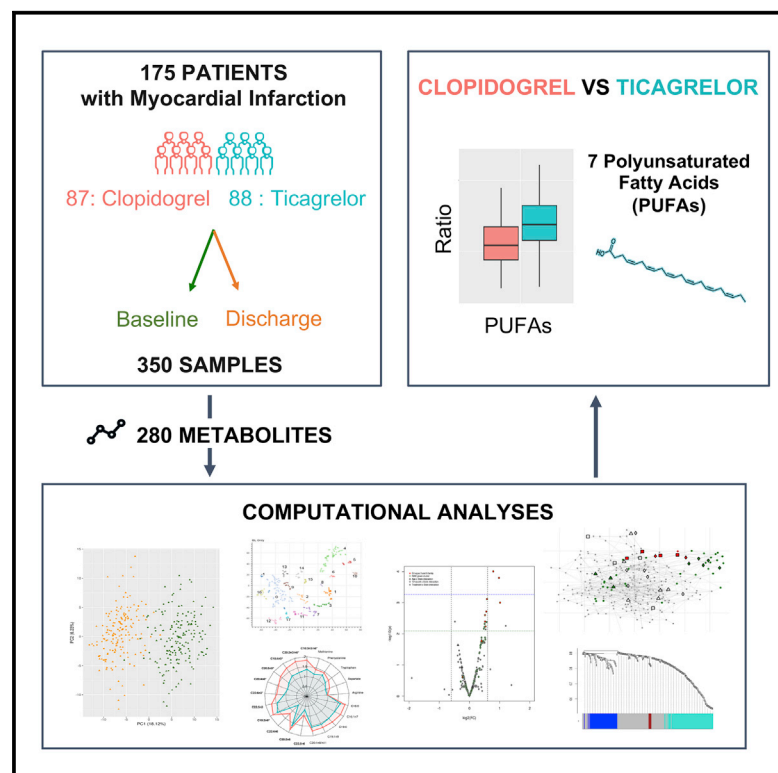


Differential modulation of polyunsaturated fatty acids in patients with myocardial infarction treated with ticagrelor or clopidogrel

Graphical abstract



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In brief

Samman et al. present a metabolomics study of 350 samples from participants hospitalized for acute coronary syndrome. The analyses reveal that polyunsaturated fatty acids levels are upregulated in patients taking ticagrelor compared to clopidogrel, highlighting a pathway of potential clinical interest in the recovery path from myocardial infarction.

Highlights

- We detect an extreme metabolomic signature of myocardial infarction (MI) in plasma
- Polyunsaturated fatty acids (PUFAs) are upregulated in patients taking ticagrelor
- PUFA metabolism is a pathway of clinical interest in the recovery path from MI
- Data science methods detect biologically meaningful patterns in metabolite signals



Article

Differential modulation of polyunsaturated fatty acids in patients with myocardial infarction treated with ticagrelor or clopidogrel

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SUMMARY

Untargeted metabolomics is used to refine the development of biomarkers for the diagnosis of cardiovascular disease. Myocardial infarction (MI) has major individual and societal consequences for patients, who remain at high risk of secondary events, despite advances in pharmacological therapy. To monitor their differential response to treatment, we performed untargeted plasma metabolomics on 175 patients from the platelet inhibition and patient outcomes (PLATO) trial treated with ticagrelor and clopidogrel, two common P₂Y₁₂ inhibitors. We identified a signature that discriminates patients, which involves polyunsaturated fatty acids (PUFAs) and particularly the omega-3 fatty acids docosahexaenoate and eicosapentaenoate. The known cardiovascular benefits of PUFAs could contribute to the efficacy of ticagrelor. Our work, beyond pointing out the high relevance of untargeted metabolomics in evaluating response to treatment, establishes PUFA metabolism as a pathway of clinical interest in the recovery path from MI.

INTRODUCTION

Myocardial infarction (MI) is a type of acute coronary syndrome (ACS) resulting from sudden rupture of plaque inside the coronary artery. It is the leading cause of mortality in most developed countries. Despite advances in treatments, patients with MI remain at a high risk of early and late secondary events. There is a great interest in finding prompt biomarkers of myocardial ischemia to help early diagnostic uncertainty, which can result in treatment delays and increased deaths. The rapid detection of minor metabolic changes due to disease and therapy may allow earlier diagnosis and better prognosis, respectively.

Metabolomics is a powerful tool in cardiovascular research¹ and has the potential to detect prompt and subtle ischemic changes before definitive injury to the heart muscle. Metabolomics can also inform response to therapy by providing molecular signatures of the effect of drugs on the heart.² It allows a systemic approach to cardiac pathologies.³ Untargeted metabolic profiling is a comprehensive approach that uses platform measurements of a large number of metabolites from different meta-

bolic pathways. Biomarker identification through plasma and serum metabolomics is a proven approach in cardiovascular research. Several studies searched for metabolites that could be identified as risk factors for MI^{4,5} and severity biomarkers of coronary artery disease.^{6,7} Furthermore, studies have shown that lipid metabolism is altered in MI;^{8,9} however, most studies remain limited in the number of patients and/or metabolites tested.^{10–14} Furthermore, age has been found to influence metabolome profiles and to impact pathophysiology of cardiovascular diseases^{15,16,17} but has not been taken into account in assessing disease severity and response to treatment.

In this study, we performed untargeted plasma metabolomics profiling on 175 patients with ST-segment elevation myocardial infarction (STEMI) from the platelet inhibition and patient outcomes (PLATO) trial.¹⁸ Each patient has been sampled twice, at baseline and at hospital discharge. Patients were treated by percutaneous coronary intervention (PCI) and received either one of two commonly used P₂Y₁₂ receptor inhibitors for prevention in ACS, ticagrelor or clopidogrel, in combination with aspirin.¹⁹ Ticagrelor, a more potent P₂Y₁₂ inhibitor, reduces the



