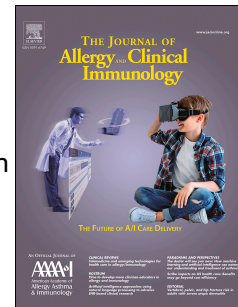


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Metabolome and lipidome derangements during a severe mast cell activation event in a patient with indolent systemic mastocytosis

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Conflicts of Interest

Thomas Boehm and Bernd Jilma are co-inventors on a patent about the use of recombinant human DAO in the treatment of conditions with excess histamine. The other authors declare they have no conflicts of interest.

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Key messages

- Depletion of ornithine, citrulline and many lysophosphatidylcholine lipid variants within a few hours during mast cell activation in a mastocytosis patient
- Reduction of arginine with increased metabolites leads to compromised nitric oxide signaling
- Histamine as possible key driver of severe clinical symptoms with rapid clinical improvement after 100-fold elevation of endogenous diamine oxidase

Capsule summary: Extensive mast cell activation leads to severe endothelial dysfunction with depletion of important amino acids in the nitric oxide signaling pathway and many lysophosphatidylcholine (LPC) variants with histamine possibly as the key driving force behind the severe clinical symptoms.

Keywords: histamine, diamine oxidase (DAO), mast cell activation syndrome (MCAS), mastocytosis, lysophosphatidylcholine (LPC), lysophosphatidic acid (LPA), citrulline, ornithine

Abbreviations:

ADMA	=	Asymmetric Dimethylarginine
aPTT	=	Activated Partial Thromboplastin Time
ATX	=	Autotaxin
DAO	=	Diamine Oxidase
eNOS	=	Endothelial Nitric Oxide Synthase
LC-MS	=	Liquid Chromatography Mass Spectrometry
LPC	=	Lysophosphatidylcholine
LPA	=	Lysophosphatidic Acid
MC	=	Mast Cell
MCAS	=	Mast Cell Activation Syndrome
MUV	=	Medical University of Vienna
SDMA	=	Symmetric Dimethylarginine
fSBL	=	Functional Small Bowel Length

ABSTRACT

Background: The number of mast cells in various organs is manifold elevated in individuals with systemic mastocytosis. Degranulation can lead to life-threatening symptomatology. No data have been published about the alterations of the metabolome and lipidome during an attack.

Objective: To analyze metabolomics and lipidomics changes during the acute phase of a severe mast cell activation event.

Methods: Forty-three metabolites and 11 lipid classes comprising 200 subvariants from multiple plasma samples in duplicate, covering 72 hours of a severe mast cell activation attack with nausea and vomiting, were compared to two baseline samples using quantitative liquid chromatography mass spectrometry.

Results: A strong enterocyte dysfunction reflected in an almost 20-fold reduction in the functional small bowel length extrapolated from strongly reduced ornithine and citrulline concentrations was very likely secondary to severe endothelial cell dysfunction with hypoperfusion and extensive vascular leakage. Highly increased histamine and lactate concentrations accompanied the peak in clinical symptoms. Elevated asymmetric and symmetric dimethylarginine combined with reduced arginine levels compromised endothelial nitric oxide synthase activity and nitric oxide signaling. Specific and extensive depletion of many lysophosphatidylcholine variants indicates localized autotaxin activation and lysophosphatidic acid release. A strong correlation of clinical parameters with histamine concentrations and symptom reduction after 100-fold elevated plasma diamine oxidase concentrations imply histamine is the key driver of the acute phase.

Conclusions: Rapid elimination of elevated histamine concentrations using recombinant human diamine oxidase, supplementation of lysophosphatidylcholine for immunomodulation, inhibition of autotaxin activity and/or blockade of lysophosphatidic acid receptors might represent new treatment options for life-threatening mast cell activation events.

INTRODUCTION

Mastocytosis is characterized by an increased number of mast cells (MC) in various tissues¹. In healthy individuals the lung, the small intestine and the skin store roughly 10, 50 and 30 mg histamine respectively (Table E3 and E4). The total amount of tissue histamine would be approximately 100 mg, with more than 90% stored in MC granules. The amount of histamine stored in circulating basophils is less than 1 mg. Based on the elevated baseline tryptase and histamine concentrations in mastocytosis patients and their correlation with the MC burden, the number of MCs in mastocytosis patients when compared to healthy individuals are at least two to ten-fold higher²⁻⁴. The elevated MC burden renders MC activation in mastocytosis patients life-threatening. Desborough et al. (1990)⁵ reported a case of systemic mastocytosis and perioperative anaphylaxis with plasma histamine concentrations greater than 500 ng/ml (4.5 μ M), which was associated with reduced vascular resistance. The mean peak histamine concentration in severe acute anaphylaxis in non-mastocytosis patients was 140 ng/ml⁶. Plasma histamine concentrations as low as 5 ng/ml start to induce significant hypotension and more than 10 ng/ml can lead to bronchospasm, arrhythmia and finally cardiac arrest^{7,8}. There is limited knowledge about definite trigger molecules in mastocytosis patients or indeed in other patients suffering from mast cell activation syndrome (MCAS). There is even less definitive knowledge regarding why some MCAS events last many hours and sometimes even days in contrast to the mainly IgE/Fc ϵ RI-mediated rapid MC degranulation during “classical anaphylaxis”. Mast cells might release inflammatory mediators not only via acute and explosive or slower piece-meal degranulation but also through the release of exosomes or other secreted extracellular vesicles^{9,10}. The number of plasma exosomes in mastocytosis patients is significantly higher compared to healthy controls, and strongly correlates with tryptase concentrations, suggesting the increased MC burden as exosome source⁹. Classification of MC activation events is currently possible following a rapid versus protracted course, or by quantifying the fast release of preformed, or slower release of freshly synthesized mediators¹¹. Nevertheless, in 25% of severe anaphylaxis cases tryptase concentrations, an accepted MC activation marker, were not elevated¹². The short half-life of histamine seems to render this potent signaling molecule relatively unsuitable for the differential diagnosis of MC activation events^{6,13}. The identification and quantification of the additional metabolites in plasma which are selectively or even specifically elevated during MC activation events might help to properly diagnose and may potentially allow us to design better preventative and therapeutic measures.

123 We are not aware of any publications describing metabolomic and lipidomic alterations
124 during severe MC activation in humans. The mediators released in the “classical” acute
125 anaphylaxis-type reactions, including leukotriene and prostaglandin lipids, have been
126 described many times^{6,13–16}, but what happens to the metabolome and lipidome when MCs are
127 activated and degranulate over many hours?
128 Here, we characterize the metabolomics and lipidomics derangements in a mastocytosis
129 patient during severe MC activation. This patient was treated in the intensive care unit and
130 had acute life-threatening symptoms for several hours.

METHODS

Mast cell activation event

The patient was diagnosed with indolent systemic mastocytosis in 2008 and is positive for the KIT D816V mutation. He is a 42 year old Caucasian male with no comorbidities. Bone marrow biopsy confirmed infiltrating mast cells staining positive for c-kit, CD25 and tryptase with minimal staining for CD2 and negative staining for CD34. This mastocytosis patient suffers from recurrent events once or twice per year. These events follow a similar course and demonstrate similar symptoms including fever, but are of varying severity. An infectious cause was not diagnosed during several severe events in the past.

The symptoms, possibly triggered by an upper respiratory tract infection (PCR negative for seasonal influenza viruses), started in the evening with 38°C fever and nausea. The patient ate for the last time at 6 PM. At midnight the patient contacted BJ because he was afraid of developing “anaphylaxis”. The patient had measured his blood pressure and heart rate, and these were still normal at that time. The symptoms worsened and the patient came to the emergency department at 6.30 AM. In all relevant figures the time of admission is represented as the 0 hour time point. Mean arterial pressure (MAP) and diastolic pressure were already reduced. The patient had vomited a few times at home, and continued to do so throughout the first few hours in the hospital. Following transfer from the emergency department the patient was treated in intensive care starting approximately 8 hours after admission. Medications, including electrolyte solutions, and the time of their administration are listed in Fig 1E. The CoolGard system (Zoll, Sunnyvale; CA) was started roughly 10 hours post admission and was used for at least 24 hours.

Most presented data were normalized to baseline (BL) values obtained either from two visits as part of the DAOMAST study, or other regular clinic visits where the patient did not have any symptoms. The DAOMAST study was a non-interventional study based on questionnaires and blood draws including 43 mastocytosis patients and is briefly described in Boehm 2019¹⁷. The visits used for BL normalization were in April 2013 and March 2014 and the event happened in February 2018. The patient provided informed consent and was included in an anaphylaxis clinical study (EC:1018/2015) approved by the Ethics Committee of the Medical University of Vienna (MUV). The BL values for blood pressure and heart rate were measured 24-times at home by the patient during a 16 day symptom-free interval in March 2018. The mean (SD) systolic and diastolic blood pressure were 124/75 (7.7; 5.4) mmHg with a mean arterial pressure (MAP) of 91 (5.9) mmHg and a heart rate of 68 (7.8).

Other measurements

The DAO, tryptase and histamine Medical University of Vienna (MUV) data were recently published¹⁷ and are included here to provide a complete picture of the MC activation event. Histamine was also measured in the metabolomics samples and showed a high correlation ($R^2 = 0.87$; $p = 0.00021$) with the state of the art histamine ELISA measurements described in Boehm 2019¹⁷. Other clinical parameters were measured in the intensive care unit and in the clinical chemistry laboratory of the Vienna General Hospital using accredited standard methods.

The functional small bowel length (fSBL) was calculated using the following equation: plasma citrulline (μM) = $0.23 \times \text{small bowel length (cm)} + 5.68 (\mu\text{M})$ ¹⁸. In this publication a total of 57 patients were included. Twenty patients suffered from transient and 37 from permanent intestinal failure. The remnant small bowel length was evaluated on radiograph films and together with the measured plasma citrulline concentrations used to generate the linear regression line. Evaluation of post-duodenal small bowel length during an operation or using radiograph films was highly correlated validating the radiograph film method ($R = 0.94$; $p < 0.0001$)¹⁸. The citrulline synthesis capacity of colonic enterocytes is at least 10-fold lower compared to the jejunum or ileum. Citrulline is not used by the intestine and is not taken up by the liver bypassing splanchnic extraction. Therefore, plasma citrulline concentrations reflect metabolic health of small bowel enterocytes.

Additional methods including Statistical Analysis are described in the Online Repository.

RESULTS

Many clinical parameters are strongly altered during mast cell activation but normalize after rapid increase in diamine oxidase (DAO) and decrease in histamine concentrations

This patient with indolent systemic mastocytosis frequently suffers from MC activation events leading to hospital admission. Under non-event conditions only the anti-histamine medications cetirizine and ranitidine are required for the patient to remain almost symptom-free. According to the patient the trigger is frequently an upper respiratory tract infection. In the described event, the patient came to the emergency department about 12 hours after the first symptoms with fever, generalized flush, nausea and vomiting.

Nevertheless, symptoms of respiratory illness such as cough or rhinitis were not present.

Three lung x-rays within the first 24 hours post admission were negative for signs of infection and lung auscultation was unremarkable. Oxygenation was > 96% for the entire hospital stay. Nonspecific signs of a gastrointestinal infection were nausea and vomiting and mild epigastric pain. Abdominal pain, defense and diarrhea were not present and consequently no stool PCR pathogen diagnosis was performed. In the evening prior to admission the patient had two soft stools similar to other episodes. Blood cultures were negative and there were no signs of urinary infection. In previous episodes with similar symptoms an infection was never diagnosed.

Various clinical parameters shown in Fig 1 and 2 strongly suggest that the peak of the MC activation event happened 6 to 8 hours post admission. The heart rate, activated partial thromboplastin time (aPTT), leukocytes, glucose and pH demonstrated maximum levels during this time window. The MC activation markers tryptase and histamine also reached their maximum values. Thereafter, lactate decreased at 1.1 mM/hour and the pH moved from 7.53 towards normal. Heart rate, diastolic and mean arterial pressure approached pre-attack values within a few hours (Fig 1). This peak is also reflected in the inverse correlation between tryptase and DAO versus histamine concentrations (Fig 2). Histamine concentrations returned to pre-event concentrations at the latest 10 hours post admission. The patient has a known allergy to non-steroidal anti-inflammatory drugs and therefore this drug class could not be used to lower body temperature, which was consistently above 38.5°C until a CoolGard system was applied. It was necessary to use the CoolGard device for more than two days. When cooling was stopped 24 hours after admission, body temperature once again increased to 39°C. Neither vasoconstrictors nor antibiotics were administered. Two to four systemic inflammatory response syndrome (SIRS) criteria were fulfilled during the first 10 hours post admission, classifying this event as SIRS. Two of the MCAS consensus criteria

(severe recurrent mast cell activation and increase in serum tryptase levels) were clearly fulfilled¹⁹. It is not possible to estimate whether Criterion C (response of symptoms to mast cell-related or mast cell-released mediator treatment modalities) was also fulfilled, because although the patient was treated with anti-histamine medications and steroids symptoms became life-threatening despite treatment. The response to treatment is not defined in the MCAS consensus criteria. Symptoms improved after approximately 8 hours post admission and therefor one could also consider Criterion C as fulfilled.

Human serum albumin and total protein concentrations decreased more significantly compared to hemoglobin and hematocrit, reflected in a separation of the two dilution curves at the peak (Fig 2, A to C). The infusion of 1000 ml electrolyte solution roughly 7 hours post-admission is seen in many figures as a decrease in concentration. Even under SIRS conditions vascular permeability of small molecular weight metabolites like amino acids versus albumin are unlikely to be equal, and therefore albumin-based hemodilution might not truly reflect vascular leakage. Binding of amino acids to albumin or other large proteins adds another level of complexity. It is unknown how fast equilibration occurs. We therefore calculated p-values using the albumin-based hemodilution curve but also calculated the dilution curve using tyrosine, which is a stable amino acid during fasting, sepsis or other stress conditions (see Online Repository). We did not correct any biochemical parameters for the dilution factors shown in Fig 2C but added the normalized albumin dilution curve to all panels as a reference where appropriate. The peak tyrosine-, albumin- and red blood cell-based percent dilutions during the first 10 hours post admission were circa 35%, 29% and 16% respectively (Fig 2).

Metabolomics data indicate severe endothelial cell dysfunction with concomitant loss of enterocyte citrulline and ornithine synthesis capabilities

Acetyl-carnitine (Ace-C; $p = 0.02$) was statistically significantly elevated concomitant with reduced carnitine (C) values. The Ace-C/C ratio was increased 4-fold ($p = 0.064$) during the peak, before decreasing rapidly to normal values 2 to 3 hours later (Fig 3, A). Creatinine was above baseline during the attack (Fig 3, B) with a corresponding decrease in the glomerular filtration rate by maximally 20% at the peak (data not shown). Contrary to tryptophan, kynurenine concentrations did not decrease despite hemodilution reflected in a 1.5-fold increased kynurenine/tryptophan ratio (Fig 3, B; $p = 0.018$).

