs 142.0% ± 25.0% vs 136.1% ± 19.7% vs 152.1% ± 35.2%), comparable mean RHI values (1.7 ± 0.4 vs 1.7 ± 0.5) when compared with diabetic controls and lower when compared with foot ulcer and healthy controls (1.7 ± 0.4 vs 2.3 ± 0.5 vs 2.2 ± 0.6) ($P <$ 0.0005), a lower mean MMSE score (26.6 ± 3.4 vs 27.5 ± 2.4 vs 28.6 ± 1.4 vs 29.2 ± 1.0; $P < 0.0005$), comparable vaspin serum levels (0.16 ± 0.11 ng/mL vs 0.21 ± 0.10 ng/mL vs 0.22 ± 0.1 ng/mL vs 0.18 ± 0.12 ng/mL), and significantly lower omentin serum levels (38.3 ± 11.7 ng/mL vs 43.2 ± 3.5 ng/mL vs 50.5 ± 8.2 ng/mL vs 58.8 ± 6.2 ng/mL; $P < 0.0005$).

Concerning brain MRI findings, PVH and DWMH distribution in patients with diabetic foot, in diabetic subjects without diabetic foot, and in control subjects are listed in Table 1.

Subjects with diabetic foot showed, in comparison with diabetic subjects without diabetic foot and in comparison with subjects with foot lesions without diabetes and in healthy controls, a higher prevalence of

Table 1. General and Demographic Variables in Subjects With Diabetic Foot, Diabetic Controls, and Healthy Patients With Diabetes

	Group 3: Patients With Diabetic Foot (n = 40)	Group 2: Diabetic Controls (n = 40)	Group 1: Controls With Foot Ulcers Without Diabetes (n = 40)	Group 0: Healthy Controls (n = 40)	*P*
Age, y (mean ± SD)	61.8 ± 8.3	61.4 ± 11.4	61.4 ± 10.9	61.0 ± 13.5	0.250
SBP, mm Hg (mean ± SD)	133.3 ± 20.2	123.5 ± 16.5	130.1 ± 9.2	124 ± 10.3	**0.007**
DBP, mm Hg (mean ± SD)	77.3 ± 10.1	70.2 ± 12.2	72.5 ± 5.7	71.5 ± 10.4	**0.003**
Weight, kg (mean ± SD)	89.5 ± 15.6	83.3 ± 16.5	73.7 ± 4.1	78.1 ± 13.2	**<0.0005**
BMI, kg/m^2 (mean ± SD)	30.2 ± 4.1	29.4 ± 5.5	26.6 ± 2.1	27.7 ± 3.6	**<0.0005**
Hb1Ac, % (mean ± SD)	8.1 ± 1.2	7.2 ± 1.4	-	—	**0.005**
Female/male, %	10/30	17/23	13/27	16/24	0.351
Hypertension, n (%)	38 (95)	29 (72.5)	10 (25)	6 (15)	**<0.0005**
Previous cardiovascular events, n (%)	10 (25)	9 (22.5)	4 (10)	0	**0.001**
Previous stroke, n (%)	4 (10)	4 (10)	2 (5)	0	0.180
Dyslipidemia, n (%)	28 (70)	20 (50)	4 (10)	2 (5)	**<0.0005**
Brain MRI findings					
PVH, n (%)					
0	6 (15)	18 (45)	39 (97.5)	34 (85)	
−1	20 (50)	16 (40)	1 (2.5)	6 (15)	**<0.0005**
−2	11 (27.5)	6 (15)	0	0	
−3	3 (7.5)	0	0	0	
DWMH					
0	18 (45)	25 (62.5)	36 (90)	36 (90)	
−1	16 (40)	8 (20)	4 (10)	4 (10)	**<0.0005**
−2	4 (10)	7 (17.5)	0	0	
−3	2 (5)	0	0	0	
Fazekas score					
0	14 (35)	20 (50)	38 (95)	32 (80)	
−1	22 (55)	15 (37.5)	2 (5)	8 (20)	**<0.0005**
−2	3 (7.5)	5 (12.5)	0	0	
−3	1 (2.5)	0	0	0	
Fazekas score (mean ± SD)	0.78 ± 0.70	0.62 ± 0.70	0.05 ± 0.22	0.20 ± 0.41	**<0.0005**
Aix, % (mean ± SD)	137.4 ± 21.3	142.0 ± 25.0	136.1 ± 19.7	152.1 ± 35.2	**0.028**
PWV, m/s (mean ± SD)	11.4 ± 4.8	9.0 ± 3.9	8.1 ± 2.4	6.8 ± 1.9	**<0.0005**
RHI (mean ± SD)	1.7 ± 0.4	1.7 ± 0.5	2.3 ± 0.5	2.2 ± 0.6	**<0.0005**
MMSE (mean ± SD)	26.6 ± 3.4	27.5 ± 2.4	28.6 ± 1.4	29.2 ± 1.0	**<0.0005**
Vaspin, ng/mL	0.16 ± 0.11	0.21 ± 0.10	0.22 ± 0.1	0.18 ± 0.1	0.632
Omentin, ng/mL	38.3 ± 11.7	43.2 ± 3.5	50.5 ± 8.2	58.8 ± 6.2	**<0.0005**
					1, 2, 3 vs 0 [a]: ***P* < 0.0005**
					3, 2 vs 1 [a]: ***P* < 0.0005**
					3 vs 2 [a]: ***P* = 0.047**