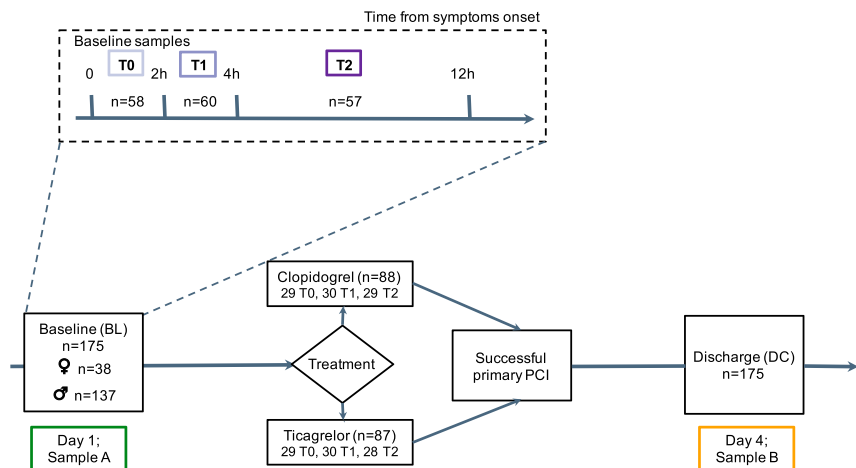


Figure 1. Study design and participant flow

Study involves 175 patients with STEMI successfully treated by primary PCI, randomized (1:1) to either clopidogrel or ticagrelor (PLATO trial; NCT00391872). All samples were taken within 12 h of symptoms onset with patients selected from three time-from-symptoms-onset to randomization categories (time points): T0, 0–2 h; T1, 2–4 h; T2, 4–12 h. Discharge plasma was sampled at hospital discharge or 4 days after the baseline sample, whichever came first. ♀/♂, female/male; PCI, percutaneous coronary intervention; PLATO, Platelet Inhibition and Patient Outcome; STEMI, ST-elevation myocardial infarction.

occurrence of ischemic events compared with clopidogrel in ACS¹⁸ and is assumed to exert pleiotropic effects through improved endothelial function,²⁰ adenosine,²¹ and other yet-unknown mechanisms. Our statistical and machine learning analyses of hundreds of metabolite concentrations in 350 samples revealed clear and global metabolome changes at baseline compared to steady state at discharge, unveiling the complete molecular signature of the acute stage of MI in human plasma. We show that this biosignature is influenced by age and physician-reported sex. Importantly, we report a differential association of polyunsaturated fatty acids (PUFAs) according to ticagrelor and clopidogrel treatment, detected in the 4 days following initiation of treatment.

RESULTS

Patient characteristics and study design

A total of 180 participants with STEMI with plasma samples available were selected for the metabolomics sub-study from the full PLATO trial population, including 142 men (79%) and 38 women (21%). To obtain a homogeneous population in terms of disease severity, we ensure that those with other major cardiac events were not selected (Figure S1). To minimize variability, stratification to clopidogrel versus ticagrelor was done in a 1:1 ratio and participants were matched by age, physician-reported male or female status (herein referred to as “sex”), diabetes mellitus, and dyslipidemia. Plasma samples were collected at baseline before P₂Y₁₂ inhibitor loading and PCI and at hospital discharge or at day 4 post-STEMI, whichever came first. To further account for the time-sensitive nature of metabolome elution from the injured myocardium, we stratified baseline samples from three time-from-symptoms-onset to randomization intervals (T0, less than 2 h; T1, 2–4 h; T2, 4–12 h; Figure 1), matched between the ticagrelor and clopidogrel arms (30 participants in each time interval in each arm). This approach was taken to obviate the need to have dense serial measurements in the same participant but to simulate a temporal profile of the human STEMI metabolome. Three participants were excluded due to insufficient quantity of plasma, and two patients were

identified as statistical outliers in the ticagrelor group, leaving 88 clopidogrel and 87 ticagrelor participants available for final analyses (Figure S2). The baseline characteristics and past medical history of those treated with clopidogrel and ticagrelor were similar (Table 1).

The biosignature of myocardial infarction is influenced by age, sex, and time from onset

We used untargeted metabolomics to ensure a wider look at human metabolic pathways, with chemically diverse metabolites from different classes. We see a clear metabolic signature of the acute stage of MI, with the levels of 72.1% (202/280) of metabolites studied differentially modulated between baseline and discharge states (model 1; false discovery rate [FDR] < 1%). We observed an increase in fatty acids levels, such as stearidonate (C18:4n3), dihomo-linoleate (C20:2n6), 10-nonadecenoate (C19:1n9), and palmitoleate (C16:1n7), as well as a marked decrease of amino acids levels, such as arginine, aspartate, tryptophan, and serine at baseline, which represent acute disease state (Figure 2B). The importance of fatty acid and amino acids metabolism/biosynthesis pathways were further highlighted by co-expression network analysis using weighted gene co-expression network analysis (WGCNA)²² followed by a metabolite set enrichment analysis (Methods S1; Figure S3; Table S2). WGCNA analyses showed that the metabolite signature of reperused STEMI differs with the time from symptoms onset (Methods S1; Figure S4), and significant disease state by time point interaction effects (model 1; FDR < 5%) were seen (Table S1). Analyses of time points distribution revealed that it was significantly confounded by age (Figure S4), such that participants of greater age were overrepresented in T0 (<2 h) compared with T1 and T2 (Kolmogorov-Smirnov test; Figure S2E). However, most interaction effects remain significant for patients from 49 to 61 years old, a subset of the cohort in which age is matched across time points. The age of patient itself was also associated with the metabolic signature of STEMI acute state, with significant disease state by age interactions (model 2a; FDR < 5%) for 10 metabolites (Table S1; Figure S4), revealing that temporal metabolite patterns post-STEMI differ according to age (Methods S1). Sex was also associated with the STEMI acute state biosignature, with significant disease state by sex interactions (model 2b; FDR < 5%) observed for four metabolites,

Table 1. Patient characteristics of matched population

	Clopidogrel (n = 90)	Ticagrelor (n = 90)	p value ^b
Demographics			
Age ^a , median (IQR), years	57 (52, 65)	57 (52, 64)	0.99
Physician-reported sex ^a (male), no. (%)	71 (78.9)	71 (78.9)	1.00
Cardiovascular risk factors, no. (%)			
Diabetes mellitus ^a	10 (11.1%)	10 (11.1%)	1.00
Dyslipidemia ^a	32 (35.6%)	32 (35.6%)	1.00
Current cigarette use	55 (61.1%)	50 (55.6%)	0.45
Hypertension	51 (56.7%)	51 (56.7%)	1.00
Medical history, no. (%)			
Previous MI	10 (11.1%)	5 (5.6%)	0.18
Previous PCI	7 (7.8%)	2 (2.2%)	0.17
Previous CABG	1 (1.1%)	1 (1.1%)	1.00
Previous CVA	2 (2.2%)	0 (0%)	0.50
Dyspnea	5 (5.6%)	4 (4.4%)	1.00
Congestive heart failure	0 (0%)	1 (1.1%)	1.00
COPD	2 (2.2%)	3 (3.3%)	1.00
Peripheral arterial disease	3 (3.3%)	4 (4.4%)	1.00
Chronic renal disease	1 (1.1%)	1 (1.1%)	1.00
Gout	1 (1.1%)	2 (2.2%)	1.00
Asthma	2 (2.2%)	0 (0%)	0.50
Presenting characteristics, median (IQR)			
Weight, kg	84 (73, 93)	82 (70, 90)	0.16
Body mass index, kg/m ²	28 (26, 31)	27 (25, 30)	0.06
Systolic BP, mmHg	132 (119, 145)	134 (120, 150)	0.34
Diastolic BP, mmHg	80 (70, 90)	80 (72, 90)	0.89
Heart rate, bpm	75 (65, 90)	75 (67, 82)	0.74

bpm, beats per minute; BP, blood pressure; CABG, coronary artery bypass graft; COPD, chronic obstructive pulmonary disease; CVA, cerebrovascular accident; IQR, interquartile range; kg, kilogram; MI, myocardial infarction; no., number of participants; PCI, percutaneous coronary intervention; years, years

^aParticipants were matched for these covariates. Data are presented for the intent-to-treat population.

