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Plasma metabolomics exhibit response to therapy in chronic thromboembolic pulmonary hypertension

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Abstract

Pulmonary hypertension is a condition with limited effective treatment options. Chronic thromboembolic pulmonary hypertension (CTEPH) is a notable exception with pulmonary endarterectomy (PEA) often proving curative. This study investigated the plasma metabolome of CTEPH patients, estimated reversibility to an effective treatment and explored the source of metabolic perturbations.

We performed untargeted analysis of plasma metabolites in CTEPH patients compared to healthy controls and disease comparators. Changes in metabolic profile were evaluated in response to PEA. A subset of patients were sampled at three anatomical locations and plasma metabolite gradients calculated.

We defined and validated altered plasma metabolite profiles in patients with CTEPH. 12 metabolites were confirmed by ROC analysis to distinguish CTEPH and both healthy (AUCs 0.64-0.94, all $p<2x10-5$) and disease controls (AUCs 0.58-0.77, all $p<0.05$. Many of the metabolic changes were notably similar to those observed in idiopathic pulmonary arterial hypertension (IPAH). Only five metabolites (5-methylthioadenosine, N1-methyladenosine, N1-methylinosine, 7-methylguanine, N-formylmethionine) distinguished CTEPH from chronic thromboembolic disease or IPAH. Significant corrections (15-100% of perturbation) in response to PEA were observed in some but not all metabolites. Anatomical sampling identified 188 plasma metabolites, with significant gradients in tryptophan, sphingomyelin, methionine, and Krebs cycle metabolites . Metabolites associated with CTEPH and gradients also showed significant associations with clinical measures of disease severity.

We identified a specific metabolic profile that distinguishes CTEPH from controls and disease comparators, despite the observation that most metabolic changes were common to both CTEPH and IPAH patients. Plasma metabolite gradients implicate cardiopulmonary tissue metabolism of metabolites associated with PH and metabolites that respond to PEA surgery could be a suitable non-invasive marker for evaluating future targeted therapeutic interventions.

Key Words: metabolomics; pulmonary hypertension; CTEPH; chronic thromboembolism

Take-Home Message: Metabolic profiles can distinguish CTEPH from controls and disease comparators, but most metabolic changes are common to both CTEPH and IPAH. Cardiopulmonary tissue metabolism is relevant to PH and metabolites do respond to PEA surgery.

Plain language summary: Metabolites, which are breakdown products such as sugars, fats and other chemicals, can be detected in blood samples. Levels of these metabolites relate to disease, and specific metabolites are shown here to relate to a disease of high blood pressure in the lungs following blood clots (CTEPH). These metabolites are produced or used by the lung

and heart and their levels change in the blood following curative surgery. This means they may be useful as a test for other successful treatments including drugs.

Introduction

Pulmonary hypertension (PH) is defined by persistent elevation of resting mean pulmonary artery pressure and is associated with an increased risk of right heart failure and premature death[1]. Progress in medical therapies for PH has been limited to pulmonary arterial hypertension (PAH) and chronic thromboembolic pulmonary hypertension (CTEPH). Moreover, this has not been related to discovery of new disease mechanisms but to improvements in targeting known pathways responsible for vasodilation and strategies related to early combination and escalation of treatments. CTEPH remains the only group of PH for which a potential cure exists, by means of a pulmonary endarterectomy that commonly normalises haemodynamics[2, 3]. This provides an invaluable opportunity to study pathobiology and response to treatment[4].

Metabolomics allows high-dimensional molecular mapping of disease presentations and the potential to define endophenotypes. We and others have previously reported the plasma metabolomic profiles of patients with idiopathic and heritable pulmonary arterial hypertension (IPAH/HPAH)[5]. Here we compare the plasma metabolomic profiles of patients with CTEPH with those of other disease and healthy controls and patients with IPAH/HPAH and seek to establish whether metabolic alterations are corrected by pulmonary endarterectomy. We also use plasma metabolome gradients between superior vena cava (SVC), pulmonary artery (PA) and radial artery (ART) to investigate the tissue of origin of any perturbation.

Methods

Study participants and sample collection

Patients attending the National Pulmonary Hypertension Service at Hammersmith Hospital, London and Royal Papworth Hospital, Cambridge, donated blood samples with informed consent and approval of local research ethics committees (Reference numbers 17/LO/0563 and 15/EE/0201). Total sample collected in the main cohorts and analysis plan are detailed in Table 1 and Figure 1 respectively.

Patients were recruited at Hammersmith Hospital London (10 December 2002 to 20 May 2019) and Papworth Hospital Cambridge (30 September 2015 to 10 January 2019) with diagnoses of CTEPH, idiopathic or heritable PAH (IPAH/HPAH). Control samples were obtained from healthy volunteers, patients with chronic thromboembolic disease (CTED)[6] and disease control individuals (DC); the latter presented as symptomatic patients who were subsequently found not to have pulmonary hypertension[5]. Additional IPAH/HPAH patients were included as a comparator group and sampled between 19 February 2014 and 24 June 2015 from other expert centres in the UK as part of the National Cohort Study of Idiopathic and Heritable Pulmonary Arterial Hypertension (ClinicalTrials.gov. Unique identifier: NCT01907295). Venous blood samples were drawn from the antecubital fossa in to EDTA Vacutainer tubes (BD, Oxford, UK), immediately inverted 8-10 times, put on ice, centrifuged (1300g, 15 minutes at 4°C) within 30 minutes, and plasma stored at -80°C until required.

Initially, a discovery cohort of 108 consecutive CTEPH patients was compared to 58 healthy controls and the results replicated in a second cohort of 92 CTEPH patients compared to a distinct healthy control group (n=63) (Figure 1). Similar proportions were deemed operable for PEA surgery (59/108 and 48/92) in the two CTEPH cohorts. To understand the specificity of any differences for CTEPH, metabolite profiles were compared with disease controls (DC n=132), patients with CTED (n=63) and IPAH/HPAH (n=433) (Table 1 and Figure 1).

In the second arm of the study, we evaluated the metabolite profiles of CTEPH patients before and after PEA surgery (Figure 1). We compared metabolite levels in CTEPH patients deemed suitable for PEA surgery (pre-PEA, n=64) with matched (based on clinical characteristics) patients sampled after PEA surgery (post-PEA, n=82, Supplementary Table 1), and then analysed differences in a separate group of 43 patients who were sampled both before and after PEA surgery. All post-PEA samples were obtained after full recovery from surgery, at median 37

months for unpaired and 5.8 months for paired samples, and both groups exhibited similar reductions in mean pulmonary artery pressures and pulmonary vascular resistance (Supplementary Table 1).

A further set of patients with diagnoses of CTEPH (n=68) or IPAH/HPAH (n=18) at Papworth Hospital were sampled during elective right heart catheterisation between 2015 and 2017, allowing simultaneous sample collection from the superior vena cava, proximal portion of pulmonary artery and radial artery, and haemodynamic measurements. Exclusion criteria included left ventricular systolic and or diastolic dysfunction, significant valvular heart disease, chronic kidney disease stage 4 or 5, chronic liver disease, liver failure or alcohol abuse, current illicit substance use, active infection and peripheral arterial vascular disease. Patients were sampled between 9:30 am and 12:30 pm.

Metabolomic analysis

Metabolomic profiling by ultra-performance liquid chromatography mass spectrometry (LC-MS) was conducted on the Discovery HD4TM Global Metabolomics platform by Metabolon, Inc. (Durham, NC, USA)[7]; data were provided as semi-quantitative metabolite levels, annotated with pathways, as previously described[5]. Glycerophospholipid groups are abbreviated as follows: glycerophosphorylcholine (GPC), glycerophosphoethanolamine (GPE), glycerophosphatidylinositol (GPI), glycerophosphatidylserine (GPS).

Statistical analysis

We pre-processed metabolite data as described previously[5]. Briefly, metabolites were normalised by Box-Cox transformations[5] and samples where metabolites were undetected were imputed with the minimum detected level for the metabolite. Only 324 non-xenobiotic metabolites detected in at least 95% of samples were included. All data were z-score transformed based on healthy control data for ease of interpretation. In order to account for any between batch variability a quantile normalisation approach was utilised, which sets the distribution of metabolite levels in each sample to the average distribution of all samples, making them directly comparable[8]. This has previously been used in metabolomics LC-MS data to minimise experimental variation due to a variety of causes, including experiments being conducted at different times[8], using more than one instrument and different sample processing procedures[9].

Initial group comparisons between controls and patients were performed using non-parametric Mann Whitney U tests (as transformations did not eliminate skew). Comparisons before and after PEA surgery in paired samples was conducted using the Wilcoxon signed rank test. Comparisons of demographic features between study groups were conducted using the Kruskal-Wallis (continuous data) or Chi-squared (categorical data) tests.

To assess the relationships between metabolite levels, diagnoses and potential confounders, regression models included preserved renal function defined as creatinine <75 µmol/L, and liver function as bilirubin <21 µmol/L[5]. In the healthy control group, preserved renal and hepatic function was assumed as clinical assay data was unavailable.

Paired Wilcoxon signed-rank test was used for comparisons of metabolites abundance between sampling sites. False discovery rate correction was used to minimise false positive rate. Baseline clinical characteristics were expressed as numbers and percentages for categorical variables and mean (standard deviation) or median [interquartile range] for continuous variables according to data distribution. Comparisons of clinical characteristics between study groups were performed with parametric and non-parametric tests as per data distribution. Data were analysed and visualised using R http://www.R-project.org/.

Pathway enrichment analysis on metabolites showing tissue gradients was performed with Fisher's exact test with all detected metabolites in each pathway as background. Undirected relevance network analysis[10] was carried out to investigate the inter-relationship between metabolites which showed gradients across sampling sites; highly correlated metabolites (Spearman’s rho>0.9) were visualized using the *tidygraph* R package. Spearman’s correlation was also performed to assess relationships between discriminatory metabolites and normalised clinically relevant (diagnostic or prognostic) variables. The results were visualised using *ggplot2*, *ggpubr*, *pROC, ggdendro*, and *egg* R packages.

Results

Study participants

Baseline characteristics and laboratory data are shown in Table 1 and Supplementary Table 1. Patients with PH show altered haemodynamics and impaired exercise capacity and an overview of the main comparison groups is given in Figure 1 with details in Table 1.

Altered plasma metabolite profiles in CTEPH patients

We first compared plasma metabolite levels in two sets of samples from pre-PEA or inoperable CTEPH patients and healthy control subjects (Figure 1). Plasma levels of 55 metabolites distinguished CTEPH patients from healthy controls in both discovery and replication analyses following Bonferroni correction (mean differences to controls ranging -0.33 to -1.53 SD and +0.84 to 2 SD, $p<1.54x10^{-4}$, Supplementary Table 2). Of these, 35 metabolites distinguished CTEPH from healthy controls after correcting for potential confounders such as age, gender, ethnicity, body mass index, creatinine, bilirubin and drug therapies ($p<0.05$, Supplementary Table 2). Age affected 17 of these metabolites, but the average effect of CTEPH was around 50-100 times greater, and gender affected 11/35 metabolites, with the effect of CTEPH being 1.5-3.2 times the effect of gender (Supplementary Table 3 and Supplementary Figure 1).

Of the 35 discriminating metabolites, a subset of 19 also distinguished CTEPH patients from disease controls after correcting for potential confounders ($p<0.05$, Figure 2); 10 were increased, including modified nucleosides (e.g. N2,N2-dimethylguanosine), monohydroxy- fatty acids and metabolites of polyamine and methionine metabolism and 9, including phosphatidylcholines, oxalate, gamma-glutamyl-epsilon-lysine and several sphingomyelins, were decreased (Supplementary Table 2). Twelve metabolites were also significantly different between CTEPH and CTED patients ($p<0.05$), the latter group being included as a control for underlying chronic thromboembolism without pulmonary hypertension and anticoagulation therapy (Table 2, Supplementary Table 2). These 12 metabolites were confirmed by ROC analysis to distinguish CTEPH and both healthy (AUCs 0.64-0.94, all $p<2x10^{-5}$) and disease controls (AUCs 0.58-0.77, all $p<0.05$, Figure 3). Sensitivities and specificities of the best cut-offs reached 57-92% and 35-94% (Supplementary Table 4). Finally, 5 metabolites significantly distinguished CTEPH from the PH comparator group of IPAH patients with the most marked difference being in 5-methylthioadenosine (MTA, Figure 2 & Figure 4A, Table 2, Supplementary Table 2).

Metabolite changes associated with PEA surgery in CTEPH patients

We hypothesised that plasma levels of some metabolites relate directly to the consequences of raised pulmonary vascular resistance (PVR) and associated right ventricle strain; if so, PEA surgery would be expected to correct a subset of altered metabolite levels in CTEPH patients.

Thirty-seven metabolites distinguished operable CTEPH patients sampled pre-PEA from those sampled post-PEA (Supplementary Table 5). Twelve of these metabolites also showed a nominally significant change in post-PEA surgery in the paired sample validation analysis (correcting 15-100% of perturbation versus healthy controls), with 7 meeting multiple test corrections including N2,N2-dimethylguanosine and sphingomyelin-(d18:1/22:1, d18:2/22:0, d16:1/24:1) (Supplementary Table 5 and Figure 4B). Taurine increased in the unpaired samples but decreased in the paired samples, suggesting this may be a false positive; the other 6 metabolites showed consistent directions of change.

Cardiopulmonary metabolism

We hypothesised that cardiac and pulmonary metabolic activity would affect the plasma metabolome and contribute to the metabolic signals observed here in CTEPH and previously in IPAH[5]. We tested this by analysing metabolite gradients across samples from three anatomical arterial and venous sites from patients with IPAH and CTEPH: the superior vena cava (SVC), pulmonary artery (PA) and radial artery (ART) (Figure 5). We found 188 metabolites with significant gradients ($p<0.05$, Supplementary Table 6) and the overlap of gradients is depicted in Figure 4. Network analysis revealed functionally related clusters of metabolites with tissue gradients that were closely correlated (Rho>0.9) (Supplementary Figure 2). Twenty-one of the metabolites we have identified as altered in CTEPH also had significant gradients (Table 3), including SVC-PA and PA-ART gradients of alpha-ketoglutarate (TCA cycle) and modified methionine metabolites, PA-ART gradients of monohydroxy fatty acids (2-hydroxypalmitate) and SVC-PA gradients of N-formylmethionine and 7-methylguanine (Table 3).

Metabolites associated with CTEPH and gradients also showed significant associations with clinical measures of disease severity, with the strongest associations observed between metabolites with SVC-PA gradients (e.g. N-formylmethionine, N-acetylmethionine and alpha-ketoglutarate) and measures of adverse clinical outcome (mRAP, cardiac output and 6MWD, effect size estimates up to +/-0.432, Supplementary Figure 3).

We performed an enrichment analysis of the metabolite pathways represented by four or more metabolites (Supplementary Table 7). TCA cycle metabolites were enriched in both SVC-PA and PA-ART gradients ($p<0.05$), whereas nicotinamide, nicotinate, phospholipid and lysoplasmalogen metabolites were enriched in PA-ART and ART-SVC gradients ($p<0.05$). Plasmalogens were enriched only in the PA-ART gradient analysis. ($p=0.032$, Supplementary Table 7).

Overlap between metabolites associated with CTEPH, response to surgery and plasma gradients between sampling sites

Overall, we detected several metabolites robustly associated with CTEPH compared to relevant controls and with response to PEA surgery and explored the association of levels of metabolites with passage of blood across different vascular beds. 11 metabolites were overlapping from these main analyses as summarised in Figure 5. In particular, sphingomyelin (d18:1/22:1, d18:2/22:0, d16:1/24:1)* and N2,N2-dimethylguanosine were associated with CTEPH and also changed post-surgery suggesting a close association with disease development and reversal.

Discussion

This comprehensive profile of plasma metabolites has identified circulating metabolites that associate with CTEPH and a subset of metabolites that change in response to an effective treatment. The metabolic profile correlates with clinical severity, which together with demonstrating changes in plasma metabolite levels across the lung and heart, provides biological plausibility. Therefore, metabolic profiling may have clinical utility as a non-invasive approach to assessing response to PH treatments.

Most of the metabolic changes seen in CTEPH were notably similar to those observed in IPAH. This included increased modified nucleosides, TCA cycle intermediates, monohydroxy fatty acids, tryptophan, polyamine and arginine metabolites, and decreased sphingomyelin, phosphocholines and steroid metabolites. Differences in metabolite levels between IPAH and CTEPH were subtle and significant for only 5 metabolites – four modified nucleosides 5-methylthioadenosine (MTA), N1-methyladenosine, N1-methylinosine, 7-methylguanine, and N-formylmethionine. Importantly some of these metabolites (7-methylguanine, N-formylmethionine) also exhibited plasma gradients from the superior vena cava to the pulmonary artery, which will include metabolites draining from the coronary sinus, indicating a potential relevance to cardiac metabolism, further supported by significant correlations with haemodynamics. RNA modifications are associated with multiple diseases ranging from various types of cancer, immune to neurodevelopmental disorders[11-14]. The dynamic and reversible nature of nucleoside modifications identifies these metabolites as candidates to monitor therapeutic response[15], as exemplified by the change in N2,N2-dimethylguanosine following PEA. While many metabolites are affected by age and sex, we found the differences associated with CTEPH were much larger and independent of these and other potential confounders.

The overlap in metabolic disturbance between PAH and CTEPH is understandable, and likely reflects common changes in cardiopulmonary structure and function[16]. Indeed, similarities between CTEPH and PAH with pulmonary arterial remodelling and endothelial cell dysfunction, as well as subsequent right ventricle remodelling are well documented[16, 17]. The implication of this is that future therapeutic strategies which act by correcting the metabolic dysfunction observed could be investigated not just in CTEPH but potentially in all forms of PH which demonstrate similar metabolic disturbances. When studying CTEPH it is challenging to dissociate the effects of PH and chronic thromboembolism, both of which can affect metabolism. To mitigate this, we included comparisons with patients with chronic thromboembolism without

PH, and patients with IPAH. While some effects of the severity and duration of thromboembolism in CTEPH patients may remain, the changes we observe are most likely driven by the haemodynamics of PH and the associated pulmonary vascular remodelling and right heart dysfunction.