Ornithine (baseline mean 106 μM ; SD = 7.8 μM), citrulline (baseline mean 27.4 μM ; SD = 0.08 μM) and lysine (baseline mean 214 μM ; SD = 38.1 μM) decreased statistically significantly with ornithine concentrations falling more than 5-fold from baseline values (Fig

3, D). The slopes for ornithine and citrulline concentration decreases are clearly steeper compared to the albumin levels. Arginine (baseline mean 69 μM ; SD = 22.4 μM) is also statistically significantly reduced more than 2-fold. The functional small bowel length (fSBL) estimated based on the citrulline concentrations is reduced almost 20-fold during the MC activation peak with lowest citrulline (SD) levels of 6.9 (0.39) $\mu\text{M}^{18,20}$ (Fig 3, D). The coefficients of variation (CV) for all duplicates used for calculating the fSBL are below 7%. This proxy for the functional integrity of the enterocytes decreased rapidly at a rate of 11 cm/hour ($R^2 = 98\%$) corresponding to 11% of BL level before reaching 6% of BL at the peak of the attack. The fSBL only returned to 60% of BL values 72 hours post admission. These metabolome derangements are also reflected in the time course of various ratios related to arginine metabolism and transport, with some ratios increasing more than 250% during the peak (Fig 3, E). Ratios have the advantage that they circumvent dilution effects assuming components show similar permeability and diffusion behavior. Since lysine decreased more compared to arginine, the arginine/lysine ratio increased 1.5-fold during the acute phase. The arginine breakdown products asymmetric and symmetric dimethylarginines (ADMA and SDMA) increased statistically highly significantly (p-value 0.0063 for ADMA and SDMA) with corresponding strong and statistically significant decreases of more than 2-fold in the arginine/ADMA and arginine/SDMA ratios before returning to above BL values after 72 hours (Fig 3, F).

The essential amino acid phenylalanine ($p = 0.01$) was the only amino acid statistically highly significantly increasing over time (Fig E15). Tyrosine concentrations follow roughly the course of albumin hemodilution albeit 10 to 20% lower and therefore the increase in the phenylalanine/tyrosine ratio is mainly driven by increased phenylalanine levels (Fig 2A and B, Fig E15). The data from other amino acids and metabolites are shown in the Online Repository Fig E15.

After 72 hours most amino acids, with the exceptions of citrulline, 4-hydroxy-proline and especially glutamate, not only returned to baseline concentrations but increased on average to 170% (Fig 3, G). Interestingly, arginine increased 250% to 173 μM , which corresponds to a 511% elevation compared to the bottom level of 34 μM during the zenith of the attack. Glutamate decreased to 31% of baseline concentrations 72 hours after admission.

Massive depletion of most lysophosphatidylcholine (LPC) lipids during mast cell activation but unchanged autotaxin (ATX) antigen concentrations

Although several of the 14 lipid classes show statistically significant increases or decreases compared to baseline using weighted average calculations (Fig 4, A and B), the differences are within +/- 40% compared to baseline. We hypothesize that the diffusion properties of lipids during vascular leakage are more similar to albumin and therefore follow the BL normalized albumin concentrations with important deviations. Many measured lipids are incorporated into large lipoprotein particles. The clear exceptions of the analyzed lipids were LPC and LPC-O variants with a massive and statistically highly significant decrease during the MC activation attack probably starting hours before the peak (Fig 4, B). Using baseline as 0 time point in the first 7 hours post admission the LPCs decrease following an exponential decay curve with a rate of 26%/hour ($R^2 = 0.99$). In total 170 μM LPCs were degraded using the baseline mean (weighted mean CV) of 202 (12.6%) μM as starting value. This is on average a 6.7-fold decline. More details about the most abundant glycerophospholipids are presented in Fig 4C to 4F. Some phosphatidylinositols (baseline mean 122 μM ; weighted CV 21%), phosphatidylethanolamines (baseline mean 23 μM ; weighted CV 24%) and phosphatidylserines (baseline mean 698 μM ; weighted CV 14%) showed the highest concentrations during the symptom peak with rapid decline over the next three hours (Fig 4E and 4F). Phosphatidylcholine (baseline mean 943 μM ; weighted CV 9%) concentrations decreased with the rate of hemodilution (Fig 4, C). The sphingomyeline variants (baseline mean 276 μM ; weighted CV 9%) are stable lipids with minimal variation compared to the albumin concentration curve (Fig E16). Many of the 36 triacylglyceride (TAG) variants showed strongly reduced concentrations in contrast to several diacylglycerides (DAG) with increased levels (Fig 4, A versus Fig E16). The different lipid classes including most LPCs returned to or exceeded baseline concentrations similar to the amino acids and other metabolites 72 hours post admission (Fig 4, A to F and Fig E16).

Fig 5A shows all LPC curves and with the exception of LPCs 20:4, 22:4, 22:5 and 22:6 all LPCs showed robust > 3-fold decreases compared to baseline concentrations. The most profound decreases of more than 10-fold were measured with the low abundant LPCs 14:0 and 20:0. The most abundant LPCs 16:0, 18:0, 18:1 and 18:2 (>90% of all PLCs) degraded with 27%/hour in the first 7 hours. Some LPEs, which are also substrates for ATX, albeit with reduced LPA generation rate compared to LPC^{21,22}, decreased more than 4-fold (Fig 5, B). The ATX antigen concentrations followed the albumin curve (Fig 5, C), whereas the plasma choline concentrations correlate with the decreased LPC levels (Fig 5, D).

The absolute concentrations (μM) of the different quantified amino acids, metabolites and lipids are provided in the Online Repository Excel File E1. All p-values comparing the

321 concentrations of metabolites, lipids and ratios during the first 10 hours with baseline are
322 provided in Table E1 and E2. The mean baseline metabolite and lipid concentrations of this
323 patient obtained 11 months apart four years earlier are quite similar to the NIST SRM1950
324 standard plasma values (Fig E11 and Fig E12).

DISCUSSION

The baseline histamine concentrations in this mastocytosis patient are circa 4 ng/ml compared to a peak of 30 ng/ml during the zenith of the MC activation event. This was reached approximately 6 to 8 hours after admission, and then dropped rapidly to concentrations approaching baseline 3 hours later. The plasma histamine concentrations in healthy individuals are ≤ 1 ng/ml. The local histamine concentrations in tissue with high mast cell density were probably manifold higher considering interstitial mean histamine concentrations of 7.4 ng/ml in normal skin²³. A few hours after admission, plasma tryptase and DAO concentrations rapidly increased with tryptase levels decreasing after the event peak. The release of tryptase and DAO is very likely accompanied by increased plasma heparin, heparan sulfate and/or serglycin concentrations. These molecules are released from MCs and possibly reflected in the 5-fold elevated aPTT at the zenith followed by a rapid decline^{1,24}. Both DAO and tryptase possess heparin/heparan sulfate-binding domains. The increased heparin-like activity in the circulation might also have released lipoprotein and hepatic lipase providing an explanation for the reduced triacylglyceride and increased diacylglyceride concentrations. Both lipases possess a heparin-binding domain.

It is tempting to speculate that histamine was the driving force behind many clinical symptoms during the acute phase 3 to 8 hours after admission. Several parameters such as histamine, tryptase, DAO, glucose or aPTT rapidly increased during this pre-peak period. Others including pH, lactate, heart rate and diastolic blood pressure returned to almost baseline values within 4 hours after the histamine peak. Elevated histamine concentrations activate several signaling pathways in endothelial cells causing not only nitric oxide synthesis, vasodilation, vascular leakage with plasma exudation and edema formation but also synthesis of prostaglandin I₂, a potent vasodilator, modified cell adhesion and loosening of adherent junctions allowing easier leukocyte migration^{25,26}. The increased permeability is reflected in the more pronounced reduction of protein versus erythrocyte concentration markers.

Temporal amino acid profiles indicate more extensive and molecular weight dependent vascular leakage beyond leakage extrapolated using albumin concentrations. The interstitial fluid compartment is at least 3-fold larger compared to the plasma volume and small metabolite concentrations might be rapidly reduced in plasma during increased vascular leakage with albumin and proteins lagging behind. Histamine-mediated endothelial cell activation with enhanced vessel permeability might be a key pathophysiological process during protracted MC activation.

Considering that most of the MCs are located in the lung, skin and small intestine (Online Repository Table E4), these organs might be severely affected by vasodilation, hypoperfusion, increased tissue pressure and hypoxia. Surprisingly, the patient did not show any bronchospasm but was suffering from a generalized flush without urticaria indicating skin hyperperfusion with minimal vessel leakage. This pattern suggests that MCs are not generally activated, and if activated, the consequences are not the same when comparing skin and intestine. Alternatively, mast cell distribution might differ between those organs in this mastocytosis patient.

The patient vomited multiple times and suffered extreme nausea. The metabolic functioning of the small intestinal enterocytes was severely compromised based on an almost 20-fold reduction in the functional integrity of the small bowel using the citrulline plasma concentrations as proxy for enterocyte “health”¹⁸. Citrulline is almost exclusively synthesized in the mitochondria of enterocytes. The lowest concentration of citrulline during the MC activation event was 6.9 μM compared to baseline concentrations of 27.4 μM . At $< 10 \mu\text{M}$ citrulline levels in-hospital survival decreased significantly in critically-ill patients²⁷.

Citrulline is synthesized from ornithine and considering that ornithine is even more decreased compared to citrulline, the primary defect might have been upstream of ornithine synthesis. Citrulline and ornithine were decreased 4- and 5.8-fold respectively at their lowest concentrations compared to baseline. The low glutamate concentrations 72 hours after admission (31% of baseline) could be caused by the intense recovery/anabolic generation of ornithine, citrulline and finally arginine using the same precursor glutamate. Enterocyte function was unlikely normalized at this time providing another explanation for the deficit in returning to normal concentrations in contrast to most other metabolites and lipids exceeding baseline levels 72 hours post admission.

The steady increase in C-reactive protein levels during the first 24 hours post admission might have been also influenced by translocation of bacteria from the lumen of the gastrointestinal tract because of severe hypoxia and possibly some apoptosis and necrosis. Histamine has been described as causing not only vascular leakage but also disruption of the epithelial cell barrier function allowing more microbial translocation²⁸. The tips of the villi of the small intestine are especially sensitive to hypoxia, because of the countercurrent blood flow anatomy²⁹. The presence of pathogen- or damage-associated molecular pattern molecules (PAMPs and DAMPs) followed by activation of pattern recognition receptors might contribute to the MCAS pathophysiology²⁵. Heparan sulfate and heparin, both released from activated mast

cells, are considered to be DAMPs activating TLR4 receptors on endothelial cells like lipopolysaccharides²⁵.

At the peak of the attack lactate concentrations were highly elevated at 7 mM but dropped rapidly at 1.1 mM/hour with a half-life of 153 minutes. The physiological half-life of increased lactate concentrations after exercise is roughly 10 to 20 minutes, an at least 7-fold faster rate³⁰. The positive correlation with decreasing histamine levels is striking. We hypothesize that because of reduced histamine concentrations, vascular leakage and edema formation decreased with increased oxygen supply, reperfusion and consequently lactate clearance. Lactate concentrations can be highly elevated in anaphylaxis. Guerci et al. (2020) published that using multivariate analysis only lactate was a predictor of death in 339 anaphylaxis cases admitted to the intensive care unit³¹. In a dog anaphylaxis model lactate strongly increased and gastrointestinal blood flow severely decreased and was still 60% reduced after 180 minutes, although the mean arterial pressure and liver blood flow had already returned to normal (Fig E14)³². The rapid normalization of the acetyl-carnitine to carnitine ratio within less than 3 hours after the symptom peak suggests that some reversible mitochondrial dysfunction was present as has been described in sepsis patients³³.

Based on the reduced arginine, elevated phenylalanine/tyrosine ratio (see below) and increased ADMA and SDMA concentrations and the respective increased/decreased ratios endothelial cells were likely severely dysfunctional with uncoupled nitric oxide synthesis and release of superoxide, a highly reactive and damaging radical. Arginine concentrations dropped to 37 μ M, which is clearly below the K_m for the CAT-1 transporter³⁴. Comparable reductions of arginine, but also citrulline and ornithine levels, have been described in sepsis patients³⁵⁻³⁷. The same has been seen in malaria with a strong correlation with lactate concentrations and endothelial cell dysfunction³⁴. In pediatric malaria, arginine, ornithine and citrulline were reduced in a fashion comparable with our data³⁸. In a meta-analysis the overall mean drop of arginine between sepsis patients and controls was 34 μ M or 41%³⁵. The lowest arginine concentration in our data corresponds to a drop of 37 μ M or 47%.

Both dimethylarginines ADMA and SDMA increased, causing a two-fold reduction in the arginine/ADMA and arginine/SDMA ratios. Both ratios correlate with morbidity and mortality in sepsis patients^{35,36,39}. A two-fold reduction increased mortality approximately 30% in sepsis patients with survivors demonstrating significantly higher ratios³⁶. ADMA is a potent inhibitor of endothelial nitric oxide synthase, whereas SDMA competes with transport of arginine into endothelial cells. The half-life of ADMA, but not SDMA, is controlled by dimethylarginine dimethylaminohydrolase (DDAH), which is sensitive to

nitrosative/oxidative stress and therefore DDAH inhibition could have been responsible for increased ADMA concentrations⁴⁰. Despite high intracellular arginine it is extracellular arginine levels which are the key determinants of eNOS activity⁴¹⁻⁴³. Plasma arginine levels were significantly reduced during the MCAS event.

The phenylalanine/tyrosine ratio increased almost 200% indicating that the phenylalanine hydroxylase was dysfunctional. This effect was likely caused by a deficit in the essential cofactor tetrahydrobiopterin (THB4). Endothelial NOS and phenylalanine hydroxylase are dependent on THB4, which is highly sensitive to nitrosative/oxidative stress⁴⁴. We did not measure nitrosative/oxidative stress, but a peak lactate concentration of at least 7 mM combined with an almost 20-fold reduction in enterocyte functional “health” and robust histamine-induced nitric oxide synthesis under hypoxic conditions with uncoupling of eNOS makes the presence of nitrosative/oxidative stress highly likely. The statistically significantly increased homocystine concentrations despite hemodilution reflect elevated oxidative stress⁴⁵.

Based on data from phenylketonuria heterozygote patients a mean (SD) peak phenylalanine/tyrosine ratio of 2.7 (0.04; normal values 1.14), also seen in human and monkey infections, and a mean (SD) peak phenylalanine²/tyrosine ratio of 260 (16.3; normal values 118) implies that the activity of the phenylalanine hydroxylase was severely compromised during the acute MCAS phase and only about 30% of normal^{46,47}. Increased phenylalanine concentrations have been described in sepsis patients^{48,49}.

The 400% increased acetyl-carnitine to carnitine ratio supports the presence of mitochondrial stress during the zenith of the MC activation event⁵⁰. Reduced THB4 availability also contributes to decreased blood pressure because efficient noradrenaline and dopamine synthesis are dependent on THB4⁴⁴. Finally, arginase in circulating neutrophils might contribute to the reduced arginine concentrations⁵¹. The number of leukocytes more than doubled compared to baseline during the attack, peaking at 14 G/L. We did not measure the percentage of activated neutrophils, but arginase is present in circulating neutrophils and the doubling of the leukocyte cell number alone could have contributed to the arginine concentration decline. The 1.5-fold elevated kynurenine/tryptophan ratio is another indication of vascular dysfunction⁵².

