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Gender-Specific Alterations in Fibrin Structure Function in Type 2 Diabetes: Associations with Cardiometabolic and Vascular Markers

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Context: Diabetes is associated with increased incidence of atherothrombotic disease. The fibrin network forms the backbone of the arterial thrombus, and fibrin clot structure determines pre-disposition to cardiovascular events.

Objectives: The aim of the study was to investigate fibrin clot structure/fibrinolysis in the largest type 2 diabetes cohort and analyze associations with cardiometabolic risk factors and vascular pathology.

Design: Clot structure/fibrinolysis was assessed in 875 participants of the Edinburgh Type 2 Diabetes Study [age, 68 (range, 60–75) yr; 450 males] by turbidimetric assays, and clots were visualized by confocal microscopy. Four parameters of clot structure/fibrinolysis were analyzed, and plasma levels of fibrinogen and plasminogen activator inhibitor-1 were studied by Clauss assay and ELISA, respectively.

Results: Clot maximum absorbance was increased in females compared with males (0.37 ± 0.005 and 0.34 ± 0.005 arbitrary unit, respectively; $P < 0.001$), and took longer to lyse (803 ± 20 and 665 ± 12 sec, respectively; $P < 0.001$). These gender differences in clot structure and fibrinolysis were still evident after correcting for fibrinogen and plasminogen activator inhibitor-1 plasma levels. A prothrombotic fibrin structure profile was associated with increased body mass index and low levels of high-density lipoprotein in women and with inadequate diabetes control in men. Clot formation time was related to previous cardiac ischemic events in both men and women after adjusting for traditional risk factors [odds ratio, 1.22 (95% confidence interval, 1.07, 1.38); and 1.33 (1.15, 1.50), respectively], and prothrombotic clots were associated with low ankle brachial index, renal impairment, and smoking, regardless of gender.

Conclusions: Women with type 2 diabetes have compact clots with compromised fibrinolysis compared with men. There are gender-specific associations between clotting parameters and cardiometabolic risk factors in this population, whereas vascular abnormalities, impaired renal function, and smoking are associated with prothrombotic clot structure profile regardless of gender. (*J Clin Endocrinol Metab* 97: E2282–E2287, 2012)

Atherothrombotic disease remains the main cause of mortality in subjects with type 2 diabetes (T2DM) (1). An occlusive arterial thrombus consists of a skeleton of fibrin fibers, the structure of which influences predisposition to cardiovascular disease (2). Only a limited number of studies investigated the effects of diabetes on fibrin structure and demonstrated an association with compact clots and resistance to fibrinolysis (3–5), although this was less clear in smaller studies involving T2DM subjects (6, 7). In addition to the limited number of samples analyzed, a collective criticism of T2DM studies is incomplete characterization of this highly heterogeneous group of individuals, making results interpretation inconclusive. Also, the relationship between clot structure and metabolic parameters, cardiovascular risk factors, and the presence of vascular pathology has not been appropriately addressed. Therefore, there is a gap in the literature in relation to characterization of clot structure in T2DM, which is crucial for understanding the role of the fibrin network in predisposition to atherothrombotic events in this common condition.

Our aim was to study fibrin clot structure/fibrinolysis in a large cohort of well-characterized T2DM subjects. We have analyzed the effects of gender, age, metabolic and cardiac parameters, current vascular pathology, and previous history of ischemic heart disease (IHD) on multiple fibrin clot parameters.

Subjects and Methods

Study population and examination

The study protocol of the Edinburgh Type 2 Diabetes Study is described elsewhere, including criteria used for the diagnosis of macrovascular complications (8). Briefly, 1066 patients with T2DM (age, 60–75 yr) were randomly recruited; they are representative of those invited to participate ($n = 5454$) (9). A total of 875 plasma samples were available for clot structure analysis.

Analysis of clot formation/lysis and plasma levels of coagulation proteins

Blood samples were taken into citrated tubes without a tourniquet after a 4-h fast, usually at midday. The first 5 ml were used for clinical tests and platelet poor plasma separated from subsequent samples with clot turbidity and lysis measurements conducted as previously described (10). Several variables were recorded that show associations with cardiovascular risk (10–13), including lag phase, clot maximum absorbance, clot formation time, and lysis time. Fibrinogen and plasminogen activator inhibitor-1 (PAI-1) plasma levels were measured as described (4).

