

Gene-Metabolite Expression in Blood Can Discriminate Allergen-Induced Isolated Early from Dual Asthmatic Responses

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Abstract

Some asthmatic individuals undergoing allergen inhalation challenge develop an isolated early response whereas others develop a dual response (early plus late response). In the present study we have used transcriptomics (microarrays) and metabolomics (mass spectrometry) of peripheral blood to identify molecular patterns that can discriminate allergen-induced isolated early from dual asthmatic responses. Peripheral blood was obtained prior to (pre-) and 2 hours post allergen inhalation challenge from 33 study participants. In an initial cohort of 14 participants, complete blood counts indicated significant differences in neutrophil and lymphocyte counts at pre-challenge between early and dual responders. At post-challenge, significant genes (*ALOX15*, *FADS2* and *LPCAT2*) and metabolites (lysolipids) were enriched in lipid metabolism pathways. Enzymes encoding for these genes are involved in membrane biogenesis and metabolism of fatty acids into pro-inflammatory and anti-inflammatory mediators. Correlation analysis indicated a strong negative correlation between *ALOX15*, *FADS2*, and *IL5RA* expression with 2-arachidonoylglycerophosphocholine levels in dual responders. However, measuring arachidonic acid and docosahexaenoic acid levels in a validation cohort of 19 participants indicated that the free form of DHA (nmoles/μg of protein) was significantly ($p=0.03$) different between early and dual responders after allergen challenge. Collectively these results may suggest an imbalance in lipid metabolism which dictates pro- (anti-) inflammatory and pro-resolving mechanisms. Future studies with larger sample sizes may reveal novel mechanisms and therapeutic targets of the late phase asthmatic response.

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Introduction

Asthma is the most common chronic lung disease, but remains poorly understood due to its complexity and heterogeneity [1]. Asthma is characterized by reversible narrowing of the airways, airway inflammation and airway remodeling [2–5]. Current physiological or functional tests such as clinical symptoms or lung function tests have not been shown to reflect airway inflammation and clinical outcome [6].

The allergen inhalation challenge is a useful clinical model in order to study the mechanisms underlying asthmatic responses [7]. The early asthmatic response (EAR) is initiated upon allergen inhalation and results in the activation of IgE-bearing cells such as mast cells and basophils [8–10]. Upon degranulation, these cells

release proinflammatory mediators such as histamine and eicosanoids triggering bronchoconstriction and increased vascular permeability [11,12]. The late asthmatic response (LAR) occurs 4 to 6 hours [13] after allergen exposure and is characterized by cellular infiltration of the airway, increased bronchovascular permeability, and mucus secretion [14]. Fifty to 60% of allergic asthmatic individuals develop both an EAR and LAR (dual responders; DRs) following allergen inhalation challenge, whereas 30 to 40% of allergic asthmatic individuals develop an isolated early response (early responders; ERs) after allergen challenge [7]. The molecular mechanisms leading to the early and late asthmatic responses are not fully understood.

Recent advancements in 'omics' technologies such as transcriptomics and metabolomics have enabled the study of complex

diseases such as asthma [15,16]. However, the success of these technologies is limited by confounding factors such as the heterogeneity of study populations, and the effect of medications on gene expression [17,18]. Therefore, careful selection of subjects and clinically relevant samples should enable greater understanding of the underlying mechanisms and reveal novel therapeutic targets for the treatment of allergic asthma.

Our laboratory has previously shown that significant changes in the whole blood transcriptome (mRNA and miRNA) of mild atopic asthmatic individuals can be identified two hours after allergen inhalation challenge [19,20]. Previous studies comparing ERs and DRs undergoing allergen inhalation challenge have investigated changes in inflammatory progenitor cells in peripheral blood [21] and sputum [22]. In addition, the change in IL-10 producing CD4⁺ cells after allergen inhalation challenge has been shown to differ between ERs and DRs [23]. In addition, we have recently reported significant differences in the plasma proteome between ERs and DRs [24]. In the present study, we demonstrate the utility of applying omics-based approaches to peripheral blood in order to identify molecular patterns that can discriminate allergen-induced isolated early from dual asthmatic responses.

Methods

Asthma Cohorts

This study was approved by the Institutional Review Boards of the participating health research institutes (McMaster University, Université Laval, and University of British Columbia). Following written informed consent, 33 individuals participated in the allergen inhalation challenge as part of the AllerGen Clinical Investigator Collaborative (CIC). All study participants had predicted FEV₁ (forced expiratory volume in one second) [25] greater than 70% and baseline methacholine PC₂₀ (the provocative concentration of methacholine causing a 20% fall in FEV₁) less than 16 mg/ml [26]. The study participants were non-smokers with stable, mild atopic asthma, free of other lung disease. Exclusion criteria included: use of inhaled corticosteroids and use of other asthma medication with the exception of infrequently used β_2 -agonists which were withheld 8 hours prior to spirometry measurements [27]. All study participants were selected due to their hypersensitivity to various allergens using skin prick tests. The discovery cohort consisted of 14 participants whereas an additional 19 participants were recruited for the validation phase.

Methacholine Challenge Test and Allergen Inhalation Challenge

A methacholine challenge test (MCT) was conducted one day prior and one day following allergen inhalation challenge. On day one, each participant underwent a MCT where methacholine [PC₂₀] was calculated using the Cockcroft equation [26]. A post MCT was conducted 24 hours after the allergen inhalation challenge in order to detect an allergen induced shift in airway hyperresponsiveness. The allergen inhalation challenge was conducted on the second day, where spirometry measurements were assessed at regular intervals for up to 7 hours, inclusive. Skin prick tests were used to determine the dose of allergen extract for inhalation. The allergen dosage was administered in a doubling dosage until a drop in FEV₁ of 20% was achieved in all participants. Study participants were classified as early responders (ERs) if the initial drop in FEV₁ resolved back to baseline within 1 to 3 hours of allergen inhalation and if the maximum drop in FEV₁ between 3 to 7 hours was less than 15%. Study participants were classified as dual responders (DRs) if in addition to the early response, the participants experienced a maximum drop in FEV₁

of 15% or greater between 3 to 7 hours of allergen inhalation. Participants with a maximum late phase drop less than 15% but required a lower dose of methacholine [PC₂₀] on the second MCT compared to the first MCT were also classified as DRs.

Blood Collection and Processing

A standard operating protocol exists for all blood collection and processing at participating AllerGen CIC sites. Ten milliliters of venous blood was obtained from each subject, prior to challenge (pre) and 2 hours post-challenge. Leukocyte-enriched (PAXgene Blood RNA tubes, PreAnalytiX, Qiagen/BD, Valencia, CA, USA) and plasma fractions (in multiple aliquots) were freshly prepared from the blood and frozen at -80°C , after which RNA was extracted using standard Qiagen kits. Complete blood counts and differentials (CBCs/differentials) were obtained at the time of each blood draw (via a 3 ml EDTA blood tube, drawn immediately prior to the PAXgene Blood RNA tube sample). CBCs/differentials were obtained for all study participants in the discovery cohort.

Transcriptomics

Whole transcript gene expression analysis of pre and post RNA samples (28 samples) of the discovery cohort was performed using Affymetrix Human Gene 1.0 ST Arrays at the Centre for Translational and Applied Genomics (CTAG) in Vancouver, British Columbia. All microarray data (CEL) files have been deposited in NCBI's Gene Expression Omnibus (GEO Series GSE40240). The 'affy' package [28] was used to import CEL files, and annotate Affymetrix IDs to gene symbols. The *farm* package (version 1.6.0) was used for preprocessing and filtering of non-informative probe sets [29,30]. Preprocessing included the Robust MultiArray Average (RMA) background correction, quantile normalization, and Factor Analysis for Robust Microarray Summarization (FARMS). The summarization step makes use of factor analysis to determine a common factor (z) in the set of probes measuring the same target gene (probe set). In the process, parameters representing the signal and noise for each probe set are calculated. Informative/Non-informative (I/NI) calls use these parameters in order to filter out probe sets with little correlation amongst their probes (non-informative probe sets). The use of I/NI-calls for filtering of probe sets avoids having to set arbitrary thresholds such as for overall variance and mean cut-offs which may eventually affect downstream analysis [31]. All software packages used to preprocess the microarray data can be accessed through Bioconductor (<http://www.bioconductor.org/>). All analyses were performed in the statistical computing program R 2.14.2 [32].

Metabolomics Using the Metabolon Platform

Metabolite profiling as well as informatics were performed by Metabolon Inc. (Durham, North Carolina, United States). Samples from the discovery cohort were extracted and prepared for analysis using Metabolon's standard solvent extraction method. The extracted samples were divided into equal parts for analysis on the GC-MS and LC-MS/MS platforms. Also included were several technical replicate samples created from a homogeneous pool, containing a small amount of all study samples. The Metabolon informatics system consisted of four major components, the Laboratory Information Management System (LIMS), the data extraction and peak-identification software, data processing tools for quality control and compound identification, and a collection of information interpretation and visualization tools. The hardware and software foundations for these informatics components were the LAN backbone, and a database server

running Oracle 10.2.0.1 Enterprise Edition. General platform methods are described in the Supplementary Information.

Lipid Profiling

Arachidonic acid (AA) and docosahexaenoic acid (DHA) were profiled using plasma samples in a validation cohort of 19 study participants. Briefly, plasma was first suspended in 1 mM butylated hydroxyanisole (BHA) in chloroform and methanol (2:1) in order to protect the integrity of the samples. Following a previously described method [33], lipids were extracted from these plasma samples using 1.9 ml of chloroform/methanol (2:1 v/v) and 1 ml of cold water. From the organic phase, aliquots were used for phospholipid analysis by thin-layer chromatography (TLC) [34]. Lipids were separated from samples by TLC and detected by iodine. After scraping from the plate, the fatty acids were esterified using diazomethane and the esters were quantified using gas chromatography/mass spectrometry (Hewlett Packard 5880A, WCOT capillary column (Supelco-10, 35 m×0.5 mm, 1 µm thick)) using commercial standards (Sigma-Aldrich, Oakville, ON, Canada). The amount of lipids per sample was normalized to the protein concentration measured by the bicinchoninic assay (BCA).

Statistical Analyses

Moderated robust regression in the Linear Models for MicroArrays (limma) package [35] was used in order to determine differential changes in gene expression between early and dual responders. Limma moderates the gene-wise residual variance by combining it with the global mean residual variance across all genes, in order to control for the number of false positive discoveries. A Benjamini Hochberg false discovery rate (FDR) [36] of 10% was used for all limma analyses. Robust regression [37] in the MASS package was used to determine differentially expressed metabolites and lipids using a p-value cut-off of 0.05. Robust test statistics were used for all analyses in order to attenuate the effects of outliers in small sample sizes. In addition, in order to avoid over fitting each variable of interest, ERs vs. DRs at pre-challenge, and at post-challenge (scaled to pre-challenge; post-challenge divided by pre-challenge levels) were considered in separate linear models. Age and sex have been adjusted for in all comparisons (discovery and validation). Regularized Canonical Correlation Analysis (RCCA), an extension of CCA [38], highlights the correlation between two data sets where the number of variables (genes/metabolites) is much larger than the sample size (see Section 3 in [39]). RCCA in the mixOmics package was used in order to identify highly correlated clusters of differentially expressed genes and metabolites in ERs and DRs, respectively. All software packages used to perform the statistical analyses can be accessed through Bioconductor (<http://www.bioconductor.org/>) and The Comprehensive R Archive Network (CRAN) (<http://cran.r-project.org/>).

