Title:
Platelet acetyl-CoA carboxylase phosphorylation: A risk stratification marker that reveals platelet-lipid interplay in coronary artery disease patients.

Supplemental Material and Methods

Reagents and Materials

Apyrase (#A6132), bovine thrombin (#T6634), ADP (#A2754), horse radish peroxidase (HRP)-conjugated anti-rabbit antibodies (#A0545), hematoxylin (#MHS32) and oil-red-O (#O0625) were purchased from Sigma. Eptifibatide (Integrilin) was obtained from GlaxoSmithKline. Collagen (#ABP-COL-1, equine tendon type 1) was obtained from American Biochemical and Pharmaceutical Ltd. Anti-phosphoACC (S79) (#3661), anti-phosphoP38 MAPK (T180/Y182) (#9211), anti-gelsolin (#12953) and anti-phosphoPKC substrate (#2261) antibodies were obtained from Cell Signaling. Bovine serum albumin (BSA) (#8076) was purchased from Roth. GOLD 96-well streptavidin SECTOR plates (#L15SA-5) and goat anti-rabbit sulfo-tag secondary antibody (#R32AB5) were purchased from MSD. A cocktail of protease and phosphatase inhibitors was purchased from Thermo Fisher Scientific. IL1β (#78034), IL6 (#78050), IL10 (#78024), IL17A (#78032), and TNFα (#78068) cytokines were purchased from STEMCELL Technologies. Oxidized choline glycerophospholipids ($OxPC^{CD36}$- KOdia-PC, CAS 439904) were purchased from Santa Cruz Biotechnology. Anti-CD36 (FA6-152, ab17044) and the corresponding isotype control (ab18449) antibodies were purchased from Abcam. APC mouse anti-human CD45 (#555485) and FITC mouse anti-human CD41a antibodies were purchased from BD biosciences for flow cytometry experiments.

LDL preparation and oxidation

Native low-density lipoproteins (LDLs) were isolated from the plasma of healthy donors by means of sequential density gradient ultracentrifugation. LDL concentration was adjusted to 1mg/mL in phosphate-buffered saline (PBS). Copper-oxidized LDLs (coxLDLs) were obtained by treating native LDLs (1mg/mL) with 10µM $CuSO_4$ for 24 hours at 37°C. The reaction was stopped on ice with 25µM butylated hydroxytoluene (Sigma-Aldrich, St. Louis, MO, USA) (1). Myeloperoxidase-oxidized LDLs (moxLDLs) were produced by mixing 1.6mg of native LDL with PBS containing 4mM HCl, 250nM myeloperoxidase, 1mM H_2O_2 and 3.4mM EDTA for 4 hours at 37°C (1,2). OxLDL and moxLDL were desalted in RPMI-1640 medium without glutamine (Lonza, Belgium) using PD-10 desalting columns (GE Healthcare, Little Chalfont, Buckinghamshire, UK). OxLDL and moxLDL were sterilized using sterile filters (0.2µm), resuspended in Tyrode's buffer and stored in the dark at 4°C. Concentrations of both types of oxidized LDL were determined using the Lowry method (3).

Clinical Cohort

Patients were classed as follows:

- Non-significant coronary artery disease (NS-CAD) if stenosis less than 50% or displayed irregularities

- Significant CAD (S-CAD) or acute coronary syndrome (ACS) classifications depended on the clinical presentation of the patient and the presence of stenosis of more than 50%. S-CAD included patients with chronic stable chest pain for more than 1 month or patients with valvular disease or heart failure. ACS included patients with unstable angina (*de novo* chest pain for < 1 month, resting angina or worsening symptoms without high sensitivity [hs]-troponin T elevation); non-ST segment elevation myocardial infarction (NSTEMI) (requiring changes in cardiac markers, including hs-troponin T elevation above the 99th percentile in addition to

recent chest pain and/or ECG changes); or ST segment elevation myocardial infarction (STEMI) treated by primary angioplasty.

Eligibility criteria

Inclusion criteria

- Patients > 18 years old

- Signed informed consent

- Angiography scheduled within the following 3 days, whatever the indication

Exclusion criteria

- Patients not able to sign the informed consent form

- Patients on anticoagulation therapy (oral or parenteral) for any reason, including heparins, fondaparinux, vitamin K antagonists, or novel oral anticoagulants

- Hemophilia or other coagulopathy

- Abnormal platelet count (< 1.5 x 10^5/µL or > 4 x 10^5/µL)

- Active neoplasia or chronic inflammatory disease

- Patients with a life expectancy of less than 3 years

- Heart transplant patients

- Active hepatitis B or C, or HIV patients

- Any contraindication to coronary angiography

Study endpoints

Primary endpoint

To demonstrate an increase in platelet ACC phosphorylation (phosphoACC) in CAD patients.

Secondary endpoints

- To correlate platelet phosphoACC with thrombin generation (ThG) markers in diseased patients.

- To determine factors predictive of increased platelet phosphoACC in the study population.

- To establish the importance of coronary and extra-coronary atherosclerotic burden in mediating an increase in platelet phosphoACC.

Estimated sample size before inclusion

Based on our preliminary data, we determined that an enrolment of 102 patients would provide a power of 80% at a significance level of 5% for detecting a difference of 0.15 AU in platelet phosphoACC in CAD patients.

Blood sampling and analysis

Human platelet isolation

Blood samples were drawn into citrated tubes (citrate phosphate dextrose adenine, S-Monovette, Sarstedt) after the sheath had been inserted at the catheterization laboratory but before any drugs had been administered. Platelet-rich plasma (PRP) was obtained by 20 minutes' centrifugation at 330 × g. Platelets were then counted with a Cell-Dyn Emerald (Abbott) and pelleted by centrifugation at 400 × g for 10 minutes in apyrase (0.5 U/mL) and eptifibatide (4µg/mL). The presence of contaminating leucocytes and platelets was tracked by flow cytometry using anti-CD45 and anti-CD41a antibodies respectively, all along the platelet isolation process from blood of 3 healthy volunteers. The platelet pellets were either immediately lyzed (400,000 platelets/µL) or stored frozen (-80°C) along with plasma samples.

Western blot analysis

The platelet pellets were lyzed in Laemmli buffer (50mM Tris-HCl, pH 6.8, 10% (v/v) glycerol, 2% (w/v) SDS, 0.01% (w/v) bromophenol blue, and 6.25% (v/v) β-mercaptoethanol). Protein extracts from whole platelet lysates for each patient were separated on polyacrylamide gels (Criterion TGX, Bio-Rad). The membranes were probed with phosphoACC (1:1000), gelsolin (1:40 000), phosphoP38, or phosphoPKC substrate (1:10 000) antibodies. HRP-conjugated

anti-rabbit (1:20 000) antibodies were diluted in 5% bovine serum albumin. Band intensities were quantified using Image J (National Institutes of Health, Bethesda, MD, USA).