Boldface type indicates statistically significant variables.

Abbreviations: Aix, augmentation index; DBP, diastolic blood pressure; SBP, systolic blood pressure.

[a] *Post hoc* analysis.

grade 2 severity of PVH (27.5% vs 15% vs 0 vs 0; $P < 0.0005$) and a higher mean Fazekas score (0.78 ± 0.70 vs 0.62 ± 0.70 vs 0.5 ± 0.22 vs 0.20 ± 0.41; $P < 0.0005$).

With regard to correlation analyses between neuroimaging, vascular variables, and cognitive variables among subjects with diabetes, we observed a significant positive correlation between PWV and PVH ($r = 0.430$; $P = 0.006$) and between Fazekas score and PWV ($r = 0.475$; $P = 0.002$), whereas among subjects with diabetic foot we observed a significant negative correlation between PVH and RHI ($r = -0.429$; $P = 0.006$) and DWMH and RHI ($r = -0.358$; $P = 0.023$) (see Tables 2 and 3).

Among subjects with foot lesions without diabetes we did not observe any relationship between arterial stiffness and endothelial function markers and brain WMH

Table 2. Correlations Among Clinical and Laboratory Variables Among Diabetic Subjects

		PVH	DWMH	FAZEKAS	PWV	RHI	MMSE
PVH	Pearson correlation	1	0.752[a]	0.830[a]	0.430[a]	-0.0.161	−0-0.151
	Sig. (two-tailed)		**<0.0001**	**<0.0001**	**0.006**	0.322	0.359
	N	40	40	40	40	40	39
DWMH	Pearson correlation	0.752[a]	1	0.755[a]	0.288	−0.053	−0.136
	Sig. (two-tailed)	**<0.0001**		**<0.0001**	0.071	0.747	0.411
	N	40	40	40	40	40	39
Fazekas score	Pearson correlation	0.830[a]	0.755[a]	1	0.475[a]	−0.249	−0.207
	Sig. (two-tailed)	**<0.0001**	**<0.0001**		**0.002**	0.121	0.207
	N	40	40	40	40	40	39
PWV	Pearson correlation	0.430[a]	0.288	0.475[a]	1	−0.060	0.040
	Sig. (two-tailed)	**0.006**	0.071	0.**002**		0.714	0.809
	N	40	40	40	40	40	39
RHI	Pearson correlation	−0.161	−0.053	−0.249	−0.060	1	0.181
	Sig. (two-tailed)	0.322	0.747	0.121	0.714		0.270
	N	40	40	40	40	40	39
MMSE	Pearson correlation	−0.151	-0.0136	−0.207	0.040	0.181	1
	Sig. (two-tailed)	0.359	0.411	0.207	0.809	0.270	
	N	39	39	39	39	39	39

Boldface type indicates statistically significant variables.