The most striking and totally unexpected finding during the course of this MC activation event is the massive depletion of most plasma LPC variants. The LPC reduction was reflected in strongly decreased choline levels but with normal circulating ATX antigen concentrations. Reduced LPC concentrations have been described in sepsis, malaria and trypanosomiasis, but the mechanisms have never been defined⁵³⁻⁵⁶. What caused the depletion of LPCs, which are

derived from phosphatidylcholine via phospholipase A₂ (PLA₂) activity? Widespread catalytic inhibition of the numerous PLA₂ enzyme variants is an option but is unlikely. It is not clear whether LPC depletion happened in the plasma or in the interstitial compartment. High local concentrations of histamine cause vessel leakage and ATX might have been readily diffused into the interstitium. In sheep intravenously administered radioactive albumin reaches 45% of plasma concentrations in lung lymphatic fluid within 4 hours without histamine and is likely to reach higher percentages more rapidly with histamine and lysophosphatidic acid (LPA), which can also induce vascular leakage⁵⁷⁻⁵⁹. Intriguingly, ATX is secreted from human gastro-intestinal MCs⁶⁰. Activation of endothelial cells exposes active integrin binding sites for ATX. The binding of ATX to cellular membranes is essential for efficient ATX activity^{61,62}. Exosomes bind ATX and it is tempting to speculate that ATX also binds to exosomes released from MCs^{9,63}. Exosome-bound ATX is active and considering that exosomes are circulating in mastocytosis patients and that the number is significantly increased during MC activation⁹, exosome-bound ATX could deplete LPC in plasma. Rodent MCs also release large extravesicular structures with heparin as the main component¹⁰. We are tempted to hypothesize that the ATX- α variant, which contains a heparin-binding domain, binds to these membrane structures and remains active²¹. This ATX variant might have been released from matrix-associated heparan-sulfate proteoglycans followed by integrin interaction with activated endothelial cells and local LPA release and LPA receptor activation. The plasma ATX concentration would not be increased. The release of exosomes or other extracellular vesicles from activated MCs might be reflected in the increased plasma concentrations of some phosphatidylinositol and phosphatidylserine variants at the zenith of the attack. This would be a rational explanation for the clear peaks during increasing hemodilution and rapid decline within a few hours after the event's peak. About 170 μ M LPCs are "missing" from the baseline LPC plasma pool degraded within a few hours. If such an amount of LPA is locally released, it will likely contribute to the pathophysiology. Lysophosphatidic acid induces not only vascular leakage and plasma exudation but also histamine release from MCs in vitro and in vivo. This positive feedback loop might significantly contribute to the severe hypoperfusion and hypoxia in the gastro-intestinal tract^{58,59} (Fig 6). This signaling lipid also up-regulates E-selectin and VCAM expression in human endothelial cells in vitro. Binding of LPA to human endothelial cells increased neutrophil/endothelial cell interactions and therefore LPA activates endothelial cells allowing more ATX binding and local ATX activity and LPA release⁶⁴.

Three important issues must be discussed. First, is the use of the two baseline samples appropriate? They were drawn four and five years earlier and 11 months apart, and were compared to four duplicate samples drawn during the attack. The use of the mean and more importantly the variance of the two baseline samples for statistical analysis is conservative because of the long time gap between withdrawals. Furthermore, it has been shown in hundreds of samples that the metabolome including lipids is highly stable in most individuals over four or even seven years^{65,66}. The mean ratios of “fresh” samples divided by 4 year old samples ($n = 1409$) are remarkably stable (Fig E13)⁶⁶. If certain metabolites are consistently unstable, the mean concentrations must be lower in the 4 year old samples and this should have resulted in ratios > 1 . Using the same logic, samples from the acute episode after baseline-normalization should have started at values $> 100\%$, but this is not what we saw. Falsely reduced levels at the 4 hour after admission time point would indicate that metabolites increase in concentration during storage. We are not aware of studies describing such an effect. Finally, instability can also not explain the steep slopes of ornithine, lysine, citrulline or many LPC variant concentrations in samples during the acute phase of the episode. These samples have been stored equally long.

We therefore believe that the use of these baseline samples is appropriate and this is also reflected in the data 72 hours after admission with most amino acids, metabolites and lipids not only returning to but exceeding the baseline concentrations.

Are the measured perturbations in the metabolome and lipidome data caused by fasting and administered steroids? Limited space does not allow for a discussion here and therefore we added published data to the Online Repository. Based on these data it is highly unlikely that the key findings are significantly influenced either by fasting and/or administered steroids. We also added short paragraphs about possible trigger molecules and therapeutic considerations to the Online Repository.

Considering the $n = 1$ nature of the study, statistical significance testing was confined to overall deviations of metabolite concentrations during the event compared to baseline levels. It is a limitation of the study that the statistical significance of differences between single values during the event remains unknown and that these differences can only be descriptively assessed.

Several mast cell activation events feature in the medical history of this patient. These have demonstrated fever, generalized flush, nausea, vomiting and hypotension but an infectious cause was never verified. We cannot exclude the influence of an infection on the metabolic and lipidomic derangements but in this event there was also no infection found. Fever can be

a prominent symptom during mast cell activation⁶⁷⁻⁷¹. In the published cases no infection was diagnosed and antibiotic treatments were ineffective. In the Stefanini 2012 case report and the 2 cases described in Hot 2009 fever of unknown origin was the only symptom finally leading to the definite diagnosis of systemic mastocytosis. In the other cases nausea, vomiting, flush, hypotension and varying additional symptoms accompanied fever. Therefore, fever possibly induced by interleukin-1 release from activated mast cells can accompany mast cell activation without involvement of an infection⁷⁰. Nausea, vomiting and epigastric pain, present in the acute phase of this mast cell activation event, can be directly explained by elevated histamine concentrations and indirectly by severely reduced blood flow in the gastrointestinal tract. In conclusion, metabolomics and lipidomics characterization of a severe MC activation event demonstrated massive depletion of LPC with release of LPA. A severe dysfunction of the small intestine with an almost 20-fold reduction in enterocyte "health" was reflected in strongly reduced citrulline and ornithine concentrations, likely secondary to endothelial cell activation and dysfunction with hypoperfusion and hypoxia. It is not surprising that in mastocytosis patients the most prominent tissue dysfunction during MCAS is located in the small intestine considering the number of MCs in this tissue (see Online Repository). A positive feedback loop might have been induced with MC degranulation causing activation of endothelial cells, which consequently bind ATX via activated integrin receptors (Fig 6). The ATX-generated LPA causes more endothelial cell but also MC activation with more histamine release. Histamine activates endothelial cells followed by increased ATX binding and local LPA generation. The definite primary trigger molecule/mechanism to start this vicious cycle is unknown, but could be derived from medications, food ingredients or infections, all known trigger entities of anaphylaxis or MC activation. In healthy subjects this positive feedback loop is rarely fully activated but in mastocytosis patients the increased MC burden with elevated histamine and in general MC mediator release might readily allow full activation of this feedback loop with life-threatening consequences. Stop signals for this vicious cycle might be LPC depletion, MC exhaustion or efficient histamine degradation via heparin-released gastro-intestinal DAO. Although derived from a single severe MC activation event, the data provide strong and sufficient rationale to test recombinant human DAO for rapid histamine degradation, to supplement citrulline/arginine to improve nitric oxide signaling, to use LPC for immunomodulation, to inhibit ATX and/or to block LPA receptor signaling as new therapeutic strategies for the treatment of severe mast cell activation events in future clinical studies.

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Author Contributions

TB conceived the idea, designed the experiments, performed data analysis and interpretation, prepared the figures and wrote the manuscript. RR performed all statistical analyses. SJ was instrumental in the DAOMAST study recruitment, data collection and data analysis. KP performed ATX antigen and data analysis. KK was responsible for metabolomics and lipidomics data generation and analysis. PV provided clinical data during regular control visits and critically read and revised the manuscript. BJ is the principal investigator in the DAOMAST and Anaphylaxis study and contributed to the writing of the manuscript. All authors read, revised and approved the final version of the manuscript.

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FIG 1. Clinical parameters during the severe MC activation event - Part 1

The means of several clinical parameters measured during regular hospital visits or during the DAOMAST study without symptoms are shown as baseline (BL); The x-axis represents BL and hours post admission; **(A)** Time course of mean ($\pm$ SD) systolic (SYS; $p = 0.56$), diastolic (DIA; $p = 0.003$), heart rate (HR; $p = 0.0001$) and mean arterial pressure (MAP, $p = 0.002$) during BL and hours post admission; BL data were derived from 24 measurements over 16 symptom-free days; The values starting 7 hours post admission were recorded in the intensive care unit and reflect mean values over 1 hour with multiple measurements. **(B)** Temperature (TEMP; °C) and lactate (LAC; mM) concentrations; **(C)** Glucose (mg/dl) and pH; the mean ($\pm$ -SD) BL glucose was derived from 4 symptom-free hospital visits; **(D)** Activated partial thromboplastin time (aPTT; sec); C-reactive protein (CRP; mg/dl); White blood cell count (Leukos; G/L); the mean ($\pm$ - SD) BL aPTT, CRP and Leukos values were derived from three visits 1, 6 and 10 months after the event; **(E)** Administered medications during the MC activation event; EL=electrolyte. **(F)** Aspartate aminotransferase (ASAT); alanine aminotransferase (ALAT) and gamma glutamyl transferase (GGT); the mean ($\pm$ - SD) BL ASAT, ALAT and GGT values were derived from the same visits as described in **(D)**.

FIG 2. Clinical parameters during the severe MC activation event - Part 2

In Panels **A** to **C** the means of three baseline (BL) values without symptoms measured during three regular hospital visits 1, 6 and 10 months post event were used as 100%. In Panels **D** and **E** the means of four different time points with two independent EDTA plasma samples drawn at the same time for each time point were normalized to the BL values derived from two visits during the DAOMAST study; **(A)** Time course of human serum albumin (HSA), total protein (TP), the mean and tyrosine (TYR) during BL without symptoms and hours post admission; **(B)** Hemoglobin (Hb), hematocrit (Hct), the mean and tyrosine (TYR); **(C)** The dilution factors (DF) using HSA and TP (DF_P; P = protein); the DF using Hb and Hct (DF_RBC; RBC = Red Blood Cell); on the 2nd y-axis the differential of the two DF curves is shown (Δ DFs); **(D)** Mean diamine oxidase (DAO, ng/ml) and tryptase (TRYP, ng/ml) concentrations (data published in Boehm 2019¹⁷); **(E)** Semiquantitative MS-based histamine (HIST) measurements as part of the metabolomics analysis performed at CeMM compared to state of the art quantitative ELISA measurements (MUV); The MUV data have been published¹⁷; 1 ng/ml HIST corresponds to 9 nM. **(F)** Correlation between CeMM and MUV measurements; $p = 0.00021$.

FIG 3. Metabolomics data during the severe MC activation event

(A) Time course of carnitine (C; $p = 0.53$) and the acyl-carnitines acetyl- (ACE; $p = 0.02$), butyryl- (BUT), propionyl- (PRO), octanoyl- (OCT) and the ratio of ACE-C/C at baseline (BL) and hours post admission; The isovaleryl-C data were almost identical to C and BUT-C (data not shown); (B) Tryptophan (TRP), kynurenine (KYN), the KYN/TRP ratio ($p = 0.02$), sarcosine (SARC; $p = 0.006$) and creatinine (CREA; $p = 0.044$); (C) Spermidine (SPD; $p = 0.02$), spermine (SPM; $p = 0.02$), γ -amino and α -amino butyric acid (GABA, AABA) and taurine (TAU); (D) Arginine (ARG; $p = 0.034$), lysine (LYS; $p = 0.006$), citrulline (CIT; $p = 0.027$), ornithine (ORN; $p = 0.019$) and functional small bowel length (fSBL); (E) Different amino acid ratios using the standard single letter amino acid code and O for ornithine and C for citrulline; (F) Asymmetric dimethyl arginine (ADMA; $p = 0.0063$), symmetric dimethyl arginine (SDMA; $p = 0.0063$); and the ARG/ADMA ($p = 0.024$) and ARG/SDMA ($p = 0.038$) ratios; The 72 hours value of the ARG/SDMA ratio is 275% (data not shown). In (A) to (F) the serum albumin BL normalized data are shown as proxy for plasma dilution, if appropriate, in the panels as dashed line; (G) Amino acid concentrations including CIT, ORN and 4H-PRO as percent of BL 72 hours after admission; The average increase of 170% compared to BL is shown as a dashed line.