^bFrom a two-sample t test.

including three sulfated sex hormones (21-hydroxypregnenolone disulfate, pregnenolone sulfate, and pregn-steroid monosulfate). One additional sulfated sex hormone was measured in our dataset, pregnen-diol disulfate, which shows a sex effect in both states, yielding a significant main effect of sex (model 2b; FDR = 0.00738). Of note, the sharp imbalance between male and female (4:1) in our cohort might lead to lower power to detect sex-specific effects in this study.

Clopidogrel and ticagrelor are differentially associated with metabolome changes

Our analyses revealed nine metabolites exhibiting discharge levels that differed significantly according to assigned treatment (clopidogrel versus ticagrelor), after accounting for their baseline value. To estimate the intra-individual change in raw metabolite concentration, we computed discharge on baseline ratio values for each metabolite. Both the normalized metabolite value and intra-individual change in metabolite levels were predictive of treatment (FDR < 5%; Table 2; Figure 3). Seven were fatty acids,

all of which were found in the PUFAs omega-3/6 biosynthesis pathways. More specifically, participants treated with ticagrelor had significantly higher plasma levels of linolenate α or γ (C18:3n3/6), dihomo-linolenate (C20:3n3/6), stearidonate (C18:4n3), docosahexaenoate (DHA) (C22:6n3), eicosapentaenoate (EPA) (C20:5n3), adrenate (C22:4n6), and arachidonate (ARA) (C20:4n6) at discharge compared with clopidogrel-treated participants (Figure 3). The estimated change in ratio values is modest but goes above 1.5 for adrenate and EPA. These fatty acids were increased at baseline compared to discharge, but before administration of the drugs, we see no significant difference between participants assigned to distinct treatment groups, as expected. Compared to the top metabolites that are highly modulated in STEMI acute state, the difference between clopidogrel and ticagrelor groups in the change in metabolite concentration across states is generally more pronounced for the PUFAs (Figure 3B). Three metabolites are specific to the omega-3 metabolism pathway (DHA, EPA, and arachidonate), although two are specific to the omega-6 pathway (ARA

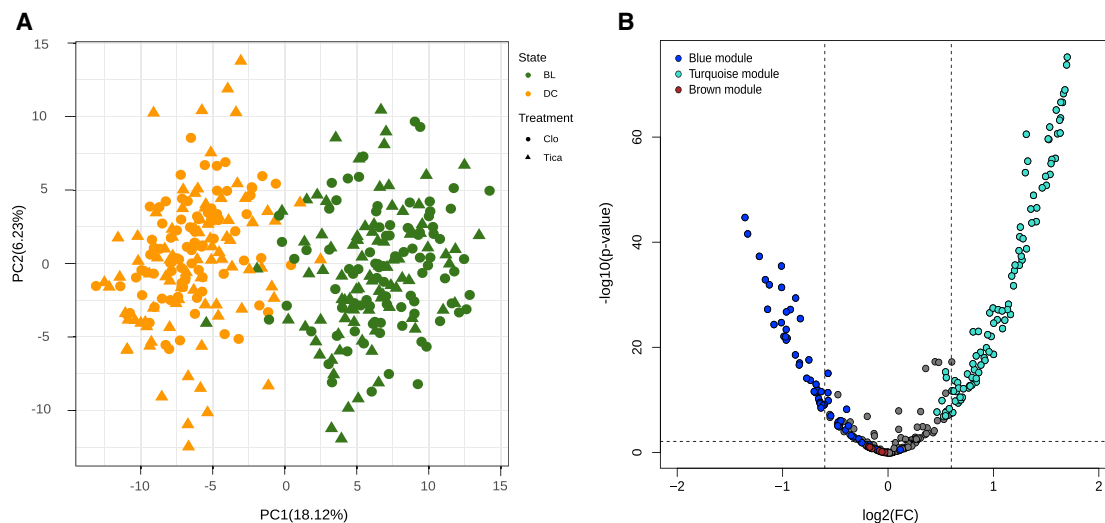


Figure 2. Global metabolomic signature of myocardial infarction

(A) First two principal-components analysis of all post-processed samples. Baseline samples are shown in green and discharge samples in orange; treatment with clopidogrel (Clo) in circles and ticagrelor (Tica) in triangles are shown. Proportion of variance explained by each PC is indicated in parentheses on the axis label. (B) Volcano plot showing significant differences between states. p values are obtained from analysis of covariance (ANCOVA) model 1 main effect on state for the full dataset. Fold changes are obtained from the LMM model's state effect estimate. The top 2 WGCNA modules, represented in blue (mostly amino acids) and in turquoise (mostly fatty acids), map to the decreased and enhanced metabolites at disease states, respectively, whereas the brown module metabolites are non-significant.

WGCNA, weighted gene correlation network analysis; BL, baseline; DC, discharge.

and adrenate; Figure S5), suggesting both pathways are implicated.