Gene-set Enrichment Analysis

Top biological functions and canonical pathways of differentially expressed genes were analyzed using Ingenuity Pathway Analysis (IPA). The filtering criteria included; organ: lung, and cells: immune cells. Gene networks consisting of hypothetical and proven relationships were also generated using IPA. Fisher's Exact Test p-value of 0.05 and Benjamini Hochberg adjusted p-value (FDR) of 0.05 were used to determine significant canonical pathways and biological pathways.

Results

Asthma Cohort

Table 1 displays the demographics for the initial 14 study participants (eight ERs and six DRs), that participated in the cat allergen inhalation challenge. Both groups were age-matched and met the initial percent drop in forced expiratory volume in one second (FEV₁) criteria; a drop of greater than 20% (Figure 1). During the late phase (between 3 to 7 hours), the maximum percent drop in FEV₁ in the DRs (21.3±3.2%) was approximately 4 times greater than that in ERs (5.1±1.4%).

Blood Differential Counts

Complete blood counts with differentials were obtained from all samples in the discovery cohort. At pre-challenge, absolute neutrophil counts were significantly ($p=0.04$) greater in ERs ($3.5\pm0.4\times10^9$ cells) compared to DRs ($2.6\pm0.5\times10^9$ cells). Figure 2 indicates that at pre-challenge, the relative levels of neutrophils were significantly ($p=0.01$) greater in ERs ($60\pm3.0\%$) compared to DRs ($50\pm4\%$), whereas relative levels of lymphocytes were significantly ($p=0.02$) reduced in ERs ($30\pm2\%$) compared to DRs ($40\pm4\%$). No significant differences in cell counts were identified post-challenge. All differential measures are presented in Table S1.

Profiling Gene Expression and Metabolite Levels

32,321 probe sets on the Human Gene ST 1.0 microarray were used to profile blood leukocyte gene expression. Pre-filtering for informative probe sets (see Methods) resulted in 770 probe sets of which 497 annotated probe sets were retained for further analysis. From the same samples, the plasma was analyzed for 292 named and 201 unnamed metabolites; only the named metabolites were kept for downstream analyses.

Differentially Expressed Genes and Metabolites at Pre-challenge

At pre-challenge, 72 (11 over and 61 under-expressed) differentially expressed probe sets in DRs relative to ERs were identified at an FDR of 10%, after adjusting for age and sex. Ingenuity Pathway Analysis (IPA) indicated Connective Tissue Disorders, Immunological Disease, Inflammatory Disease and Skeletal and Muscular Disorder as the top biological functions at an FDR of 5% (Figure S1). Significant genes, such as Interleukin 8 receptor, alpha (*CXCR1*), Nicotinamide phosphoribosyltransferase (*NAMPT*), C-C chemokine receptor type 7 (*CCR7*) and Lymphoid enhancer-binding factor 1 (*LEF1*) are abundantly expressed by neutrophils and lymphocytes. In fact the differences in gene expression between ERs and DRs are consistent with the differences in cell-type frequencies between ERs and DRs. (Figure 2). Therefore it is likely that the differential gene expression is confounded by the white blood cell count differences between ERs and DRs at pre-challenge rather than true differences in cellular gene expression.

Eight metabolites were found differentially expressed ($p<0.05$) between ERs and DRs at pre-challenge. Plasma levels of six metabolites including bilirubin, 4-vinyl phenol sulphate, 2-arachidonoylglycerophosphocholine, methionine, N-acetylglycine and malate were significantly elevated in DRs compared to ERs. Methyl palmitate and mannose were significantly under-expressed in DRs relative to ERs.

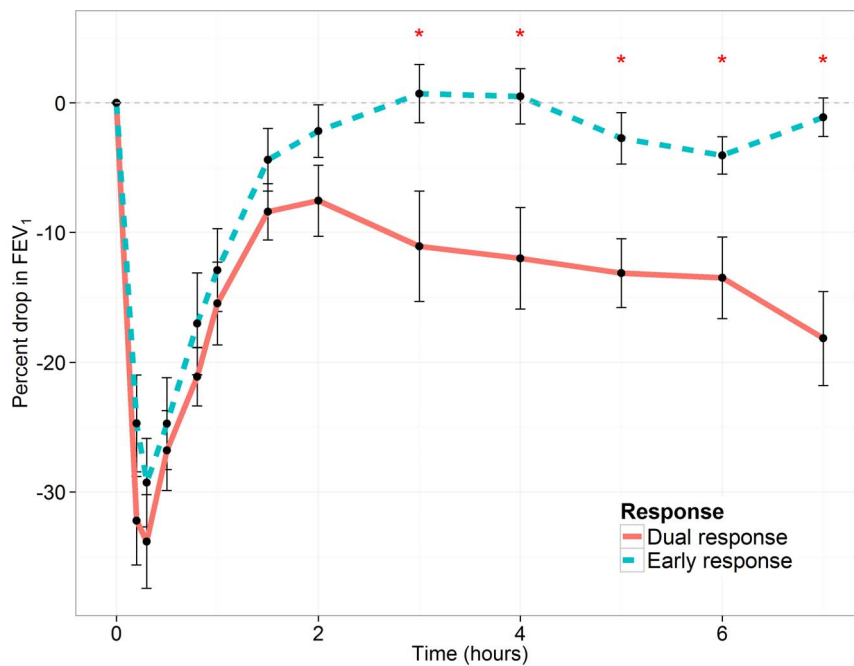


Figure 1. Lung function during allergen inhalation challenge. Forced expiratory volume in 1 second (FEV₁) measurements at 0.2 h, 0.3 h, 0.5 h, 0.8 h, 1 h, 1.5 h, 2 h, 3 h, 4 h, 5 h, 6 h and 7 h for eight early and six dual responders. FEV₁ measurements are statistically different (*p<0.05) between early and dual responders at each of the time points between three and seven hours inclusive. The p-value for each time point comparing ERs and DRs was computed using a robust linear model (see Methods).
doi:10.1371/journal.pone.0067907.g001

Table 1. Demographics of study participants of the discovery cohort.

Patient ID	Age (year)	Sex (M:F)	Pre [PC ₂₀] (mg/ mL)	Post [PC ₂₀] (mg/ mL)	Allergen Induced Shift ^a	% Fall in FEV1	
						Early	Late
ER							
1	28	F	12.8	ND	ND	−20.3	−4.8
2	34	F	2.8	6.1	0.4	−21	−1.5
3	27	M	4.5	1.8	2.5	−34.4	0
4	42	F	5.3	8.6	0.6	−42.1	−11.1
5	29	F	0.4	ND	ND	−44.3	0
6	31	M	11.8	16	0.7	−24.2	−7.5
7	28	F	9.4	16	0.6	−27.1	−7.1
8	42	M	0.1	ND	ND	−23	−9
Mean ± SE	32.6±2.2	3:5	2.8 ^a	7.5 ^a	1.0±0.4	−29.6±3.2	−5.1±1.4
DR							
9	23	F	0.3	0.2	1.5	−38.9	−31.8
10	26	F	5.1	1.5	3.4	−31.4	−14.9
11	49	F	3.6	1.0	3.6	−25.3	−12.6
12	26	M	0.9	1.0	0.9	−31.5	−15.6
13	27	F	0.6	0.1	6	−48.3	−25.8
14	52	F	ND	ND	ND	−33	−27
Mean ± SE	33.8±3.3	1:5	1.3 ^a	0.5 ^a	3.0±0.8	−34.7±3.2	−21.3±3.2 ^c

ND - Not determined; all study participants were challenged with cat allergen.

^a: geometric mean (PC₂₀ values are measured on a log scale);

^b: [PC₂₀]_{pre}/[PC₂₀]_{post}.

^cp<0.05 versus ER group.

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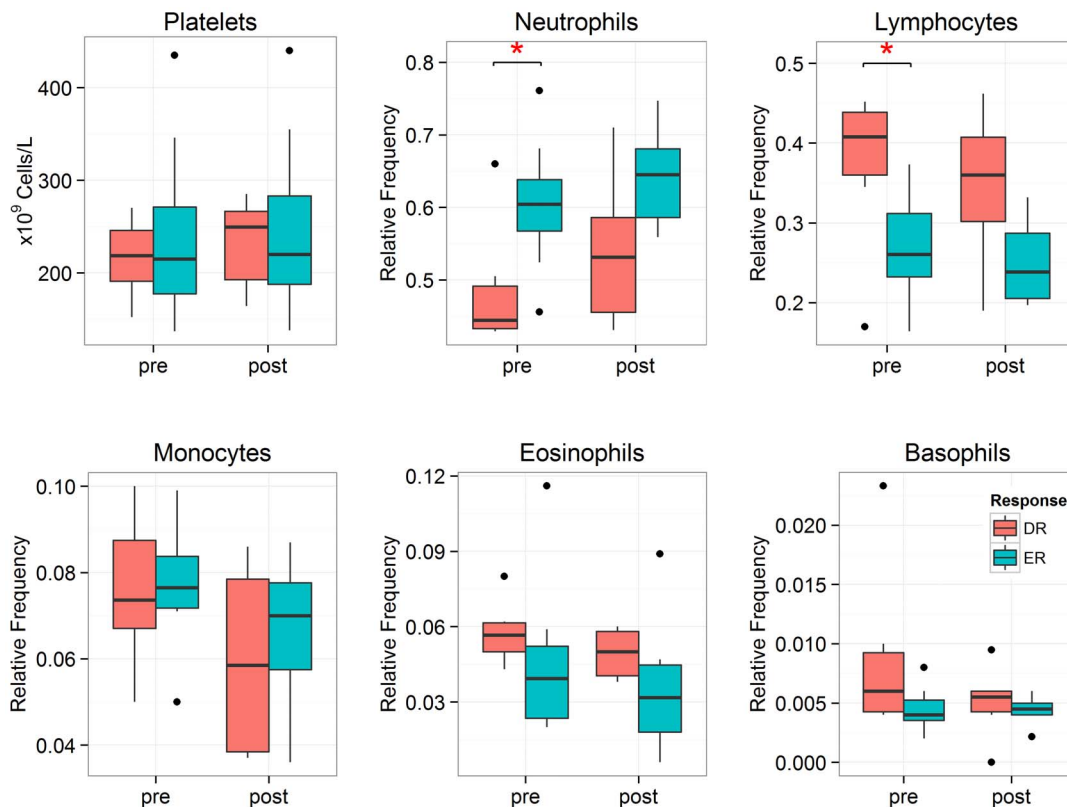