Electrochemiluminescence analysis (ECLIA)

To facilitate the quantitative assessment of platelet phosphoACC in patients, we optimized the ECLIA technique from MSD. Since ACC contains biotin, phosphoACC quantification was based on the interaction between streptavidin and its biotin group. GOLD 96-well streptavidin SECTOR plate wells (MSD) were blocked for 1 hour, washed and incubated with protein extracts (1µg/µL) from the patients' platelets, which had been previously lyzed in 50mM Tris-HCl, pH 7.5, 1mM EGTA, 1mM EDTA, 1% Triton X 100, 0.27M sucrose, 0.1% β-mercaptoethanol, 0.1% SDS and a cocktail of protease and phosphatase inhibitors (1:100). The plate was incubated at room temperature with shaking for 1 hour. After washing, primary rabbit anti-phosphoACC antibody was added (1:250) and incubated overnight at 4°C with shaking. After washing, goat anti-rabbit sulfo-tag secondary antibody (1:250) was added and incubated at room temperature with shaking for 1 hour. The plates were read on the SECTOR Imager 2400 (Meso Scale Discovery) immediately after the read buffer had been added. A positive control of washed platelets from healthy volunteers stimulated with thrombin (0.5 U/mL) for 2 minutes was used as a benchmark in all experiments.

ThG, oxLDL and platelet reactivity measurement

ThG was assessed in citrated plasma by measuring D-dimer levels using the automated latex-enhanced immunoassay (HemosIL D-dimer HS, Werfen, Milan, Italy), and thrombin anti-thrombin complex (TATc) and fragment 1.2 (F1.2) using the ELISA method (Siemens Healthcare Diagnostics), according to the manufacturers' protocols. OxLDL levels were measured using the ELISA method with the 4E6 monoclonal antibody (Mercodia, Uppsala, Sweden). Platelet reactivity was also evaluated in all patients by the multiple electrode platelet aggregometry method (Multiplate® Analyzer, Roche Diagnostics).

Platelet lipid extraction and analysis

Lipids were extracted using the methyl-tert-butyl ether (MTBE) method (5,6). Platelets were transferred to a 2mL Eppendorf vial to which 100µL of water and 100µL of an Internal Standard Mix (Sciex, Nieuwekerk aan den Ijsel, Netherlands) were added. Next, 160µL of methanol and 500µL of MTBE were added. The extracts were then shaken at room temperature for 30 minutes. Then, 200µL of water was added, after which the samples were briefly vortexed and then centrifuged at 16 100 × g for 3 minutes. The upper organic layer (400µL) was then transferred to a fresh glass vial. Another 500µL of MTBE, 100µL of methanol and 100µL of water were added to the original Eppendorf vials and the extraction was repeated. The combined organic extracts were concentrated under a gentle stream of nitrogen and reconstituted in 250µL of running buffer (10mM ammonium acetate in 50:50 dichloromethane:methanol).

Lipidomic analysis was carried out using the commercial Lipidyzer platform according to the manufacturer's instructions (Sciex) (7). Lipid analysis was performed in flow-injection mode. The lipid classes were separated using differential mobility spectroscopy (7), followed by tandem mass spectrometric lipid species analysis on a QTrap 5500 (Sciex) in multiple reaction monitoring mode. The lipid species were identified and quantified based on characteristic mass spectrometric transitions. The Lipidyzer software automatically calculated lipid species concentrations. All samples were analyzed in a randomized fashion. Control and fortified plasma samples were analyzed daily as quality controls. The relative standard deviations (RSD) of the quality control samples were below 15% for all lipid classes, except for sphingomyelin, where a RSD of 25% was observed.

Experimental dataset

Atherosclerotic mouse model

We studied platelet phosphoACC in 20 to 24-week-old female SR-B1$^{flox/flox}$/ApoE$^{-/-}$ hypercholesterolemic mice. The animals were randomized to receive either 24 weeks of standard chow diet or 12 weeks of a chow diet followed by 12 weeks of a Western diet (1.25% cholesterol, 16% cocoa butter, U8220 version 151, Safe, Augy, France), while 24-week-old female C57BL6 mice were similarly randomized as above and were used as controls. On the day of sacrifice, the mice were bled under ketamine and xylazine anesthesia from the retro-orbital plexus. Blood was processed for platelet isolation as described below. All animals were kept in a 12:12 hour light/dark cycle with free access to food and water. Animal procedures and protocols were approved by local authorities (Comité d'éthique facultaire pour l'expérimentation animale, 2012/UCL/MD/003 and 2016/UCL/MD/027) and performed in accordance with the "Guide for the Care and Use of Laboratory Animals" published by the US National Institutes of Health (publication no. 85-23, revised 1996).

Murine platelet preparation

Blood was collected in EDTA-K tubes (Sarstedt) containing citrate-dextrose solution (1:8), apyrase (1 U/mL) and eptifibatide (4µg/mL). PRP was obtained by centrifugation at 100 × g for 5 minutes. Platelets were counted with a Cell-Dyn Emerald (Abbott). After centrifugation at 400 × g for 5 minutes, platelet pellets were lyzed in Laemmli buffer for Western blotting (400,000 platelets/µL).

Murine atherosclerosis analysis

Murine hearts and aortic roots were isolated, embedded in optimum cutting temperature (OCT) compound (Tissue-Tek, Sakura, Torrance, CA, USA) and frozen to quantify atherosclerosis at the aortic sinus. Cryostat sections (10µm) were then cut, fixed with 4% formaldehyde, stained with 0.3% oil-red-O and counterstained with hematoxylin. Lesion sizes were quantified using

a Leica SCN400 image analysis program and calculated by dividing the surface area of the lesion by the total surface area of the vessel.

Ex vivo *human platelet signaling*

Human platelet preparation

Blood from healthy volunteers was processed as previously described (8). Platelet pellets were washed in modified Tyrode's buffer (135mM NaCl, 12mM $NaHCO_3$, 2.9mM KCl, 0.3mM Na_2HPO_4, 1mM $MgCl_2$, 10mM Hepes, 5mM D-glucose, 0.35% bovine serum albumin, pH 7.4, 37°C) before being suspended to a density of 4.0 x 10^5 platelets/μL. Platelets were treated with selected cytokines for 5 minutes at 37°C and stimulated (in 2mM Ca^{2+}) with thrombin, collagen or ADP for 2 minutes at 37°C, or coxLDL or moxLDL for 5 minutes at 37°C (as stated in the figure legends), prior to lysis with Laemmli buffer for western blot analysis. Gelsolin was used as the loading control.

