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Conflicts of interest:

63 CSW received grant support from Allergopharma, PLS Design as well as Zeller AG and 64 received speaker honoraria from Allergopharma. All authors have no conflict of interest 65 in relation to this work.

ABSTRACT

69 Background

Eicosanoid lipid mediators play key roles in type 2 immune responses, e.g. in allergy and asthma. Macrophages represent major producers of eicosanoids and they are key effector cells of type 2 immunity. We aimed to comprehensively track eicosanoid profiles during type 2 immune responses to house dust mite (HDM) or helminth infection and to identify mechanisms and functions of eicosanoid reprogramming in human macrophages.

77 Methods

We established an LC-MS/MS workflow for the quantification of 52 oxylipins to analyze mediator profiles in human monocyte derived macrophages (MDM) stimulated with HDM and during allergic airway inflammation (AAI) or nematode infection in mice. Expression of eicosanoid enzymes was studied by qPCR and western blot and cytokine production was assessed by multiplex assays.

84 Results

Short (24h) exposure of alveolar-like MDM (aMDM) to HDM suppressed 5-LOX expression and product formation, while triggering prostanoid (thromboxane and prostaglandin D₂ and E₂) production. This eicosanoid reprogramming was p38dependent, but Dectin-2-independent. HDM also induced pro-inflammatory cytokine production, but reduced granulocyte recruitment by aMDM. In contrast, high levels of cysteinyl leukotrienes (cysLTs) and 12-/15-LOX metabolites were produced in the airways during AAI or nematode infection in mice.

93 Conclusion

Our findings show that a short exposure to allergens as well as ongoing type 2 immune
responses are characterized by a fundamental reprogramming of the lipid mediator
metabolism with macrophages representing particularly plastic responder cells.
Targeting mediator reprogramming in airway macrophages may represent a viable
approach to prevent pathogenic lipid mediator profiles in allergy or asthma.

100 Word count: 3641

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2	100		
<u>з</u>	102	Key words	
- 5	103	Elcosanoids; house di	ust mite; LC-MS/MS; macrophages; type 2 inflammation
6	104		
7	105	Abbreviations:	
8	106	5-LOX	5-lipoxygenase
9	107	COX	cyclooxygenase
10	108	cysLTs	cysteinyl leukotrienes
11	109	IS	internal standard
12	110	LTA₄H	leukotriene A ₄ hydrolase
13	111	LTB ₄	leukotriene B ₄
14	112	LTC₄S	leukotriene C ₄ synthase
15	113	LC-MS/MS	liquid chromatography tandem mass spectrometry
16	114	MDM	monocyte derived macrophages
17	115	PGs	prostaglandins
18	116	PMN	polymorphnuclear leukocytes
19	117	SPM	specialized pro-resolving mediator
20	118		
21	119		
22	120	INTRODUCTION	
23	121		
24	122	Lipid mediators gove	ern immune responses in a multitude of infectious or chronic
25	123	inflammatory settings	(1) In alleray and asthma prostanoids and leukotrienes (ITs)
26	123	derived from the poly	unsaturated fatty acid (PLIFA) arachidonic acid ($\Delta \Delta$) drive hallmark
27	124	type 2 immune resr	consets such as eosinophil accumulation (2.3) $\Delta\Delta$ metabolites
28	125	(eicosanoids) have	also been suggested to contribute to type 2 immunity during
29	120	nometodo infection (4	also been suggested to contribute to type 2 initiality during
30	127		-o). Despite these important initiationological functions, lew studies
31	128	This is possibly due t	y assessed lipid mediator profiles during type 2 immune responses.
32	129	This is possibly due t	to the infilted availability of adequate LC-MS/MS worknows, which
33	130	are required for the s	simultaneous quantification of a multitude of structurally similar but
34	131	functionally distinct	mediators. Indeed, most immunological studies in allergy or
35	132	nematode infection	nave used immunoassays to quantity less than a handful of
36	133	mediators (5,7,8). How	wever, LC-MS/MS analysis of 18 elcosanoids in macrophages from
37	134	nematode infected m	ice suggested abundant and plastic eicosanoid production during
38	135	type 2 immune respon	nses (6). In addition, a number of studies have applied LC-MS/MS
39	136	approaches to quanti	ify up to 88 lipid mediators in ex vivo samples from allergy and
40	137	asthma patients (9–1	2). Moreover, using macrophages as a model system, targeted
41	138	lipidomics approaches	s were applied to quantify more than 100 eicosanoid metabolites
42	139	(13). Due to their	plasticity and abundant expression of eicosanoid biosynthetic
43	140	pathways, macrophag	ges present an attractive cellular model to study lipid mediator
44	141	production in immuno	plogical settings (14). In the context of inflammasome activation,
45	142	targeted lipidomics w	orkflows allowed for the characterization of an "eicosanoid storm"
40	143	during macrophage	activation (15,16). However, despite these recent advances in
4/	144	lipidomics technologie	es, information about the lipid mediator profiles in type 2 immune
48	145	responses remains so	carce.
49 50	146	Here, we have esta	ablished a targeted lipidomics workflow for the simultaneous
50	147	quantification of 52	oxylipins from several PUFAs (AA, LA, DHA). We applied this
50	148	workflow to demonst	trate that HDM exposure of human macrophages results in a
52	149	pronounced eicosand	bid reprogramming, characterized by high levels of prostanoids
54	150	(particularly thrombo	(xane) but low levels of 5-I OX products. This eicosanoid
55	151	reprogramming was o	dependent on p38 MAPK activation but independent of Dectin-2
56	152	We further show that	HDM-driven eicosanoid reprogramming occurs on the mRNA and
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protein level and is associated with the production of pro-inflammatory cytokines and chemokines. However, HDM-exposed macrophages showed a reduced chemotactic potential towards granulocytes, correlating with suppressed LTB₄ production. Together, these findings suggest that HDM induces a pro-inflammatory macrophage phenotype with impaired effector function. Finally, we quantified mediator profiles in bronchoalveolar lavage fluid (BALF) from HDM-sensitized and nematode-infected mice, thus revealing profound changes in COX- and LOX metabolites during type 2 immune responses in In summary, these data show that the AA metabolism is fundamentally vivo. reprogrammed during type 2 immune responses and suggest macrophage reprogramming as an attractive target in type 2 inflammation.

MATERIALS AND METHODS

Animal experiments were performed according to institutional guidelines and to Swiss federal and cantonal laws on animal protection.

Material

Eicosanoids, PUFAs and deuterated internal standards (IS) were purchased from Cayman Chemical (Ann Arbour, MI, USA). An analyte and IS working solution was prepared as shown in Table S1/S2. LC-grade solvents (2-propanol, Carl Roth (Karlsruhe, Germany), acetonitrile, Thermo Fisher Scientific (Waltham, MA, USA), methanol, Applichem (Darmstadt, Germany)) and ultrapure H₂O (supplied through a MilliQ system (Merck Millipore, Darmstadt, Germany)) were used for mobile phase preparation.

Isolation and culture of polymorphnuclear leukocytes (PMN) and peripheral blood mononuclear Cells (PBMC)

Written informed consent in accordance with the Declaration of Helsinki was obtained from healthy volunteers before blood collection, which had been approved by the local ethics committee at the Technical University of Munich. PMN and PBMC were isolated and cultured in medium containing 10% heat-inactivated FBS and monocytes were differentiated to aMDM as described previously (17,18). Supernatants were stored at -80°C in 50% MeOH for LC-MS/MS or undiluted for cytokine analysis.

Chemotaxis Assay

PMN were incubated for 30 min at 37°C with pooled conditioned medium of aMDM ± HDM ± indomethacin (100µM, Cayman Chemical) ± DBM-1285. 2x10⁵ PMN were transferred to transwells (3µm pore size, Corning, NY, USA) and allowed to migrate for 3h at 37°C towards conditioned medium containing chemoattractants: 2 ng/ml LTB₄, Cayman Chemical; 20ng/ml IL-8; 2ng/ml CCL5, both Miltenyi Biotec. Migrated PMN were counted microscopically.

In vivo model of N. brasiliensis infection

Mice were infected subcutaneously with 200 larvae of N. brasiliensis (Nb), and BALF was collected on day 5 post infection as previously described (19,20).

In vivo model of HDM-induced allergic airway inflammation

C57BL/6J mice were sensitized by bilateral intranasal (i.n.) instillations of extract from Dermatophagoides farinae ("HDM") (1µg in 20µl PBS; Stallergenes). Challenges were performed on days 8-11 with 10µg HDM extract. Three days after the final challenge,

BALF (600ul) was collected, equal volumes of methanol were added and samples were frozen immediately at -80°C until further processing.

Real-Time PCR

aMDM were lysed in RLT Buffer (Qiagen, Hilden, Germany) with 1% β-Mercaptoethanol (Merck Millipore,), followed by RNA extraction (Zymo Research, Irvine, CA, USA) and reverse transcription according to the manufacturer's instructions (Thermo Fisher Scientific). gPCR analysis was performed as described previously (primers shown in Table S3) (18).

Western blotting

Western blotting was performed similarly to previously published procedures (18). A detailed procedure can be found in the supplement.

Multiplex Cytokine Assay and ELISA

Multiplex cytokine assays were performed as detailed in the supplement.

Sample Preparation for LC-MS/MS

Samples for method validation were prepared as triplicates in medium/MeOH (1:1) or PBS/MeOH (1:1) with an analyte concentration of 0.1, 1 or 10ng/ml (10x higher concentrations for PUFAs (Table S1/S2)). Automated solid phase extractions were performed with a Microlab STAR robot (Hamilton, Bonaduz, Switzerland). Prior to extraction all samples were diluted with H₂O to a MeOH content of 15% and 10µl of IS stock solution was added. Samples were extracted using Strata-X 96-well plates (30 mg. Phenomenex, Aschaffenburg, Germany) and eluted with MeOH. Samples were evaporated to dryness under N₂ stream and redissolved in 100 μ l MeOH/H₂O (1:1).

LC-MS/MS lipid mediator analysis

Chromatographic separation of eicosanoids was achieved with a 1260 Series HPLC (Agilent, Waldbronn, Germany) using a Kinetex C18 reversed phase column (2.6µm, 100 x 2.1mm, Phenomenex) with a SecurityGuard Ultra Cartridge C18 (Phenomenex) precolumn. The Sciex QTRAP 5500 mass spectrometer (Sciex, Darmstadt, Germany), equipped with a Turbo-V[™] ion source, was operated in negative ionization mode. Identification of metabolites was achieved via retention time and scheduled multiple reaction monitoring (sMRM). Unique Q1/Q3 transitions were selected for each analyte by using single analyte injections and comparison with the literature (14). Analytes with identical MRM transitions were differentiated by retention time (Figure S1). A more detailed method description can be found in the supplement.

Data Analysis

All data were analyzed using GraphPad Prism 6 (GraphPad Software, La Jolla, CA, USA) or R 3.4.3 (21). For LC-MS/MS analysis all samples were normalized to their RNA content. Data were analyzed using Wilcoxon-, Friedman or Kruskal-Wallis test with respective post-hoc test as specified in the Figure legends and considered statistically significant if p<0.05.

RESULTS

Lipid mediators involved in type 2 immune responses can be detected with high accuracy, precision and recovery by LC-MS/MS

Quantification of lipid mediators in type 2 immune settings has resulted in discrepancies. depending on the analytical method (EIA or LC-MS/MS) (5,22). Thus, we compared these methods for leukotrienes, PGE_2 and TXB_2 in supernatants of human PMN and aMDM. Quantification by EIA showed higher variability, particularly for LTs: SD=8.48 (EIA) vs. SD=0.72 (LC-MS/MS) for LTB₄; SD=6.44 (EIA) vs. SD=0.23 (LC-MS/MS) for cysLTs. Levels obtained by EIA were also significantly higher as compared to LC-MS/MS and did not correspond well to AA-metabolizing enzymes (Figure 1A/ 4) (17). Thus, we established an LC-MS/MS workflow for the comprehensive and simultaneous quantification of PUFA metabolites involved in type 2 inflammation (Figure 1B/S1, Tables S4-S9). At 1ng/ml we could detect 36 metabolites according to FDA guidelines (accuracy: ±15%, RSD <20%) (Table S4). This included eicosanoids (LTs, TXB₂, PGD₂) as well as specialized pro-resolving mediators (SPMs) (resolvin E1/D1 (RvE1/RvD1) and protectin D1 (PDX)) (Figure 1C/D, Table S4). The recovery ranged from 69-127% for key lipid mediators of type 2 inflammation with a matrix effect in a similar range (Figure 1E, Table S5). Thus, at concentrations \geq 1ng/ml eicosanoid mediators of type 2 immunity (LTs, TXB₂, PGD₂) and several SPMs could be quantified with good accuracy, precision

Zymosan exposure reprograms the eicosanoid metabolism of myeloid cells

In order to validate our LC-MS/MS workflow in a well-characterized cellular model, we processed and analyzed culture supernatants from human PMN that were either left untreated or exposed for 24h to zymosan prepared from fungal cell walls. First, a pool of PMN supernatants was measured in three technical replicates (Figure 2A-E) and second, levels of eicosanoids produced by PMN from different individuals (n=5) were analyzed separately (Figure 2F). Untreated PMN produced mainly 5-LOX metabolites (5-HETE and LTB₄) at a concentration of around 1.4 ng/ml and low levels of cysLTs (Figure 2A). PMN preparations contained neutrophils and eosinophils and thus had the capacity to generate LTs and 15-LOX metabolites (Figure 2A/B). Treatment with zymosan resulted in reprogramming of the eicosanoid metabolism, characterized by reduced production of LTB₄, cysLTs and 5-HETE (p=0.06) (Figure 2A/D/E/F). In contrast, zymosan exposure triggered the formation of COX-metabolites with a five-fold increase in TXB₂ levels. Additionally, zymosan-exposed PMN released PGE₂ and PGF₂ α that were undetectable in unstimulated PMN (Figure 2C). Taken together, lipid mediator class-switching could be tracked by the developed LC-MS/MS workflow, allowing us to reveal previously reported as well as unprecedented zymosan-induced changes in the eicosanoid profile (23,24).

and recovery.

TGFβ1 induces a macrophage phenotype that resembles alveolar macrophages and resists IL-4 mediated regulation of eicosanoid pathways

Based on recent studies showing key roles for GM-CSF and TGF^{β1} in alveolar macrophage (AM) differentiation (25,26) we differentiated human monocytes into alveolar-like macrophages (aMDM) and characterized their eicosanoid profile. At baseline, aMDM expressed high levels of 5-LOX and its respective oxylipin products (Figure 3A/4). In addition, aMDM expressed higher levels of 5-LOX and IL-1ß as compared to MDM, suggesting that they adapted features of AM (Figure S2A/B) (27,28). IL-4 is known to reprogram the AA metabolism of macrophages by inducing 15-LOX, but suppressing 5-LOX and COX. We confirmed the IL-4-triggered induction of ALOX15 in MDM from most donors during differentiation in the absence of TGF β 1 (Figure S2C). However, IL-4 had no significant impact on the eicosanoid profile of aMDM (Figure 3A-C), suggesting that aMDM resist IL-4-driven induction of 15-LOX as well as suppression

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3	304	of COX and 5-LOX. Indeed, PTGS2, PTGES, ALOX5, and ALOX15 mRNA levels
4	305	remained unaffected by IL-4 (Figure 4A).
5	306	
6	307	HDM exposure decreases 5-LOX- but increases COX metabolism in human
7	308	alveolar-like macrophages via p38 MAPK
8	309	Next, we assessed the eicosanoid profile of aMDM during 24-96 h exposure to IL-4 and
9	310	HDM After 24h of HDM+II -4 exposure formation of 5-I OX products (I TB ₄ and 5-HETE)
10	311	was reduced (Figure 3A) Contrary to the effect on 5-LOX HDM+II -4 stimulation
11	312	resulted in an increase of prostanoids (Figure 3B/S3A). In line with the LC-MS/MS data
12	313	5-I OX mRNA levels were reduced by II -4+HDM while COX-2 was induced (Figure 4A)
13	314	At later time points, we observed only minor changes in eicosanoid concentrations with
14 1 <i>Г</i>	315	the exception of PGD, which decreased after prolonged HDM exposure (Figure S3A)
15	316	and 5-HETE and I TB, which initially decreased but increased back to control levels at
10	317	966 (Figure S3B/S3C)
17	318	In the absence of IL 4. HDM also triggered prostanoid production, while 5 LOX products
10	210	were reduced in aMDM from 5 out of 7 denors (Figure 2D/E)
20	220	To identify the mechanisme underlying this cicesenoid representation we first
20	520 221	noutralized Destin 2, which has been described as the major C type lestin resenter
27	221	neutralized Declin-z, which has been described as the major C-type lectin receptor recognizing UDM (7). However, blocking Declin 2 did not interfere with UDM triggered
23	322	recognizing HDM (7). However, blocking Declin-z did hot interfere with HDM-triggered
24	323 224	The finance of the second seco
25	324 225	ILR-4 or addition of polymyxin B to inactivate LPS did not affect mediator
26	325	reprogramming by HDM (Figure S4). As the MAP kinase p38 has been implicated in the
27	326	regulation of eleosanoid pathways (29), we assessed p38 phosphorylation in response
28	327	to HDM. Levels of phosphorylated p38 were increased in HDM-exposed as compared to
29	328	unstimulated aMDM and prostanoid formation was significantly reduced when
30	329	macrophages were co-incubated with HDM and a p38 inhibitor (Figure 3F/G). In
31	330	addition, p38 inhibition during HDM exposure restored 5-LOX product formation (Figure
32	331	3F, S5A).
33	332	We further examined the effect of HDM on mRNA and protein levels of COX and LOX
34	333	pathway enzymes. PTGES (mPGES1) and PTGS2 (COX-2) were increased, while
35	334	ALOX5 (5-LOX) expression was down-regulated by HDM on both transcript and protein
36	335	level (Figure 4B/C). M2 polarization markers were either significantly reduced (ALOX15)
37	336	or unaffected by HDM exposure (TGM2) (Figure 4A-C). Altogether, HDM-induced
38	337	eicosanoid reprogramming likely occurred as a result of profound changes in the
39	338	expression of eicosanoid pathway genes.
40	339	

HDM exposure triggers the production of proinflammatory cytokines and chemokines

To study whether allergen-driven eicosanoid reprogramming was associated with an altered cytokine profile, we performed multiplex bead assays of supernatants from aMDM. We observed a significant increase in proinflammatory cytokines (IL-6, IL-1β, TNFα and IL-12 p70) after 24h HDM exposure (Figure 5A/B). HDM also triggered the release of chemokines (CXCL9/10, IL-8) involved in granulocyte recruitment (Figure 5A). Similar to the effects on eicosanoid reprogramming, p38 inhibition reduced the HDM-induced production of IL-6 and TNF α , while Dectin-2 neutralization did not affect the expression of these cytokines (Figure 5C/D, S5B).

HDM-exposed macrophages have a reduced capacity to recruit granulocytes

The recruitment of inflammatory neutrophils and eosinophils is a hallmark response of asthma. Thus, we addressed the functional consequence of HDM-driven mediator reprogramming by performing chemotaxis assays with human PMN. Migration of PMN

towards a chemoattractant mixture was diminished if PMN were exposed to supernatant from aMDM stimulated with HDM as compared to supernatant from unstimulated aMDM (Figure 5E). Addition of the COX inhibitor indomethacin during HDM stimulation did not affect the HDM-triggered reduction in chemotaxis, suggesting that prostanoids were not responsible for this effect. In contrast, p38 inhibition could restore PMN chemotaxis, correlating with increased LTB₄ levels (Figure S5A, S5C) and reduced IL-6, TNFα concentrations (Figure S5B).

Taken together, HDM-exposure induced a pro-inflammatory macrophage phenotype characterized by abundant production of bronchoconstrictive thromboxane and TNF α , but low production of LTB₄ and impaired chemotactic potential.