Figure 2. Complete blood counts. Relative frequencies of various leukocytes obtained at pre and post-challenge. The relative neutrophil counts were significantly ($p=0.01$) elevated in ERs compared to DRs, whereas the relative lymphocyte counts were significantly reduced ($p=0.02$) in ERs compared to DRs at pre-challenge. The p-value was computed using a robust linear model (see Methods); * denotes $p<0.05$. doi:10.1371/journal.pone.0067907.g002

Differentially Expressed Genes and Metabolites at Post-challenge

25 probe sets were differentially expressed post-challenge at an FDR of 10%, after adjusting for age and sex (Table S2A). Six of these probe sets were up-regulated whereas 4 probe sets were down-regulated in both ERs and DRs after challenge. The gene encoding RPL9 (ribosomal protein L9) was up-regulated in ERs and down-regulated in DRs at post-challenge. The remaining 14 genes were down-regulated in ERs and up-regulated in DRs after challenge. IPA revealed Lipid Metabolism ($FDR<5\%$) and Linoleic Acid Metabolism ($p<0.05$) as the top biological function and Canonical Metabolism, respectively (Figure S2). Figure 3 shows a network of the differentially expressed genes consisting of the Interleukin 5 receptor alpha (*IL5RA*), Fatty acid desaturase 2 (*FADS2*), Arachidonate 15-lipoxygenase (*ALOX15*), and Cold shock domain protein A (*CSDA*). *FADS2* ($\Delta 6$ -fatty acid desaturase) is a key enzyme in the biosynthesis of ω -6 and ω -3 fatty acids such as arachidonic acid (AA) and docosahexaenoic acid (DHA) [40]. *ALOX15* (15-LOX-1) can metabolize AA into lipoxins which have anti-inflammatory properties [41]. 15-LOX-1 can also produce pro-inflammatory mediators such as eoxins by metabolizing arachidonic acid in eosinophils and mast cells [42]. In addition, 15-LOX-1 can also metabolize DHA into 17-series DHA metabolites such as resolvins and protectins which have anti-inflammatory and pro-resolving properties and are more potent than metabolites derived from AA [43]. Table S2A indicates that Lysophosphatidylcholine acyltransferase 2 (*LPCAT2*), which is involved in membrane biogenesis [44] was differentially expressed between ERs and DRs at post-challenge.

Eleven metabolites were differentially expressed ($p<0.05$) post-challenge (Table S2B). Four of these metabolites were up-regulated whereas 2 metabolites were down-regulated after challenge in both ERs and DRs. The metabolite 1-pentadecanoylglycerophosphocholine was up-regulated in ERs and down-regulated in DRs after challenge. There were 4 metabolites that decreased in ERs after challenge and increased in DRs after challenge. The list of 11 differentially expressed metabolites was mainly enriched with lipid and amino acid metabolism pathways. Lipids were either lysolipids such as 1-pentadecanoylglycerophosphocholine, 2-arachidonoylglycerophosphocholine, 1-linoleoylglycerophosphocholine or sterols/steroids such as andro steroid monosulfate 1, cortisol and 7- α -hydroxy-3-oxo-4-cholestenate (7-Hoca). The amino acid metabolism pathway consisted of metabolites such as 4-hydroxyphenylacetate, 3-methyl-2-oxobutyrate and cysteine. Interestingly, the polypeptide bradykinin, hydroxy-pro (3) increased in DRs and decreased in ERs in response to allergen challenge.

Regularized Canonical Correlation Analysis (RCCA) was used to highlight clusters of correlated genes and metabolites. The ratio between pre and post gene expression levels (post/pre) was calculated for the 25 and 11 differentially expressed genes and metabolites for 14 responders resulting in 2 datasets; $\Delta G_{25,14}$ (25 significant genes for 14 responders) and $\Delta M_{11,14}$ (11 significant metabolites for 14 responders). Figure 4 displays the correlations between ΔG and ΔM independently for ERs (Figure 4A; $\Delta G_{25,8}$ and $\Delta M_{11,8}$) and DRs (Figure 4B; $\Delta G_{25,6}$ and $\Delta M_{11,6}$) using a correlation cut-off of 0.5. RCCA indicated greater numbers of highly correlated ($r>0.5$) gene-metabolite clusters in dual

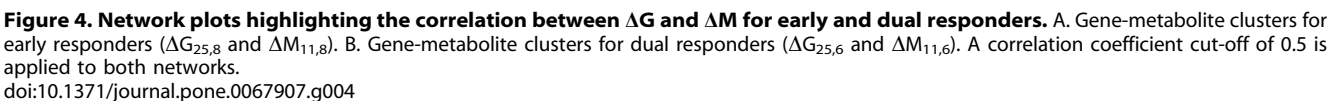


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blood cells may differ between early and dual responders. However, validation of the underlying mechanism is beyond the scope of this study. Instead we looked to see how general mediators of inflammation which are also components of cellular membranes such as arachidonic acid (pro- and anti-inflammatory) and docosahexaenoic acid (anti-inflammatory and pro-resolving) respond to allergen inhalation challenge.

ω -6 and ω -3 Polyunsaturated Fatty Acids

Both free and phospholipid forms of arachidonic acid (AA) and docosahexaenoic acid (DHA) were measured in a validation cohort of 19 (8 ERs and 11 DRs) study participants challenged with various allergens including cat, grass and ragweed (Table 2). The free fatty acid form is the fatty acid bound to albumin or on the surface of lipoproteins and usually refers to the metabolized lipid either from the hydrolysis of phospholipids or other complex lipids. The phospholipid (membrane bound) form is another structure which combines two fatty acids, usually at the *sn* position 2 in AA or DHA. Lipid levels were compared between ERs and DRs at post-challenge (scaled to pre-challenge) using a robust linear model adjusting for age and sex. At post-challenge, neither forms of AA were significant (Table S3), whereas only the free form of DHA was significant ($p = 0.033$) between ERs and DRs. Figure 5 shows that the free form of DHA decreases in ERs post-challenge compared to baseline levels whereas little change occurs in DRs.

Discussion

Asthma is a complex disease consisting of multiple sub-phenotypes such that current therapies perform well in some phenotypes compared to others [45]. Therefore, well characterized study cohorts are required to identify new molecular targets of asthma. Despite the limited sample size of the present study, many

potentially confounding factors have been accounted for through careful selection of study participants. The participants were selected from a homogenous group of non-smoking individuals with stable, mild, atopic asthma and free of other lung diseases. Detailed characterization of the participants was based on the development of allergen-induced bronchoconstriction, and airway responsiveness to methacholine. Although airway samples are commonly used to study the pathobiology of respiratory disease, such samples are obtained using invasive procedures. In the present study, we demonstrate the utility of applying 'omics' based approaches to peripheral blood in order to discriminate allergen-induced early from dual asthmatic responders (ERs from DRs).

Although many genes were differentially expressed between ERs and DRs prior to (pre-) allergen inhalation challenge, this may be influenced by significant differences in cellular frequencies between ERs and DRs (Figure 2). Figure 2 shows that the cellular frequencies of ERs and DRs move in parallel from pre to post-challenge, thus comparing post-challenge levels (scaled to pre-challenge levels, see Methods) was deemed less likely to be influenced by differences in cell-type proportions. Therefore greater focus was directed towards identifying significant molecular differences between ERs and DRs in response to the allergen inhalation challenge. These molecular targets may serve as effective tools for the diagnostic monitoring of asthmatic responses in clinical trials or as therapeutics targets for the attenuation of the late phase asthmatic response.

Differentially expressed genes at post-challenge were enriched for lipid metabolism and linoleic acid metabolism pathways (Figure S2). Figure 3 depicts genes within these pathways such as *FADS2* and *ALOX15* as well as *IL5RA*, which may suggest that these expression differences may be originating from eosinophils. Table S2A indicates that *EMR1*, an eosinophil-specific receptor was differentially expressed between ERs and DRs at post-challenge [46]. The mechanism of IL-5 on eosinophil survival

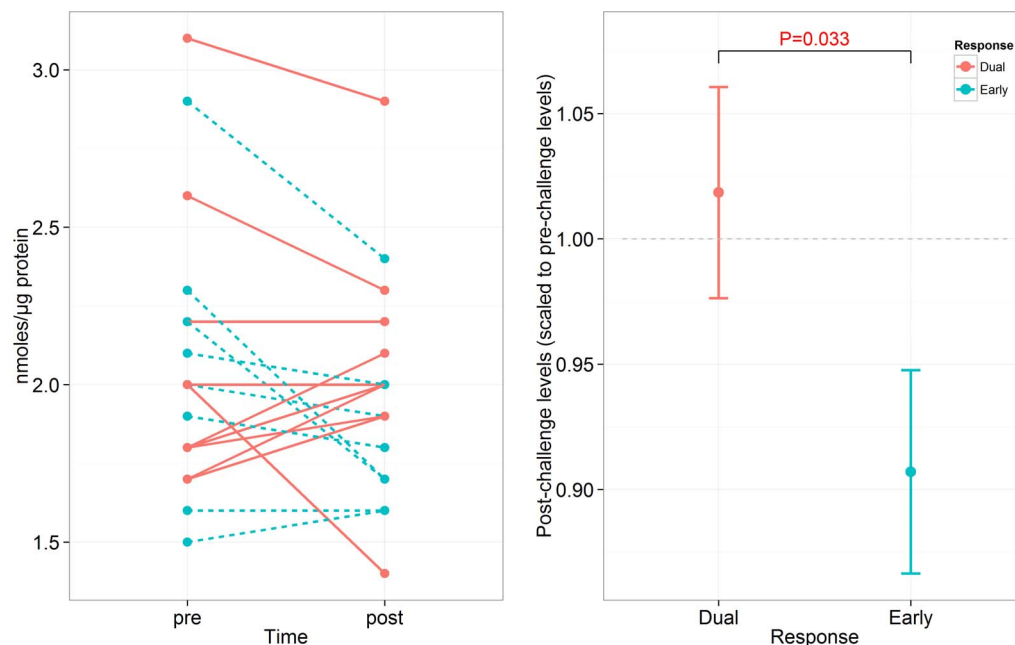


Figure 5. Levels of free docosahexaenoic acid in the plasma of early and dual responders undergoing allergen inhalation challenge. Levels of DHA (left) decrease in early responders from pre to post-challenge whereas no consistent change is observed in dual responders following allergen challenge. At post-challenge (scaled to pre-challenge levels), DHA was differentially expressed ($p = 0.033$) between ERs and DRs (right). The p-value was computed using a robust linear model (see Methods). doi:10.1371/journal.pone.0067907.g005

Table 2. Demographics of study participants of the validation cohort.