Modulation of three additional metabolites was significantly impacted by treatment (Table 2). Levels of androsteroid monosulfate 2 (ASM) were markedly lower at discharge among participants treated with clopidogrel but did not vary significantly between baseline and discharge among ticagrelor-treated patients. 2-hydroxybutyrate (AHB) levels were higher in the ticagrelor-treated group but only among patients aged 49–61 years

(Methods S1). Finally, we identified a significant sex by treatment interaction for palmitoylcarnitine (Table S1), consistent with a sexual dimorphism previously reported regarding to acylcarnitines metabolism.²³

To visualize these treatment associations across the whole metabolome, we used t-distributed stochastic neighbor embeddings (t-SNEs) on metabolites, a dimensionality reduction technique that can reveal hidden structure in metabolite profiles.²⁴ This methodology is an alternative to the hierarchical clustering

Table 2. Treatment interaction effects

Metabolites	t-SNE group	ANCOVA state × treatment effect FDR	LOGIT FC	LOGIT FDR
Linolenate α or γ C18:3n3-n6 ^a	4	0.035	1.432	0.067
Dihomo-linolenate C20:3n3-n6 ^a	4	0.034	1.430	0.075
Stearidonate C18:4n3	4	0.013	2.024	0.028
Docosahexaenoate (DHA) C22:6n3	4	0.029	1.474	0.067
Eicosapentaenoate (EPA) C20:5n3	4	0.001	1.973	0.016
Arachidonate (ARA) C20:4n6	4	0.029	1.499	0.043
Adrenate C22:4n6	4	0.146	1.730	0.040
2-hydroxybutyrate (AHB) ^b	11	0.043	0.916	0.815
Andro steroid monosulfate 2	12	0.005	1.414	0.071

Metabolites significantly modulated with treatment (FDR < 5%) using a three-way ANCOVA model (model 1) and the LOGIT model are shown, for which we also report ratio fold change (FC) between treatment groups. Both models include four covariates (age, physician-reported sex, diabetes, and dyslipidemia). Seven out of nine metabolites belong to the omega-3/6 fatty acid biosynthesis pathway. t-SNE groups refer to the ones labeled in Figure 4A. ANCOVA, analysis of covariance; FC, fold change; FDR, false discovery rate; LOGIT, logistic regression; t-SNE, t-distributed stochastic neighbor embedding.

^aMetabolites in the top 10 of baseline versus discharge effects.

^bMetabolite significant for age as main effect. FDR value shown is from the ANCOVA model applied to the sub-dataset (FDR = 0.345 for the full dataset).

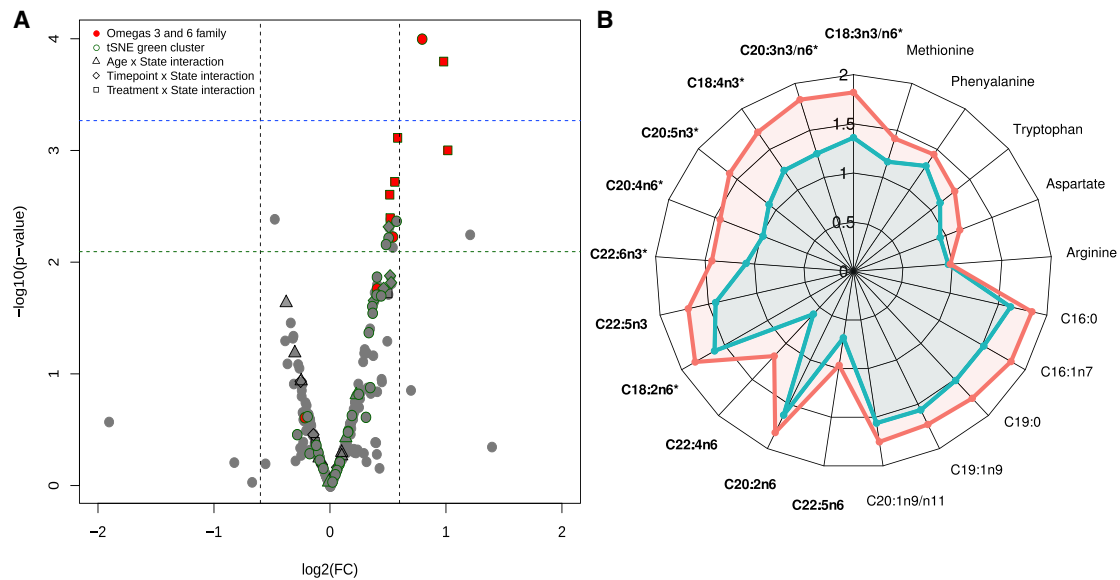


Figure 3. Omega-3/6 metabolites associated with treatment

(A) Volcano plot showing significant differences in metabolite ratios discharge on baseline ratios between treatment groups. p values and fold changes are obtained from the LOGIT model's treatment effect estimation.

(B) Radar plot showing the change in metabolite concentration between baseline and discharge states, with the ticagrelor arm (blue) showing a smaller change compared to clopidogrel arm (red). PUFAs as well as the top 10 increased and decreased metabolites in myocardial infarction are shown. PUFAs, polyunsaturated fatty acids.

WGCNA approach that allows to easily visualize the correlation structure of the metabolome at finer scale without assumption of collinearity. This analysis highlighted 18 clusters of correlated metabolites at baseline (Figure 4A), with most clusters containing metabolites from related metabolic pathways (Data S3; Methods S1). Very few clusters were conserved at discharge (Figure S6), confirming the major remodeling of the whole plasma metabolome between these two states. The t-SNE grouping at baseline between treatment groups showed no major differences, which is expected and reassuring, given that the effect of treatment randomization should only be seen at discharge. This analysis highlighted 18 clusters of metabolites at baseline (Figure 4A), with very few clusters conserved at discharge (Figure S6). The t-SNE grouping at baseline between treatment groups showed no major differences. The interacting metabolites presented above tended to cluster together at baseline, with a unique t-SNE group (no. 4; colored in green) that included many of the metabolites reported in Tables 2 and S1 (14/33; $p < 10^{-4}$; permutation test). Furthermore, 12 out of the top 15 metabolites differentially modulated between treatments (Figure 3A) are found in this t-SNE cluster ($p < 10^{-4}$; permutation test). This green cluster was conserved at discharge for the clopidogrel group (Figure 4B), revealing that the correlation network between these metabolites remained stable, whereas the ticagrelor group was highly remodeled, with a split between lysoglycerophospholipids and fatty acids (Figure 4C). Both can be derived from the hydrolysis of glycerophospholipids through the action of the phospholipase A2. To understand the relationship between these two sub-pathways and treatment-associated metabolite levels, we derived an interaction network (Figures 4D and S7) using a machine learning strategy RFPimp, which allows us to further analyze the correla-

tion structure between metabolites (Methods S1). This analysis revealed that the omega-3/6 metabolites formed a hub between glycerophosphocholine metabolites and fatty acids, possibly responsible for the ticagrelor-specific split at discharge. We noted that AHB is one of the most connected metabolites in this network, implying that its modulation may have several downstream effects and that, to the contrary, ASM appeared as an outlier metabolite, very sparsely connected to the rest of the metabolome.

DISCUSSION

In this study, we found differential associations of key PUFAs metabolites according to clopidogrel and ticagrelor treatment, two P_2Y_{12} inhibitors commonly used in the treatment of ACS. This is the largest plasma metabolomics study comparing disease state and post-treatment changes, revealing that P_2Y_{12} inhibitors may exert differential effects on systemic metabolism and that ticagrelor modulates circulating levels of PUFAs in the early days following a reperfused STEMI. Furthermore, we applied advanced tools for data mining and visualization to this dataset, which lead to the discovery of a cluster of metabolites (green cluster; Figure 4) that includes the PUFAs, in a way that is analytically independent of the single-metabolite multivariate approaches used, adding further evidence that this pathway differentially modulated between treatments.