Distinct eicosanoid profiles are induced during the type 2 immune response to HDM or nematode infection in the airways

To assess whether eicosanoid reprogramming is a general feature of type 2 immune responses, we characterized lipid mediator profiles in the airways of HDM-sensitized or nematode-infected mice. When comparing eicosanoid profiles after sensitization and challenge with HDM or infection with the lung-migrating nematode Nippostrongylus brasiliensis (Nb), we observed an abundant formation of prostanoids in BALF from Nb-infected, but not from HDM-sensitized mice (p < 0.05 for all COX metabolites) (Figure 6A). In addition, no prostanoids could be detected in the BALF of naïve mice (Figure 6A). In contrast, cysLTs were detectable in the airways of Nb-infected as well as of HDM-sensitized mice (Figure 6B). Moreover, high levels of 12-/15-LOX metabolites (particularly 12-HETE and 13-HODE) were produced in the airways of Nb-infected and HDM-sensitized mice (Figure 6B/C). LA-derived metabolites (9-/13-HODE, 9.10-/11.13-DiHOME) were synthesized in similar quantities as compared to AA-metabolites in the airways after challenge with HDM or infection with Nb with a tendency for higher levels in Nb-infected mice (p=0.025 for 9,10 DiHOME, p=0.124 for 9-HODE). Finally, BALF from Nb-infected mice also contained detectable levels of SPMs (17-HDHA and RvD2) (Figure 6D). Thus, lipid mediator reprogramming occurs during the type 2 immune response to HDM or nematode parasites in the airways in vivo with partially distinct profiles. The induction of the COX- and simultaneous suppression of the 5-LOX pathway may represent an early response of macrophages in type 2 immune settings, which then governs the ensuing type 2 immune response to allergens or helminth infection.

DISCUSSION

Eicosanoid lipid mediators play central roles in type 2 immune responses, particularly in allergic inflammation. Thus, the comprehensive assessment of eicosanoid profiles in settings of type 2 inflammation can provide important information about the ensuing immune response and the functional plasticity of the cell types involved. Here, we describe an LC-MS/MS workflow, which allowed us to characterize eicosanoid reprogramming in two distinct settings of type 2 inflammation. First, we show that the lipid mediator metabolism of human alveolar-like macrophages (aMDM) is highly responsive to allergen-driven reprogramming. Second, we describe profound changes in lipid mediator profiles during the type 2 immune response to HDM or nematode infection in vivo.

Using a newly developed LC-MS/MS workflow, up to 52 oxylipins could be quantified in cell culture supernatants and biological samples from the airways. To our knowledge, this represents one of the largest oxylipin panels that has been validated and applied in the context of type 2 immune responses. This workflow, allowed for the sensitive and reliable quantification of central eicosanoid mediators of type 2 inflammation (e.g. LTs, TXB_2 , PGD₂), whilst the accuracy should be improved for other mediators (e.g. PGE₂).

- To initially validate the LC-MS/MS workflow, we studied zymosan-triggered eicosanoid reprogramming in human PMN. At baseline, stimulation with Ca²⁺ ionophore resulted in the release of 5-HETE and LTs, which is consistent with previous studies (30,31). In keeping with the literature, zymosan induced a shift in the eicosanoid metabolism. characterized by higher amounts of prostanoids (32). Previous studies largely focused on the acute effects of zymosan or HDM and showed that both stimuli could trigger LT production by myeloid cells, when applied for short times (2-60 min) (7,33). Here, we focused on the prolonged exposure to TLR2/ Dectin ligands (zymosan and HDM) as they are involved in the initiation of type 2 inflammation (2,8,34). Lipid mediator class-switching from 5-LOX to COX metabolites occurred for both stimuli, thus suggesting that lipid mediator reprogramming during type 2 inflammation happens analogous to settings of type 1 inflammation (16).
- The induction of prostanoids and suppression of 5-LOX metabolites appears to be a common feature of macrophages in type 2 immune settings in response to allergens, IL-4 or nematode infection. Indeed, the reduced production of 5-LOX metabolites could be a result of high levels of IL-4 produced by T_{H2} cells, ILC2s and/ or basophils (35) as IL-4 is known to suppress 5-LOX expression in various cell types, including macrophages (17,36). In a model of filarial nematode infection, eicosanoid reprogramming in nematode-elicited macrophages was shown to depend on IL-4 receptor signaling (6). In line with this study, we confirmed the induction of prostanoids for two a different nematode parasites, thus suggesting that activation of the COX pathway is a general feature of the immune response to nematodes. Recently, soluble egg antigen of a distinct helminth species (the trematode Schistosoma mansoni) was reported to induce PGE₂, which contributed to T_{H2} polarization (37). This suggests an important functional role of prostanoids during the type 2 immune response to helminth infection.
- The plasticity of macrophages and their extraordinary capacity to produce lipid mediators suggests that these cells are key drivers of eicosanoid reprogramming in type 2 immunity. During allergen-triggered type 2 immune responses in the airways, the macrophage pool consists of resident alveolar macrophages (AMs) and macrophages derived from recruited monocytes (38). We used aMDM (differentiated in the presence of GM-CSF and TGF β 1) as a cellular model to mimic this mixed macrophage population. Although aMDM may not fully recapitulate macrophages in the lung, these cells showed several typical features of AMs, including high baseline expression of LT-biosynthetic enzymes and of the pro-inflammatory cytokine IL-1 β (27,28).
- We particularly focused on HDM extract as a trigger of type 2 inflammation with well-established functional roles for lipid mediators (2,8,39). Exposure of aMDM to HDM for 24-96h resulted in a dynamic mediator class switching of LOX and COX metabolites. While the production of regulatory mediators (e.g. PGE₂) peaked after 48h of HDM exposure, pro-inflammatory 5-LOX metabolites were initially suppressed, but increased back to baseline over time. This may explain why cysLTs were formed in the airways of HDM-sensitized mice during a two-week model of allergic airway inflammation. However, in addition to macrophages, other cell types including eosinophils and airway epithelial cells can contribute to the formation of LOX metabolites (including 5-LOX-derived cysLTs and 12/15-LOX derived HETEs and HODEs) during HDM-triggered airway inflammation in vivo (18). Nevertheless, macrophages likely represent a major source of lipid mediators during the initial exposure to HDM as they are abundant in the airways and highly express or readily upregulate LOX and COX enzymes.
- Given the production of several neutrophil-chemotactic factors by HDM-exposed aMDM. we hypothesized that aMDM would show an increased potential to trigger granulocyte chemotaxis after HDM exposure. However, in line with previous in vivo studies, secretions from HDM-exposed aMDM rather tended to decrease granulocyte chemotaxis

(40). This may result in impaired host defense and thus increased susceptibility to infections, which is a common complication in asthmatic patients (41). To address the functional contribution of COX metabolites, we studied granulocyte chemotaxis in the presence of secretions from HDM-exposed aMDM, which had been treated with the COX inhibitor indomethacin. However, COX inhibition did not affect the chemotactic potential of HDM-exposed aMDM. Instead, a p38 inhibitor restored LTB₄ production and neutrophil chemotaxis, suggesting that the reduced production of the neutrophil chemoattractant LTB₄ by HDM-exposed aMDM may contribute to the impaired chemotactic potential.

Contrary to previous reports of Dectin-2 as an essential HDM receptor (7,8), we did not observe a reduction of HDM-triggered prostanoid or cytokine production when neutralizing Dectin-2. However, this may be due to the timing of Dectin-2 ligation as previous studies were focused on acute responses (20-60 minutes) after HDM exposure. Indeed, while the initial response might depend on Dectin-2, other mechanisms likely drive mediator reprogramming during longer exposure. Our results suggest that p38 activation by a Dectin-2 and TLR-2/-4-independent mechanism contributed to eicosanoid and cytokine reprogramming in macrophages.

Taken together, HDM exposure induced a potentially pathogenic macrophage phenotype, characterized by abundant production of prostanoids (particularly TXB₂) and pro-inflammatory cytokines (particularly TNFα). Given that several of the HDM-triggered macrophage mediators are implicated in severe, steroid-resistant airway inflammation, mediator reprogramming in macrophages should be explored as a therapeutic target in therapy-resistant allergy and asthma.

FIGURE LEGENDS

Figure 1. Lipid mediators involved in type 2 immune responses can be detected with high accuracy, precision and recovery by LC-MS/MS.

A Levels of major bioactive eicosanoids (mean + SD) in supernatants from PMN (n=5) or MDM (n=11-30) quantified by EIA or LC-MS/MS; B Sample preparation workflow; C Accuracy (%) at three different concentrations for key eicosanoids, shown as mean + SD. Dotted lines: ± 15% range; D Precision calculated as relative standard deviation (RSD) (%), shown as mean. Dotted lines: 15% and 20% RSD; E Recovery at 1 ng/ml. Dotted lines: ± 15% range. Samples in C-E were extracted and measured in triplicates on the same day. Statistical significance was determined using Wilcoxon test.

Figure 2. Zymosan triggers eicosanoid reprogramming in human granulocytes.

A Heatmap of LC-MS/MS data for human PMN (pool of n=6 donors) ± zymosan, analyzed as three technical replicates. B Neutrophil (left) or eosinophils (right) stained for 5-LOX and LTA4H or LTC4S and 15-LOX, respectively. Blue: DAPI (nuclei). C-E Levels of COX metabolites (C), leukotrienes (D) and HETEs (E) produced by PMN, presented as mean + SD (pool of n=6, measured in triplicates). F Levels of prostaglandins, leukotrienes and HETEs produced after 24h ± zymosan (n=5). Statistical significance was determined using Wilcoxon test.

Figure 3. House dust mite extract triggers COX- but suppresses 5-LOX metabolism in human alveolar-like macrophages via p38 MAPK.

A-C LC-MS/MS data for 5-LOX (A), COX (B), or 15-LOX (C) metabolites of aMDM, stimulated or not with 10ng/ml IL-4 ± 10µg/ml HDM (n=4); D-F LC-MS/MS data for COX

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3	508	(D) or 5/15-LOX metabolites (E), (n=7) and of aMDM pre-incubated with p38 inhibitor or
4	509	Dectin-2 neutralizing antibody (F) before HDM exposure (n=5). G Representative WB for
5	510	total and phosphorylated p38 in aMDM (n=3). Data are shown as mean + SD; statistical
6	511	significance was determined using Kruskal-Wallis test with Dunn's correction (A-C, F) or
7	512	Wilcoxon test (D).
8	513	
9	514	Figure 4 HDM-driven eicosanoid reprogramming occurs on the mRNA and protein
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11	516	A and B relative gene expression of aMDM stimulated or not with 10ng/ml II -4 + 10
12	517	μ and \mathbf{B} relative gene expression of ambin stimulated of not with rong/mine- μ role u/mine- μ (A) or with 10µg/mi HDM (n=9) (B) for 24 h; C protein levels
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14	510	and representative we images (lower panels) of an approximative we images (lower panels) of a MDM + 10ug/ml HDM for 24b (n=5.7). Data are shown as mean + SD: Statistical
15	520	awiDivi \pm 10µy/iii 11Divi 101 2411 (11–5-7). Data are shown as mean \pm 5D, Statistical significance was determined using Wilcoven test
10	520	significance was determined using wilcoxon test.
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18	522	Figure 5. HDM exposure triggers the production of pro-inflammatory cytokines
19	523	and chemokines, but reduces the granulocyte-chemotactic potential of human
20	524	macrophages.
21	525	A Overview of cytokine levels [ng/ml], B INF α , IL-12 p70 and IL-27 (mean + SD) for
22	526	aMDM from 10 different blood donors \pm 10µg/ml HDM for 24h; C concentration (n=10)
25	527	and D gene expression (n=6) of IL-6 and TNF α in aMDM pre-incubated with p38 inhibitor
24	528	VX702 or Dectin-2 neutralizing antibody before HDM exposure; E Percentage of
25	529	granulocytes migrating towards supernatants (SN) of aMDM \pm 10µg/ml HDM \pm 100µM
20	530	indomethacin (n=7). Statistical significance was determined using Wilcoxon test (B-D) or
27	531	Friedman test with Dunn's correction (E).
20	532	
29	533	Figure 6. Distinct eicosanoid profiles are induced during the type 2 immune
30	534	response to HDM or nematode infection in the airways.
37	535	A-D LC-MS/MS analysis of prostanoids (A), LOX-metabolites of AA (B), LA metabolites
32	536	(C) and SPMs (D) in BALF from HDM-sensitized or Nb-infected mice (n=3-6),
34	537	representative data from two independent experiments are presented as mean + SD.
35	538	Dotted lines represent levels for naïve mice. Statistical significance was determined
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- med experiments: FDRH, AF, MH, DT, TF, PH, MRJ, FA; Analyzed data: FDRH, H, DT, JEvB; Designed the study: JEvB, JA, NLH, CBSW; Wrote the manuscript: FDRH, AF.
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Figure 1. Lipid mediators involved in type 2 immune responses can be detected with high accuracy, precision and recovery by LC-MS/MS.

A Levels of major bioactive eicosanoids (mean + SD) in supernatants from PMN (n=5) or MDM (n=11-30) quantified by EIA or LC-MS/MS; B Sample preparation workflow; C Accuracy (%) at three different concentrations for key eicosanoids, shown as mean + SD. Dotted lines: ± 15% range; D Precision calculated as relative standard deviation (RSD) (%), shown as mean. Dotted lines: 15% and 20% RSD; E Recovery at 1 ng/ml. Dotted lines: ± 15% range. Samples in C-E were extracted and measured in triplicates on the same day. Statistical significance was determined using Wilcoxon test.



Figure 2. Zymosan triggers eicosanoid reprogramming in human granulocytes.
A Heatmap of LC-MS/MS data for human PMN (pool of n=6 donors) ± zymosan, analyzed as three technical replicates. B Neutrophil (left) or eosinophils (right) stained for 5-LOX and LTA4H or LTC4S and 15-LOX, respectively. Blue: DAPI (nuclei). C-E Levels of COX metabolites (C), leukotrienes (D) and HETEs (E) produced by PMN, presented as mean + SD (pool of n=6, measured in triplicates). F Levels of prostaglandins, leukotrienes and HETEs produced after 24h ± zymosan (n=5). Statistical significance was determined using Wilcoxon test.

186x138mm (300 x 300 DPI)



Figure 3. House dust mite extract triggers COX- but suppresses 5-LOX metabolism in human alveolar-like macrophages via p38 MAPK.

A-C LC-MS/MS data for 5-LOX (A), COX (B), or 15-LOX (C) metabolites of aMDM, stimulated or not with 10ng/ml IL-4 \pm 10µg/ml HDM (n=4); D-F LC-MS/MS data for COX (D) or 5/15-LOX metabolites (E), (n=7) and of aMDM pre-incubated with p38 inhibitor or Dectin-2 neutralizing antibody (F) before HDM exposure (n=5). G Representative WB for total and phosphorylated p38 in aMDM (n=3). Data are shown as mean + SD; statistical significance was determined using Kruskal-Wallis test with Dunn's correction (A-C, F) or Wilcoxon test (D).



Figure 4. HDM-driven eicosanoid reprogramming occurs on the mRNA and protein level. A and B relative gene expression of aMDM stimulated or not with 10ng/ml IL-4 \pm 10 µg/ml HDM, (n=7) (A) or with 10µg/ml HDM (n=9) (B) for 24 h; C protein levels normalized to β-actin (upper panels) and representative WB images (lower panels) of aMDM \pm 10µg/ml HDM for 24h (n=5-7). Data are shown as mean + SD; Statistical significance was determined using Wilcoxon test.



Figure 5. HDM exposure triggers the production of pro-inflammatory cytokines and chemokines, but reduces the granulocyte-chemotactic potential of human macrophages.

A Overview of cytokine levels [ng/ml], B TNFa, IL-12 p70 and IL-27 (mean + SD) for aMDM from 10 different blood donors \pm 10µg/ml HDM for 24h; C concentration (n=10) and D gene expression (n=6) of IL-6 and TNFa in aMDM pre-incubated with p38 inhibitor VX702 or Dectin-2 neutralizing antibody before HDM exposure; E Percentage of granulocytes migrating towards supernatants (SN) of aMDM \pm 10µg/ml HDM \pm 100µM indomethacin (n=7). Statistical significance was determined using Wilcoxon test (B-D) or Friedman test with Dunn's correction (E).





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Figure 6. Distinct eicosanoid profiles are induced during the type 2 immune response to HDM or nematode infection in the airways.

A-D LC-MS/MS analysis of prostanoids (A), LOX-metabolites of AA (B), LA metabolites (C) and SPMs (D) in BALF from HDM-sensitized or Nb-infected mice (n=3-6), representative data from two independent experiments are presented as mean + SD. Dotted lines represent levels for naïve mice. Statistical significance was determined using Kruskal-Wallis test with Dunn's correction

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Allergy





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5-LOX

*<u>p=.0</u>2

5-HETE

ctrl - SN HDM - SN

HDM+DBM-1285 - SN

ctrl

HDM

HDM+DBM-1285



Α

1

2 1

0

500-

400-

300

200

100

0

HDM

DBM-1285

+

-

В

conc. [ng/ml]

***<u>p=.</u>0002

T

PGE₂

IL-6

*<u>p=.03</u>

*<u>*p<.</u>01

conc. [ng/ml]

prostanoids

****p=.0005

PGD₂

30.

20

10

ctrl 0

+ + + + - +

p<u>=.0</u>69

****p<u>=.0</u>001

*p<u>=.0</u>3

L 10

 TXB_2

n.d. ctrl

TNFα

*<u>p=.03</u>

25.

20

15

5

C

С

% of migrated cells

150

100

50

0

LTB₄

House dust mite drives pro-inflammatory eicosanoid reprogramming and macrophage effector functions

House dust mite reprograms eicosanoid metabolism

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*these authors contributed equally

Supplemental material

Methods

Isolation and culture of polymorphnuclear leukocytes (PMN) and peripheral blood mononuclear Cells (PBMC)

PMN and PBMC were isolated and cultured as previously described in medium containing 10 % heat-inactivated FBS (18,19). PMN were stimulated with 50 µg/ml zymosan (24h), followed by 5 µM Ca²⁺-ionophore A23187 (10 min) (both Sigma Aldrich, Darmstadt, Germany). Monocytes were differentiated to aMDM as described previously (17,18). Cells were stimulated with *Dermatophagoides farinae* (HDM, 10 µg/ml, Stallergenes, Antony, France; LPS content: 549 EU/ml, determined by Pierce endotoxin quantification kit, Thermo Fisher Scientific), IL-4 (10 ng/ml, Miltenyi Biotec) or both for 24 h. A p38 inhibitor (VX-702, 1 µM, Cayman or DBM-1285 dihydrochloride, 1 µM, Tocris Bioscience, Bristol, UK), a Dectin-2-neutralizing antibody (10 µg/ml, Invivogen, Toulouse, France), anti-TLR2 (10 µg/ml, Invivogen) or anti-TLR4 antibody (10 µg/ml, Invivogen) was added 2h before or polymyxin B (5 µg/ml, Sigma Aldrich) concurrently with HDM stimulation. Before harvest aMDM were stimulated with Ca²⁺-ionophore A23187 in the same manner as PMN. Supernatants were stored at -80°C in 50% MeOH for LC-MS/MS or undiluted for cytokine analysis.

Multiplex Cytokine Assay and ELISA

Multiplex cytokine assays (Magnetic Luminex Assay for Eotaxin (CCL11), GRO α (CXCL1), GRO β (CXCL2), IL-1 β , IL-18, IL-6, TARC (CCL17), IP-10 (CXCL10), IL-8 (CXCL8), IL-10, IL-27, TNF α , RANTES (CCL5), ITAC-1 (CXCL11), MIG (CXCL9), IL-12 p70, IL-33, R&D Systems, Minneapolis, MN, USA) were performed according to the manufacturer's instructions on a Bio Plex 200 System (Bio-Rad, Munich, Germany). ELISAs for human TNF α (R&D Systems) or IL-6 (BD Biosciences, San Diego, CA, USA) or eicosanoid EIAs (LTB₄, cysLTs, PGE₂, TXB₂; Cayman Chemical) were performed according to the manufacturers' instructions.