We explored the metabolites altered in CTEPH patients sampled post-PEA compared to pre-operative cases and were able to verify correction of six metabolites which report on relevant pathways in patients sampled both prior to and after full recovery from PEA. This included two sphingomyelins which through structural and signalling roles, including cell cholesterol and plasma membrane homeostasis, play an important role in cardiovascular health[18]; here we also show significant inverse correlations with hemodynamic parameters. Reduced alpha-tocopherol, a potent antioxidant and cytoprotective agent that inhibits platelet aggregation and promotes vasodilation[19, 20] and is reduced in the failing right ventricle[21], was also corrected post-surgery. 3- ureidopropionate, a pyrimidine breakdown product which can inhibit complex V of the respiratory complex chain[22] was also decreased back towards normal levels post-PEA. The modified nucleoside N2,N2-dimethylguanosine, which could reflect stress or hyperproliferation of vascular cells, was partially corrected by surgery, adding to its utility as a risk marker already established in PAH[5]. The responsiveness of these markers to successful therapy in CTEPH is encouraging for their utility in monitoring successful treatment in other forms of PH.

By sampling PH patients at different anatomic locations, we aimed to characterise alterations in the plasma metabolome across tissues, in particular the heart and lung. In the PA-arterial gradients we also saw enrichment in nicotinamide/nicotinate (1-methylnicotinamide has anti-thrombotic activity[23]), phospholipid, lysoplasmalogen and plasmalogen (anti-oxidants[24] which can be targeted by hypoxia-induced phospholipases[25]) metabolites, reflecting at least in part, the metabolic activity of the lung. In gradients from the PA-arterial and SVC-PA samples we found enrichment of TCA cycle metabolites such as alpha-ketoglutarate, which was also elevated in PH patients. Previous metabolomic[5, 26] and imaging studies[27] have demonstrated disrupted bioenergetics in IPAH and CTEPH. Accumulation of TCA cycle intermediates is consistent with reduced mitochondrial glucose oxidation, previously reported in PAH and a therapeutic target[28]. Mitochondrial dysfunction in pulmonary artery cells[29], right ventricle[30] and peripheral organs[31] points toward multi-organ energetic reprogramming[32] and is now considered an important component of the pathophysiology of PAH. Our data suggest this may also be an important feature of CTEPH.

During exercise, fit individuals elevate plasma glycerol (lipolysis), fatty acid entry to the TCA cycle (pantothenate) and expand the TCA cycle intermediate pool[33]. In patients with oxidative phosphorylation dysfunction (mitochondrial/McArdle disease) these responses to increased demand on skeletal muscle are not maintained[34]. Equally, disruption of TCA intermediates and purine metabolites is associated with RV-PV dysfunction in PH[35] and RV fatty acid metabolism is perturbed[36]. We found metabolites in these, and other (modified nucleosides and lysophospholipids), pathways were associated with disease severity and exercise performance in CTEPH patients and further studies such as skeletal muscle biopsy metabolomics may be required to fully appreciate the tissue specificity of these changes. Similarly, differential metabolic response to environmental interventions (diet, exercise programs) can shed new light on the impact on lifestyle modifications on disease trajectory [37, 38].

While well established in heart failure[39] there is also a growing body of evidence that perturbations in systemic metabolism are involved in the pathogenesis of PAH and CTEPH[40]. This appears to include a role for the gut microbiome in PAH[41] with some bacterial taxa enriched in PAH stool samples and associated microbial metabolite changes in PAH patients[42]. In line with these findings we also show here perturbations and significant systemic gradients of microbial metabolites, including those involved in tryptophan, sphingomyelins and phosphatidylcholine metabolism.

The strengths of this study include the large sample size, stringent sampling and processing conditions, inclusion of disease controls, comprehensive clinical assessment, including near normalisation of pulmonary haemodynamics, and its untargeted approach to assessing a wide range of plasma metabolites. There were also limitations. Plasma samples were taken at advanced stages of CTEPH, which makes it difficult to distinguish causative from compensatory changes. The influence of current medical therapies on metabolic profiles was also not assessed. Sampling directly from the coronary sinus could better characterise trans-cardiac metabolism in future studies. Reduced plasma albumin levels in chronic diseases such as CTEPH more closely represent inflammation and thus have limited utility in estimating nutritional status. For optimal clinical utility, the effects of diurnal variation and diet, through collection of accurate nutritional data, on specific metabolic profiles will need to be better understood, but some confidence can be taken from pilot data from CTEPH patients sampled in a fasting state who demonstrated several similar perturbations[26].

Conclusion

We identified a metabolic profile that separates CTEPH from healthy and disease controls but the overlap in metabolic disturbance between PAH and CTEPH likely reflects common changes in cardiopulmonary structure and function. Plasma metabolite gradients implicate cardiopulmonary tissue metabolism of metabolites associated with PH. Metabolites that respond to surgery with improvement in pulmonary haemodynamics could be a suitable non-invasive marker for evaluating future targeted therapeutic interventions in pulmonary hypertension.

Author's contributions: Conception and design: CR, JPZ, MT, ES, KZ; Analysis and interpretation: ES and CR; Drafting the manuscript, making tables and figures: ES and CR; Data collection: ES, KZ, PG, DT and JEC; Revising the manuscript for important intellectual content: ES, CR, MW, JW, MT, LSH, NWM and JPZ. All authors approved the final draft of the manuscript.

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Table 1. Main Cohort Characteristics.

	HC N=121	DC N=132	CTED N=63	IPAH N=433	CTEPH -Discovery N=108	CTEPH -Replication N=92	Patients sampled at 3 locations N=86
Demographics							
Age at sampling [years]	51 [37;57]	59 [43;69]	60 [45;70]	54 [41;67]	68 [56;76]	66 [53;77]	64 [50;71]
Sex: Female	78 (64%)	90 (68%)	27 (43%)	305 (70%)	41 (38%)	56 (61%)	42 (49%)
Ethnicity: European	66 (55%)	57 (43%)	33 (66%)	358 (83%)	82 (76%)	68 (74%)	74 (86%)
BMI [kg/m^2]	25 [24;30]	27 [24;30]	30 [26;34]	28 [24;32]	27 [24;30]	28 [25;32]	29 [25;34]
WHO functional class:							
I	N/D	N/D	4 (17%)	28 (7%)	2 (2%)	7 (9%)	5 (6%)
II	N/D	N/D	12 (52%)	105 (26%)	23 (22%)	13 (16%)	30 (35%)
III	N/D	N/D	7 (30%)	238 (58%)	73 (69%)	53 (67%)	48 (56%)
IV	N/D	N/D	0 (0%)	38 (9%)	8 (8%)	6 (8%)	3 (3%)
Six-minute walk test [m]	N/D	N/D	387 [312;452]	336 [187;420]	282 [146;384]	218 [96;352]	352 [260;436]
Creatinine [mmol/l]	N/D	71 [63;89]	78 [72;86]	83 [69;104]	84 [70;106]	88 [74;103]	88 [72;103]
Bilirubin [umol/l]	N/D	9 [7;14]	9 [7;13]	11 [8;17]	12 [9;19]	12 [9;20]	11 [8;14]
Albumin [g/l]	N/D	40 [38;42]	40 [39;42]	40 [37;44]	38 [35;40]	38 [36;40]	38 [36;40]
CRP [mg/l]	N/D	3 [1;6]	2 [1;3]	4 [2;7]	3 [2;7]	5 [3;11]	2 [1;7]
Haemodynamics at diagnosis							
mRAP [mmHg]	N/D	6 [4;9]	6 [4;8]	9 [6;13]	9 [6;12]	9 [6;13]	8 [5;11]
mPAP [mmHg]	N/D	20 [16;23]	20 [17;22]	53 [44;62]	41 [33;54]	45 [34;54]	38 [33;44]
mPAWP [mmHg]	N/D	11 [9;14]	10 [8;13]	10 [7;13]	12 [9;14]	11 [8;13]	10 [8;13]
PVR [WU]	N/D	1.7 [1.1;2.5]	1.8 [1.2;2.3]	11.1 [6.8;15.7]	8.0 [4.9;11.3]	7.9 [4.8;11.6]	5.5 [4.0;8.1]
CO [L/min]	N/D	4.8 [3.7;5.9]	5.2 [4.5;6.0]	3.8 [3.0;4.8]	3.9 [3.3;4.6]	4.0 [3.0;4.8]	4.6 [4.1;5.7]
Comorbidities and medication							
COPD	0 (0%)	20 (15%)	4 (6%)	65 (15%)	8 (7%)	12 (13%)	8 (9%)
Diabetes	0 (0%)	15 (11%)	5 (8%)	82 (19%)	11 (10%)	8 (9%)	9 (10%)
Atherosclerosis	0 (0%)	14 (11%)	2 (4%)	59 (14%)	32 (37%)	17 (18%)	20 (23%)
Atrial arrhythmia	0 (0%)	22 (17%)	3 (5%)	57 (13%)	20 (19%)	22 (24%)	8 (9%)
Hypertension	0 (0%)	39 (30%)	12 (19%)	103 (24%)	36 (33%)	26 (28%)	29 (34%)
Dyslipidemia	0 (0%)	16 (12%)	13 (21%)	44 (10%)	22 (20%)	14 (15%)	25 (29%)
PDE-5 inhibitors	0 (0%)	0 (0%)	1 (2%)	283 (65%)	40 (39%)	34 (37%)	27 (31%)
ERA	0 (0%)	0 (0%)	0 (0%)	232 (54%)	28 (27%)	21 (23%)	15 (17%)
Riociguat	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (3%)
Prostanoid	0 (0%)	0 (0%)	0 (0%)	78 (18%)	2 (2%)	1 (1%)	4 (5%)
Anticoagulation	0 (0%)	44 (33%)	27 (84%)	291 (67%)	104 (96%)	87 (95%)	Stopped for RHC
Loop diuretic	0 (0%)	24 (18%)	5 (8%)	228 (53%)	47 (44%)	46 (50%)	46 (53%)
Potassium sparing diuretic	0 (0%)	6 (5%)	2 (3%)	104 (24%)	24 (22%)	26 (28%)	17 (20%)
Statin	0 (0%)	40 (30%)	11 (22%)	112 (26%)	38 (42%)	35 (38%)	25 (29%)
CCB	0 (0%)	22 (17%)	5 (8%)	75 (17%)	4 (4%)	9 (10%)	12 (14%)
Digoxin	0 (0%)	11 (8%)	1 (2%)	68 (16%)	7 (6%)	8 (9%)	3 (3%)
Anti-diabetic drugs	0 (0%)	13 (10%)	4 (9%)	62 (14%)	11 (13%)	8 (9%)	9 (10%)
Iron supplementation	0 (0%)	7 (5%)	1 (2%)	50 (12%)	8 (10%)	6 (7%)	7 (8%)
ACEi	0 (0%)	44 (33%)	11 (17%)	100 (23%)	30 (28%)	26 (28%)	12 (14%)

Means and standard deviations or counts are given. Co-morbidities and drug therapy are shown as the percentage of patients with those co-morbidities or on each agent (%). Ethnicity is shown for subjects who self-declared. A further group of 82 CTEPH patients sampled only after PEA surgery are detailed in Supplementary Table 1. BMI, body mass index; CTED, chronic thromboembolic disease; CTEPH, chronic thromboembolic pulmonary hypertension; IPAH, idiopathic and heritable pulmonary arterial hypertension; COPD, chronic obstructive pulmonary disease; PDE-5, phosphodiesterase 5; ERA, endothelin receptor

antagonists; CCB, calcium channel blocker; ACEi, angiotensin converting enzyme inhibitors; mRAP, mean right atrial pressure; mPAP, mean pulmonary artery pressure; mPAWP, mean pulmonary artery wedge pressure; PVR, pulmonary vascular resistance; CO, cardiac output; WHO, World Health Organisation;

Table 2 - Metabolites distinguishing chronic thromboembolic pulmonary hypertension from healthy and disease controls.

Metabolite	Metabolic pathway	Discovery			Replication			Linear regression with confounders		Comparator groups			
		CTEPH mean (SD)	HC mean (SD)	**Sig.**	CTEPH mean (SD)	HC mean (SD)	**Sig.**	HC vs CTEPH **Sig.**	DC vs CTEPH **Sig.**	CTED mean (SD)	**Sig.**	IPAH mean (SD)	**Sig.**
Significant in all analyses													
5-methylthioadenosine (MTA)	Polyamine Metabolism	1.72 (0.84)	0.07 (1.22)	**5.00E-17**	1.55 (0.91)	0.32 (1.12)	**6.95E-12**	**5.21E-05**	**0.0009**	1.34 (0.73)	**0.0019**	0.86 (1.26)	**3.75E-13**
N1-methyladenosine	Purine Metabolism, Adenine containing	1.58 (0.78)	0.03 (1.01)	**3.02E-17**	1.67 (0.61)	0.4 (1.1)	**8.10E-14**	**4.55E-05**	**0.0105**	0.91 (1.08)	**2.07E-06**	1.21 (0.93)	**1.56E-06**
N1-methylinosine	Purine Metabolism, (Hypo)Xanthine/Inosine containing	1.64 (1.54)	0 (1.1)	**1.51E-12**	1.91 (1.45)	0.41 (1.06)	**8.70E-13**	**7.20E-05**	**2.35E-05**	1.59 (1.11)	**0.0406**	1.65 (1.11)	**0.008**
7-methylguanine	Purine Metabolism, Guanine containing	1.23 (1.09)	0.01 (1.16)	**8.47E-10**	1.27 (1.25)	0.45 (1.19)	**0.0001**	**0.0007**	**0.0099**	0.54 (1.08)	**4.18E-05**	0.95 (1.28)	**0.019**
N-formylmethionine	Methionine, Cysteine, SAM and Taurine Metabolism	1.45 (0.88)	0.05 (1.02)	**6.10E-14**	1.51 (0.78)	0.36 (1.11)	**4.27E-11**	**0.0024**	**0.0042**	1.22 (0.78)	**0.014**	1.31 (0.86)	**0.0406**
Significant versus HC, DC and CTED													
sphingomyelin (d18:1/20:0, d16:1/22:0)*	Sphingomyelins	-0.91 (0.75)	0.3 (1.19)	**2.52E-10**	-0.71 (0.7)	0.1 (1.06)	**7.45E-07**	**9.64E-05**	**0.0402**	-0.28 (0.96)	**7.81E-05**	-0.93 (0.91)	0.0655
1-stearoyl-2-arachidonoyl-GPC (18:0/20:4)	Phosphatidylcholine (PC)	-0.69 (0.62)	0.14 (1.19)	**3.28E-06**	-0.53 (0.69)	0.26 (1.16)	**4.62E-06**	**0.002**	**0.0007**	-0.25 (0.75)	**0.0005**	-0.48 (0.85)	0.0989
N2,N2-dimethylguanosine	Purine Metabolism, Guanine containing	2 (0.69)	0.15 (0.99)	**8.50E-21**	1.9 (0.79)	0.28 (1.18)	**5.02E-15**	**4.73E-06**	**1.10E-06**	1.35 (0.82)	**6.44E-07**	1.81 (0.9)	0.1246
sphingomyelin (d18:1/21:0, d17:1/22:0, d16:1/23:0)*	Sphingomyelins	-0.87 (0.9)	0.36 (1.18)	**8.60E-11**	-0.56 (0.74)	0.17 (1.03)	**1.53E-06**	**1.25E-05**	**0.0161**	-0.15 (1.32)	**0.0032**	-0.77 (0.76)	0.3203
N-acetylmethionine	Methionine, Cysteine, SAM and Taurine Metabolism	1.17 (0.76)	0.18 (1.09)	**1.42E-10**	1.16 (0.75)	0.21 (1.16)	**1.00E-08**	**0.0286**	**0.031**	0.44 (1.03)	**7.22E-08**	1.12 (0.7)	0.3691
1-linoleoyl-2-arachidonoyl-GPC (18:2/20:4n6)*	Phosphatidylcholine (PC)	-0.78 (0.63)	0.13 (1.2)	**1.87E-09**	-0.54 (0.55)	0.32 (1.22)	**2.20E-10**	**0.001**	**0.0116**	-0.3 (0.66)	**9.38E-05**	-0.7 (0.92)	0.5379
pseudouridine	Pyrimidine Metabolism, Uracil containing	1.63 (0.78)	0.19 (0.97)	**8.02E-17**	1.67 (0.63)	0.26 (1.26)	**2.29E-13**	**0.0018**	**0.0425**	1.28 (0.82)	**0.0024**	1.57 (1.01)	0.8223

Metabolites that are significantly different in CTEPH compared with both healthy and disease controls, independent of confounders and significantly different in CTEPH compared to CTED. Mean values are given, and the data is scaled to the healthy control group. *Metabolite name**: probable metabolite identity, but unconfirmed (see methods). GPC, glycerophosphocholine; DC, disease controls; CTED, chronic thromboembolic disease (without PH); CTEPH, chronic thromboembolic pulmonary hypertension; IPAH/HPAH, idiopathic and heritable pulmonary arterial hypertension.

Biochemical	Sub-pathway	Super-pathway	Gradients					
			Pulmonary Artery Radial Artery (PA-ART)		Superior Vena Cava - Pulmonary Artery (SVC-PA)		Radial Artery – Superior Vena Cava (ART-SVC)	
			FC	FDR p-value	FC	FDR p-value	FC	FDR p-value
alpha-ketoglutarate	TCA Cycle	Energy	-0.184	<0.001	-0.269	<0.001	-0.453	<0.001
N-acetylmethionine	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	0.087	0.038	-0.152	<0.001		
oxalate (ethanedioate)	Ascorbate and Aldarate Metabolism	Cofactors and Vitamins	-0.288	<0.001			-0.282	<0.001
glycerate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	Carbohydrate	-0.36	<0.001			-0.403	<0.001
2-hydroxypalmitate	Fatty Acid, Monohydroxy	Lipid	-0.304	0.002			-0.414	<0.001
N-acetylvaline	Leucine, Isoleucine and Valine Metabolism	Amino Acid	0.118	0.003			0.052	0.026
N-formylmethionine	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid			-0.185	<0.001		
7-methylguanine	Purine Metabolism, Guanine containing	Nucleotide			-0.165	<0.001		
sphingomyelin (d18:2/23:0, d18:1/23:1, d17:1/24:1)*	Sphingomyelins	Lipid					0.119	<0.001
gamma-glutamyl-epsilon-lysine	Gamma-glutamyl Amino Acid	Peptide					-0.265	0.002
sphingomyelin (d18:1/21:0, d17:1/22:0, d16:1/23:0)*	Sphingomyelins	Lipid					0.085	0.005
N-acetylserine	Glycine, Serine and Threonine Metabolism	Amino Acid					-0.108	0.008
1,2-dilinoleoyl-GPC (18:2/18:2)	Phosphatidylcholine (PC)	Lipid					0.1	0.008
androsterone sulfate	Androgenic Steroids	Lipid					0.067	0.012
1-stearoyl-2-arachidonoyl-GPC (18:0/20:4)	Phosphatidylcholine (PC)	Lipid					0.145	0.013
sphingomyelin (d18:1/22:1, d18:2/22:0, d16:1/24:1)*	Sphingomyelins	Lipid					0.11	0.018
tryptophan	Tryptophan Metabolism	Amino Acid					0.109	0.018
N-acetylalanine	Alanine and Aspartate Metabolism	Amino Acid					-0.128	0.022
1-linoleoyl-2-arachidonoyl-GPC (18:2/20:4n6)*	Phosphatidylcholine (PC)	Lipid					0.099	0.03
N-acetylphenylalanine	Phenylalanine Metabolism	Amino Acid					0.092	0.033
sphingomyelin (d18:1/17:0, d17:1/18:0, d19:1/16:0)	Sphingomyelins	Lipid					0.083	0.045
1-(1-enyl-palmitoyl)-2-linoleoyl-GPC (P-16:0/18:2)*	Plasmalogen	Lipid					0.068	0.046

Table 3. Metabolites that associate with CTEPH and show significant gradients between sampling sites.