Laser scanning confocal microscopy

Fibrin clots were prepared from pooled plasma of randomly selected samples (10 female and 10 male subjects) as described (4).

Statistical analysis

Exploratory analysis was done using SPSS program version 16 (SPSS Inc., Chicago, IL). Statistical modeling was done using R program version 2.15 (r-project.org). Between-group comparisons of normally distributed variables were carried out using t test, whereas comparisons of nonnormally distributed variables were carried out by Mann-Whitney test. Differences between categorical variables were analyzed by χ^2 test. Logistic and multiple regression models were used to identify clotting parameters associated with vascular disease and cardiometabolic factors associated with clotting parameters.

Results

Subject characteristics

Demographic/clinical characteristics of the study population are summarized in Table 1.

Female gender is associated with a prothrombotic fibrin network profile

Female gender was associated with longer clot formation time compared with males, which may be related to higher maximum absorbance and/or longer clot lysis time (Table 1). Plasma fibrinogen levels were higher in females than males, but differences in fibrin clot parameters remained after adjusting for protein levels. Similarly, PAI-1 was higher in women than men, and the difference in lysis time was still present after adjusting for PAI-1 levels. Gender differences in clot structure remained after correcting for body mass index (BMI), indicating that these are not related to higher BMI in women. Differences between male and female clots were further visually confirmed by laser scanning confocal microscopy (Fig. 1A, *upper panel*).

Clot formation time showed significant correlations with both fibrinogen and PAI-1 levels ($P < 0.001$ for both), indicating that it reflects both thrombosis and fibrinolysis potential. For comparison, lysis time mainly correlated with PAI-1 levels, whereas clot maximum absorbance correlated with fibrinogen levels. Given gender differences, further analysis was conducted separately for men and women.

Older age is associated with denser clots and enhanced fibrinolysis in men

In men, clot maximum absorbance increased with age, whereas lysis shortened, associated with a trend toward higher PAI-1 plasma levels in younger men (921 ± 71 and 738 ± 64 pg/ml for lower and higher tertiles of age, respectively; $P = 0.06$). The effect of age was independent of vascular pathology measured as ankle brachial index (ABI) or intima media thickness (IMT) (detailed in the section entitled *Clot structure/fibrinolysis is altered in the presence of clinical and subclinical vascular disease*). In

TABLE 1. Clinical characteristics, clot structure parameters, and coagulation factor plasma levels by gender

Variable	Male	Female	P value
n	450	425	0.39
Age, yr (range)	68.2 (60–75)	67.9 (60–75)	0.34
Duration of T2DM (yr)	8.33 ± 0.31	7.75 ± 0.31	0.31
Current smokers, n (%)	65 (14)	47 (11)	0.27
Vascular parameters			
IHD, n (%)	164 (36)	100 (24)	<0.001
Systolic BP (mm Hg)	133 ± 0.7	133 ± 0.8	0.58
Diastolic BP (mm Hg)	70 ± 0.4	68 ± 0.4	<0.001
IMT (mm)	1.04 ± 0.01	0.96 ± 0.01	<0.001
ABI	1.01 ± 0.01	0.96 ± 0.007	<0.001
Anthropometrics			
BMI (kg/m ²)	30.2 ± 0.2	32.5 ± 0.3	<0.001
Waist circumference (cm)	107.9 ± 0.5	113.8 ± 0.6	0.004
Waist/hip ratio	1.00 ± 0.003	0.93 ± 0.003	<0.001
Metabolic and renal			
HbA1c (%)	7.33 ± 0.06	7.46 ± 0.06	0.16
Plasma glucose (mmol/liter)	7.6 ± 0.1	7.5 ± 0.1	0.56
Total cholesterol (mmol/liter)	4.2 ± 0.04	4.5 ± 0.05	<0.001
HDL cholesterol (mmol/liter)	1.16 ± 0.016	1.34 ± 0.017	<0.001
eGFR (ml/min · m ²)	66.0 ± 0.7	61.7 ± 0.7	<0.001
Treatment, n (%)			
Metformin	285 (63)	240 (56)	0.09
Aspirin	310 (69)	261 (61)	0.06
Insulin	43 (10)	51 (12)	0.39
Thiazolidinediones	72 (16)	73 (17)	0.77
ACEi + ARB	317 (70)	274 (64)	0.13
Statins	332 (74)	316 (74)	0.91
Sulfonylurea	125 (28)	114 (27)	0.84
Nitrates	85 (19)	69 (16)	0.43
Clot structure parameters			
Lag phase (sec)	528 ± 6	520 ± 6	0.37
Maximum absorbance (au)	0.34 ± 0.005	0.37 ± 0.005	<0.001
Clot formation time (sec)	516 ± 7	562 ± 6	<0.001
Lysis time (sec)	665 ± 12	803 ± 20	<0.001
Plasma protein levels			
Fibrinogen (mg/ml)	3.50 ± 0.03	3.77 ± 0.03	<0.01
PAI-1 (pg/ml)	797 ± 38	1081 ± 50	<0.01

