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Impaired glucose metabolism and type 2 diabetes are associated with hypercoagulability: potential role of central adiposity and low-grade inflammation – The Hoorn Study ☆

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ABSTRACT

Introduction: Type 2 diabetes (DM2) is associated with greater risk for cardiovascular disease (CVD), which may, at least partially, be explained by prothrombotic alterations. We therefore investigated; first, the extent to which individuals with impaired glucose metabolism (IGM) and/or DM2 had greater levels of thrombin generation than those with normal glucose metabolism (NGM); and second, whether any differences were independent of other cardiovascular risk factors, such as smoking, hypertension, dyslipidaemia, (micro) albuminuria, glycemic control and (central) adiposity, and/or were potentially 'mediated' by low-grade inflammation (high-sensitivity C-reactive protein (hsCRP)).

Materials and methods: We studied 744 individuals from the Hoorn Study (275 NGM, 176 IGM and 293 DM2, mean age 68.6 ± 7.1 years). Thrombin generation in platelet-poor plasma was measured using the Calibrated Automated Thrombogram and three parameters were derived: lag time, peak height and endogenous thrombin potential (ETP). Data were analyzed with multiple linear regression analyses.

Results: After adjustment for age, sex, prior CVD and smoking status, individuals with IGM or DM2 had a longer lag time [ß = 0.14 min (95% CI: 0.02; 0.26)], higher peak height [ß = 7.29 nM (−1.33; 15.91)] and ETP [ß = 35.65nM*min (0.97; 70.34)] than those with NGM. These differences were attenuated to ß = 0.06 min (−0.07; 0.19), 3.82 nM (−5.46; 13.10) and 16.34 nM*min (−20.92; 53.59), respectively, when further adjusted for waist circumference and hsCRP.

Conclusion: Individuals with IGM or DM2 had up to 4% higher thrombin generation compared with NGM, which may be explained, to a great extent, by the greater levels of central adiposity and related low-grade inflammation characterizing these individuals.

Individuals with type 2 diabetes (DM2) have a 2- to 3-fold higher risk for cardiovascular disease (CVD) as compared with non-diabetic individuals [1]. A substantial portion of diabetes-related macrovascular complications is due to atherothrombotic events [2] which could, at least in part, be explained by an association between impaired glucose metabolism and thrombin generation. Indeed, thrombin plays a central role in the coagulation cascade [3,4] and elevated levels of thrombin-antithrombin (TAT) complexes and prothrombin 1.2 fragments (both *in vivo* markers of thrombin generation) have been reported in patients with DM2 [5–7].

Recently, several new methods have become available which quantitatively measure thrombin generation, *in vitro*, after activation of the coagulation cascade with tissue factor (TF), phospholipids and

Abbreviations: ANOVA, analysis of variance; BMI, body mass index; CAT, calibrated automated thrombogram; CV, coefficient of variation; CVD, cardiovascular disease; DBP, diastolic blood pressure; DM2, Diabetes Mellitus type 2; ETP, endogenous thrombin potential; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; IGM, impaired glucose metabolism; LDL, low-density lipoprotein; NGM, normal glucose metabolism; PAI-1, plasminogen activator inhibitor 1; PPP, platelet poor plasma; SBP, systolic blood pressure; SPSS, Statistical Package for Social Sciences; TAT, thrombin-antithrombin complexes; TF, tissue factor; TFPI, tissue factor pathway inhibitor.

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calciumchloride ($CaCl_2$) [8]. The Calibrated Automated Thrombogram (CAT) is one such method [9,10]. Specifically, it generates a thrombin generation curve that estimates the overall plasma coagulability potential. The endogenous thrombin potential (ETP), or the area under this curve, represents the total amount of active thrombin that can be formed after activation of the coagulation cascade. Measured as such, the ETP has been shown to be a good indicator of a hypercoagulable state in, for example, individuals using oral contraceptives, or who had prior venous thromboembolism [9,11–13]. Because the CAT method mimics the overall potential to clot whenever a thrombogenic stimulus (e.g. TF release after plaque rupture) appears, it is thought to provide a more reliable estimation of coagulation under pathophysiological conditions than the single point measurement of activation markers such as prothrombin 1.2 fragments or TAT complexes [14].

