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ORIGINAL RESEARCH

Association of Global Coagulation Profiles With Cardiovascular Risk Factors and Atherosclerosis: A Sex Disaggregated Analysis From the BioHEART-CT Study

Katharine A. Kott, BSc, MBBS; Marie-Christine Morel-Kopp, PhD; Stephen T. Vernon, MBBS; Yuki Takagi, PhD; Belinda A. Di Bartolo, PhD; Karlheinz Peter, MD, PhD; Jean Y. Yang, PhD; Stuart M. Grieve, MBBS, DPhil (Oxon); Christopher Ward, MBBS, PhD; Gemma A. Figtree, MBBS, DPhil (Oxon)

BACKGROUND: Although the association between dysregulated coagulation and atherosclerosis is well recognized, individual assays have been of minimal value in understanding disease susceptibility. Here we investigated the association of global coagulation profiles with coronary artery disease with consideration of sex differences.

METHODS AND RESULTS: The study included patients from the BioHEART-CT (The BioHEART Study: Assessing Patients With Suspected Cardiovascular Disease for New Disease Markers and Risk Factors) biobank who had computed tomography coronary angiograms scored for coronary artery calcium score (CACS) and Gensini score. The cohort included 206 adult patients who were referred for clinically indicated computed tomography coronary angiography and had a median of 2 major cardiac risk factors; 50% were women and the average age was 62.6 years (±9.9 years). The overall hemostatic potential (OHP) and calibrated automated thrombography generation assays were performed on platelet-poor plasma. CACS and Gensini score in men were significantly correlated in bivariate analysis with measures from the OHP assay, and regression models predicting disease severity by CACS or Gensini score were improved by adding the OHP assay variables in men but not in women. The calibrated automated thrombography generation assay demonstrated a more hypercoagulable profile in women than in men. The OHP assay showed hypercoagulable profiles in women with hyperlipidemia and men with obesity.

CONCLUSIONS: The OHP assay identified hypercoagulable profiles associated with different risk factors for each sex and was associated with CACS and Gensini score severity in men, emphasizing the associations between increased fibrin generation and reduced fibrinolysis with cardiac risk factors and early atherosclerosis.

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Key Words: atherosclerosis ■ hypercoagulability ■ risk factors ■ sex

Coronary artery disease (CAD) remains the largest global cause of years of life lost in adults,[1] impacting both men and women, persisting as a challenge even in countries with top-tier medical systems and comprehensive primary and secondary prevention strategies.[2] This persistence of cardiovascular mortality despite aggressive management of known risk factors has prompted a search for novel disease

Corresponding to: Gemma Figtree, MBBS, DPhil (Oxon), Level 12, Cardiothoracic and Vascular Health, Kolling Institute of Medical Research, 10 Westbourne Street, St Leonards, 2065, Australia. E-mail: gemma.figtree@sydney.edu.au

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CLINICAL PERSPECTIVE

What is New?

- Global coagulation profiles were determined for >200 patients from the BioHEART-CT (The BioHEART Study: Assessing Patients With Suspected Cardiovascular Disease for New Disease Markers and Risk Factors) cohort using the calibrated automated thrombography assay to assess thrombin generation and the overall hemostatic potential (OHP) assay to assess fibrin generation. Sex-segregated analysis demonstrated baseline hypercoagulable profiles in the calibrated automated thrombography assay in women but no differences in the OHP assay.
- Hypercoagulable OHP profiles were associated with obesity in men and hyperlipidemia in women, and OHP measures were found to correlate with coronary artery disease severity scores in men but not women. The addition of the OHP assay to basic risk models improved the ability of the model to improve prediction of disease severity in men but not in women.

What are the Clinical Implications?

- These results highlight the importance of further work in the study of sex-specific biomarkers and risk factors for coronary artery disease, searching for underlying mechanisms and identifying the potential prognostic significance to better inform sex-specific risk scoring in the future.

Nonstandard Abbreviations and Acronyms

BioHEART-CT	The BioHEART Study: Assessing Patients With Suspected Cardiovascular Disease for New Disease Markers and Risk Factors
CACS	coronary artery calcium score
CAT	calibrated automated thrombography
CTCA	computed tomography coronary angiography
ETP	endogenous thrombin potential
OCP	overall coagulation potential
OD	optical density
OFP	overall fibrinolysis potential
OHP	overall hemostatic potential
PPP	platelet-poor plasma
SPS	soft plaque score

mechanisms that could be contributing to individual disease susceptibility––mechanisms potentially untreated by current approaches.

Dysregulated coagulation is thought to have a significant relationship with atherosclerosis, as vascular inflammation triggers activation of platelets and coagulation factors, and intraplaque microhemorrhages with subsequent healing have been shown to contribute to plaque progression.[3] Studies of apolipoprotein E knockout atherosclerosis-prone mice have demonstrated this relationship experimentally. When the apolipoprotein E knockout is combined with a phenotype that has a 50% reduction in prothrombin expression, substantial decreases in plaque formation are observed.[4] In addition, when the apolipoprotein E background is combined with a hypercoagulable phenotype caused by a thrombomodulin mutation, severe atherosclerosis develops.[4] While this role of coagulation is well recognized, we are lacking sensitive markers to reflect this relationship. Markers that specifically represent the biological link between coagulation and atherosclerosis formation could be applied to the identification of individual disease risk, allowing for improved precision approaches to prevention.

Studies of coagulation profiles in patients with CAD have shown that some specific measures of hemostatic factors such as fibrinogen and D-dimer have significant associations with cardiac events, as do some of the common hemostatic gene polymorphisms; however, the effect sizes have been small and the accuracy of clinical risk prediction has been limited.[5] Unfortunately, routinely available measures of coagulation such as the activated partial thromboplastin time and international normalized ratio do not reflect many of these atherosclerosis-associated factor abnormalities. Tests that instead incorporate the cumulative effects of these factors and polymorphisms while assessing the more physiological processes of thrombin and fibrin generation may be more suitable for integrated CAD risk prediction.

Rather than looking at individual factor levels and enzyme activities, the overall hemostatic potential (OHP) assay is a global test of hemostatic function, which, although it is not an assay validated for routine clinical use, has been applied in multiple clinical situations with previous studies identifying differences in patients with mild or severe hemophilia A,[6] in patients with antiphospholipid antibody syndrome,[7] in long-term survivors of acute pulmonary embolism,[8] in patients following orthopedic trauma,[9] in patients with schizophrenia taking antipsychotic therapy,[10] in patients with severe CAD awaiting bypass surgery,[11] and in patients with symptomatic CAD.[12,13] The test can be easily performed on platelet-poor plasma (PPP) by measuring the optical density of cross-linked fibrin over time, producing curves that demonstrate fibrin

generation triggered by thrombin, and fibrinolysis as triggered by tissue plasminogen activator.[14] These data are complemented by the calibrated automated thrombography (CAT) assay,[15] which reports measures from a thrombin generation curve. The CAT assay has been more extensively studied in the CAD context,[16–18] but again is not validated for routine clinical use. Both assays can be performed on appropriately stored frozen samples.

To assess whether these global assays could detect important alterations in coagulation profiles relevant to atherosclerosis, we utilized the large and well-characterized BioHEART-CT (The BioHEART Study: Assessing Patients With Suspected Cardiovascular Disease for New Disease Markers and Risk Factors) cohort and accompanying biobank.[19] This biobank employs advanced computed tomography coronary angiography (CTCA) imaging analysis to patients with suspected CAD, providing the opportunity to associate novel coagulation measures with atherosclerotic burden and plaque characteristics. The cohort also has the benefit of using these gold-standard techniques to identify patients who have no visible atherosclerosis on CTCA, minimizing "contamination" of the healthy control group. Additionally, the size of the cohort allowed us to add to the existing knowledge base describing how clinical and demographic factors relate to OHP and CAT coagulation profiles. This is particularly important with respect to sex, which has been shown to influence global coagulation assays[20,21] and to play a key role in the biology and outcomes of CAD.[22–24]

In this study we examined the primary hypothesis that global coagulation profiles as measured by CAT and OHP assays were associated with the presence and severity of CAD, in a sex-dependent manner. Secondary exploratory analyses were performed to determine the association of hypercoagulable profiles with cardiovascular risk factors.

METHODS

The data that support this study are available from the corresponding author upon reasonable request.

Study Population

The protocol for the BioHEART-CT biobank (Australia New Zealand Clinical Trials Registry ANZTR12618001322224) has been previously described in detail[19] and recruited patients presenting for clinically indicated CTCA from 2015 onwards at multiple sites in Sydney, Australia. The study was approved by the Northern Sydney Local Health District Human Research Ethics Committee (HREC/17/HAWKE/343), and study participants provided informed written consent. Patients were referred from a variety of sources, including emergency department presentations and referrals from general practice and cardiologist departments. Patients were included if they could provide informed consent and were aged ≥18 years. Patients who were unwilling or unable to participate in follow-up were excluded. Patient data were collected at the time of recruitment, including demographic information, smoking history, medical history, medication history and family history of premature CAD. Samples were excluded if they appeared hemolyzed or the clinical history indicated an anticoagulant medication was being taken. The OHP and CAT assays are performed on PPP, thus antiplatelet medications have been previously shown not to impact the OHP or CAT assay results,[12] and patients taking these were included. Current smoking was defined as having regularly smoked within the past 12 months, and significant smoking history was defined as a pack-year history of ≥10. A family history of ischemic heart disease was considered significant if it was in a first-degree relative aged <60 years. In addition to significant smoking history, the standard modifiable cardiovascular risk factors were self-reported as a history of being diagnosed with hypertension, hyperlipidemia, or diabetes mellitus. Additional biochemical data such as fasting lipid profiles and glycated hemoglobin measurements were not available.