FIG 4. Lipidomics data during the severe MC activation event

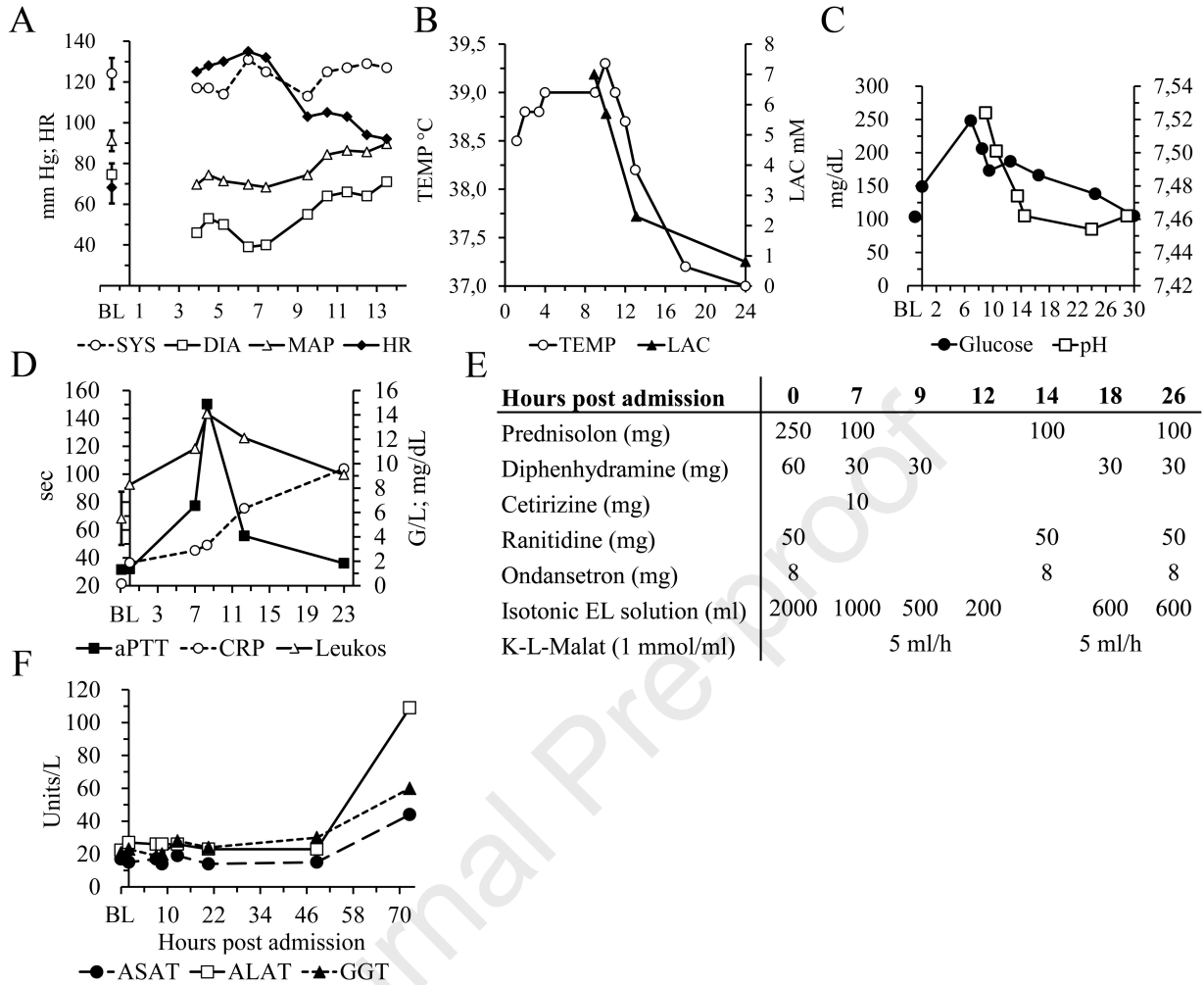
(A) Time course of the weighted means of lipid class subclass variants normalized to baseline (BL); the numbers before the lipid class represent the number of included subclass variants; cholesteryl esters (CEs; $p = 0.14$); ceramides (CERs; $p = 0.35$); diacylglycerides (DAGs; $p = 0.012$); sphingomyelins (SMs; $p = 0.03$); triacylglycerides (TAGs; $p = 0.006$); L_exCE represents all lipids except the CE class; (B) Weighted mean data of lysophosphatidylcholines (LPC; $p = 0.007$; LPC-O; $p = 0.006$); lysophosphatidylethanolamines (LPE; $p = 0.006$); phosphatidylcholines (PC; $p = 0.07$; PC-O; 0.26); phosphatidylethanolamines (PE; $p = 0.008$; PE-O; $p = 0.016$); phosphatidylinositols (PI; $p = 0.1$) and phosphatidylserines (PS; $p = 0.03$); (C) Selected PCs representing 89% of all PCs; (D) Selected PEs representing 84% of all PEs; PE 37:7 is not shown because it is almost identical to PE 40:4; (E) All PIs; (F) Selected PSs representing 97% of all PSs; The serum albumin BL normalized data (dashed line) are shown as proxy for plasma dilution.

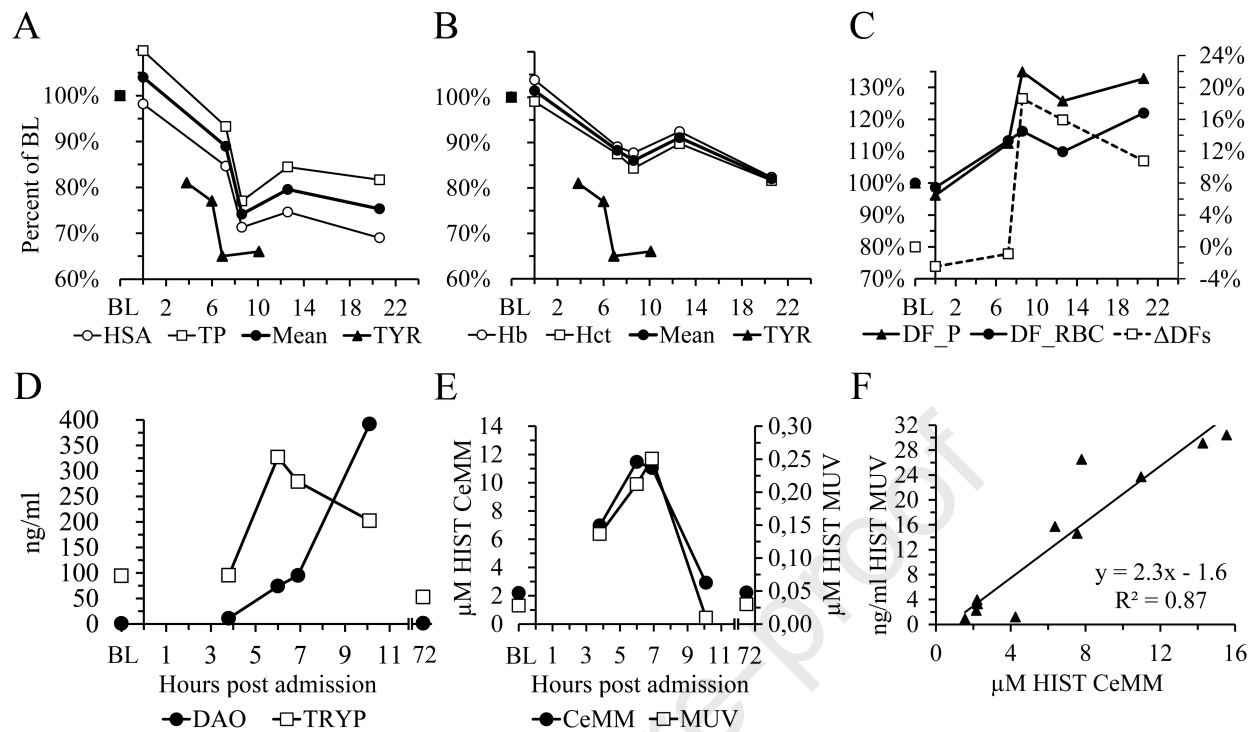
FIG 5. Substantial decrease of LPCs and choline during MC activation with unchanged plasma ATX concentrations

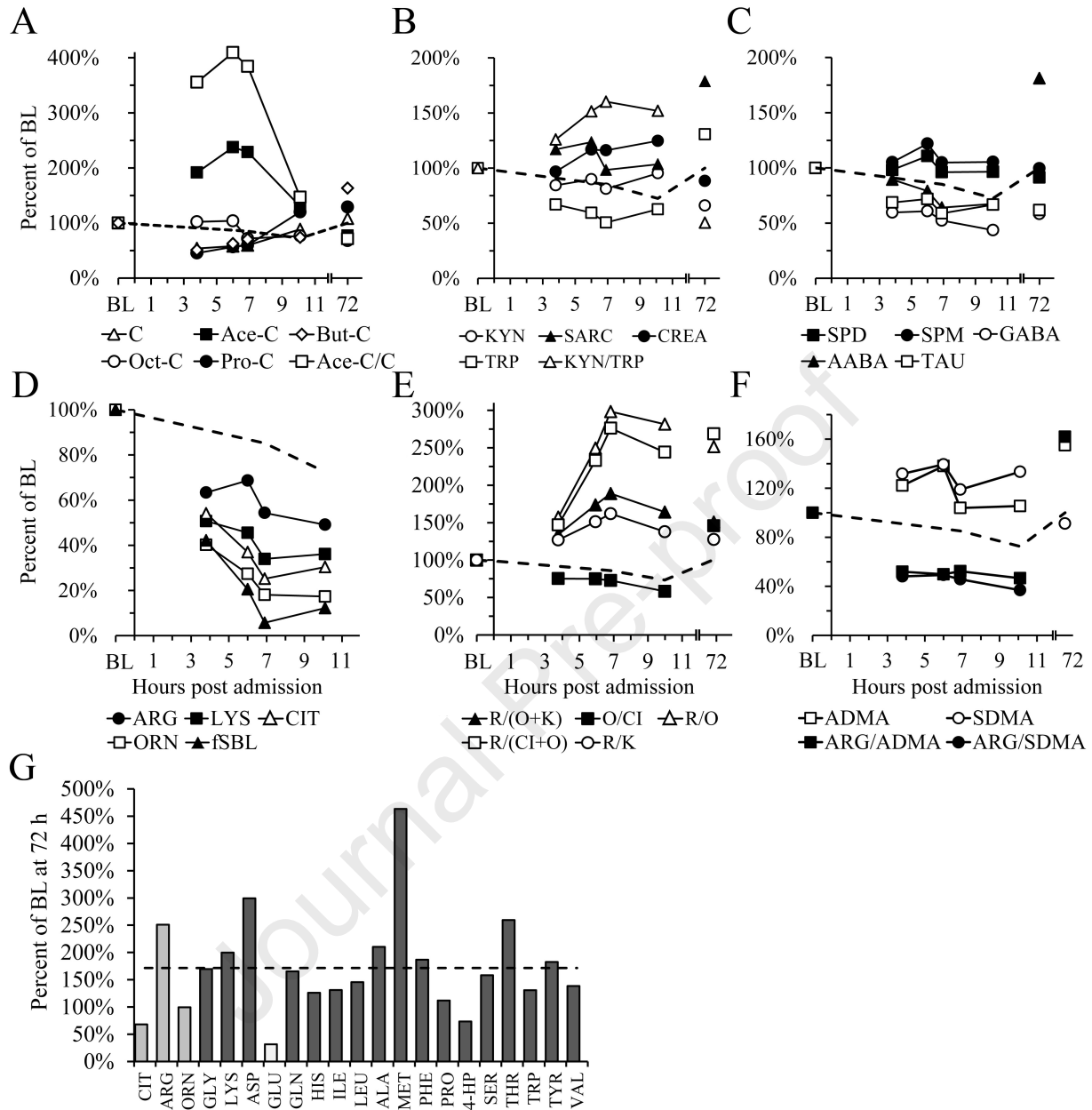
(A) Fold-decreases normalized to baseline (BL) values of all LPCs and all LPC-Os; The bold empty squares ($\square$) represent the weighted means of all LPCs except the four lowest LPCs not changing relevantly during the event; The bold empty circles ($\circ$) represent the weighted means of all measured LPCs; the two highest and four lowest LPCs are labeled below the graph; **(B)** Fold-decreases compared to BL of the 6 measured LPEs; The sum of all LPE variants is 8.5 μ M. **(C)** ATX concentrations were measured in duplicate in two independent experiments; An additional sample at 3 hours post admission was also included; The means ($\pm$ SD) of the two means determined in duplicate in two independent experiments are presented; In **(C)** and **(D)** the albumin concentration curve as percent of BL was added; The 72 hour albumin value was not measured and assumed it to be 100%. **(D)** The means ($\pm$ -SD) of the choline counts per second (cps) from the MS data; p-value = 0.0004; on the 2nd y-axis the cps data were normalized and shown as percent of BL.

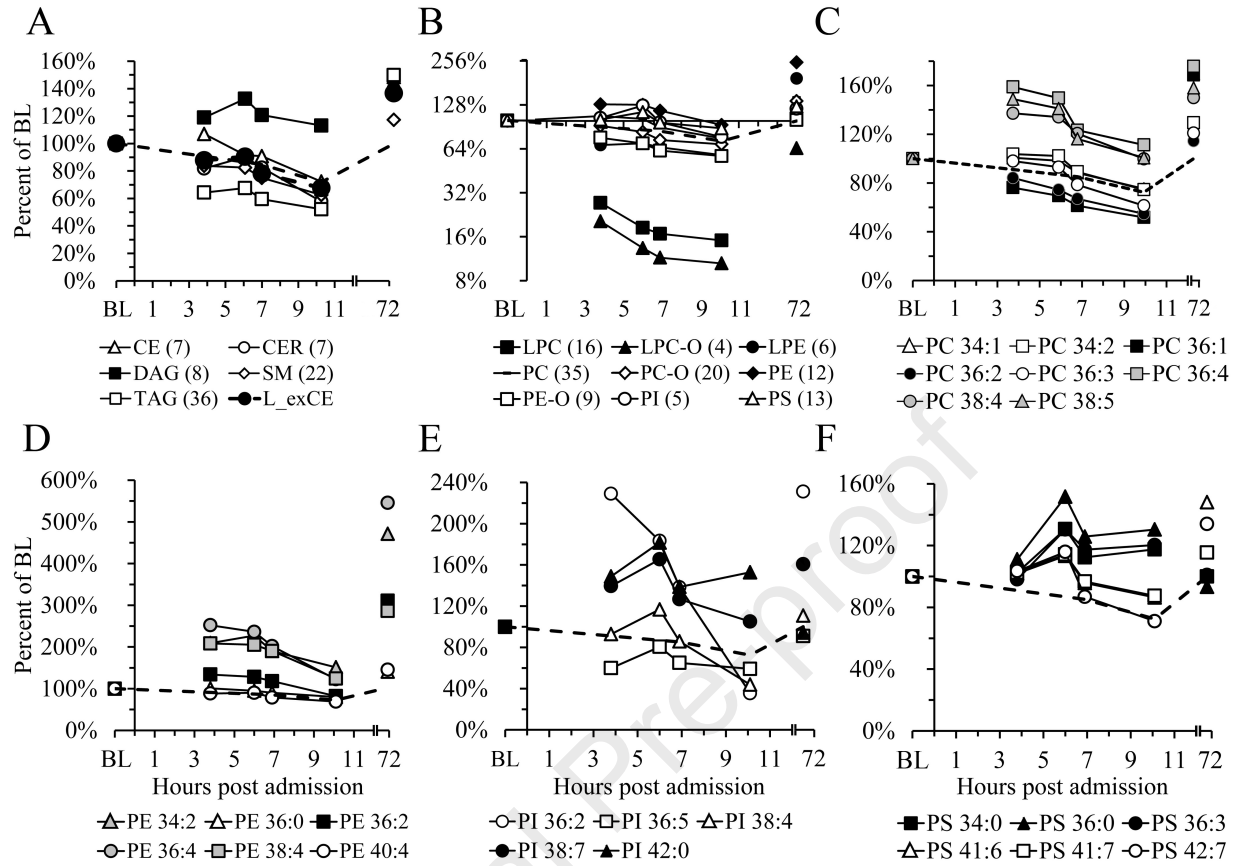
FIG 6. Possible feedback loop causing extensive mast cell and endothelial cell activation