	Age (year)	Sex (M:F)	Allergen	Pre [PC ₂₀] (mg/ mL)	Post [PC ₂₀] (mg/ mL)	Allergen Induced Shift ^b	% Fall in FEV1	
							Early	Late
ER								
1	25	M	Timothy Grass	0.08	ND	ND	−25.24	−11
2	36	F	Timothy Grass	0.64	ND	ND	−19.66	4.41
3	21	F	Ragweed	6.96	2.93	0.421	−33.1	2.11
4	33	M	Grass	13.3	21.1	1.587	−50	−2.5
5	21	F	Cat	0.59	ND	ND	−39.66	−1.72
6	30	F	Grass	1.81	ND	ND	−39.8	−11.7
7	21	F	Cat	9.6	ND	ND	−44.96	−12.4
8	43	M	Cat	1.94	ND	ND	−25.6	−10.8
Mean ± SE	28.8±2.9	3:5		1.8 ^a	7.9 ^a	1.0±0.3	−34.8±3.8	−5.5±2.4
DR								
9	56	F	Cat	0.16	0.3	1.875	−23.22	−21.3
10	37	M	Timothy Grass	3.26	0.08	0.025	−60.61	−36.9
11	48	M	Orchard Grass	0.28	0.79	2.821	−25.16	−19.4
12	44	M	Cat hair	1.45	0.36	0.248	−41.33	−21.3
13	40	M	Grass	6.9	2.38	0.345	−21.21	−17.6
14	19	M	Grass	15.56	2.36	0.152	−42.99	−26.2
15	21	M	Ragweed	1.5	1.15	0.767	−55.73	−16.3
16	21	F	Cat	0.42	0.23	0.548	−44.06	−31.6
17	43	F	Cat	0.28	0.18	0.643	−26.92	−17.3
18	21	F	Cat	5.25	3.78	0.72	−47.22	−11.5
19	20	F	Grass	5.34	1.64	0.307	−23.61	−16.7
Mean ± SE	33.6±4.1	6:5		1.5 ^a	0.7 ^a	0.8±0.3	−37.5±4.2	−21.5±2.2 ^c

^ageometric mean (PC₂₀ values are measured on a log scale);^b[PC₂₀]_{pre}/[PC₂₀]_{post};^cp<0.001 versus ER group.

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involves the arachidonate pathway where phosphorylation of cytosolic phospholipase A₂ α (cPLA₂-α) induces up-regulation of arachidonic acid levels in eosinophils [47]. Allergen inhalation challenge stimulates these pathways in circulating blood eosinophils [47]. FADS2 is a key enzyme in the biogenesis of arachidonic acid and docosahexaenoic acid [48]. ALOX15 can produce pro-inflammatory mediators such as eoxins [42] and anti-inflammatory mediators such as lipoxins from arachidonic acid [49], as well as pro-resolving mediators such as resolvins and protectins from docosahexaenoic acid [41,50]. Previous studies analyzing lipid mediators in bronchoalveolar lavage fluid have identified significant differences in ALOX15 metabolites between mild asthmatics and controls [42,43]. The latter study indicated higher levels of ALOX15 metabolites in mild allergic asthmatics compared to healthy controls, and this difference increased following allergen challenge [43]. This is clear evidence that blood is a useful medium to depict lung inflammation since divergent oxylipin profiles can be observed in individuals with mild disease.

Lipid metabolism was also enriched in the list of significant metabolites at post-challenge. Cortisol, an immunosuppressive and anti-inflammatory molecule decreased in ERs and increased in DRs after challenge. Cortisol bound to carrier proteins in the blood can be released at the site of inflammation by the catalytic activity of neutrophil elastase [51]. This result may suggest that the uptake of plasma cortisol in ERs leads to reduced inflammation

whereas this mechanism is impaired in DRs. Many lysolipids which are important components of cellular membranes were differentially regulated between ERs and DRs after challenge (Table S2B). The release and subsequent metabolism of these lipids into pro- or anti-inflammatory mediators may dictate the associated clinical response. Bradykinin, hydroxy-pro(3) was differentially expressed between ERs and DRs at post-challenge; increase in DRs and decrease in ERs from pre- to post-challenge. This result supports the well established role of bradykinin in asthma as a bronchoconstrictor and vasodilator [52]. We have previously shown that Kininogen (KN1), which is a precursor of bradykinin decreases in DRs and increases in ERs after challenge [24]. Therefore, plasma metabolomics reinforces the genomics analysis and identifies other biologically relevant molecules.

Although each data set (transcriptomics and metabolomics) is useful in identifying differences between ERs and DRs, considering each data set independently may result in some loss of information as both data sets were obtained from the same individuals. Current bioinformatics tools contain poor annotations for many of the differentially expressed metabolites; therefore integration with the transcriptomic data was approached using statistical means. Correlation analysis identified interesting patterns between differentially expressed genes and metabolites in ERs and DRs at post-challenge. Figure 4B shows that genes encoding for enzymes that can metabolize (*ALOX15*) or produce

(*FADS2*) arachidonic acid were negatively correlated with the arachidonic acid lysolipid (2-arachidonoylglycerophosphocholine) in DRs after challenge. Although this network is biologically plausible, experimental work to validate the interactions amongst these molecules in peripheral blood is beyond the scope of this study.

Interestingly, in the validation cohort of 19 study participants (8 ERs and 11 DRs), docosahexaenoic acid instead of arachidonic acid was differentially expressed between ERs and DRs at post-challenge. The free form of DHA was down-regulated in ERs post challenge compared to pre-challenge whereas little change occurred in DRs from pre- to post-challenge (Figure 5). It may be possible that DHA is being taken up and metabolized in ERs leading to the resolution of inflammation [43,50,53] whereas no such effect occurs in DRs within the 2 hour time frame. However, this result should be approached with caution as various forms (essential fatty acids, long chain fatty acids, and lysolipids) of arachidonic acid and docosahexaenoic acid were profiled in the discovery cohort and were not significantly different between ERs and DRs at post-challenge (Table S4). Other limiting factors of the validation cohort apart from the limited sample size include the use of various allergens (Table 2), and incomplete data on complete blood cell counts. The potency of different allergens is variable [54] and can dictate the type of response an individual may experience. For example, allergen inhalation challenge using house dust mite mainly elicits the dual response [55] (also seen in our data sets). Frequencies of white blood cell counts may influence plasma lysolipid levels and their metabolism can influence subsequent clinical phenotypes. In addition, measuring the specific arachidonic acid lysolipid (2-arachidonoylglycerophosphocholine) may be a more appropriate validation. However the differential expression of DHA may suggest that mechanisms involved in the resolution of inflammation may be significantly dysregulated between ERs and DRs resulting in their distinct clinical phenotypes.

To our knowledge, this study is the first of its kind to apply 'omics' methodologies to peripheral blood and identify molecular differences between two asthma phenotypes. These differences consist of factors associated with both systemic and airway inflammation. Although this study does not directly evaluate the impact of allergen inhalation on the airways, the downstream effects of this lung insult can provide significant evidence in understanding the molecular mechanisms underlying the early and late asthmatic responses. Our study has shown that careful selection of study participants is effective in detecting molecular differences between ERs and DRs. The use of multiple data sets from the same individuals can identify common biological functions and mechanisms and integration of these data sets may narrow down the number of generated hypotheses. Although many gene-metabolite clusters were identified, one biologically plausible gene-metabolite cluster was identified in DRs. Similar studies with larger sample sizes may reveal novel mechanisms, and targets for the pathogenesis and treatment of the late phase asthmatic response.

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Supporting Information

Figure S1 Top biological functions and canonical pathways at pre-challenge. The FDR, affy IDs and fold-change of genes were uploaded into IPA after which the following filters were applied: organ: lung, and cells: immune cells and FDR cut-off of 10%. (DOCX)

Figure S2 Top biological functions and canonical pathways for differentially expressed (FDR = 10%) genes at post-challenge. (See Figure S1 for filtering criteria). (DOCX)

Table S1 Complete blood counts and differentials. The Mean $\pm$ SE of each cell count and differential at pre-challenge in early and dual responders. The Mean $\pm$ SE of each cell count and differential at post-challenge (levels scaled to pre-challenge levels) in early and dual responders. (DOCX)

Table S2 Differentially expressed genes and metabolites at post-challenge (scaled to pre-challenge levels; post divided by pre levels). A. Differentially expressed genes at post-challenge (FDR<10%). B. Differentially expressed metabolites at post-challenge (p-value<0.05). (DOCX)

Table S3 Differentially expressed lipids in the validation cohort. Levels (Mean $\pm$ SE) of arachidonic acid and docosahexaenoic at post-challenge (levels scaled to pre-challenge levels) in early and dual responders. (DOCX)

Table S4 Named metabolites profiled in this study. Metabolites highlighted in red are differentially expressed at p<0.05. (PDF)

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Author Contributions

Conceived and designed the experiments: GMG PMO JMF L-PB DR SJT. Analyzed the data: AS SHYK GMG GW CK DR SJT. Wrote the paper: AS MY SHYK JR GMG PMO JMF RS L-PB GW CK JBD DR SJT.

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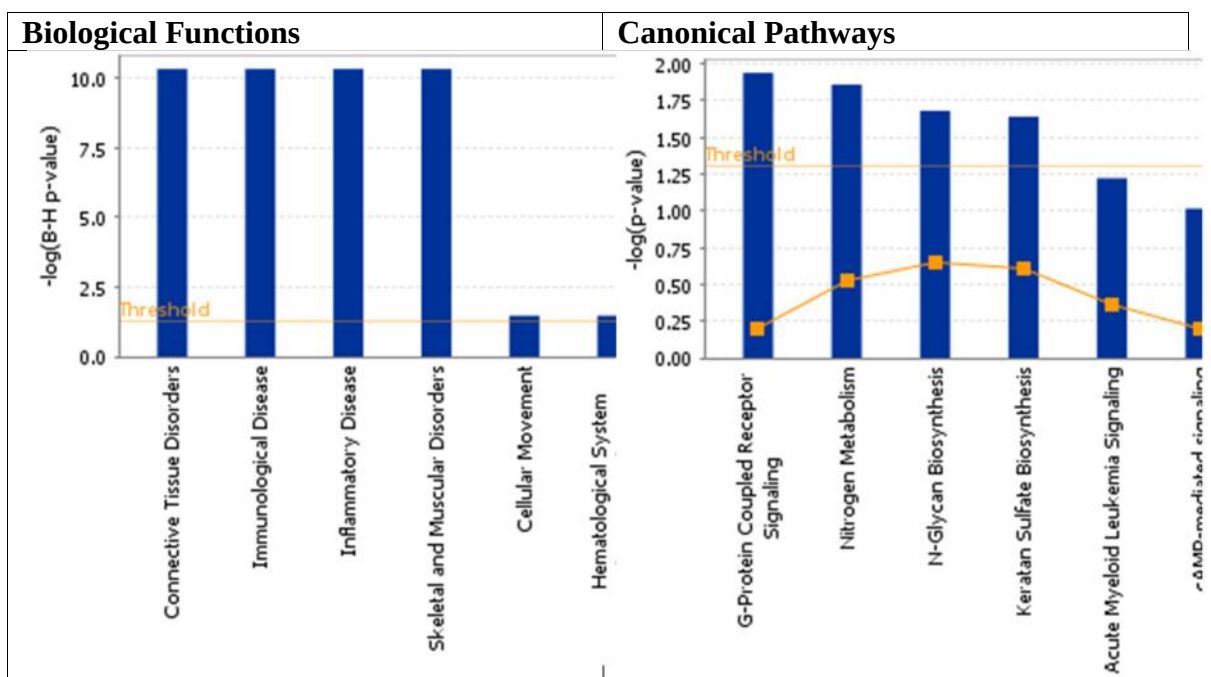


Figure S1: Top biological functions and canonical pathways at pre-challenge. The FDR, affy IDs and fold-change of genes were uploaded into IPA after which the following filters were applied: organ: lung, and cells: immune cells and FDR cut-off of 10%.