Abbreviations: FC - fold change; SVC - superior vena cava; PA - pulmonary artery; ART - radial artery; FDR - false discovery rate (corrected p-values displayed)

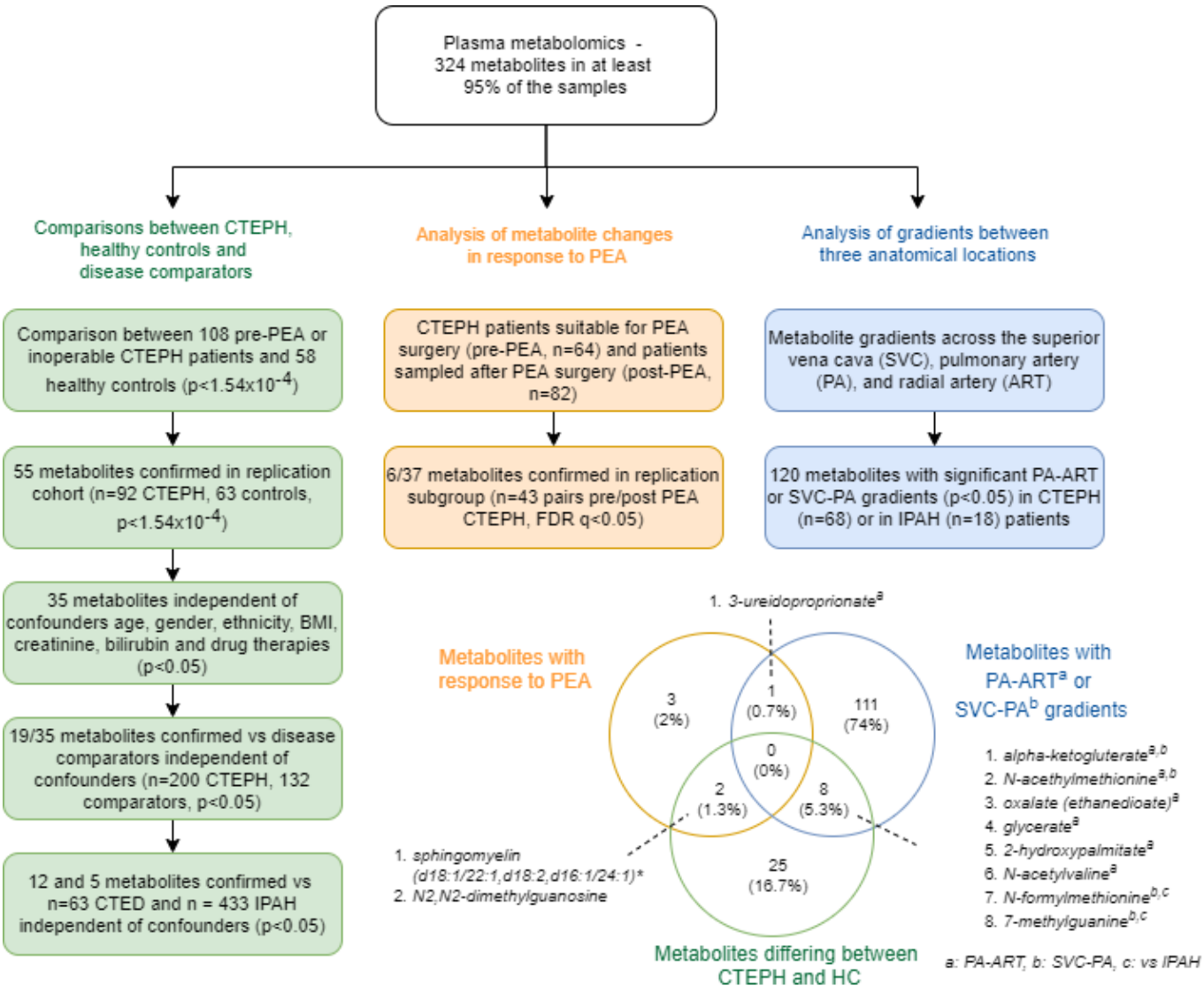


Figure 1. Main analyses study flowchart and overlap of metabolites identified in the main analyses. Individuals analysed consisted of patients with chronic thromboembolic pulmonary hypertension (CTEPH), healthy controls (HC), disease comparators (DC - referrals found not to have PH or those with chronic thromboembolic disease (CTED) but not PH) and patients with idiopathic or hereditary pulmonary arterial hypertension (IPAH/HPAH). Patients with inoperable CTEPH or those sampled before pulmonary endarterectomy (PEA) were used in the main analyses. 43 patients were also sampled after PEA, while a further 82 patients consented for sampling only post-PEA. Venn diagram depicts overlap in metabolites identified by comparisons of CTEPH compared to healthy controls, CTEPH patients analysed before and after PEA surgery, and plasma gradients across tissue vascular beds relevant to PH, specifically the pulmonary artery to radial artery (PA-ART[a]) and superior vena cava to pulmonary artery (SVC-PA[b]) gradients. Metabolites which also differed in the analysis of CTEPH against IPAH patients are indicated by superscript c.

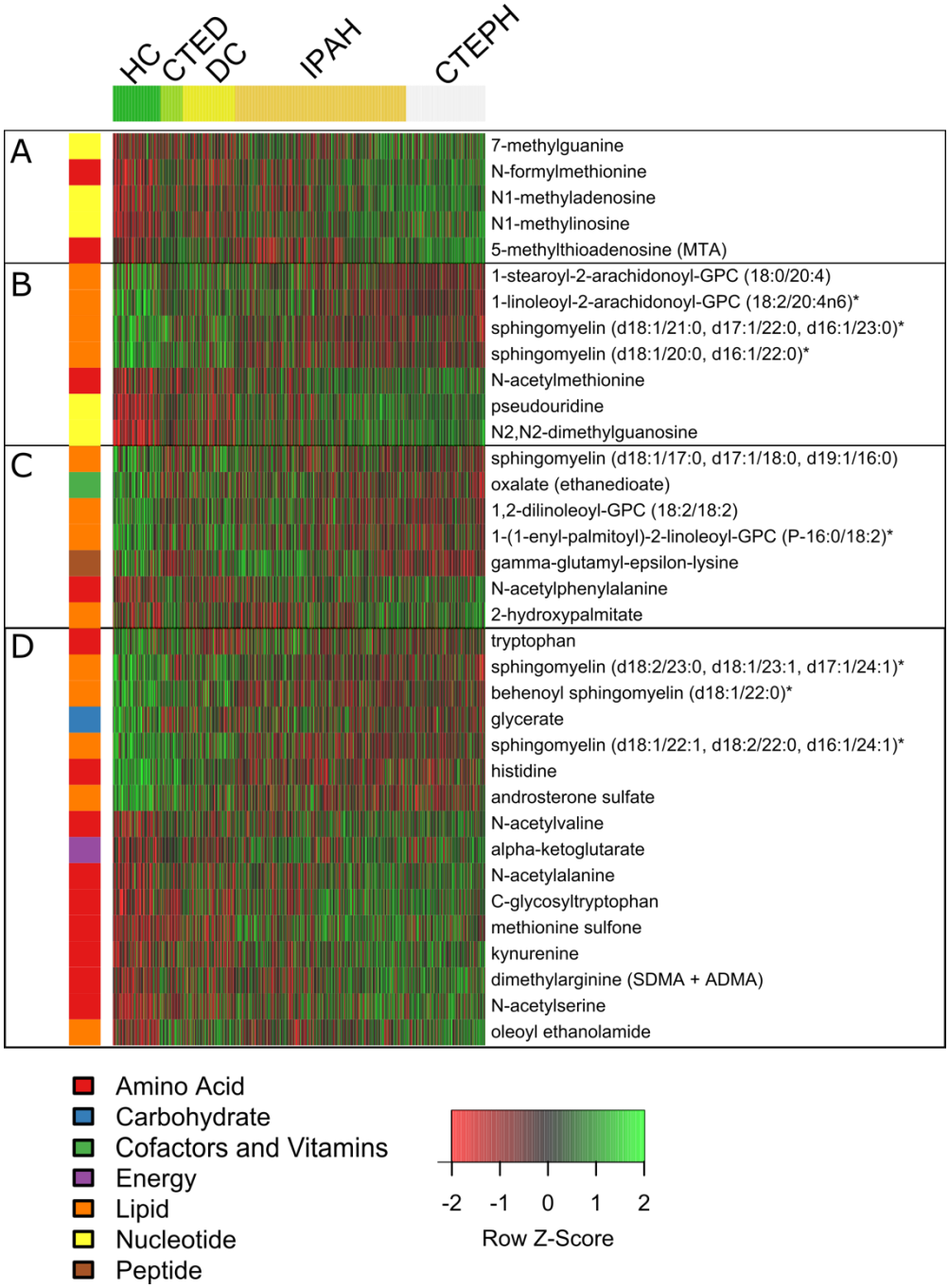
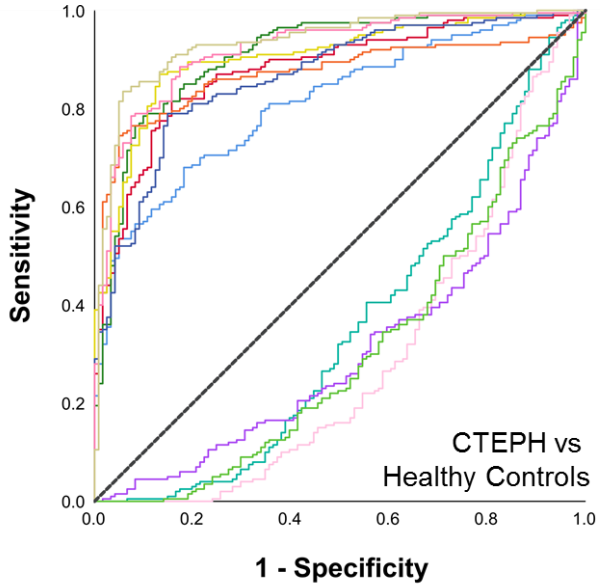
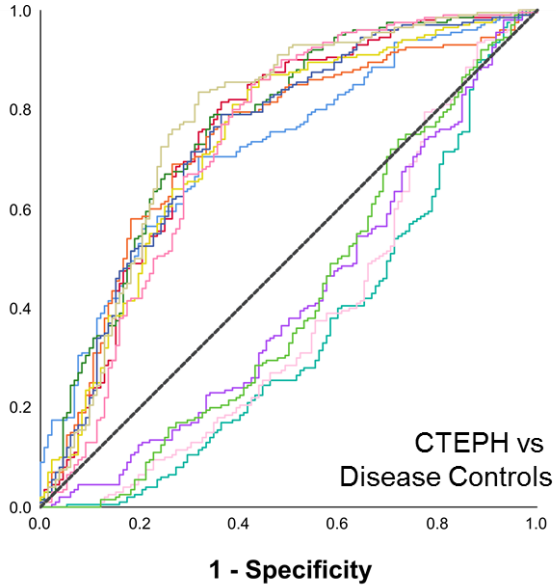
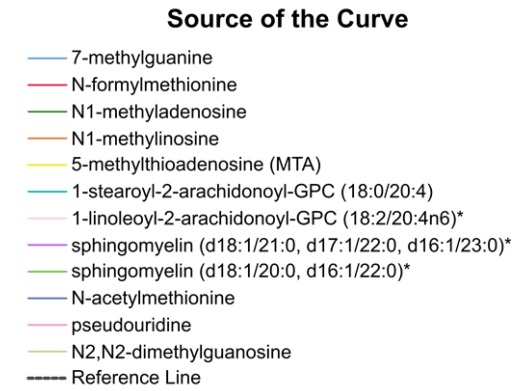


Figure 2. **Heatmap of 35 metabolites that distinguish CTEPH patients from healthy controls and disease comparators independent of confounders.** A. Metabolites distinguishing CTEPH from all other groups, B. distinguishing CTEPH from healthy controls (HC), disease controls (DC) and chronic thromboembolic disease (CTED), C. distinguishing CTEPH from HC and DC independent of confounders, D. distinguishing CTEPH from HC independent of confounders







	CTEPH vs healthy controls (n=200/121)				CTEPH vs disease controls (n=200/132)			
	Area Under the Curve	95% Confidence Interval			Area Under the Curve	95% Confidence Interval		
Test Result Variable(s)		Lower	Upper	Sig.		Lower	Upper	Sig.
7-methylguanine	0.812	0.766	0.858	7.94E-21	0.718	0.663	0.774	1.63E-11
N-formylmethionine	0.878	0.84	0.916	8.1E-30	0.746	0.689	0.803	2.96E-14
N1-methyladenosine	0.909	0.875	0.943	1.12E-34	0.76	0.706	0.815	1E-15
N1-methylinosine	0.871	0.831	0.911	7.68E-29	0.731	0.674	0.787	1.14E-12

5-methylthioadenosine (MTA)	0.904	0.87	0.937	7.99E-34	0.732	0.675	0.79	7.63E-13
1-stearoyl-2-arachidonoyl-GPC (18:0/20:4)	0.356	0.291	0.421	1.5E-05	0.349	0.286	0.411	3.1E-06
1-linoleoyl-2-arachidonoyl-GPC (18:2/20:4n6)*	0.291	0.229	0.354	3.8E-10	0.384	0.319	0.449	0.000333
sphingomyelin (d18:1/21:0, d17:1/22:0, d16:1/23:0)*	0.31	0.251	0.369	1.17E-08	0.417	0.353	0.481	0.010438
sphingomyelin (d18:1/20:0, d16:1/22:0)*	0.308	0.248	0.368	7.73E-09	0.416	0.351	0.481	0.009658
N-acetylmethionine	0.864	0.825	0.904	7.01E-28	0.739	0.682	0.795	1.74E-13
pseudouridine	0.92	0.89	0.95	1.6E-36	0.73	0.67	0.789	1.45E-12
N2,N2-dimethylguanosine	0.936	0.908	0.963	4.03E-39	0.769	0.713	0.825	1.16E-16

Figure 3 – ROC analysis of key metabolites distinguishing CTEPH from healthy controls and disease controls. Receiver operating characteristic curves demonstrate ability of metabolites to distinguish CTEPH from healthy controls (left-hand plot) and disease controls (right-hand plot) with areas under the curve shown in table below.

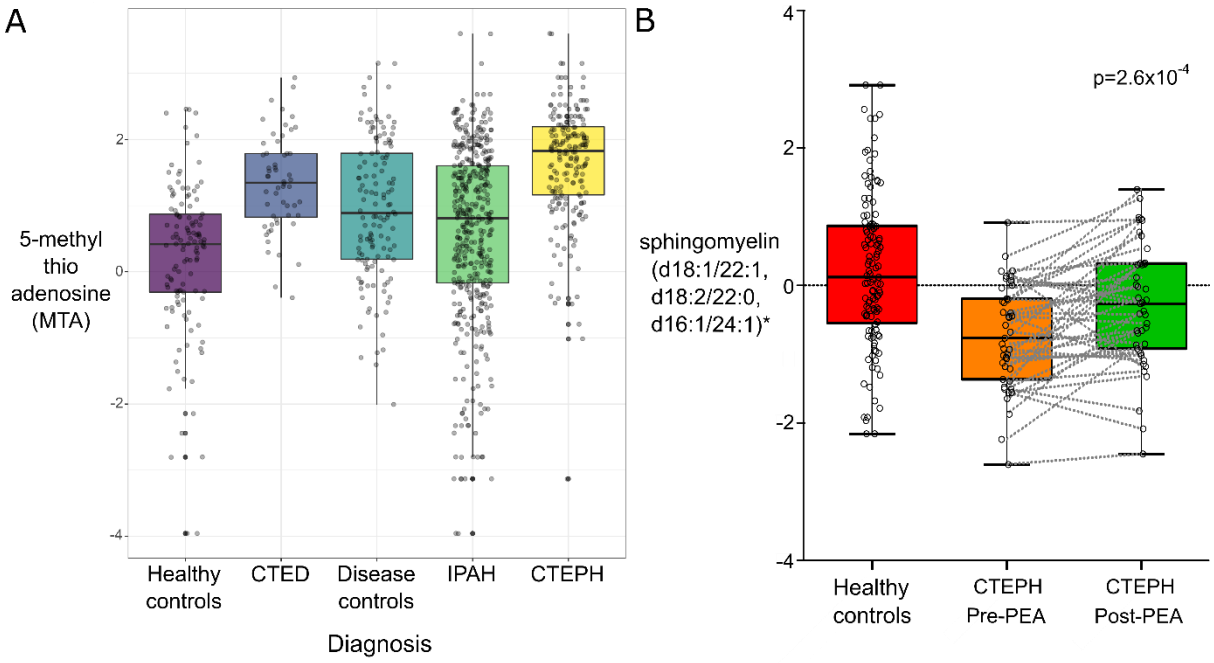


Figure 4 – Box and dot-plots of plasma levels of key metabolites. A. 5-methylthioadenosine (MTA) in healthy controls (HC), chronic thromboembolic disease (CTED) patients without PH, disease controls (DC), idiopathic pulmonary arterial hypertension (IPAH) and chronic thromboembolic pulmonary hypertension (CTEPH). Levels in CTEPH are significantly different compared to HC ($p=5.2x10^{-5}$) and DC ($p=9.3x10^{-4}$) after correcting for confounders and versus CTED ($p=1.9x10^{-3}$) and IPAH ($p=3.8x10^{-13}$). B. Boxplot of sphingomyelin (d18:1/22:1, d18:0/22:0, d16:1/24:1) levels in paired plasma samples from 43 CTEPH patients, taken before (Pre-PEA) and after surgery (Post-PEA), compared with healthy controls.