LC-MS/MS lipid mediator analysis

Lipid mediators were eluted with a gradient consisting of mobile phase A H_2O /acetonitrile/acetic acid (70:30:0.01, v/v/v) and mobile phase B 2-propanol. After 1 min of 100% A, the solvent was decreased to 33% within 1.5 min, held isocratic for 7.5 min. Over 2 min B was increased to 100% and held for 2.5 min. The flow-rate was set to 450 µl/min and reduced to 400 µl/min, when the gradient had reached 100% 2-propanol.

After every scheduled measurement, a 5 min clean-run was performed, ramping from acetonitrile/2-propanol (3:1) over 3.5 min to H₂O/acetonitrile/acetic acid (70:30:0.01, v/v/v), which was maintained for another 1.5 min, at an overall flow rate of 300 µl/min.

The column oven was operated at 40 °C. Samples (20 μ I) were injected by an HTC PAL auto-sampler (CTC Analytics, Zwingen, Switzerland), set to 4 °C. Mass spectrometric parameters were set to: curtain gas 40 psi, ionspray voltage -4000 V, source temperature 500 °C, ion source gas 1 with 50 psi and ion source gas 2 with 40 psi. Declustering potential (DP), collision energy (CE), and cell exit potential (CXP) were optimized for each sMRM. sMRMs were measured within a 90 s time window.

The calibration curve for each analyte was obtained using an analyte stock solution at concentrations of 0.005, 0.01, 0.05, 0.1, 0.5, 1, 5, 10, and 50 ng/ml with constant levels of IS (1 ng/ml), prepared in medium/MeOH (1:1) and extracted as specified for the samples. Concentrations for PUFAs were 10x higher. The area under the curve of each metabolites was linked to its respective internal standard area to obtain the area ratio. Analyte individual calibration curves were obtained by plotting the area ratio against concentrations.

Acquisition of LC-MS/MS data was performed using Analyst Software 1.6.3 followed by quantification with MultiQuant Software 3.0.2 (both Sciex, Darmstadt, Germany).

Method Validation

To calculate the recovery for each metabolite, the analyte response of medium samples, medium:MeOH (1:1, v/v) spiked with analyte mix and extracted as a sample, was compared to not extracted samples, the same concentration of analyte mix spiked into MeOH:H₂O (1:1, v/v), without being extracted. The pure matrix effect was calculated as the ratio between the metabolite response in medium and the response in PBS (PBS:MeOH (1:1, v/v) spiked with analyte mix and extracted) extracted samples. Recovery and matrix effects for each analyte x were calculated as follows for each analyte:

$$Recovery (\%) = \frac{area \ ratio_{medium} (x)}{area \ ratio_{not \ extracted \ sample} (x)}$$
$$Matrix \ effect (\%) = \frac{area \ ratio_{medium} (x)}{area \ ratio_{PBS} (x)}$$

Accuracy and precision were determined by extracting the calibration curve 4-times. The accuracy was calculated as the ratio between the measured concentration in the samples and the theoretical concentration.

$$Accuracy (\%) = \frac{Concentration \ measured(x)}{Concentration \ spiked(x)}$$

The precision, estimating the variance of the extraction, was calculated as the relative standard deviation (RSD):

$$RSD(\%) = \frac{sd_c(x)}{mean_c(x)}$$

The limit of detection (LOD), the smallest concentration that can be distinguished from zero, was determined as signal to noise ratio (S/N) > 3 and the lower limit of quantification (LLOQ) was defined by a precision <20% of the quadruplicate calibration curve.

Stability

The analyte stability at 4 °C was obtained by consecutive measurements after 24 h and 48 h of the same sample, left in the autosampler. The reproducibility of the measurement was assessed by comparing calibration curves for extracted samples analyzed on three different days.

Linearity

The linearity of the method for each analyte was determined by calculating the Pearson correlation coefficient (R-value) of the calibration curve. R-values of all analytes were greater than 0.995.

Inter-day variability

Inter-day precision and accuracy were assessed on three consecutive days. Precision varied between 2% and 61% at 0.1 ng/ml, while at higher concentrations only 2 metabolites showed an RSD >20%. Inter-day accuracy varied between 85% - 230% over all concentration levels.

Tables (legends see below)

- 1) Analyte stock solutions with MRM parameters
- 2) Internal standard stock solution with MRM parameters
- 3) Primers for qPCR
- 4) Intraday accuracy and precision (RSD)
- 5) Recovery and Matrix Effect
- 6) 48 h Stability at 4 °C
- 7) Interday variability of precision and accuracy
- 8) LOD/LOQ + Linearity
- 9) LC-MS/MS data comparison ("Frankfurt vs. Munich panel")

Western blotting

Cells were lysed in RIPA buffer (Thermo Scientific, Waltham, MA, USA) supplemented with protease inhibitor (cOmplete tablets EDTA free, EASYpack, Roche Diagnostics, Mannheim, Germany) and phosphatase inhibitor (PhosSTOP tablets, EASYpack, Roche Diagnostics) in concentrations as indicated by the manufacturer. Protein concentration was assessed by Pierce BCA Protein Assay kit (Thermo Fisher Scientific) and lysates diluted to equal concentrations in deionized H₂O. Samples were heated under reducing conditions and run on Bolt 4-12% Bis-Tris Plus 12-well gels (Invitrogen, Thermo Fisher Scientific) for 60 minutes with constant voltage at 125 V using a Mini Gel Tank system (GE Healthcare Life Technologies, Freiburg, Germany). Western blotting was performed on an Immobilon-P Transfer membrane (Merck Chemical, Darmstadt, Germany) followed by blocking in 5% nonfat milk (AppliChem, Darmstadt, Germany) in TRISbuffered saline with 0.5% Tween-20 (TBS-T, EMD Millipore, Billerica, MA, USA). Primary antibodies (goat-anti-COX2: Cayman Chemical, Ann Arbor, MI, USA, rabbit-anti-5-LOX, a kind gift or Dr. Olof P. Rådmark, Karolinska Institutet, Stockholm, Sweden rabbit-anti-TGM2: Cell Signaling, Danvers, MA, USA, mouse-anti- β -actin: Sigma Aldrich, Darmstadt. Germany) were diluted in 5% non-fat milk and membranes were incubated overnight. After washing in TBS-T, membranes were incubated in appropriate dilutions of the secondary HRP-linked antibody (goat-anti-rabbit IgG, goat-anti-mouse IgG, Santa Cruz, Dallas, TX, USA or donkey-anti-goat IgG, Novus Biologicals, Abingdon, United Kingdom) and detection was performed by using SuperSignal West Femto Maximum

Sensitivity Substrate (Thermo Scientific) or Amersham ECL Prime Western Blotting Detection Reagent (GE Healthcare Life Technologies) on an Intas ECL Chemocam Imager (Intas Science Imaging Instruments, Goettingen, Germany). Recorded images were analysed using LabImage 1D software (Kapelan Bio-Imaging, Leipzig, Germany).

Sample preparation and LC-MS/MS lipid mediator analysis ("Frankfurt panel")

Quantification of HETE and LTB4 was done in principle as described previously.¹ For analysis of 5-HETE, 12-HETE, 15-HETE and LTB₄, 150 - 200 µl supernatant were spiked with the corresponding deuterated internal standards and extracted by liquid-liquid-extraction using ethyl acetate. Analytes were separated using a Gemini NX C18 RP-LC-column (150 mm × 2 mm I.D., 5 µm particle size and 110 Å pore size from Phenomenex, Aschaffenburg, Germany) under gradient conditions with H₂O and acetonitrile as mobile phases, both containing 0.01% ammonia solution. The LC system was coupled to a mass spectrometer 5500 QTrap (Sciex, Darmstadt, Germany) equipped with a Turbo-V-source operating in negative electrospray ionization mode. Data Acquisition was done using Analyst Software V 1.6 and quantification was performed with MultiQuant Software V 3.0 (Sciex) employing the internal standard method (isotope dilution mass spectrometry).

For the analysis of prostanoids, 200 µl supernatant were spiked with isotopically labeled internal standards (PGE₂-d4, PGD₂-d4, TXB₂-d4, PGF₂α-d4, 6-keto PGF₁α-d4), 100 µl EDTA solution (0.15M) and 600 µl ethyl acetate. Samples were vortexed and centrifuged at 20,000 g for 5 min. The organic phase was removed, and the extraction was repeated with 600 µl ethyl acetate. The organic fractions were evaporated at a temperature of 45°C under a gentle stream of nitrogen. The residues were reconstituted with 50 µl of acetonitrile/H₂O/formic acid (20:80:0.0025, v/v/v) and transferred to glass vials.

The LC-MS/MS analysis was carried out using an Agilent 1290 Infinity LC system (Agilent, Waldbronn, Germany) coupled to a hybrid triple guadrupole linear ion trap mass spectrometer QTRAP 6500+ (Sciex) equipped with a Turbo-V-source operating in negative ESI mode. The chromatographic separation was conducted using a Synergi Hydro-RP column (150 × 2 mm, 4 µm particle size and 80 Å pore size; Phenomenex). A gradient program was employed at a flow rate of 300 µl/min. Mobile phase A was H₂O/formic acid (100:0.0025, v/v) and mobile phase B was acetonitrile/formic acid (100:0.0025, v/v). The analytes were separated under gradient conditions within 16 min. The injection volume was 10 µl. The gradient program started with 90% A for 1 min, then mobile phase A was decreased to 60% within 1 min, held for 1 min, further decreased to 50% within 1 min and held for 2 min. Within 2 min, mobile phase A was further decreased to 10% and held for 1 min. Within 1 min, the initial conditions were restored and the column was re-equilibrated for 6 min. Mass spectrometric parameters were set as follows: lonspray voltage -4500 V, source temperature 500 °C, curtain gas 40 psi, nebulizer gas 40 psi and Turbo heater gas 60 psi. Both quadrupoles were running at unit resolution.

For analysis and quantification, Analyst Software 1.6 and Multiquant Software 3.0 (both Sciex) were used, employing the internal standard method (isotope dilution mass spectrometry). Calibration curves were constructed using linear regression with $1/x^2$ weighting.

Supplemental figure and table legends

Fig S1. LC-MS/MS spectrum of the 52 metabolites as labeled in the figure at a concentration of 1 ng/ml (10 ng/ml for PUFAs)

Fig S2. Gene expression profile and effect of IL-4 in MDM differentiated in the absence of TGF β 1 A Gene expression profile of MDM differentiated in the presence of GM-CSF ± TGF- β 1 (n=7) B Gene expression of *ALOX5* and *IL1B* normalized to *GAPDH* expression of MDM differentiated ± TGF- β 1 C Gene expression normalized to *GAPDH* expression of MDM differentiated with GM-CSF for 6 days then stimulated with 10 ng/ml IL-4 for 24h (n=6). Data are presented as mean + SD. Statistical significance was determined using Wilcoxon test.

Fig S3. Time course of eicosanoid production by human aMDM during stimulation with IL-4 or HDM+IL-4

A – **C** Time course of prostanoids (A), 5-HETE, 5-oxoETE and 15-HETE (B), leukotrienes (C) in supernatants of aMDM stimulated or not with 10 ng/ml IL-4 +/- 10 μ g/ml HDM for 24h, 48h, 72h or 96h (n=7)

Fig S4. Mediator reprogramming by HDM does not depend on LPS or TLR2/4 signaling.

A-B COX and 5-LOX products (A), IL-6 and TNF α (B) formed by aMDM ± HDM ± antiTLR4 (10 µg/ml)/TLR2 (10 µg/ml) or polymyxin B (5 µg/ml) (n=5). Data shown as mean + SD. Statistical significance was determined using Friedmann test with Dunn's post test.

Fig S5. p38 MAPK mediates eicosanoid reprogramming, cytokine induction and the chemotaxic potential of human aMDM. A-B COX and 5-LOX products (A), IL-6 and TNF α (B) from aMDM ± 10 µg/ml HDM ± 1 µmol/l DBM-1285 (n=6), C Percentage of granulocytes migrating towards pooled supernatants (SN) of aMDM ± 10 µg/ml HDM ± 1 µmol/l DBM (n=5). Data are presented as mean + SD. Statistical significance was determined using Friedmann test with Dunn's post test (A) or Wilcoxon test (B).

Table S1. Analyte stock solutions with MRM parameters; DP: declustering potential, CE: collision energy and CXP: collision cell exit potential

 Table
 S2.
 Internal
 standard
 stock
 solutions
 with
 MRM
 parameters;
 DP:

 declustering potential, CE: collision energy and CXP: collision cell exit potential

 Table S3. Forward and reverse primers for qPCR

Table S4. Intraday accuracy and precision (RSD) of the LC-MS/MS panel at different concentration levels (0.1, 1 and 10 ng/ml, 10x higher for PUFAs, n = 3)

 Table S5. Recovery and matrix effect at a concentration of 1 ng/ml

Table S6. Accuracy und precision/RSD after 48h at 4°C at a concentration of 0.1, 1 and 10 ng/ml; PUFAs are 10x higher concentrated

Table S7: Inter-3-day variability of accuracy and precision/RSD at 0.1, 1 and 10 ng/ml; PUFAs are 10x higher concentrated

Table S8. Limit of detection (LOD) and lower limit of quantitation (LLOQ) with correlation coefficient

Table S9. Comparison of Frankfurt and Munich LC-MS/MS panel; shown is mean ± SD of 6 different blood donors

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Allergy

Metabolite	Stock Concentration (ng/µl)	Q1 (m/z)	Q3 (m/z)	RT (min)	DP (V)	CE (V)	CXP (V
±11-HDHA	1	343.418	149.1	9.4	-20	-18	-13
±11,12-DHET	1	337.14	167.1	6.7	-60	-24	-17
±12,13-DiHOME	1	313.083	183	6	-70	-30	-9
±13-HDHA	1	343.273	192.9	8.9	-10	-18	-9
±17-HDHA	1	343.258	244.9	8.6	-100	-16	-11
±18-HEPE	1	317.208	259.2	6.9	-65	-14	-7
±4-HDHA	1	343.27	101	11	-25	-18	-7
±5,6-DHET	1	337.149	144.9	7.6	-80	-24	-13
±8(9)-DHET	1	337.328	126.9	7	-80	-26	-23
±9-HETE	1	319.19	167.2	9.7	-10	-20	-15
±9,10-DiHOME	1	313.186	201.1	6.2	-85	-28	-11
11(12)-EET	1	319.19	167	11.4	-65	-20	-27
11(S)-HETE	1	319.091	166.7	9.1	-95	-20	-11
12-oxo-ETE	1	317.112	153.2	9.2	-90	-20	-19
12(S)-HEPE	1	317.113	178.8	7.6	-35	-18	-25
12(S)-HETE	1	319.3	178.7	9.4	-70	-18	-7
13(S)-HODE	1	295.065	195.1	8.2	-15	-24	-21
14(15)-EET	1	319,152	218.8	10.4	-60	-16	-29
14(15)-EpETE	1	317 139	207	86	-50	-18	-21
15-0x0-ETE	1	317 124	113.2	8.5	-120	-22	-13
15(S)-HEPE	1	317 235	218.9	72	-75	-16	-17
15(S)-HETE	1	319 152	218.9	8.6	-35	-18	-39
15(S)-HETrE	1	321 322	221.0	9.0	-15	-20	11
15(S)-HnETE	1	335.079	112.0	10	-55		_9
20-HETE	1	319.09	245	74		-20	
5-0X0ETE	1	317 025	203	10.8	-65	_22	_35
5(6)-FET	1	319.025	191.2	10.0	-05	_14	
5(0)-LLT 5(0)-HEPE	1	317 266	115.1	8	-25	-14	_15
5(S)-HETE	1	310 101	114.0	10.3	-35	-10	
5(S) HnETE	1	335.064	166.0	0.5	-15	-10	15
6 keto PCE.a	1	360.004	162.0	3.0 1.4	-5	-22	10
$\frac{0-ke_{1}0-r}{8}$ is a PCE of	1	353.16	102.9	25	125	-30	10
8/0) EET	1	310 152	155	2.5	130	-50	-13
	1	205.001	171.2	8.4	-130	-10	-13
	1	335 12	105	5.4	-110	-22	-23
	1	624 207	272.1	5.0	-90	-20	-25
	1	405 204	177	5.5	-100	-30	-11
	1	495.204	222	5.5	-05	-20	-21
	1	430.207	114.0	0.4	-70	-24	-39
	1	250.14	177.0	4.5	-50	-20	-1
	1	250.14	177.2	5.5	-120	-20	-23
	1	309.14	153.1	0.0	-95	-20	-13 11
		301.122	271.1	4.1	-60	-22	-11
		351.05/	2/1.3	3.8	-20	-20	-9
		353.143	193.1	3.4	-130	-34	-21
		3/5.143	141	4.5	-/5	-20	-13
		349.087	161	1.6	-90	-26	-19
	1	369.082	169.1	2.5	-45	-24	-21
AA	10	303.153	258.9	12.7	-95	-26	-13
ALA	10	277.155	233.2	12.4	-140	-18	-11

 Table S1: Analyte stock with MRM parameters: DP: declustering potential, CE: collision energy and CXP: collision cell exit potential

DGLA	10	305.156	261.2	12.8	-110	-22	-9
DHA	10	327.122	283.2	12.6	-110	-14	-11
EPA	10	300.992	257	12.4	-5	-16	-25

Table S2: Internal standard stock with MRM parameters; DP: declustering potential, CE: collision energy and CXP: collision cell exit potential

Metabolite	Stock Concentration (ng/µl)	Q1 (m/z)	Q3 (m/z)	RT (min)	DP (V)	CE (V)	CXP (V)
11,12-DHET- d11	1	348.22	167.1	6.6	-80	-26	-9
8,9-DHET-d11	1	348.143	127	7	-85	-28	-13
11(12)-EET-d11	1	330.226	167	11.3	-70	-18	-13
12(S)-HETE-d8	1	327.119	184.5	9.2	-90	-20	-9
13(S)-HODE-d4	1	299.174	198.1	8.1	-95	-24	-11
15(S)-HETE-d8	1	327.122	226.1	8.4	-30	-18	-9
5-oxoETE-d7	1	324.177	210.2	10.8	-85	-24	-13
5(S)-HETE-d8	1	327.167	116	10.1	-15	-18	-7
6-keto-PGF₁α- d4	1	373.079	167	1.4	-70	-36	-7
LTB ₄ -d4	1	339.12	197	5.6	-90	-20	-25
PGD ₂ -d4	1	355.105	275.3	4.1	-70	-22	-5
PGE ₂ -d4	1	355.173	275.2	3.8	-75	-24	-11
PGF ₂ α-d4	1	357.212	313.3	3.4	-65	-24	-35
RvD1-d5	1	380.196	141	4.5	-155	-20	-17
TXB ₂ -d4	1	373.201	173	2.5	-60	-22	-21
AA-d8	10	311.09	267 🧹	12.6	-95	-26	-13
EPA-d5	10	306.246	262.2	12.4	-30	-16	-9
Table S3: Forward and reverse primers for gPCR							

Table S3: Forward and reverse primers for qPCR

Gene	Forward Primer (5' - 3')	Reverse Primer (5' - 3')
PTGS2	GCTGGAACATGGAATTACCCA	CTTTCTGTACTGCGGGTGGAA
PTGES	TCAAGATGTACGTGGTGGCC	GAAAGGAGTAGACGAAGCCCAG
ALOX5	GATTGTCCCCATTGCCATCC	AGAAGGTGGGTGATGGTCTG
ALOX15	GGACACTTGATGGCTGAGGT	GTATCGCAGGTGGGGAATTA
TGM2	AGGCCCGTTTTCCACTAAGA	AGCAAAATGAAGTGGCCCAG
GAPDH	GAAGGTGAAGGTCGGAGT	GAAGATGGTGATGGGATTTC