Data are presented as mean ± SEM or absolute number (percentage). To control for false discovery rate, adjustments have been made for multiple testing. BP, Blood pressure; ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker.

women, age had no effect on clot structure parameters (Fig. 1A) or plasma levels of clotting factors.

Association of anthropometric, metabolic, and renal factors with clot structure parameters

Waist circumference (WCF)

Clot maximum absorbance was increased in the highest tertile of WCFs compared with the lowest in women, and lysis time was longer, which did not apply to men (Fig. 1B). Similar results were obtained for BMI (data not shown).

Glycated hemoglobin (HbA1c)

Glycemic control affected clot density in men only because maximum absorbance was increased in the highest tertile of HbA1c compared with the lowest (Fig. 1B).

High-density lipoprotein (HDL) cholesterol

The lowest HDL tertile in women showed longer clot formation time compared with highest tertile, with a

similar pattern observed for maximum absorbance and lysis time. HDL levels failed to correlate with clotting parameters in men (Fig. 1B).

Renal factors

Clot formation time was longer and maximum absorbance higher in the lowest estimated glomerular filtration rate (eGFR) tertile compared with the highest in both men and women (Fig. 1B).

Smoking has additional effect on clot structure parameters in T2DM

Maximum absorbance was higher in current smokers than nonsmokers in both men [0.37 ± 0.014 and 0.32 ± 0.009 arbitrary unit (au); $P = 0.008$] and women (0.41 ± 0.018 and 0.37 ± 0.009 au; $P = 0.04$). In men, time to full clot formation was longer in smokers than nonsmokers (572 ± 16 and 509 ± 11 sec; $P = 0.002$), whereas in