Only one small case-control study has investigated the association between DM2 and thrombin generation according to this method [15]. However, no studies have been performed at the population level. Establishing such an association is a necessary step to ascertain its potential value as a tool to estimate atherothrombotic risk in individuals with impaired glucose metabolism (IGM) and/or DM2. Furthermore, from an etiological point of view, it is not clear whether any such association is independent of other risk factors that typically characterize these individuals (e.g. hypertension and central adiposity) or, alternatively, is explained by these risk factors and their associated pathophysiological mechanisms. Central adiposity and related low-grade inflammation are likely candidates in this regard [16]. Indeed, individuals with IGM and DM2 have higher levels of high-sensitivity C-reactive protein (hsCRP) which are, to a great extent, attributable to their higher levels of (central) adiposity [17]. In addition, *in vivo*, hsCRP has been associated with increased thrombin generation (assessed by activation markers) [18].

In view of these considerations, we investigated, in a population-based cohort, first, whether individuals with IGM and/or DM2 had greater levels of thrombin generation in plasma than those with NGM; and second, whether any such differences were independent of other cardiovascular risk factors and/or explained by (adiposity-related) low-grade inflammation (hsCRP).

Materials and methods

Study population

For the present investigation, we used data from the 2000 Hoorn Study follow-up examination and, to increase the number of individuals with DM-2, data from The Hoorn Diabetes Screening Study, both of which were population-based and have been described in detail elsewhere [16,19,20]. Briefly, the Hoorn Study is a cohort study of glucose metabolism in the general population (n = 2484), which started in 1989 [20]. In 2000, a follow-up examination was carried out among all surviving participants who had given their permission to be recontacted. We invited all those who had diabetes, as determined by an oral glucose tolerance test, or who were treated for diabetes at the previous 1996 follow-up (n = 176). Next, we invited random samples of individuals with normal glucose metabolism (NGM) (n = 705) and IGM (n = 193). Of 1074 individuals thus invited, 648 (60%) participated [19]. Additionally, we invited 217 individuals with DM-2 from the Hoorn Screening Study [21], of whom 188 (87%) participated. All participants underwent a glucose tolerance test, except those with previously diagnosed diabetes (n = 67). Data on 14 individuals were missing because of logistical problems. Glucose tolerance was defined according to the 1999 WHO criteria [22]. Altogether, the eligible cohort thus consisted of 822 individuals. For the present study, participants using cumarin derivatives (n = 25) and/or hormonal replacement therapy (n = 3) and/or having missing data on thrombin generation parameters (n = 54) and/or in whom glucose metabolism status could not be ascertained (n = 14) were excluded. The present study therefore consisted of 744 individuals: 275 with normal glucose metabolism (NGM), 176 with IGM (including those with impaired fasting glucose and/or impaired glucose tolerance) and 293 with DM2.

The Ethical Review Committee of the VU University Medical Centre approved the study protocol and all participants gave their written informed consent.