A sample size of ≈50 patients with CAD has been previously used to show highly significant differences in the OHP assay,[12] and smaller sample sizes of 20 to 30 have been used in other clinical conditions.[25,26] To improve the power of subgroup analyses, a sample size of 100 was chosen. Because of baseline sex differences in coagulation profiles previously seen in global coagulation assays,[20,21] a patient cohort with an equal sex distribution was chosen for this study, bringing the total to 200. The total study cohort comprised an initial sex-balanced pilot study, which selected for extreme phenotypes (n=79), and was expanded to a total of 206 patients (103 men, 103 women) with some enrichment for current smokers and patients with diabetes mellitus as outlined in Figure S1.

Imaging Analysis

CTCA images were acquired on a 256-slice scanner using standard clinical protocols, with reconstructions created using vendor-specific software. Scans were overseen and dual-reported by an accredited cardiologist and radiologist. Heart rate optimization was achieved using oral metoprolol or ivabradine as clinically appropriate, with doses adjusted based on patient body weight and blood pressure. If heart rate was sufficiently controlled, the study was performed prospectively, otherwise retrospective acquisition was employed. Vasodilation with sublingual nitroglycerine

(600–800 μg) was given immediately before intravenous contrast. Radiation doses were minimized as per current recommendations.[27] CTCAs were scored to assess plaque composition as well as stenosis, as outlined in Figure 1A. Coronary artery calcium score (CACS) was generated using standard scanner-associated

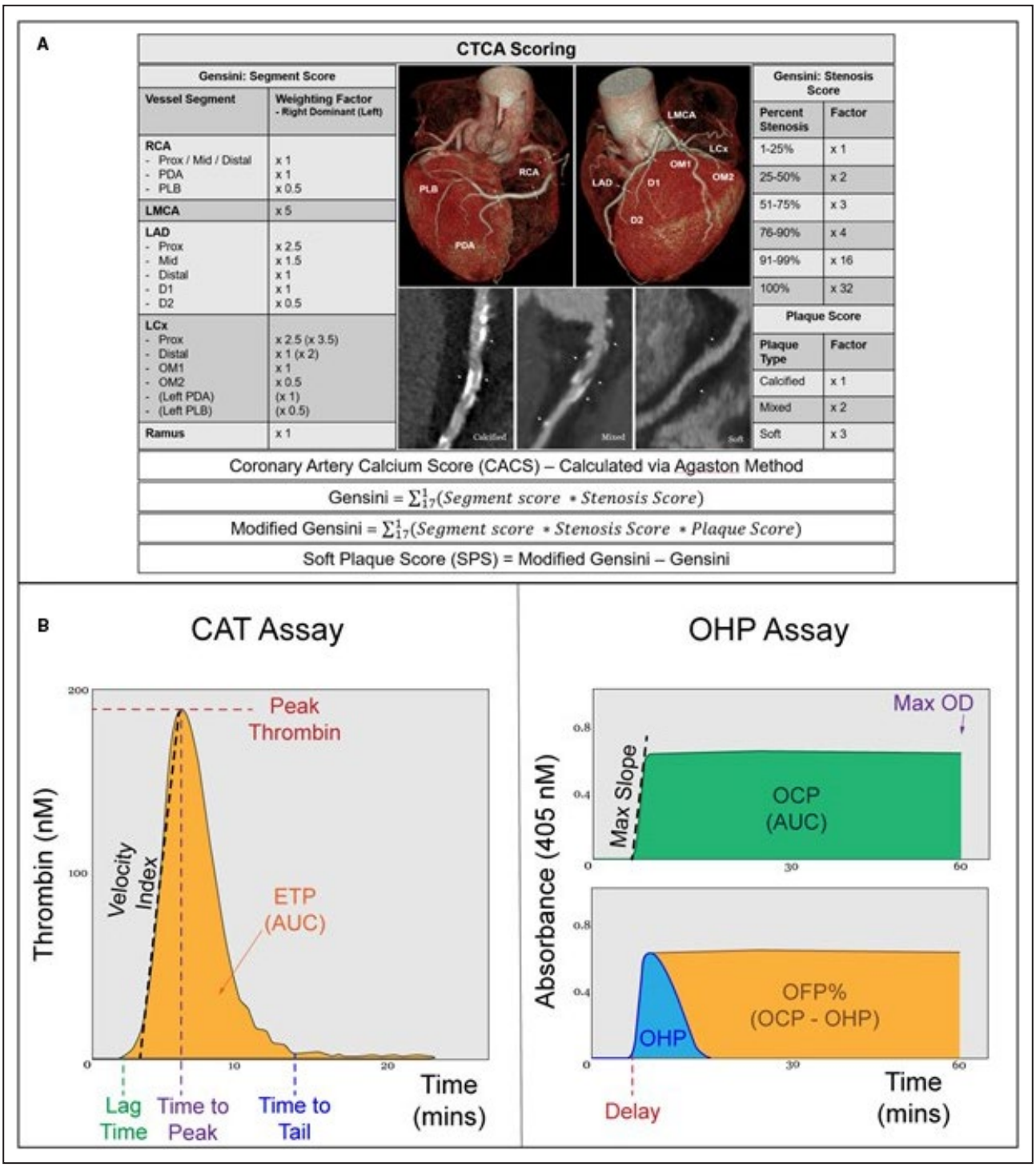

Figure 1. Imaging analysis and hematological assay curves.
A, Scoring systems used in BioHEART-CT (The BioHEART Study: Assessing Patients With Suspected Cardiovascular Disease for New Disease Markers and Risk Factors) for semiquantitative plaque analysis. **B**, Thrombin generation curve from the calibrated automated thrombography (CAT) assay showing derived variables. **C**, Fibrin generation (top panel) and generation and lysis (bottom panel) curves from the overall haemostatic potential (OHP) assay showing derived variables. AUC indicates area under the curve; ETP, endogenous thrombin potential; OCP, overall coagulation potential; OD, optical density; and OFP, overall fibrinolysis potential.

software via the Agatston method[28] for each CTCA. Further analysis of each coronary tree was performed using the standard 17-segment model[29] of coronary artery anatomy, with stenosis per segment graded according to the Gensini scoring system.[30] A modified Gensini score was then calculated by incorporating a multiplier for the composition of the plaque in each segment: calcified plaque=1, mixed plaque=2, and noncalcified (soft) plaque=3. A soft plaque score (SPS) was generated by subtracting the modified Gensini score from the Gensini score.

Biological Samples and Analysis

Peripheral venous blood samples were collected following insertion of the intravenous cannula for the CTCA, transferred into sodium citrate pathology tubes, and then transported to the pathology department on ice. The tubes were centrifuged for 15 minutes at 4 °C, then the plasma supernatant was transferred to a fresh tube and centrifuged under the same conditions for a second time to create PPP. Samples were aliquoted and stored at −80 °C until analysis.

CAT Assay

Frozen samples were thawed at 37 °C and thrombin generation was measured using the fluorometric CAT method[31] as previously described.[13] Briefly, 80 µL of PPP was added to round-bottom 96-well plates (Immulon 2HB, Thermofisher) containing 20 µL of calibrator or PPP reagent (Stago). Thrombin generation was initiated by the addition of 20 µL of $CaCl_2$ and fluorogenic thrombin substrate (Z-Gly-Gly-Arg-AMC, Merck). Fluorescence was continuously measured over 60 minutes on a Fluoroskan Ascent plate reader (Thermofisher). All samples were run in triplicate with curves and results automatically generated by Thrombinoscope software (Stago). Variables derived from the thrombin generation curve include the time-to-curve onset (lag time), initial slope and overall ascending slope of the curve (velocity index), peak thrombin absorbance (peak), time taken to reach the peak (time to peak), area under the thrombin curve (endogenous thrombin potential [ETP]), and the time taken to reach the thrombin curve tail (time to tail) (Figure 1B). α_2-Macroglobulin levels were also calculated.

OHP Assay

Frozen samples were thawed at 37 °C and the fibrin generation and lysis assay was immediately performed as previously described.[25] Briefly, 75 µL of PPP and 75 µL of Tris buffer (Tris 66 mM, NaCl 130 mM, $CaCl_2$ 33 mM; pH 7.0) were added to the wells of a 96 microtiter plate (Falcon flat bottom 353195) and kinetic absorbance was measured at 390 nm over 60 minutes using a Powerwave XS Biotek microplate reader (Biotek). Each test was performed in duplicate. For the fibrin generation assay, 0.06 IU/mL of thrombin (Dade-Behring) was added to the buffer, and 0.06 IU/mL of thrombin and 300 ng/mL of recombinant tissue plasminogen activator (alteplase, Boehringer Ingelheim International) was added for the fibrin lysis assay. The overall coagulation potential (OCP) represents the area under the fibrin generation curve, and the overall OHP represents the area under the fibrin generation and lysis curve. The overall fibrinolysis potential (OFP) represents the difference between the OCP and OHP and is expressed as a percentage of the OCP. The OFP45 removes the impact of lag time differences and is calculated over 45 minutes from the start of the fibrin generation curve. Additional data derived from the fibrin time curve include maximum optical density, maximum slope, and delay in the onset of fibrin generation (Figure 1C).

Statistical Analysis

Data are presented as frequencies and percentages for categorical variables, means and SDs for normally distributed continuous variables, and medians with interquartile ranges for non-normally distributed continuous variables. Subgroup analysis of baseline CAT and OHP assay results between the sexes was performed using unpaired *t* tests for continuous variables and Fisher exact tests for categorical variables. A 2-tailed *P* value <0.05 was considered statistically significant. ANCOVA was used to assess for the impact of age on the baseline CAT and OHP results.