References

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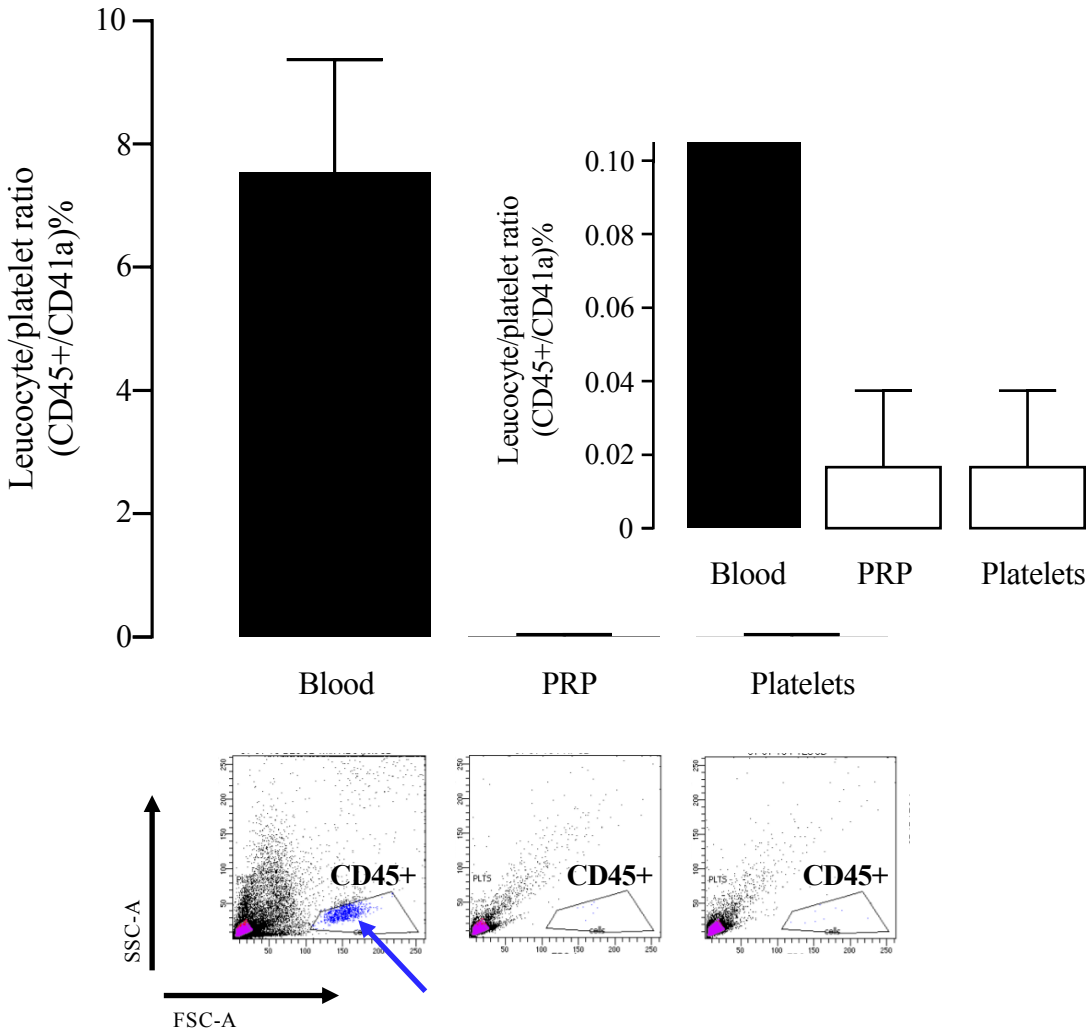
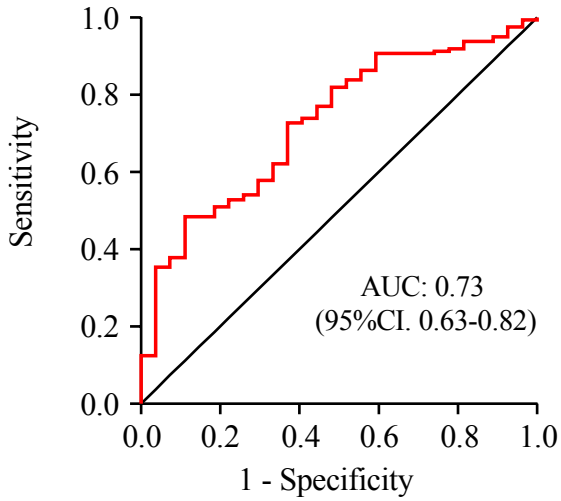
Supplemental Tables

Supplemental Figure 1



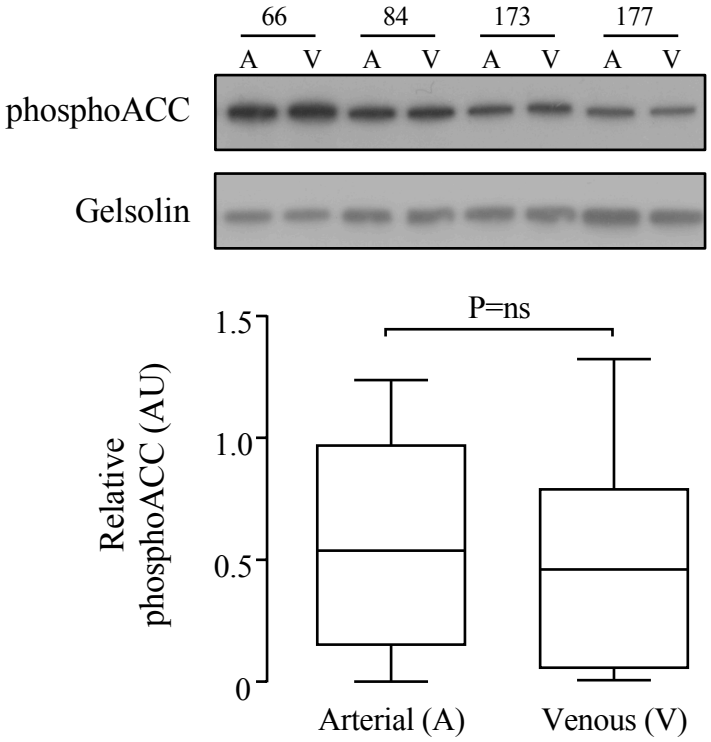
Supplemental Figure 1. Leukocytes in platelet preparation.
Blood samples from healthy volunteers (n=3) were processed for platelet isolation. Leucocytes (CD45+) and platelets (CD41a+) were detected by flow cytometry in whole blood samples, platelet rich-plasma et platelet samples (used for phosphoACC analysis). Box-plots represents mean ± SD. Blue arrow indicates leukocyte population
FSC-A: forward-scattered light-area, PRP: platelet rich-plasma, SSC-A: side-scattered light-area.