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7 8	Concentration	0.1 n	g/ml	1 ng	g/ml	10 n	g/ml
9	Metabolites	Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%)
10	±11-HDHA	95.26	45.26	121.52	15.42	121.64	11.70
11	+11.12-DHET	114.55	27.47	109.46	14.02	91.52	14.20
12	+12 13-DiHOME	NA	NA	103.87	13.80	104.35	18.12
13	+13-HDHA	NA	NA	97.90	13 64	103 55	13 31
14	+17-HDHA	140.48	53 19	110 44	2 88	112 29	10.95
15	+18-HEPE	NA	NA	104 93	11 59	101 24	13.04
16	+4-HDHA	155.63	24 76	117 30	19.22	105.17	13.64
1/	+5 6-DHFT	88.30	39.87	106 59	7 23	95.46	12 44
18	+8(9)-DHFT	NA	NA	95.82	14 73	97 25	13 44
19	+9-HFTF	96.67	37.91	126.38	14 66	121 23	12 55
20	+9 10-DiHOME	85.87	48.05	88.60	14 29	79.86	19 75
21	11(12)-FFT	47.85	80.53	106.23	12.94	96.02	11 55
22	11(S)-HETE	119 79	23 47	100.35	16.36	88.94	14 76
23	12-0x0-ETE	276.86	5.94	59.81	26.45	26.17	79.04
24	12(S)-HEPE	59.16	98.57	101.71	14.13	96.00	13.21
25	12(S)-HETE	89.67	59.94	109.23	17.98	107.72	19.02
26	13(S)-HODE	60.27	99.28	104.32	15.45	98.05	13.79
27	14(15)-EET	NA	NA	82.28	19.54	96.21	11.39
28	14(15)-EpETE	NA	NA	53.59	47.43	99.43	9.59
29	15-oxo-ETE	52.15	41.83	101.50	9.74	96.07	14.01
30	15(S)-HEPE	18.35	114.94	101.20	13.46	100.33	11.79
31	15(S)-HETE	177.23	11.66	98.63	14.42	96.39	11.67
32	15(S)-HETrE	NA	NA	93.77	20.74	93.86	16.04
33	15(S)-HpETE	NA	NA	NA	NA	26.82	78.79
24 25	20-HETE	NA	NA	156.46	15.19	119.87	27.95
35 26	5-oxoETE	144.23	31.83	107.97	9.11	91.01	10.92
20 27	5(6)-EET	33.03	74.44	108.11	87.12	61.18	101.98
27 20	5(S)-HEPE	66.64	61.40	122.21	6.17	112.28	14.19
20	5(S)-HETE	83.48	83.86	102.42	18.91	91.63	13.80
39	5(S)-HpETE	NA	NA	NA	NA	NA	NA
40	6-keto-PGF _{1alpha}	124.16	3.38	143.04	7.08	119.90	10.69
41	8-iso-PGF _{2alpha}	51.63	21.08	109.82	21.53	110.56	13.18
42	8(9)-EET	79.63	10.88	102.80	12.53	100.64	10.85
45	9(S)-HODE	85.22	59.03	106.24	15.77	92.55	14.48
45	LTB ₄	106.03	22.11	98.02	10.87	86.87	14.46
46	LTC ₄	368.38	6.49	128.66	15.88	96.54	5.73
47	LTD ₄	29.24	99.62	105.70	12.55	97.71	7.24
48	LTE ₄	50.86	97.21	110.17	13.90	95.36	9.43
49	LXA4	42.19	73.46	82.47	13.64	79.78	23.44
50	MAR1	NA	NA	205.38	15.28	138.94	27.30
51	PDX	NA	NA	100.06	16.37	111.18	11.09
52	PGD ₂	NA	NA	106.52	18.57	104.22	5.07
53	PGE ₂	34.18	74.36	67.28	12.89	58.92	11.58
54	PGF _{2alpha}	NA	NA	99.12	12.34	97.98	13.33
55	RvD1	59.84	46.36	98.35	13.55	91.53	15.97
56	RvE1	179.13	12.56	117.52	18.10	119.98	7.01
57	TXB ₂	125.72	26.63	112.53	2.47	97.03	4.52
58	AA	NA	NA	1108.92	39.52	142.08	47.69
59	ALA	NA	NA	82.53	NA	97.06	27.37
60	DGLA	NA	NA	400.56	19.18	117.29	24.34
	DHA	NA NA	NA	2938.46	44.93	188.60	73.28

NANA59.36NA82.43e S4: Accuracy and precision (RSD) at different concentration levels; 0.1, 1 and 10 nidual extractions
idual extractions

 Allergy

Table S5: Recovery and matrix effect at 1 ng/ml (n=3 separate extractions)

$\begin{array}{c c c c c c c c c c c c c c c c c c c $	4		·	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	5	Metabolite	Recovery ± SD	Matrix Effect ± SD
7 $\pm 11,12-DHET$ 118.03 ± 24.79 96.27 ± 20.22 8 $\pm 12,13-DiHOME$ 158.56 ± 51.27 112.19 ± 36.28 9 $\pm 13-HDHA$ 111.10 ± 22.94 94.03 ± 19.42 10 $\pm 17-HDHA$ 138.36 ± 24.21 108.51 ± 18.99 11 $\pm 16-HEPE$ 128.26 ± 26.47 93.40 ± 19.28 12 $\pm 4-HDHA$ 251.65 ± 42.3 210.88 ± 45.45 13 $\pm 5.6-DHET$ 144.45 ± 34.95 96.86 ± 23.44 14 $\pm 8(9)$ -DHET 126.03 ± 29.79 101.01 ± 23.73 15 $\pm 9-HETE$ 166.06 ± 25.38 112.10 ± 17.13 16 ± 9.10 -DiHOME 130.83 ± 46.62 87.33 ± 31.11 17 11(2)-EET 116.46 ± 22.45 92.55 ± 17.84 18 11(5)-HETE 126.72 ± 28.44 105.81 ± 22.31 19 12-cox-6FE 6.76 ± 2.71 27.40 ± 15.01 20 12(S)-HETE 181.59 ± 34.31 131.64 ± 24.87 21 12(S)-HETE 109.40 ± 22.31 86.10 ± 17.56 22 13(S)-HODE 105.21 ± 23.5 1002.76 ± 20.5 <	6	±11-HDHA	148.75 ± 34.92	108.16 ± 25.39
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	7	±11,12-DHET	118.03 ± 24.79	96.27 ± 20.22
9 ± 13 -HDHA 111.10 ± 22.94 94.03 ± 19.42 10 ± 17 -HDHA 138.36 ± 24.21 108.51 ± 18.99 11 ± 18 -HEPE 128.26 ± 26.47 93.40 ± 19.28 12 ± 4 -HDHA 251.65 ± 54.23 210.88 ± 45.45 13 ± 5.6 -DHET 144.45 ± 34.95 96.86 ± 23.44 14 $\pm 8(9)$ -DHET 126.83 ± 29.79 101.01 ± 23.73 15 ± 9 -HETE 166.06 ± 25.38 112.10 ± 17.13 16 ± 9.10 -DiHOME 130.83 ± 46.62 87.33 ± 31.11 17 11(12)-EET 116.46 ± 22.45 92.55 ± 17.84 18 11(S)-HETE 122.57 ± 25.84 105.81 ± 22.31 19 12-0x0-ETE 6.76 ± 2.71 27.40 ± 15.01 20 12(S)-HEPE 146.17 ± 38.96 113.53 ± 30.26 21 12(S)-HETE 181.59 ± 34.31 131.64 ± 24.87 23 13(S)-HODE 105.21 ± 23.16 103.45 ± 22.77 24 14(15)-EET 109.40 ± 22.31 86.10 ± 17.56 25 15-0x0-ETE 121.15 ± 27.23 98.26 ± 22.08 26 15(S)-HETE 134.76 ± 31.85 106.58 ± 25.01 27 15(S)-HETE 123.92 ± 22.27 88.43 ± 15.89 28 15(S)-HETE 134.76 ± 31.85 105.58 ± 25.01 29 15(S)-HETE 134.76 ± 31.85 105.58 ± 25.01 29 15(S)-HETE 134.76 ± 31.85 105.58 ± 25.01 29 15(S)-HETE 134.76 ± 31.85 99.73 ± 21.47 30 20-HETE 141.68 ± 35.5 96.21 ± 24.17 31 5-0x0ETE 68.73 ± 18.59 89.37 ± 24.17 32 5(G)-HETE 190.21 ± 40.9 116.98 ± 25.15 33 5(S)-HETE 190.21 ± 40.9 116.98 ± 25.15 35 5(S)-HETE 190.21 ± 40.9 116.98 ± 25.15 35 5(S)-HETE 190.21 ± 40.9 116.98 ± 25.15 35 5(S)-HETE 133.69 ± 9.45 192.92 ± 27.43 36 6-keto-PGF ₁ a 136.13 ± 26.51 92.98 ± 18.11 37 8-iso-PGF ₂ a 312.89 ± 66.64 160.30 ± 3.414 38 8(9)-EET 118.49 ± 27.25 92.00 ± 21.16 39 9(S)-HODE 132.93 ± 29.76 102.12 ± 22.86 40 LTB ₆ 120.93 ± 31.32 91.29 ± 23.64 41 LTC ₄ 81.91 ± 24.97 75.42 ± 22.92 42 LTD ₄ 88.45 ± 25.56 76.89 ± 22.22 43 LTE ₄ 82.85 ± 23.48 79.60 ± 22.52 44 LXA4 131.71 ± 27.76 99.16 ± 20.93 45 MAR1 13.83.66 ± 37.96 96.30 ± 27.55 46 PDX 133.66 ± 37.96 96.30 ± 27.55 47 PGD ₂ 68.76 ± 22.87 82.16 ± 27.33 48 PGE ₂ 123.64 ± 20.24 92.57 ± 15.16 49 PGF ₂ a 123.64 ± 20.24 92.57 ± 15.16 49 PGF ₂ a 123	8	±12,13-DiHOME	158.56 ± 51.27	112.19 ± 36.28
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	9	±13-HDHA	111.10 ± 22.94	94.03 ± 19.42
11 ± 12 ± 4 -HDHA 251.65 \pm 26.47 93.40 \pm 19.28 12 ± 4 -HDHA 251.65 \pm 54.23 210.88 \pm 45.45 13 ± 5.6 -DHET 144.45 \pm 34.95 96.86 \pm 23.44 14 \pm (9)-DHET 126.83 \pm 29.79 101.01 \pm 23.73 15 ± 9.16 -DiHOME 130.83 \pm 46.62 87.33 \pm 31.11 17 11(12)-EET 116.46 \pm 22.45 92.55 \pm 17.84 18 11(S)-HETE 122.57 \pm 25.84 105.81 \pm 22.31 19 12-cox-ETE 6.76 \pm 2.71 27.40 \pm 15.01 20 12(S)-HETE 181.59 \pm 34.31 131.64 \pm 24.87 21 12(S)-HETE 199.40 \pm 22.31 86.10 \pm 17.56 24 14(15)-EET 102.87 \pm 32.5 102.76 \pm 20.5 24 14(15)-EET 109.40 \pm 22.31 86.10 \pm 17.56 25 15-cox-ETE 121.15 \pm 27.23 98.26 \pm 22.08 26 15(S)-HETE 123.92 \pm 22.27 88.43 \pm 15.89 28 15(S)-HETE 123.92 \pm 22.27 88.43 \pm 15.89 28 15(S)-HETE 123.47 \pm 31.85 100.82 \pm 26.17 </td <td>10</td> <td>±17-HDHA</td> <td>138.36 ± 24.21</td> <td>108.51 ± 18.99</td>	10	±17-HDHA	138.36 ± 24.21	108.51 ± 18.99
12 ± 4 -HDHA 251.65 ± 54.23 210.88 ± 45.45 13 ± 5.6 -DHET 144.45 ± 34.95 96.86 ± 23.44 14 $\pm 8(9)$ -DHET 126.83 ± 29.79 101.01 ± 23.73 15 ± 9 -HETE 166.06 ± 25.38 112.10 ± 17.13 16 ± 9.10 -DIHOME 130.83 ± 46.62 87.33 ± 31.11 17 $111(2)$ -EET 116.46 ± 22.45 92.55 ± 17.84 18 $11(5)$ -HETE 122.57 ± 25.84 105.81 ± 22.31 19 12 -coxo-ETE 6.76 ± 2.71 27.40 ± 15.01 20 $12(S)$ -HEPE 146.17 ± 38.96 113.53 ± 30.26 21 $12(S)$ -HEPE 105.21 ± 23.16 103.45 ± 22.77 23 $14(15)$ -EET 102.67 ± 23.5 102.76 ± 20.8 24 $14(15)$ -EET 102.47 ± 32.5 102.76 ± 20.8 25 15 -coxo-ETE 121.15 ± 27.23 98.26 ± 22.08 26 $15(S)$ -HETE 123.92 ± 22.27 88.43 ± 15.89 27 $15(S)$ -HETE 123.92 ± 22.77 88.43 ± 15.89 28 $15(S)$ -HETE 123.92 ± 32.5 105.52 ± 25.0	11	±18-HEPE	128.26 ± 26.47	93.40 ± 19.28
13 $\pm 5, 6$ -DHET $144, 45 \pm 34, 95$ $96, 86 \pm 23, 44$ 14 $\pm 8(9)$ -DHET $126, 83 \pm 29, 79$ $101.01 \pm 23. 73$ 15 ± 9 -HETE $166, 06 \pm 25, 38$ $112.10 \pm 17. 13$ 16 $\pm 9, 10$ -DiHOME 130.83 ± 46.62 $87.33 \pm 31. 11$ 17 $11(12)$ -EET 116.46 ± 22.45 $92.55 \pm 17. 84$ 18 $11(5)$ -HETE 122.57 ± 25.84 $105.81 \pm 22. 31$ 19 12 -cox-ETE 6.76 ± 2.71 27.40 ± 15.01 20 $12(S)$ -HEPE 146.17 ± 38.96 113.53 ± 30.26 21 $12(S)$ -HETE 181.59 ± 34.31 131.64 ± 24.87 22 $13(S)$ -HODE 105.21 ± 23.16 103.45 ± 22.77 23 $14(15)$ -EET 109.40 ± 22.31 86.10 ± 17.56 24 $14(15)$ -EET 109.40 ± 22.31 86.10 ± 17.56 25 15 -cox-ETE $121.15 \pm 27. 23$ 98.26 ± 22.08 26 $15(S)$ -HETE 136.27 ± 33.54 106.32 ± 26.17 27 $15(S)$ -HETE 136.37 ± 30.5 96.21 ± 24.17 30 20 -HETE 141.68 ± 35.5 <td< td=""><td>12</td><td>±4-HDHA</td><td>251.65 ± 54.23</td><td>210.88 ± 45.45</td></td<>	12	±4-HDHA	251.65 ± 54.23	210.88 ± 45.45
14 $\pm 8(9)$ -DHET126.83 ± 29.79 101.01 ± 23.73 15 ± 9.10 -DiHOME130.83 ± 46.62 87.33 ± 31.11 16 ± 9.10 -DiHOME130.83 ± 46.62 87.33 ± 31.11 1711(12)-EET116.46 ± 22.45 92.55 ± 17.84 1811(S)-HETE122.57 ± 25.84 105.81 ± 22.31 1912-coxo-ETE 6.76 ± 2.71 27.40 ± 15.01 2012(S)-HEPE146.17 ± 38.96 113.53 ± 30.26 2112(S)-HETE181.59 ± 34.31 131.64 ± 24.87 2213(S)-HODE105.21 ± 23.16 103.45 ± 22.77 2314(15)-EET109.40 ± 22.31 86.10 ± 17.56 2414(15)-EET109.40 ± 22.31 86.10 ± 17.56 2515-oxo-ETE121.15 ± 27.23 98.26 ± 22.08 2615(S)-HETE123.92 ± 22.27 88.43 ± 15.89 2815(S)-HETE123.92 ± 22.27 88.43 ± 15.89 2815(S)-HETE134.78 ± 31.85 105.85 ± 25.01 2915(S)-HETE134.78 ± 31.85 90.21 ± 24.11 315-oxETE68.73 ± 18.59 89.37 ± 24.17 325(S)-HETE190.21 ± 40.9 116.98 ± 25.15 335(S)-HETE130.21 ± 25.56 97.79 ± 27.43 46(S)-HETE136.13 ± 26.51 92.98 ± 18.11 336(-keto-PGF ₁ a136.13 ± 26.51 92.98 ± 18.11 346(9)-EET132.93 ± 29.76 102.12 ± 22.86 40LTE ₄ 82.85 ± 23.48 79.60 ± 22.27 <	13	±5,6-DHET	144.45 ± 34.95	96.86 ± 23.44
15 ± 9 -HETE 166.06 ± 25.38 112.10 ± 17.13 16 ± 9.10 -DiHOME 130.83 ± 46.62 87.33 ± 31.11 17 11(12)-EET 116.46 ± 22.45 92.55 ± 17.84 18 11(5)-HETE 122.57 ± 25.84 105.81 ± 22.31 19 12-0x0-ETE 6.76 ± 2.71 27.40 ± 15.01 20 12(S)-HETE 146.17 ± 38.96 113.53 ± 30.26 21 12(S)-HETE 105.21 ± 23.16 103.45 ± 22.77 23 14(15)-EET 109.40 ± 22.31 86.10 ± 17.56 24 14(15)-EET 109.40 ± 22.21 88.64 ± 22.08 25 15-0x0-ETE 109.40 ± 22.27 88.43 ± 15.89 26 15(S)-HETE 123.92 ± 22.27 88.43 ± 15.89 27 15(S)-HETE 134.78 ± 31.85 106.32 ± 26.17 28 15(S)-HETE 134.78 ± 31.85 105.85 ± 25.01 29 15(S)-HETE 134.78 ± 31.85 105.85 ± 25.01 29 15(S)-HETE 134.78 ± 31.85 106.45 ± 33.33 30 20-HETE 141.68 ± 35.5 96.21 ± 24.17	14	±8(9)-DHET	126.83 ± 29.79	101.01 ± 23.73
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	15	±9-HETE	166.06 ± 25.38	112.10 ± 17.13
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	16	±9,10-DiHOME	130.83 ± 46.62	87.33 ± 31.11
1811(S)-HETE122.57 \pm 25.84105.81 \pm 22.311912-oxo-ETE 6.76 ± 2.71 27.40 \pm 15.012012(S)-HEPE146.17 \pm 38.96113.53 \pm 30.262112(S)-HETE181.59 \pm 34.31131.64 \pm 24.872213(S)-HODE105.21 \pm 23.16103.45 \pm 22.772314(15)-EET102.87 \pm 32.5102.76 \pm 20.52414(15)-EPETE109.40 \pm 22.3186.10 \pm 17.562515-oxo-ETE121.15 \pm 27.2398.26 \pm 22.082615(S)-HETE123.92 \pm 22.2788.43 \pm 15.892815(S)-HETE123.92 \pm 22.2788.43 \pm 15.892915(S)-HETE134.78 \pm 31.85106.32 \pm 26.172915(S)-HETE141.68 \pm 35.596.21 \pm 24.173020-HETE141.68 \pm 35.596.21 \pm 24.17315-oxoETE68.73 \pm 18.5989.37 \pm 24.17325(G)-EET25.03 \pm 7.83170.46 \pm 53.33335(S)-HETE190.21 \pm 40.9116.98 \pm 25.15345(S)-HETE130.61 \pm 24.91105.81 \pm 22.27345(S)-HETE130.21 \pm 40.9116.98 \pm 25.15355(S)-HETE138.69 \pm 97.79 \pm 27.43366-keto-PGF10132.93 \pm 92.76102.12 \pm 22.86378-iso-PGF20312.89 \pm 66.64160.30 \pm 34.14388(9)-ET118.49 \pm 27.2592.00 \pm 21.16399(S)-HODE132.93 \pm 29.76102.12 \pm 22.8641LTC481.9	17	11(12)-EET	116.46 ± 22.45	92.55 ± 17.84
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	18	11(S)-HETE	122.57 ± 25.84	105.81 ± 22.31
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	19	12-oxo-ETE	6.76 ± 2.71	27.40 ± 15.01
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	20	12(S)-HEPE	146.17 ± 38.96	113.53 ± 30.26
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	21	12(S)-HETE	181.59 ± 34.31	131.64 ± 24.87
23 $14(15)-EET$ 162.87 ± 32.5 102.76 ± 20.5 24 $14(15)-EpETE$ 109.40 ± 22.31 86.10 ± 17.56 25 15 -oxo-ETE 121.15 ± 27.23 98.26 ± 22.08 26 $15(S)-HEPE$ 136.25 ± 33.54 106.32 ± 26.17 27 $15(S)-HETE$ 123.92 ± 22.27 88.43 ± 15.89 28 $15(S)-HETE$ 123.92 ± 22.77 88.43 ± 15.89 29 $15(S)-HETE$ 123.92 ± 22.27 88.43 ± 15.89 20 $15(S)-HETE$ 141.68 ± 35.5 96.21 ± 24.17 30 20 -HETE 141.68 ± 35.5 96.21 ± 24.11 31 5 -oxoETE 68.73 ± 18.59 89.37 ± 24.17 32 $5(6)$ -EET 25.03 ± 7.83 170.46 ± 53.33 33 $5(S)$ -HEPE 114.64 ± 24.15 105.71 ± 22.27 34 $5(S)$ -HETE 190.21 ± 40.9 116.98 ± 25.15 35 $5(S)$ -HETE 130.21 ± 40.9 116.98 ± 25.15 36 6 -keto-PGF ₁ α 132.89 ± 66.64 160.30 ± 34.14 37 8 -iso-PGF ₂ α 312.89 ± 66.64 160.30 ± 34.14 38 89 -PGF ₂ α 312.89 ± 66.64 160.30 ± 24.16 39 $9(S)$ -HODE 132.93 ± 29.76 102.12 ± 22.86 41 LTC_4 81.91 ± 24.9 75.42 ± 22.92 42 LTD_4 88.45 ± 25.56 76.89 ± 22.26 44 $LXA4$ 131.71 ± 27.76 99.16 ± 20.9 45MAR1 138.35 ± 36.68 103.82 ± 27.52 46PDX 133.66 ± 37.96 96.30 ± 27.52 <td>22</td> <td>13(S)-HODE</td> <td>105.21 ± 23.16</td> <td>103.45 ± 22.77</td>	22	13(S)-HODE	105.21 ± 23.16	103.45 ± 22.77
24 $14(15)$ -EpETE 109.40 ± 22.31 86.10 ± 17.56 25 15 -oxo-ETE 121.15 ± 27.23 98.26 ± 22.08 26 $15(S)$ -HEPE 136.25 ± 33.54 106.32 ± 26.17 27 $15(S)$ -HETE 123.92 ± 22.27 88.43 ± 15.89 28 $15(S)$ -HETE 134.78 ± 31.85 105.85 ± 25.01 29 $15(S)$ -HETE 134.78 ± 31.85 105.85 ± 25.01 29 $15(S)$ -HETE 141.68 ± 35.5 96.21 ± 24.11 30 20 -HETE 141.68 ± 35.5 96.21 ± 24.17 31 5 -oxoETE 68.73 ± 18.59 89.37 ± 24.17 32 $5(6)$ -ET 25.03 ± 7.83 170.46 ± 53.33 33 $5(S)$ -HETE 190.21 ± 40.9 116.98 ± 25.13 34 $5(S)$ -HETE 190.21 ± 40.9 116.98 ± 25.13 35 $5(S)$ -HETE 190.21 ± 40.9 116.98 ± 25.13 36 6 -keto-PGF ₁ a 136.13 ± 26.51 92.98 ± 18.11 37 8 -iso-PGF ₂ a 312.89 ± 66.64 160.30 ± 34.14 38 $8(9)$ -ET 118.49 ± 27.25 92.00 ± 21.16 39 $9(S)$ -HODE 132.93 ± 29.76 102.12 ± 22.86 40 LTB_4 120.93 ± 31.32 91.29 ± 23.64 41 LTC_4 81.91 ± 24.9 75.42 ± 22.92 42 LTD_4 88.45 ± 25.56 76.89 ± 22.22 43 LTE_4 82.85 ± 23.48 79.60 ± 22.56 44 $LXA4$ 131.71 ± 27.76 99.16 ± 20.9 45MAR1 138.35 ± 36.68 103.82 ± 27.52 46	23	14(15)-EET	162.87 ± 32.5	102.76 ± 20.5
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	24	14(15)-EpETE	109.40 ± 22.31	86.10 ± 17.56
2615(S)-HEPE136.25 ± 33.54106.32 ± 26.172715(S)-HETE123.92 ± 22.2788.43 ± 15.892815(S)-HETE134.78 ± 31.85105.85 ± 25.012915(S)-HPETENANA3020-HETE141.68 ± 35.596.21 ± 24.11315-0x0ETE68.73 ± 18.5989.37 ± 24.17325(6)-EET25.03 ± 7.83170.46 ± 53.33335(S)-HEPE114.64 ± 24.15105.71 ± 22.27345(S)-HETE190.21 ± 40.9116.98 ± 25.15355(S)-HETE190.21 ± 40.9116.98 ± 25.15366-keto-PGF1a136.13 ± 26.5192.98 ± 18.11378-iso-PGF2a312.89 ± 66.64160.30 ± 34.14388(9)-EET118.49 ± 27.2592.00 ± 21.16399(S)-HODE132.93 ± 29.76102.12 ± 22.8641LTC481.91 ± 24.975.42 ± 22.9242LTB4120.93 ± 31.3291.29 ± 23.6441LTC488.45 ± 25.5676.89 ± 22.2243LTE482.85 ± 23.4879.60 ± 22.5644LXA4131.71 ± 27.7699.16 ± 20.945MAR1138.35 ± 36.68103.82 ± 27.5246PDX133.66 ± 37.9696.30 ± 27.3547PGD268.76 ± 22.8782.16 ± 27.3348PGE2123.64 ± 20.2492.57 ± 15.1649PGF2a123.64 ± 20.2492.57 ± 15.1649PGF2a123.64 ± 66.65447.00 ± 94.8550RvD1116.	25	15-oxo-ETE	121.15 ± 27.23	98.26 ± 22.08
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	26	15(S)-HEPE	136.25 ± 33.54	106.32 ± 26.17
2815(S)-HETrE134.78 ± 31.85105.85 ± 25.012915(S)-HPETENANA3020-HETE141.68 ± 35.596.21 ± 24.11315-0x0ETE68.73 ± 18.5989.37 ± 24.17325(6)-EET25.03 ± 7.83170.46 ± 53.33335(S)-HEPE114.64 ± 24.15105.71 ± 22.27345(S)-HETE190.21 ± 40.9116.98 ± 25.15355(S)-HETE136.13 ± 26.5192.98 ± 18.11366-keto-PGF1a136.13 ± 26.5192.98 ± 18.11378-iso-PGF2a312.89 ± 66.64160.30 ± 34.14388(9)-EET118.49 ± 27.2592.00 ± 21.1699(S)-HODE132.93 ± 29.76102.12 ± 22.8641LTC481.91 ± 24.975.42 ± 22.9242LTD488.45 ± 25.5676.89 ± 22.2243LTE482.85 ± 23.4879.60 ± 22.5644LXA4131.71 ± 27.7699.16 ± 20.945MAR1138.35 ± 36.68103.82 ± 27.5246PDX133.66 ± 37.9696.30 ± 27.3547PGD268.76 ± 22.8782.16 ± 27.3348PGE2123.64 ± 20.2492.57 ± 15.1649PGF2a127.10 ± 30.7198.41 ± 23.7850RvD1116.18 ± 23.2489.99 ± 17.9251RvE1170.89 ± 28.2699.91 ± 16.5252TXB2118.69 ± 22.5196.24 ± 18.5253AA785.36 ± 166.65447.00 ± 94.8554ALA146.64 ± 32.26<	27	15(S)-HETE	123.92 ± 22.27	88.43 ± 15.89
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	28	15(S)-HETrE	134.78 ± 31.85	105.85 ± 25.01
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	29	15(S)-HpETE	NA	NA
315-0x0ETE 68.73 ± 18.59 89.37 ± 24.17 32 $5(6)$ -EET 25.03 ± 7.83 170.46 ± 53.33 33 $5(S)$ -HEPE 114.64 ± 24.15 105.71 ± 22.27 34 $5(S)$ -HETE 190.21 ± 40.9 116.98 ± 25.15 35 $5(S)$ -HETE 190.21 ± 40.9 116.98 ± 25.15 36 6 -keto-PGF1a 136.13 ± 26.51 92.98 ± 18.11 37 8 -iso-PGF2a 312.89 ± 66.64 160.30 ± 34.14 38 $8(9)$ -EET 118.49 ± 27.25 92.00 ± 21.16 39 $9(S)$ -HODE 132.93 ± 29.76 102.12 ± 22.86 40 LTB_4 120.93 ± 31.32 91.29 ± 23.64 41 LTC_4 81.91 ± 24.9 75.42 ± 22.92 42 LTD_4 88.45 ± 25.56 76.89 ± 22.22 43 LTE_4 82.85 ± 23.48 79.60 ± 22.56 44 $LXA4$ 131.71 ± 27.76 99.16 ± 20.9 45MAR1 138.35 ± 36.68 103.82 ± 27.52 46PDX 133.66 ± 37.96 96.30 ± 27.35 47PGD2 68.76 ± 22.87 82.16 ± 27.33 48PGE2 123.64 ± 20.24 92.57 ± 15.16 49PGF2a 123.64 ± 20.24 92.57 ± 15.16 49PGF2a 123.64 ± 20.24 99.91 ± 16.52 51RvD1 116.18 ± 23.24 89.59 ± 17.92 51RvE1 170.89 ± 28.26 99.91 ± 16.52 52TXB2 118.69 ± 22.51 96.24 ± 18.52 53AA 785.36 ± 166.65 447.00 ± 94.85	30	20-HETE	141.68 ± 35.5	96.21 + 24.11
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	31	5-oxoETE	68.73 + 18.59	89.37 + 24.17
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	32	5(6)-FFT	25 03 + 7 83	170 46 + 53 33
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	33	5(S)-HEPE	114.64 + 24.15	105.71 + 22.27
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	34	5(S)-HETE	190.21 + 40.9	116.98 + 25.15
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	35	5(S)-HpFTF	33 69 + 9 45	97 79 + 27 43
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	36	6-keto-PGF₁α	136 13 + 26 51	92 98 + 18 11
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	37	8-iso-PGE ₂ α	312 89 + 66 64	160 30 + 34 14
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	38	8(9)-FFT	118 49 + 27 25	92 00 + 21 16
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	39	9(S)-HODF	132 93 + 29 76	102 12 + 22 86
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54 7140.04 ± 32.20 714.73 ± 23.24 55 DGLA 316.64 ± 47.09 208.02 ± 30.94 56 DHA 410.34 ± 95.54 219.43 ± 51.09 57 EPA 335.53 ± 73.83 241.74 ± 53.19	57		146 64 + 22 26	11/ 75 ± 95.00
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50 510 410.34 ± 93.54 219.43 ± 51.09 57 EPA 335.53 ± 73.83 241.74 ± 53.19	55		110 31 ± 05 51	200.02 ± 50.94
5/ LFA 555.55 ± 75.65 241.74 ± 55.19	50	ΕΡΔ	335 52 ± 72 22	213.43 ± 31.09 2/1 7/ ± 52.10
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Table S6: Accuracy und precision/RSD after 48 h at 4 $^{\circ}$ C at a concentration of 0.1, 1 and 10 ng/ml; PUFAs are 10x higher concentrated (n =3)