The associations between the assay variables and CAD were assessed using a 2-part model to avoid transformation of the CAD scores that would limit the interpretability of the results. First the incidence of disease was assessed using a logistic regression model. The dependent variable was presence of CAD as defined by a Gensini score >0. Second, the relationship with disease severity was assessed using linear regression of the nonzero disease score. The models were adjusted for age, body mass index, hypertension, diabetes mellitus, hyperlipidemia, and significant smoking history. Next, to assess for independent associations between the coagulation assays and CAD measures, bivariate correlations between adjusted unstandardized residual disease scores and the coagulation assay variables were performed. Bivariate correlations of continuous variables are presented as Pearson coefficients with associated 2-tailed *P* values.

To demonstrate the potential additional contribution of the OHP and CAT assays to the predictive value of the standard cardiac risk factors for each sex, 4 linear regression models were performed. Model 1 incorporates age, body mass index, hypertension, hyperlipidemia,

diabetes mellitus, current or significant smoking history, and family history of ischemic heart disease. Model 2 uses the same risk factors and adds in all of the CAT assay variables. Model 3 includes all standard risk factors and all OHP assay variables. Model 4 incorporates all risk factors, CAT assay variables, and OHP assay variables. The R^2, adjusted R^2, F value, and P value were calculated for each model, and the P value for model change was calculated for models 2 to 4 with comparison to model 1. The 4 models were reproduced for each disease score and each sex.

The association of CAT and OHP measures with cardiac risk factors was examined in men and women using unpaired t tests for continuous variables and Fisher exact tests for categorical variables. A 2-tailed P value <0.05 was considered statistically significant. Cohen d was calculated to demonstrate the magnitude of the difference of means between patients with and without each risk factor for each sex.

All data were analyzed in IBM SPSS Statistics, version 26, release 26.0.0.0.

RESULTS

Cohort Demographics and Clinical Characteristics

The clinical characteristics, demographics, and disease burden of the 206 patients are shown in Table 1. Previous studies have found that women have more prothrombotic parameters in the CAT assay, independent of hormonal status.[21] Therefore, in our study, we first evaluated the sexes separately to guide further analysis.

In our study population, women were slightly older than men, with an average age of 64.1 years compared with 61.1 years (P=0.032). There were more men with a significant smoking history (39.8% versus 24.3%, P=0.025). There were no other significant differences in the distribution of risk factors for CAD or in the use of preventative cardiac medications between the sexes. Disease severity in the cohort comprised ≈30% with no evidence of CAD (Gensini score=0), 40% with nonobstructive disease (stenosis 0%–50% in all vessels), and 30% with obstructive disease (>50% stenosis in ≥1 vessels). Men had higher median disease scores in all measures, but this was statistically significant only for the Gensini score (7.5 versus 6.5, P=0.040). There were no significant differences between the sexes in terms of patients with "healthy" arteries (Gensini=0) and those with obstructive disease >50% stenosis.

Sex and Age Differences in OHP and CAT Assay Results

On average, women had a more hypercoagulable profile than men as assessed by the CAT assay. Women had significantly higher peak thrombin readings, a longer time-to-peak thrombin formation, a higher velocity index, a shorter time to tail, and a higher α_2-macroglobulin level compared with their male counterparts (Table 2). ETP and initial slope also trended higher in women, although this was not statistically significant. These results were not significantly altered when adjusted for age by ANCOVA. Average CAT and OHP time curves for each sex are shown in Figure 2, illustrating that the changes are consistent with women having a more hypercoagulable profile than men. In contrast, the OHP assay was less varied between the

Table 1. Cohort Clinical Characteristics and Disease Burden

Demographics, Risk Factors, and Medications	Men	Women	*P* Value
No.	103	103	
Age, mean (SD), y*	61.1 (10.1)*	64.1 (9.6)*	0.032*
BMI, mean (SD), kg/m^2	27.7 (4.69)	27.45 (5.96)	0.729
Hypertension, n (%)	48 (46.6)	52 (50.5)	0.676
Diabetes mellitus, n (%)	22 (21.4)	27 (26.2)	0.513
Hyperlipidemia, n (%)	61 (59.2)	63 (61.2)	0.887
Significant smoking history (>10 pack-y), n (%)*	41 (39.8)*	25 (24.3)*	0.025*
Standard modifiable cardiovascular risk factors (hypertension/T2DM/ hyperlipidemia/significant smoking), mean (SD)	1.67 (1.18)	1.62 (1.07)	0.758
0, n (%)	22 (21.4)	18 (17.5)	
1, n (%)	23 (22.3)	29 (28.2)	
2, n (%)	30 (29.1)	32 (31.1)	
3, n (%)	23 (22.3)	22 (21.4)	
4, n (%)	5 (4.9)	2 (1.9)	
Current smoking, n (%)	13 (12.6)	12 (11.7)	1.000
Significant family history of ischemic heart disease, n (%)	19 (18.4)	24 (23.3)	0.493
Antiplatelet, n (%)	24 (23.3)	36 (35)	0.091
Statin, n (%)	42 (40.8)	43 (41.7)	1.000
β-Blocker, n (%)	14 (13.6)	7 (6.8)	0.166
ACEI/ARB, n (%)	37 (35.9)	43 (41.7)	0.475
CACS, median (IQR)	41.4 (0–371.7)	25.8 (0–208.4)	0.106
Gensini score, median (IQR)*	7.5 (0–20.0)*	6.5 (0–13.5)*	0.040*
SPS, median (IQR)	5.0 (0–20.5)	3.5 (0–14.0)	0.133
Healthy (Gensini=0), n (%)	31 (30.1)	36 (35.0)	0.457
Obstructive disease 50 (stenosis >50% in any vessel), n (%)	33 (32.0)	29 (28.2)	0.543

Values are given as number (percentage), mean (SD), or median (interquartile range [IQR]) as noted.

ACEI indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CACS, coronary artery calcium score; SPS, soft plaque score; and T2DM, type 2 diabetes mellitus.

* P<0.05.

Table 2. Sex Differences in the CAT and OHP Assay Results

Assay Variables	Men	Women	*P* Value
No.	103	103	
CAT assay			
Lag time	4.07 (1.00)	3.90 (0.87)	0.195
ETP	1511.4 (313.9)	1547.2 (280.7)	0.389
Peak	212.7 (59.3)*	239.3 (60.3)*	0.002*
Time to peak	8.84 (1.89)*	8.22 (1.49)*	0.010*
Velocity index	49.9 (23.7)*	61.9 (29.8)*	0.002
Tail	25.7 (3.2)*	24.2 (2.6)*	<0.001*
α_2-Macroglobulin	17.2 (7.6)*	20.0 (8.1)*	0.010*
Initial slope	26.7 (3.2)	27.4 (3.1)	0.085
OHP assay			
OHP	9.76 (4.28)	10.76 (4.70)	0.110
OCP	38.4 (11.8)	39.9 (11.3)	0.331
OFP%	74.3 (7.5)	73.2 (8.3)	0.287
OFP45'	70.5 (8.3)	68.7 (9.5)	0.140
Delay in the onset of fibrin generation	8.21 (2.40)	7.65 (2.05)	0.068
Maximum optical density	0.799 (0.250)	0.792 (0.253)	0.838
Maximum slope	194.0 (70.6)*	213.4 (67.7)*	0.046*

Values are given as means with SDs. CAT indicates calibrated automated thrombography; ETP, endogenous thrombin potential; OCP, overall coagulation potential; OFP, overall fibrinolysis potential; and OHP, overall hemostatic potential.

* $P<0.05$.

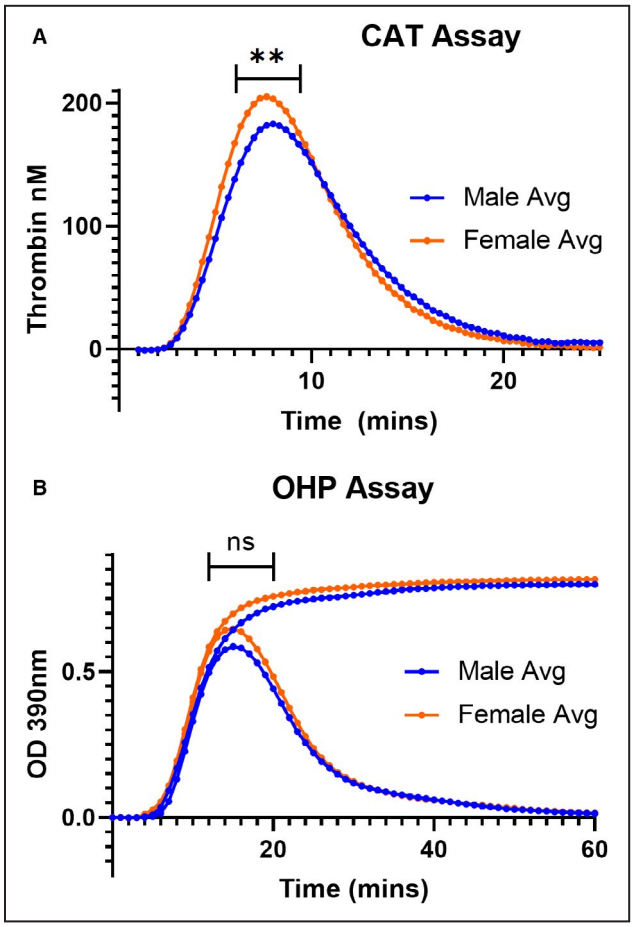

Figure 2. Sex differences in global coagulation curves. **A**, Mean thrombin generation curve for each sex showing significant differences in peak, time to peak, velocity index, and tail. See Table 2. **B**, Mean fibrin generation and lysis curves for men and women showing no significant differences between sexes. $^{**}P < 0.01$. CAT indicates calibrated automated thrombography; ns, not significant; OD, optical density; and OHP, overall hemostatic potential.

sexes, with only the maximum slope reading being significantly higher in women.