Supplemental Figure 2



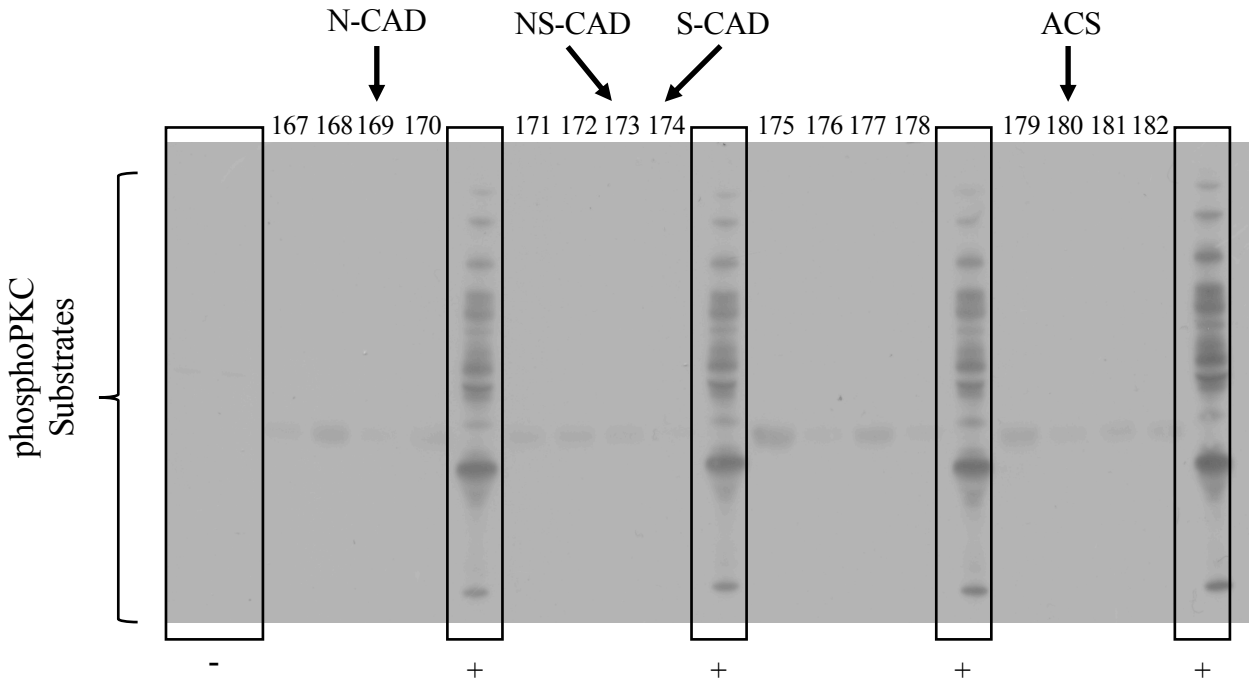
Supplemental Figure 2. ROC curve analysis of platelet phosphoACC (CAD vs N-CAD).
ROC curve analysis of platelet phosphoACC (CAD vs. N-CAD) to estimate the optimal threshold (0.5 AU) for a maximal sensitivity (48 %) and specificity (90 %).
AUC: area under the curve, CAD: coronary artery disease, CI: confidence interval, N-CAD: absence of coronary artery disease, ROC: receiver operating characteristic.

Supplemental Figure 3



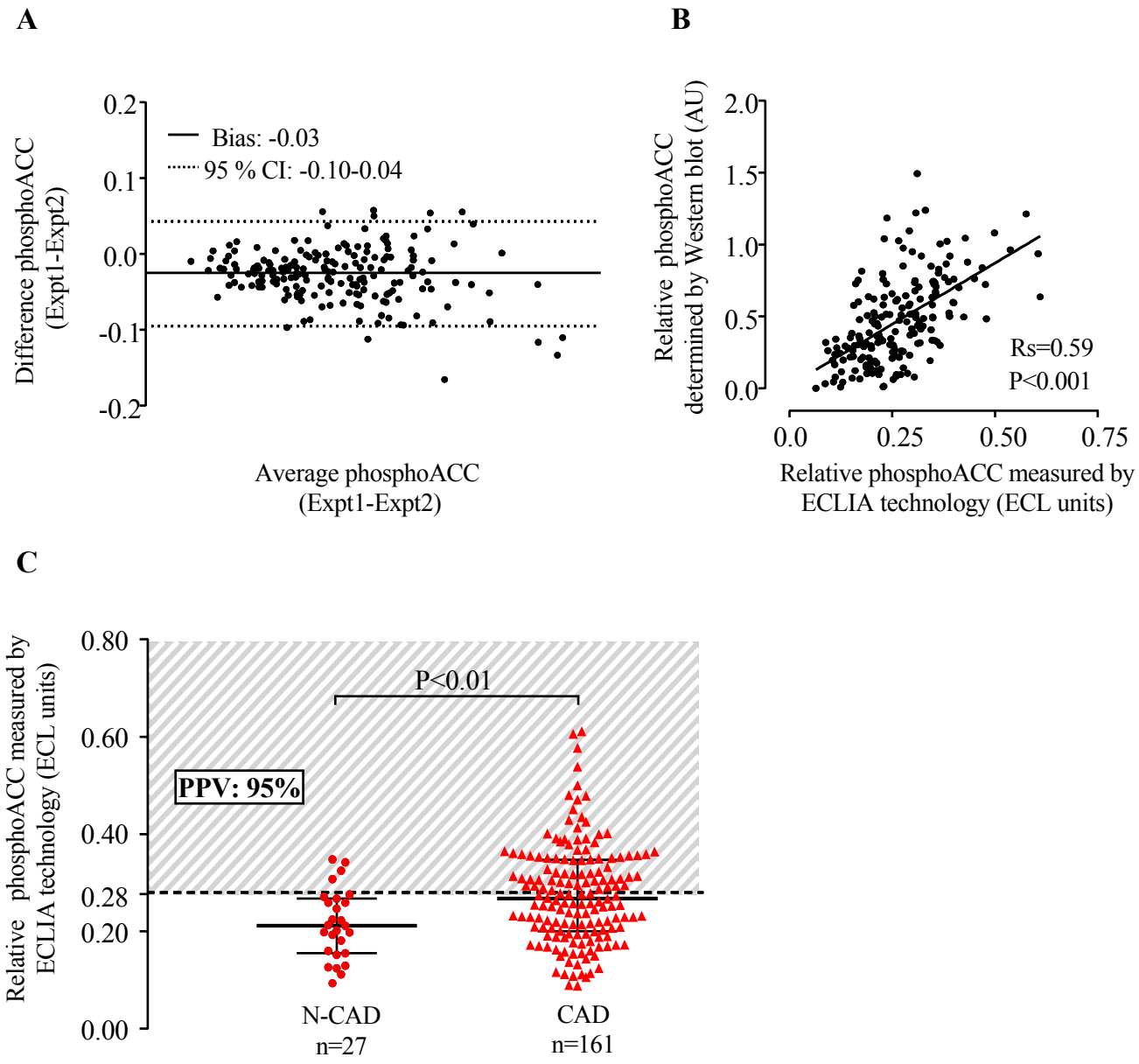
Supplemental Figure 3. Arterio-venous difference in platelet ACC phosphorylation.
Platelet phosphoACC was detected in platelets from venous (V) and arterial (A) blood samples drawn simultaneously from central venous and arterial access site respectively, during left and right catheterization (in 8 patients from ACCTHEROMA cohort). Representative western blot analysis is shown on the top panel and quantification, on bottom panel. Gelsolin was used as loading control. Box-and-whisker plots represents median (interquartile range: P25-P75) and extremes of the distribution.
A: arterial, A.U.: arbitrary unit, V: venous.