Abbreviations: Aix: augmentation index; Sig., significance.

[a]Correlation is significant at the 0.01 level (two-tailed).

markers, but only a negative correlation between MMSE and DWMH and Fazekas score and between RHI and PWV (see Table 3).

At multivariable analysis of predictive variables of diabetes, after correction for confounding factors, we showed that, among diabetic subjects, DBP (OR, 0.88; $P = 0.008$), BMI (OR, 1.20; $P = 0.049$), and MMSE (OR, 0.37; $P = 0.002$) were significantly associated with diabetes, whereas among subjects with diabetic foot, DBP (OR, 0.84; $P = 0.001$), BMI (OR, 1.28; $P = 0.019$), MMSE (OR, 0.36; $P = 0.003$), PWV (OR, 1.45; $P = 0.015$), and PVH (OR, 106.6; $P = 0.001$) were significantly associated with diabetic foot presence (see Table 4).

Discussion

We report that subjects with DFS in comparison with control subjects with diabetes without diabetic foot and healthy controls with and without foot lesions had higher

Table 3. Correlations Among Clinical and Laboratory Variables Among Subjects With Diabetic Foot

		PVH	DWMH	FAZEKAS	PWM	RHI	MMSE
PVH	Pearson correlation	1	0.702[a]	0.697[a]	−0.143	−0.429[a]	−0.143
	Sig. (two-tailed)		**<0.0001**	**<0.0001**	0.379	**0.006**	0.380
	N	40	40	40	40	40	40
DWMH	Pearson correlation	0.702[a]	1	0.602[a]	−0.042	−0.358[b]	−0.094
	Sig. (two-tailed)	**<0.0001**		**<0.0001**	0.796	**0.023**	0.565
	N	40	40	40	40	40	40
Fazekas score	Pearson correlation	0.697[a]	0.602[a]	1	0.027	−0.277	0.028
	Sig. (two-tailed)	**<0.0001**	**<0.0001**		0.867	0.084	0.866
	N	40	40	40	40	40	40
PWV	Pearson correlation	−0.143	−0.042	0.027	1	0.082	−0.031
	Sig. (two-tailed)	0.379	0.796	0.867		0.615	0.848
	N	40	40	40	40	40	40
RHI	Pearson correlation	-0.0429[a]	−0.358[b]	−0.277	0.082	1	0.020
	Sig. (two-tailed)	**0.006**	**0.023**	0.084	0.615		0.904
	N	40	40	40	40	40	40
MMSE	Pearson correlation	−0.143	−0.094	0.028	−0.031	0.020	1
	Sig. (two-tailed)	0.380	0.565	0.866	0.848	0.904	
	N	40	40	40	40	40	40

Boldface type indicates statistically significant variables.

Abbreviations: Aix, augmentation index; Sig., significance.

[a]Correlation is significant at the 0.01 level (two-tailed).

[b]Correlation is significant at the 0.05 level (two-tailed).