6		Accuracy		Accuracy		Accuracy	
7	Metabolites	(%)	RSD (%)	(%)	RSD (%)	(%)	RSD (%)
8	concentration	0.1 ng	g/ml	1 ng	g/ml	10 ng	g/ml
9	±11-HDHA	1	1	81.97	1.71	106.30	3.31
10	±11,12-DHET	14.45	121.38	93.16	1.18	114.49	2.17
11	±12,13-DiHOME	95.82	4.85	81.85	0.69	104.79	2.06
12	±13-HDHA	69.08	12.16	80.67	1.94	107.75	4.82
13	±17-HDHA	/	/	81.25	9.46	104.86	4.49
14	±18-HEPE	/	/	77.80	5.35	106.41	3.22
15	±4-HDHA	9.34	79.80	74.67	5.42	105.21	4.80
16	±5,6-DHET	91.07	6.48	94.39	0.37	126.88	2.58
17	±8(9)-DHET	93.83	10.55	81.04	1.61	110.97	3.48
18	±9-HETE	98.17	4.01	89.75	0.45	108.58	4.59
19	±9,10-DiHOME	93.61	7.10	86.21	2.69	110.46	2.16
20	11(12)-EET	16.38	1	78.01	5.31	104.23	2.20
21	11(S)-HETE	/		86.91	2.52	107.20	4.12
22	12-oxo-ETE	8.88	7.86	38.33	7.90	58.59	3.53
23	12(S)-HEPE	100.63	6.19	79.00	5.40	108.31	1.31
24	12(S)-HETE	89.63	2.50	54.16	6.54	104.59	4.06
25	13(S)-HODE	87.70	4.16	86.02	1.72	107.66	2.87
26	14(15)-EET	4.95	1	87.83	0.69	108.09	7.81
27	14(15)-EpETE	41.15	46.16	89.36	4.65	106.74	5.28
28	15-oxo-ETE	101.07	9.48	88.80	1.54	103.02	7.01
29	15(S)-HEPE	1	/	86.10	1.55	107.15	0.21
30	15(S)-HETE	489.52	5.98	217.44	2.00	103.00	2.73
31	15(S)-HETrE	92.07	5.57	89.35	2.85	107.80	2.60
32	15(S)-HpETE	46.20	3.10	78.55	6.44	114.89	7.94
33	20-HETE	25.37	4.26	311.67	0.57	177.37	3.15
34	5-oxoETE	31.30	25.67	79.96	5.41	110.53	2.44
35	5(6)-EET	259.67	/	155.06	59.20	98.35	104.00
36	5(S)-HEPE	100.56	8.21	85.99	6.41	108.03	3.69
37	5(S)-HETE	/	/	98.21	3.80	106.58	2.23
38	5(S)-HpETE	37.53	36.77	/		117.13	5.31
39	6-keto-PGF₁α	185.28	22.91	333.08	3.21	/ 184.08	0.92
40	8-iso-PGF ₂ α	86.35	7.62	97.69	2.65	113.37	4.97
41	8(9)-EET	3.01	/	83.15	9.74	109.94	1.15
42	9(S)-HODE	92.17	8.14	96.40	0.98	109.02	2.79
43	LTB ₄	36.48	11.50	87.25	1.85	106.77	1.32
44	LTC ₄	87.73	6.46	60.09	14.77	95.68	5.38
45	LTD ₄	102.99	1.30	80.80	0.59	107.33	2.76
46	LTE ₄	91.40	2.57	90.12	3.42	119.59	4.24
47	LXA4	14.83	/	84.79	4.75	103.93	2.41
48	MAR1	396.85	1.41	398.73	3.21	211.92	4.23
49	PDX	90.21	4.62	92.74	1.53	115.08	1.53
50	PGD ₂	85.04	4.41	97.10	0.98	109.62	2.13
51	PGE ₂	94.22	8.66	92.20	0.74	109.63	2.74
52	PGF₂α	86.21	4.13	92.01	0.80	112.21	2.68
53	RvD1	86.59	3.25	84.60	1.72	104.69	2.99
54	RvE1	382.67	16.31	370.75	7.25	191.90	1.41
55	TXB ₂	98.71	4.77	83.93	1.18	101.07	3.57
56	AA	96.85	15.07	98.72	2.11	111.05	2.66
57	ALA	76.26	7.09	121.03	3.22	128.08	2.53

	40.07	,	04.07	4.00	104.04	
DGLA	107.96	0.37	69.02	4.99	57.28	<u></u> 5
EPA	125.55	2.74	125.82	0.96	134.70	2.
		I		I	I	

Metabolites	Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%
concentration	(<i>1</i> 0)	a/ml	(<i>1</i> no	/ml	10 nc	1/ml
	1/13 02	30.76	103.40	8 70	103.82	/нн Л
	110.68	1.03	102.49	6.10	100.02	+ 2
±12.13 DiHOME	140.53	1.95	102.03	4.62	100.39	
	140.55	29.11	117.10	4.02	07.64	6
	136.02	15.60	106.30	3 10	101.04	0
	1/18 1/	10.58	110.39	0.00	101.04	۲ ۶
	202 72	23 32	10.29	23.01	120.00	17
	1/1 55	10.66	106.84	20.01	120.00	۱۱ ج
+8(9)-DHET	130 38	20.59	106.18	8 78	100.03	
	107.88	10.70	84.90	1/ 8/	87 / 0	20
	172 42	16.70	116 61	10.02	101 21	- 20
11(12) EET	1/2.42	16.30	113.63	2.28	101.21	r G
	142.04	28.43	107.75	6.61	104.05	
12-0Y0-ETE	144.99 ΝΔ	20.45 NA	107.73	47.04	90.25	15
12-0X0-LTL 12(S)_HEPE	166 38	24.30	113.60	3 10	90.23	40
	230.28	60.98	101 30	1.03	101 57	
	166 54	7 11	101.39	6.18	101.37	
14(15)_EET	94.60	15.53	112 70	13.23	102.73	
14(15)-EDETE	140.26	28.50	103.28	9.02	101.73	
15 ovo ETE	152 30	13 / 3	113.20	2.92	104.94	
15/0X0-LTL 15/0X0-LTL	130.70	0.05	102.36	8 71	100.03	
	138.53	16 73	102.30	1 0.71	111 00	
15(S)-HETrE	138 79	27.16	112.88	8.87	106.01	
15(S)-HpETE	NA	NA	125.00	NA	62 15	86
20-HETE	105.16	21 19	104.98	7 27	104 69	
5-0x0FTF	128 17	10 70	103.40	10.31	114 85	24
5(6)-FFT	139.76	23.86	105.10	12.07	91 75	 1/
5(S)-HEPE	149 54	7 15	106.00	2.94	110.07	1(
5(S)-HETE	231 21	44 70	113 16	7 13	101 12	
5(S)-HpETE	NA	NA	NA	NA	NA	
6-keto-PGE₁α	102.01	12 82	91.50	6 4 9	96.75	(
8-iso-PGF ₂ α	113.92	9.52	103.22	7.63	103.40	1!
8(9)-EET	150.18	16.08	111.40	2.65	112.48	
9(S)-HODE	172.33	24.73	105.72	6.30	102.03	4
LTB ₄	130.61	22.99	107.42	7.22	100.07	
LTC₄	119.86	32.20	101.37	8.37	101.13	
LTD ₄	140.30	31.13	107.71	6.63	100.06	
LTE4	120.35	24.06	109.84	3.07	103.58	
LXA4	134.56	7.52	111.38	5.75	108.98	8
MAR1	135.97	21.48	104.56	8.88	102.06	
PDX	130.64	18.81	105.48	13.01	101.45	
PGD ₂	131.20	21.28	103.53	7.04	100.79	
PGE ₂	125.20	22.41	109.18	5.68	103.26	
PGF ₂ α	147.94	21.55	115.13	4.68	105.34	4
RvD1	126.87	20.61	104.69	3.22	101.95	
RvE1	98.36	3.04	98.72	5.59	95.94	14
TXB ₂	125.13	20.40	104.07	2.74	106.39	-
AA	349.17	26.16	120.76	40.71	125.30	2!
ΑΙΑ	216.22	55 50	109 50	12 29	96 96	(

Table S7: Inter-3-day variability of accuracy and precision/RSD at 0.1, 1 and 10 ng/ml; PUFAs are 10x higher concentrated (n=3)