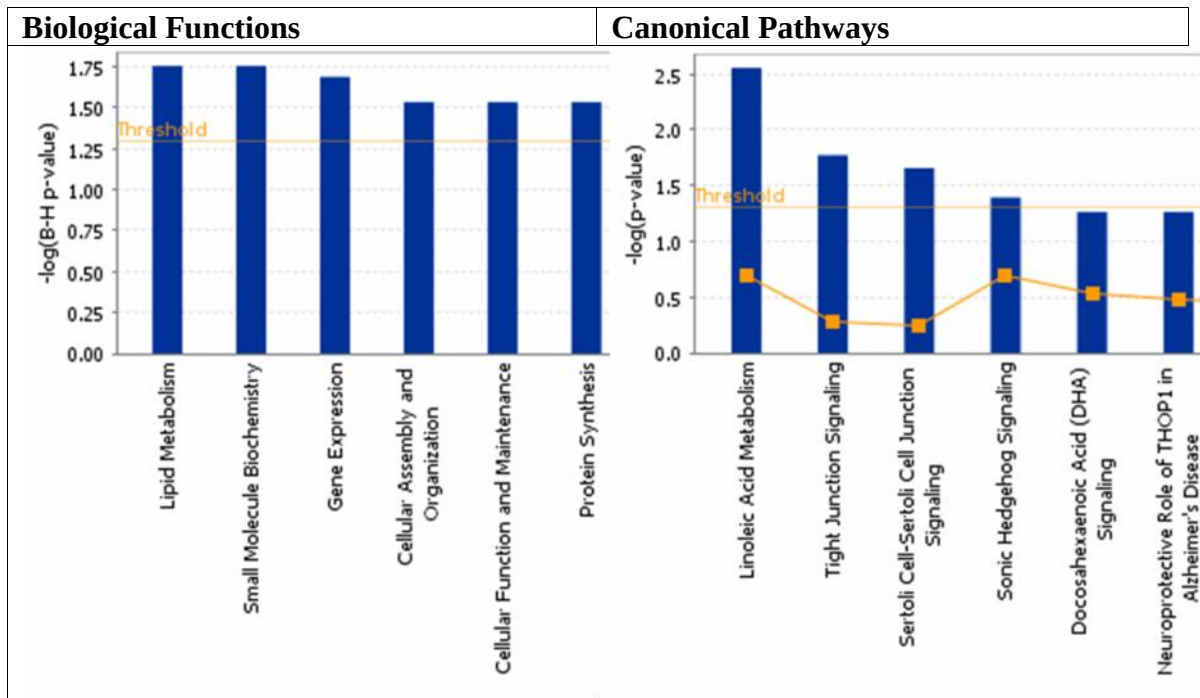


Figure S2: Top biological functions and canonical pathways for differentially expressed (FDR=10%) genes at post-challenge (see Figure S1 for filtering criteria).

Table S1: Complete blood counts and differentials

	Pre-challenge			Post-challenge		
	ER (Mean±SE)	DR (Mean±SE)	p- value	FC* in ER (Mean±SE)	FC in DR (Mean±SE)	p- value
Erythrocytes..(10 ¹² cells/L)	4.6±0.13	4.5±0.1	0.59	-1.01±0.011	-1.00±0.010	0.90
Hemoglobin(g/L)	137.5±3.43	133.8±4.94	0.31	-1.01±0.009	-1.00±0.009	0.83
Hematocrit L/L	0.4±0.01	0.4±0.02	0.57	-1.01±0.011	1.00±0.010	0.97
Mean.Cell.Volume (fL)	87.5±1.73	88.3±1.87	0.77	1.00±0.004	1.00±0.004	0.84
Mean.Cell.HB (pg/cell)	29.9±0.68	29.8±0.65	0.76	-1.00±0.004	1.00±0.005	0.89
Mean.Cell.HB.Conc (g/L)	342±4.87	337.3±1.96	0.57	-1.01±0.003	1.00±0.005	0.41
Red.Cell.Distr..Width (% CV)	13.4±0.3	13.4±0.14	0.95	-1.02±0.018	1.01±0.020	0.33
Platelets (10 ⁹ cells/L)	242±35.87	216±17.98	0.95	1.04±0.019	1.08±0.039	0.39
Leukocytes (10 ⁹ cells/L)	5.8±0.49	5±0.42	0.32	1.14±0.046	1.12±0.076	0.51
Neutrophils (10 ⁹ cells/L)	3.5±0.37	2.6±0.47	0.04	1.22±0.085	1.17±0.093	0.44
Lymphocytes (10 ⁹ cells/L)	1.6±0.2	1.9±0.2	0.13	1.07±0.059	-1.02±0.059	0.40
Monocytes (10 ⁹ cells/L)	0.4±0.05	0.4±0.05	0.39	-1.04±0.072	-1.06±0.13	0.64
Eosinophils (10 ⁹ cells/L)	0.3±0.05	0.4±0.11	0.40	-1.19±0.128	-1.21±0.072	0.36
Basophils (10 ⁹ cells/L)	0.001±0	0±0.02	0.41	ND†	ND	0.15
Relative.Neutrophils (%)	0.6±0.03	0.5±0.04	0.01	1.07±0.035	1.12±0.066	0.69
Relative.Lymphocytes (%)	0.3±0.02	0.4±0.04	0.02	-1.04±0.048	-1.05±0.071	0.89
Relative.Monocytes (%)	0.1±0.01	0.1±0.01	0.84	-1.15±0.058	-1.28±0.099	0.37
Relative.Eosinophils (%)	0.05±0.01	0.1±0.01	0.07	-1.38±0.076	-1.15±0.073	0.72
Relative.Basophils (%)	0.004±0.001	0.01±0.003	0.12	1.12±0.152	-1.27±0.200	0.31

*Levels at post-challenge are scaled to pre-challenge levels;

FC = post/pre if FC>0, FC = -1/(post/pre)

†ND (Not determined): division by zero cell-type frequency

Table S2. Differentially expressed genes and metabolites at post-challenge (scaled to pre-challenge levels, ratio of post to pre levels).

A. Differentially expressed genes in the interaction (FDR<0.1).

		FC* in ERs	FC in DRs	P -Value	BH-FDR
8011680	ALOX15	-1.034±0.053	-1.007±0.013	1.37E-06	0.000681
8085062	IL5RA	-1.072±0.008	-1.022±0.005	5.80E-06	0.001
8124527	HIST1H1B	-1.008±0.009	1.026±0.016	1.25E-05	0.002
7916862	WLS	1.011±0.011	1.036±0.018	1.38E-05	0.002
8047443	STRADB	-1.021±0.007	1.011±0.005	0.000193	0.019
7940565	FADS2	-1.032±0.013	1.015±0.008	0.000254	0.020
7995697	LPCAT2	-1.009±0.005	1.015±0.011	0.00028	0.020
8161288	CNTNAP3	1.017±0.012	1.058±0.024	0.000422	0.026
7953901	CLEC12A	-1.030±0.015	1.018±0.018	0.000609	0.034
8166632	GK	1.007±0.011	1.029±0.018	0.00087	0.043
8174361	TSC22D3	-1.037±0.006	-1.014±0.011	0.001	0.046
8169365	TMEM164	-1.004±0.006	1.016±0.007	0.001	0.046
8176709	CYorf15B	-1.003±0.018	1.028±0.038	0.001	0.050
8007607	RUNDC3A	-1.021±0.005	1.014±0.019	0.001	0.050
8161460	CNTNAP3	1.037±0.015	1.081±0.032	0.002	0.058
8025103	EMR1	-1.040±0.005	-1.007±0.009	0.002	0.063
7988033	EPB42	-1.028±0.008	1.015±0.010	0.002	0.063
8164596	C9orf78	-1.016±0.007	1.003±0.002	0.002	0.063
7921873	FCGR3A	1.011±0.007	1.020±0.011	0.003	0.084
8153652	SHARPIN	-1.024±0.008	1.009±0.007	0.004	0.087
8135378	PRKAR2B	-1.015±0.008	1.021±0.005	0.004	0.087
7961230	CSDA	-1.027±0.007	1.008±0.009	0.004	0.093
7929052	IFIT3	1.000±0.01	1.033±0.023	0.004	0.093
8171834	RPL9	1.022±0.02	-1.029±0.010	0.005	0.093
8157264	SLC31A2	-1.019±0.005	1.009±0.012	0.005	0.093

*Levels at post-challenge are scaled to pre-challenge levels;

FC = post/pre if FC>0, FC = -1/(post/pre)

B. Differentially expressed metabolites at post-challenge (p-value<0.05).

BIOCHEMICAL	SUB_PATHWAY	HMDB	FC* in ERs*	FC in DRs	P -Value
andro steroid monosulfate 1*	Sterol/Steroid	HMDB02759	1.003±0.120	1.054±0.202	0.002
4-hydroxyphenylacetate	Phenylalanine & tyrosine metabolism	HMDB00020	-1.519±0.210	1.226±0.414	0.006
thymol sulfate	Food component/Plant	HMDB01878	-1.085±0.146	-1.381±0.317	0.014
1-pentadecanoylglycerophosphocholine*	Lysolipid		1.404±0.569	-1.005±0.300	0.014
2-arachidonoylglycerophosphocholine*	Lysolipid		3.950±2.892	1.181±0.436	0.014
cortisol	Sterol/Steroid	HMDB00063	-1.560±0.210	1.008±0.400	0.017

3-methyl-2-oxobutyrates	Valine, leucine and isoleucine metabolism	HMDB00019	-1.048±0.407	1.307±0.341	0.018
1-linoleoylglycerophosphocholine	Lysolipid		1.046±0.192	1.185±0.255	0.019
7- α -hydroxy-3-oxo-4-cholestenoate (7-Hoca)	Sterol/Steroid	HMDB12458	1.188±0.207	1.416±0.253	0.031
cysteine	Cysteine, methionine, SAM, taurine metabolism	HMDB00574	-1.037±0.215	-1.404±0.110	0.041
bradykinin, hydroxy-pro(3)	Polypeptide	HMDB11728	-1.344±0.241	2.788±3.447	0.043

*Levels at post-challenge are scaled to pre-challenge levels;
FC = post/pre if FC>0, FC = -1/(post/pre)

Table S3: Differentially expressed lipids in the validation cohort.