The impact of age on the CAT and OHP assay variables was also assessed in both sexes (Table S1). In the CAT assay, α_2-macroglobulin was found to have a weak association with age (Pearson correlation 0.225, P=0.022) in men but not in women. None of the other CAT assay variables were significantly correlated with age in either sex. In the OHP assay, the maximum slope value was weakly correlated with age in both men (Pearson correlation 0.207, P=0.036) and women (Pearson correlation 0.229, P=0.020), but no other significant associations with age were identified.

OHP/CAT Associations With CAD

The potential prognostic value of the OHP and CAT assay variables for CAD incidence was assessed by logistic regression, and no significant associations were identified (Table S2). The OHP and CAT assay variables were then assessed for association with CAD severity by linear regression using the nonzero disease scores, as shown in Table 3. OCP and maximum optical density were significantly correlated with CACS in men after adjustment (Pearson correlations: 0.333 [P=0.005] and 0.339 [P=0.004], respectively). A similar but more modest association was seen with adjusted Gensini score for both OCP and maximum optical density (Pearson correlations: 0.285 [P=0.0015] and 0.238 [P=0.044], respectively). There were no significant associations between global coagulation parameters and disease burden in women. No significant associations were seen with adjusted SPS in either men or women.

Linear regression models were then performed and compared with the predictive value of each assay in addition to the standard cardiovascular risk factors (Table S3 and Figure 3). For men, all 4 models were significantly predictive of CACS severity, the models that incorporated the OHP variables (3 and 4) were predictive of Gensini severity, and no model reached statistical significance for predictions of SPS severity. In women,

Table 3. Correlations Between Adjusted Disease Severity and OHP and CAT Assay Variables

Bivariate Correlation: Adjusted Linear Regression	CACS				Gensini Score				SPS			
	Men: Pearson Coefficient	*P* Value	Women: Pearson Coefficient	*P* Value	Men: Pearson Coefficient	*P* Value	Women: Pearson Coefficient	*P* Value	Men: Pearson Coefficient	*P* Value	Women: Pearson Coefficient	*P* Value
No.	103		103		103		103		103		103	
CAT assay												
Lag time	0.079	0.519	−0.132	0.295	0.064	0.596	−0.08.	0.503	0.028	0.830	−0.070	0.602
ETP	0.197	0.104	0.116	0.359	−0.013	0.913	0.169	0.171	−0.091	0.484	0.151	0.259
Peak	0.043	0.724	−0.023	0.855	−0.057	0.634	0.057	0.647	−0.128	0.322	0.127	0.341
Time to peak	0.116	0.342	0.019	0.880	0.050	0.675	0.045	0.720	−0.001	0.992	−0.017	0.898
Velocity index	−0.024	0.847	−0.103	0.412	−0.030	0.802	−0.009	0.941	−0.027	0.834	0.109	0.414
Tail	0.021	0.086	−0.101	0.424	0.183	0.124	0.089	0.475	0.126	0.330	0.078	0.560
α_2-Macroglobulin	0.065	0.605	0.001	0.992	−0.055	0.645	0.132	0.286	−0.181	0.158	0.114	0.396
Initial slope	0.041	0.739	−0.066	0.604	0.133	0.264	−0.232	0.059	0.031	0.811	−0.187	0.159
OHP assay												
OHP	0.212	0.083	−0.048	0.702	0.195	0.103	−0.048	0.699	0.151	0.246	−0.132	0.323
OCP	0.333*	0.005*	0.057	0.652	0.285*	0.015*	−0.015	0.907	0.202	0.115	−0.092	0.493
OFP%	0.026	0.830	0.056	0.657	0.009	0.939	0.018	0.885	0.002	0.986	0.065	0.630
OFP45'	0.020	0.873	0.075	0.551	0.014	0.909	0.013	0.918	0.007	0.960	0.053	0.694
Delay in the onset of fibrin generation	−0.139	0.253	0.100	0.428	−0.042	0.729	0.006	0.965	−0.039	0.765	−0.060	0.655
Maximum optical density	0.339*	0.004*	0.148	0.239	0.238*	0.044*	0.028	0.821	0.141	0.273	−0.083	0.537
Maximum slope	0.112	0.358	−0.190	0.130	0.039	0.745	−0.126	0.310	−0.011	0.930	−0.176	0.186

CACS indicates coronary artery calcium score; CAT, calibrated automated thrombography; ETP, endogenous thrombin potential; OCP, overall coagulation potential; OFP, overall fibrinolysis potential; OHP, overall hemostatic potential; and SPS, soft plaque score.

* $P<0.05$.

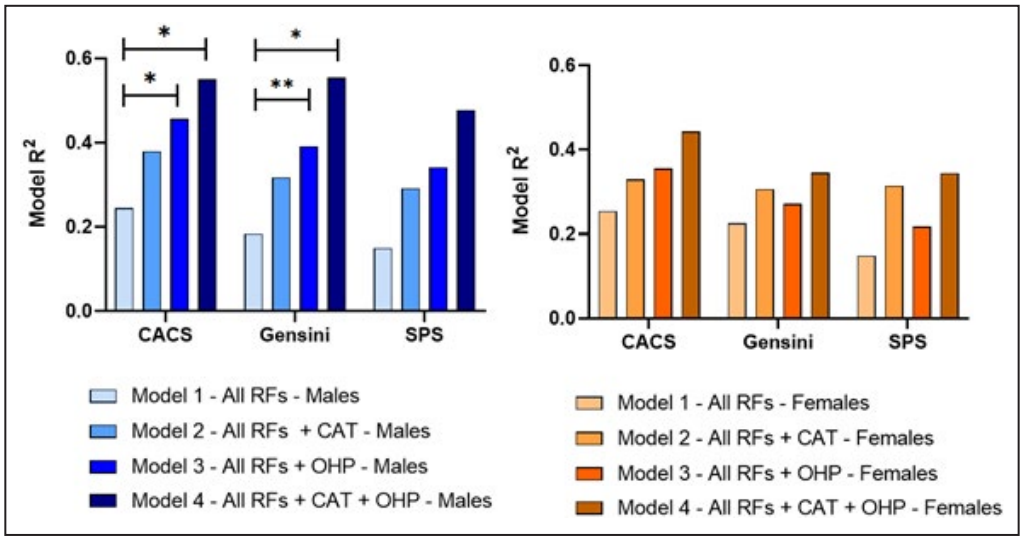

Figure 3. Comparison of model R^2 values for cardiovascular disease prediction for each sex.
Bar graph of model R^2 values for 4 linear regression models of cardiovascular risk showing the *P* value for model change across multiple disease scores for each sex. See Table S3. $^*P < 0.05$, $^{**}P < 0.01$. CACS indicates coronary artery calcium score; CAT, calibrated automated thrombography; RFs, cardiovascular risk factors (age, body mass index, hypertension, hyperlipidemia, diabetes mellitus, current or significant smoking history, family history of ischemic heart disease); OHP, overall hemostatic potential; and SPS, soft plaque score.

only model 1 was significantly predictive of CACS and Gensini severity, with no significant improvements in the model when the CAT and OHP assay results were included. None of the models in women were predictive of SPS severity.

Statistically significant improvements between the models are shown in Figure 3. The addition of the OHP assay variables to the model in men significantly improved the R^2 for both CACS and Gensini severity but not for SPS. No significant improvements were seen in women with the addition of the CAT and OHP assay variables.

OHP/CAT Associations With Risk Factors for CAD

The relationship between the assay variables and cardiac risk factors is summarized in Figure 4, which shows a *P* value heat map indicating the significant changes seen in each variable for each sex and risk factor. The color represents whether the alteration in each assay variable corresponded with hypercoagulability or hypocoagulability, as well as the significance of the association, as shown in the legend and in Table S4. The effect size is indicated by the absolute value of Cohen d.

The relationship between each individual cardiac risk factor and the CAT and OHP variables was examined separately, with the results presented tabularly in the supplemental material. Hypertension was associated with a hypocoagulable profile in some of the CAT assay variables in women but not in men (Table S5). There was no association of hypertension with the OHP assay variables. Diabetes mellitus was associated with a mixed hypercoagulable and hypocoagulable profile in the CAT assay in men, and only hypocoagulable trends were seen in women (Table S6). There was no significant association of diabetes mellitus with the OHP assay variables. A significant smoking history was associated with some scattered hypocoagulable results in the CAT assay, primarily in women (Table S7). There was no association of significant smoking and the OHP assay results. Patients with a significant family history of ischemic heart disease (Table S8) were found to have no significant associations with any of the assay variables assessed.