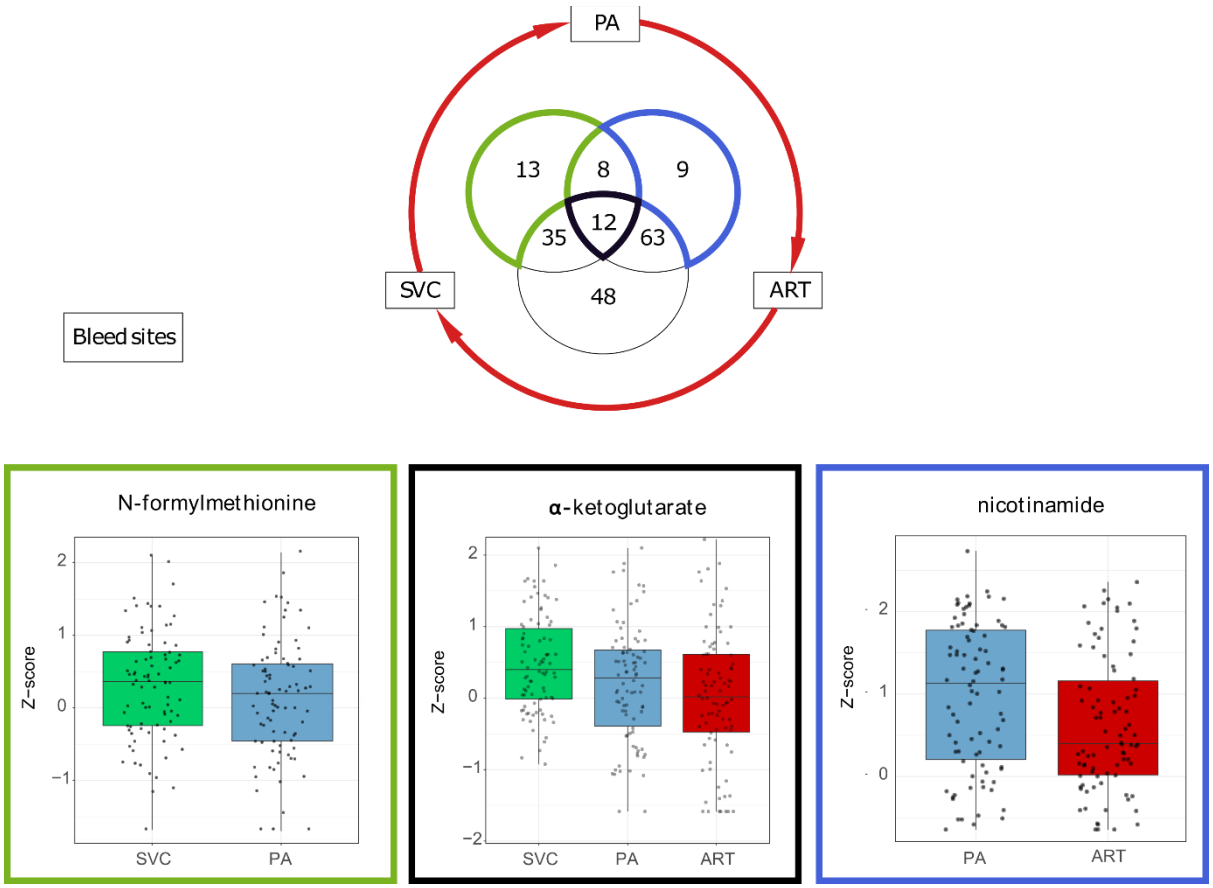


Figure 5. Overlap in metabolites showing significant PA-ART, SVC-PA and ART-SVC gradients. Boxplots show data for example metabolites from the SVC-PA - (green outline) and PA-ART - (blue outline) specific results and α-ketoglutarate which was significant across all three gradients. SVC, superior vena cava, PA, pulmonary artery, ART, radial artery.

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Online data supplement – Supplementary methods, tables and figures

Metabolomics in CTEPH by Swietlik et al.

Supplementary Methods

Assessment of operability

All patients with chronic thromboembolic pulmonary hypertension (CTEPH) included in the study were assessed in pulmonary endarterectomy (PEA) multidisciplinary team meetings (MDT) involving at least one cardiothoracic surgeon, cardiothoracic radiologist and chest physician. Technical operability was established based on surgical accessibility of thromboembolic material assessed by CT pulmonary angiogram and ventilation perfusion scan, in conjunction with thrombus burden and PVR.

PEA surgical procedure

PEA involves removal of obstructive thromboembolic material from the pulmonary arteries including the intima and superficial media in order to reduce PVR, decrease right ventricle (RV) afterload and improve ventilation-perfusion mismatch. PEA was performed through median sternotomy with cardiopulmonary bypass (CPB) enabling hypothermia to 20℃and safe circulatory arrest(1). Deep hypothermic circulatory arrest (DHCA) provided a clear operating field and was limited to 20 minutes intervals on each side. The identification of endarterectomy plane was followed by distal dissection to remove endarterectomy specimen as previously described(2, 3).

Right heart catheterisation (RHC) procedure and sampling at 3 anatomical locations

All patients sampled at 3 anatomical locations had elective RHC performed via right internal jugular (RIJ) vein. The access area was cleaned with 2% chlorhexidine and infiltrated with 6 to 9 ml of local aesthetic (1% lignocaine), position of RIJ was confirmed with ultrasound under sterile conditions. 7 French sheath was introduced to the RIJ using a Seldinger technique. Pressure transducer was set to zero at mid-thoracic level(1). Swan Ganz catheter was inserted and the position of the catheter was confirmed by pressure trace and fluoroscopy screening. First sample was obtained from SVC with the catheter tip just above right atrium entry, subsequently catheter was advanced and RA pressure was measured. Next the catheter was floated to the RV, where RV systolic pressure (RVSP) and end-diastolic pressure were recorded. Upon advancing the catheter to pulmonary artery (PA) systolic and diastolic pressure were measured (sPAP and dPAP) and the PA blood sample was obtained for the metabolomic analysis. After that the balloon was inflated and advanced to the distal portion of pulmonary artery in order to obtain pulmonary artery wedge pressure (PAWP). Subsequently cardiac output was measured

using thermodilution technique. Saturation run was performed at the end of RHC while removing the catheter, 3 ml samples for oximetry analysis were taken from PA, RV, RA and SVC. Finally, peripheral arterial sample for metabolomics analysis was obtained from distal portion of radial artery.

	Operable CTEPH sampled pre-PEA	Operable CTEPH sampled post-PEA	Operable CTEPH with paired samples pre/post-PEA	p-value
	N=64	N=82	N=43	
Baseline characteristics				
Age at sampling [years]	65 [50;74]	67 [54;75]	63 [55;72]	0.416
Time from sampling to PEA [months]	8.7[12.7;6.9]		7.7[5.6;9.9]	
Sex: F	23 (36%)	37 (45%)	15 (35%)	0.406
Ethnicity: European	41 (64%)	69 (84%)	37 (86%)	0.005
BMI [kg/m^2]	28 [25;31]	29 [25;33]	28 [25;33]	0.617
WHO functional class:				0.022
I	4 (7%)	12 (15%)	1 (2%)	
II	15 (25%)	29 (36%)	8 (19%)	
III	39 (65%)	35 (44%)	31 (72%)	
IV	2 (3%)	4 (5%)	3 (7%)	
6MWD [m]	240 [96;384]	382 [286;427]	336 [240;363]	0.002
Creatinine [mmol/l]	90 [73;103]	79 [70;90]	86 [75;100]	0.063
Bilirubin [μmol/l]	12 [9;19]	10 [8;14]	15 [9;20]	0.056
mRAP[mmHg]	8 [6;13]	10 [7;14]	8 [6;13]	0.076
mPAP[mmHg]	44 (14)	48 (11)	43 (12)	0.075
mPAWP[mmHg]	12 [9;16]	13 [10;15]	10 [9;12]	0.064
PVR[WU]	7.1 [4.5;10.6]	7.8 [5.2;11.5]	7.9 [5.9;10.3]	0.807
CO[L/min]	4.1 [3.4;5.0]	3.8 [2.9;4.8]	4.0 [3.5;4.5]	0.494
COPD	7 (11%)	12 (15%)	3 (7%)	0.437
Diabetes	5 (8%)	5 (6%)	6 (14%)	0.330
Atherosclerosis	17 (27%)	20 (24%)	12 (55%)	0.019
Atrial arrhythmia	11 (17%)	23 (28%)	8 (19%)	0.238
Hypertension	19 (30%)	26 (32%)	9 (21%)	0.435
Dyslipidemia	17 (27%)	12 (15%)	11 (26%)	0.156
Post pulmonary endarterectomy				
Time from PEA to sampling [months]		37[11.8;65.2]	5.8[4.7;11.9]	
mPAP [mmHg]		28 (11)	28 (10)	
PVR [WU]		1[0.99;3.9]	1.8 [0.97;3.4]	

Supplementary Table 1. Cohort Characteristics for CTEPH analysis. Significance is shown using Kruskal-Wallis (continuous) and Chi-squared tests (categorical) showing only minor differences in baseline characteristics between groups. Means and standard deviations, median and IQR and counts are given. BMI, body mass index; WHO, World Health Organisation; 6MWD, six-minute walk distance; mRAP, mean right atrial pressure; mPAP, mean pulmonary artery pressure; mPAWP, mean pulmonary artery wedge pressure; PVR, pulmonary artery resistance; CO, cardiac output;

COPD, chronic obstructive pulmonary disease; PEA, pulmonary endarterectomy. Ethnicity is shown for subjects who self-declared.

		Discovery			Validation			Linear regression with confounders			Comparator groups			
Metabolite	**Metabolic pathway**	**CTEPH**	**HC**	**Sig.**	**CTEPH**	**HC**	**Sig.**	**HC vs CTEPH**	**Main confounder vs HC**	**DC vs CTEPH**	**CTED**	**Sig.**	**IPAH**	**Sig.**
		mean (SD)	mean (SD)		mean (SD)	mean (SD)		**Sig.**		**Sig.**	mean (SD)		mean (SD)	
Significant in all analyses														
5-methylthioadenosine (MTA)	Polyamine Metabolism	1.72 (0.84)	0.07 (1.22)	**5.00E-17**	1.55 (0.91)	0.32 (1.12)	**6.95E-12**	**5.21E-05**		**0.0009**	1.34 (0.73)	**0.0019**	0.86 (1.26)	**3.75E-13**
N1-methyladenosine	Purine Metabolism, Adenine containing	1.58 (0.78)	0.03 (1.01)	**3.02E-17**	1.67 (0.61)	0.4 (1.1)	**8.10E-14**	**4.55E-05**		**0.0105**	0.91 (1.08)	**2.07E-06**	1.21 (0.93)	**1.56E-06**
N1-methylinosine	Purine Metabolism, (Hypo)Xanthine/Inosine containing	1.64 (1.54)	0 (1.1)	**1.51E-12**	1.91 (1.45)	0.41 (1.06)	**8.70E-13**	**7.20E-05**		**2.35E-05**	1.59 (1.11)	**0.0406**	1.65 (1.11)	**0.008**
7-methylguanine	Purine Metabolism, Guanine containing	1.23 (1.09)	0.01 (1.16)	**8.47E-10**	1.27 (1.25)	0.45 (1.19)	**0.0001**	**0.0007**		**0.0099**	0.54 (1.08)	**4.18E-05**	0.95 (1.28)	**0.019**
N-formylmethionine	Methionine, Cysteine, SAM and Taurine Metabolism	1.45 (0.88)	0.05 (1.02)	**6.10E-14**	1.51 (0.78)	0.36 (1.11)	**4.27E-11**	**0.0024**		**0.0042**	1.22 (0.78)	**0.014**	1.31 (0.86)	**0.0406**
Significant versus HC, DC and CTED														
sphingomyelin (d18:1/20:0, d16:1/22:0)*	Sphingomyelins	-0.91 (0.75)	0.3 (1.19)	**2.52E-10**	-0.71 (0.7)	0.1 (1.06)	**7.45E-07**	**9.64E-05**		**0.0402**	-0.28 (0.96)	**7.81E-05**	-0.93 (0.91)	0.0655
1-stearoyl-2-arachidonoyl-GPC (18:0/20:4)	Phosphatidylcholine (PC)	-0.69 (0.62)	0.14 (1.19)	**3.28E-06**	-0.53 (0.69)	0.26 (1.16)	**4.62E-06**	**0.002**		**0.0007**	-0.25 (0.75)	**0.0005**	-0.48 (0.85)	0.0989
N2,N2-dimethylguanosine	Purine Metabolism, Guanine containing	2 (0.69)	0.15 (0.99)	**8.50E-21**	1.9 (0.79)	0.28 (1.18)	**5.02E-15**	**4.73E-06**		**1.10E-06**	1.35 (0.82)	**6.44E-07**	1.81 (0.9)	0.1246
sphingomyelin (d18:1/21:0, d17:1/22:0, d16:1/23:0)*	Sphingomyelins	-0.87 (0.9)	0.36 (1.18)	**8.60E-11**	-0.56 (0.74)	0.17 (1.03)	**1.53E-06**	**1.25E-05**		**0.0161**	-0.15 (1.32)	**0.0032**	-0.77 (0.76)	0.3203
N-acetylmethionine	Methionine, Cysteine, SAM and Taurine Metabolism	1.17 (0.76)	0.18 (1.09)	**1.42E-10**	1.16 (0.75)	0.21 (1.16)	**1.00E-08**	**0.0286**		**0.031**	0.44 (1.03)	**7.22E-08**	1.12 (0.7)	0.3691
1-linoleoyl-2-arachidonoyl-GPC (18:2/20:4n6)*	Phosphatidylcholine (PC)	-0.78 (0.63)	0.13 (1.2)	**1.87E-09**	-0.54 (0.55)	0.32 (1.22)	**2.20E-10**	**0.001**		**0.0116**	-0.3 (0.66)	**9.38E-05**	-0.7 (0.92)	0.5379
pseudouridine	Pyrimidine Metabolism, Uracil containing	1.63 (0.78)	0.19 (0.97)	**8.02E-17**	1.67 (0.63)	0.26 (1.26)	**2.29E-13**	**0.0018**		**0.0425**	1.28 (0.82)	**0.0024**	1.57 (1.01)	0.8223
Significant versus HC, DC independent of confounders														
1,2-dilinoleoyl-GPC (18:2/18:2)	Phosphatidylcholine (PC)	-0.78 (0.75)	0.11 (1.25)	**5.91E-07**	-0.7 (0.67)	0.36 (1)	**7.68E-11**	**0.0025**		**0.0069**	-0.54 (0.79)	0.1446	-0.58 (0.93)	0.1023

N-acetylphenylalanine	Phenylalanine Metabolism	1 (0.94)	0.11 (1.11)	**9.94E-08**	0.98 (1.05)	0.27 (1.13)	**5.42E-05**	**0.0217**		**0.0029**	1.11 (0.88)	0.3722	0.85 (0.94)	0.1535
gamma-glutamyl-epsilon-lysine	Gamma-glutamyl Amino Acid	-1.06 (1.03)	0.33 (1.35)	**2.38E-09**	-0.98 (1.05)	0.05 (1.43)	**7.68E-06**	**0.0169**		**0.0464**	-1.11 (0.91)	0.4414	-0.35 (1.15)	1.82E-09
sphingomyelin (d18:1/17:0, d17:1/18:0, d19:1/16:0)	Sphingomyelins	-0.7 (1)	0.35 (1.15)	**4.63E-08**	-0.54 (0.85)	0.14 (1.21)	**0.0001**	**0.0239**		**0.0273**	-0.66 (1.19)	0.5115	-0.5 (1.05)	0.4819
oxalate (ethanedioate)	Ascorbate and Aldarate Metabolism	-0.7 (1.07)	0.28 (1)	**4.13E-08**	-0.92 (0.91)	0.18 (1.29)	**8.36E-08**	**0.0087**		**0.0023**	-0.8 (1.25)	0.5194	-0.54 (1.04)	0.009
1-(1-enyl-palmitoyl)-2-linoleoyl-GPC (P-16:0/18:2)*	Plasmalogen	-0.95 (1.06)	0.27 (1.17)	**3.17E-09**	-1.08 (1.24)	0.16 (1.21)	**6.04E-08**	**0.0065**		**0.0446**	-0.87 (1.33)	0.6299	-0.99 (1.12)	0.5948
2-hydroxypalmitate	Fatty Acid, Monohydroxy	1.08 (0.86)	0.29 (1.12)	**8.56E-06**	0.94 (0.99)	0.2 (1.19)	**5.34E-05**	**0.0002**		**0.0005**	1.01 (0.91)	0.909	0.85 (0.95)	0.052
Significant versus HC independent of confounders														
sphingomyelin (d18:1/22:1, d18:2/22:0, d16:1/24:1)*	Sphingomyelins	-0.87 (0.81)	0.34 (1.24)	**7.45E-10**	-0.71 (0.82)	0.1 (1)	**5.21E-07**	**0.0002**		0.0549	0 (0.86)	8.20E-09	-0.98 (0.97)	0.018
dimethylarginine (SDMA + ADMA)	Urea cycle; Arginine and Proline Metabolism	1.3 (1.02)	0.08 (1.09)	**1.04E-10**	1.24 (0.89)	0.34 (1.04)	**4.49E-08**	**0.0003**		0.0596	0.86 (1.07)	0.0043	1.07 (1.02)	0.0264
methionine sulfone	Methionine, Cysteine, SAM and Taurine Metabolism	1.22 (1.39)	0.22 (1.1)	**1.30E-05**	1.58 (1.3)	0.17 (1.14)	**6.98E-10**	**0.0025**		0.0606	0.28 (1.26)	3.34E-07	1.75 (1.27)	0.006
kynurenine	Tryptophan Metabolism	1.24 (0.95)	0.12 (1.13)	**1.62E-08**	1.37 (1.12)	0.31 (1.31)	**1.42E-06**	**0.0159**		0.0694	0.88 (1.38)	0.1004	1.25 (1.11)	0.6286
androsterone sulfate	Androgenic Steroids	-1.28 (1.28)	0.26 (1.19)	**2.59E-11**	-1.43 (1.43)	0.2 (1.47)	**4.26E-10**	**0.0005**		0.0728	-0.45 (1.5)	2.57E-05	-1.28 (1.36)	0.5555
sphingomyelin (d18:2/23:0, d18:1/23:1, d17:1/24:1)*	Sphingomyelins	-0.61 (0.81)	0.39 (1.05)	**1.90E-10**	-0.33 (0.68)	0.18 (0.9)	**9.03E-06**	**0.0002**		0.075	-0.33 (1.12)	0.286	-0.48 (0.66)	0.9952
histidine	Histidine Metabolism	-1.27 (1.33)	0.19 (1.03)	**7.41E-11**	-1.53 (1.5)	0.26 (1.32)	**6.33E-11**	**0.039**		0.0869	-0.14 (1.34)	1.85E-08	-1.53 (1.33)	0.4164
alpha-ketoglutarate	TCA Cycle	1.06 (1.27)	0.25 (1.18)	**3.44E-07**	1.05 (1.31)	0.25 (1.44)	**0.0001**	**0.0274**		0.1066	1.02 (0.7)	0.0712	1.13 (1.18)	0.7675
oleoyl ethanolamide	Endocannabinoid	1.57 (0.85)	0.38 (1.19)	**5.06E-10**	1.17 (1.13)	0.04 (1.13)	**3.47E-09**	**0.0001**		0.177	1.45 (1.08)	0.6262	0.95 (0.97)	8.56E-08
glycerate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	-0.72 (0.99)	0.48 (1.15)	**1.88E-10**	-0.91 (0.95)	-0.06 (1.26)	**2.38E-06**	**0.029**		0.2477	-1 (1.25)	0.0857	-0.57 (1.05)	0.0087
C-glycosyltryptophan	Tryptophan Metabolism	1.2 (0.75)	0.17 (0.92)	**3.47E-11**	1.31 (0.81)	0.24 (1.15)	**3.97E-09**	**0.0062**		0.3142	0.24 (0.97)	6.11E-12	1.27 (0.94)	0.5221
N-acetylserine	Glycine, Serine and Threonine Metabolism	1.49 (1.04)	0.09 (0.93)	**2.19E-12**	1.54 (1.08)	0.37 (1.18)	**3.28E-08**	**0.0271**		0.3199	0.76 (1.05)	5.36E-06	1.41 (1.17)	0.5923