Thrombin generation parameters

Venous blood samples were collected in 2000–2001 and 3.2% citrated platelet-poor plasmas (PPP) were prepared by two-step centrifugation: at 4000 ×*g* for 15 minutes followed by centrifugation at 10000 ×*g* for 5 minutes. Plasmas were stored at −80 °C until analysis in 2008 and had not been thawed before. Thrombin generation in PPP was measured using the CAT-method [10], which employs low-affinity fluorogenic substrate for thrombin (Z-Gly-Gly-Arg-AMC) to continuously monitor thrombin activity in clotting plasma. Thrombin generation was assessed according to manufacturer's instructions (Thrombinoscope BV) in a 96-well plate fluorometer (Ascent Reader, Thermolabsystems OY, Helsinki, Finland) as previously described [23].Briefly, thrombin generation was determined in the presence of 1 pM TF and 4 uM phospholipids in the absence and presence of 0.55 nM recombinant soluble thrombomodulin (Paion GmbH, Aachen, Germany). The 1 pM TF trigger was a commercial product (PPP Reagent Low; Thrombinoscope BV, Maastricht, the Netherlands). Three parameters were derived from these analyses, i.e. lag time (min), peak height (nM) and ETP (nM*min). A thrombomodulin ratio was calculated by dividing the ETP and peak height in the presence by those in the absence of thrombomodulin. This ratio indicates the percentage inhibition of thrombin generation by activation of the anti-coagulant protein C pathway. At our lab, the intra-assay coefficients of variation (CVs) are <5% [13] and the inter-assay CVs , obtained in 2 lots of normal-pool plasma in 25 independent runs during the present study, were <11% for all thrombin generation parameters (calculated using the EP Evaluator 8.0.0.90 software).

Other measurements

Health status, medical history, medication use and smoking habits were assessed by questionnaires [20]. We measured waist circumference, body mass index (BMI), systolic (SBP) and diastolic blood pressure (DBP), levels plasma glucose (fasting and post-load), HbA1c, total, high- (HDL) and low-density lipoprotein (LDL) cholesterol, triglycerides, urinary albumin-to-creatinine ratio and hsCRP as described in detail elsewhere [20,24,25]. Hypertension was defined as SBP ≥140 mmHg and/or DBP ≥90 mmHg and/or the use of anti-hypertensive drugs [26]. Participants were classified as having microalbuminuria if the albumin-to-creatinine ratio was ≥2.0 mg/mmol [24]. Prior CVD was defined as described previously [16].

Statistical analyses

Variables with a skewed distribution (i.e. triglycerides and hsCRP) were log-transformed prior to further analyses. Comparisons of the clinical characteristics according to individuals' glucose metabolism status were investigated by means of analyses of variance (ANOVA) for continuous variables and chi-squared tests for frequency data.

We used multiple linear regression analyses to investigate the differences in thrombin generation parameters between individuals with IGM or DM2 vs. NGM (reference group). These analyses were first adjusted for age, sex, prior CVD and smoking status (model 1), and additionally for measures of blood pressure, dyslipidemia, (micro)albuminuria, glycemic control, (central) adiposity and low-grade inflammation (models 2 to 7). In addition, we investigated

Table 1
Clinical characteristics according to glucose metabolism status.

	NGM (n = 275)	IGM (n = 176)	DM2 (n = 293)	*p* for linear trend
Age (years)	68.5 ± 6.0	70.3 ± 6.5	67.6 ± 8.0	0.099
Sex (% male)	47.3	50.6	50.9	0.397
Fasting glucose (mmol/l)	5.42 ± 0.37	6.08 ± 0.48	7.71 ± 1.80	<0.001
Glycated haemoglobin (%)	5.68 ± 0.41	5.88 ± 0.39	6.61 ± 0.92	<0.001
Body mass index (kg/m^2)	26.2 ± 3.3	27.8 ± 4.1	29.1 ± 4.6	<0.001
Waist circumference (mm)	90.7 ± 11.0	97.3 ± 10.8	101.1 ± 12.0	<0.001
Total cholesterol (mmol/l)	5.79 ± 1.03	5.79 ± 1.02	5.53 ± 1.06	0.003
LDL-cholesterol (mmol/l)	3.71 ± 0.91	3.68 ± 0.92	3.46 ± 0.92	0.001
HDL-cholesterol (mmol/l)	1.51 ± 0.41	1.44 ± 0.40	1.24 ± 0.34	<0.001
Total-to-HDL cholesterol ratio	4.05 ± 1.09	4.32 ± 1.39	4.71 ± 1.32	<0.001
Triglycerides (mmol/l)	1.20 [0.90-1.50]	1.30 [1.00-1.70]	1.60 [1.20-2.20]	<0.001
Lipid lowering drugs (%)	12.4	15.9	19.9	0.016
Systolic blood pressure (mmHg)	137 ± 20	143 ± 18	146 ± 21	<0.001
Diastolic blood pressure (mmHg)	81 ± 11	83 ± 10	85 ± 10	<0.001
Mean arterial pressure (mmHg)	95 ± 11	100 ± 10	102 ± 11	<0.001
Use of antihypertensives (%)	23.4	37.5	50.3	<0.001
Microalbuminuria (%)	9.8	15.9	18.8	0.003
Smoking (%)	13.9	17.6	12.1	0.525
Prior CVD (%)	45.1	50.0	53.2	0.053
Use of Platelet aggregation inhibitors (%)	13.2	16.5	19.8	0.038
High-sensitivity C-reactive protein (mg/l)	1.52 [0.80; 3.14]	2.19 [1.29; 4.61]	3.14 [1.42; 7.00]	<0.001