More robust coagulation profile shifts were seen in association with hyperlipidemia and obesity. Patients who had a history of hyperlipidemia had hypercoagulable changes in the OHP assay results, with most differences seen in women (Table S9). Men with hyperlipidaemia (n=61) compared with those with normolipidemia (n=42) had a significantly higher maximum slope (207.4±78.9 versus 174.5±51.4, *P*=0.020). In contrast, nearly the entire OHP assay was shifted to a hypercoagulable profile in women with hyperlipidemia (n=63) when compared with those without hyperlipidemia (n=40), with significant increases in OHP (11.7±4.8 versus 9.2±4.1, *P*=0.007), OCP (41.8±10.7 versus 37.0±12.0, *P*=0.038), and maximum slope (224.5±65.6 versus 195.8±68.2, *P*=0.035); a significant decrease in OFP% (71.9±8.4 versus 75.2±7.8, *P*=0.050); and a

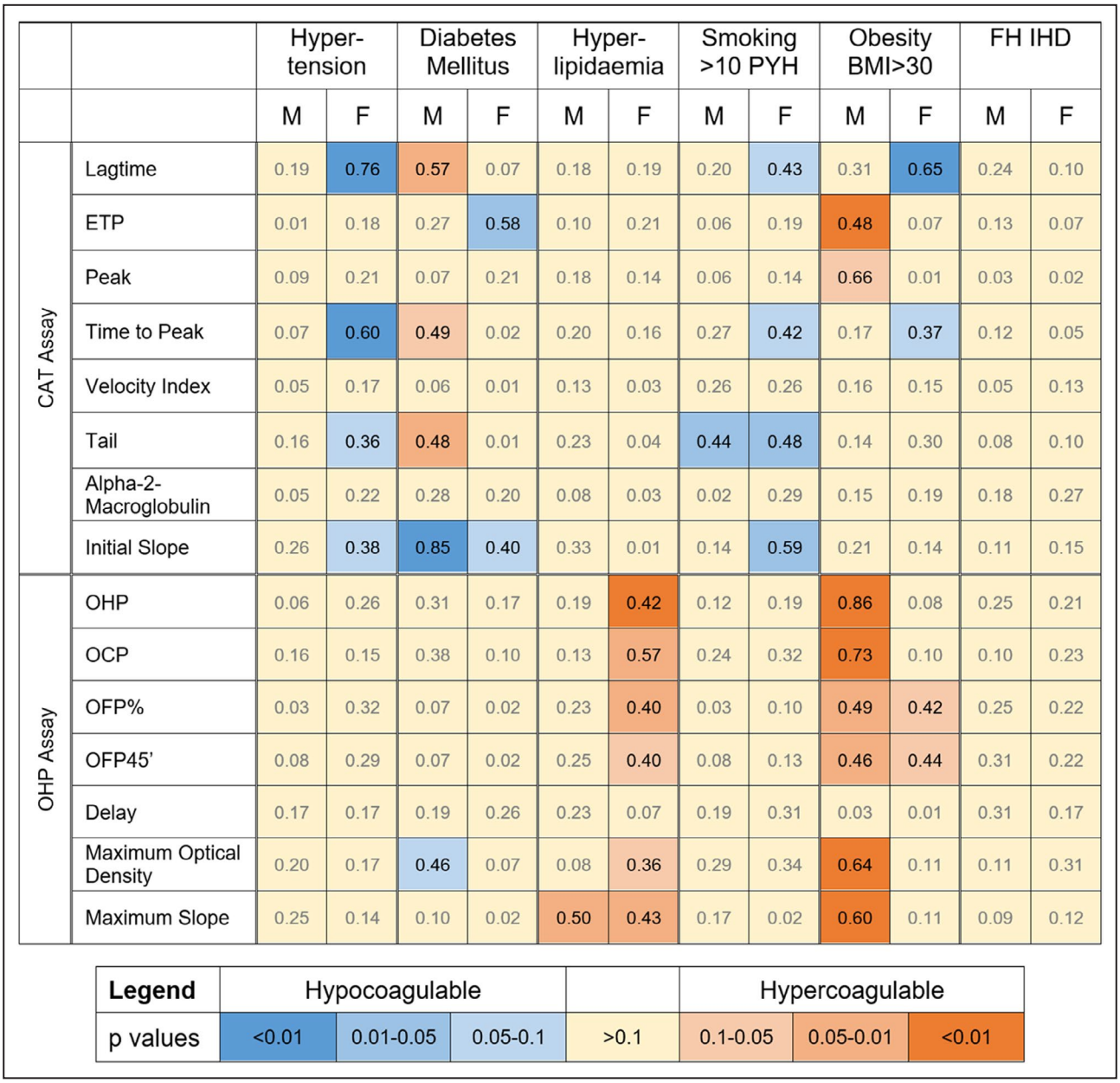

Figure 4. ***P*** **value heat map of significant associations between cardiac risk factors and overall hemostatic potential (OHP) and calibrated automated thrombography (CAT) assay variables showing effect size as Cohen d.**
Each cell shows the effect size as the absolute value of Cohen d for the comparison of those with each risk factor to those without, colored by whether the change was hypocoagulable (blue) or hypercoagulable (red) and *P* value significance. See Tables S4–S9. BMI indicates body mass index; ETP, endogenous thrombin potential; OFP, overall fibrinolysis potential; and PYH, pack-year history.

trend towards increased maximum optical density and decreased OFP45'.

A similar sex difference was seen in association with obesity (Table S10) where a hypercoagulable profile was seen particularly in men. Obese men (n=26) when compared with nonobese men (n=77) had a significantly higher peak thrombin (232.5±47.1 versus 206.0±62.4, *P*=0.004), OHP (12.55±5.37 versus 8.80±3.38, *P*=0.002), OCP (45.1±14.9 versus 36.1±9.6, *P*=0.007), maximum optical density (0.922±0.292 versus 0.757±0.220, *P*=0.003), and maximum slope (226.9±84.2 versus 182.9±62.1, *P*=0.005), as well as significantly lower OFP% (71.5±8.7 versus 75.3±6.9, *P*=0.024) and OFP45' (67.6±9.3 versus 71.5±7.7, *P*=0.037), and a trend towards a higher ETP. The changes in women were far less extensive, with only a trend towards lower OFP% and OFP45' being hypercoagulable, and a hypocoagulable trend towards a lower time to peak.

DISCUSSION

In this study we assessed over a hundred men and a hundred women by the comprehensive OHP and CAT assays to determine their global coagulation profiles and identify potential associations with cardiac risk factors and atherosclerosis burden. We have confirmed the results of previous studies,[20,21] which showed that sex is associated with differences in global assays of coagulation, here seen most significantly in the CAT assay. Subsequently, we have systematically reported the numerous sex-specific associations of cardiovascular risk factors with the individual coagulation assay variables. While we observed no significant differences in the global coagulation profiles with the presence or absence of CAD, there was a moderately strong association with the quantitative measures of CACS and Gensini score in men that remained significant after adjustment for age, body mass index, and cardiac risk factors. Finally, we showed that addition of the OHP assay variables improved the ability of basic risk models to predict disease severity in men but not in women.

Coagulation profiles in patients with CAD in our study were only found to demonstrate increased potential for fibrin generation in men with calcified CAD, and our results here differ somewhat from the published literature. There is one previous study that looked at the OHP and CAT assay in 56 patients with stable angina and found global changes of hypercoagulability and hypofibrinolysis when compared with healthy pooled plasma.[12] However, the demographics of the patients used in the pooled plasma sample significantly differed from the CAD group, as the healthy controls were predominantly women and an average of 28 years younger than the diseased group. This may account for the observed differences in the OHP assay. While we did not find a global profile shift towards hypercoagulability, the robust associations we identified between OCP and maximum fibrin concentration with CACS and Gensini score in men remained significant after adjustment for age, body mass index, and multiple risk factors, and are in line with the previous study's findings. This was complimented by the addition of the OHP assay results to disease severity models, which improved prediction of CACS and Gensini score in men but not in women.

In contrast, our CAT assay results showed no association between increased thrombin generation and the presence or burden of CAD. However, the findings of previous studies using the CAT assay to assess patients with atherosclerosis have been mixed. Thrombin generation was increased in patients with stable angina,[12] around the time of myocardial infarction,[18] and elevated activity may persist in the months following infarction.[32] Conversely, other studies have demonstrated that CAT assay results did not predict severity of stenosis or prognosis in patients with coronary disease without myocardial infarction,[17] and one CTCA-based study identified a U-shaped association between ETP and CAD severity defined by CACS, with a significant drop in ETP observed in the group with mild CAD.[16] As our patients did not have infarctions and largely had early CAD, our results are consistent with the majority of these previous data.

Our data demonstrate that hypercoagulability is more predictive of CAD severity in men than in women, and while activation of coagulation plays a key role in atherosclerosis-related events regardless of sex, clinical manifestations of atherosclerosis are different in women and this relationship with coagulation may impact disease progression. In recent years, studies into sex-specific differences in atherosclerosis have shown that women have a higher prevalence of nonobstructive CAD[33] and have smaller,[34] less vulnerable[35,36] plaques, which are associated with microvascular dysfunction. Larger, more rupture-prone plaques may put men at more risk of disease progression in the hypercoagulable context, and this may explain the sex difference identified in the risk models.

Within the sex-disaggregated analysis of the cardiac risk factors, the most striking hypercoagulable profiles we observed were in women with hyperlipidemia and in men with obesity. Women with hyperlipidemia in our study were found to have changes in the OHP assay but not the CAT assay. Fibrin generation in the context of hyperlipidemia has never been previously explored, and as such our OHP results are highly novel. Thrombin generation, however, has been assessed in the past. In a study of 448 patients using a thrombin generation assay similar to the CAT assay, lag time, time to peak, peak thrombin, and ETP were found to have weak to moderate positive correlations with triglycerides, total cholesterol, and low-density lipoprotein cholesterol, and a similarly sized negative correlation with high-density lipoprotein cholesterol.[37] Our results from the CAT assay did not show similar changes in association with self-reported diagnosis of hyperlipidemia; however, the mentioned study showed that the bulk of the effect was in those with abnormal lipid readings.[37] In our study, we were limited by a clinical diagnosis of hyperlipidemia without fasting lipid values, and our findings in the CAT assay may be explained by less extreme lipid readings, as most patients were on treatment with statins.