Table 4. Multivariable Analysis of Variables Predictive of Diabetes and Diabetic Foot Presence

	OR	95% CI Exp(B)	*P*
Diabetic subjects			
PAS	0.997	0.92–1.08	0.941
PAD	**0.865**	**0.76–0.98**	**0.029**
BMI	**1.291**	**1.04–1.59**	**0.019**
MMSE	**0.459**	**0.22–0.92**	**0.030**
PWV	1.243	0.90–1.71	0.183
RHI	**0.196**	**0.04–0.86**	**0.032**
PVH	3.764	0.17–0.82	0.400
DWMH	18.874	0.38–918.52	0.138
Fazekas	0.213	0.007–6.37	0.379
Subjects with diabetic foot			
AGE	1.060	0.96–1.16	0.239
PAS	1.051	0.96–1.14	0.265
PAD	**0.818**	**0.71–0.932**	**0.004**
BMI	**1.320**	**1.04–1.66**	**0.019**
MMSE	**0.446**	**0.21–0.91**	**0.027**
PWV	1.398	0.99–1.96	0.052
RHI	0.230	0.03–1.35	0.104
PVH	**51.908**	**2.02–1330.19**	**0.017**
DWMH	9.678	0.175–534.48	0.267
Fazekas	0.052	0.001–1.83	0.104

The reference category is healthy subjects. Boldface type indicates statistically significant variables.

Abbreviations: AGE, advanced glycation end products; Aix, augmentation index; DBP, diastolic blood pressure; PAD, peripheral artery disease; SBP, systolic blood pressure.

mean values of PWV, lower mean values of RHI, and lower mean MMSE scores and that there is a negative correlation between the white matter lesion markers and the endothelial index. To the best of our knowledge, only a few studies (12, 35–37) have evaluated surrogated markers of cardiovascular disease such as arterial stiffness and endothelial function indexes in diabetic foot subjects, whereas no study has evaluated in these subjects the correlation between vascular health indexes, brain small vessel disease findings such as WMHs, and serum markers of cardiometabolic risk such as some adipokine serum levels.

It has been reported that T2DM is linked to microcirculatory impairment and endothelial dysfunction (35). These pathologic changes cause microvascular dysfunction such as nephropathy and retinopathy and foot complications (35, 36). Furthermore, it has been speculated that arterial stiffness leads to generalized microvascular dysfunction and that individuals with T2DM are particularly prone to the detrimental effects of arterial stiffness (37).

We report a higher mean value of PWV indicative of higher arterial stiffness degree in subjects with diabetic foot in comparison with control subjects with diabetes and nondiabetic subjects with and without foot complications. We also reported significantly lower mean values of RHI in subjects with diabetes with diabetic foot in comparison with controls with and without foot lesions but not in comparison with subjects with diabetes without foot injury. It has been hypothesized that individuals with T2DM are particularly prone to the detrimental effects of endothelial dysfunction (38–40), a key mechanism in the pathogenesis of atherothrombosis, and that this may explain the increased cardiovascular events risk in diabetes. Furthermore, a bidirectional association between endothelial dysfunction and T2DM, in which endothelial dysfunction plays the role as both cause (35, 40) and consequence (41) of T2DM, has been reported.

Diabetes causes endothelial dysfunction by means the formation of advanced glycation end products, accumulation of glucose, and increased oxidative stress. Alternatively, endothelial dysfunction causes or aggravates T2DM by impairing the timely access of glucose and insulin to their target tissues.

These issues explain our findings that corroborate the concept of DFS as a clinical condition characterized by a higher degree of vascular disease, as indicated by our findings of a higher degree of arterial stiffness, endothelial dysfunction, and brain small vessel disease in terms of WMH severity and MMSE impairment. These results may be related to a possible impact of our research on clinical practice suggesting a more aggressive therapeutic strategy of cardiovascular prevention in subjects with DFS by means of cardiovascular active drugs such as angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, statins, and antiplatelets. Furthermore, our findings showing a negative correlation between PVH and RHI indicate the advisability to evaluate vascular health with noninvasive tools such as RH-PAT in diabetic subjects with foot injury. We suggest that a noninvasive evaluation of endothelial function in DFS subjects could precociously show a degree of endothelial dysfunction, and this issue may represent a possible recommendation in clinical practice.

In our subjects with diabetic foot in comparison with control subjects we reported comparable vaspin serum levels and significantly lower omentin serum levels.