Results are expressed as percentages (%), mean ± SD, or median [inter-quartile range].
NGM: normal glucose metabolism; IGM: impaired glucose metabolism; DM2: type 2 diabetes mellitus; LDL: low density lipoprotein; HDL: high density lipoprotein; CVD: cardiovascular disease.

whether the attenuation by low-grade inflammation was adiposity-driven by adding adjustments for both waist circumference and hsCRP (model 8). In all these analyses individuals with IGM and DM2 were combined into one group, since the parameters of thrombin generation examined herein did not substantially differ between these two groups. In all analyses described above, dependent variables were the thrombin generation parameters obtained in both the absence and presence of thrombomodulin.

Statistical significance was set at a *p*-value of <0.05. All analyses were performed with the use of the Statistical Package for Social Sciences (SPSS) for Windows, version 15.0 (SPSS Inc., Chicago, IL, USA).

Results

Participants who were excluded from the analyses (n = 78) were younger, more often obese and more often had diabetes than the ones included (data not shown). The clinical characteristics of the participants included (n = 744) are shown in Table 1 according to their glucose metabolism status. A deteriorating glucose metabolism status was associated with a larger waist circumference and higher levels of dyslipidemia, blood pressure, microalbuminuria, hsCRP and prior CVD. In addition, participants with IGM and DM2 more often used lipid-lowering drugs and platelet aggregation inhibitors those with NGM.

Levels of lag time, peak height and ETP assessed in the absence of thrombomodulin were comparable in individuals with IGM and DM2 and greater in these individuals than in those with NGM (Table 2, Fig. 1). Activating the anticoagulant protein C pathway by adding thrombomodulin did not affect the absolute values of lag time but led to lower absolute values of peak height and ETP, which were reduced by approximately 14 and 35%, respectively, in all groups (Table 2). Despite the relatively higher values in individuals with IGM and DM2 under these conditions, peak height and ETP did not differ significantly from those of individuals with NGM, however lag time remained significantly prolonged. The thrombomodulin ratio of peak height and ETP did not materially differ between the 3 groups (Table 2).

Associations between glucose metabolism status and thrombin generation parameters

After adjustment for age, sex, prior CVD and smoking status, individuals with IGM or DM2 had a significantly prolonged lag time, a

Table 2
Thrombin generation parameters according to glucose metabolism status.

	NGM (n = 275)	IGM (n = 176)	DM2 (n = 293)	p-value (IGM + DM2 vs. NGM)
Analyses without thrombomodulin				
Lag time (min)	3.98 ± 0.83	4.10 ± 0.80	4.14 ± 0.77	0.021
Peak Height (nM)	182 ± 57	191 ± 58	189 ± 59	0.067
ETP (nM*min)	1166 ± 222	1218 ± 237	1192 ± 238	0.034
Analyses with thrombomodulin				
Lag time (min)	3.91 ± 0.68	4.06 ± 0.71	4.06 ± 0.76	0.005
Peak height (nM)	159 ± 60	166 ± 60	167 ± 65	0.104
ETP (nM*min)	776 ± 253	810 ± 256	807 ± 261	0.106
Thrombomodulin ratio				
TM ratio Peak height n = 737	0.86 ± 0.11	0.86 ± 0.10	0.86 ± 0.14	0.867
TM ratio ETP n = 737	0.65 ± 0.14	0.65 ± 0.14	0.66 ± 0.15	0.559