Whereas most of the PUFAs are derived from nutrition,²⁵ given the short time frame between randomization and treatment response in this study (maximum 4 days), higher circulating n3 and n6 PUFAs are unlikely to have resulted from a facilitated nutritional uptake. The higher plasma concentration of

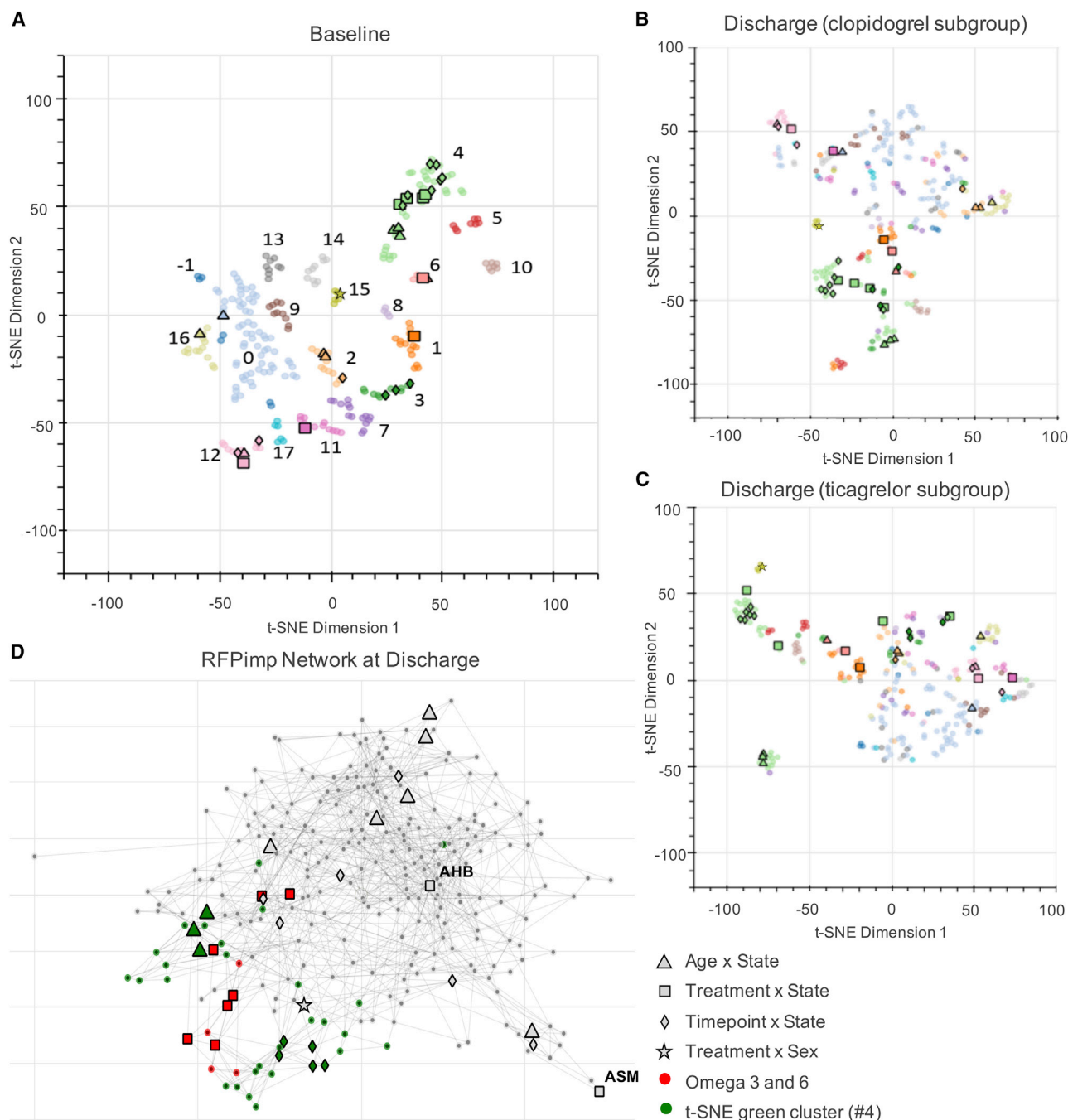


Figure 4. Clustering and network analyses

(A–C) Analyses of metabolites using t-SNE for all patients at baseline (A), clopidogrel patients at discharge (B), and ticagrelor patients at discharge (C) show grouping of metabolites with significant interaction effects (symbols) at baseline (group 4), maintained at discharge in clopidogrel patients but split in ticagrelor patients.

(D) RFPimp is used to construct a metabolite network, highlighting metabolites part of the t-SNE group 4 (green) and omega 3/6 metabolites (red). The “sex” variable is physician reported.

AHB, 2-hydroxybutyrate; ASM, androsteroid monosulfate 2; t-SNE, t-distributed stochastic neighbor embedding.

α -linoleate (ALA) (C18:3n3), a compound that is not endogenously produced,²⁶ found among ticagrelor-treated patients can be related to the potentiation of pharmacodynamics pro-

cesses, including gastrointestinal absorption, lymphatic transport, or liver metabolism.²⁶ A *de novo* biosynthesis of n3-PUFAs²⁵ from ALA is possible through the sequential action of