In humans, vaspin expression as mRNA was detected in human visceral and subcutaneous adipose tissue (42), and it has been reported that vaspin has a role in the adipoinsular axis and may be associated with insulin resistance in subjects with obesity, including patients with T2DM (43). Nevertheless, vaspin has been shown to improve glucose tolerance and insulin sensitivity significantly in mice and to be positively associated with obesity-related diseases in humans (44). Some findings could indicate that the levels of serum vaspin may change with the progression of diabetes (21). Vaspin may increase at the beginning of diabetes and decrease with worsening of diabetes in humans.

Our findings of no significant difference with regard of vaspin levels in subjects with diabetes with and without diabetic foot, as well as our findings of significantly lower omentin serum levels in subjects with diabetic foot, are not easy to explain. No studies had evaluated the different expression of adiponectins with regard of vascular disease degree in subjects with diabetes, and thus our findings could appear as original. The different role of vaspin and omentin in subjects with DFS may be due to the different pathogenetic roles of these two adiponectins in vascular abnormalities related to diabetic foot presence. In particular, a recent study (45) reported a significant correlation between serum vaspin and leptin concentrations indicating that serum vaspin concentration reflects body fat mass in humans and that vaspin may have a compensatory role in insulin resistance in human obesity-associated diseases. Recent studies suggest a more strict and direct link between omentin serum levels and cardiovascular risk. Yue *et al.* (46) showed that omentin could be a biomarker for stroke and its severity, because higher levels of this molecule were inversely correlated with plaque instability. Another recent study suggested a strong association between decreased serum omentin levels and peripheral arterial disease and its severity (20). A further study showed that after adjusting for cardiovascular risk factors, a decreased omentin level was found to be an independent predictor of angiographic coronary artery disease (21). Omentin is strictly inversely correlated with BMI, endothelial dysfunction, and the degree of insulin resistance, typically elevated in DFS patients by several microvascular damage. These issues may explain our divergent results with regard to vaspin and omentin in subjects with DFS indicating a more direct role of omentin serum levels in vessel protection also in subjects with diabetes with foot complications.

Furthermore, our patients with DFS also showed higher values of BMI and PWV in comparison with subjects with diabetes without foot injury, but no significant difference in RHI value. We suggest that higher values of BMI and massive fat adipose tissue, on an equivalent state of endothelial dysfunction but on a different milieu of insulin resistance, can explain why omentin serum levels are lower in subjects with DFS than in subjects with diabetes (47, 48).

Nevertheless, we sought a possible relationship between the lower serum levels of omentin and the higher degree of macrovascular damage such as diabetic foot. We also sought to evaluate a possible relationship in the context of microvascular injury between lower omentin serum levels and a higher degree of white matter lesions in the brain of patients with diabetes with foot injury.

Our study highlighted a possible interplay between brain PVHs and DFS and, to our knowledge, this is an original finding. With regard of brain MRI findings and periventricular and deep white matter hyperintensity distribution we showed a higher degree of severity of periventricular white matter lesions in our patients with diabetic foot compared with subjects with diabetes without diabetic foot to subjects with foot lesions without diabetes and to healthy subjects.

No study has yet analyzed the burden of microvascular brain damage in subjects with diabetes with foot complications, whereas some studies evaluated white matter lesion prevalence in subjects with diabetes. A study by Jongen *et al.* (48) showed that T2DM was associated with a smaller volume of gray matter and with larger lateral ventricle volume and with larger white matter lesion volume, whereas white matter volume was not affected. Consistent with these findings, more recent studies (49, 50) showed that patients with T2DM had an increased progression of brain atrophy at follow-up compared with control subjects and no difference in progression of WMH volume or infarction.

In our study, we report that patients with diabetes with diabetic foot in comparison with subjects with diabetes without foot complication showed a higher degree of extension of PVHs, and this finding could suggest that DFS represents an additive vascular risk factor also in the clinical context of microvascular brain damage. Thus, patients with diabetic foot in comparison with subjects with diabetes appear more likely to develop white matter hyperintensity, and this finding seems consistent with our findings of a higher prevalence of markers of vascular damage and of lower omentin serum levels.