Supplemental Figure 4



Supplemental Figure 4. PKC substrates phosphorylation in ACCTHEROMA cohort.
Representative western blot of platelet phosphoPKC substrates of 16 consecutive patients (their corresponding phosphoACC is shown in Figure 3A). (-) negative control corresponding to washed unstimulated platelets from healthy volunteers. (+) positive controls corresponding to washed platelets from healthy volunteer, stimulated *ex-vivo* with thrombin 0.5 U/ml for 2 min.
ACS: acute coronary syndrome, CAD: coronary artery disease, M: molecular weight marker, N-CAD: absence of coronary artery disease, NS-CAD: coronary artery disease without significant stenosis, phosphoPKC substrates: phosphorylation of protein kinase C substrates, S-CAD: coronary artery disease with at least one lesion > 50 %.

Supplemental Figure 5



Supplemental Figure 5. Detection of platelet phosphoACC by electrochemiluminescence.
(A) Bland-Altmann analysis of two separate quantifications (Expt1-Expt2) of phosphoACC by electrochemiluminescence. (B) Correlation between western blot quantifications and electrochemiluminescence results for platelet phosphoACC (Rs=Spearman coefficient). (C) Electrochemiluminescence quantification of platelet phosphoACC in N-CAD and CAD patients. PPV of optimal threshold (0.28 ECL units for a sensitivity of 47 % and specificity of 85 %) for CAD is indicated on the graph. Red dots (N-CAD, reference population) or triangles (CAD patients) represent individual values. Medians with interquartile range are presented.
AU: arbitrary unit, CAD: coronary artery disease, CI: confidence interval, ECL: electrochemiluminescence, ECLIA: electrochemiluminescence immunoassay, Expt: experiment, N-CAD: absence of coronary artery disease, PPV: positive predictive value.

Supplemental Table 1: Prediction of ischemic events by platelet phosphoACC in multivariate Cox regression analysis

	Univariable Analysis		Multivariable Analysis	
Variable	**HR (95% CI)**	**P value**	**HR (95% CI)**	**P value**
Previous history of MI	**1.55 [1.15-2.08]**	**0.004**	**3.17 [1.38-7.29]**	**0.006**
ASCVD score	**1.37 [1.00-1.88]**	**0.048**	**10.07 [1.88-54.02]**	**0.007**
ACS at inclusion time	1.42 [1.01-1.99]	0.045		
Platelet count ($x10^3$) /µl	1.45 [1.03-2.04]	0.034		
Creatinine (mg/dl)	0.90 [0.46-1.76]	0.76		
HsCRP (log-transformed)	1.41 [0.96-2.09]	0.083		
Non-HDL (mg/dL)	0.82 [0.54-1.25]	0.35		
TG/HDL-C ratio (log-transformed)	0.90 [0.6-1.36]	0.63		
D-dimer (log-transformed)	1.36 [1.01-1.83]	0.043		
Platelet phosphoACC	**1.44 [1.02-2.03]**	**0.036**	**3.66 [1.11-12.12]**	**0.034**