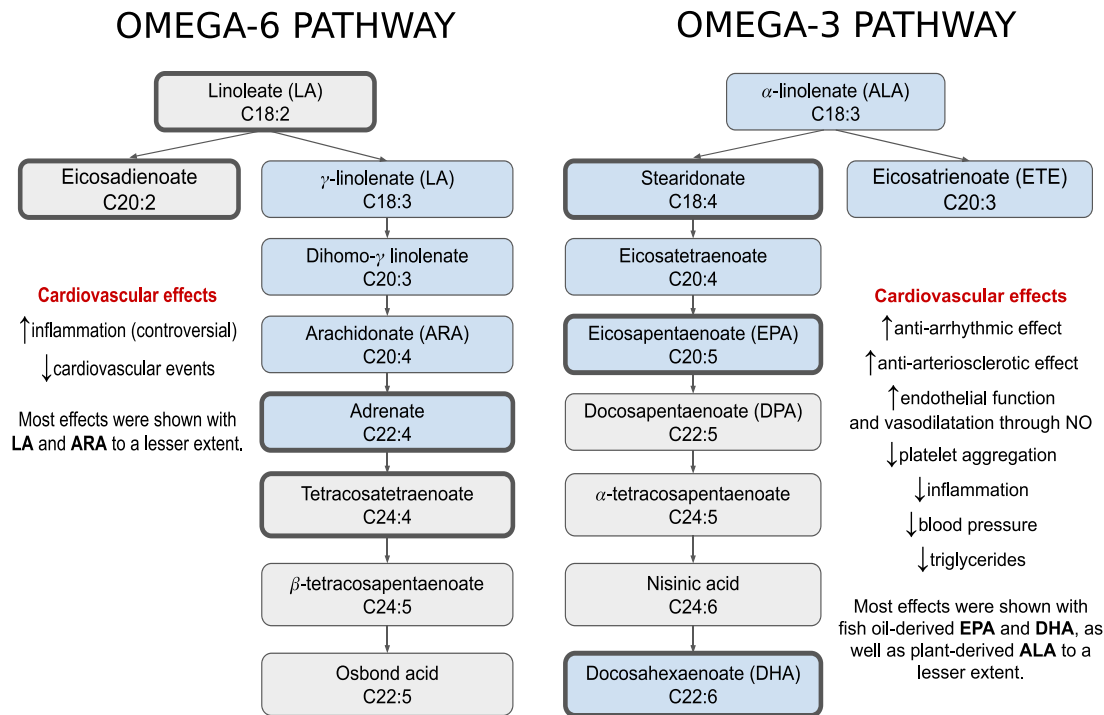


Figure 5. Omega-3 and omega-6 PUFA pathways

Cardiovascular effects of omega-3/6 metabolites noted in sources from the text are shown in this figure. Metabolites found to be significantly modulated by treatment are shown in light blue boxes. The boxes with bold borders are highlighting discriminant metabolites between omega-3 and omega-6 pathways.

elongases and desaturases predominantly in the liver to form EPA (C20:5n3) and docosapentaenoic acid (DPA) (C22:5n3), while the formation of DHA (C22:6n3) requires one cycle of peroxisomal β -oxidation.²⁵ Thereby, it could be that ticagrelor stimulates the hepatic enzymatic processes in the synthesis pathway of PUFAs and promotes the peroxisomal formation of DHA. Alternatively, PUFAs may be released from cells through the action of phospholipases, particularly phospholipase A2 that catalyzes the hydrolysis of sn-2 ester bond position of glycerophospholipids liberating lysoglycerophospholipids and fatty acids, including PUFAs.²⁷ This hypothesis is in line with our t-SNE analysis, in which the green cluster split is specific to the ticagrelor group (Figure 4C). Hence, ticagrelor could act as an activator of the phospholipase A2 activity in the liver and possibly other tissues. This premise is reinforced by recent literature showing that ticagrelor may increase myocardial cytosolic phospholipase A2 activity.²⁸ Additionally, it is well known that clopidogrel is a prodrug bioactivated by several cytochrome P450 enzymes in the liver, including CYP2C19,²⁹ which is a key enzyme responsible for metabolizing linoleic and arachidonic acids.³⁰ There is a possibility that clopidogrel loading and maintenance doses divert CYP2C19 from its principal epoxide activity on long-chain PUFAs and therefore result in a reduction in circulating these metabolites. Such an interaction has not been described as of yet.

Platelet activation induces remarkable changes in their lipids.³¹ Key regulator of these lipidomic modifications in platelets are cytosolic³¹ and calcium-independent phospholipase A₂

(cPLA₂),³² known to be strongly inhibited by increases in cyclic AMP.³³ Through inhibition of P₂Y₁₂ receptors, as well as potentiation through adenosine receptors, ticagrelor is known to induce marked increases in cAMP levels,³⁴ which in turn could modulate platelet cPLA₂ activity. *In vitro* studies have further shown that ticagrelor, but not thienopyridines, affect prostanoid formation, most clearly TxA₂ formation, even in the absence of aspirin.³⁵ Thus, it is possible that ticagrelor induces intracellular lipidomic changes in platelets through both regulation of PUFA availability in plasma and modulation of intracellular phospholipase activity. These potential additional platelet-modulating mechanisms are worthy of further exploration, and additional *in vitro* experiments exposing human platelets to either ticagrelor or clopidogrel, followed by metabolome profiling, would be extremely helpful in order to understand their role.

We hypothesize that ticagrelor's early modulation of circulating levels of PUFAs is favorable for future cardiovascular outcomes. Recently, a meta-analysis of 13 randomized studies using marine supplementation of n3 PUFAs showed lower risk of cardiovascular adverse events, namely MI, coronary heart disease, and overall cardiovascular disease.³⁶ PUFAs have also been linked to cardiovascular benefits in several studies.³⁷ Patients with increased tissue and adipose levels of linoleate (LA) (C18:2n6) and potentially its downstream metabolite (Figure 5), ARA (C20:4n6), had a reduced incidence of major cardiovascular events.³⁸

Several precautions were taken to minimize biases and confounding factors while designing the study. Samples used in this study were obtained from a multicenter, double-blind,

randomized controlled trial, and each participant served as his own control in our analyses, which minimizes the signal-to-noise of inter-individual biological variability. Participants were matched between treatment groups for physician-reported sex, despite the cohort being predominantly composed of men, and for diabetes mellitus and dyslipidemia, two conditions known to deeply impact the metabolome.¹ Additional analyses with targeted metabolomics will be necessary to confirm our hypothesis³⁷ and refine our understanding of pathophysiological processes involved. In particular, because lipid metabolism is markedly regulated by the activity level of the sympathetic nervous system, which also depends, among other factors, on disease severity, information about the clinical course of the participants, including ejection fraction, left ventricular pressure, and arrhythmias, would strengthen interpretation of the data in future studies.

Limitations of study

Our observations remain liable to certain limitations. The lack of individual-level information on several clinical risk factors or other potential confounders (e.g., blood pressure [BP], BMI, statin or aspirin dose, center, and country) is a limitation of the study. We also did not have a replication cohort or an internal sub-cohort to validate on. Per design, we were not able to obtain metabolomics signature prior to STEMI and at steady state several weeks or months following the event; therefore, all results here compare patients with acute MI at baseline and then stabilized after treatment. Moreover, despite matching for diabetic status, which controls for insulin³⁹ used in hospital, our study may involve interactions with other medications that have known metabolic effects, such as statins,⁴⁰ because most PLATO participants were treated with a statin at discharge (89%). Similarly, we cannot exclude an interaction or synergy between P₂Y₁₂ inhibitors and aspirin (taken by all participants)¹⁹ in the observed metabolomics patterns or with medication used at discharge, for example, for beta blockers and angiotensin-converting enzyme inhibitors or angiotensin-II receptor blockers (88%–89% of PLATO participants).¹⁸ Identification of exogenous metabolites from diet or pharmacotherapy³ among participants, prior to analysis (e.g., omega-3 supplements), would allow better detection of meaningful biological variations in future studies. We underline also that we analyzed peripheral plasma and not coronary sinus or myocardial samples, meaning that we cannot imply cause and effects at the myocardial level.