N-acetylvaline	Leucine, Isoleucine and Valine Metabolism	0.96 (0.82)	0.1 (1.11)	**1.33E-08**	1.01 (0.69)	0.26 (0.98)	**1.24E-07**	**0.0397**		0.3923	0.86 (0.75)	0.2182	0.91 (0.89)	0.5789
behenoyl sphingomyelin (d18:1/22:0)*	Sphingomyelins	-0.68 (0.67)	0.34 (0.98)	**2.37E-11**	-0.47 (0.63)	0.19 (1.06)	**4.16E-06**	**0.0049**		0.4009	-0.1 (0.67)	1.62E-06	-0.65 (0.78)	0.0769
N-acetylalanine	Alanine and Aspartate Metabolism	1.1 (0.95)	0.15 (0.94)	**1.52E-10**	1.18 (0.79)	0.28 (1.17)	**5.16E-07**	**0.0423**		0.5963	0.8 (0.89)	0.0025	1 (0.95)	0.249
tryptophan	Tryptophan Metabolism	-0.44 (0.87)	0.22 (1.12)	**9.54E-05**	-0.48 (0.91)	0.22 (1.31)	**0.0001**	**0.0405**		0.6793	0.05 (0.98)	0.0003	-0.28 (1.07)	0.0634
Significant versus HC but dependent on confounders														
1-linoleoyl-GPC (18:2)	Lysophospholipid	-0.92 (0.68)	0.16 (1.2)	**1.95E-09**	-0.86 (0.68)	0.28 (1.2)	**1.09E-09**	0.0662	Age	0.7221	-0.7 (1.04)	0.1358	-1.07 (1.05)	0.0082
citrate	TCA Cycle	1.58 (1.13)	-0.01 (1.15)	**2.63E-12**	1.33 (1.15)	0.41 (1.12)	**6.09E-06**	0.0775	PDE5 inhibitors	0.0486	1.1 (1.18)	0.038	0.93 (1.24)	5.14E-06
1-palmitoyl-GPC (16:0)	Lysophospholipid	-0.84 (0.64)	0.24 (1.08)	**8.40E-11**	-0.9 (0.74)	0.21 (1.29)	**1.37E-09**	0.0972	Bilirubin	0.0031	-0.13 (0.78)	5.49E-10	-0.69 (0.91)	0.0722
malate	TCA Cycle	1.34 (0.8)	0 (1.37)	**3.98E-11**	1.31 (0.88)	0.35 (0.83)	**6.46E-10**	0.1074	Age	0.013	0.53 (0.81)	3.46E-09	1.32 (0.93)	0.9231
4-acetamidobutanoate	Polyamine Metabolism	1.57 (1.2)	0.14 (0.98)	**7.11E-13**	1.58 (1.13)	0.28 (1.22)	**2.32E-09**	0.1192	Age	0.6594	0.35 (1.73)	5.58E-07	1.63 (1.22)	0.4758
erythronate*	Aminosugar Metabolism	0.89 (0.79)	0.11 (0.94)	**3.97E-07**	1.07 (0.77)	0.25 (1.21)	**2.47E-06**	0.1233	Diuretics	0.7631	0.58 (1.04)	0.0077	0.94 (0.99)	0.7118
leucine	Leucine, Isoleucine and Valine Metabolism	-0.59 (1.09)	0.15 (1.02)	**0.0001**	-0.54 (1.02)	0.27 (1.23)	**3.20E-05**	0.1256	Gender	0.3889	0.35 (0.99)	7.31E-08	-0.59 (1.01)	0.9785
1-stearoyl-2-linoleoyl-GPC (18:0/18:2)*	Phosphatidylcholine (PC)	-0.78 (0.9)	0.11 (1.1)	**7.41E-07**	-0.64 (0.87)	0.32 (1.17)	**4.07E-07**	0.1405	Statins	0.0817	-0.33 (0.63)	0.0006	-0.59 (1.05)	0.2836
arginine	Urea cycle; Arginine and Proline Metabolism	-1.16 (1.05)	0.22 (1.2)	**2.79E-11**	-1.11 (0.82)	0.26 (1.19)	**1.02E-12**	0.1406	Age	0.0507	-0.95 (1.38)	0.0765	-1.1 (1.15)	0.9618
1-stearoyl-GPC (18:0)	Lysophospholipid	-0.9 (0.63)	0.21 (1.18)	**2.05E-09**	-0.84 (0.73)	0.24 (1.24)	**4.38E-09**	0.1595	Bilirubin	0.1739	-0.18 (0.92)	1.25E-07	-0.73 (0.84)	0.2863
homoarginine	Urea cycle; Arginine and Proline Metabolism	-0.8 (1.11)	0.24 (1.13)	**8.63E-08**	-1.18 (1.04)	0.23 (1.46)	**9.63E-10**	0.1746	Age	0.8135	0.1 (1.1)	5.25E-09	-0.99 (1.14)	0.8129
N-acetylneuraminate	Aminosugar Metabolism	0.87 (0.71)	0.08 (1.14)	**5.10E-06**	0.87 (0.85)	0.28 (1.03)	**6.04E-05**	0.181	Age	0.0414	-0.08 (0.72)	2.74E-13	0.91 (0.77)	0.7097
orotidine	Pyrimidine Metabolism, Orotate containing	0.92 (0.72)	0.1 (1.12)	**1.14E-07**	1.03 (0.66)	0.27 (1.21)	**2.56E-06**	0.2776	Gender	0.19	0.45 (0.98)	1.10E-05	1.1 (0.82)	0.0021

N6-carbamoylthreonyladenosine	Purine Metabolism, Adenine containing	1.35 (1.01)	0.14 (1.06)	**1.54E-12**	1.43 (0.89)	0.32 (1.3)	**1.18E-09**	0.2958	Age	0.1371	1.11 (0.84)	0.0028	1.32 (0.89)	0.2009
1-(1-enyl-palmitoyl)-GPC (P-16:0)*	Lysoplasmalogen	-0.51 (0.9)	0.28 (1.29)	**4.64E-05**	-0.63 (1)	0.18 (1.06)	**2.36E-06**	0.4844	Antidiabetic	0.9232	0.02 (0.96)	9.18E-05	-0.57 (0.89)	0.6294
methionine sulfoxide	Methionine, Cysteine, SAM and Taurine Metabolism	-0.61 (0.84)	0.12 (1.08)	**4.23E-06**	-0.61 (0.98)	0.23 (1.27)	**2.27E-05**	0.4864	Gender	0.3433	-0.68 (0.77)	0.6097	-0.11 (0.87)	2.02E-09
asparagine	Alanine and Aspartate Metabolism	-0.6 (0.95)	0.19 (1.12)	**1.02E-05**	-0.87 (0.83)	0.2 (1.4)	**2.87E-07**	0.5035	Age	0.0657	-0.59 (1.05)	0.549	-0.87 (1.02)	0.1567
1-arachidonoyl-GPC (20:4n6)*	Lysophospholipid	-0.6 (1.04)	0.12 (1.4)	**3.57E-05**	-0.54 (1.13)	0.25 (1.11)	**4.12E-05**	0.5114	Statins	0.9221	0.26 (1.04)	2.27E-06	-0.85 (1.07)	0.0125
3-hydroxy-3-methylglutarate	Mevalonate Metabolism	0.92 (0.73)	0.11 (1.11)	**3.50E-07**	0.84 (0.9)	0.21 (1.13)	**0.0001**	0.653	Age	0.5855	0.19 (0.84)	9.72E-09	0.93 (0.81)	0.5036
vanillylmandelate (VMA)	Tyrosine Metabolism	1.53 (1.21)	0.01 (1.13)	**9.88E-12**	1.34 (1.27)	0.4 (1.11)	**5.16E-07**	0.8066	Age	0.1236	0.54 (1.08)	5.68E-07	1.31 (1.3)	0.3765

Supplementary Table 2. Altered plasma metabolite profiles in CTEPH patients.

Metabolites distinguishing CTEPH from healthy and disease controls. 55 metabolites that are significantly different between CTEPH and healthy controls in a discovery and validation cohort ($p<1.54x10^{-4}$) are shown. Mean values are given and the data is scaled to the healthy control group. Significance from linear regression is shown (p value), and for metabolites with $p>0.05$ in CTEPH HC linear regression, the significant confounder is shown. Significance is also shown for Mann Whitney U test between all CTEPH patients versus CTED and PE patients. *probable metabolite identity, but unconfirmed (see methods). DM, diabetes; GPC, glycerophosphocholine; HC, healthy controls; DC, disease controls; BMI, body mass index; CTED, chronic thromboembolic disease; PE, pulmonary embolism.

Metabolite	Metabolite family	Metabolic pathway	Effect of Age	SE	Sig	Effect of male sex	SE	Sig.	Effect of CTEPH vs HC	SE	Sig.
N1-methylinosine	Nucleotide	Purine Metabolism, (Hypo)Xanthine/Inosine containing	0.02	0.01	0.013	0.31	0.18	0.086	2.03	0.50	7E-05
5-methylthioadenosine (MTA)	Amino Acid	Polyamine Metabolism	0.00	0.01	0.561	0.33	0.15	0.029	1.72	0.42	5E-05
N1-methyladenosine	Nucleotide	Purine Metabolism, Adenine containing	0.01	0.00	0.05	-0.08	0.13	0.55	1.48	0.35	5E-05
7-methylguanine	Nucleotide	Purine Metabolism, Guanine containing	0.01	0.01	0.046	0.24	0.15	0.114	1.46	0.43	0.0007
N-formylmethionine	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism	0.01	0.00	0.002	0.11	0.13	0.409	1.11	0.36	0.0024
N2,N2-dimethylguanosine	Nucleotide	Purine Metabolism, Guanine containing	0.02	0.00	1E-04	-0.07	0.12	0.55	1.55	0.33	5E-06
pseudouridine	Nucleotide	Pyrimidine Metabolism, Uracil containing	0.01	0.00	6E-04	0.16	0.13	0.214	1.10	0.35	0.0018
N-acetylmethionine	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism	0.00	0.00	0.375	-0.19	0.14	0.164	0.84	0.38	0.0286
1-stearoyl-2-arachidonoyl-GPC (18:0/20:4)	Lipid	Phosphatidylcholine (PC)	-0.01	0.00	0.056	-0.08	0.12	0.528	-1.05	0.33	0.002
1-linoleoyl-2-arachidonoyl-GPC (18:2/20:4n6)*	Lipid	Phosphatidylcholine (PC)	-0.02	0.00	7E-07	0.04	0.13	0.768	-1.17	0.35	0.001
sphingomyelin (d18:1/20:0, d16:1/22:0)*	Lipid	Sphingomyelins	0.00	0.00	0.572	-0.39	0.11	8E-04	-1.26	0.32	1E-04

sphingomyelin (d18:1/21:0, d17:1/22:0, d16:1/23:0)*	Lipid	Sphingomyelins	0.00	0.00	0.647	-0.57	0.12	6E-06	-1.53	0.34	1E-05
2-hydroxypalmitate	Lipid	Fatty Acid, Monohydroxy	0.01	0.00	0.229	-0.02	0.15	0.889	1.52	0.41	0.0002
N-acetylphenylalanine	Amino Acid	Phenylalanine Metabolism	0.00	0.01	0.898	0.25	0.16	0.126	1.03	0.45	0.0217
sphingomyelin (d18:1/17:0, d17:1/18:0, d19:1/16:0)	Lipid	Sphingomyelins	0.01	0.00	0.304	-0.59	0.15	7E-05	-0.92	0.41	0.0239
1,2-dilinoleoyl-GPC (18:2/18:2)	Lipid	Phosphatidylcholine (PC)	-0.01	0.00	7E-04	-0.12	0.13	0.34	-1.09	0.36	0.0025
gamma-glutamyl-epsilon-lysine	Peptide	Gamma-glutamyl Amino Acid	-0.01	0.01	0.225	0.03	0.17	0.864	-1.14	0.47	0.0169
oxalate (ethanedioate)	Cofactors and Vitamins	Ascorbate and Aldarate Metabolism	0.00	0.01	0.436	-0.32	0.16	0.044	-1.18	0.44	0.0087
1-(1-enyl-palmitoyl)-2-linoleoyl-GPC (P-16:0/18:2)*	Lipid	Plasmalogen	-0.01	0.01	0.024	-0.55	0.16	5E-04	-1.19	0.43	0.0065
oleoyl ethanolamide	Lipid	Endocannabinoid	0.00	0.00	0.395	-0.25	0.14	0.071	1.50	0.38	0.0001
dimethylarginine (SDMA + ADMA)	Amino Acid	Urea cycle; Arginine and Proline Metabolism	0.02	0.00	0.001	0.03	0.14	0.813	1.45	0.39	0.0003
methionine sulfone	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism	0.01	0.01	0.28	-0.48	0.17	0.004	1.41	0.46	0.0025
alpha-ketoglutarate	Energy	TCA Cycle	0.00	0.01	0.77	-0.20	0.19	0.3	1.19	0.54	0.0274
kynurenine	Amino Acid	Tryptophan Metabolism	0.01	0.01	0.279	0.26	0.16	0.098	1.06	0.44	0.0159
C-glycosyltryptophan	Amino Acid	Tryptophan Metabolism	0.02	0.00	3E-05	-0.17	0.12	0.169	0.94	0.34	0.0062
N-acetylserine	Amino Acid	Glycine, Serine and Threonine Metabolism	0.02	0.01	1E-04	0.11	0.15	0.473	0.93	0.42	0.0271
N-acetylalanine	Amino Acid	Alanine and Aspartate Metabolism	0.01	0.00	0.005	0.03	0.13	0.808	0.77	0.37	0.0423

N-acetylvaline	Amino Acid	Leucine, Isoleucine and Valine Metabolism	0.01	0.00	0.069	0.07	0.13	0.594	0.74	0.36	0.0397
tryptophan	Amino Acid	Tryptophan Metabolism	-0.02	0.00	6E-07	0.18	0.13	0.188	-0.76	0.37	0.0405
behenoyl sphingomyelin (d18:1/22:0)*	Lipid	Sphingomyelins	-0.01	0.00	0.013	-0.30	0.11	0.006	-0.86	0.30	0.0049
histidine	Amino Acid	Histidine Metabolism	-0.03	0.01	1E-06	0.05	0.17	0.769	-1.00	0.48	0.039
sphingomyelin (d18:2/23:0, d18:1/23:1, d17:1/24:1)*	Lipid	Sphingomyelins	0.00	0.00	0.414	-0.81	0.10	5E-15	-1.00	0.27	0.0002
glycerate	Carbohydrate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	0.00	0.01	0.871	-0.09	0.17	0.57	-1.02	0.46	0.029
sphingomyelin (d18:1/22:1, d18:2/22:0, d16:1/24:1)*	Lipid	Sphingomyelins	0.00	0.00	0.636	-0.81	0.11	2E-11	-1.19	0.31	0.0002
androsterone sulfate	Lipid	Androgenic Steroids	-0.02	0.01	0.003	0.84	0.17	3E-06	-1.71	0.48	0.0005

Supplementary Table 3 – Effect of age and sex on metabolites associated with CTEPH Linear regression analyses were used to model age and sex effects in healthy controls and CTEPH patients correcting for CTEPH status and confounders.