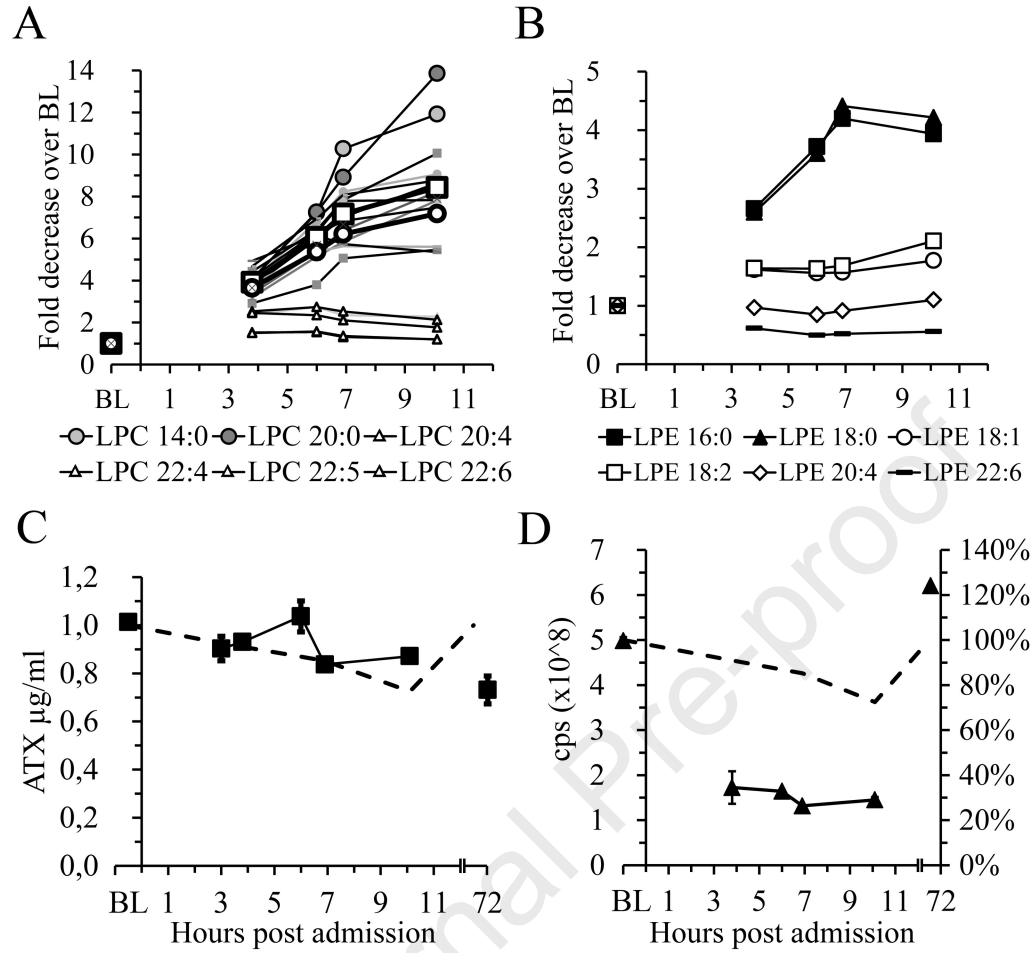
*But DAO activity compromised¹⁷; LPC = Lysophosphatidylcholine; LPA = Lysophosphatidic acid; HIS = Histamine, DAO = Diamine oxidase; EC = Endothelial cells; MC = Mast cells; CIT = Citrulline; ORN = Ornithine; ATX = Autotaxin; IF = Interstitial fluid.