D <u>DLA 2246.77 134.75 91.21 63.96 68.53 68.</u> D <u>HA 10738.50 142.89 1448.4 61.05 185.00 16.</u> EPA 296.48 56.65 120.56 44.51 85.10 16.							
296.48 10/36.30 142.33 1445.42 114.03 112.63 44 EPA 296.48 56.65 120.56 44.51 85.10 16.4	DGLA	2846.77	134.75	91.21	63.96	68.53	68.78
torpeer Review	EPA	296.48	56.65	120.56	44.51	85.10	16.89

	Metabolite	LOD (ng/ml)	LLOQ (ng/ml)	Correlation Coefficient
	±11-HDHA	0.05	0.5	0.99999
	±11,12-DHET	0.005	0.05	0.99999
	±12,13-DiHOME	0.005	0.5	0.99993
	±13-HDHA	0.1	1	0.99978
)	±17-HDHA	0.005	1	0.99999
, 	+18-HEPE	0.05	0.5	0.99993
,	+4-HDHA	0.5	1	1
-	+5 6-DHET	0.05	0.05	1
_	+8(9)-DHFT	0.005	0.5	0 99992
	+9-HETE	0.005	0.5	0.99999
	+9 10-DiHOME	0.000	1	0.9999
,	11(12)-FET	0.005	0.5	0.0000
,	11(S)_HETE	0.005	0.5	0.00003
))		0.005	0.05	0.99993
	12-0X0-ETE	0.5	0.5	0.99977
,	12(S)-HEFE	0.0	1	0.99998
		0.005		0.99999
-		0.1	0.5	0.99999
	14(15)-EE1	0.01	0.5	0.99989
ł	14(15)-EpETE	0.1	0.5	0.99885
	15-0x0-EIE	0.05	0.5	0.99995
	15(S)-HEPE	0.05	0.5	0.99992
	15(S)-HETE	0.25	0.05	0.99994
	15(S)-HETrE	0.1	10	0.99984
	15(S)-HpETE	0.005	NA	0.99958
	20-HETE	0.01	1	0.99946
	5-oxoETE	0.005	0.05	0.99992
	5(6)-EET	0.01	NA	0.99955
	5(S)-HEPE	0.05	0.05	0.99999
	5(S)-HETE	0.5	0.05	1
	5(S)-HpETE	NA	NA	N/A
	6-keto-PGF ₁ α	0.01	0.1	0.99958
	8-iso-PGF ₂ α	0.005	0.5	0.99951
	8(9)-EET	0.05	0.1	0.99994
	9(S)-HODE	0.1	0.05	0.99998
	LTB₄	0.005	0.05	0.99998
	LTC ₄	0.005	0.005	0.99959
	LTD ₄	0.005	0.5	0.99988
		0.005	0.5	0.99998
	1 XA4	0.005	0.5	0 99996
	MAR1	0.005	1	0 99988
	PDX	0.005	0.5	0.00000
,	PGD	0.005	0.5	0.00040
	PGE-	0.005	0.5	0.000/7
		0.005	0.5	0.00005
		0.005	0.5	0.99990
,		0.005	0.0	0.99999
		0.05	0.05	0.99999
		0.005	0.25	0.99996
		500	100	0.99948
		50	100	0.9995
)	DGLA	50	1	0.99986
I	DHA	500	NA	0.99568
1		50	100	0 00011

Table S8: Limit of detection (LOD) and lower limit of quantitation (LLOQ) with correlation coefficient

to per period

Table S9: Comparison of Frankfurt and Munich LC-MS/MS panels, data are shown as mean ± SD for 6 different blood donors.

Metabolite	Frankfurt (ng/ml) (method 2)	Munich (ng/ml) (method 1)
PGD ₂	0.009 ± 0.005	0.012 ± 0.008
PGE ₂	0.012 ± 0.012	0.008 ± 0.007
PGF₂α	0.018 ± 0.017	0.050 ± 0.065
LTB ₄	9.061 ± 4.342	29.097 ± 34.562
TXB ₂	0.298 ± 0.380	0.186 ± 0.210
5-HETE	8.411 ± 9.181	11.913 ± 12.668
15-HETE	0.237 ± 0.547	0.110 ± 0.066

Table S10: Levels of eicosanoids detected in BALF or intestinal culture supernatant of naïve mice or mice infected with Nippostrongylus brasiliensis (Nb) or Heligmosomoides polygyrus bakeri (Hpb); concentrations in pg/ml ± SD, n=5 for naïve mice and n=3-4 for infected mice

Motabolito	BALF,	BALF,	intestine,	intestine,
Metabolite	naïve	helminth (Nb)	naïve	helminth (Hpb)
PGE ₂	<lod< td=""><td>122.0 ± 68.0</td><td>16.9 ± 7.0</td><td>16600 ± 4862</td></lod<>	122.0 ± 68.0	16.9 ± 7.0	16600 ± 4862
PGD ₂	<lod< td=""><td>64.5 ± 1.0</td><td>25.6 ± 11.6</td><td>511.9 ± 149.3</td></lod<>	64.5 ± 1.0	25.6 ± 11.6	511.9 ± 149.3
TXB ₂	<lod< td=""><td>66.9 ± 17.2</td><td>9.1 ± 3.7</td><td>4180 ± 518.6</td></lod<>	66.9 ± 17.2	9.1 ± 3.7	4 180 ± 518.6
PGF _{2a}	<lod< td=""><td>62.5 ± 4.4</td><td>0.2 ± 0.2</td><td>28490 ± 8135</td></lod<>	62.5 ± 4.4	0.2 ± 0.2	28490 ± 8135
LTB ₄	4 6.0 ± 64. 1	<mark>100.8 ±</mark> 49.5	<lod< td=""><td><lod< td=""></lod<></td></lod<>	<lod< td=""></lod<>
5-HETE	12.0 ± 24.0	<lod< td=""><td>0.3 ± 0.4</td><td><lod< td=""></lod<></td></lod<>	0.3 ± 0.4	<lod< td=""></lod<>
12-HETE	233.0 ± 63.7	1995 ± 2355	17.3 ± 12.1	60070 ± 23620
15-HETE	26.8 ± 31.7	141.2 ± 161.8	2.3 ± 1.6	73120 ± 16380

Table S1: A CXP: collisio	nalyte stock with on cell exit potentia	MRM para al	meters: DP:	declusterin	g potential,	CE: collisior	n energy and	d
	Ctool							

6		Stock						
7 8	Metabolite	Concentration (ng/µl)	Q1 (m/z)	Q3 (m/z)	RT (min)	DP (V)	CE (V)	CXP (V)
9	±11-HDHA	1	343.418	149.1	9.4	-20	-18	-13
10	±11,12-DHET	1	337.14	167.1	6.7	-60	-24	-17
11	±12,13-DiHOME	1	313.083	183	6	-70	-30	-9
12	±13-HDHA	1	343.273	192.9	8.9	-10	-18	-9
13	±17-HDHA	1	343.258	244.9	8.6	-100	-16	-11
14	±18-HEPE	1	317.208	259.2	6.9	-65	-14	-7
15	±4-HDHA	1	343.27	101	11	-25	-18	-7
16	±5,6-DHET	1	337.149	144.9	7.6	-80	-24	-13
17	±8(9)-DHET	1	337.328	126.9	7	-80	-26	-23
18	±9-HETE	1	<u>31</u> 9.19	167.2	9.7	-10	-20	-15
19	±9,10-DiHOME	1	313.186	201.1	6.2	-85	-28	-11
20	11(12)-EET	1	319.19	167	11.4	-65	-20	-27
21	11(S)-HETE	1	319.091	166.7	9.1	-95	-20	-11
22	12-oxo-ETE	1	317.112	153.2	9.2	-90	-20	-19
23	12(S)-HEPE	1	317.113	178.8	7.6	-35	-18	-25
24	12(S)-HETE	1	319.3	178.7	9.4	-70	-18	-7
25	13(S)-HODE	1	295.065	195.1	8.2	-15	-24	-21
26	14(15)-EET	1	319.152	218.8	10.4	-60	-16	-29
27	14(15)-EpETE	1	317.139	207	8.6	-50	-18	-21
28	15-oxo-ETE	1	317.124	113.2	8.5	-120	-22	-13
29	15(S)-HEPE	1	317.235	218.9	7.2	-75	-16	-17
30	15(S)-HETE	1	319.152	218.9	8.6	-35	-18	-39
31	15(S)-HETrE	1	321.322	221.2 🧹	9.9	-15	-20	-11
32	15(S)-HpETE	1	335.079	112.9	10	-55	-18	-9
33	20-HETE	1	319.09	245	7.4	-145	-20	-11
34	5-oxoETE	1	317.025	203	10.8	-65	-22	-35
35	5(6)-EET	1	319.045	191.2	11.7	-25	-14	-1
36	5(S)-HEPE	1	317.266	115.1	8	-95	-18	-15
37	5(S)-HETE	1	319.101	114.9	10.3	-15	-18	-7
38	5(S)-HpETE	1	335.064	166.9	9.6	-5	-22	-15
39	<u>6-keto-PGF₁α</u>	1	369.09	162.9	1.4	-65	-36	-19
40	8-iso-PGF ₂ α	1	353.16	193	2.5	-125	-38	-19
41	8(9)-EE1	1	319.152	155	11.6	-130	-16	-15
42	9(S)-HODE	1	295.091	171.2	8.4	-110	-22	-23
43		1	335.12	195	5.6	-90	-20	-25
44		1	624.207	272.1	5.3	-100	-30	-11
45		1	495.204	1//	5.5	-65	-26	-21
46		1	438.207	333	5.4	-70	-24	-39
47		1	351.123	114.9	4.5	-50	-20	-/
48	MAR1	1	359.14	177.2	5.5	-120	-20	-23
49		1	359.14	153.1	5.5	-95	-20	-15
50			351.122	2/1.1	4.1	-00	-22	-11
51			351.057	2/1.3	3.ð	-20	-20	-9
52			303.143	193.1	3.4	-130	-34	-21
53			3/5.143	141	4.5	-/5	-20	-13
54			349.087		1.0	-90	-20	-19
55		10	309.082	109.1	2.3 12.7	-40	-24	-21
56		10	303.153	200.9	12.1	-90	-20	-13
57	ALA	10	211.100	233.2	12.4	-140	-10	-11

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DGLA	10	305.156	261.2	12.8	-110	-22	-9
DHA	10	327.122	283.2	12.6	-110	-14	-11
EPA	10	300.992	257	12.4	-5	-16	-25

Table S2: Internal standard stock with MRM parameters; DP: declustering potential, CE: collision energy and CXP: collision cell exit potential

Metabolite	Stock Concentration (ng/µl)	Q1 (m/z)	Q3 (m/z)	RT (min)	DP (V)	CE (V)	CXP (V)	
11,12-DHET- d11	1	348.22	167.1	6.6	-80	-26	-9	
8,9-DHET-d11	1	348.143	127	7	-85	-28	-13	
11(12)-EET-d11	1	330.226	167	11.3	-70	-18	-13	
12(S)-HETE-d8	1	327.119	184.5	9.2	-90	-20	-9	
13(S)-HODE-d4	1	299.174	198.1	8.1	-95	-24	-11	
15(S)-HETE-d8	1	327.122	226.1	8.4	-30	-18	-9	
5-oxoETE-d7	1	324.177	210.2	10.8	-85	-24	-13	
5(S)-HETE-d8	1	327.167	116	10.1	-15	-18	-7	
6-keto-PGF₁α- d4	1	373.079	167	1.4	-70	-36	-7	
LTB ₄ -d4	1	339.12	197	5.6	-90	-20	-25	
PGD ₂ -d4	1	355.105	275.3	4.1	-70	-22	-5	
PGE ₂ -d4	1	355.173	275.2	3.8	-75	-24	-11	
PGF₂α-d4	1	357.212	313.3	3.4	-65	-24	-35	
RvD1-d5	1	380.196	141	4.5	-155	-20	-17	
TXB ₂ -d4	1	373.201	173	2.5	-60	-22	-21	
AA-d8	10	311.09	267 🧹	12.6	-95	-26	-13	
EPA-d5	10	306.246	262.2	12.4	-30	-16	-9	

Table S3: Forward and reverse primers for qPCR

Gene	Forward Primer (5' - 3')	Reverse Primer (5' - 3')
PTGS2	GCTGGAACATGGAATTACCCA	CTTTCTGTACTGCGGGTGGAA
PTGES	TCAAGATGTACGTGGTGGCC	GAAAGGAGTAGACGAAGCCCAG
ALOX5	GATTGTCCCCATTGCCATCC	AGAAGGTGGGTGATGGTCTG
ALOX15	GGACACTTGATGGCTGAGGT	GTATCGCAGGTGGGGAATTA
TGM2	AGGCCCGTTTTCCACTAAGA	AGCAAAATGAAGTGGCCCAG
GAPDH	GAAGGTGAAGGTCGGAGT	GAAGATGGTGATGGGATTTC

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Concentration	0.1 n	g/ml	1 ng	g/ml	10 n	g/ml
Metabolites	Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%)
±11-HDHA	95.26	45.26	121.52	15.42	121.64	11.70
±11,12-DHET	114.55	27.47	109.46	14.02	91.52	14.20
±12,13-DiHOME	NA	NA	103.87	13.80	104.35	18.12
±13-HDHA	NA	NA	97.90	13.64	103.55	13.31
±17-HDHA	140.48	53.19	110.44	2.88	112.29	10.95
±18-HEPE	NA	NA	104.93	11.59	101.24	13.04
±4-HDHA	155.63	24.76	117.30	19.22	105.17	13.64
±5,6-DHET	88.30	39.87	106.59	7.23	95.46	12.44
±8(9)-DHET	NA	NA	95.82	14.73	97.25	13.44
±9-HETE	96.67	37.91	126.38	14.66	121.23	12.55
±9,10-DiHOME	85.87	48.05	88.60	14.29	79.86	19.75
11(12)-EET	47.85	80.53	106.23	12.94	96.02	11.55
11(S)-HETE	119.79	23.47	100.35	16.36	88.94	14.76
12-oxo-ETE	276.86	5.94	59.81	26.45	26.17	79.04
12(S)-HEPE	59.16	98.57	101.71	14.13	96.00	13.21
12(S)-HETE	89.67	59.94	109.23	17.98	107.72	19.02
13(S)-HODE	60.27	99.28	104.32	15.45	98.05	13.79
14(15)-EET	NA	NA	82.28	19.54	96.21	11.39
14(15)-EpETE	NA	NA	53.59	47.43	99.43	9.59
15-oxo-ETE	52.15	41.83	101.50	9.74	96.07	14.01
15(S)-HEPE	18.35	114.94	101.20	13.46	100.33	11.79
15(S)-HETE	177.23	11.66	98.63	14.42	96.39	11.67
15(S)-HETrE	NA	NA	93.77	20.74	93.86	16.04
15(S)-HpETE	NA	NA	NA	NA	26.82	78.79
20-HETE	NA	NA	156.46	15.19	119.87	27.95
5-oxoETE	144.23	31.83	107.97	9.11	91.01	10.92
5(6)-EET	33.03	74.44	108.11	87.12	61.18	101.98
5(S)-HEPE	66.64	61.40	122.21	6.17	112.28	14.19
5(S)-HETE	83.48	83.86	102.42	18.91	91.63	13.80
5(S)-HpETE	NA	NA	NA	NA	NA	NA
6-keto-PGF _{1alpha}	124.16	3.38	143.04	7.08	119.90	10.69
8-iso-PGF _{2alpha}	51.63	21.08	109.82	21.53	110.56	13.18
8(9)-EET	79.63	10.88	102.80	12.53	100.64	10.85
9(S)-HODE	85.22	59.03	106.24	15.77	92.55	14.48
LTB ₄	106.03	22.11	98.02	10.87	86.87	14.46
LTC ₄	368.38	6.49	128.66	15.88	96.54	5.73
LTD ₄	29.24	99.62	105.70	12.55	97.71	7.24
LTE ₄	50.86	97.21	110.17	13.90	95.36	9.43
LXA4	42.19	73.46	82.47	13.64	79.78	23.44
MAR1	NA	NA	205.38	15.28	138.94	27.30
PDX	NA	NA	100.06	16.37	111.18	11.09
PGD ₂	NA	NA	106.52	18.57	104.22	5.07
PGE ₂	34.18	74.36	67.28	12.89	58.92	11.58
PGF _{2alpha}	NA	NA	99.12	12.34	97.98	13.33
RvD1	59.84	46.36	98.35	13.55	91.53	15.97
RvE1	179.13	12.56	117.52	18.10	119.98	7.01
TXB ₂	125.72	26.63	112.53	2.47	97.03	4.52
AA	NA	NA	1108.92	39.52	142.08	47.69
ALA	NA	NA	82.53	NA	97.06	27.37
DGLA	NA	NĀ	400.56	19.18	117.29	24.34
DHA	NA	NA	2938.46	44.93	188.60	73.28

EPA		NA	NA	59.36	NA	82.43
Table S4: Accu	racy and p	orecision (R	SD) at differ	ent concenti	ation levels; 0	.1, 1 and 1
individual extrac	lions					

Metabolite	Recovery ± SD	Matrix Effect ± SD
11-HDHA	148.75 ± 34.92	108.16 ± 25.39
±11,12-DHET	118.03 ± 24.79	96.27 ± 20.22
±12,13-DiHOME	158.56 ± 51.27	112.19 ± 36.28
±13-HDHA	111.10 ± 22.94	94.03 ± 19.42
±17-HDHA	138.36 ± 24.21	108.51 ± 18.99
±18-HEPE	128.26 ± 26.47	93.40 ± 19.28
±4-HDHA	251.65 ± 54.23	210.88 ± 45.45
±5,6-DHET	144.45 ± 34.95	96.86 ± 23.44
±8(9)-DHET	126.83 ± 29.79	101.01 ± 23.73
±9-HETE	166.06 ± 25.38	112.10 ± 17.13
±9,10-DiHOME	130.83 ± 46.62	87.33 ± 31.11
11(12)-EET	116.46 ± 22.45	92.55 ± 17.84
11(S)-HETE	122.57 ± 25.84	105.81 ± 22.31
12-oxo-ETE	6.76 ± 2.71	27.40 ± 15.01
12(S)-HEPE	146.17 ± 38.96	113.53 ± 30.26
12(S)-HETE	181.59 ± 34.31	131.64 ± 24.87
13(S)-HODE	105.21 ± 23.16	103.45 ± 22.77
14(15)-EET	162.87 + 32.5	102.76 + 20.5
14(15)-EpETE	109 40 + 22 31	86 10 + 17 56
15-0x0-FTF	121 15 + 27 23	98 26 + 22 08
15(S)-HEPE	136 25 + 33 54	106.32 + 26.17
15(S)-HETE	123 92 + 22 27	88 43 + 15 89
15(S)-HETrE	134 78 + 31 85	105 85 + 25 01
15(S)-HpETE	<u>104.70 ± 01.00</u> ΝΔ	100.00 ± 20.01 ΝΔ
20-HETE	141 68 + 35 5	96 21 + 24 11
5-0x0FTF	68 73 + 18 59	89 37 + 24 17
5(6)-FFT	25 03 + 7 83	170 46 + 53 33
5(S)-HEPE	114 64 + 24 15	105 71 + 22 27
5(S)-HETE	190 21 + 40 9	116 98 + 25 15
5(S)-HnETE	33 69 + 9 45	97 79 + 27 43
6-keto-PGE.a	136 13 + 26 51	<u>97.79 ± 27.45</u> 02.08 ± 18.11
8-iso-PGE-a	312 80 + 66 64	<u> </u>
8(9)-FFT	118 49 + 27 25	92 00 + 21 16
	132 03 + 20 76	102 12 + 22 86
	120 03 + 31 32	01 20 + 22 6/
	81 01 + 2/ 0	75 42 + 22 02
	88 45 + 25 56	76 80 + 22 22
	82 85 + 23 / 8	79 60 + 22 56
	131 71 + 27 76	00 16 + 20 0
	138 35 + 36 68	<u> </u>
	133 66 ± 37 06	<u>105.02 ± 27.32</u> 06.30 ± 27.35
	68 76 ± 27.90	82 16 + 27 22
	123.64 ± 20.24	02.10 ± 27.33
	123.04 ± 20.24 127.10 ± 20.71	92.37 ± 13.10
	$\frac{121.10 \pm 30.11}{116.10 \pm 32.24}$	90.41 ± 23./0
	110.10 ± 23.24	09.09 ± 17.92
	1/0.89 ± 28.26	99.91 ± 16.52
	110.09 ± 22.51	90.24 ± 18.52
AA	/ 85.36 ± 166.65	447.00 ± 94.85
ALA	146.64 ± 32.26	114./5 ± 25.24
DGLA	316.64 ± 47.09	208.02 ± 30.94
	410.34 ± 95.54	219.43 ± 51.09
FPA	335 53 + 73 83	241 74 + 53 19