Results are expressed as mean ± SD.
NGM: normal glucose metabolism; IGM: impaired glucose metabolism; DM2: type 2 diabetes mellitus; ETP endogenous thrombin potential; TM: thrombomodulin.

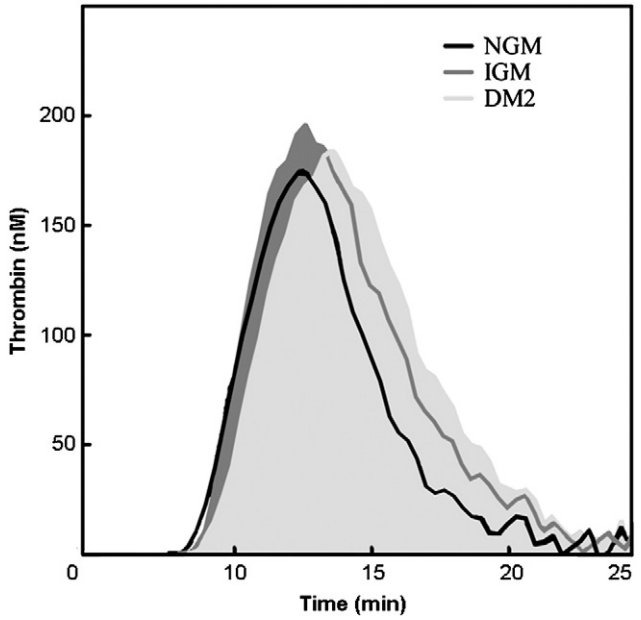

Fig. 1. Thrombin generation curves according to glucose metabolism status i.e. Normal glucose metabolism (NGM), impaired glucose metabolism (IGM) or type 2 diabetes mellitus (DM2).

higher peak height and larger ETP as compared with NGM (Table 3, model 1). In relative terms, these differences were small and equivalent to approximately a 4% longer lag time, a 4% higher peak height and a 3% higher ETP in individuals with IGM or DM as compared with those with NGM. In addition, the differences in peak height and ETP were attenuated to a great extent when further adjusted for waist circumference (model 6) and hsCRP (model 7), whereas adjustments for the other risk factors considered did not materially affect these results (models 2 to 5). When differences between groups were adjusted for both waist circumference and hsCRP (model 8), results were similar to those obtained in model 7, thus suggesting that any 'mediation' by low-grade inflammation was adiposity-driven. In contrast to the above, the differences in lag time were mainly attenuated by dyslipidaemia.

Similar findings were obtained when analyses compared the parameters of thrombin generation obtained in the presence of recombinant thrombomodulin (second part Table 3).

Additional analyses

Additional adjustment for use of platelet aggregation inhibitors, or lipid- , blood pressure- or glucose-lowering drugs did not materially affect any of the reported associations (data not shown).

We also investigated whether the association between glucose metabolism status and thrombin generation parameters differed between men and women or between different age categories, by adding interaction terms to our models. However, no such interactions were found (p-values for interaction all >0.10).

Discussion

The main findings of this study were two-fold: first, individuals with IGM and DM2 had comparable levels and both had higher levels of thrombin generation than those with NGM, but the magnitude of these differences was relatively small (up to 4%). Second, central adiposity and related low-grade inflammation explained to a great extent the association between glucose tolerance status and thrombin generation, i.e., peak height and ETP.