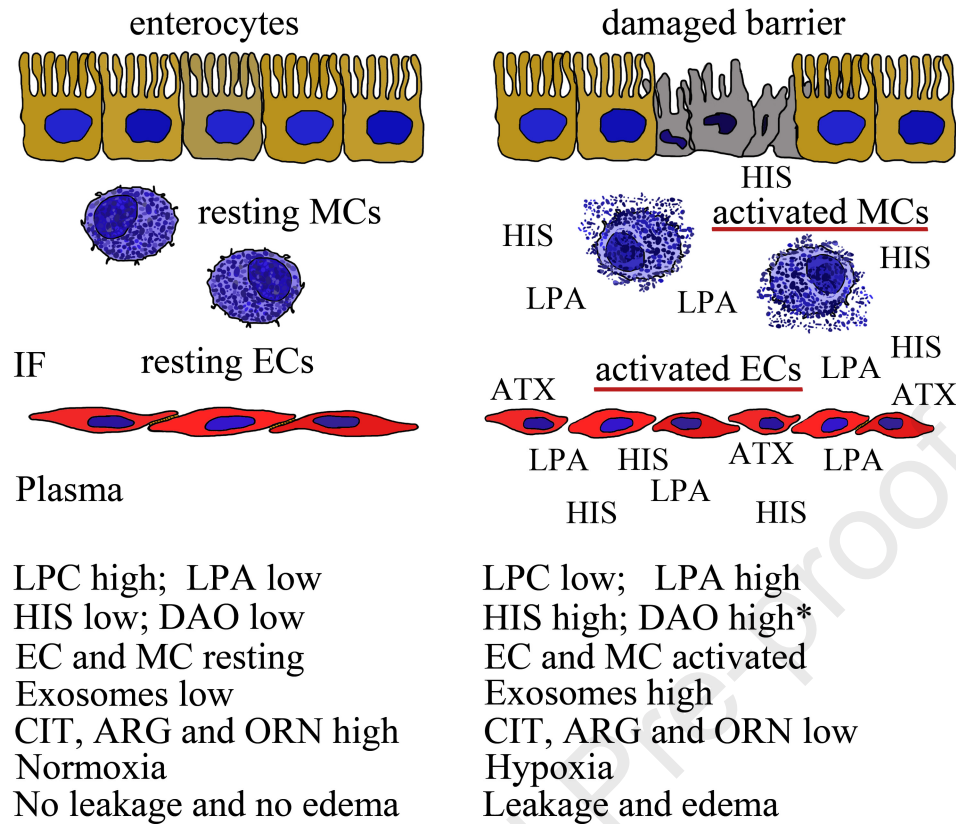


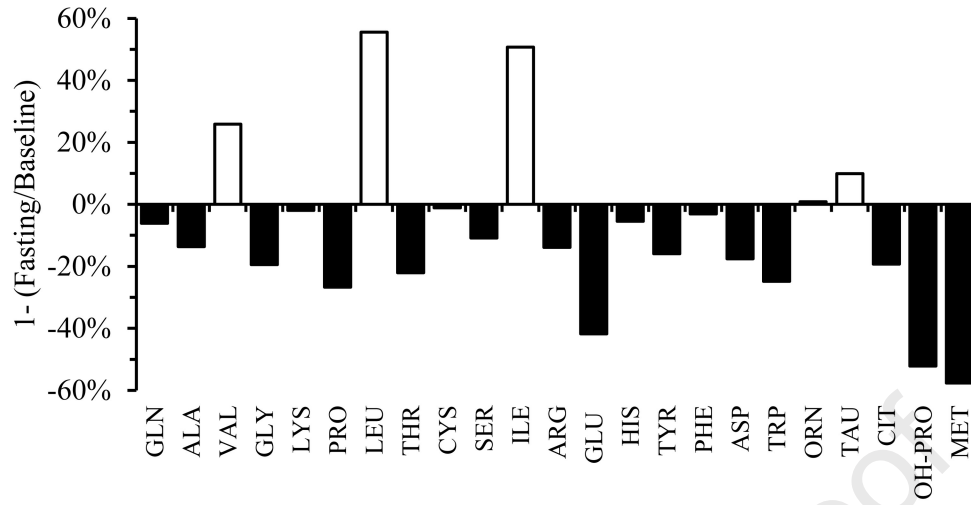


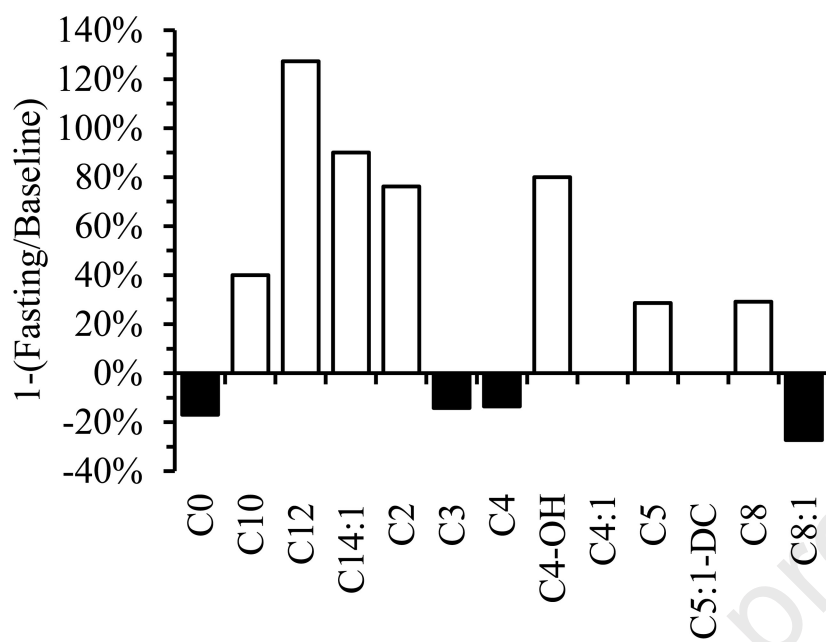


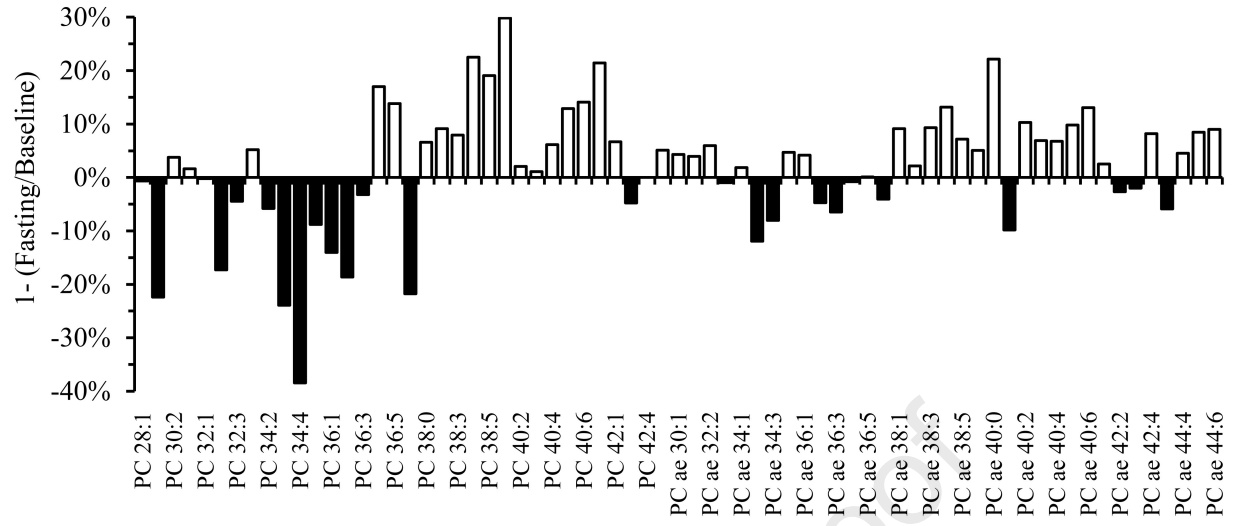


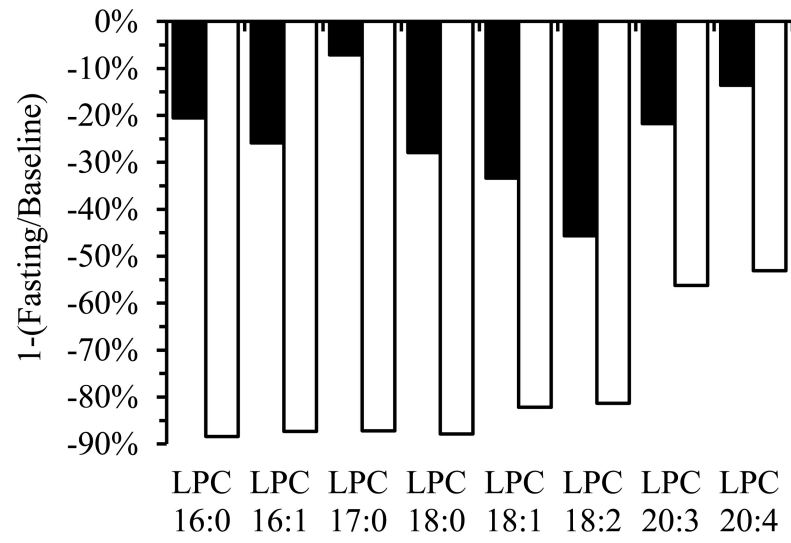


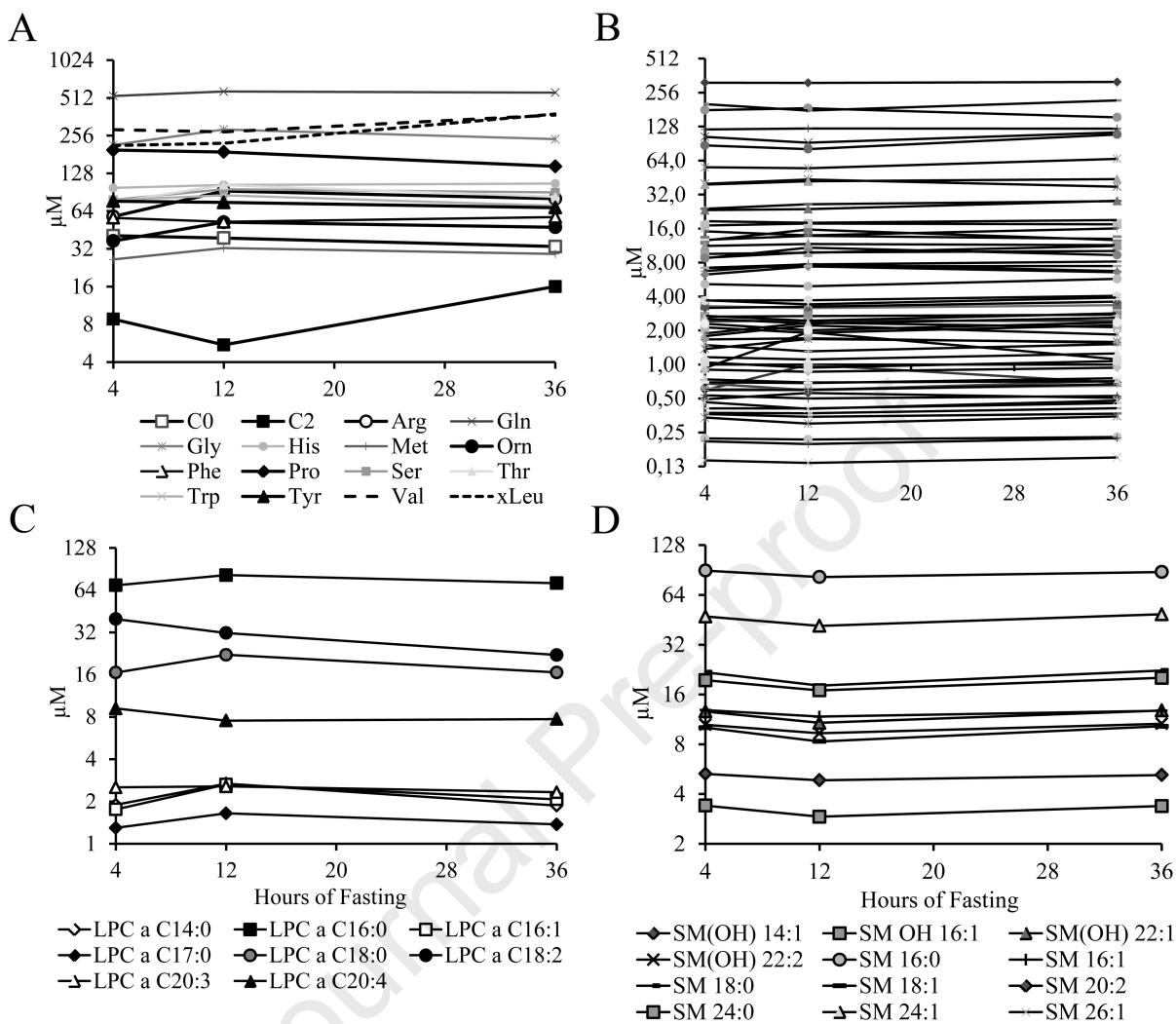


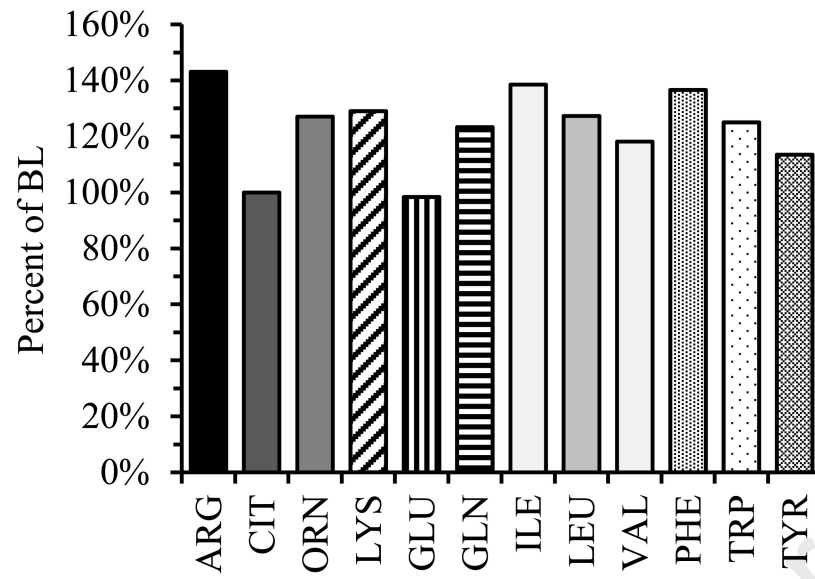


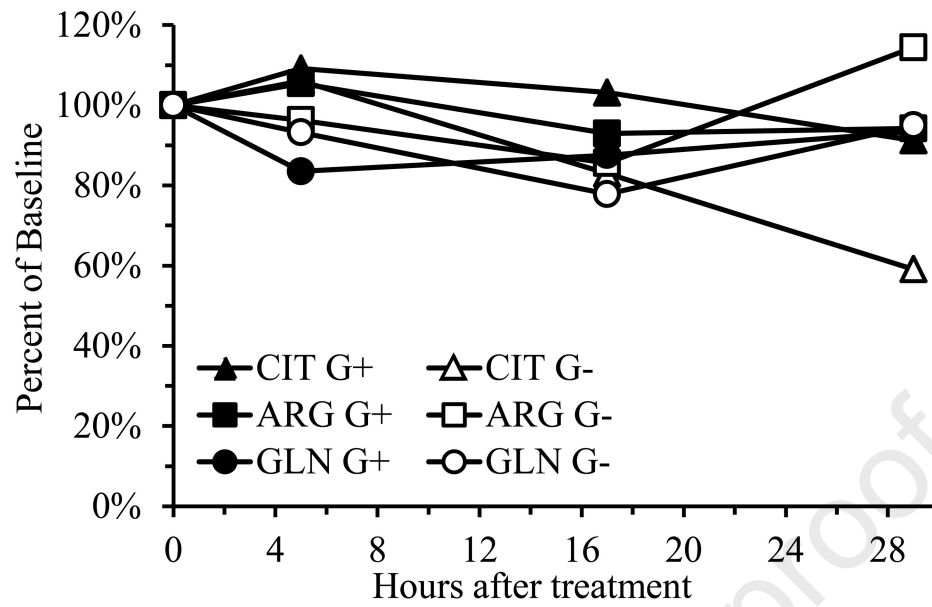


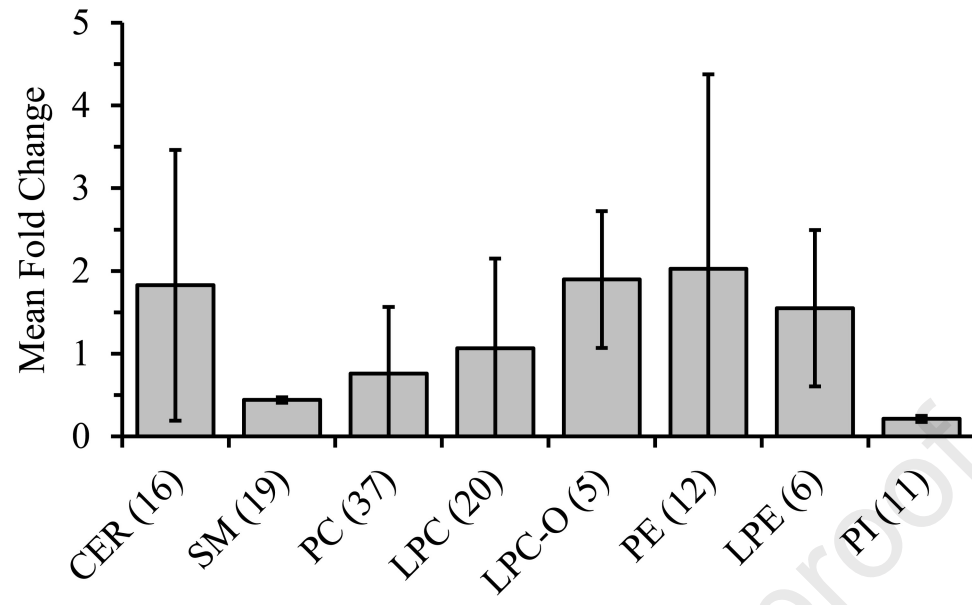


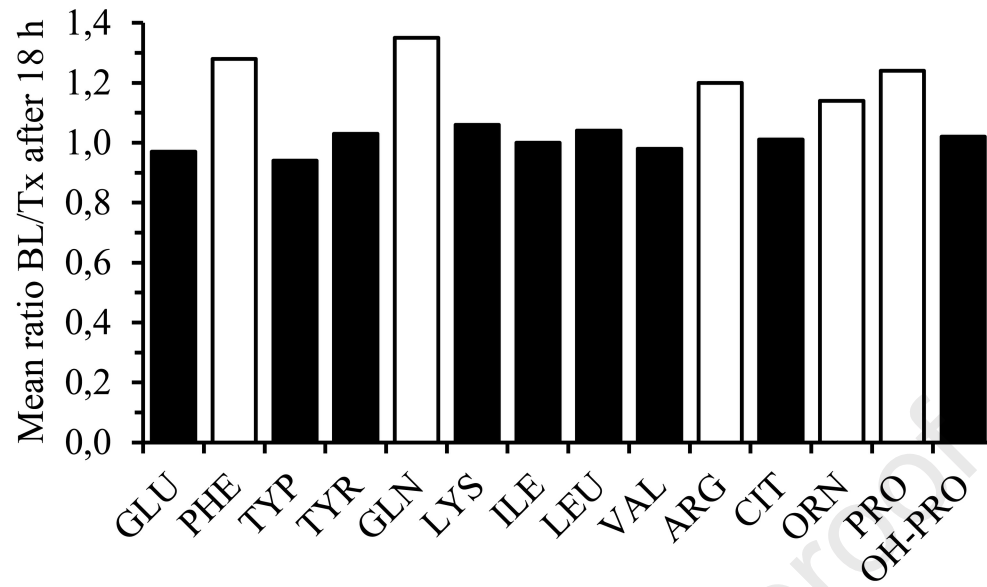


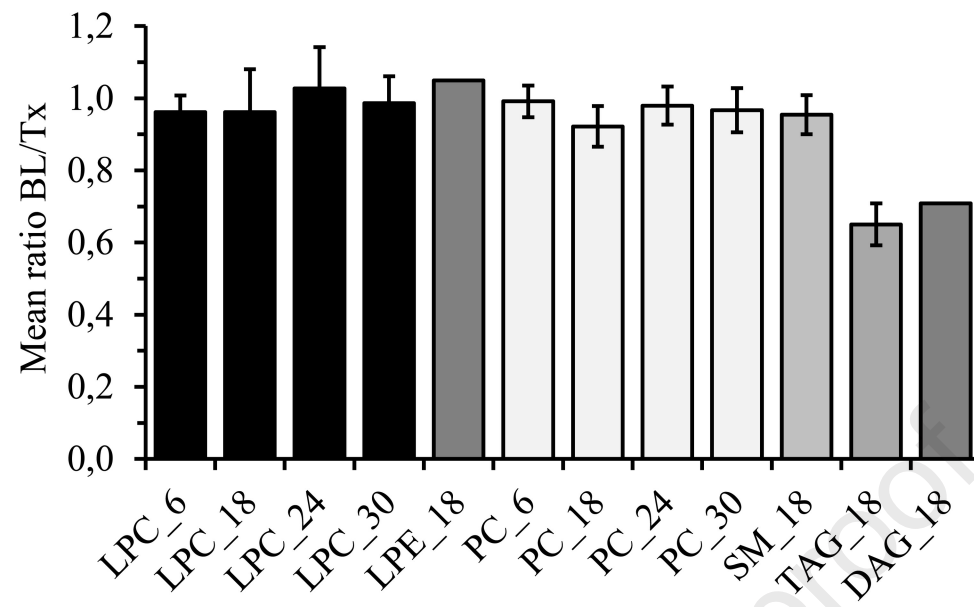


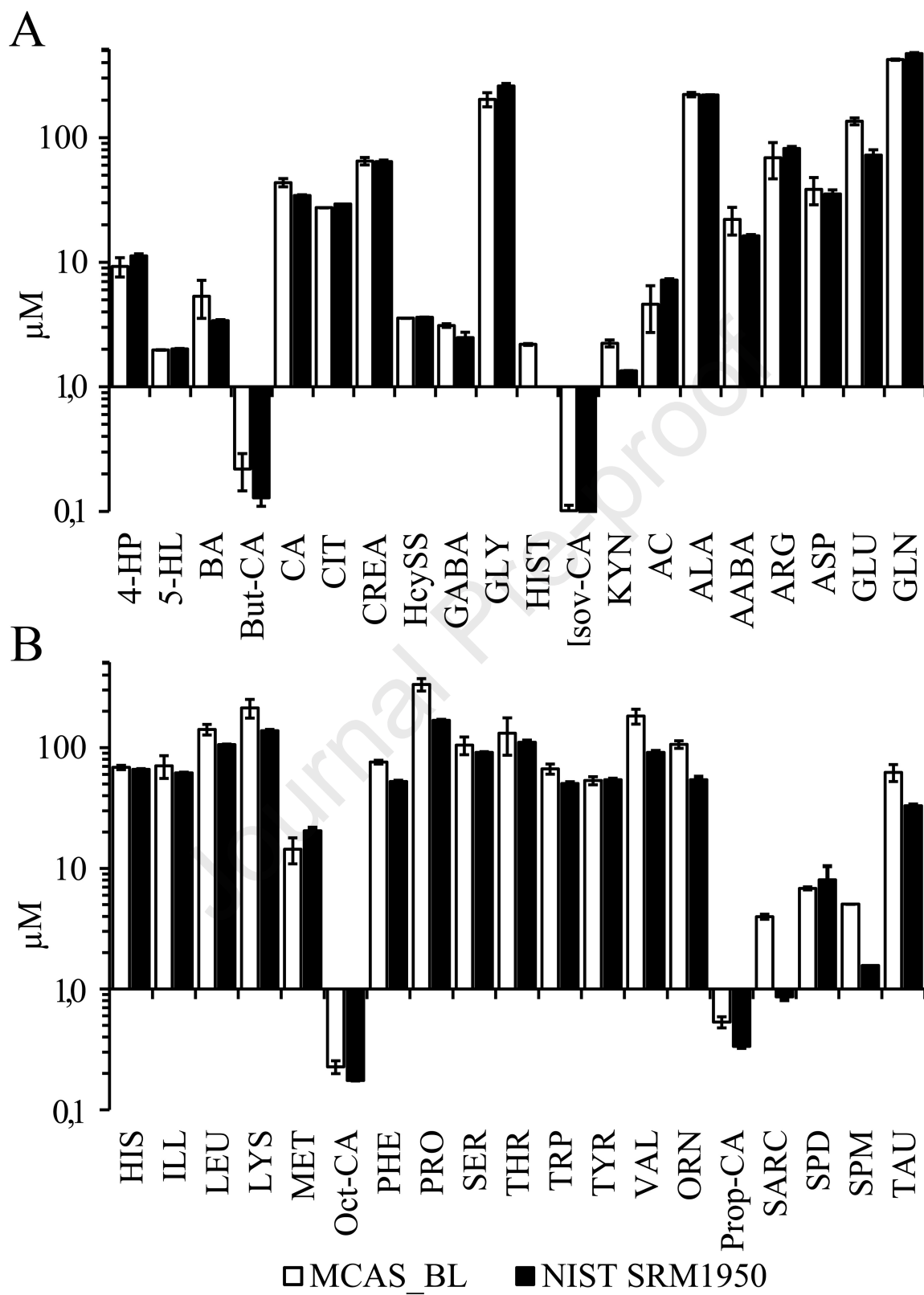


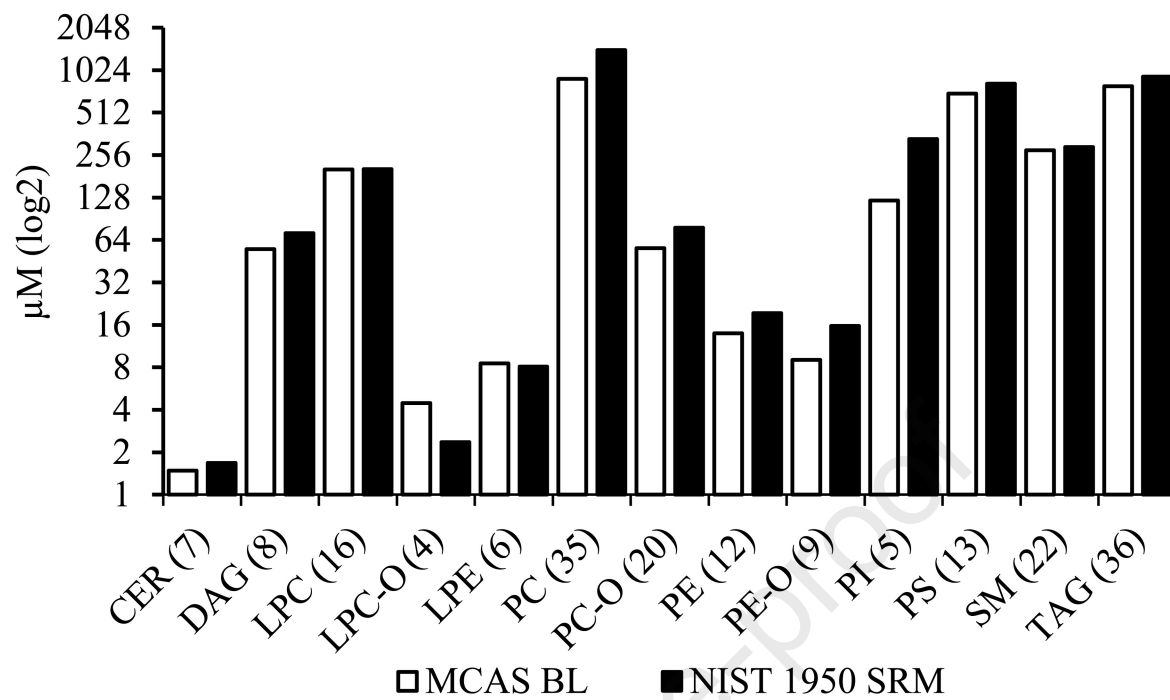


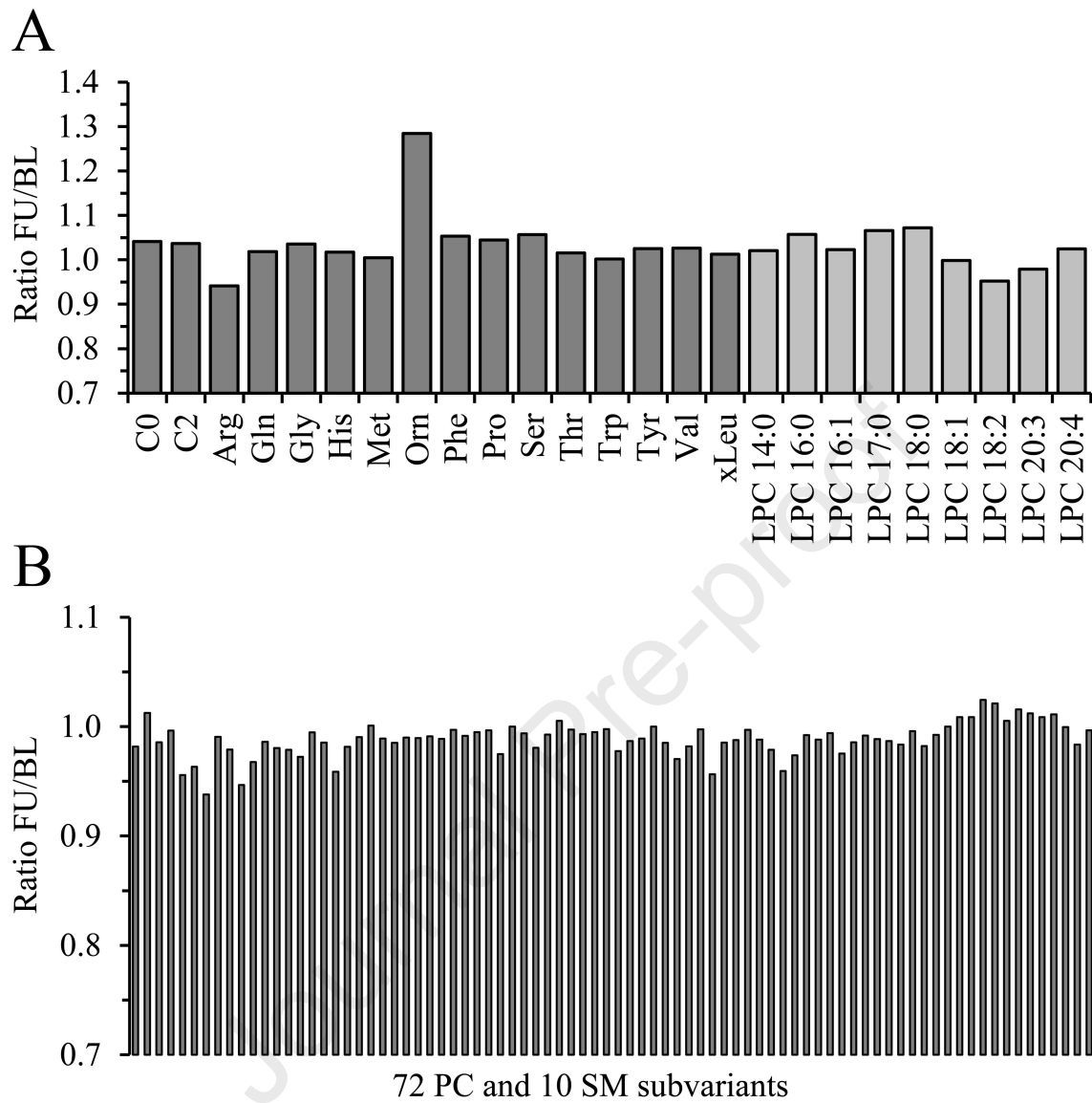


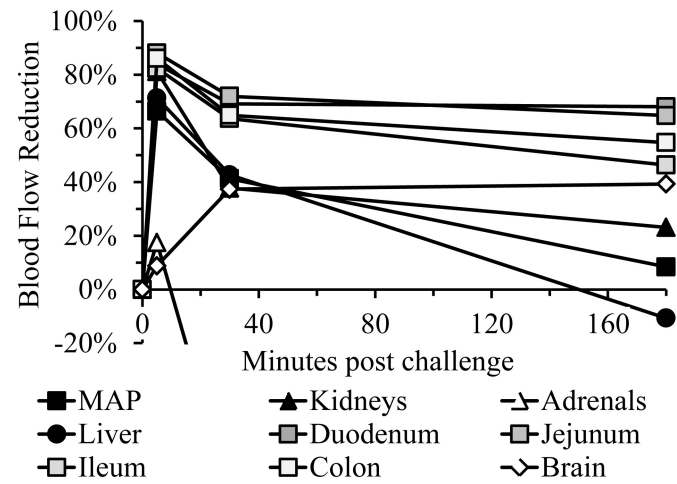


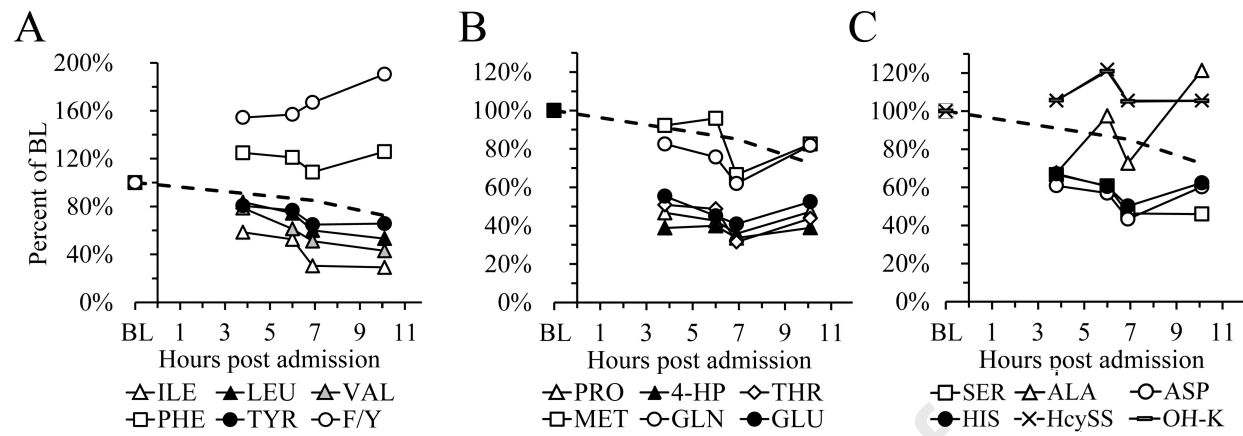


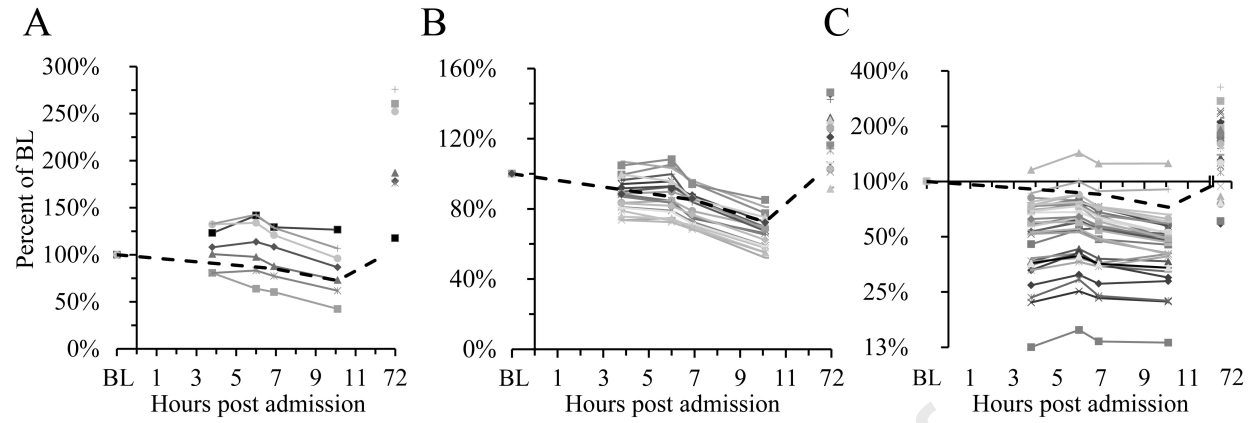












Online Repository to:**Metabolome and lipidome derangements during a severe mast cell activation event in a patient with indolent systemic mastocytosis**

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Additional methods from the main manuscript:

Lipidome and metabolome analysis

For metabolomics and lipidomics analysis we used EDTA plasma from two BL visits during the DAOMAST study and four different time points during the attack. At each time point two independent EDTA plasma samples were collected with and without a final concentration of 20 μ M diminazene aceturate, which was added to empty EDTA plasma collection tubes before the blood draw. Diminazene aceturate was added to inhibit DAO from degrading histamine. The 10% volume increase caused by adding the diminazene solution was corrected in the final analysis data set. The last sample was drawn as a single sample 72 hours after admission to hospital. In total 11 EDTA plasma samples drawn at five different time points in duplicate and one singlicate sample were analyzed in the same liquid chromatography mass spectrometry (LC-MS) run. All blood samples from the DAOMAST study and the MC activation event were immediately placed on ice, plasma was derived within 30 minutes and then frozen at -80°C.

For choline presentation the raw counts per second (cps) were used. Asymmetric and symmetric dimethylarginines (ADMA and SDMA) were quantified using the arginine standard.

Lipidome analysis

For lipid extraction 20 μ l of the samples were transferred into a glass vial and 10 μ l internal standard solution (SPLASH® Lipidomix®, Avanti Polar Lipids, Alabaster; AL) and 140 μ l methanol were added. After vortexing 500 μ l methyl-tert-butyl-ether (MTBE) were added and the mixture was incubated in a shaker for 10 min at room temperature (RT). A phase separation was induced by adding 130 μ l MS-grade water. After 10 min of incubation at RT, the samples were centrifuged at 1000 g for 10 min. An aliquot of 450 μ l of the upper phase was collected and dried under nitrogen. The samples were reconstituted in 200 μ l methanol and used for LC-MS analysis.

The LC-MS analysis was performed using a Vanquish UHPLC system (TFS = Thermo Fisher Scientific, Waltham; MA) combined with an Orbitrap Fusion™ Lumos™ Tribrid™ mass spectrometer (TFS, Waltham; MA). Lipid separation was performed by reversed phase chromatography employing an Accucore C18, 2.6 μ m, 150 x 2 mm (TFS, Waltham; MA) analytical column at a column temperature of 35°C. As mobile phase A an acetonitrile/water (50/50, v/v) solution containing 10 mM ammonium formate and 0.1 % formic acid was used.