CTEPH (n=200) vs HC (n=121)	Area Under the Curve	95% Confidence Interval					
Test Result Variable(s)		Lower	Upper	Sig.	Best cut-off	Sensitivity	Specificity
7-methylguanine	0.812	0.766	0.858	7.94E-21	0.83	0.68	0.818
N-formylmethionine	0.878	0.84	0.916	8.1E-30	0.94	0.815	0.843
N1-methyladenosine	0.909	0.875	0.943	1.12E-34	1.14	0.785	0.901
N1-methylinosine	0.871	0.831	0.911	7.68E-29	1.29	0.745	0.95
5-methylthioadenosine (MTA)	0.904	0.87	0.937	7.99E-34	0.89	0.87	0.868
1-stearoyl-2-arachidonoyl-GPC (18:0/20:4)	0.356	0.291	0.421	1.5E-05	0.44	0.92	0.347
1-linoleoyl-2-arachidonoyl-GPC (18:2/20:4n6)*	0.291	0.229	0.354	3.8E-10	0.08	0.84	0.521
sphingomyelin (d18:1/21:0, d17:1/22:0, d16:1/23:0)*	0.31	0.251	0.369	1.17E-08	-0.51	0.565	0.752
sphingomyelin (d18:1/20:0, d16:1/22:0)*	0.308	0.248	0.368	7.73E-09	-0.43	0.63	0.669
N-acetylmethionine	0.864	0.825	0.904	7.01E-28	0.83	0.79	0.851

pseudouridine	0.92	0.89	0.95	1.6E-36	0.83	0.885	0.826
N2,N2-dimethylguanosine	0.936	0.908	0.963	4.03E-39	1.35	0.835	0.942

CTEPH (n=200) vs DC (n=132)	Area Under the Curve	95% Confidence Interval					
Test Result Variable(s)		Lower	Upper	Sig.	Best cut-off	Sensitivity	Specificity
7-methylguanine	0.718	0.663	0.774	1.63E-11	0.74	0.705	0.682
N-formylmethionine	0.746	0.689	0.803	2.96E-14	0.94	0.815	0.636
N1-methyladenosine	0.76	0.706	0.815	1E-15	1.14	0.785	0.659
N1-methylinosine	0.731	0.674	0.787	1.14E-12	1.65	0.69	0.735
5-methylthioadenosine (MTA)	0.732	0.675	0.79	7.63E-13	1.16	0.81	0.614
1-stearoyl-2-arachidonoyl-GPC (18:0/20:4)	0.349	0.286	0.411	3.1E-06	-0.13	0.72	0.553
1-linoleoyl-2-arachidonoyl-GPC (18:2/20:4n6)*	0.384	0.319	0.449	0.000333	-0.46	0.585	0.652
sphingomyelin (d18:1/21:0, d17:1/22:0, d16:1/23:0)*	0.417	0.353	0.481	0.010438	-0.05	0.74	0.439
sphingomyelin (d18:1/20:0, d16:1/22:0)*	0.416	0.351	0.481	0.009658	-0.21	0.695	0.5
N-acetylmethionine	0.739	0.682	0.795	1.74E-13	0.83	0.79	0.636

pseudouridine	0.73	0.67	0.789	1.45E-12	0.97	0.86	0.568
N2,N2-dimethylguanosine	0.769	0.713	0.825	1.16E-16	1.35	0.835	0.682

Supplementary Table 4 – ROC analysis and best cut-offs for metabolites which distinguish CTEPH from healthy and disease controls and CTED patients. Best cut-offs derived using the Youden Index of specificity+sensitivity. Areas under the curve below 0.5 indicate that lower levels of the metabolite are associated with CTEPH – comparable AUCs can be calculated by subtracting these from 1.

Metabolite	Super metabolic pathway	Sub metabolic pathway	CTEPH operable	CTEPH-PostPEA	Sig.	Paired CTEPH, pre-PEA	Paired CTEPH, post-PEA	Sig.	q FDR
sphingomyelin (d18:1/22:1, d18:2/22:0, d16:1/24:1)*	Lipid	Sphingomyelins	-0.85 (0.88)	-0.59 (0.87)	0.043	-0.84	-0.38	0.0002	**0.0075**
taurine	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism	-0.35 (0.9)	-0.01 (1.12)	0.0407	0.43	-0.22	0.0007	**0.0131**
sphingomyelin (d18:1/14:0, d16:1/16:0)*	Lipid	Sphingomyelins	-0.45 (0.63)	-0.16 (0.67)	0.0064	-0.61	-0.31	0.0014	**0.0133**
3-ureidopropionate	Nucleotide	Pyrimidine Metabolism, Uracil containing	0.22 (1.56)	-0.03 (1.15)	0.038	1.20	0.74	0.0014	**0.0133**
alpha-tocopherol	Cofactors and Vitamins	Tocopherol Metabolism	0.29 (1.02)	0.62 (1.19)	0.05	0.12	0.65	0.0035	**0.0257**
N2,N2-dimethylguanosine	Nucleotide	Purine Metabolism, Guanine containing	1.95 (0.8)	1.68 (0.79)	0.0312	2.17	1.85	0.0045	**0.0278**
beta-hydroxyisovalerate	Amino Acid	Leucine, Isoleucine and Valine Metabolism	0.47 (1.29)	-0.07 (1.11)	0.0065	0.33	-0.12	0.009	**0.0478**
sphingomyelin (d18:1/21:0, d17:1/22:0, d16:1/23:0)*	Lipid	Sphingomyelins	-0.75 (0.79)	-0.39 (0.88)	0.0277	-1.04	-0.65	0.0122	0.05
cholesterol	Lipid	Sterol	-0.19 (1.19)	0.24 (1.14)	0.013	-0.45	0.00	0.0133	0.05
1-palmitoyl-2-stearoyl-GPC (16:0/18:0)	Lipid	Phosphatidylcholine (PC)	0.14 (0.89)	0.57 (1.12)	0.0129	0.45	0.03	0.0138	0.05
1,2-dipalmitoyl-GPC (16:0/16:0)	Lipid	Phosphatidylcholine (PC)	-0.16 (1.01)	0.27 (1.17)	0.0236	0.87	0.53	0.0162	0.05
1-(1-enyl-palmitoyl)-2-arachidonoyl-GPE (P-16:0/20:4)*	Lipid	Plasmalogen	-0.08 (1.09)	0.3 (1.05)	0.0282	-0.02	-0.42	0.0379	0.12
1-(1-enyl-palmitoyl)-GPE (P-16:0)*	Lipid	Lysoplasmalogen	-0.18 (1.04)	0.22 (0.97)	0.0219	-0.03	-0.38	0.06	0.18
1-(1-enyl-stearoyl)-2-arachidonoyl-GPE (P-18:0/20:4)*	Lipid	Plasmalogen	-0.21 (0.88)	0.18 (0.94)	0.0104	-0.17	-0.46	0.07	0.19
N-acetyltryptophan	Amino Acid	Tryptophan Metabolism	0.72 (1.11)	0.16 (1.06)	0.004	0.59	0.32	0.08	0.19
N-acetylneuraminate	Carbohydrate	Aminosugar Metabolism	0.65 (0.82)	0.91 (0.79)	0.0451	0.90	0.66	0.08	0.19
1-(1-enyl-palmitoyl)-2-oleoyl-GPE (P-16:0/18:1)*	Lipid	Plasmalogen	-0.01 (1.05)	0.29 (1.01)	0.0289	-0.01	-0.37	0.09	0.19
behenoyl sphingomyelin (d18:1/22:0)*	Lipid	Sphingomyelins	-0.6 (0.67)	-0.38 (0.76)	0.05	-0.65	-0.45	0.09	0.19
thyroxine	Amino Acid	Tyrosine Metabolism	-0.33 (1.17)	0.1 (1.32)	0.0416	-0.10	0.17	0.11	0.19

cysteine-glutathione disulfide	Amino Acid	Glutathione Metabolism	0.11 (1.52)	0.91 (1.45)	0.0026	-0.10	0.37	0.11	0.19
alpha-ketoglutarate	Energy	TCA Cycle	1.16 (1.07)	0.48 (1.37)	0.0038	1.01	1.37	0.11	0.19
1-(1-enyl-oleoyl)-GPE (P-18:1)*	Lipid	Lysoplasmalogen	-0.13 (0.95)	0.26 (0.89)	0.009	0.05	-0.21	0.13	0.22
5,6-dihydrothymine	Nucleotide	Pyrimidine Metabolism, Thymine containing	0.95 (1.04)	0.62 (1.21)	0.042	1.52	1.29	0.14	0.23
1-(1-enyl-stearoyl)-GPE (P-18:0)*	Lipid	Lysoplasmalogen	-0.45 (1.07)	0.06 (0.96)	0.003	-0.19	-0.45	0.16	0.24
N-acetylphenylalanine	Amino Acid	Phenylalanine Metabolism	1.04 (0.99)	0.73 (1.05)	0.0242	1.17	1.02	0.17	0.24
palmitoyl dihydrosphingomyelin (d18:0/16:0)*	Lipid	Dihydrosphingomyelins	-0.61 (0.98)	-0.24 (1.09)	0.0115	-0.49	-0.28	0.17	0.24
cysteine s-sulfate	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism	0.44 (1.25)	0.1 (1.2)	0.0409	-1.16	-0.89	0.18	0.25
palmitoyl sphingomyelin (d18:1/16:0)	Lipid	Sphingomyelins	-0.3 (1.04)	0 (1.06)	0.0493	-0.17	-0.01	0.29	0.38
glucuronate	Carbohydrate	Aminosugar Metabolism	0.51 (0.96)	0.07 (1.06)	0.0106	0.66	0.51	0.30	0.38
1-methylnicotinamide	Cofactors and Vitamins	Nicotinate and Nicotinamide Metabolism	-0.4 (0.9)	0.07 (0.82)	0.0016	-0.03	-0.20	0.33	0.40
glycerophosphoethanolamine	Lipid	Phospholipid Metabolism	0.07 (1)	0.39 (1.01)	0.0432	0.08	-0.08	0.36	0.43
imidazole propionate	Amino Acid	Histidine Metabolism	1.03 (0.92)	0.2 (1.58)	0.0007	0.49	0.64	0.37	0.43
glycoursodeoxycholate	Lipid	Secondary Bile Acid Metabolism	0.28 (1.15)	-0.27 (1.15)	0.0077	0.09	0.25	0.40	0.44
cysteine	Amino Acid	Methionine, Cysteine, SAM and Taurine Metabolism	-0.56 (1.36)	-0.13 (1.35)	0.0151	-1.15	-1.29	0.58	0.64
N-acetylglutamate	Amino Acid	Glutamate Metabolism	0.52 (0.52)	0.31 (0.52)	0.0242	0.58	0.54	0.61	0.65
1-stearoyl-GPE (18:0)	Lipid	Lysophospholipid	-0.33 (0.94)	-0.03 (0.92)	0.0405	-0.26	-0.23	0.82	0.84
2-hydroxy-3-methylvalerate	Amino Acid	Leucine, Isoleucine and Valine Metabolism	0.52 (1.05)	0.07 (1.32)	0.0493	0.15	0.16	0.96	0.96

Supplementary Table 5. Changes in metabolomic profile pre- and post-PEA.

Metabolites different between patients with operable CTEPH sampled before surgery and patients sampled post-PEA surgery. Median metabolite levels (z-scores relative to healthy controls) are shown for independent patients before or after PEA surgery, and for a group of 43 patients sampled before and after surgery. Significance shown is for Mann Whitney U tests or paired t-tests, as appropriate. FDR, false discovery rate corrected p-value

Biochemical	Sub-pathway	Super-pathway	Gradients					
			PA-ART.		SVC-PA		PA-SVC	
			FC	FDR p-value	FC	FDR p-value	FC	FDR p-value
Gradients across all sampling sites								
1-methylnicotinamide	Nicotinate and Nicotinamide Metabolism	Cofactors and Vitamins	0.151	6.3668E-06	-0.23	4.8183E-09	-0.079	0.02913395
malate	TCA Cycle	Energy	-0.239	9.3537E-05	-0.264	2.5687E-10	-0.503	5.9814E-12
alpha-ketoglutarate	TCA Cycle	Energy	-0.184	0.00025164	-0.269	8.8517E-08	-0.453	1.8931E-09
arachidonate (20:4n6)	Polyunsaturated Fatty Acid (n3 and n6)	Lipid	-0.241	0.00052156	-0.171	0.00067213	-0.413	1.1959E-08
2-hydroxybutyrate/2-hydroxyisobutyrate	Glutathione Metabolism	Amino Acid	0.073	0.00091228	0.08	0.00298024	0.153	1.6462E-06
glutamate	Glutamate Metabolism	Amino Acid	-0.239	0.00122588	0.998	7.0141E-12	0.759	3.1009E-10
ornithine	Urea cycle; Arginine and Proline Metabolism	Amino Acid	0.142	0.0035656	0.116	0.00596847	0.258	5.1913E-07
glucose	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	Carbohydrate	-0.115	0.00646371	0.198	3.8473E-06	0.083	0.04600271
eicosapentaenoate (EPA; 20:5n3)	Polyunsaturated Fatty Acid (n3 and n6)	Lipid	-0.127	0.01166334	-0.138	7.3513E-05	-0.265	6.6222E-08
dihomo-linolenate (20:3n3 or n6)	Polyunsaturated Fatty Acid (n3 and n6)	Lipid	-0.151	0.02202636	-0.142	0.00081195	-0.293	1.6462E-06
fumarate	TCA Cycle	Energy	-0.145	0.03812286	-0.346	2.1222E-09	-0.492	5.15E-09
stearidonate (18:4n3)	Polyunsaturated Fatty Acid (n3 and n6)	Lipid	0.078	0.04989586	-0.154	3.2923E-07	-0.076	0.00224968
Gradients between PA-ART and SVC-PA samples								
S-1-pyrroline-5-carboxylate	Glutamate Metabolism	Amino Acid	-0.261	1.6353E-05	0.235	0.0004531		

citrate	TCA Cycle	Energy	0.284	0.00021356	-0.32	1.9078E-07		
hypoxanthine	Purine Metabolism, (Hypo)Xanthine/Inosine containing	Nucleotide	0.246	0.0003471	-0.227	0.00025109		
4-hydroxyphenylpyruvate	Tyrosine Metabolism	Amino Acid	0.238	0.00067592	-0.132	0.01580435		
aspartate	Alanine and Aspartate Metabolism	Amino Acid	-0.318	0.00251419	0.404	2.3314E-05		
methionine sulfoxide	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	-0.143	0.00576202	0.08	0.04780915		
mannose	Fructose, Mannose and Galactose Metabolism	Carbohydrate	-0.089	0.03252006	0.128	0.00201883		
N-acetylmethionine	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	0.087	0.03793341	-0.152	2.6986E-06		
Gradients between PA-ART and ART-SVC samples								
palmitoylcholine	Fatty Acid Metabolism (Acyl Choline)	Lipid	-0.803	2.3129E-09			-0.927	2.1208E-10
glycerophosphorylcholine (GPC)	Phospholipid Metabolism	Lipid	-0.721	6.4598E-09			-0.787	2.0185E-10
sarcosine	Glycine, Serine and Threonine Metabolism	Amino Acid	0.631	1.8199E-08			0.713	1.8931E-09
erythronate*	Aminosugar Metabolism	Carbohydrate	-0.29	3.0496E-08			-0.286	5.15E-09
3-hydroxy-3-methylglutarate	Mevalonate Metabolism	Lipid	-0.378	3.9233E-08			-0.426	2.8368E-10
1-(1-enyl-palmitoyl)-GPE (P-16:0)*	Lysoplasmalogen	Lipid	-0.681	1.3054E-06			-0.604	5.7047E-05
glycerophosphoethanolamine	Phospholipid Metabolism	Lipid	-0.646	1.3149E-06			-0.499	0.00016003
oxalate (ethanedioate)	Ascorbate and Aldarate Metabolism	Cofactors and Vitamins	-0.288	1.3149E-06			-0.282	6.2582E-06
glycerate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	Carbohydrate	-0.36	2.363E-06			-0.403	3.3828E-07
1-stearoyl-GPE (18:0)	Lysophospholipid	Lipid	-0.416	6.3668E-06			-0.337	0.0007122
1-linoleoyl-GPC (18:2)	Lysophospholipid	Lipid	-0.396	1.3154E-05			-0.387	6.2582E-06
hypotaurine	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	-0.485	1.5989E-05			-0.557	8.8499E-07
threonate	Ascorbate and Aldarate Metabolism	Cofactors and Vitamins	-0.381	1.5989E-05			-0.34	8.1514E-06
1-(1-enyl-stearoyl)-GPE (P-18:0)*	Lysoplasmalogen	Lipid	-0.569	1.9284E-05			-0.437	0.00166427
1-oleoyl-GPC (18:1)	Lysophospholipid	Lipid	-0.392	2.685E-05			-0.356	3.4486E-05

myo-inositol	Inositol Metabolism	Lipid	-0.318	3.0697E-05			-0.347	4.7834E-07
1-arachidonoyl-GPC (20:4n6)*	Lysophospholipid	Lipid	-0.491	3.4957E-05			-0.501	6.9484E-06
1-linolenoyl-GPC (18:3)*	Lysophospholipid	Lipid	-0.27	3.7291E-05			-0.269	5.1503E-05
1-(1-enyl-oleoyl)-GPE (P-18:1)*	Lysoplasmalogen	Lipid	-0.578	4.0065E-05			-0.529	0.00077158
glucuronate	Aminosugar Metabolism	Carbohydrate	-0.279	8.7596E-05			-0.324	6.1505E-06
cysteine s-sulfate	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	0.412	9.3537E-05			0.428	7.5481E-05
phenol sulfate	Tyrosine Metabolism	Amino Acid	0.058	9.6634E-05			0.071	4.0266E-05
1-stearoyl-GPI (18:0)	Lysophospholipid	Lipid	-0.546	0.00014489			-0.54	3.11E-05
3-methylhistidine	Histidine Metabolism	Amino Acid	0.106	0.0001818			0.095	9.5465E-05
taurine	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid	-0.527	0.00021015			-0.394	0.01136516
1-palmitoyl-2-palmitoleoyl-GPC (16:0/16:1)*	Phosphatidylcholine (PC)	Lipid	0.134	0.00036363			0.128	6.2582E-06
arabonate/xylonate	Pentose Metabolism	Carbohydrate	-0.28	0.00039401			-0.333	6.6796E-07
1-palmitoleoyl-GPC (16:1)*	Lysophospholipid	Lipid	-0.23	0.00039401			-0.168	0.00334584
1-arachidonoyl-GPI (20:4)*	Lysophospholipid	Lipid	-0.457	0.00041187			-0.519	3.826E-06
phosphoethanolamine	Phospholipid Metabolism	Lipid	-0.469	0.00067592			-0.322	0.02584305
cysteine-glutathione disulfide	Glutathione Metabolism	Amino Acid	0.211	0.00067592			0.157	0.02344767
1-stearoyl-GPC (18:0)	Lysophospholipid	Lipid	-0.269	0.00087333			-0.261	7.5481E-05
adenosine 5'-monophosphate (AMP)	Purine Metabolism, Adenine containing	Nucleotide	-0.301	0.0016345			-0.4	0.00024698
ribonate	Pentose Metabolism	Carbohydrate	-0.254	0.00171284			-0.195	0.01520143
2-hydroxypalmitate	Fatty Acid, Monohydroxy	Lipid	-0.304	0.00174563			-0.414	6.2582E-06
N-acetylneuraminate	Aminosugar Metabolism	Carbohydrate	-0.297	0.0019371			-0.233	0.03142122
1-oleoyl-GPE (18:1)	Lysophospholipid	Lipid	-0.123	0.00251419			-0.085	0.03894162
1-(1-enyl-palmitoyl)-2-arachidonoyl-GPE (P-16:0/20:4)*	Plasmalogen	Lipid	-0.293	0.00282943			-0.212	0.03515882
N-acetylvaline	Leucine, Isoleucine and Valine Metabolism	Amino Acid	0.118	0.00282943			0.052	0.02630446
1-linoleoyl-GPI (18:2)*	Lysophospholipid	Lipid	-0.325	0.00293949			-0.354	6.3924E-05