The modest elevations in thrombin generation parameters suggest that thrombin generation in PPP only slightly contributes to the hypercoagulable state in IGM and DM2. This observation is in line with a recently published case-control study in DM2 patients that also reported minimal differences between DM2 patients compared with healthy controls [15]. Alterations in primary hemostasis and/or the fibrinolytic system may thus be more important in establishing the hypercoagulable state in IGM and/or DM2 than those reflected by the thrombin generation assay on which we focused herein. For example, DM2 is associated with increased platelet activation and aggregation and von Willebrand factor levels [27,28] and plasminogen activator inhibitor (PAI)-1 levels are elevated in DM2, probably due to an increased hepatic synthesis [29]. PAI-1 inhibits fibrinolysis, which is associated with more resistant clot formation and possibly plays a role in vascular complications such as atherothrombosis.

The thrombin generation parameters were elevated to a similar extent in individuals with IGM and DM2, which is in line with a

Table 3
Thrombin generation parameters according to glucose metabolism status.

		Lag time (min)		Peak height (nM)		ETP (nM*min)	
Model	Adjustments	β	95%CI	β	95%CI	β	95%CI
Without thrombomodulin							
0	Crude	0.14*	0.02; 0.26	7.98	−0.63; 16.58	36.75*	2.17; 71.34
1	Age, sex, prior CVD, smoking	0.14*	0.02; 0.26	7.29	−1.33; 15.91	35.65*	0.97; 70.34
2	Model 1 + SBP	0.13*	0.01; 0.25	6.42	−2.34; 15.18	32.80	−2.46; 68.07
3	Model 1 + total cholesterol/ HDL ratio and ln-triglycerides	0.04	−0.08; 0.16	8.16	−0.79; 17.11	34.29	−1.88; 70.47
4	Model 1 + microalbuminuria	0.12*	0.01; 0.24	7.10	−1.57; 15.76	34.31	−0.56; 69.17
5	Model 1 + HbA1c	0.07	−0.06; 0.20	6.95	−2.49; 16.39	41.54*	3.56; 79.52
6	Model 1 + waist circumference	0.09	−0.04; 0.22	5.48	−3.76; 14.71	23.85	−13.26; 60.97
7	Model 1 + ln-hsCRP	0.09	−0.03; 0.21	4.57	−4.22; 13.36	22.90	−12.40; 58.19
8	Model 1 + waist circumference and ln-hsCRP	0.06	−0.07; 0.19	3.82	−5.46; 13.10	16.34	−20.92; 53.59
With thrombomodulin							
0	Crude	0.15†	0.04; 0.26	7.54	−1.66; 16.74	31.21	−6.90; 69.33
1	Age, sex, prior CVD, smoking	0.15†	0.04; 0.25	6.80	−2.41; 16.02	27.89	−10.28; 66.03
2	Model 1 + SBP	0.13*	0.03; 0.24	6.18	−3.19; 15.55	26.39	−12.42; 65.21
3	Model 1 + total cholesterol/ HDL ratio and ln-triglycerides	0.07	−0.04; 0.18	7.95	−1.62; 17.53	34.87	−4.86; 74.60
4	Model 1 + microalbuminuria	0.13*	0.02; 0.23	6.78	−2.48; 16.04	27.42	−10.95; 65.79
5	Model 1 + HbA1c	0.08	−0.03; 0.20	6.60	−3.49; 16.69	32.42	−9.37; 74.21
6	Model 1 + waist circumference	0.09	−0.03; 0.20	5.15	−4.72; 15.02	22.07	−18.83; 62.97
7	Model 1 + ln-hsCRP	0.09	−0.02; 0.20	4.26	−5.15; 13.66	17.15	−21.80; 56.10
8	Model 1 + waist circumference and ln-hsCRP	0.06	−0.06; 0.17	3.60	−6.34; 13.53	15.43	−25.71; 56.56

Regression coefficient (β) with respective 95%CI indicates the difference in thrombin generation parameter (in respective units) in IGM/DM2 vs. NGM. *$p<0.05$, †$p<0.01$.

previous study [30]. These findings support the 'ticking clock hypothesis' suggesting that hemostatic disturbances may be present even before fully developed diabetes [31].