Table S5: Recovery and matrix effect at 1 ng/ml (n=3 separate extractions)

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Table S6: Accuracy und precision/RSD after 48 h at 4 °C at a concentration of 0.1, 1 and 10 ng/ml; PUFAs are 10x higher concentrated (n = 3)

6		Accuracy		Accuracy		Accuracy	
7	Metabolites	(%)	RSD (%)	(%)	RSD (%)	(%)	RSD (%)
8	concentration	0.1 ng	g/ml	1 ng	g/ml	10 ng	g/ml
9	±11-HDHA	/	/	81.97	1.71	106.30	3.31
10	±11,12-DHET	14.45	121.38	93.16	1.18	114.49	2.17
11	±12,13-DiHOME	95.82	4.85	81.85	0.69	104.79	2.06
12	±13-HDHA	69.08	12.16	80.67	1.94	107.75	4.82
13	±17-HDHA	/	/	81.25	9.46	104.86	4.49
14	±18-HEPE	/		77.80	5.35	106.41	3.22
15	±4-HDHA	9.34	79.80	74.67	5.42	105.21	4.80
16	±5,6-DHET	91.07	6.48	94.39	0.37	126.88	2.58
17	±8(9)-DHET	93.83	10.55	81.04	1.61	110.97	3.48
18	±9-HETE	98.17	4.01	89.75	0.45	108.58	4.59
19	±9.10-DiHOME	93.61	7.10	86.21	2.69	110.46	2.16
20	11(12)-EET	16.38	1	78.01	5.31	104.23	2.20
20	11(S)-HETE	/		86.91	2.52	107.20	4.12
21	12-0x0-ETE	8.88	7.86	38.33	7.90	58.59	3.53
22	12(S)-HEPE	100.63	6 19	79.00	5 40	108.31	1 31
23	12(S)-HETE	89.63	2 50	54 16	6 54	104 59	4 06
27	13(S)-HODE	87 70	4 16	86.02	1 72	107.66	2 87
25	14(15)-FFT	4 95		87.83	0.69	108.09	7 81
20	14(15)-EpETE	41 15	46 16	89.36	4 65	106.00	5 28
27	15-0x0-ETE	101.07	9.48	88.80	1.54	103.02	7 01
20	15(S)-HEPE	/	/	86.10	1.51	107.15	0.21
29	15(S)-HETE	489 52	5.98	217 44	2.00	107.10	2 73
50 21	15(S)-HETrE	92.07	5.50	89 35	2.00	103.00	2.70
22	15(S)-HpETE	46.20	3 10	78.55	6.44	11/ 80	7 0/
3Z		25.37	4.26	311.67	0.77	177.37	3 15
33		31.30	25.67	70.06	5.41	110.53	2.13
34 25	5-0X0LTL 5(6) EET	250.67	23.07	155.06	50.20	08.35	104.00
35		209.07	9.21	155.00	59.20	109.03	104.00
36		100.50	0.21	00.99	2 90	106.03	3.09
3/		27.52	26 77	90.21	3.00	117.12	5.21
38	$5(3)$ - $\Pi P \Box T \Box$	105.00	22.01	/	2.21	101.00	0.02
39		100.20	22.91	07.60	3.21	112.27	0.92
40	$\frac{6-150-FGF_2u}{2(0)}$	00.33	1.02	97.09	2.03	113.37	4.97
41		3.01	0 1 1	03.13	9.74	109.94	1.13
42		92.17	0.14	90.40	0.90	109.02	2.79
43		30.40	6.46	60.00	1.00	100.77	<u>ا.32</u>
44		07.73	0.40	00.09	14.77	95.00	0.30
45		102.99	1.30	00.00	0.59	107.33	2.70
46		91.40	2.57	90.12	3.42	119.59	4.24
47		14.83	/	84.79	4.75	103.93	2.41
48		390.85	1.41	398.73	3.21	211.92	4.23
49	PDX	90.21	4.62	92.74	1.53	115.08	1.53
50	PGD ₂	85.04	4.41	97.10	0.98	109.62	2.13
51	PGE ₂	94.22	8.66	92.20	0.74	109.63	2.74
52	$PGF_2\alpha$	86.21	4.13	92.01	0.80	112.21	2.68
53		86.59	3.25	84.60	1.72	104.69	2.99
54		382.67	16.31	370.75	7.25	191.90	1.41
55	1 XB ₂	98.71	4.77	83.93	1.18	101.07	3.57
56	AA	96.85	15.07	98.72	2.11	111.05	2.66
57	ALA	76.26	7.09	121.03	3.22	128.08	2.53
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Allergy

DGLA	19.67	/	91.67	4.99	104.61	
DHA	107.96	0.37	69.02	4.96	57.28	
EPA	125.55	2.74	125.82	0.96	134.70	

Metabolites	Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%)
concentration	0.1 n	a/ml	1 nc	u/ml	10 nc	1/ml
±11-HDHA	143.02	30.76	103.49	8.79	103.82	4.95
±11.12-DHET	119.68	1.93	102.83	6.10	100.59	2.20
+12,13-DiHOME	140.53	14.74	102.67	4.62	100.21	1.90
+13-HDHA	180.04	28.11	117.19	13.53	97.64	6.61
+17-HDHA	136.92	15 69	106.39	3 10	101.04	4 52
+18-HFPF	148 14	10.58	110 29	0.99	103 43	5.22
+4-HDHA	202 72	23.32	109.19	23.01	120.00	17.31
+5 6-DHFT	141.55	19.66	106.84	4 04	100.89	3.81
+8(9)-DHET	130.38	20.59	106.01	8.78	101.00	5.02
+9-HFTF	107.88	10.70	84 90	14 84	87.49	20.97
	172 42	16.70	116.61	10.92	101 21	7.68
11(12)_FET	1/2.42	16.30	113.63	2.28	101.21	6 25
	144.00	28.43	107.75	6.61	104.05	1 78
12 ovo ETE	144.55 NA	20.43	107.73	47.04	00.25	45.12
12-0X0-ETE	166.29	24.20	112.60	47.04	90.23	40.1Z
	100.30	24.39	101.20	3.10	90.01	0.20
	230.28	00.98	101.39	1.03	101.57	3.30
	100.54	1.11	103.40	0.18	102.73	2.53
14(15)-EET	94.60	15.53	112.79	13.23	101.73	6.35
14(15)-EPETE	140.26	28.50	103.28	9.92	104.94	5.53
15-0X0-EIE	152.39	13.43	113.81	2.28	108.03	6.57
15(S)-HEPE	130.70	9.05	102.36	8.71	101.82	3.17
15(S)-HETE	138.53	16.73	107.79	4.94	111.99	8.42
15(S)-HETrE	138.79	27.16	112.88	8.87	106.01	9.23
15(S)-HpETE	NA	NA	125.99	NA NA	62.15	86.12
20-HETE	105.16	21.19	104.98	7.27	104.69	1.98
5-oxoETE	128.17	10.70	103.40	10.31	114.85	24.63
5(6)-EET	139.76	23.86	105.50	12.07	91.75	14.08
5(S)-HEPE	149.54	7.15	106.10	2.94	110.07	10.56
5(S)-HETE	231.21	44.70	113.16	7.13	101.12	4.38
5(S)-HpETE	NA	NA	NA	NA	NA	NA
6-keto-PGF₁α	102.01	12.82	91.50	6.49	96.75	9.37
8-iso-PGF ₂ α	113.92	9.52	103.22	7.63	103.40	15.29
8(9)-EET	150.18	16.08	111.40	2.65	112.48	9.48
9(S)-HODE	172.33	24.73	105.72	6.30	102.03	4.08
LTB ₄	130.61	22.99	107.42	7.22	100.07	2.30
LTC ₄	119.86	32.20	101.37	8.37	101.13	3.95
LTD ₄	140.30	31.13	107.71	6.63	100.06	3.07
LTE ₄	120.35	24.06	109.84	3.07	103.58	5.93
LXA4	134.56	7.52	111.38	5.75	108.98	8.84
MAR1	135.97	21.48	104.56	8.88	102.06	3.55
PDX	130.64	18.81	105.48	13.01	101.45	1.54
PGD ₂	131.20	21.28	103.53	7.04	100.79	3.62
PGE ₂	125.20	22.41	109.18	5.68	103.26	3.79
<u>−</u> PGF₂α	147.94	21.55	115.13	4.68	105.34	4.77
RvD1	126.87	20.61	104.69	3.22	101.95	2.58
RvE1	98.36	3 04	98 72	5 59	95.94	14 67
TXB ₂	125 13	20 40	104 07	2 74	106 39	7 99
AA	349 17	26.16	120 76	40 71	125 30	25.53
ALA	216 22	55 50	109 50	12 29	96.96	0.97
	210.22	00.00	100.00	12.23	00.00	0.07

Table S7: Inter-3-day variability of accuracy and precision/RSD at 0.1, 1 and 10 ng/ml; PUFAs are 10x higher concentrated (n=3)