The second notable hypercoagulable profile in our study was seen in both the OHP and CAT assay results in men with obesity. There are limited data in the literature relating to this association. One previous study looking at body mass in detail found an association between central obesity and higher levels of thrombin generation in elderly women but not in men, which was postulated to be explained by adiposity-related low-grade

inflammation.[38] Patients with bariatric surgery in general have also been found to have more hypercoagulability on thromboelastography assays,[39] but the differences between the sexes in this context have not been previously assessed. Here, we saw more of a significant shift in the fibrin generation assay results in men, and to our knowledge this has not been previously shown.

This study had a number of strengths, including the sample size and the detailed CAD phenotyping obtained by analysis of the CTCA imaging to compare patients who had no visible CAD with those with early atherosclerosis. However, there were some limitations. The disease scoring systems used in this study are based on visual detection of epicardial atherosclerotic plaque and do not account for microvascular disease. While women are more likely than men to have microvascular disease as an underlying contributor to a presentation with symptomatic CAD, this is unlikely to explain differences related to epicardial CAD defined by CTCA. The dichotomous description of the risk factor variables does not incorporate the continuous nature of these conditions, and there is a gradient of severity that will not be captured using this approach. Future research incorporating fasting lipids, hyperglycemic markers, anthropometric measurements such as waist circumference, and a greater number of current smokers would be beneficial to clarify some of the significant findings and trends identified in the present study. Furthermore, the potential prognostic value of these types of detailed coagulation measures can be further assessed as the BioHEART-CT study continues, and association with cardiovascular outcome events can be incorporated.

CONCLUSIONS

The CAT and OHP assays have highlighted the significant differences in global coagulation that exist between the sexes and have identified interesting hypercoagulable changes in men with obesity and those who have atherosclerosis, and in women with hyperlipidemia. These differential associations warrant further study in regard to mechanism and potential prognostic significance and reinforce the need to consider the biological differences between men and women in the study of coagulation and CAD. Further discovery of sex-specific differences in biomarkers and risk factors may improve risk scoring, better enabling precision preventative and therapeutic strategies in the future.

ARTICLE INFORMATION

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Affiliations

Cardiovascular Discovery Group, Kolling Institute of Medical Research, University of Sydney, Sydney, Australia (K.A.K., S.T.V., B.A.D.B., G.A.F.); Department of Cardiology, Royal North Shore Hospital, Sydney, Australia (K.A.K., S.T.V., G.A.F.); Northern Clinical School, Faculty of Medicine and Health, University of Sydney, Sydney, Australia (K.A.K., S.T.V., S.M.G., C.W., G.A.F.); Department of Haematology and Transfusion Medicine, Royal North Shore Hospital, Sydney, Australia (M.M., C.W.); Northern Blood Research Centre, Kolling Institute of Medical Research, University of Sydney, Sydney, Australia (M.M., Y.T., C.W.); Atherothrombosis and Vascular Biology Laboratory, Baker Heart and Diabetes Institute, Melbourne, Australia (K.P.); Charles Perkins Centre (J.Y.Y., G.A.F.); and School of Mathematics and Statistics (J.Y.Y.), University of Sydney, Sydney, Australia; Department of Radiology, Royal Price Alfred Hospital, Sydney, Australia (S.M.G.); and Imaging and Phenotyping Laboratory, Charles Perkins Centre, Faculty of Medicine and Health, University of SydneyAustralia, (S.M.G.).

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Disclosures

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Supplementary Material

Tables S1-S10
Figure S1

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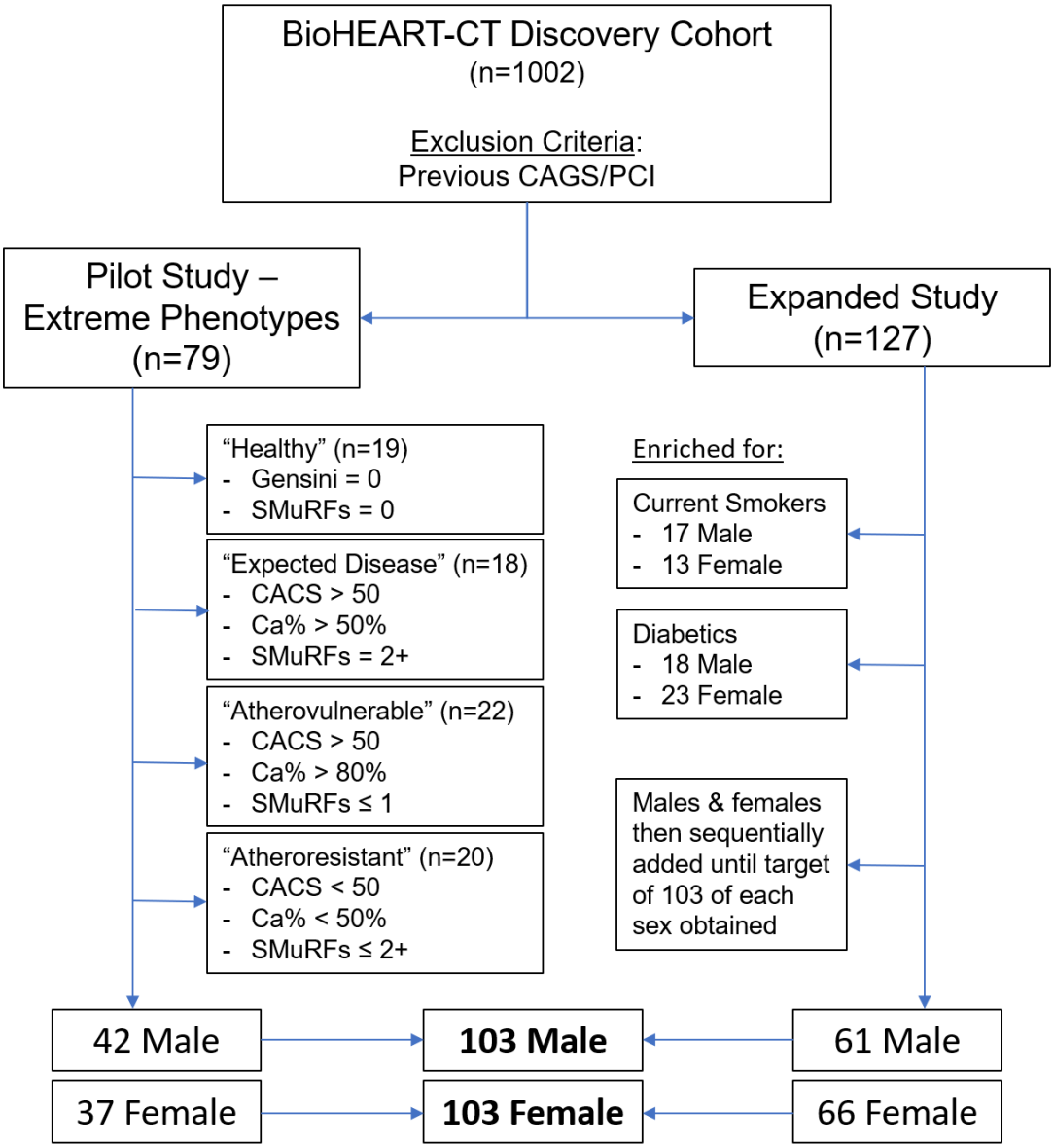
SUPPLEMENTAL MATERIAL

Figure S1. Patient selection from the larger BioHEART-CT cohort.



CAGS indicates coronary artery graft surgery; PCI, percutaneous coronary intervention; CACS, coronary artery calcium score; Ca%, calcium percentile – normalised for age and sex; SMuRFs, standard modifiable cardiovascular risk factors

Table S1. Correlation of CAT & OHP Assay Variables with Age.

Bivariate Correlation – Age	**Males – Pearson Coefficient**	**P**	**Females – Pearson Coefficient**	**P**
Number	103		103	
CAT Assay				
Lagtime	0.053	0.598	0.161	0.104
ETP	-0.010	0.920	-0.080	0.424
Peak	0.103	0.300	-0.038	0.705
Time to Peak	-0.066	0.505	0.082	0.413
Velocity Index	0.193	0.051	-0.021	0.833
Tail	-0.004	0.966	0.034	0.734
Alpha-2-Macroglobulin	**0.225**	**0.022**	0.045	0.655
Initial Slope	0.152	0.124	0.161	0.105
OHP Assay				
OHP	0.112	0.261	0.088	0.377
OCP	0.080	0.419	0.166	0.094
OFP%	-0.095	0.343	-0.019	0.849
OFP45’	-0.118	0.236	-0.026	0.797
Delay	-0.190	0.055	-0.085	0.392
Maximum Optical Density	0.070	0.480	0.134	0.178
Maximum Slope	**0.207**	**0.036**	**0.229**	**0.020**

CAT indicates calibrated automated thrombin; ETP, endogenous thrombin potential; OCP, overall coagulation potential; OD, optical density; OFP, overall fibrinolysis potential; OHP, overall haemostatic potential.

Table S2. Correlations Between Adjusted Disease Incidence and OHP and CAT Assay Variables.

Bivariate Correlation – Adjusted Logistic Regression (Gensini > 0)	**Males – Pearson Coefficient**	**P**	**Females – Pearson Coefficient**	**P**
Number	103		103	
CAT Assay				
Lagtime	0.069	0.488	-0.066	0.506
ETP	-0.088	0.375	0.161	0.105
Peak	-0.035	0.726	0.097	0.330
Time to Peak	0.063	0.529	-0.044	0.662
Velocity Index	0.007	0.942	0.025	0.799
Tail	0.077	0.440	-0.029	0.768
Alpha-2-Macroglobulin	-0.070	0.485	0.049	0.620
Initial Slope	0.019	0.853	-0.079	0.428
OHP Assay				
OHP	0.066	0.511	0.039	0.695
OCP	-0.005	0.962	-0.008	0.939
OFP%	-0.090	0.368	-0.046	0.645
OFP45’	-0.070	0.486	-0.026	0.796
Delay	0.093	0.352	0.083	0.403
Max OD	0.009	0.928	-0.029	0.774
Max Slope	0.000	0.996	0.029	0.768

CAT indicates calibrated automated thrombin; ETP, endogenous thrombin potential; OCP, overall coagulation potential; OD, optical density; OFP, overall fibrinolysis potential; OHP, overall haemostatic potential.