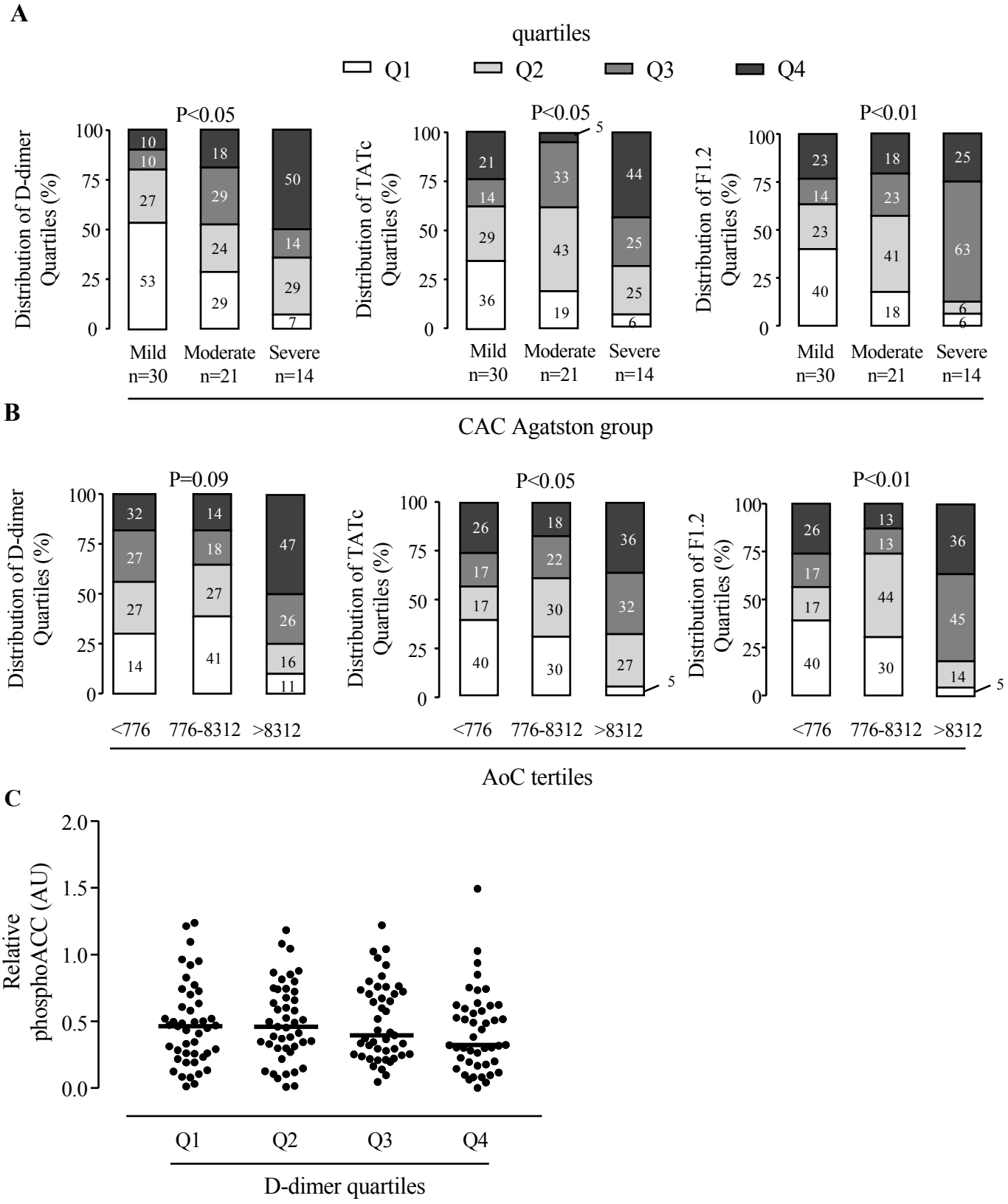
Ischemic events include cardiovascular death and recurrent myocardial infarction/revascularization procedures. Statistical significance when $p<0.05$, CI: confidence interval, HR: hazard ratio. ACS: Acute Coronary Syndrome, ASCVD score: 10-year risk of atherosclerotic cardiovascular disease (2013 ACC/AHA Guideline on the Assessment of Cardiovascular Risk), hsCRP: high-sensitive C-reactive protein, HDL-C: high-density lipoprotein cholesterol, MI: myocardial infarction, TG: triglycerides.

Supplemental Table 2: Multivariable linear regression model of factors associated with CAC

Variable	Univariable			Multivariable (global $R^2 = 0.47$, $p < 0.001$)	
	R^2	**β Coefficient** (95% CI)	**P value**	**β Coefficient** (95% CI)	**P value**
Age	**0.219**	**0.03 (0.01-0.04)**	**<0.001**	**0.21 (0.01-0.04)**	**0.011**
Sex (male)	0.025	0.24 (-0.14-0.61)	0.22		
Diabetes	0.001	0.05 (-0.40-0.50)	0.82		
Hypertension	0.137	0.58 (0.21-0.96)	0.003		
Non-HDLc (mg/dl)	0.004	-0.001(-0.005-0.003)	0.62		
D-dimer (log-transformed)	**0.127**	**0.95 (0.17-1.72)**	**0.018**	**0.87 (0.17-1.56)**	**0.017**
Platelet phosphoACC	**0.088**	**2.27 (0.38-4.16)**	**0.019**	**3.61 (1.57-5.65)**	**0.001**

Statistical significance when P<0.05. CAC: coronary artery calcification, CI: confidence interval, HDLc: high-density lipoprotein cholesterol.

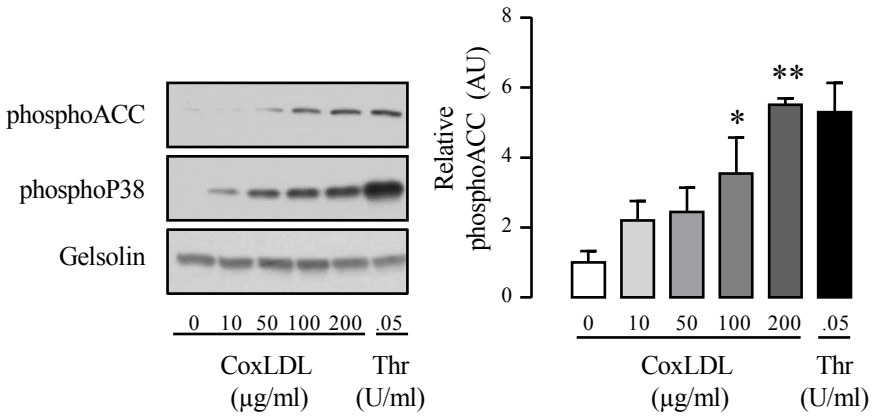
Supplemental Figure 6



Supplemental Figure 6. Association between thrombin generation markers and the severity of atherosclerotic burden.

(A, B) Distribution of D-dimers, TATc and F1.2 quartiles among CAC Agatston score groups (A) or AoC score tertiles (B). CAC was classified as mild, moderate or severe if the CAC Agatston score was below 100, between 100-400 or above 400, respectively. (C) Correlation between D-dimer quartiles and platelet phosphoACC in the entire cohort (P=ns). Black dots represent individual values. Medians are presented. AoC: extra-coronary calcification score, AU: arbitrary unit, CAC: coronary artery calcification, F1.2: fragment 1.2, Q: quartile, TATc: thrombin anti-thrombin complex.