allantoin	Purine Metabolism, (Hypo)Xanthine/Inosine containing	Nucleotide	-0.137	0.00313861			-0.117	0.00162672
3-carboxy-4-methyl-5-propyl-2-furanpropanoate (CMPF)	Fatty Acid, Dicarboxylate	Lipid	0.046	0.00348162			0.067	0.00026166
1-myristoyl-2-palmitoyl-GPC (14:0/16:0)	Phosphatidylcholine (PC)	Lipid	0.101	0.00646447			0.129	0.00018146
ethylmalonate	Leucine, Isoleucine and Valine Metabolism	Amino Acid	-0.08	0.00737992			-0.092	0.00021416
asparagine	Alanine and Aspartate Metabolism	Amino Acid	-0.139	0.00751376			-0.216	2.7952E-05
mannitol/sorbitol	Fructose, Mannose and Galactose Metabolism	Carbohydrate	-0.08	0.00767081			-0.058	0.00654856
arabitol/xylitol	Pentose Metabolism	Carbohydrate	-0.1	0.00932002			-0.092	0.00588643
trigonelline (N'-methylnicotinate)	Nicotinate and Nicotinamide Metabolism	Cofactors and Vitamins	0.054	0.01432868			0.049	0.0056586
biliverdin	Hemoglobin and Porphyrin Metabolism	Cofactors and Vitamins	0.124	0.01824202			0.143	0.00642229
1-palmitoyl-GPC (16:0)	Lysophospholipid	Lipid	-0.236	0.02202636			-0.189	0.03328623
1-stearoyl-2-arachidonoyl-GPE (18:0/20:4)	Phosphatidylethanolamine (PE)	Lipid	-0.18	0.02202636			-0.149	0.02584305
1-palmitoyl-2-linoleoyl-GPC (16:0/18:2)	Phosphatidylcholine (PC)	Lipid	0.132	0.02921371			0.214	0.00057078
N1-Methyl-2-pyridone-5-carboxamide	Nicotinate and Nicotinamide Metabolism	Cofactors and Vitamins	0.059	0.02964567			0.056	0.01237472
quinolinate	Nicotinate and Nicotinamide Metabolism	Cofactors and Vitamins	-0.087	0.03223069			-0.091	0.0342017
16a-hydroxy DHEA 3-sulfate	Androgenic Steroids	Lipid	0.042	0.03252006			0.05	0.0097586
1-stearoyl-2-linoleoyl-GPC (18:0/18:2)*	Phosphatidylcholine (PC)	Lipid	0.13	0.03567173			0.15	0.01612179
3-ureidopropionate	Pyrimidine Metabolism, Uracil containing	Nucleotide	-0.18	0.03573385			-0.181	0.018683
1,5-anhydroglucitol (1,5-AG)	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	Carbohydrate	0.062	0.03793341			0.103	0.00018146
pyridoxate	Vitamin B6 Metabolism	Cofactors and Vitamins	0.069	0.03812286			0.092	0.00247425
1-palmitoyl-2-arachidonoyl-GPI (16:0/20:4)*	Phosphatidylinositol (PI)	Lipid	0.109	0.04000976			0.119	0.0216564
1-stearoyl-2-docosahexaenoyl-GPE (18:0/22:6)*	Phosphatidylethanolamine (PE)	Lipid	0.108	0.04170345			0.107	0.00095383
1-(1-enyl-palmitoyl)-GPC (P-16:0)*	Lysoplasmalogen	Lipid	-0.173	0.04611967			-0.237	0.02347027
phenyllactate (PLA)	Phenylalanine Metabolism	Amino Acid	0.09	0.04664857			0.081	0.02263569
Gradients between SVC-PA and ART-SVC samples								
5,6-dihydrothymine	Pyrimidine Metabolism,	Nucleotide			-0.702	6.1669E-11	-0.548	3.5702E-09

	Thymine containing							
myristoleate (14:1n5)	Long Chain Fatty Acid	Lipid			-0.215	2.2062E-09	-0.138	9.9553E-06
succinate	TCA Cycle	Energy			-0.561	5.6399E-09	-0.518	4.7923E-06
5-dodecenoate (12:1n7)	Medium Chain Fatty Acid	Lipid			-0.188	1.4414E-08	-0.115	2.7952E-05
glycerol	Glycerolipid Metabolism	Lipid			-0.351	4.492E-08	-0.346	5.2117E-06
orotate	Pyrimidine Metabolism, Orotate containing	Nucleotide			-0.182	6.148E-08	-0.265	2.4564E-09
laurate (12:0)	Medium Chain Fatty Acid	Lipid			-0.18	4.0707E-07	-0.247	2.9391E-09
myristate (14:0)	Long Chain Fatty Acid	Lipid			-0.189	4.6872E-07	-0.123	0.00057078
7-alpha-hydroxy-3-oxo-4-cholestenoate (7-Hoca)	Sterol	Lipid			-0.308	7.0852E-07	-0.284	1.4406E-08
linolenate [alpha or gamma; (18:3n3 or 6)]	Polyunsaturated Fatty Acid (n3 and n6)	Lipid			-0.144	1.61E-06	-0.07	0.00959807
3-hydroxylaurate	Fatty Acid, Monohydroxy	Lipid			-0.174	4.7676E-06	-0.151	6.3924E-05
gamma-glutamylglutamate	Gamma-glutamyl Amino Acid	Peptide			0.406	2.2447E-05	0.536	6.0645E-08
xanthine	Purine Metabolism, (Hypo)Xanthine/Inosine containing	Nucleotide			-0.12	2.7189E-05	-0.096	7.5074E-05
3-hydroxybutyrate (BHBA)	Ketone Bodies	Lipid			0.128	3.1815E-05	0.191	1.7422E-06
creatine	Creatine Metabolism	Amino Acid			0.135	5.4254E-05	0.154	7.5074E-05
choline	Phospholipid Metabolism	Lipid			-0.182	7.0016E-05	-0.127	0.00450938
caprate (10:0)	Medium Chain Fatty Acid	Lipid			-0.114	7.1324E-05	-0.106	0.00017611
glutamine	Glutamate Metabolism	Amino Acid			-0.241	0.00014192	-0.165	0.0216564
docosapentaenoate (n3 DPA; 22:5n3)	Polyunsaturated Fatty Acid (n3 and n6)	Lipid			-0.151	0.00037289	-0.154	0.00088718
linoleate (18:2n6)	Polyunsaturated Fatty Acid (n3 and n6)	Lipid			-0.128	0.00038275	-0.086	0.01338683
5-oxoproline	Glutathione Metabolism	Amino Acid			-0.204	0.00053368	-0.188	0.0088785
trans-4-hydroxyproline	Urea cycle; Arginine and Proline Metabolism	Amino Acid			-0.11	0.00067213	-0.124	5.7976E-05
citrulline	Urea cycle; Arginine and Proline Metabolism	Amino Acid			0.163	0.00076679	0.151	0.00331986
alanine	Alanine and Aspartate Metabolism	Amino Acid			-0.223	0.0008132	-0.211	0.00196605
docosahexaenoate (DHA; 22:6n3)	Polyunsaturated Fatty Acid (n3 and n6)	Lipid			-0.159	0.00094698	-0.228	6.2582E-06

deoxycarnitine	Carnitine Metabolism	Lipid			-0.12	0.00160152	-0.087	0.02712928
guanidinoacetate	Creatine Metabolism	Amino Acid			-0.118	0.00199572	-0.2	6.5855E-05
caprylate (8:0)	Medium Chain Fatty Acid	Lipid			-0.169	0.0036433	-0.133	0.03840176
serine	Glycine, Serine and Threonine Metabolism	Amino Acid			0.14	0.00935717	0.112	0.00725554
pentadecanoate (15:0)	Long Chain Fatty Acid	Lipid			-0.157	0.01427051	-0.093	0.04481935
3-methyl-2-oxovalerate	Leucine, Isoleucine and Valine Metabolism	Amino Acid			0.143	0.01624892	0.123	0.03395985
indoleacetate	Tryptophan Metabolism	Amino Acid			0.054	0.01785248	0.076	0.00272741
phosphate	Oxidative Phosphorylation	Energy			0.164	0.01927463	0.274	5.7047E-05
13-HODE + 9-HODE	Fatty Acid, Monohydroxy	Lipid			-0.13	0.02857954	-0.184	0.00280084
4-methyl-2-oxopentanoate	Leucine, Isoleucine and Valine Metabolism	Amino Acid			0.081	0.03931529	0.102	0.01237472
Gradients between PA-ART samples								
choline phosphate	Phospholipid Metabolism	Lipid	-0.471	0.0007693				
1-(1-enyl-stearoyl)-2-arachidonoyl-GPE (P-18:0/20:4)*	Plasmalogen	Lipid	-0.294	0.00168983				
nicotinamide	Nicotinate and Nicotinamide Metabolism	Cofactors and Vitamins	-0.394	0.00535394				
homoarginine	Urea cycle; Arginine and Proline Metabolism	Amino Acid	0.092	0.00576202				
1-palmitoyl-GPE (16:0)	Lysophospholipid	Lipid	-0.205	0.00646447				
bilirubin (E,E)*	Hemoglobin and Porphyrin Metabolism	Cofactors and Vitamins	-0.207	0.00978439				
1-(1-enyl-palmitoyl)-2-oleoyl-GPE (P-16:0/18:1)*	Plasmalogen	Lipid	-0.199	0.01436973				
betaine	Glycine, Serine and Threonine Metabolism	Amino Acid	0.089	0.0280844				
1-arachidonoyl-GPE (20:4n6)*	Lysophospholipid	Lipid	-0.123	0.03247541				
Gradients between SVC-PA samples								
lactate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	Carbohydrate			-0.215	2.1564E-06		
N-formylmethionine	Methionine, Cysteine, SAM and Taurine Metabolism	Amino Acid			-0.185	6.8059E-06		
7-methylguanine	Purine Metabolism, Guanine containing	Nucleotide			-0.165	0.00016298		
3-(4-hydroxyphenyl)lactate	Tyrosine Metabolism	Amino Acid			-0.124	0.00040297		

N-acetylputrescine	Polyamine Metabolism	Amino Acid			-0.109	0.0004531		
isoleucine	Leucine, Isoleucine and Valine Metabolism	Amino Acid			0.171	0.00144109		
palmitoleate (16:1n7)	Long Chain Fatty Acid	Lipid			-0.116	0.00152404		
10-heptadecenoate (17:1n7)	Long Chain Fatty Acid	Lipid			-0.109	0.00201883		
3-hydroxy-2-ethylpropionate	Leucine, Isoleucine and Valine Metabolism	Amino Acid			-0.201	0.0043224		
3-hydroxydecanoate	Fatty Acid, Monohydroxy	Lipid			-0.091	0.00465296		
trans-urocanate	Histidine Metabolism	Amino Acid			-0.343	0.01140618		
1-palmitoyl-2-stearoyl-GPC (16:0/18:0)	Phosphatidylcholine (PC)	Lipid			0.182	0.01629051		
3-hydroxyoctanoate	Fatty Acid, Monohydroxy	Lipid			-0.097	0.02857954		
Gradients between ART-SVC samples								
leucine	Leucine, Isoleucine and Valine Metabolism	Amino Acid					0.174	0.00028644
1-stearoyl-2-docosahexaenoyl-GPC (18:0/22:6)	Phosphatidylcholine (PC)	Lipid					0.126	0.0003638
sphingomyelin (d18:2/23:0, d18:1/23:1, d17:1/24:1)*	Sphingomyelins	Lipid					0.119	0.0009553
vanillylmandelate (VMA)	Tyrosine Metabolism	Amino Acid					0.118	0.00216943
gamma-glutamyl-epsilon-lysine	Gamma-glutamyl Amino Acid	Peptide					-0.265	0.00228894
1-palmitoyl-2-oleoyl-GPC (16:0/18:1)	Phosphatidylcholine (PC)	Lipid					0.177	0.00241612
imidazole propionate	Histidine Metabolism	Amino Acid					0.129	0.00246402
1-stearoyl-2-oleoyl-GPC (18:0/18:1)	Phosphatidylcholine (PC)	Lipid					0.173	0.00255738
1-linoleoyl-2-linolenoyl-GPC (18:2/18:3)*	Phosphatidylcholine (PC)	Lipid					0.122	0.00267674
gamma-glutamylmethionine	Gamma-glutamyl Amino Acid	Peptide					-0.199	0.00298366
1-myristoyl-2-arachidonoyl-GPC (14:0/20:4)*	Phosphatidylcholine (PC)	Lipid					0.116	0.00331986
1-(1-enyl-stearoyl)-2-linoleoyl-GPE (P-18:0/18:2)*	Plasmalogen	Lipid					0.086	0.00399784
sphingomyelin (d18:1/21:0, d17:1/22:0, d16:1/23:0)*	Sphingomyelins	Lipid					0.085	0.00520102
1-palmitoyl-2-docosahexaenoyl-GPE (16:0/22:6)*	Phosphatidylethanolamine (PE)	Lipid					0.083	0.00586853
1-oleoyl-2-docosahexaenoyl-GPC (18:1/22:6)*	Phosphatidylcholine (PC)	Lipid					0.18	0.00725554
N-acetylserine	Glycine, Serine and Threonine Metabolism	Amino Acid					-0.108	0.00791607

1,2-dilinoleoyl-GPC (18:2/18:2)	Phosphatidylcholine (PC)	Lipid					0.1	0.00828729
phenylpyruvate	Phenylalanine Metabolism	Amino Acid					0.215	0.00918305
glycochenodeoxycholate glucuronide (1)	Primary Bile Acid Metabolism	Lipid					0.066	0.00952848
N-acetyltryptophan	Tryptophan Metabolism	Amino Acid					0.12	0.01174907
androsterone sulfate	Androgenic Steroids	Lipid					0.067	0.01181751
indolepropionate	Tryptophan Metabolism	Amino Acid					0.052	0.01237472
1-stearoyl-2-arachidonoyl-GPC (18:0/20:4)	Phosphatidylcholine (PC)	Lipid					0.145	0.01262466
glycocholenate sulfate*	Secondary Bile Acid Metabolism	Lipid					0.095	0.01447754
1-palmitoyl-2-linoleoyl-GPE (16:0/18:2)	Phosphatidylethanolamine (PE)	Lipid					0.07	0.01520143
sphingomyelin (d18:1/18:1, d18:2/18:0)	Sphingomyelins	Lipid					0.076	0.01520143
sphingomyelin (d18:1/20:1, d18:2/20:0)*	Sphingomyelins	Lipid					0.094	0.01547065
orotidine	Pyrimidine Metabolism, Orotate containing	Nucleotide					-0.06	0.01618316
gamma-glutamylglutamine	Gamma-glutamyl Amino Acid	Peptide					-0.202	0.01651043
sphingomyelin (d18:1/22:1, d18:2/22:0, d16:1/24:1)*	Sphingomyelins	Lipid					0.11	0.01774506
tryptophan	Tryptophan Metabolism	Amino Acid					0.109	0.01784552
1-(1-enyl-palmitoyl)-2-arachidonoyl-GPC (P-16:0/20:4)*	Plasmalogen	Lipid					0.114	0.01790998
2-hydroxystearate	Fatty Acid, Monohydroxy	Lipid					-0.238	0.02142168
N-acetylalanine	Alanine and Aspartate Metabolism	Amino Acid					-0.128	0.0222828
trimethylamine N-oxide	Phospholipid Metabolism	Lipid					0.056	0.02263569
alpha-hydroxyisovalerate	Leucine, Isoleucine and Valine Metabolism	Amino Acid					0.057	0.02375697
1-palmitoyl-2-docosahexaenoyl-GPC (16:0/22:6)	Phosphatidylcholine (PC)	Lipid					0.126	0.02492348
uridine	Pyrimidine Metabolism, Uracil containing	Nucleotide					0.095	0.02766267
N-methylproline	Urea cycle; Arginine and Proline Metabolism	Amino Acid					0.041	0.02913395
1-linoleoyl-2-arachidonoyl-GPC (18:2/20:4n6)*	Phosphatidylcholine (PC)	Lipid					0.099	0.03010652
N-acetylphenylalanine	Phenylalanine Metabolism	Amino Acid					0.092	0.03270972
3-methylglutarylcarnitine (2)	Leucine, Isoleucine and Valine Metabolism	Amino Acid					0.079	0.03413624

sphingomyelin (d18:2/24:1, d18:1/24:2)*	Sphingomyelins	Lipid					0.099	0.0342017
1,2-dipalmitoyl-GPC (16:0/16:0)	Phosphatidylcholine (PC)	Lipid					0.145	0.03528075
phenylacetylglutamine	Acetylated Peptides	Peptide					0.063	0.03894162
2-hydroxy-3-methylvalerate	Leucine, Isoleucine and Valine Metabolism	Amino Acid					0.091	0.04416028
sphingomyelin (d18:1/17:0, d17:1/18:0, d19:1/16:0)	Sphingomyelins	Lipid					0.083	0.04456191
1-(1-enyl-palmitoyl)-2-linoleoyl-GPC (P-16:0/18:2)*	Plasmalogen	Lipid					0.068	0.0462457