	FC* in ERs	FC in DRs	P -Value
DHA free (nmoles./μ gram.of.protein)	-1.10±0.04	1.02±0.04	0.03
AA phospholipid (nmoles./μ gram.of.protein)	-1.08±0.05	1.02±0.06	0.21
AA free (nmoles./μ gram.of.protein)	1.22±0.20	1.41±0.12	0.59
DHA phospholipid (nmoles./μ gram.of.protein)	1.05±0.06	1.02±0.06	0.63

*Levels at post-challenge are scaled to pre-challenge levels;

FC = post/pre if FC>0, FC = -1/(post/pre)

Named metabolites profiled in this study. Shaded cells indicate $p \leq 0.05$									
PATHWAY SORT	SUPER PATHWAY	SUB PATHWAY	BIOCHEMICAL NAME	PLATFORM	COMP ID	KEGG	HMDB	Pre-challenge	Post-challenge
1	Amino acid	Glycine, serine and threonine metabolism	glycine	GC/MS	32338	C00037	HMDB00123	0.9773	0.1069
3			dimethylglycine	GC/MS	5086	C01026	HMDB00092	0.8356	0.0759
4			N-acetylglycine	GC/MS	27710		HMDB00532	0.0224	0.0621
5			N-formylglycine	GC/MS	1596			0.3479	0.1218
6			beta-hydroxypropyruvate	GC/MS	15686	C00168	HMDB01352	0.6966	0.5392
7			serine	GC/MS	32315	C00065	HMDB03406	0.3766	0.8752
9			N-acetylserine	GC/MS	37076		HMDB02931	0.9043	0.3175
16			threonine	LC/MS pos	1284	C00188	HMDB00167	0.2985	0.6786
20			N-(2-furoyl)glycine	LC/MS pos	31536		HMDB00439	0.1544	0.1544
21			betaine	LC/MS pos	3141	C00719	HMDB00043	0.4175	0.9807
23		Alanine and aspartate metabolism	alanine	GC/MS	32339	C00041	HMDB00161	0.9031	0.7872
24			beta-alanine	GC/MS	35838	C00099	HMDB00056	0.2604	0.0579
26			N-acetylalanine	LC/MS neg	1585	C02847	HMDB00766	0.2504	0.4600
27			N-acetyl-beta-alanine	LC/MS pos	37432	C01073		0.2991	0.4536
29			aspartate	GC/MS	35834	C00049	HMDB00191	0.7561	0.8588
33			asparagine	GC/MS	34283	C00152	HMDB00168	0.5249	0.9715
38		Glutamate metabolism	glutamate	GC/MS	32322	C00025	HMDB03339	0.5697	0.5616
42			glutamine	LC/MS pos	53	C00064	HMDB00641	0.3835	0.8355
43			pyroglutamine*	LC/MS pos	32672			0.0830	0.7976
48		Histidine metabolism	histidine	LC/MS neg	59	C00135	HMDB00177	0.8068	0.4715
51			3-methylhistidine	LC/MS neg	15677	C01152	HMDB00479	0.8158	0.7155
66		Lysine metabolism	lysine	GC/MS	35836	C00047	HMDB00182	0.6664	0.4579
69			pipecolate	LC/MS pos	1444	C00408	HMDB00070	0.5233	0.4734
74			N6-acetyllysine	LC/MS pos	36752	C02727	HMDB00206	0.6733	0.8080
75			glutaroyl carnitine	LC/MS pos	35439		HMDB13130	0.9684	0.0910
77		Phenylalanine & tyrosine metabolism	phenyllactate (PLA)	LC/MS neg	22130	C05607	HMDB00779	0.2980	0.4201
78			phenylalanine	LC/MS pos	64	C00079	HMDB00159	0.2444	0.9616
80			phenylacetate	LC/MS neg	15958	C07086	HMDB00209	0.4940	0.5339
82			p-cresol sulfate	LC/MS neg	36103	C01468		0.9233	0.5269
90			tyrosine	LC/MS pos	1299	C00082	HMDB00158	0.2655	0.3479
91			3-(4-hydroxyphenyl)lactate	LC/MS neg	32197	C03672	HMDB00755	0.8469	0.1321
97			4-hydroxyphenylpyruvate	LC/MS neg	1669	C01179	HMDB00707	0.8648	0.6273
106			4-hydroxyphenylacetate	GC/MS	541	C00642	HMDB00020	0.9462	0.0055
110			3-methoxytyrosine	LC/MS pos	12017		HMDB01434	0.8608	0.9903
115			phenylacetylglutamine	LC/MS pos	35126	C05597	HMDB06344	0.8936	0.4842
119		Tryptophan metabolism	3-phenylpropionate (hydrocinnamate)	LC/MS neg	15749	C05629	HMDB00764	0.8407	0.9535
121			phenol sulfate	LC/MS neg	32553	C02180		0.6961	0.0977
125			kynurenine	LC/MS pos	15140	C00328	HMDB00684	0.6616	0.8830
126			tryptophan	LC/MS pos	54	C00078	HMDB00929	0.3733	0.7068
127			indolelactate	GC/MS	18349	C02043	HMDB00671	0.0827	0.2156
129			indoleacetate	LC/MS pos	27513	C00954	HMDB00197	0.1009	0.7881
131			tryptophan betaine	LC/MS pos	37097	C09213		0.7485	0.5376
138			serotonin (5HT)	LC/MS pos	2342	C00780	HMDB00259	0.1883	0.3511
141			C-glycosyltryptophan*	LC/MS pos	32675			0.8916	0.3842
147			3-indoxyl sulfate	LC/MS neg	27672		HMDB00682	0.4038	0.6606
148			indolepropionate	GC/MS	32405		HMDB02302	0.9499	0.5636
149			3-methyl-2-oxobutyrate	LC/MS neg	21047	C00141	HMDB00019	0.0554	0.0180
150			3-methyl-2-oxovalerate	LC/MS neg	15676	C00671	HMDB03736	0.3628	0.6419
155			beta-hydroxyisovalerate	LC/MS neg	12129		HMDB00754	0.4131	0.9643
156			alpha-hydroxyisocaproate	GC/MS	22132	C03264	HMDB00746	0.4949	0.1493
157			isoleucine	LC/MS pos	1125	C00407	HMDB00172	0.3452	0.3935

158	Valine, leucine and isoleucine metabolism	leucine	LC/MS pos	60	C00123	HMDB00687	0.0520	0.1468	
167		valine	LC/MS pos	1649	C00183	HMDB00883	0.1200	0.1324	
168		2-hydroxyisobutyrate	GC/MS	22030		HMDB00729	0.7945	0.5942	
170		4-methyl-2-oxopentanoate	LC/MS neg	22116	C00233	HMDB00695	0.2985	0.2668	
175		alpha-hydroxyisovalerate	GC/MS	33937		HMDB00407	0.9553	0.6551	
177		isobutyrylcarnitine	LC/MS pos	33441		HMDB00736	0.2272	0.9941	
179		2-methylbutyrylcarnitine	LC/MS pos	35431		HMDB00378	0.6827	0.6512	
181		isovalerylcarnitine	LC/MS pos	34407		HMDB00688	0.1703	0.8730	
182		hydroxyisovaleroyl carnitine	LC/MS pos	35433			0.9060	0.8655	
188		Cysteine, methionine, SAM, taurine metabolism	cysteine	GC/MS	31453	C00097	HMDB00574	0.1033	0.0410
194	N-formylmethionine		LC/MS neg	2829	C03145	HMDB01015	0.9585	0.8707	
199	methionine		LC/MS pos	1302	C00073	HMDB00696	0.0039	0.1310	
201	N-acetylmethionine		LC/MS neg	1589	C02712	HMDB11745	0.7183	0.9075	
202	alpha-ketobutyrate		LC/MS neg	4968	C00109	HMDB00005	0.5096	0.8342	
203	2-hydroxybutyrate (AHB)		GC/MS	21044	C05984	HMDB00008	0.2699	0.7103	
213	Urea cycle; arginine-, proline-, metabolism	arginine	LC/MS neg	1638	C00062	HMDB00517	0.6091	0.9850	
216		ornithine	GC/MS	35832	C00077	HMDB03374	0.5529	0.8294	
217		urea	GC/MS	1670	C00086	HMDB00294	0.6564	0.8730	
218		proline	LC/MS pos	1898	C00148	HMDB00162	0.6887	0.9377	
220		citrulline	LC/MS pos	2132	C00327	HMDB00904	0.8528	0.1334	
221		N-acetylornithine	LC/MS pos	15630	C00437	HMDB03357	0.3731	0.5547	
224		trans-4-hydroxyproline	GC/MS	32319	C01157	HMDB00725	0.5184	0.7295	
232	Creatine metabolism	creatine	LC/MS pos	27718	C00300	HMDB00064	0.9177	0.5397	
233		creatinine	LC/MS pos	513	C00791	HMDB00562	0.3472	0.1890	
235	Butanoate metabolism	2-aminobutyrate	GC/MS	32309	C02261	HMDB00650	0.8138	0.8277	
247	Guanidino and acetamido metabolism	4-acetamidobutanoate	LC/MS pos	1558	C02946	HMDB03681	0.4512	0.9619	
253	Glutathione metabolism	5-oxoproline	LC/MS neg	1494	C01879	HMDB00267	0.2157	0.2836	
298	Peptide	Dipeptide	pro-hydroxy-pro	LC/MS pos	35127		HMDB06695	0.7856	0.2311
321		gamma-glutamyl	gamma-glutamylvaline	LC/MS pos	32393		HMDB11172	0.5871	0.8673
323			gamma-glutamylleucine	LC/MS pos	18369		HMDB11171	0.8830	0.3906
324			gamma-glutamylisoleucine*	LC/MS pos	34456		HMDB11170	0.3643	0.6019
327			gamma-glutamylmethionine	LC/MS pos	37539			0.1446	0.6047
331			gamma-glutamylphenylalanine	LC/MS pos	33422		HMDB00594	0.3695	0.2189
332			gamma-glutamyltyrosine	LC/MS pos	2734			0.2090	0.8757
336			gamma-glutamylalanine	LC/MS pos	37063			0.3655	0.6283
337		Polypeptide	bradykinin	LC/MS pos	22154	C00306	HMDB04246	0.3800	0.0547
338			bradykinin, hydroxy-pro(3)	LC/MS pos	33962		HMDB11728	0.7089	0.0429
339			bradykinin, des-arg(9)	LC/MS pos	34420	C00306	HMDB04246	0.1848	0.3271
343			HWESASXX*	LC/MS pos	32836			0.4784	0.2194
345			[H]HWESASLLR[OH]	LC/MS pos	33964			0.8241	0.4578
354		Fibrinogen cleavage peptide	ADSGEGDFXAEGGGVR*	LC/MS pos	33084			0.3214	0.2859
372	Carbohydrate	Aminosugars metabolism	erythronate*	GC/MS	33477		HMDB00613	0.4497	0.1625
389		Fructose, mannose, galactose, starch, and sucrose metabolism	fructose	GC/MS	31266	C00095	HMDB00660	0.9890	0.3352
398			mannitol	GC/MS	15335	C00392	HMDB00765	0.8050	0.9718
402			mannose	GC/MS	584	C00159	HMDB00169	0.0403	0.1649
430		Glycolysis, gluconeogenesis, pyruvate metabolism	1,5-anhydroglucitol (1,5-AG)	GC/MS	20675	C07326	HMDB02712	0.5548	0.4131
432			glycerate	GC/MS	1572	C00258	HMDB00139	0.1548	0.4634
436			glucose	GC/MS	20488	C00293	HMDB00122	0.3029	0.4384
444			3-phosphoglycerate	GC/MS	1414	C00597	HMDB00807	0.3059	0.6315
450			pyruvate	GC/MS	599	C00022	HMDB00243	0.4391	0.2392
451			lactate	GC/MS	527	C00186	HMDB00190	0.3466	0.9505
463		Nucleotide sugars, pentose metabolism	threitol	GC/MS	35854	C16884	HMDB04136	0.1165	0.6685
480			arabinose	GC/MS	575	C00181	HMDB00646	0.0599	0.3859