Mobile phase B consisted of acetonitrile/isopropanol/water (10/88/2, v/v/v) containing 10 mM ammonium formate and 0.1% formic acid. The flow rate was set to 400 μ l/min. A gradient of mobile phase B was applied to ensure optimal separation of the analyzed lipid species. The mass spectrometer was operated in electrospray ionization (ESI)-positive and ESI-negative mode, capillary voltage 3500 V (positive) and 3000 V (negative), vaporize temperature 320°C, ion transfer tube temperature 285°C, sheath gas 60 arbitrary units, auxiliary gas 20 arbitrary units and sweep gas 1 arbitrary unit. The Orbitrap MS scan mode at 120000 mass resolution was used for lipid detection. The scan range was set to 250-1200 m/z for both positive and negative ionization mode. The automatic gain control (AGC) target was set to 2.0e5 and the intensity threshold to 5.0e3. The data dependent MS2 scan using high energy collision-induced dissociation (HCD) and collision-induced dissociation (CID) with fixed collision energy mode set to 35%. The data analysis was performed using the TraceFinder 4.1 software (TFS, Waltham; MA). Lipid class specific isotopically-labeled internal standard signals were used to obtain semi-quantitative values for lipids.

Metabolome analysis

Ten μ l of samples was mixed with 10 μ l of an isotopically-labeled internal standard mixture in a hydrophobic 96-well filter plate. Aliquots of 300 μ l of methanol were added and mixed for 20 min at 450 rpm. Afterwards the sample extracts were collected in a 96-well plate by centrifugation of the filter plate for 5 min at 500 g. A Vanquish UHPLC system (TFS, Waltham; MA) coupled with an Orbitrap Q Exactive (TFS, Waltham; MA) mass spectrometer was used for the LC-MS analysis. The chromatographic separation for samples was carried out on an ACQUITY UPLC BEH Amide, 1.7 μ m, 2.1 x 100 mm analytical column (Waters Corporation, Milford; MA) equipped with a VanGuard: BEH C18, 2.1 x 5mm pre-column (Waters Corporation, Milford; MA). The column was maintained at a temperature of 40°C and 2 μ l of the samples was injected per run. The mobile phase A was 0.15% formic acid (v/v) in water and mobile phase B was 0.15% formic acid (v/v) in 85% acetonitrile (v/v) with 10 mM ammonium formate. The gradient elution with a flow rate 0.4 ml/min was performed with a total analysis time of 17 min. The Orbitrap Q Exactive (TFS, Waltham; MA) mass spectrometer was operated in an ESI-positive mode with spray voltage 3.5 kV, auxiliary gas heater temperature 400°C, capillary temperature 350°C and auxiliary gas flow rate 12. The metabolites of interest were analyzed using a full MS scan mode with a scan range of 50 to 400 m/z, resolution 35000, AGC target 1.0e6 and a maximum integration time of 50 ms. The TraceFinder 4.1 software was used for the data processing. Seven-point linear calibration

curves with internal standardization and 1/x weighing was constructed for the quantification of metabolites.