Table S3. Comparison of Models Predicting Disease Severity.

	Males					Females				
	R^2	**Adjusted R^2**	**F Value**	**P**	**P for Model Change**	**R^2**	**Adjusted R^2**	**F Value**	**P**	**P for Model Change**
CACS										
Model 1	**0.245**	**0.144**	**2.432**	**0.026**		**0.254**	**0.147**	**2.383**	**0.028**	
Model 2	**0.380**	**0.189**	**1.990**	**0.032**	0.213	0.329	0.105	1.470	0.151	0.716
Model 3	**0.457**	**0.301**	**2.919**	**0.002**	**0.013**	0.355	0.158	1.802	0.062	0.377
Model 4	**0.550**	**0.314**	**2.335**	**0.008**	**0.040**	0.443	0.130	1.416	0.163	0.545
Gensini										
Model 1	0.184	0.081	1.777	0.099		**0.225**	**0.118**	**2.100**	**0.050**	
Model 2	0.318	0.119	1.602	0.099	0.240	0.306	0.084	1.378	0.191	0.661
Model 3	**0.392**	**0.227**	**2.368**	**0.010**	**0.019**	0.271	0.056	1.262	0.260	0.858
Model 4	**0.554**	**0.335**	**2.535**	**0.003**	**0.007**	0.346	-0.004	0.989	0.497	0.908
SPS										
Model 1	0.150	0.022	1.168	0.311		0.149	0.010	1.071	0.399	
Model 2	0.292	0.041	1.161	0.334	0.361	0.314	0.046	1.172	0.329	0.305
Model 3	0.342	0.123	1.560	0.125	0.109	0.217	-0.063	0.775	0.696	0.814
Model 4	0.478	0.176	1.584	0.060	0.058	0.345	-0.067	0.837	0.692	0.742

Model 1: all cardiac risk factors (RFs) including age, BMI, hypertension, hyperlipidaemia, diabetes mellitus, current or significant smoking history and family history of ischaemic heart disease; Model 2: all RFs + CAT assay; Model 3: all RFs + OHP assay; Model 4: all RFs + CAT + OHP assays. CACS indicates coronary artery calcium score; CAT, calibrated automated thrombin; RFs, cardiovascular risk factors; OHP, overall haemostatic potential; SPS, soft plaque score.

Table S4. Direction of Variable Changes in Hypercoagulable and Hypocoagulable States.

	Hypercoagulable	Hypocoagulable
CAT Assay		
Lagtime	Decreased	Increased
ETP	Increased	Decreased
Peak	Increased	Decreased
ttPeak	Decreased	Increased
VI	Increased	Decreased
Tail	Decreased	Increased
A2M	Decreased	Increased
OHP Assay		
OHP	Increased	Decreased
OCP	Increased	Decreased
OFP%	Decreased	Increased
OFP45'	Decreased	Increased
Delay	Decreased	Increased
Max OD	Increased	Decreased
Max Slope	Increased	Decreased

A2M indicates alpha-2-macroglobulin; CAT, calibrated automated thrombin; ETP, endogenous thrombin potential; OCP, overall coagulation potential; OD, optical density; OFP, overall fibrinolysis potential; OHP, overall haemostatic potential; ttPeak, time to peak; VI, velocity index.

Table S5. Sex-Specific Differences in CAT & OHP Assay Results for Hypertension.

	Male			**Female**		
Assay Variables	**Normotensive**	**Hypertensive**	**P**	**Normotensive**	**Hypertensive**	**P**
Number	55	48		51	52	
CAT Assay						
Lagtime	3.98 (0.93)	4.18 (1.01)	0.325	**3.59 (0.71)**	**4.21 (0.92)**	**<0.001**
ETP	1512.9 (307.7)	1509.7 (324.2)	0.959	1572.1 (297.7)	1522.8 (263.5)	0.375
Peak	215.1 (60.7)	210.0 (59.3)	0.668	245.6 (57.9)	233.2 (62.6)	0.299
ttPeak	8.78 (1.88)	8.91 (1.92)	0.733	**7.79 (1.39)**	**8.65 (1.47)**	**0.003**
VI	50.4 (24.5)	49.3 (23.0)	0.812	64.5 (28.4)	59.4 (31.2)	0.390
Tail	25.4 (3.2)	26.0 (3.3)	0.411	23.7 (2.4)	24.6 (2.8)	0.073
A2M	17.4 (7.8)	17.0 (7.5)	0.786	20.9 (7.4)	19.2 (8.6)	0.278
Initial Slope	26.3 (3.2)	27.1 (3.3)	0.187	26.9 (3.2)	28.0 (2.9)	0.057
OHP Assay						
OHP	9.65 (4.05)	9.89 (4.57)	0.780	10.2 (5.0)	11.4 (4.3)	0.194
OCP	37.5 (11.1)	39.4 (12.5)	0.406	39.1 (11.0)	40.8 (11.6)	0.438
OFP%	74.2 (6.7)	74.5 (8.4)	0.873	74.5 (7.9)	71.9 (8.5)	0.110
OFP45’	70.2 (7.7)	70.9 (9.1)	0.704	70.1 (9.2)	67.3 (9.7)	0.148
Delay	8.03 (2.43)	8.44 (2.37)	0.384	7.48 (2.17)	7.82 (1.92)	0.398
Max OD	0.775 (0.224)	0.826 (0.267)	0.310	0.770 (0.247)	0.813 (0.261)	0.403
Max Slope	185.6 (61.3)	203.5 (79.6)	0.201	208.7 (59.4)	217.9 (75.3)	0.494

Values are given as means with standard deviations. CAT indicates calibrated automated thrombin; ETP, endogenous thrombin potential; OCP, overall coagulation potential; OD, optical density; OFP, overall fibrinolysis potential; OHP, overall haemostatic potential.

Table S6. Sex-Specific Differences in CAT & OHP Assay Results for Diabetes.

	Male			**Female**		
Assay Variables	**Non-Diabetic**	**Diabetic**	**P**	**Non-Diabetic**	**Diabetic**	**P**
Number	81	22		76	27	
CAT Assay						
Lagtime	**4.18 (1.03)**	**3.67 (0.80)**	**0.032**	3.89 (0.81)	3.95 (1.04)	0.759
ETP	1528.3 (327.2)	1449.0 (256.4)	0.296	**1588.6 (275.3)**	**1430.7 (267.0)**	**0.011**
Peak	213.6 (62.4)	209.8 (50.6)	0.793	242.8 (57.6)	229.6 (67.7)	0.332
ttPeak	9.01 (1.96)	8.18 (1.45)	0.065	8.23 (1.40)	8.20 (1.73)	0.936
VI	49.6 (23.4)	51.1 (25.4)	0.787	62.0 (28.7)	61.6 (33.2)	0.949
Tail	26.0 (3.4)	24.6 (2.1)	0.029	24.2 (2.4)	24.2 (3.2)	0.965
A2M	16.7 (7.4)	18.9 (8.5)	0.229	20.5 (7.8)	18.8 (8.8)	0.361
Initial Slope	**26.1 (3.1)**	**28.7 (2.8)**	**0.001**	27.1 (2.9)	28.4 (3.5)	0.066
OHP Assay						
OHP	10.0 (4.4)	8.6 (3.8)	0.216	11.0 (4.8)	10.2 (4.4)	0.468
OCP	39.3 (11.6)	34.8 (12.1)	0.112	40.2 (10.6)	39.0 (13.3)	0.634
OFP%	74.2 (7.8)	74.7 (6.7)	0.797	73.1 (8.0)	73.3 (9.3)	0.933
OFP45’	70.4 (8.5)	71.0 (7.5)	0.778	68.7 (8.8	68.5 (11.3)	0.921
Delay	8.13 (2.51)	8.55 (1.96)	0.463	7.80 (1.86)	7.23 (2.50)	0.287
Max OD	0.822 (0.254)	0.714 (0.219)	0.072	0.797 (0.240)	0.778 (0.292)	0.739
Max Slope	195.5 (69.3)	188.5 (76.7)	0.682	213.8 (61.1)	212.2 (85.0)	0.916

Values are given as means with standard deviations. CAT indicates calibrated automated thrombin; ETP, endogenous thrombin potential; OCP, overall coagulation potential; OD, optical density; OFP, overall fibrinolysis potential; OHP, overall haemostatic potential.

Table S7. Sex-Specific Differences in CAT & OHP Assay Results for Significant Smoking History.