Supplemental Figure 7



Supplemental Figure 7. Platelet phosphoACC-induced by CoxLDL.
Platelet phosphoACC and phosphoP38 were detected in washed platelets from healthy volunteers, after treatment with varying concentrations of CoxLDL (10-200 µg/ml) for 5 min or with thrombin (0.05 U/ml) for 2 min, which was used as reference. Gelsolin was used as loading control. Representative western blots and quantifications are shown. Data are expressed as mean±SEM. Significance was determined by 1-way ANOVA with bonferroni post hoc analysis. *P<0.05, **P<0.01, relative to unstimulated platelets.
AU: arbitrary unit, CoxLDL: copper-oxidized low-density lipoprotein, Thr: thrombin

Supplemental Table 3. Baseline characteristics of the lipidomics cohort

	All N = 31	Low phosphoACC N = 12	High phosphoACC N = 19	P
Clinical Characteristics				
Age, years	61.4 ± 10.6	56.2 ± 10.4	64.7 ± 9.5	0.025*
Male sex, n (%)	19 (61.3)	6 (50)	13 (68.4)	0.52†
BMI, kg/m^2	28.4 ± 4.2	27.3 ± 4.7	29.1 ± 3.9	0.25
Hypertension, n (%)	15 (48.4)	5 (41.7)	9 (52.6)	0.82†
Smoking, n (%)	18 (58.1)	8 (66.7)	10 (52.6)	0.70†
Diabetes, n (%)	6 (19.4)	1 (8.3)	5 (26.3)	0.45†
Prior history of CAD, n (%)	10 (32.3)	0 (0)	10 (52.6)	0.004*,†
- MI, n (%)	7 (22.6)	0 (0)	7 (36.8)	0.038*,†
- PCI, n (%)	10 (32.3)	0 (0)	10 (32.3)	0.004*,†
- CABG, n (%)	1 (3.2)	0 (0)	1 (5.3)	> 0.99†
Aortic valve disease, n (%)	3 (9.7)	2 (16.7)	1 (5.3)	0.66†
Mitral valve disease, n (%)	2 (6.5)	2 (16.7)	0 (0)	0.28†
ACS, n (%)	12 (38.7)	0 (0)	12 (63.2)	<0.001*,†
Lab results				
Creatinine, (mg/dL)	1.0 (0.8-1.2)	0.9 (0.8-1.3)	1.0 (0.9-1.2)	0.68
CRI, n (%)	2 (6.5)	2 (16.7)	0 (0)	0.30†
Total cholesterol, (mg/dL)	167 ± 47	175 ± 66	163 ± 34	0.51
Non-HDL, (mg/dL)	119 ± 42	122 ± 54	117 ± 35	0.78
Triglycerides, (mg/dL)	125 ± 54	98 ± 44	141 ± 53	0.034*
Medication				
ACEi/ARB, n (%)	13 (41.9)	4 (33.3)	9 (47.4)	0.70†
Beta-blockers, n (%)	14 (45.2)	5 (41.7)	9 (47.4)	> 0.99†
Lipid-lowering treatment, n (%)	18 (58.1)	4 (33.3)	14 (73.7)	0.065†
Aspirin, n (%)	23 (74.2)	6 (50)	17 (89.5)	0.044*,†
Dual antiplatelet therapy, n (%)	6 (19.4)	0 (0)	6 (31.6)	0.074†
- Clopidogrel, n (%)	2 (6.5)	0 (0)	2 (10.5)	0.74†
- Ticagrelor, n (%)	3 (9.7)	0 (0)	3 (15.8)	0.43†
- Prasugrel, n (%)	1 (3.2)	0 (0)	1 (5.3)	>0.99†
Platelet phosphoACC	0.8 (0.1-1.0)	0.10 (0.08-0.13)	0.96 (0.85-1.03)	<0.001

Values are mean ± SD, n (%) or median (25th – 75th percentile). ACEi/ARB: angiotensin converting enzyme inhibitor/angiotensin receptor blocker, ACS: acute coronary syndrome, BMI: body mass index, CABG: coronary artery bypass graft, CAD: coronary artery disease, CRI: chronic renal insufficiency, hsCRP: high sensitivity C reactive protein, LDL: low-density lipoprotein, MI: myocardial infarction, PCI: percutaneous coronary intervention, P: P value. Statistical differences between groups are denoted as * between low- and high-phosphoACC. † denotes calculated mid-P value (Fischer's test).

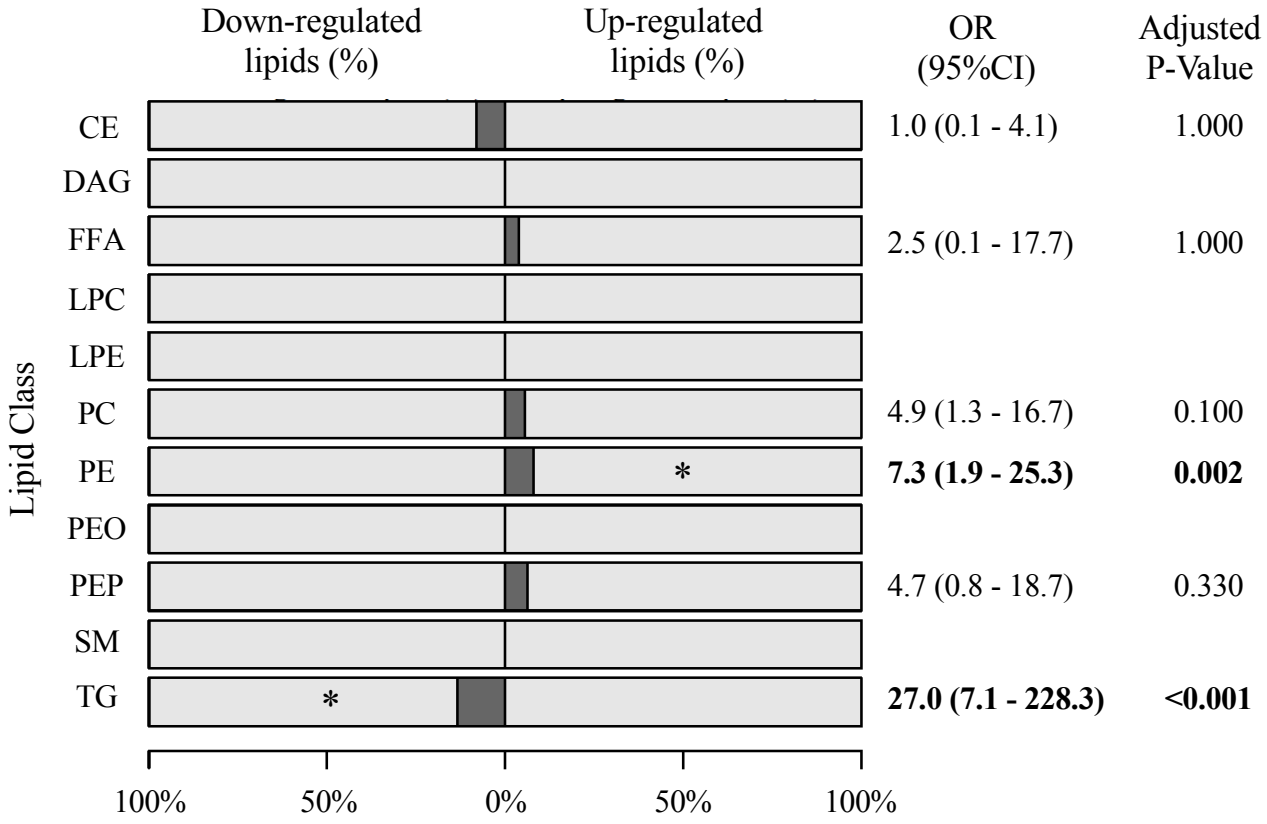
Supplemental Table 4. List of the significantly down-regulated TG lipid species in platelets with increased phosphoACC.