Conclusions

The acute stage of myocardial infarction is associated with strong disruption of the circulating metabolite network. After consideration of physician-reported sex, age, and time from symptoms onset to randomization, ticagrelor is shown to be associated with increased concentrations of omega-3/6 fatty acids compared to clopidogrel in STEMI patients. This increase in circulating PUFAs was observed in the early days after the initiation of ticagrelor uptake, suggesting an early treatment effect. The discovery of PUFA metabolism as a pathway of interest in the recovery path from MI needs additional research to clarify the long-term clinical implications of this finding.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.xcrm.2021.100299>.

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AUTHOR CONTRIBUTIONS

The authors are solely responsible for the design and conduct of this study, all study analyses, the drafting and editing of this paper, and its final contents. E.M.J. and K.S. conceived the study. P.M., J.-C.G., and E.T.E. analyzed the data under the supervision of J.G.H., who designed the bioinformatics and statistical analyses. M.R. contributed extensively to interpretation of the metabolomics data. M.L. advised on platelet biology and helped interpret results. M.Y.C., R.D.L., M.L.N., T.Y.W., L.K.N., and R.C.B. helped with conception and data acquisition. J.G.H., K.S., P.M., M.R., and E.M.J. wrote the manuscript, and all co-authors revised it substantially.

DECLARATION OF INTERESTS

J.G.H. has received speaker honoraria from Dalcov and District 3 Innovation Centre. R.C.B. has received grants from AstraZeneca during the conduct of the study and has received personal fees from Ionis, Akcea, and Novartis outside the submitted work. M.Y.C. has received research support and consultation honoraria from AstraZeneca. E.M.J. is supported by research grants from Les Fonds la Recherche du Québec en Santé (FRQS) and the Canadian Institutes for Health Research (CIHR). R.D.L. has received grants from AstraZeneca during the conduct of the study; has consulted for Bayer, Boehringer Ingelheim, Daiichi Sankyo, Merck, and Portola; and has received grants and consulted for Sanofi, Bristol-Myers Squibb, Glaxo Smith Kline, Medtronic,

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Plasma samples from patients successfully revascularized by primary PCI for a STEMI within 12 hours of symptoms onset treated with clopidogrel (baseline and discharge time points)	PLATO trial	[NCT00391872]
Plasma samples from patients successfully revascularized by primary PCI for a STEMI within 12 hours of symptoms onset treated with ticagrelor (baseline and discharge time points)	PLATO trial	[NCT00391872]
Chemicals, peptides, and recombinant proteins		
Water and methanol both containing 0.1% Formic acid	This paper	N/A
Water and methanol both containing 6.5 mM Ammonium Bicarbonate	This paper	N/A
Critical commercial assays		
Non-targeted mass spectrometry methods; LC/ML and GC/MS	Metabolon inc.	N/A
Dried nitrogen	N/A	N/A
Bistrimethyl-silyl-trifluoroacetamide (BSTFA)	N/A	N/A
5% phenyl and the temperature ramp was from 40° to 300°C	N/A	N/A
Deposited data		
Analyzed data	This paper	https://mhi-omics.org/research-projects/metabolomics
Full dataset (Mendeley)	This paper	https://doi.org/10.17632/xp89jmbtjn.1
Software and algorithms		
Metabolon informatics LAN backbone database (running on Oracle 10.2.0.1)	Metabolon Inc.	N/A
MetaboAnalyst	Chong et al. ⁴¹	https://www.metaboanalyst.ca/
Metabolite Set Enrichment Analysis (MSEA) provided in MetaboAnalyst	Chong et al. ⁴¹	https://www.metaboanalyst.ca/
Weighted Gene Co-expression Network Analysis (WGCNA) version 1.64-1	Langfelder et al. ²²	https://cran.r-project.org/web/packages/WGCNA/index.html
DBSCAN as provided in Scikit-Learn v0.20	Ester et al. ⁴² ; Pedregosa et al. ⁴³	https://scikit-learn.org/stable/index.html
t-SNE as provided in Scikit-Learn v0.20	Pedregosa et al. ⁴² ; Laurens van der Maaten et al. ²⁴	https://scikit-learn.org/stable/index.html
RFPimp python package v1.3.2	Terence Parr	https://github.com/parr/random-forest-importances
R statistical software 3.6.3	R Foundation for Statistical Computing	https://www.r-project.org/
NetworkX python package v2.5	Hagberge et al. ⁴⁴	https://networkx.org/
Other		
Metabolon LIMS system	Metabolon Inc.	N/A
MicroLab STAR® system	Hamilton Company	N/A
TurboVap®	Zymark	N/A

(Continued on next page)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Waters ACQUITY UPLC	N/A	N/A
Thermo-Finnigan LTQ mass spectrometer	N/A	N/A
Thermo-Finnigan Trace DSQ fast-scanning single-quadrupole mass spectrometer	N/A	N/A

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Julie Hussin (julie.hussin@umontreal.ca).

Materials availability

This study did not generate new unique reagents.

Data and code availability

The summary statistics generated during this study are available on our website (<https://mhi-omics.org/research-projects/metabolomics/>). All figures are available in interactive mode on the same website to allow explorative interrogation of the data. The normalized metabolomic data have been deposited to Mendeley Data: <https://doi.org/10.17632/xp89jmbtjn.1>. The code generated during this study is available on GitHub (<https://github.com/HussinLab/metabo2020>). The raw metabolomics dataset is available from the Lead Contact on request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Plasma samples were obtained from 180 participants in the PLATelet inhibition and patient Outcomes (PLATO) trial [NCT00391872] (Table 1; Figure S1), 90 from each arm (clopidogrel and ticagrelor). The design and results of the PLATO trial were reported previously.^{18,19} The PLATO trial was approved by the institutional review board (IRB) at each participating center, and all participants provided written informed consent. The plasma samples were obtained from PLATO participants specifically consenting to future non-genetic biomarker analyses. The transfer of patient-level information and plasma samples has been approved by the steering committee of the PLATO trial and the sponsor and was overseen by the Duke University's IRB. Age, physician-reported sex (referred to in Results as 'sex'), diabetes mellitus, and dyslipidemia status were the only variables for which we had patient-level information in this PLATO metabolomics sub-study. Metabolomics and bioinformatics analyses were approved and overseen by the IRB at the Montreal Heart Institute. This sub-study was sponsored and funded by AstraZeneca, which played no role in the design of the protocol or in the management or analysis of the data.