Our multivariable analysis of predictive variables of diabetes showed that among subjects with diabetes, diastolic blood pressure, BMI, MMSE mean score, and mean value of the RHI were significantly associated with diabetic foot presence.

These findings further corroborate the reported relationship between some cardiovascular risk factors such as diastolic blood pressure and BMI and both diabetes and diabetes foot, whereas the relationships between RHI and diabetes and PWH and diabetic foot seem to indicate the different expression of vascular damage in patients with diabetes without and with foot complications. Diabetes seems to be more associated with endothelial function disturbance in comparison with patients with diabetic foot who exhibit a more strict association with microvascular brain damage, as indicated by our significant reported association with PVHs.

With regard to correlation analysis between neuroimaging findings and vascular and cognitive variables among subjects with diabetes, we observed a significant

positive correlation between PWV and PVHs and between Fazekas score and PWVs, whereas among subjects with diabetic foot we observed a significant negative correlation between PVHs and RHI and DWMHs and RHI.

These correlation analyses corroborate the possible role of white matter lesions as surrogate markers of vascular damage, with a positive linear correlation between the chosen markers of brain white matter abnormality and arterial stiffness indexes such as PWV, and a negative correlation between endothelial function markers and both PVHs and DWMHs.

We did not observe any correlation between the chosen adipokine serum levels and indexes of vascular and microvascular damage, such as arterial stiffness and endothelial and brain small vessel markers, and thus our findings do not seem consistent also in the context of DFS, with other findings indicating a possible role of adipokine serum levels as possible predictive factors of vascular damage as observed in diabetes without foot complications. A recent study (51) showed that patients with T2D have significantly lower levels of adiponectin and that adipokines correlate with indicators of vascular damage and could contribute to cardiovascular risk in patients with T2D.

White matter lesions are mainly due to arteriosclerosis of cerebral small vessels, and this leads to a impaired hematic supply to white substance and its consequent necrosis and rarefaction.

This issue may explain our finding of no correlation between lower serum concentrations of omentin and WMHs. Nevertheless, arteriosclerosis is strictly linked to endothelial dysfunction and arterial stiffness, and this may explain our finding of a significant positive correlation between PWV and PVH in subjects with diabetes and the negative correlation between RHI and PVH and DWMH in subjects with diabetes with foot injury.

Thus, our findings seem to indicate that the cardiovascular predictive role of adipokines is not maintained in the clinical context of DFS, and this result is consistent with our findings of a higher degree of periventricular WMH indicating a lower degree of vascular protection of omentin in the setting of brain small vessel disease in the context of microvascular damage. Future studies will be designed to better analyze this issue, but our findings may suggest some implications on clinical practice underlining the importance of a direct vascular protection in comparison with therapeutic strategies addressing adipoinflammatory dysfunction and the adipokine axis in subjects with DFS.

Limitations

The cross-sectional analysis is a limitation because it does not allow conclusions on cause–effect relationships, and the relatively small sample size may also limit conclusions.

Conclusions

Thus, we can conclude that evaluation of white matter lesion prevalence and brain distribution is advisable in patients with diabetic foot because the presence of foot complications in these subjects seems to make these patients more prone, in comparison with subjects with diabetes without foot complications, to white matter lesion incidence.

Acknowledgments

Author Contributions: A.T., A. Caronia, G. Guercio, C.M., A.D., M.P., C.B., R.P., and I.S. researched data; A.T., A.D., and A.P. wrote the manuscript; A.T., A. Casuccio, R.P., C.M., G. Guercio, M.C., G. Gulotta, A.P., and V.D. reviewed/edited manuscript; and A.T., A.D., A. Casuccio, R.P., and A.P. contributed to discussions.

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Disclosure Summary: The authors have nothing to disclose.

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