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Allergy

DGLA	2846.77	134.75	91.21	63.96	68.53	6
DHA	10738.50	142.89	1448.42	114.05	118.85	
EPA	296.48	56.65	120.56	44.51	85.10	1

	4				
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	5	Metabolite	LOD (ng/ml)	LLOQ (ng/ml)	Correlation Coefficient
7 $\pm 11,12-DHET$ 0.005 0.05 0.99993 9 $\pm 12,13-DHOME$ 0.005 0.5 0.99993 10 $\pm 17+HDHA$ 0.01 1 0.99993 11 $\pm 18-HEPE$ 0.05 0.5 0.99993 12 $\pm 4-HDHA$ 0.5 1 1 13 $\pm 5.6-DHET$ 0.05 0.5 0.99992 14 $\pm 8(9)-DHET$ 0.005 0.5 0.99992 15 $\pm 9-HETE$ 0.005 0.5 0.99993 16 $\pm 9,10-DHOME$ 0.1 1 0.99993 11(12)-EET 0.005 0.5 0.99993 11(12)-HETE 0.005 1 0.99993 12(S)-HETE 0.05 1 0.99993 13(S)-HODE 0.1 0.5 0.99993 14(15)-EPTE 0.1 0.5 0.99992 13(S)-HODE 0.1 0.5 0.99992 15(S)-HEPE 0.05 0.5 0.99992	6	±11-HDHA	0.05	0.5	0.99999
8 $\pm 12,13-DHOME$ 0.005 0.5 0.99978 10 $\pm 17.HDHA$ 0.1 1 0.99978 11 $\pm 17.HDHA$ 0.05 1 0.99978 12 $\pm 41.HDHA$ 0.5 1 1 13 $\pm 5.6-DHET$ 0.05 0.05 1 14 $\pm 8(9)-DHET$ 0.005 0.5 0.99992 15 $\pm 9.HETE$ 0.005 0.5 0.99991 16 $\pm 9.(D-DHCME$ 0.1 1 0.9992 17 11(12)-EET 0.005 0.5 0.99993 19 12-cox-FTE 0.5 1 0.99983 11 12(S)-HETE 0.1 0.5 0.99993 12(S)-HETE 0.1 0.5 0.99983 13(S)-HODE 0.1 0.5 0.99983 14(15)-EET 0.01 0.5 0.99984 14(15)-EET 0.05 0.5 0.99984 15(S)-HETE 0.25 0.05 0.99984	7	±11,12-DHET	0.005	0.05	0.99999
9 ± 13 -HDHA 0.1 1 0.99978 10 ± 17 -HDHA 0.005 1 0.99983 12 ± 4 -HDHA 0.5 1 1 13 ± 5.0 -DHET 0.05 0.5 0.99983 14 $\pm 8(9$ -DHET 0.005 0.5 0.99992 15 ± 9 -HETE 0.005 0.5 0.99993 16 ± 9 ,10-DHOME 0.1 1 0.99993 11(S)-HETE 0.005 0.5 0.99993 12(S)-HETE 0.05 1 0.99992 13(S)-HODE 0.1 0.5 0.99993 14(15)-EPTE 0.1 0.5 0.99992 13(S)-HODE 0.1 0.5 0.99983 14(15)-EPTE 0.1 0.5 0.99992 13(S)-HETE 0.05 0.5 0.99984 14(15)-EPTE 0.1 1.0 0.99984 15(S)-HETE 0.05 0.5 0.99984 15(S)-HETE 0.05	8	±12,13-DiHOME	0.005	0.5	0.99993
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	9	±13-HDHA	0.1	1	0.99978
11 ± 18 -HEPE 0.05 0.5 0.99983 12 ± 4 -HDHA 0.5 1 1 13 ± 5 -0-HET 0.05 0.05 1 14 $\pm 8(9)$ -DHET 0.005 0.5 0.99999 15 ± 9 -HETE 0.005 0.5 0.99999 16 ± 9 -HETE 0.005 0.5 0.99999 11(2)-HETE 0.005 0.5 0.99993 11(2)-HETE 0.005 1 0.99998 11(S)-HETE 0.005 1 0.99999 12(S)-HEPE 0.5 1 0.99999 14(15)-EDT 0.01 0.5 0.99989 14(15)-EDT 0.01 0.5 0.99985 15(S)-HEPE 0.05 0.5 0.99992 15(S)-HEPE 0.05 NA 0.99984 15(S)-HEPE 0.05 NA 0.99983 15(S)-HEPE 0.05 NA 0.99984 15(S)-HEPE 0.05 0.05 0.9	10	±17-HDHA	0.005	1	0.99999
12 $\frac{1}{45,6-DHET}$ 0.05 1 1 13 $\frac{1}{45,6-DHET}$ 0.05 0.05 1 14 $\frac{1}{8}(9)-DHET$ 0.005 0.5 0.99992 15 $\frac{1}{9}$ -HETE 0.005 0.5 0.99991 16 $\frac{1}{9}$ -HETE 0.005 0.5 0.99991 11(12)-EET 0.005 0.5 0.99993 11(12)-EET 0.005 1 0.99998 12(S)-HODE 0.1 0.5 0.99998 12(S)-HODE 0.1 0.5 0.99998 13(S)-HODE 0.1 0.5 0.99989 14(15)-EPETE 0.1 0.5 0.99988 15(S)-HETE 0.25 0.05 0.99984 15(S)-HETE 0.25 0.05 0.99984 15(S)-HETE 0.25 0.05 0.99984 15(S)-HETE 0.05 0.05 0.99984 15(S)-HETE 0.05 0.05 0.99984 15(S)-HETE 0.05 0.05	11	±18-HEPE	0.05	0.5	0.99993
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	12	±4-HDHA	0.5	1	1
14 $\pm 8(9)$ -DHET 0.005 0.5 0.99992 15 ± 9 -HETE 0.005 0.5 0.99991 16 ± 9 -IO-DiHOME 0.1 1 0.09991 11(12)-EET 0.005 0.5 0.999931 12:0x0-ETE 0.5 1 0.99992 12:(S)-HETE 0.005 1 0.999991 12:(S)-HETE 0.01 0.5 0.999992 13:(S)-HODE 0.1 0.5 0.999992 14:(15)-EET 0.01 0.5 0.99992 14:(15)-EET 0.01 0.5 0.999885 15:(S)-HETE 0.05 0.5 0.99992 15:(S)-HETE 0.05 0.5 0.99992 15:(S)-HETE 0.05 0.5 0.99994 15:(S)-HETE 0.005 NA 0.99983 0:(HETE 0.01 NA 0.99985 15:(S)-HETE 0.01 NA 0.99985 15:(S)-HETE 0.05 0.05 0.99991	13	±5,6-DHET	0.05	0.05	1
15 $\pm 9.10-\text{DiHOME}$ 0.1 1 0.99999 16 $\pm 9.10-\text{DiHOME}$ 0.1 1 0.99999 17 11(12)-EET 0.005 0.05 0.99999 18 11(12)-HETE 0.005 0.05 0.99999 12-0x0-ETE 0.5 0.5 0.99997 20 12(S)-HEPE 0.5 1 0.99999 21 12(S)-HETE 0.01 0.5 0.99999 22 13(S)-HODE 0.1 0.5 0.99999 23 14(15)-EET 0.01 0.5 0.999885 25 15-0x0-ETE 0.05 0.5 0.999982 26 15(S)-HETE 0.1 10 0.99988 27 15(S)-HETE 0.05 NA 0.99988 30 20-HETE 0.01 A 0.99985 31 5-0x0ETE 0.005 NA 0.99985 32 5(G)-HETE 0.5 0.05 1 33 5(14	±8(9)-DHET	0.005	0.5	0.99992
16 ± 9.10 -DiHOME 0.1 1 0.9999 17 11(2)-ET 0.005 0.5 0.99993 11(2)-HETE 0.05 0.05 0.99993 12-oxo-ETE 0.5 1 0.99998 21 12(5)-HETE 0.05 1 0.99999 21 12(5)-HETE 0.01 0.5 0.99999 23 14(15)-EET 0.1 0.5 0.99999 24 14(15)-EET 0.1 0.5 0.99986 24 14(15)-EET 0.1 0.5 0.99986 25 15-oxo-ETE 0.05 0.5 0.99992 26 15(S)-HETE 0.25 0.05 0.99994 27 15(S)-HETE 0.05 NA 0.99994 26 0.5 0.99994 15(S)-HETE 0.05 0.99994 30 20-HETE 0.01 1 1 0.99945 31 5(S)-HETE 0.5 0.05 0.99994 32 <td>15</td> <td>±9-HETE</td> <td>0.005</td> <td>0.5</td> <td>0.99999</td>	15	±9-HETE	0.005	0.5	0.99999
11 11(12)-EET 0.005 0.5 0.99999 11(5)-HETE 0.005 0.05 0.99993 12-oxo-ETE 0.5 1 0.99998 12(S)-HEPE 0.5 1 0.99998 12(S)-HETE 0.005 1 0.99999 13(S)-HODE 0.1 0.5 0.99999 14(15)-EET 0.01 0.5 0.99989 14(15)-EET 0.01 0.5 0.99989 14(15)-EETE 0.1 0.5 0.99981 15(S)-HETE 0.05 0.5 0.99994 15(S)-HETE 0.05 0.5 0.99994 15(S)-HETE 0.01 1 0.99984 15(S)-HETE 0.005 NA 0.99984 15(S)-HETE 0.005 0.05 0.99992 15(S)-HETE 0.005 0.05 0.99992 15(S)-HETE 0.005 0.05 0.99992 15(S)-HETE 0.05 0.05 0.99992 15(S)-HETE 0.05 <	16	±9,10-DiHOME	0.1	1	0.9999
18 11(S)-HETE 0.005 0.05 0.99933 12-0xo-ETE 0.5 0.5 0.999971 20 12(S)-HEPE 0.5 1 0.99998 21 12(S)-HETE 0.005 1 0.99999 23 14(15)-EET 0.01 0.5 0.99999 24 14(15)-EET 0.01 0.5 0.99999 25 15-0xo-ETE 0.05 0.5 0.99995 26 15(S)-HEPE 0.05 0.5 0.99994 27 15(S)-HEPE 0.05 NA 0.99994 28 15(S)-HEPE 0.05 NA 0.99994 29 15(S)-HETE 0.01 1 0.99946 31 5-0xoETE 0.005 NA 0.99992 32 5(6)-EET 0.01 NA 0.99992 33 5(S)-HETE NA NA 0.99993 34 5(S)-HETE NA NA NA 35 5(S)-HETE	17	11(12)-EET	0.005	0.5	0.99999
19 12-oxo-ETE 0.5 0.5 0.99977 20 12(S)-HEPE 0.5 1 0.99998 21 12(S)-HETE 0.005 1 0.99999 23 14(15)-EET 0.01 0.5 0.99989 24 14(15)-EET 0.1 0.5 0.99985 25 15-oxo-ETE 0.05 0.5 0.99992 26 15(S)-HEPE 0.05 0.5 0.99992 27 15(S)-HETE 0.1 10 0.99984 28 15(S)-HETE 0.005 NA 0.99998 29 15(S)-HETE 0.005 NA 0.99984 20-HETE 0.01 1 0.99946 21 5-oxoETE 0.005 0.05 0.99992 31 5-oxoETE 0.05 0.05 1 33 5(S)-HETE NA NA N/A 34 5(S)-HETE 0.05 0.1 0.99998 37 8-iso-PGF ₂ a	18	11(S)-HETE	0.005	0.05	0.99993
20 $12(S)-HEPE$ 0.5 1 0.99998 21 $12(S)-HETE$ 0.005 1 0.99999 22 $13(S)-HODE$ 0.1 0.5 0.99999 23 $14(15)-EET$ 0.01 0.5 0.99989 24 $14(15)-EET$ 0.01 0.5 0.99985 25 15 -cox-ETE 0.05 0.5 0.99992 26 $15(S)-HEPE$ 0.05 0.5 0.99992 26 $15(S)-HEPE$ 0.05 0.99994 28 $15(S)-HEPE$ 0.005 NA 0.99994 29 $15(S)-HETE$ 0.01 1 0.99946 30 $20-HETE$ 0.01 NA 0.99946 31 5 -coeETE 0.005 0.05 0.99992 35 $5(S)-HETE$ 0.05 0.05 0.99998 33 $5(S)-HETE$ 0.05 0.5 0.99998 34 $5(S)-HETE$ 0.05	19	12-oxo-ETE	0.5	0.5	0.99977
12(S)-HETE 0.005 1 0.99999 13(S)-HODE 0.1 0.5 0.99999 14(15)-EET 0.01 0.5 0.99988 14(15)-EETE 0.1 0.5 0.99988 14(15)-EETE 0.05 0.5 0.99988 15(S)-HETE 0.05 0.5 0.99992 15(S)-HETE 0.05 0.5 0.99994 15(S)-HETE 0.05 NA 0.99984 20 15(S)-HETE 0.01 1 0.99984 30 20-HETE 0.01 1 0.99984 30 20-HETE 0.01 NA 0.99953 31 5-0x0ETE 0.005 0.05 0.99992 32(S)-HEPE 0.05 0.05 1 0.99953 33 5(S)-HEPE 0.05 0.05 1 35 5(S)-HEPE 0.05 0.5 0.99951 36 6-keto-PGF ₁ a 0.01 0.1 0.99998 37 8-iso-PGF ₂ a	20	12(S)-HEPE	0.5	1	0.99998
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	21	12(S)-HETE	0.005	1	0.99999
14(15)-EET 0.01 0.5 0.99889 24 14(15)-EpETE 0.1 0.5 0.99885 25 15-oxo-ETE 0.05 0.5 0.99992 26 15(S)-HEPE 0.05 0.5 0.99992 27 15(S)-HETE 0.25 0.05 0.99994 28 15(S)-HETE 0.1 10 0.99984 29 15(S)-HETE 0.005 NA 0.99958 30 20-HETE 0.005 0.05 0.99992 31 5-oxoETE 0.005 0.05 0.99993 32 5(6)-HET 0.01 NA 0.99953 33 5(S)-HEPE 0.05 0.05 0.99999 34 5(S)-HEPE 0.5 0.05 1 35 5(S)-HEPE NA NA NA 36 6(S-HEP NA NA N/A 36 6(S-HEPE 0.05 0.1 0.99958 37 8-iso-PGF ₂ a <t< td=""><td>22</td><td>13(S)-HODE</td><td>0.1</td><td>0.5</td><td>0.99999</td></t<>	22	13(S)-HODE	0.1	0.5	0.99999
24 14(15)-EpETE 0.1 0.5 0.99885 25 15-0x0-ETE 0.05 0.5 0.99995 26 15(S)-HEPE 0.05 0.5 0.99992 27 15(S)-HETE 0.25 0.05 0.99994 28 15(S)-HETE 0.1 10 0.99984 29 15(S)-HETE 0.005 NA 0.99995 30 20-HETE 0.01 1 0.99946 31 5-0x0ETE 0.005 0.05 0.99995 32 5(6)-EET 0.01 NA 0.99955 33 5(S)-HEPE 0.5 0.05 1 34 5(S)-HETE NA NA N/A 35 5(S)-HETE NA NA N/A 36 6-keto-PGF ₁ a 0.01 0.1 0.99995 37 8-iso-PGF ₂ a 0.005 0.1 0.99998 41 LTC4 0.005 0.5 0.99998 41 LTC4<	23	14(15)-EET	0.01	0.5	0.99989
25 15-0x0-ETE 0.05 0.5 0.99995 26 15(S)-HETE 0.05 0.5 0.99992 27 15(S)-HETE 0.25 0.05 0.99994 28 15(S)-HETE 0.1 10 0.99984 29 15(S)-HETE 0.01 1 0.99984 30 20-HETE 0.01 1 0.99992 31 5-0x0ETE 0.005 0.05 0.99992 32 5(6)-HEPE 0.01 NA 0.99958 33 5(S)-HEPE 0.05 0.05 0.99999 34 5(S)-HEPE 0.5 0.05 1 35 5(S)-HETE NA NA N/A 36 6-keto-PGF_1a 0.01 0.1 0.99958 37 8-iso-PGF_2a 0.005 0.05 0.99998 40 LTE4 0.005 0.5 0.99998 41 LTC4 0.005 0.5 0.99998 42 LTD4<	24	14(15)-EpETE	0.1	0.5	0.99885
26 15(S)-HEPE 0.05 0.5 0.99992 27 15(S)-HETE 0.25 0.05 0.99994 28 15(S)-HETE 0.1 10 0.99984 29 15(S)-HETE 0.005 NA 0.99984 29 15(S)-HPETE 0.005 NA 0.99985 30 20-HETE 0.01 1 0.99946 31 5-0x0ETE 0.005 0.05 0.99992 32 5(S)-HEPE 0.05 0.05 0.99995 33 5(S)-HEPE 0.05 0.05 1 34 5(S)-HEPE 0.5 0.05 1 35 5(S)-HEPE NA NA N/A 36 6-keto-PGF ₂ a 0.005 0.5 0.99995 38 8(9-EET 0.05 0.1 0.99994 39 9(S)-HODE 0.1 0.05 0.99998 41 LTC4 0.005 0.5 0.99998 42 LTD4 <td>25</td> <td>15-oxo-ETE</td> <td>0.05</td> <td>0.5</td> <td>0.99995</td>	25	15-oxo-ETE	0.05	0.5	0.99995
27 15(S)-HETE 0.25 0.05 0.99994 28 15(S)-HETE 0.1 10 0.99984 29 15(S)-HETE 0.005 NA 0.99986 30 20-HETE 0.01 1 0.99986 31 5-oxoETE 0.005 0.05 0.99992 32 5(6)-EET 0.01 NA 0.99995 33 5(S)-HEPE 0.05 0.05 0.99999 34 5(S)-HETE 0.5 0.05 1 35 5(S)-HETE 0.5 0.05 1 36 6-keto-PGF ₁ a 0.01 0.1 0.99958 37 8-iso-PGF ₂ a 0.005 0.5 0.99998 40 LTB ₄ 0.005 0.05 0.99998 41 LTC ₄ 0.005 0.5 0.99998 42 LTD ₄ 0.005 0.5 0.99998 43 LTE ₄ 0.005 0.5 0.99998 44 LXA	26	15(S)-HEPE	0.05	0.5	0.99992
28 15(S)-HETrE 0.0 10 0.99984 29 15(S)-HETrE 0.005 NA 0.99958 30 20-HETE 0.01 1 0.99946 31 5-0x0ETE 0.005 0.05 0.99992 32 5(6)-EET 0.01 NA 0.99955 33 5(S)-HEPE 0.05 0.05 0.99999 34 5(S)-HETE 0.5 0.05 1 35 5(S)-HETE NA NA N/A 36 6-keto-PGF ₁ a 0.01 0.1 0.99958 37 8-iso-PGF ₂ a 0.005 0.5 0.99951 38 8(9)-EET 0.05 0.1 0.99994 39 9(S)-HODE 0.1 0.05 0.99998 41 LTC4 0.005 0.05 0.99998 42 LTD4 0.005 0.5 0.99988 43 LTE4 0.005 0.5 0.99998 44 LXA4	27	15(S)-HETE	0.25	0.05	0.99994
Initial <	28	15(S)-HETrE	0.1	10	0 99984
100 100 100 100 1000 20 100 1 0.005 0.005 0.99992 31 $5-0x0ETE$ 0.01 NA 0.99992 32 $5(6)$ -ET 0.01 NA 0.99992 33 $5(5)$ -HEPE 0.05 0.05 0.99999 34 $5(S)$ -HEPE 0.5 0.05 0.99999 34 $5(S)$ -HETE NA NA N/A 36 6 -keto-PGF ₁ α 0.01 0.1 0.99958 37 8 -iso-PGF ₂ α 0.005 0.5 0.999951 38 $8(9)$ -EET 0.05 0.1 0.99998 40 LTB ₄ 0.005 0.05 0.99998 41 LTC ₄ 0.005 0.5 0.99998 41 LTC ₄ 0.005 0.5 0.99998 44 LXA4 0.005 0.5 0.99998 44	29	15(S)-HpETE	0.005	NA	0 99958
Solution District District District District 31 $5 - xx ETE$ 0.005 0.09992 0.99992 32 $5(6)$ -EET 0.01 NA 0.99992 33 $5(S)$ -HEPE 0.05 0.05 0.999993 34 $5(S)$ -HETE 0.5 0.05 1 35 $5(S)$ -HETE NA NA N/A 36 6 -keto-PGF ₂ a 0.01 0.1 0.999951 37 8 -iso-PGF ₂ a 0.005 0.5 0.999941 39 $9(S)$ -HODE 0.1 0.05 0.999981 40 LTB ₄ 0.005 0.05 0.999981 41 LTC ₄ 0.005 0.05 0.999981 42 LTD ₄ 0.005 0.5 0.999981 43 LTE ₄ 0.005 0.5 0.999981 44 LXA4 0.005 0.5 0.999981 45 MAR1 0	30	20-HETE	0.01	1	0 99946
32 32 32 33 33 33 33 33 33 33 35 <t< td=""><td>31</td><td>5-0x0FTF</td><td>0.005</td><td>0.05</td><td>0 99992</td></t<>	31	5-0x0FTF	0.005	0.05	0 99992
33 $5(S)$ -HEPE 0.05 0.05 0.99999 34 $5(S)$ -HEPE 0.05 0.05 0.99999 34 $5(S)$ -HEPE 0.05 0.05 1 35 $5(S)$ -HETE NA NA N/A 36 6 -keto-PGF ₁ α 0.01 0.1 0.99998 37 8 -iso-PGF ₂ α 0.005 0.5 0.999951 38 $8(9)$ -EET 0.05 0.1 0.99994 39 $9(S)$ -HODE 0.1 0.05 0.99998 40 LTB ₄ 0.005 0.05 0.99998 41 LTC ₄ 0.005 0.5 0.99998 41 LTC ₄ 0.005 0.5 0.99998 42 LTD ₄ 0.005 0.5 0.99998 43 LTE ₄ 0.005 0.5 0.99998 44 LXA4 0.005 0.5 0.99998 47 PGD ₂	32	5(6)-FFT	0.01	NA	0 99955
34 $5(5)$ -HETE 0.5 0.05 $0.00000000000000000000000000000000000$	33	5(S)-HEPE	0.05	0.05	0 99999
$O(y)$ $O(y)$ $O(y)$ $O(y)$ $O(y)$ 35 $5(S)$ -HpETE NA NA N/A 36 6 -keto-PGF_1\alpha 0.01 0.1 0.99958 37 8 -iso-PGF_2\alpha 0.005 0.5 0.99951 38 $8(9)$ -EET 0.05 0.1 0.99994 39 $9(S)$ -HODE 0.1 0.05 0.99998 40 LTB_4 0.005 0.05 0.99998 41 LTC_4 0.005 0.5 0.99998 42 LTD_4 0.005 0.5 0.99998 43 LTE_4 0.005 0.5 0.99998 44 LXA4 0.005 1 0.99988 46 PDX 0.005 0.5 0.999975 47 PGD_2 0.005 0.5 0.999975 48 PGE_2 0.005 0.5 0.999995 50 RvD1 0.005 0.5 0.999999 51 RvD1	34	5(S)-HETE	0.5	0.05	
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	35	5(S)-HpETE	NA	NA	N/A
37 8-iso-PGF ₂ a 0.005 0.5 0.99951 38 8(9)-EET 0.05 0.1 0.99994 39 9(S)-HODE 0.1 0.05 0.99998 40 LTB ₄ 0.005 0.05 0.99998 41 LTC ₄ 0.005 0.005 0.99998 42 LTD ₄ 0.005 0.5 0.99998 43 LTE ₄ 0.005 0.5 0.99998 44 LXA4 0.005 0.5 0.99998 45 MAR1 0.005 0.5 0.99998 46 PDX 0.005 0.5 0.99998 47 PGD ₂ 0.005 0.5 0.99998 48 PGE ₂ 0.005 0.5 0.999975 49 PGE ₂ a 0.005 0.5 0.999995 50 RvD1 0.005 0.5 0.999995 51 RVD1 0.005 0.5 0.999995 52 TXB ₂	36	6-keto-PGF ₁ α	0.01	0.1	0.99958
38 $8(9)$ -EET 0.05 0.1 0.99994 39 $9(S)$ -HODE 0.1 0.05 0.99998 40 LTB_4 0.005 0.005 0.99998 41 LTC_4 0.005 0.005 0.99998 41 LTC_4 0.005 0.005 0.99998 42 LTD_4 0.005 0.5 0.99998 43 LTE_4 0.005 0.5 0.99998 44 $LXA4$ 0.005 0.5 0.99998 45 MAR1 0.005 0.5 0.99998 46 PDX 0.005 0.5 0.999975 46 PGE ₂ 0.005 0.5 0.999995 50 RvE1 $0.$	37	8-iso-PGF ₂ α	0.005	0.5	0.99951
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	38	8(9)-EET	0.05	0.1	0.99994
GG_{2}	39	9(S)-HODE	0.1	0.05	0.99998
LTC40.0050.0050.0995941LTC40.0050.050.99988LTD40.0050.50.9998843LTE40.0050.50.9999844LXA40.0050.50.9999845MAR10.00510.9998846PDX0.0050.50.99994547PGD20.0050.50.99997548PGE20.0050.50.9999550RvD10.0050.50.99999551RvE10.0050.50.99999552TXB20.0050.250.99999553AA5001000.9994854ALA501000.9999555DGLA5010.9998656DHA500NA0.9956857EPA501000.99911	40	LTB	0.005	0.05	0,99998
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	41		0.005	0.005	0.99959
LTE_4 0.005 0.5 0.99998 43 LTE_4 0.005 0.5 0.99998 44 $LXA4$ 0.005 0.5 0.99996 45MAR1 0.005 1 0.99988 46PDX 0.005 0.5 0.99945 47PGD2 0.005 0.5 0.99975 48PGE2 0.005 0.5 0.99947 49PGF2\alpha 0.005 0.5 0.99947 50RvD1 0.005 0.5 0.99995 51RvE1 0.05 0.95 0.99999 52TXB2 0.005 0.25 0.99996 53AA 500 100 0.99948 54ALA 50 100 0.99986 55DGLA 50 100 0.99986 56DHA 500 NA 0.99568 57EPA 50 100 0.99911	42		0.005	0.5	0.99988
III = 1 + 1 + 1 + 1 + 1 + 1 + 1 + 1 + 1 + 1	43		0.005	0.5	0 99998
A5MAR10.00510.0998846PDX0.0050.50.9994547PGD20.0050.50.9997548PGE20.0050.50.9994749PGF2a0.0050.50.9999550RvD10.0050.50.9999551RvE10.0050.50.9999952TXB20.0050.250.9999653AA5001000.9994854ALA501000.999555DGLA501000.9998656DHA500NA0.9956857EPA501000.99911	44	L XA4	0.005	0.5	0,99996
A6PDX0.0050.50.09945 46 PDZ0.0050.50.99945 47 PGD20.0050.50.99975 48 PGE20.0050.50.99947 49 PGF2a0.0050.50.99995 50 RvD10.0050.50.99995 51 RvE10.0050.050.99999 51 RvE10.0050.050.99999 52 TXB20.0050.250.99996 53 AA5001000.99948 54 ALA501000.9995 55 DGLA5010.99986 56 DHA500NA0.99568 57 EPA501000.99911	45	MAR1	0.005	1	0.99988
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	46	PDX	0.005	0.5	0.99945
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	47	PGD ₂	0.005	0.5	0.99975
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	48	PGE ₂	0.005	0.5	0.99947
RvD1 0.005 0.5 0.99999 51 RvE1 0.05 0.05 0.99999 52 TXB2 0.005 0.25 0.99996 53 AA 500 100 0.99995 54 ALA 500 100 0.9995 55 DGLA 500 1 0.99986 56 DHA 500 NA 0.99568 57 EPA 50 100 0.99911	49	PGF ₂ a	0.005	0.5	0.99995
RvE1 0.05 0.05 0.99999 52 TXB2 0.005 0.25 0.99996 53 AA 500 100 0.99948 54 ALA 500 100 0.9995 55 DGLA 50 1 0.99986 56 DHA 500 NA 0.99568 57 EPA 50 100 0.99911	50	RvD1	0.005	0.5	0.99999
TXB2 0.005 0.25 0.99996 53 AA 500 100 0.99948 54 ALA 50 100 0.9995 55 DGLA 50 1 0.99986 56 DHA 500 NA 0.99568 57 EPA 50 100 0.99911	51	RvE1	0.05	0.05	0.99999
53 AA 500 100 0.99948 54 ALA 500 100 0.9995 55 DGLA 500 1 0.99986 56 DHA 500 NA 0.99568 57 EPA 50 100 0.99911	52	TXB ₂	0.005	0.25	0.99996
54 ALA 50 100 0.9995 55 DGLA 50 1 0.99986 56 DHA 500 NA 0.99568 57 EPA 50 100 0.99911	53	AA	500	100	0.99948
55 DGLA 50 1 0.99986 56 DHA 500 NA 0.99568 57 EPA 50 100 0.99911	54	ALA	50	100	0,9995
56 DHA 500 NA 0.99568 57 EPA 50 100 0.99911	55	DGLA	50	1	0,99986
57 EPA 50 100 0.99911	56	DHA	500	NA	0.99568
	57	EPA	50	100	0.99911

Table S8: Limit of detection (LOD) and lower limit of quantitation (LLOQ) with correlation coefficient

Allergy

to per period

Table S9: Comparison of Frankfurt and Munich LC-MS/MS panels	s, data	are shown	as mean	± SD for
6 different blood donors.				

Metabolite	Frankfurt (ng/ml) (method 2)	Munich (ng/ml) (method 1)
PGD ₂	0.009 ± 0.005	0.012 ± 0.008
PGE ₂	0.012 ± 0.012	0.008 ± 0.007
PGF₂α	0.018 ± 0.017	0.050 ± 0.065
LTB ₄	9.061 ± 4.342	29.097 ± 34.562
TXB ₂	0.298 ± 0.380	0.186 ± 0.210
5-HETE	8.411 ± 9.181	11.913 ± 12.668
15-HETE	0.237 ± 0.547	0.110 ± 0.066

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