483			xylose	GC/MS	15835	C00181	HMDB00098	0.1063	0.8267
491			citrate	GC/MS	1564	C00158	HMDB00094	0.6547	0.6418
500			alpha-ketoglutarate	GC/MS	33453	C00026	HMDB00208	0.5142	0.5710
501			succinate	GC/MS	1437	C00042	HMDB00254	0.1856	0.9019
502			succinylcarnitine	LC/MS pos	37058			0.1438	0.9271
504			fumarate	GC/MS	1643	C00122	HMDB00134	0.7652	0.4707
507	Energy	Krebs cycle	malate	GC/MS	1303	C00149	HMDB00156	0.0391	0.3967
508			oxaloacetate	GC/MS	35252	C00036	HMDB00223	0.6949	0.3560
510			acetylphosphate	GC/MS	15488	C00227	HMDB01494	0.0927	0.1210
511		Oxidative phosphorylation	phosphate	GC/MS	11438	C00009	HMDB01429	0.3211	0.1994
512			pyrophosphate (PPi)	GC/MS	2078	C00013	HMDB00250	0.1891	0.5469
512.5			linoleate (18:2n6)	LC/MS neg	1105	C01595	HMDB00673	0.7682	0.7085
516			linolenate [alpha or gamma; (18:3n3 or 6)]	LC/MS neg	34035	C06427	HMDB01388	0.9461	0.5141
517			dihomo-linolenate (20:3n3 or n6)	LC/MS neg	35718	C03242	HMDB02925	0.7007	0.6917
518		Essential fatty acid	eicosapentaenoate (EPA; 20:5n3)	LC/MS neg	18467	C06428	HMDB01999	0.7624	0.4712
519			docosapentaenoate (n3 DPA; 22:5n3)	LC/MS neg	32504	C16513	HMDB01976	0.3751	0.8930
520			docosapentaenoate (n6 DPA; 22:5n6)	LC/MS neg	37478	C06429	HMDB13123	0.7621	0.9600
521			docosahexaenoate (DHA; 22:6n3)	LC/MS neg	19323	C06429	HMDB02183	0.3600	0.9474
525			caproate (6:0)	LC/MS neg	32489	C01585	HMDB00535	0.9521	0.3943
526			heptanoate (7:0)	LC/MS neg	1644	C17714	HMDB00666	0.7261	0.4266
527			caprylate (8:0)	LC/MS neg	32492	C06423	HMDB00482	0.1309	0.2609
528		Medium chain fatty acid	pelargonate (9:0)	LC/MS neg	12035	C01601	HMDB00847	0.1613	0.9166
529			caprate (10:0)	LC/MS neg	1642	C01571	HMDB00511	0.0846	0.3541
530			undecanoate (11:0)	LC/MS neg	12067		HMDB00947	0.5550	0.7635
532			laurate (12:0)	LC/MS neg	1645	C02679	HMDB00638	0.5890	0.9480
533			5-dodecenoate (12:1n7)	LC/MS neg	33968		HMDB00529	0.8778	0.8268
535			myristate (14:0)	LC/MS neg	1365	C06424	HMDB00806	0.8935	0.8214
536			myristoleate (14:1n5)	LC/MS neg	32418	C08322	HMDB02000	0.9724	0.8216
537			pentadecanoate (15:0)	GC/MS	1361	C16537	HMDB00826	0.3042	0.3084
538			palmitate (16:0)	LC/MS neg	1336	C00249	HMDB00220	0.6613	0.6888
539			palmitoleate (16:1n7)	LC/MS neg	33447	C08362	HMDB03229	0.8542	0.6879
541			margarate (17:0)	LC/MS neg	1121		HMDB02259	0.6536	0.8285
542			10-heptadecenoate (17:1n7)	LC/MS neg	33971			0.8764	0.8806
543		Long chain fatty acid	stearate (18:0)	LC/MS neg	1358	C01530	HMDB00827	0.7956	0.8359
545			oleate (18:1n9)	LC/MS neg	1359	C00712	HMDB00207	0.6821	0.6317
552			stearidonate (18:4n3)	LC/MS neg	33969	C16300	HMDB06547	0.8244	0.7118
554			10-nonadecenoate (19:1n9)	LC/MS neg	33972			0.8642	0.8754
559			eicosenoate (20:1n9 or 11)	LC/MS neg	33587		HMDB02231	0.8121	0.4688
561			dihomo-linoleate (20:2n6)	LC/MS neg	17805	C16525		0.5942	0.8796
565			arachidonate (20:4n6)	LC/MS neg	1110	C00219	HMDB01043	0.3492	0.5287
569			docosadienoate (22:2n6)	LC/MS neg	32415	C16533		0.4470	0.4746
571			adrenate (22:4n6)	LC/MS neg	32980	C16527	HMDB02226	0.2385	0.9591
594		Fatty acid, monohydroxy	3-hydroxyoctanoate	LC/MS neg	22001		HMDB01954	0.5509	0.5146
602			2-hydroxystearate	LC/MS neg	17945	C03045		0.2663	0.9601
604			2-hydroxypalmitate	LC/MS neg	35675			0.6356	0.6455
610			2-hydroxyglutarate	GC/MS	37253	C02630	HMDB00606	0.2886	0.8328
616			azelate (nonanedioate)	LC/MS neg	18362	C08261	HMDB00784	0.5695	0.3440
617			dodecanedioate	LC/MS neg	32388	C02678	HMDB00623	0.9032	0.2109
618		Fatty acid, dicarboxylate	tetradecanedioate	LC/MS neg	35669		HMDB00872	0.2839	0.8029
619			hexadecanedioate	LC/MS neg	35678		HMDB00672	0.0568	0.6005
620			octadecanedioate	LC/MS neg	36754		HMDB00782	0.1449	0.4704
622			3-carboxy-4-methyl-5-propyl-2-furanpropanoate (CMPF)	LC/MS neg	31787			0.4614	0.3934
632.65		Fatty acid, branched	methyl palmitate (15 or 2)	LC/MS neg	38768			0.0045	0.3873
632.7			17-methylstearate	LC/MS neg	38296			0.7361	0.9754
683		Fatty acid metabolism (also	propionylcarnitine	LC/MS pos	32452	C03017	HMDB00824	0.5445	0.9620

685	Lipid	BCAA metabolism)	butyrylcarnitine	LC/MS pos	32412	C02862	HMDB02013	0.4105	0.3262
689		Fatty acid metabolism	isovalerate	LC/MS neg	34732	C08262	HMDB00718	0.3983	0.9205
691		Carnitine metabolism	deoxycarnitine	LC/MS pos	36747	C01181	HMDB01161	0.9535	0.2169
692			carnitine	LC/MS pos	15500	C00487	HMDB00062	0.6641	0.2499
693			3-dehydrocarnitine*	LC/MS pos	32654	C02636	HMDB12154	0.9160	0.7743
694			acetylcarnitine	LC/MS pos	32198	C02571	HMDB00201	0.9495	0.4166
696			hexanoylcarnitine	LC/MS pos	32328	C01585	HMDB00705	0.3508	0.8426
698			octanoylcarnitine	LC/MS pos	33936	C02838	HMDB00791	0.2684	0.6043
700			decanoylcarnitine	LC/MS pos	33941	C03299	HMDB00651	0.3632	0.3629
701.5			cis-4-decenoyl carnitine	LC/MS pos	38178			0.5578	0.4604
702			laurylcarnitine	LC/MS pos	34534		HMDB02250	0.1741	0.2557
705			palmitoylcarnitine	LC/MS pos	22189	C02990	HMDB00222	0.5745	0.2505
707			oleoylcarnitine	LC/MS pos	35160		HMDB05065	0.5822	0.8518
709		Bile acid metabolism	cholate	LC/MS neg	22842	C00695	HMDB00619	0.3594	0.9987
712			glycocholate	LC/MS neg	18476	C01921	HMDB00138	0.7774	0.5188
719			deoxycholate	LC/MS neg	1114	C04483	HMDB00626	0.2636	0.8695
723			glycochenodeoxycholate	LC/MS neg	32346	C05466	HMDB00637	0.7799	0.6578
725			glycolithocholate sulfate*	LC/MS neg	32620	C11301	HMDB02639	0.1897	0.7785
727			tauro lithocholate 3-sulfate	LC/MS neg	36850	C03642	HMDB02580	0.9284	0.4337
741			glycochenate sulfate*	LC/MS neg	32599			0.8451	0.5513
742			taurochenate sulfate*	LC/MS neg	32807			0.2133	0.3040
742.1			glycoursodeoxycholate	LC/MS neg	39379		HMDB00708	0.9403	0.6737
750		Glycerolipid metabolism	glycerol	GC/MS	15122	C00116	HMDB00131	0.5552	0.7552
751			choline	LC/MS pos	15506	C00114	HMDB00097	0.6618	0.3496
752			glycerol 3-phosphate (G3P)	GC/MS	15365	C00093	HMDB00126	0.1960	0.6379
753			glycerophosphorylcholine (GPC)	LC/MS pos	15990	C00670	HMDB00086	0.2295	0.7966
760		Inositol metabolism	myo-inositol	GC/MS	19934	C00137	HMDB00211	0.9573	0.8610
761			chiro-inositol	GC/MS	37112			0.2569	0.8320
769		Ketone bodies	3-hydroxybutyrate (BHBA)	GC/MS	542	C01089	HMDB00357	0.2980	0.5416
771			1,2-propanediol	GC/MS	38002	C00717.C02912.C00583.C01506.C02917	HMDB01881	0.9966	0.8412
775		Lysolipid	1-palmitoylglycerophosphoethanolamine	LC/MS neg	35631		HMDB11503	0.3341	0.4953
776			2-palmitoylglycerophosphoethanolamine*	LC/MS neg	35688			0.4160	0.6591
780			1-stearoylglycerophosphoethanolamine	LC/MS neg	34416		HMDB11130	0.2123	0.9103
781			1-oleoylglycerophosphoethanolamine	LC/MS neg	35628		HMDB11506	0.1014	0.8832
782			2-oleoylglycerophosphoethanolamine*	LC/MS neg	35687			0.5255	0.3438
783			1-linoleoylglycerophosphoethanolamine*	LC/MS neg	32635		HMDB11507	0.1848	0.7754
784			2-linoleoylglycerophosphoethanolamine*	LC/MS neg	36593			0.2086	0.5761
785			1-arachidonoylglycerophosphoethanolamine*	LC/MS neg	35186		HMDB11517	0.4125	0.9407
786			2-arachidonoylglycerophosphoethanolamine*	LC/MS neg	32815			0.7576	0.3346
791			1-myristoylglycerophosphocholine	LC/MS pos	35626		HMDB10379	0.8213	0.2310
793			1-pentadecanoylglycerophosphocholine*	LC/MS pos	37418			0.3997	0.0142
794			1-palmitoylglycerophosphocholine	LC/MS pos	33955			0.1798	0.1368
795			2-palmitoylglycerophosphocholine*	LC/MS pos	35253			0.6198	0.3514
796			1-palmitoleoylglycerophosphocholine*	LC/MS pos	33230			0.9632	0.6232
798			1-heptadecanoylglycerophosphocholine	LC/MS pos	33957		HMDB12108	0.7068	0.4604
799			1-stearoylglycerophosphocholine	LC/MS pos	33961			0.3368	0.1491
800			2-stearoylglycerophosphocholine*	LC/MS pos	35255			0.4747	0.6119
801			1-oleoylglycerophosphocholine	LC/MS pos	33960			0.5362	0.1938
802			2-oleoylglycerophosphocholine*	LC/MS pos	35254			0.9242	0.8482
803			1-linoleoylglycerophosphocholine	LC/MS pos	34419	C04100		0.0834	0.0194
804			2-linoleoylglycerophosphocholine*	LC/MS neg	38087			0.1441	0.8527
806			1-eicosadienoylglycerophosphocholine*	LC/MS pos	33871			0.6331	0.4013
807			1-eicosatrienoylglycerophosphocholine*	LC/MS pos	33821			0.3641	0.5863
809			1-arachidonoylglycerophosphocholine*	LC/MS pos	33228	C05208		0.7989	0.3767
810			2-arachidonoylglycerophosphocholine*	LC/MS pos	35256			0.0035	0.0143
811			1-docosapentaenoylglycerophosphocholine*	LC/MS pos	37231			0.6429	0.3310
813			1-docosahexaenoylglycerophosphocholine*	LC/MS pos	33822			0.8332	0.7376
818			1-stearoylglycerophosphoinositol	LC/MS neg	19324			0.3272	0.6597
821			1-arachidonoylglycerophosphoinositol*	LC/MS neg	34214			0.2258	0.3443
825.5			1-palmitoylplasmenylethanolamine*	LC/MS neg	39270			0.2408	0.1363