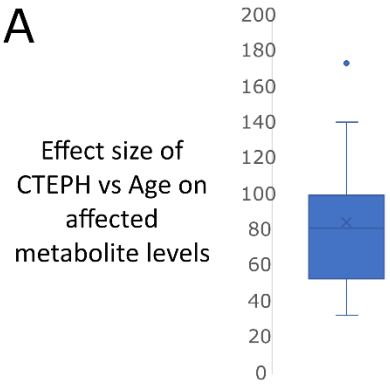
Supplementary Table 6. Full list of metabolites with nominally significant tissue gradients

Sub-pathway	Metabolites tested in the pathway	SVC-PA	Sig.	PA-ART	Sig.	ART-SVC	Sig.	SVC-PA only	Sig.	PA-ART only	Sig.	ART-SVC only	Sig.
Pathway showing enrichment													
Polyunsaturated Fatty Acid (n3 and n6)	12	8	0.0002	4	0.31	8	0.0241						
TCA Cycle	6	5	0.0008	4	0.0243	4	0.1						
Medium Chain Fatty Acid	5	4	0.0038			4	0.0431						
Phosphatidylcholine (PC)	17	1	0.33	4	1	15	0.0000	1	0.5			11	0
Nicotinate and Nicotinamide Metabolism	5	1	1	5	0.0005	4	0.0431			1	0.13		
Phospholipid Metabolism	6	1	1	4	0.0243	5	0.0166			1	0.15	1	0.57
Lysophospholipid	23			14	0.0000	12	0.07			2	0.12		
Lysoplasmalogen	4			4	0.0024	4	0.0116						
Aminosugar Metabolism	4			3	0.0363	3	0.11						
Pentose Metabolism	4			3	0.0363	3	0.11						
Plasmalogen	11			3	0.71	4	0.76			2	0.0322	3	0.16
Secondary Bile Acid Metabolism	15					1	0.0261					1	0.7
Sphingomyelins	16					7	0.42					7	0.0019
Pathways not showing enrichment													
Long Chain Fatty Acid	14	5	0.08			3	0.41	2	0.1				
Glutamate Metabolism	9	3	0.2	2	1	2	0.72						
Purine Metabolism, (Hypo)Xanthine/Inosine containing	6	2	0.28	2	0.62	2	1						
Tryptophan Metabolism	14	1	0.48			4	1					3	0.41
Fructose, Mannose and Galactose Metabolism	4	1	0.54	2	0.22	1	1						
Sterol	4	1	0.54			1	1						
Histidine Metabolism	11	1	0.7	1	0.47	2	0.35	1	0.36			1	1

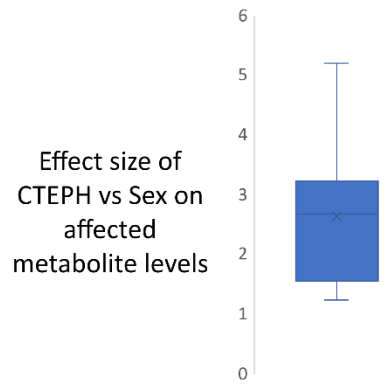
Leucine, Isoleucine and Valine Metabolism	21	4	0.77	2	0.19	8	0.64	2	0.19			4	0.5
Urea cycle; Arginine and Proline Metabolism	16	3	1	2	0.54	4	0.6			1	0.37	1	0.71
Methionine, Cysteine, SAM and Taurine Metabolism	17	3	1	5	0.55	3	0.2	1	0.5				
Tyrosine Metabolism	14	2	1	2	0.74	2	0.16	1	0.43			1	1
Glycine, Serine and Threonine Metabolism	10	1	1	2	1	3	1			1	0.25	1	1
Polyamine Metabolism	5	1	1					1	0.18				
Androgenic Steroids	11			1	0.47	2	0.35					1	1
Hemoglobin and Porphyrin Metabolism	7			2	0.66	1	0.43			1	0.18		
Fatty Acid, Dicarboxylate	4			1	1	1	1						
Phosphatidylinositol (PI)	5			1	1	1	1						
Purine Metabolism, Adenine containing	4			1	1	1	1						
Primary Bile Acid Metabolism	9					1	0.28					1	1
Acetylated Peptides	4					1	1					1	0.43

Supplementary Table 7. Enrichment analysis of all pathways. Enrichment was first tested for metabolites with gradients in each analysis, irrespective of overlap between analyses, and then in those metabolites specifically with gradients in only one of the three analyses, SVC-PA, PA-ART or ART-SVC.

A

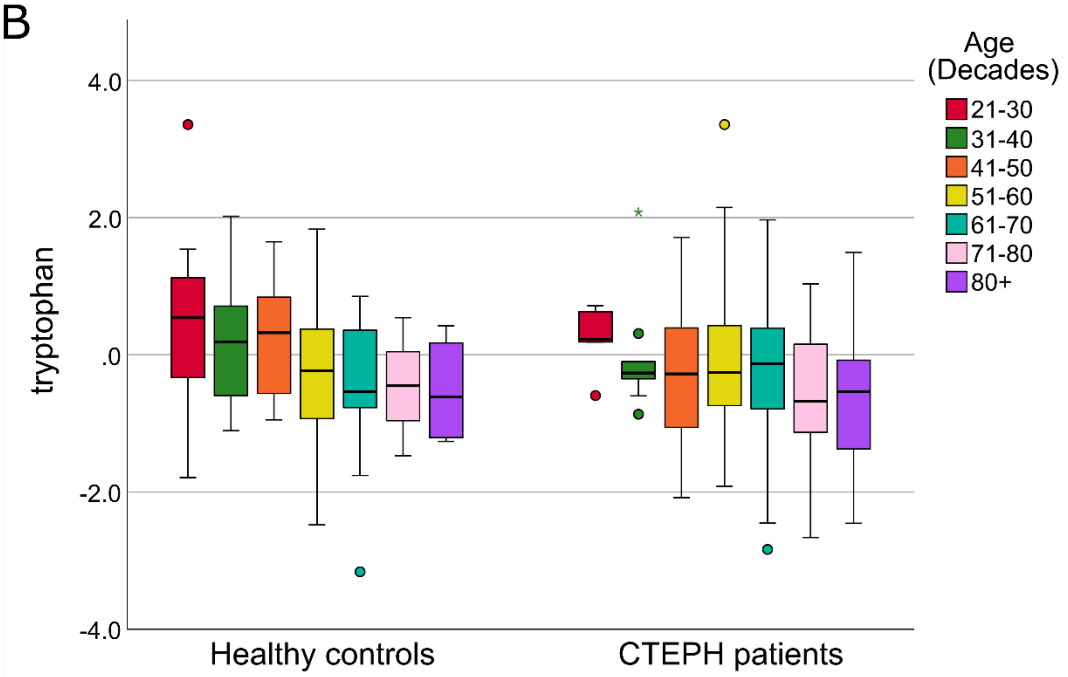


17/35 affected by age (p<0.05)

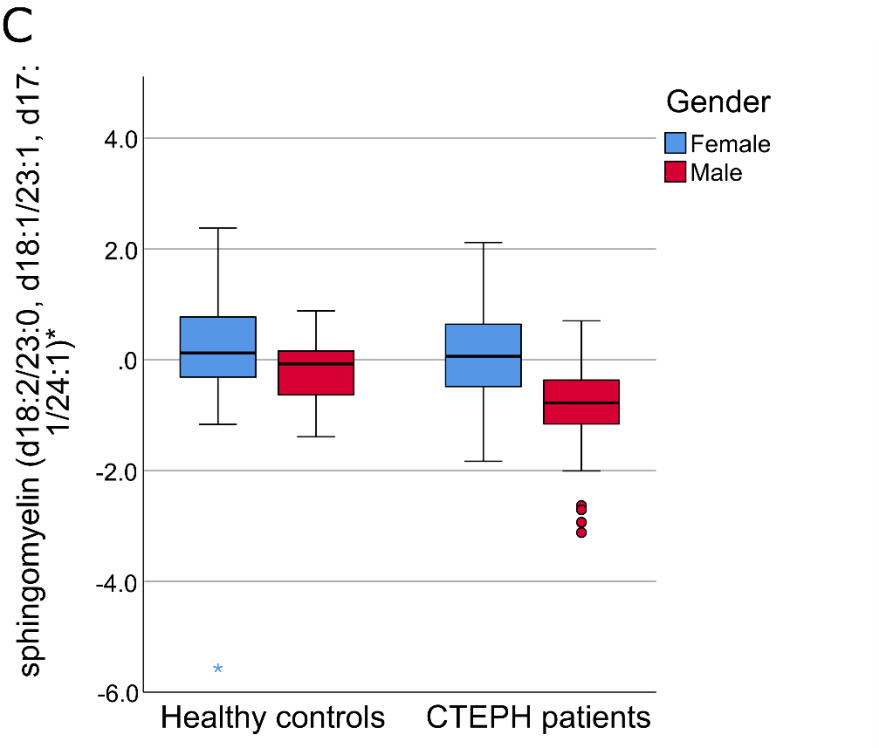


11/35 affected by sex (p<0.05)

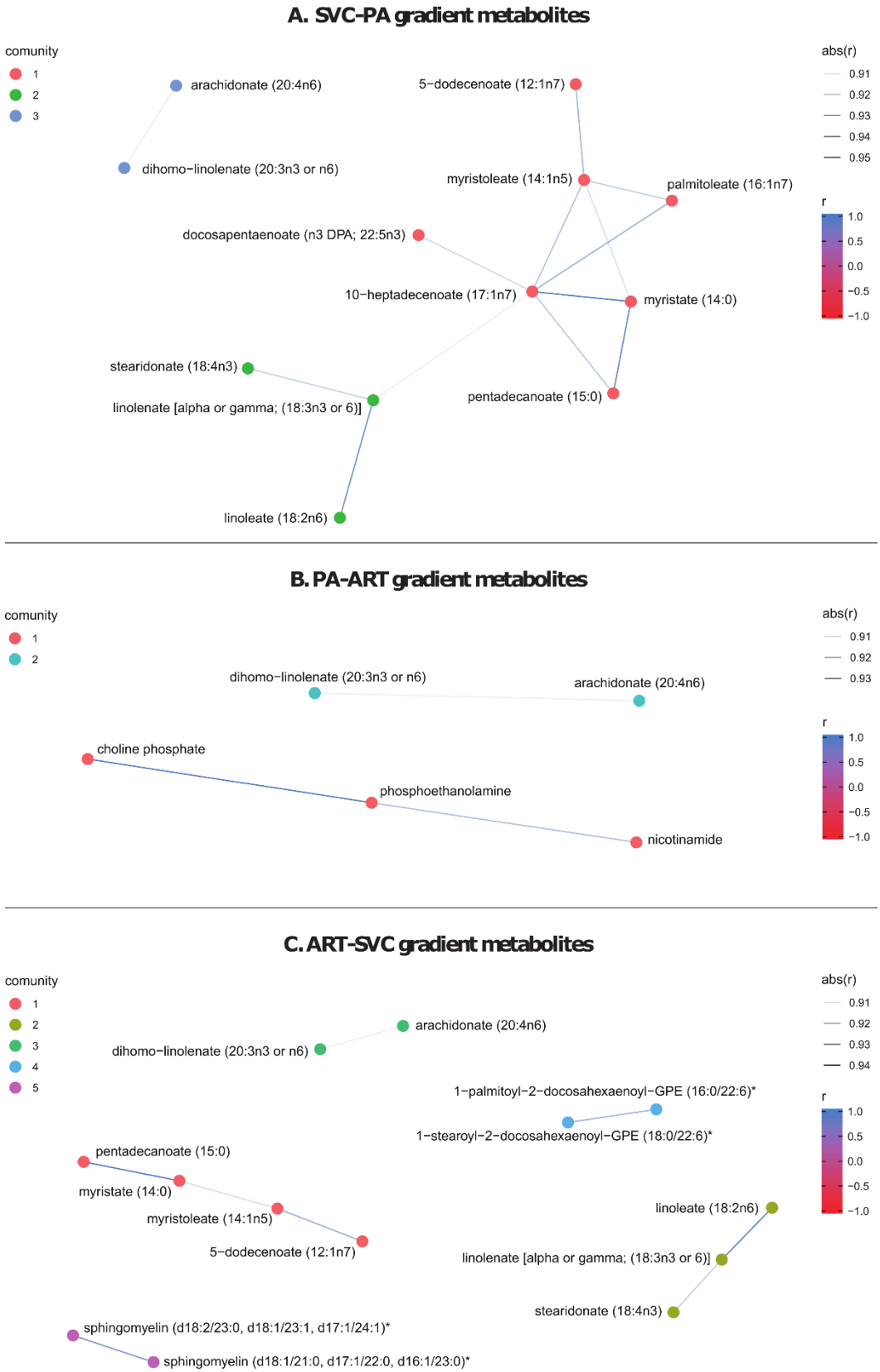
B



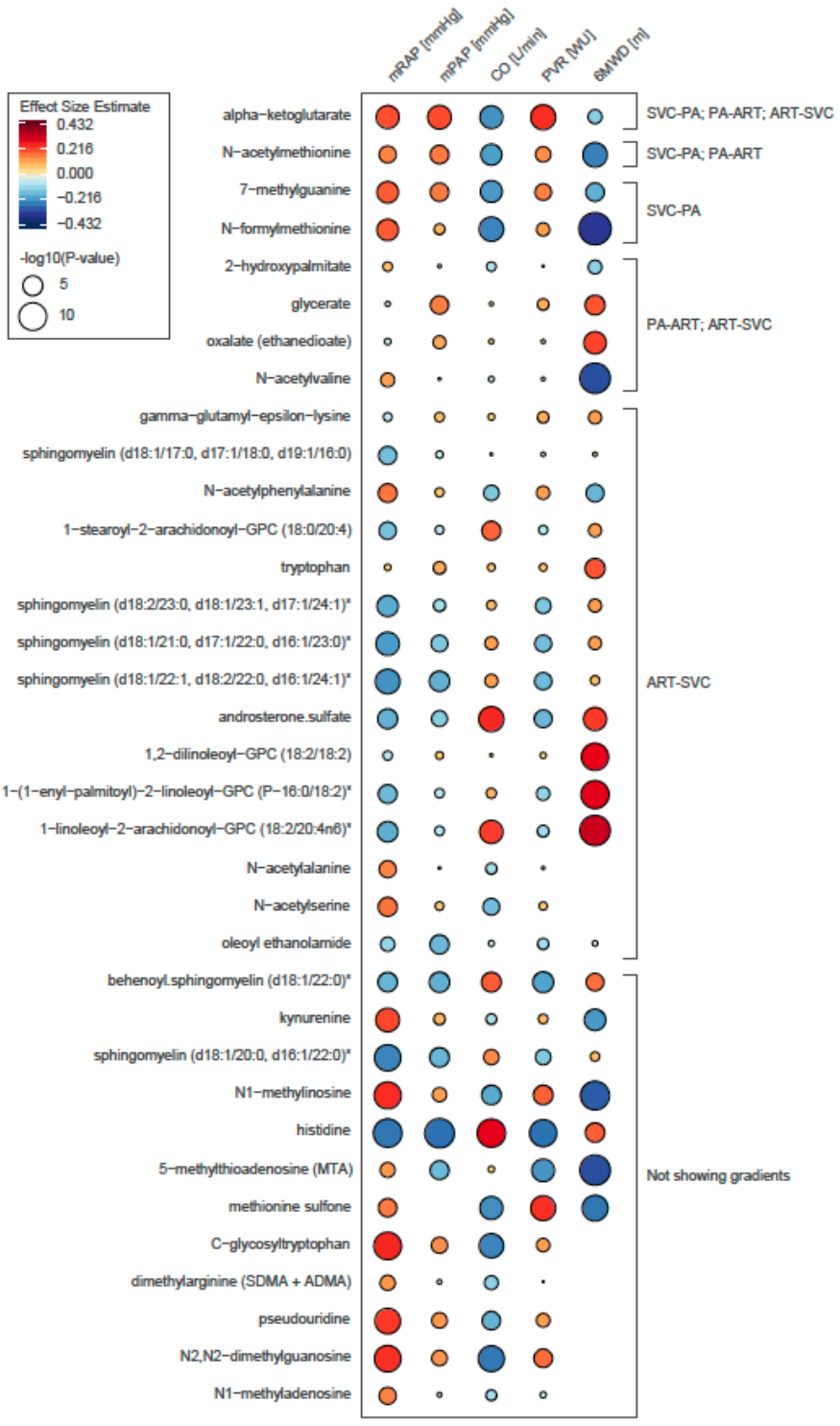
C



Supplementary Figure 1 – Effect of age and gender on metabolites associated with CTEPH. A. Ratio of effect sizes of CTEPH (versus healthy controls) compared to age and gender for metabolites significantly affected by age or gender, indicating generally larger (>1 ratio) effect of CTEPH status. B. Boxplot of CTEPH-associated metabolite most affected by age, tryptophan, in healthy controls and CTEPH patients divided by age in decades. C. Boxplot of CTEPH-associated metabolite most affected by gender, sphingomyelin (d18:2/23:0, d18.1/23:1, d17:1/24:1)*, in healthy controls and CTEPH patients divided by self-reported gender.



Supplementary Figure 2. Relevance network analysis. Network of highly correlated (r>0.9) metabolites showing SVC-PA (A), PA-ART (B) or ART-SVC (C) gradients.



Supplementary Figure 3. Associations between clinical variables and plasma metabolites that distinguish CTEPH from healthy and disease controls.

Directionality and magnitude of estimates are presented by colour fill scale (red for positive and blue for negative directionality), corresponding p-values are represented by circle size.

Supplementary References

1. Rosenkranz S, Preston IR. Right heart catheterisation: best practice and pitfalls in pulmonary hypertension. Eur Respir Rev. 2015;24(138):642-52.

2. Shenoy V, Anton JM, Collard CD, Youngblood SC. Pulmonary thromboendarterectomy for chronic thromboembolic pulmonary hypertension. Anesthesiology. 2014;120(5):1255-61.

3. Jamieson SW, Kapelanski DP, Sakakibara N, Manecke GR, Thistlethwaite PA, Kerr KM, et al. Pulmonary endarterectomy: experience and lessons learned in 1,500 cases. Ann Thorac Surg. 2003;76(5):1457-62; discussion 62-4.