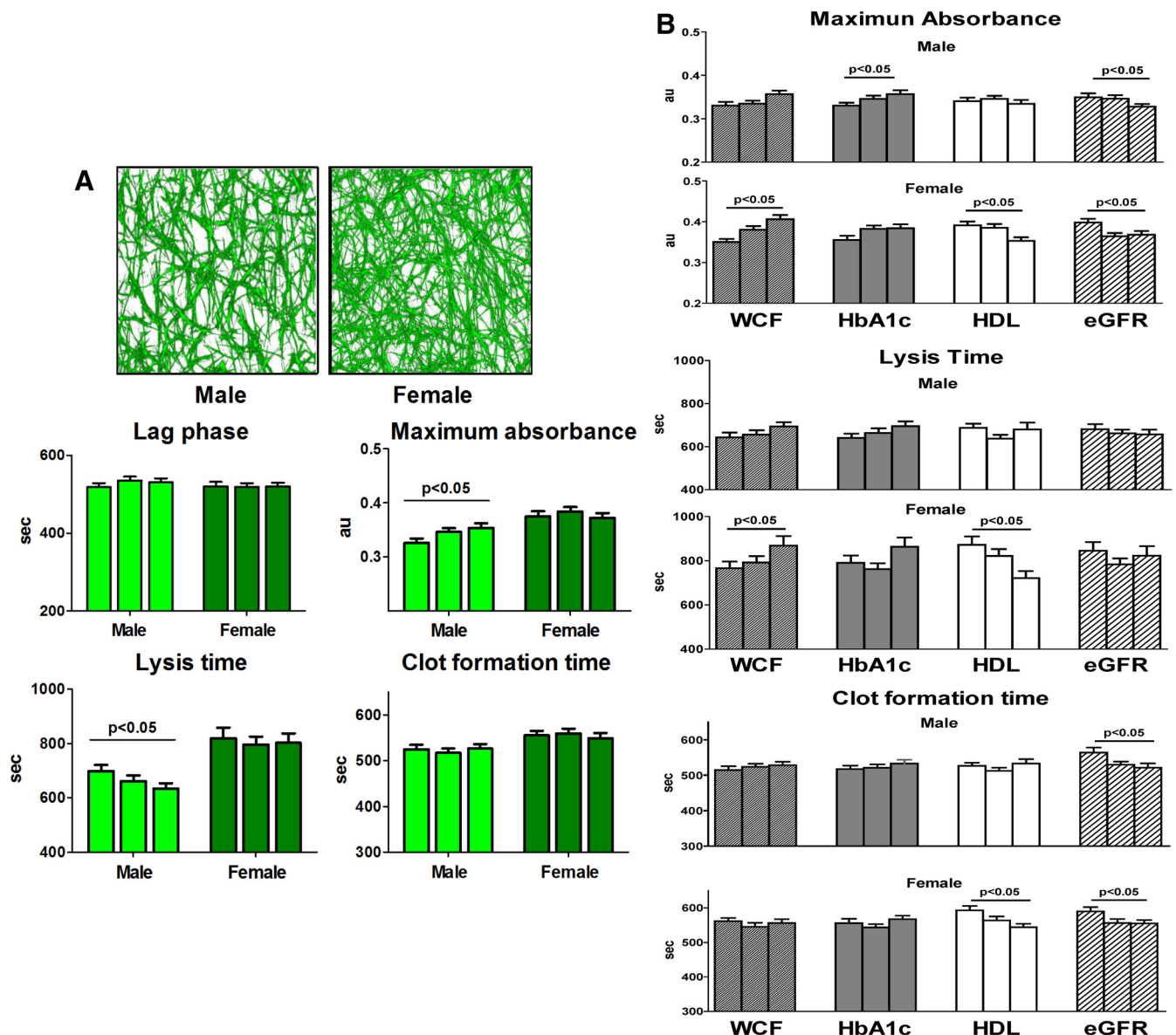


FIG. 1. The effects of gender, age, anthropometric, metabolic, and vascular parameters on clot structure fibrinolysis in T2DM. A, Upper panel represents confocal microscopy of clots made from pooled plasma samples of male and female subjects (n = 10 each). Lower panels show the effects of age, ranked in ascending tertiles, on clot structure parameters. B, Clot structure parameters in relation to WCF, HbA1c, HDL cholesterol, and eGFR, ranked in ascending tertiles.

women only a trend was observed (586 ± 20 and 551 ± 8 sec; $P = 0.06$).

Clot structure/fibrinolysis is altered in the presence of clinical and subclinical vascular disease

Ischemic heart disease

Men with IHD (n = 164) had higher clot maximum absorbance compared with those without (0.35 ± 0.008 and 0.33 ± 0.006 au; $P < 0.05$) and longer clot formation time (573 ± 15 and 522 ± 7 sec; $P < 0.05$), with a trend detected in women (n = 100; 575 ± 18 and 548 ± 6 sec; $P = 0.07$).

Ankle brachial index

Comparing the lowest and highest tertile of ABI, differences in clot structure parameters were found regard-

less of gender (Supplemental Fig. 1, published on The Endocrine Society’s Journals Online web site at <http://jcem.endojournals.org>).

Carotid IMT

IMT demonstrated no significant associations with clot structure parameters in men or women (Supplemental Fig. 1).