The prolonged lag time in IGM and DM2 may be explained by elevated tissue factor pathway inhibitor (TFPI) levels, which is one of the main determinants of lag time at the low TF concentrations used in our study [23]. In addition, previous studies have shown a positive association between glucose metabolism status and TFPI [30,32]. In the present study, the association between glucose metabolism status and lag time was partially attenuated by dyslipidemia, which is in line with previous studies that have shown a positive association between dyslipidemia and TFPI [33,34].

The associations between glucose metabolism status and peak height and ETP were to a great extent attenuated by waist circumference, suggesting that central adiposity may be a common antecedent of both IGM/DM2 and increased thrombin generation. We have previously shown in this cohort that individuals with a central pattern of fat distribution indeed had higher levels of thrombin generation in plasma, which were to a great extent explained by adiposity-related increases in hsCRP [16]. Furthermore, a recent meta-analysis supports these findings by suggesting that the elevated hsCRP levels in DM2 are probably due to central adiposity [17]. One proposed mechanism by which hsCRP, or low-grade inflammation in general, may lead to increased thrombin generation may be by inducing endothelial dysfunction which in turn leads to increased TF expression by monocytes [35], a phenomenon which is only observed in the presence of and through direct interaction with other leukocytes [36]. Taken together, our findings thus suggest that adiposity-related low-grade inflammation and not hyperglycemia *per se* might be one of the main driving factors behind the association between glucose metabolism status and thrombin generation in PPP. In addition, previous studies have not found an association between glycemic control and prothrombin 1.2 fragments and/or TAT-complexes [37,38]. However, these studies did find an association between glycemic control and thrombin generation in platelet-rich plasma, suggesting that associations between glycemic control and thrombin generation might mainly be a platelet-dependent phenomenon [39].

There are some limitations to our study that need to be mentioned. First, due to its cross-sectional design, any inferences about causality should be made with caution. Second, as we studied a relatively elderly population, we cannot fully exclude the possibility that selective mortality in the diabetes group (i.e. many 'healthy' survivors included in this study) may have explained their comparable (instead of higher) levels of thrombin generation parameters as compared with the individuals with IGM. Third, we studied a Caucasian, elderly population and it remains to be established whether these results can be generalized to other ethnicities and/or to younger individuals. Fourth, measures of thrombin generation were performed in plasma samples that were stored for 7 to 8 years. Although in our lab we have been able to rule out effects of approximately one year of storage time on ex vivo thrombin generation, long term effects, if any, are largely unknown. If long term storage introduced a systematic error in measures of thrombin generation, we can rule out its influence on the differences found between the groups examined in the present study, since storage time was similar for all plasma aliquots. If random errors were introduced, or even if storage time reduced the variation of the measures as captured by our assay, then the differences between groups reported herein were most likely underestimated. Finally, it should be noted that, in the present cohort, it is unknown which individuals carried the factor V Leiden mutation. In general, these individuals will have a higher thrombin generation due to activated protein C resistance and an association between DM2 and the factor V Leiden mutation has been previously reported [40]. However, several other studies, including a Dutch one, did not find any association between DM2 and factor V Leiden mutation [41–43]. So far, no studies have investigated the association between DM2 and acquired activated protein C resistance. We have shown herein that activating the protein C pathway by adding thrombomodulin did not materially change the strength of the associations between glucose metabolism status and peak height or ETP, which indicates that there were no abnormalities in protein C activation in individuals with IGM or DM2.

In conclusion, we have shown that IGM and DM2 are associated with higher thrombin generation in PPP, which can be explained, to a large extent, by their greater levels of central adiposity and related low-grade inflammation. It remains to be established whether the assessment of thrombin generation in PPP constitutes a useful method for the characterization of hypercoagulability, relevant to arterial and venous thrombosis, in these individuals, since the magnitude of the differences was relatively small.

Conflict of interest statement

None.

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