	Male			**Female**		
Assay Variables	**No Smoking History**	**>10 Pack Year Smoking Hx**	**P**	**No Smoking History**	**>10 Pack Year Smoking Hx**	**P**
Number	62	41		78	25	
CAT Assay						
Lagtime	3.99 (0.99)	4.20 (1.02)	0.312	3.81 (0.82)	4.19 (0.99)	0.055
ETP	1504.2 (269.4)	1522.3 (374.7)	0.777	1537.3 (272.2)	1578.2 (309.4)	0.529
Peak	217.2 (56.8)	206.0 (64.3)	0.357	242.1 (58.7)	230.6 (65.7)	0.407
ttPeak	8.64 (1.85)	9.14 (1.93)	0.184	8.07 (1.39)	8.71 (1.68)	0.057
VI	52.3 (24.5)	46.3 (22.3)	0.213	63.7 (30.2)	56.1 (28.6)	0.269
Tail	**25.1 (3.0)**	**26.5 (3.4)**	**0.031**	**23.9 (2.5)**	**25.1 (2.8)**	**0.032**
A2M	17.3 (7.9)	17.1 (7.3)	0.910	20.6 (8.3)	18.3 (7.3)	0.225
Initial Slope	26.5 (3.2)	26.9 (3.3)	0.501	**27.0 (3.1)**	**28.8 (2.8)**	**0.014**
OHP Assay						
OHP	9.6 (4.1)	10.1 (4.6)	0.555	10.5 (4.6)	11.4 (5.0)	0.411
OCP	37.2 (10.4)	40.1 (13.5)	0.222	39.1 (11.5)	42.6 (10.6)	0.173
OFP%	74.3 (7.9)	41.5 (7.0)	0.892	73.0 (8.3)	73.8 (8.5)	0.657
OFP45’	70.3 (8.8)	70.9 (7.5)	0.691	68.4 (9.5)	69.6 (9.4)	0.587
Delay	8.03 (2.21)	8.50 (2.67)	0.332	7.50 (2.13)	8.11 (1.73)	0.201
Max OD	0.770 (0.223)	0.843 (0.282)	0.146	0.772 (0.262)	0.854 (0.220)	0.162
Max Slope	189.1 (61.0)	201.4 (83.3)	0.388	213.0 (71.0)	214.3 (58.6)	0.936

Values are given as means with standard deviations. CAT indicates calibrated automated thrombin; ETP, endogenous thrombin potential; OCP, overall coagulation potential; OD, optical density; OFP, overall fibrinolysis potential; OHP, overall haemostatic potential.

Table S8. Sex-Specific Differences in CAT & OHP Assay Results for Family History of Premature IHD.

	Male			Female		
Assay Variables	**No Family History**	**Family History of Premature IHD**	**P**	**No Family History**	**Family History of Premature IHD**	**P**
Number	84	19		79	24	
CAT Assay						
Lagtime	4.03 (0.96)	4.28 (1.18)	0.323	3.92 (0.90)	3.84 (0.79)	0.687
ETP	1519.0 (317.0)	1477.7 (306.0)	0.607	1548.5 (295.1)	1542.8 (232.3)	0.931
Peak	212.5 (58.2)	214.1 (68.1)	0.917	240.3 (62.6)	236.0 (53.2)	0.761
ttPeak	8.79 (1.84)	9.04 (2.15)	0.607	8.2 (1.5)	8.3 (1.3)	0.844
VI	49.6 (23.4)	50.9 (26.9)	0.830	62.8 (29.9)	58.8 (30.0)	0.565
Tail	25.7 (3.1)	25.4 (3.8)	0.735	24.2 (2.8)	24.0 (2.0)	0.699
A2M	17.5 (7.1)	15.9 (9.8)	0.522	19.6 (8.5)	21.6 (6.3)	0.288
Initial Slope	26.7 (3.3)	26.4 (3.0)	0.678	27.3 (3.2)	27.8 (2.8)	0.538
OHP Assay						
OHP	9.9 (4.5)	8.9 (3.1)	0.380	11.0 (4.8)	10.0 (4.2)	0.381
OCP	38.1 (11.7)	39.3 (12.1)	0.697	40.5 (12.0)	38.1 (8.9)	0.361
OFP%	74.0 (7.2)	76.0 (8.9)	0.301	72.8 (8.5)	74.5 (7.5)	0.369
OFP45’	70.0 (8.0)	72.8 (9.5)	0.193	68.2 (9.8)	70.2 (8.5)	0.361
Delay	8.07 (2.29)	8.87 (2.83)	0.196	7.58 (2.14)	8.90 (1.73)	0.501
Max OD	0.794 (0.254)	0.821 (0.234)	0.667	0.809 (0.261)	0.734 (0.226)	0.202
Max Slope	195.3 (68.9)	188.3 (79.7)	0.698	215.2 (70.5)	207.2 (58.8)	0.614

Values are given as means with standard deviations. CAT indicates calibrated automated thrombin; ETP, endogenous thrombin potential; OCP, overall coagulation potential; OD, optical density; OFP, overall fibrinolysis potential; OHP, overall haemostatic potential.

Table S9. Sex-Specific Differences in CAT & OHP Assay Results for Hyperlipidaemia.

	Male			**Female**		
Assay Variables	**Normolipidaemic**	**Hyperlipidaemic**	**P**	**Normolipidaemic**	**Hyperlipidaemic**	**P**
Number	42	61		40	63	
CAT Assay						
Lagtime	4.18 (1.08)	4.00 (0.95)	0.375	3.80 (1.04)	3.97 (0.75)	0.348
ETP	1492.5 (371.1)	1524.4 (270.2)	0.614	1510.0 (299.3)	1570.8 (267.9)	0.286
Peak	206.4 (67.8)	217.2 (53.8)	0.371	234.3 (59.1)	242.5 (61.4)	0.503
ttPeak	9.06 (1.98)	8.68 (1.82)	0.319	8.10 (1.73)	8.32 (1.31)	0.430
VI	48.0 (26.4)	51.2 (21.9)	0.507	61.3 (25.2)	62.2 (32.6)	0.882
Tail	26.1 (3.62)	25.4 (2.89)	0.243	24.2 (3.2)	24.1 (2.2)	0.858
A2M	17.6 (7.8)	16.9 (7.5)	0.687	20.2 (8.9)	19.9 (7.5)	0.877
Initial Slope	26.1 (3.0)	27.1 (3.3)	0.104	27.5 (3.0)	27.4 (3.2)	0.957
OHP Assay						
OHP	9.3 (3.9)	10.1 (4.5)	0.367	**9.2 (4.1)**	**11.7 (4.8)**	**0.007**
OCP	37.5 (10.1)	39.0 (12.8)	0.525	**37.0 (12.0)**	**41.8 (10.7)**	**0.038**
OFP%	75.3 (5.9)	73.7 (8.5)	0.278	**75.2 (7.8)**	**71.9 (8.4)**	**0.050**
OFP45'	71.7 (6.9)	69.7 (9.1)	0.236	**70.9 (8.6)**	**67.3 (9.8)**	**0.054**
Delay	8.55 (2.67)	7.99 (2.12)	0.245	7.74 (1.95)	7.59 (2.12)	0.720
Max OD	0.787 (0.215)	0.807 (0.272)	0.706	0.736 (0.255)	0.827 (0.249)	0.075
Max Slope	**174.5 (51.4)**	**207.4 (78.9)**	**0.020**	**195.8 (68.2)**	**224.5 (65.6)**	**0.035**

Values are given as means with standard deviations. CAT indicates calibrated automated thrombin; ETP, endogenous thrombin potential; OCP, overall coagulation potential; OD, optical density; OFP, overall fibrinolysis potential; OHP, overall haemostatic potential.

Table S10. Sex-Specific Differences in CAT & OHP Assay Results for Obesity.

	Male			**Female**		
Assay Variables	**Non-Obese**	**Obese**	**P**	**Non-Obese**	**Obese**	**P**
Number	77	26		72	31	
CAT Assay						
Lagtime	4.00 (1.09)	4.27 (0.67)	0.233	**3.73 (0.75)**	**4.30 (1.00)**	**0.002**
ETP	**1459.6 (293.5)**	**1664.7 (327.8)**	**0.004**	1546.6 (287.0)	1548.7 (270.0)	0.725
Peak	**206.0 (62.4)**	**232.5 (47.1)**	**0.051**	237.9 (57.2)	242.5 (68.1)	0.972
ttPeak	8.76 (2.01)	9.06 (1.44)	0.483	8.05 (1.39)	8.62 (1.63)	0.075
VI	49.0 (25.1)	52.6 (19.4)	0.509	60.5 (26.8)	65.2 (36.1)	0.457
Tail	25.6 (3.4)	26.0 (2.5)	0.574	23.9 (2.4)	24.7 (3.1)	0.148
A2M	17.5 (7.8)	16.4 (7.2)	0.527	20.5 (8.0)	19.0 (8.2)	0.384
Initial Slope	26.5 (3.3)	27.2 (2.9)	0.376	27.3 (3.1)	27.7 (3.2)	0.517
OHP Assay						
OHP	**8.80 (3.38)**	**12.55 (5.37)**	**0.002**	10.6 (4.6)	11.0 (5.0)	0.692
OCP	**36.1 (9.6)**	**45.1 (14.9)**	**0.007**	40.3 (9.9)	39.1 (14.3)	0.629
OFP%	**75.3 (6.9)**	**71.5 (8.7)**	**0.024**	74.3 (7.0)	70.6 (10.3)	0.077
OFP45'	**71.5 (7.7)**	**67.6 (9.3)**	**0.037**	70.0 (7.9)	65.7 (12.0)	0.076
Delay	8.20 (2.48)	8.27 (2.19)	0.906	7.66 (2.10)	7.63 (1.95)	0.962
Max OD	**0.757 (0.220)**	**0.922 (0.292)**	**0.003**	0.801 (0.228)	0.771 (0.309)	0.589
Max Slope	**182.9 (62.1)**	**226.9 (84.2)**	**0.005**	210.8 (53.7)	219.2 (93.4)	0.646

Values are given as means with standard deviations. CAT indicates calibrated automated thrombin; ETP, endogenous thrombin potential; OCP, overall coagulation potential; OD, optical density; OFP, overall fibrinolysis potential; OHP, overall haemostatic potential.