METHOD DETAILS

Study design

Participants with plasma samples available were selected for the metabolomics sub-study from the full PLATO trial population if they: 1) were successfully revascularized by primary percutaneous coronary intervention (PCI) for an ST-segment elevation myocardial infarction (STEMI) within 12 hours of symptoms onset; 2) were treated with their assigned P₂Y₁₂ inhibitor (per protocol); 3) experienced no hemodynamic instability or other major cardiac events during their index hospital stay; and 4) consented to participate in the overall PLATO biomarker sub-study, with a complete (baseline and discharge) sample set. To minimize variability, stratification to clopidogrel versus ticagrelor was done in a 1:1 ratio and participants were matched by age, physician-reported sex, diabetes mellitus, and dyslipidemia. Almost 80% of our cohort are men. Plasma samples were collected at 1) baseline before P₂Y₁₂ inhibitor loading and reperfusion, and at 2) hospital discharge or at day 4 post-STEMI, whichever came first, and stored at -80°C. Patients were stratified based on timing of sample collection at baseline grouped in three time-from-symptoms-onset to randomization intervals (T0, less than 2 hours; T1, 2 to 4 hours; T2, 4 to 12 hours) (Figure S1).

Metabolomics dataset

EDTA-anti-coagulated blood samples were obtained via a direct venous puncture from patients providing informed consent for the PLATO metabolomics sub-study. Plasma was stored at -80°C at Uppsala Clinical Research Centre.^{45,46} For our study, frozen plasma samples were transferred to Metabolon inc. (Durham, NC, USA) and stored at -80°C until analysis. The metabolomic profiles were assessed in new aliquots, which had never been used to avoid freeze-defreeze cycles, by Metabolon Inc. using a non-targeted mass spectrometry (MS)-based approach. Detailed sample preparation methods are in Methods S1. The paired (baseline and

discharge) samples were done in tandem, the same number of clopidogrel and ticagrelor samples were run each day (12 days) and time-from-symptoms-onset were also balanced as much as possible each day (Figure S2F). Data pre-treatment procedures, such as nonlinear retention time alignment, peak discrimination, filtering, matching, and identification were performed. This comprehensive analysis allowed the identification of 489 named metabolites through liquid chromatography and gas chromatography, both combined with MS (LC/MS and GC/MS, respectively). Only compounds of known identity were analyzed. Metabolites with more than 10% missing values were removed (Figure S2A). The remaining missing data were imputed using the minimum approach (half of the smallest positive value) through MetaboAnalyst⁴¹. Near-constant metabolites, with low interquartile range (IQR), likely to be non-informative variables throughout experimental conditions were removed (Methods S1). Two samples were identified as outliers using principal component analysis (PCA) scores plot and removed with their paired sample (Figure S2B). After quality control (QC), we analyzed a total of 280 metabolites in 350 high quality samples from 175 participants. These 175 participants constituted the inception cohort for all further bioanalyses. To estimate the intra-individual change in raw metabolite concentration, we computed discharge on baseline ratio values for each metabolite. To reduce biases due to highly variable metabolites' range, raw concentrations were subjected to auto-scaling and were log transformed (referred herein as "normalized metabolite concentration"). PCA was then performed and highlighted a clear differential clustering between baseline and discharge groups (Figure 2A) but no batch effects (Figure S2E). We performed Metabolite Set Enrichment Analyses (MSEA) with MetaboAnalyst using database resources, including the KEGG and Human Metabolome Databases (HMDB), to identify enriched metabolic pathways.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical modeling

A linear mixed model (LMM) was used to estimate the fold change (FC) of metabolites that differed significantly between baseline and discharge groups using covariate-adjusted concentrations and considering pairing as a random effect. A logistic regression model (LOGIT) was used to test the effect of intra-individual metabolite concentration change on differential treatment, such that FC from this model represent the change in ratio values (discharge over baseline) between clopidogrel and ticagrelor groups. To further assess the influence of the drug received (clopidogrel/ticagrelor), and its interaction terms with time point and disease state, on normalized metabolites concentration (Y), we used a mixed analysis of covariance (ANCOVA) model, including physician-reported sex (s), age (a), diabetes (db), dyslipidemia (dl) as covariates (Model 1):

$$Y \sim \text{treatment} * \text{state} * \text{timepoint} + a + s + db + dl + \text{Error}(\text{subject})$$

Individual patient effect (*subject*) was added as a random effect, to take pairing of samples into account. This model was applied to the full dataset, composed of all samples passing QC, and a reduced dataset, which included patients from 49 to 61 years old, an age group in which there is no difference in age distribution across time points (Figure S2C; Methods S1). To further consider the influence of age (Model 2a) and physician-reported sex (Model 2b), we used additional ANCOVA models that test for interactions terms with treatment on Y, controlling for the same covariates as above (see Methods S1). Benjamini and Hochberg's method was used to compute false discovery rate (FDR) adjusted p values. All analyses are done using R 3.6.3. For each metabolite, FC between groups and FDR values for the main effects (LMM and LOGIT models, Data S1) and interaction terms (ANCOVA models, Data S2) are provided as supplemental datasets on our website (<https://mhi-omics.org/research-projects/metabolomics/>).

Clustering and network-based analyses

We used a network-based method to define clusters (or modules) of highly correlated metabolites called Weighted Gene Co-expression Network Analysis (WGCNA)²² (Methods S1). Additionally, we computed the Kendall correlation between metabolites, and used the t-distributed Stochastic Neighbor Embedding (t-SNE) to inspect the correlation patterns between metabolites (hyper parameters: perplexity = 7; learning rate = 200; 5,000 training iterations).²⁴ To visualize clusters of metabolites, we used Density-Based Spatial Clustering of Applications with Noise (DBSCAN,⁴² hyper parameters: epsilon = 2; minimum number of samples by cluster = 7). We used the python package Scikit-Learn⁴³ implementation for both algorithms. We built metabolite networks based on inter-dependencies between metabolites using the RFPimp (<https://github.com/part/random-forest-importances>) and NetworkX⁴⁴ python package (Methods S1). Permutation tests were used to assess the significance of metabolite grouping and empirical p values on 10,000 samples are reported in all cases.

ADDITIONAL RESOURCES

All figures are available in interactive mode to allow explorative interrogation of the data on our website (<https://mhi-omics.org/research-projects/metabolomics/>). Supplemental datasets can also be found on the same website. The PLATO study ClinicalTrials.gov identifier (NCT number) is NCT00391872.