To assess the role of clot structure in IHD in the presence of traditional risk factors (history of smoking, total cholesterol, HDL, systolic and diastolic blood pressure, HbA1c, and age), multiple regression models were used, to include each clotting parameter and fibrinogen/PAI-1 levels. Clot formation time was associated with previous IHD

in men and women [odds ratio, 1.22 (95% confidence interval, 1.07, 1.38) and 1.33 (1.15, 1.50), for 100 sec change, respectively; $P < 0.01$ for both] (Supplemental Fig. 2).

Discussion

In addition to the large cohort, an advantage of this work is the dynamic analysis of clot formation/lysis, giving a global assessment of fibrin-related thrombosis risk, which studies investigating plasma levels/activity of coagulation proteins fail to provide (14). Several novel findings emerge from this work: 1) women with T2DM have a prothrombotic fibrin profile that exhibits gender-specific associations with anthropometric and metabolic factors; 2) individuals with a history of vascular ischemia or evidence of subclinical vascular disease have prothrombotic clots; and 3) impaired renal function or smoking is associated with additional adverse effects on clot structure and fibrinolysis in T2DM.

The denser fibrin clots and resistance to fibrinolysis in women, which is not fully explained by fibrinogen or PAI-1 levels, may contribute to the loss of cardiovascular protection in females with diabetes (15). Gender differences in clotting parameters extended to metabolic factors because raised WCF/BMI and low HDL were associated with prothrombotic clot phenotype in women, whereas hyperglycemia was associated with an adverse clot profile in men. Age also had a gender effect, with higher clot maximum absorbance in older men coupled with a paradoxical enhancement in fibrinolysis, which may be related to a trend toward lower PAI-1 levels, an observation that has been recently documented (16). Clinically, these data suggest that women with T2DM and increased WCF/BMI or low HDL and men with poor glycemic control are at higher atherothrombotic risk and may require more aggressive antithrombotic therapy. In contrast, enhanced fibrinolysis in older men with T2DM may be associated with increased bleeding risk, calling for careful assessment of antithrombotic therapy. This proposes individualized antithrombotic therapy in T2DM to maximize benefits and minimize bleeding risks.

IHD was associated with prothrombotic clots, supporting previous work in mixed diabetic and nondiabetic populations (12, 13). Clot formation time was an independent predictor of previous ischemic history in both men and women, with a 1% increase in this clotting parameter associated with a 1% increase in odds ratio for IHD, indicating that this measure may be an additional novel marker of cardiovascular disease. The association between vascular pathology and clot structure parameters

was further evidenced by the relationship between ABI and clot structure. This supports clinical studies demonstrating high risk of vascular occlusive events in individuals with peripheral artery disease (17). In contrast, IMT failed to show associations with clot structure parameters. The discrepancy between ABI and IMT is not unprecedented and can be explained by the relatively weak correlation between these vascular parameters, which can be influenced by the IMT measurement taken (18, 19). In addition to vascular factors, low eGFR and smoking were associated with deleterious effects on clot structure, supporting previous findings (11, 20) and suggesting additional prothrombotic effects for impaired renal function and smoking in diabetes.

Although these findings have potential clinical implications, there are limitations to be acknowledged. First, investigating plasma fibrin networks *ex vivo* ignores the potential influence of platelets, endothelial, or smooth muscle cells on clot properties. Second, the study was carried out at a single time point, and therefore cause-effect relationship is not proven and further longitudinal work is needed to assess the role of clot structure in predicting vascular events in T2DM, a study that is currently ongoing. Third, it is unclear whether demonstrated gender differences are specific for the population studied and whether healthy individuals display similar findings. However, the study aim was to comprehensively characterize clot structure parameters in T2DM, and recruiting a large control group devoid of clinical disease has practical limitations, given the age of the cohort studied.

In conclusion, females with T2DM display a prothrombotic fibrin profile compared with males, and gender differences are evident in relation to cardiometabolic markers. This suggests that fibrin-related thrombosis risk should be assessed separately for men and women with T2DM, which may translate clinically into gender-specific treatment strategies. Of the parameters studied, clot formation time may represent a novel cardiovascular risk predictor in T2DM, but prospective studies are required to test this marker. Our findings also indicate that peripheral arterial disease, renal impairment, and smoking all confer additional adverse thrombotic effects in T2DM.

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