864		Sphingolipid	palmitoyl sphingomyelin	GC/MS	37506			0.6271	0.6529
865			stearyl sphingomyelin	GC/MS	19503	C00550	HMDB01348	0.6340	0.5647
881		Sterol/Steroid	cholesterol	GC/MS	63	C00187	HMDB00067	0.1719	0.7538
891			dehydroisoandrosterone sulfate (DHEA-S)	LC/MS neg	32425	C04555	HMDB01032	0.2637	0.7153
892			epiandrosterone sulfate	LC/MS neg	33973	C07635	HMDB00365	0.9708	0.5426
895			androstosterone sulfate	LC/MS neg	31591	C00523	HMDB02759	0.4674	0.9342
912			cortisol	LC/MS pos	1712	C00735	HMDB00063	0.1519	0.0172
914			cortisone	LC/MS pos	1769	C00762	HMDB02802	0.3326	0.7336
934			7-alpha-hydroxy-3-oxo-4-cholestenoate (7-Hoca)	LC/MS neg	36776	C17337	HMDB12458	0.1507	0.0307
935			4-androsten-3beta,17beta-diol disulfate 1*	LC/MS neg	37202		HMDB03818	0.4169	0.5788
936			4-androsten-3beta,17beta-diol disulfate 2*	LC/MS neg	37203		HMDB03818	0.6962	0.1373
937			5alpha-androstan-3beta,17beta-diol disulfate	LC/MS neg	37190		HMDB00493	0.4365	0.7424
938			5alpha-pregnan-3beta,20alpha-diol disulfate	LC/MS neg	37198			0.6946	0.2313
940			pregnen-diol disulfate*	LC/MS neg	32562	C05484	HMDB04025	0.4167	0.1645
941			pregn steroid monosulfate*	LC/MS neg	32619	C18044	HMDB00774	0.8218	0.1556
942			andro steroid monosulfate 1*	LC/MS neg	32827	C04555	HMDB02759	0.4867	0.0019
943			andro steroid monosulfate 2*	LC/MS neg	32792	C04555	HMDB02759	0.8246	0.8564
944			21-hydroxypregnenolone disulfate	LC/MS neg	37173	C05485	HMDB04026	0.4763	0.1523
946.4			pregnenolone sulfate	LC/MS neg	38170		HMDB00774	0.7701	0.4721
950	Nucleotide		Purine metabolism, (hypo)xanthine/inosine containing	hypoxanthine	GC/MS	3127	C00262	HMDB00157	0.9266
958		Purine metabolism, adenine containing	N1-methyladenosine	LC/MS pos	15650	C02494	HMDB03331	0.6718	0.9425
994		Purine metabolism, urate metabolism	urate	LC/MS neg	1604	C00366	HMDB00289	0.5912	0.6914
995			allantoin	GC/MS	1107	C02350	HMDB00462	0.5811	0.7090
1024		Pyrimidine metabolism, uracil containing	uridine	LC/MS neg	606	C00299	HMDB00296	0.4419	0.9065
1025			pseudouridine	LC/MS neg	33442	C02067	HMDB00767	0.8546	0.0953
1036		Purine and pyrimidine metabolism	methylphosphate	GC/MS	37070			0.1963	0.3040
1042	Cofactors and vitamins	Ascorbate and aldarate metabolism	threonate	GC/MS	27738	C01620	HMDB00943	0.4325	0.8531
1055		Hemoglobin and porphyrin	heme*	LC/MS pos	32593	C00032	HMDB03178	0.9972	0.5354
1056		Hemoglobin and porphyrin metabolism	bilirubin (Z,Z)	LC/MS neg	27716	C00486	HMDB00054	0.1036	0.8803
1057			bilirubin (E,E)*	LC/MS pos	32586			0.0002	0.5194
1059			biliverdin	LC/MS neg	2137	C00500	HMDB01008	0.4405	0.4311
1063		Nicotinate and nicotinamide metabolism	nicotinamide	LC/MS pos	594	C00153	HMDB01406	0.5907	0.9073
1084			trigonelline (N'-methylnicotinate)	LC/MS pos	32401	C01004	HMDB00875	0.4790	0.7500
1085		Pantothenate and CoA metabolism	pantothenate	LC/MS pos	1508	C00864	HMDB00210	0.3307	0.6305
1105		Tocopherol metabolism	alpha-tocopherol	GC/MS	1561	C02477	HMDB01893	0.9622	0.6682
1106			beta-tocopherol	GC/MS	35702	C14152	HMDB06335	0.8902	0.7042
1108			gamma-tocopherol	GC/MS	33420	C02483	HMDB01492	0.2469	0.0848
1117		Vitamin B6 metabolism	pyridoxate	LC/MS neg	31555	C00847	HMDB00017	0.8019	0.3829
1123	Benzoate metabolism	hippurate	LC/MS neg	15753	C01586	HMDB00714	0.3676	0.9387	
1127		4-hydroxyhippurate	LC/MS neg	35527			0.2969	0.7926	
1134		catechol sulfate	LC/MS neg	35320	C00090		0.4287	0.4776	
1135		benzoate	GC/MS	15778	C00180	HMDB01870	0.1790	0.7350	
1147		4-ethylphenylsulfate	LC/MS neg	36099	C13637		0.9064	0.4160	
1148		4-vinylphenol sulfate	LC/MS neg	36098	C05627	HMDB04072	0.0002	0.3965	
1151		Chemical	glycolate (hydroxyacetate)	GC/MS	15737	C00160	HMDB00115	0.7324	0.9966
1152	iminodiacetate (IDA)		GC/MS	21025		HMDB11753	0.5378	0.2340	
1157	glycerol 2-phosphate		GC/MS	27728	C02979.D01488	HMDB02520	0.1901	0.5172	
1191.5	Isobar: 2-propylpentanoic acid, 2-ethylhexanoic acid		LC/MS neg	35490			0.8846	0.5770	
1212		salicylate	LC/MS neg	1515	C00805	HMDB01895	0.9799	0.3249	
1216		4-acetaminophen sulfate	LC/MS neg	37475	C06804	HMDB01859	0.7846	0.8057	

1217	Xenobiotics	Drug	4-acetamidophenol	GC/MS	12032	C06804	HMDB01859	0.7846	0.8057
1218			p-acetamidophenylglucuronide	LC/MS neg	33423		HMDB10316	0.7846	0.8058
1219			2-hydroxyacetaminophen sulfate*	LC/MS neg	33173			0.7846	0.8057
1220			2-methoxyacetaminophen sulfate*	LC/MS neg	33178			0.7846	0.8057
1221			3-(cystein-S-yl)acetaminophen*	LC/MS pos	34365			0.7846	0.8058
1222			2-methoxyacetaminophen glucuronide*	LC/MS pos	33161			0.7846	0.8058
1265.3		Drug	citalopram	LC/MS pos	38651	C07572	HMDB05038	0.8596	0.9915
1268.9			olanzapine	LC/MS pos	35971	C07322	HMDB05012	0.3647	0.3647
1269		EDTA	EDTA	LC/MS neg	32511	C00284		0.1903	0.8665
1272		Food component/Plant	tartarate	GC/MS	15336	C00898	HMDB00956	0.7028	0.4926
1286			quinat	GC/MS	18335	C00296	HMDB03072	0.6853	0.2794
1301			piperine	LC/MS pos	33935	C03882		0.6784	0.8327
1328			thymol sulfate	LC/MS neg	36095	C09908	HMDB01878	0.7195	0.0135
1341.04			stachydrine	LC/MS pos	34384	C10172	HMDB04827	0.2951	0.7620
1341.05			homostachydrine*	LC/MS pos	33009	C08283	HMDB04827	0.4564	0.0962
1343		Xanthine metabolism	caffeine	LC/MS pos	569	C07481	HMDB01847	0.5617	0.7568
1344			paraxanthine	LC/MS pos	18254	C13747	HMDB01860	0.7181	0.0771
1345			theobromine	LC/MS pos	18392	C07480	HMDB02825	0.4912	0.6404
1346			theophylline	LC/MS neg	18394	C07130	HMDB01889	0.5548	0.2235
1349			1,7-dimethylurate	LC/MS neg	34400	C16356	HMDB11103	0.2629	0.3429
1353			3-methylxanthine	LC/MS pos	32445	C16357	HMDB01886	0.8913	0.2391
1354			7-methylxanthine	LC/MS pos	34390	C16353	HMDB01991	0.9423	0.2887
1360		Sugar, sugar substitute, starch	erythritol	GC/MS	20699	C00503	HMDB02994	0.5261	0.5325