Lipid Species	N data	Log FC	P	Adj P
TAG40.0.FA16.0	23	-1.383	0.002	0.038
TAG42.0.FA14.0	30	-1.657	0.001	0.027
TAG42.0.FA16.0	31	-1.824	0.000	0.025
TAG42.1.FA14.0	20	-1.351	0.000	0.025
TAG42.1.FA16.0	26	-1.262	0.003	0.042
TAG42.1.FA18.1	28	-1.493	0.001	0.025
TAG44.0.FA14.0	31	-1.588	0.001	0.025
TAG44.0.FA16.0	31	-1.438	0.002	0.038
TAG44.0.FA18.0	30	-1.301	0.001	0.031
TAG44.1.FA12.0	31	-1.416	0.004	0.043
TAG44.1.FA14.0	31	-1.635	0.000	0.025
TAG44.1.FA14.1	18	-1.041	0.004	0.047
TAG44.1.FA16.0	31	-1.319	0.001	0.025
TAG44.1.FA16.1	31	-1.289	0.002	0.038
TAG44.1.FA18.1	31	-1.480	0.000	0.025
TAG44.2.FA12.0	20	-1.162	0.002	0.038
TAG44.2.FA16.0	31	-1.380	0.000	0.025
TAG44.2.FA16.1	21	-0.866	0.003	0.041
TAG44.2.FA18.1	29	-1.417	0.001	0.025
TAG44.2.FA18.2	31	-1.514	0.001	0.025
TAG44.3.FA18.2	14	-0.915	0.001	0.025
TAG45.0.FA14.0	29	-1.320	0.001	0.025
TAG45.0.FA15.0	27	-1.311	0.000	0.025
TAG45.0.FA16.0	31	-1.472	0.000	0.025
TAG45.1.FA15.0	18	-1.139	0.000	0.025
TAG45.1.FA16.0	24	-0.945	0.001	0.027
TAG45.1.FA18.1	23	-0.983	0.001	0.028
TAG46.0.FA14.0	31	-1.171	0.002	0.040
TAG46.0.FA16.0	31	-1.140	0.004	0.043
TAG46.0.FA18.0	31	-1.232	0.003	0.040
TAG46.1.FA14.1	31	-1.167	0.003	0.042
TAG46.1.FA18.0	30	-1.301	0.001	0.025
TAG46.1.FA18.1	31	-1.180	0.003	0.042
TAG46.2.FA14.0	31	-1.273	0.001	0.025
TAG46.2.FA18.1	31	-1.209	0.001	0.027
TAG46.2.FA18.2	31	-1.188	0.005	0.050
TAG46.3.FA14.0	20	-1.148	0.002	0.038
TAG46.3.FA14.1	16	-0.802	0.004	0.047
TAG46.3.FA16.0	26	-0.900	0.004	0.047
TAG46.3.FA16.1	26	-0.844	0.003	0.041
TAG46.3.FA18.1	30	-1.222	0.001	0.028
TAG46.3.FA18.2	30	-1.196	0.001	0.027
TAG46.4.FA18.2	16	-1.056	0.000	0.025
TAG47.0.FA14.0	29	-0.968	0.004	0.047
TAG47.0.FA15.0	31	-1.103	0.002	0.038
TAG47.0.FA16.0	31	-1.166	0.002	0.038
TAG47.0.FA17.0	31	-0.787	0.001	0.031
TAG47.1.FA14.0	31	-1.106	0.004	0.043
TAG47.1.FA15.0	31	-1.116	0.002	0.040

TAG47.1.FA16.0	31	-1.094	0.003	0.040
TAG47.1.FA16.1	31	-0.839	0.002	0.038
TAG47.1.FA17.0	24	-0.910	0.002	0.038
TAG47.1.FA18.1	31	-1.257	0.001	0.025
TAG47.2.FA14.0	26	-1.026	0.001	0.025
TAG47.2.FA15.0	29	-1.091	0.000	0.025
TAG47.2.FA16.1	30	-1.222	0.000	0.025
TAG47.2.FA18.1	30	-0.954	0.001	0.027
TAG47.2.FA18.2	29	-1.042	0.001	0.025
TAG48.0.FA18.0	31	-0.932	0.005	0.050
TAG48.2.FA18.2	31	-0.962	0.003	0.040
TAG48.3.FA16.0	31	-0.976	0.005	0.048
TAG49.0.FA15.0	31	-0.836	0.004	0.047
TAG49.2.FA18.2	31	-0.977	0.003	0.041
TAG49.3.FA18.3	25	-0.871	0.002	0.040
TAG50.2.FA18.0	31	-0.722	0.003	0.042
TAG50.4.FA14.1	31	-0.737	0.003	0.040

Analysis was performed by a multivariate logistic regression model. Statistical significance was determined using the Benjamini-Hochberg correction procedure with a false discovery rate < 0.05. TG lipid species are arranged from top to bottom in order of increasing number of carbons in the TG structure. Only TG with significantly adjusted P values (<0.05) are shown (n=66).
Adj P: adjusted P value, FC: fold-change, n: number of data analyzed, P: P value.

Supplemental Figure 8



Supplemental Figure 8. Lipid class enrichment analysis from the lipidomic study.
Bars (dark grey) represent lipid classes with differentially regulated lipid species with respect to increased platelet phosphoACC. * denotes classes with significantly up- or down-regulated lipid species. OR and adjusted P-value derived from Fisher's exact test are represented.
CE: cholesterol ester, CI: confidence interval, DAG: diacylglycerol, FFA: free fatty acid, LPC: lysophosphatidylcholine, LPE: lysophosphatidylethanolamine, OR: odds ratio, PC: phosphatidylcholine, PE: phosphatidylethanolamine, PEO: plasmenyl phosphatidylethanolamine, PEP: plasmalogen phosphatidylethanolamine, SM: sphingomyelin, TG: triglycerides