Other measurements

Autotaxin (ATX) antigen was determined in duplicate using the ENPP-2 ELISA from R&D Systems (DENP20; Bio-Techne Ltd., Abingdon, UK) with modifications. The instructions in the manual do not recommend using citrate or EDTA plasma because calcium ions are necessary for the kit to perform properly. We therefore used the low cross buffer (LCB) from CANDOR Bioscience (Wangen, Germany) instead of the buffers RD1-34 and RD5-10. EDTA plasma samples were diluted 1 to 30 in LCB and 150 μ l added to the anti-ATX antibody pre-coated microtiter plates. The remaining steps followed the manual. The ENPP-2 ELISA was repeated and the means ($\pm$ SD) of the two means derived from duplicates are presented. The R^2 of the two correlations between absorption units and ATX standard concentrations were >0.98 and the mean coefficient of variation (CV) of the ATX standard concentrations between 3 and 50 ng/ml was 2.9% and 2.1% in the two independent experiments. All measured values were between 20 and 50 ng/ml of the standard curve before dilution corrections.

Statistical analysis

For amino acids, metabolites, amino acid/metabolite ratios and lipids the mean of duplicate measurements at baseline (BL), 3.8, 6.0, 6.9 and 10.1 hours post admission (0 hour) were used for further calculations. The 72 hour time-point was not included. The lipids within the same class but different fatty acid residues were pooled by summing the measured concentrations of the various subtypes.

To assess deviations of the amino acid/metabolite ratios the concentrations of two amino acids or metabolites during the attack were compared to the baseline ratios. The null hypothesis of equal mean values of the ratio prior to and during the attack was tested. This was performed using a two-sample t-test in a general linear model framework, which considers possible correlation of values measured during the attack-phase with short time intervals. We assumed an autoregressive correlation structure during the attack, meaning that the correlation between two values with time-interval δ is given by ρ^δ . The correlation parameter ρ was estimated by maximum-likelihood under the assumption of a linear time-trend and normally distributed data. Baseline values were regarded as independent because they were obtained with sufficient time distance. Using the same method, the mean values of systolic, diastolic, mean

arterial blood pressure and heart rate during the attack were compared to the baseline means. For calculating the significance of various ratios and cardiovascular parameters hemodilution/vascular leakage adjustment was not necessary.

To assess alterations in the concentration of amino acids, metabolites and lipids during the attack (most data points in Fig 3 and 4), we first calculated the relative change from baseline by dividing each value obtained during the attack by the mean of the corresponding baseline measurements. Then the relative changes were normalized by calculating the respective ratio to tyrosine for each time-point during the attack. Tyrosine is a very stable amino acid with minimal alterations during fasting, SIRS or sepsis conditions, and might better reflect vascular leakage compared to albumin (see Online Repository for further details and references). Albumin was used as reference for changes in lipid levels and as a secondary reference for changes in amino acid/metabolite levels (see Online Repository). This normalization procedure was required to account for hemodilution and vascular leakage. Before hypothesis testing the ratios were log-transformed to improve their distributional properties. The null hypothesis was that the log-transformed ratios have a mean of zero and was tested using a one-sample t-test in a general linear model framework. We again assumed an autoregressive correlation structure and the estimation of the correlation parameter was performed by maximum-likelihood under the assumption of a linear time-trend and normally distributed data.

To account for multiple testing we applied the Benjamini-Hochberg adjustment to the 70 p-values using tyrosine- and albumin-adjustment for amino acids/metabolites and lipids respectively (42 amino acids/metabolites, 14 lipids, 10 ratios and 4 cardiovascular parameters). The p-values of the secondary comparison of amino acid and metabolite levels with albumin-based hemodilution/vascular leakage adjustment were not included. The Benjamini-Hochberg adjusted p-values for these secondary comparisons using albumin-adjustment were calculated separately using 71 p-values (71 because tyrosine was included). Adjusted p-values below 0.05 were considered statistically significant controlling the false discovery rate at the 5% level. All calculations were performed using the statistical computing environment R 4.0. Selected p-values are integrated into the text and figure legends. All p-values are listed in the Online Repository Tables E1 and E2.

The p-values for non-lipid metabolites in the main manuscript are only based on the tyrosine hemodilution curve. For the lipids albumin was used for comparison, because lipids are not

freely diffusible, instead they are integrated into lipoprotein particles or exosomes or other suitable amphipathic transport vehicles.

Additional comments to some Figure legends in the main manuscript

FIG. 2

The coefficients of variation (CV) of all 4 baseline (BL) parameters (HSA [human serum albumin], TP [total protein], Hb [hemoglobin] and Hct [hematocrit]) determined three times over 9 months were < 4%. The 72 hours post admission time point was a singlicate; Except for Panel F the x-axis represents hours post admission.

FIG. 3

The mean of two baseline (BL) values without symptoms drawn four years earlier 11 months apart were used as 100%. The means of four different time points with two independent EDTA plasma samples for each time point drawn at the same time were normalized to the BL values; The 72 hours post admission time point data were derived from a single plasma sample; The 72 hour time point for albumin was not measured but assumed to be 100%; The x-axis in all panels except F represents hours post admission and the y-axis percent of BL; p-values correspond to comparison with the tyrosine (TYR) concentrations except for ratios.

FIG. 4

The basic method to generate these panels is similar to the description in FIG 3; The x-axis in all panels represents baseline (BL) and hours post admission and the y-axis percent of BL; p-values correspond to comparison with albumin concentrations.

Concentrations of quantified amino acids, metabolites and lipids

All concentrations of the different amino acids, metabolites and lipids used for calculations are provided in the Online Repository Excel File E1.

Summary of all p-values comparing metabolite and lipid concentrations with the albumin and tyrosine concentration curves during the first 10 hours of the event

Albumin concentrations are normally used to estimate hemodilution or hemoconcentration, vascular leakage or edema formation but albumin is a large molecule and the concentrations between plasma and interstitial fluid compartment unlikely equilibrate immediately. When we analyzed the data it immediately became obvious that during the acute phase with high histamine concentrations metabolite concentrations dropped more rapidly compared to albumin. The most reasonable explanation would be that an acute increase in permeability/vascular leakage “moved” small molecules like amino acids and other

metabolites into the interstitial fluid more quickly than they “moved” albumin. We did not see this phenomenon with lipids because hydrophobic lipids do not freely diffuse. They are mostly integrated into lipoprotein particles manifold larger compared to albumin. Consequently we compared metabolite with albumin concentrations but we also used tyrosine concentrations to calculate statistical significance because this amino acid is stable during fasting, sepsis or systemic inflammatory response syndrome (SIRS). During 36 hours of fasting, tyrosine was statistically not significantly reduced by 16%¹. After steroid treatment the ratio of treated versus untreated after 18 hours was 1.03². Tyrosine concentrations did not change in sepsis or during stress, today classified as SIRS^{3,4}.

TABLE E1: p-values of metabolite concentrations compared to albumin and tyrosine concentrations during the acute phase of the MCAS event

Amino acid/Metabolite	Abbreviation	Adjusted p-value HSA	Adjusted p-value TYR	Direction
<u>4-Hydroxyproline</u>	<u>4H-PRO</u>	<u>0.0100</u>	<u>0.0063</u>	<u>DOWN</u>
<u>5-Hydroxylysine</u>	<u>5H-LYS</u>	<u>0.0327</u>	<u>0.0185</u>	<u>UP</u>
Beta-Alanine	B-ALA	0.5474	0.4629	
Butyrylcarnitine	BUT-C	0.1712	0.4844	
Carnitine	C	0.2913	0.5259	
<u>Citrulline</u>	<u>CIT</u>	<u>0.0280</u>	<u>0.0270</u>	<u>DOWN</u>
<u>Creatinine</u>	<u>CREA</u>	<u>0.0708</u>	<u>0.0447</u>	<u>UP</u>
<u>DL-Homocystine</u>	<u>HcySS</u>	<u>0.0327</u>	<u>0.0185</u>	<u>UP</u>
<u>Gamma-Aminobutyric acid</u>	<u>GABA</u>	<u>0.0100</u>	<u>0.0270</u>	<u>DOWN</u>
Glycine	GLY	0.0522	0.1259	
<u>Histamine</u>	<u>HIST</u>	<u>0.0466</u>	<u>0.0467</u>	<u>UP</u>
Isovalerylcarnitine	ISO-C	0.4462	0.9758	
Kynurenine	KYN	0.6201	0.0818	
<u>L-Acetylcarnitine</u>	<u>ACE-C</u>	<u>0.0225</u>	<u>0.0197</u>	<u>UP</u>
L-Alanine	ALA	0.8355	0.3490	
L-Alpha-aminobutyric acid	AABA	0.1712	0.3390	
<u>L-Arginine</u>	<u>ARG</u>	<u>0.0189</u>	<u>0.0338</u>	<u>DOWN</u>
L-Asparagine	ASP	0.0457	0.0507	
<u>L-Glutamic acid</u>	<u>GLU</u>	<u>0.0327</u>	<u>0.0284</u>	<u>DOWN</u>
L-Glutamine	GLN	0.3633	0.5821	
L-Histidine	HIS	0.0457	0.0673	
<u>L-Isoleucine</u>	<u>ILL</u>	<u>0.0327</u>	<u>0.0380</u>	<u>DOWN</u>
L-Leucine	LEU	0.0532	0.3193	
<u>L-Lysine</u>	<u>LYS</u>	<u>0.0142</u>	<u>0.0063</u>	<u>DOWN</u>
L-Methionine	MET	0.9860	0.0689	
<u>L-Octanoylcarnitine</u>	<u>OCT-C</u>	<u>0.6201</u>	<u>0.0380</u>	<u>UP</u>
<u>L-Phenylalanine</u>	<u>PHE</u>	<u>0.0315</u>	<u>0.0102</u>	<u>UP</u>
<u>L-Proline</u>	<u>PRO</u>	<u>0.0224</u>	<u>0.0185</u>	<u>DOWN</u>
<u>L-Serine</u>	<u>SER</u>	<u>0.0225</u>	<u>0.0185</u>	<u>DOWN</u>
<u>L-Threonine</u>	<u>THR</u>	<u>0.0274</u>	<u>0.0185</u>	<u>DOWN</u>
L-Tryptophan	TRP	0.0466	0.0725	
L-Tyrosine	TYR	0.0517	NA	
L-Valine	VAL	0.0457	0.0891	
<u>Ornithine</u>	<u>ORN</u>	<u>0.0195</u>	<u>0.0185</u>	<u>DOWN</u>
Propionylcarnitine	PRO-C	0.5197	0.7801	
<u>Sarcosine (N-methyl glycine)</u>	<u>SARC</u>	<u>0.0311</u>	<u>0.0058</u>	<u>UP</u>
<u>Spermidine</u>	<u>SPD</u>	<u>0.0522</u>	<u>0.0236</u>	<u>UP</u>
<u>Spermine</u>	<u>SPM</u>	<u>0.0327</u>	<u>0.0185</u>	<u>UP</u>
Taurine	TAU	0.0520	0.1474	
<u>Asymmetric dimethyl-arginine</u>	<u>ADMA</u>	<u>0.0274</u>	<u>0.0063</u>	<u>UP</u>
<u>Symmetric dimethyl-arginine</u>	<u>SDMA</u>	<u>0.0212</u>	<u>0.0063</u>	<u>UP</u>
<u>Choline</u>	<u>CHOL</u>	<u>0.0095</u>	<u>0.0004</u>	<u>DOWN</u>

Bold = p-value <0.05; bold and underlined = p-value <0.01; UP = Increased and DOWN = decreased concentration time course compared to albumin or tyrosine concentrations; na = not applicable; All p-values have been adjusted for multiple testing using the method of Benjamini-Hochberg.

TABLE E2: p-values of lipid concentrations compared to albumin and p-values of ratios during the acute phase of the MCAS event

Amino acid/metabolite ratios	Abbreviation	Adjusted p-value	Direction
Kynurenine/Tryptophan ratio	KYN/TRP	0.018	UP
Acetylcarnitine/Carnitine ratio	Ace-C/C	0.064	
Ornithine/Citrulline ratio	O/CI	0.027	DOWN
Arginine/Ornithine ratio	R/O	0.053	
Arginine/Lysine ratio	R/K	0.053	
Arginine/(Ornithine + Lysine) ratio	R/(O + K)	0.055	
Arginine/(Citrulline + Ornithine) ratio	R/(Ci + O)	0.063	
Phenylalanine/Tyrosine ratio	PHE.TYR.ratio	0.018	UP
Arginine/ADMA	ARG/ADMA	0.024	DOWN
Arginine/SDMA	ARG./SDMA	0.038	DOWN
Lipids	Abbreviation	Adjusted p-value HSA	Direction
Cholesteryl esters	CE	0.1378	
Ceramides	CER	0.3482	
Diacylglycerides	DAG	0.0115	UP
Sphingomyelins	SM	0.0342	DOWN
Triacylglycerides	TAG	0.0063	DOWN
Lysophosphatidylcholines	LPC	0.0067	DOWN
Lysophosphatidylcholines	LPC-O	0.0063	DOWN
Lysophosphatidylethanolamines	LPE	0.0063	DOWN
Phosphatidylcholines	PC	0.0689	DOWN
Phosphatidylcholines	PC-O	0.2575	
Phosphatidylethanolamines	PE	0.0082	UP
Phosphatidylethanolamines	PE-O	0.0160	DOWN
Phosphatidylinositols	PI	0.0995	
Phosphatidylserines	PS	0.0332	UP

Bold = p-value <0.05; bold and underlined = p-value <0.01; UP = Increased and DOWN = decreased concentration time course compared to albumin concentrations for lipids. Ratios do not need to be normalized using albumin or tyrosine; All p-values have been adjusted for multiple testing using the method of Benjamini-Hochberg.

Influence of fasting and prednisolone

Effect of fasting on the metabolome and lipidome

The mastocytosis patient was fasting for roughly 20 hours from the first symptoms to the improvement in clinical parameters after the peak 8 hours post admission. In obese subjects who had been fasting for 3 to 5 days only the branched chain amino acids valine, leucine and isoleucine increased significantly, whereas arginine, ornithine and citrulline decreased by less than 20% with no changes in phenylalanine and tyrosine⁵. In the mast cell activation syndrome (MCAS) event ornithine, citrulline, arginine, valine and leucine strongly decreased (Fig 3 main manuscript). Two comprehensive metabolomics and lipidomics data sets featuring healthy volunteers who have been fasting for 36 hours have recently been published by Krug (2012)⁶ and Rubio-Aliaga (2011)¹. The patterns in comparison with the MCAS event are only minimally congruent. In the following figure (Fig E1) the amino acid changes after 36 hours of fasting are shown using the data from Rubio-Aliaga's (2011) supplement¹. The baseline plasma samples were drawn after overnight fasting and fasting was continued for another 24 hours. Arginine, citrulline and ornithine changed minimally. The only amino acids which increased were the branched chain amino acids valine, leucine and isoleucine. This is comparable to the Felig et al. (1969) study⁵. Importantly, ornithine does not change at all with citrulline showing a 19% reduction. The reductions in proline and hydroxyl-proline are also seen during the MCAS event. Methionine followed albumin-based hemodilution during MCAS, whereas it decreased by more than 50% during fasting. In sepsis methionine is sometimes greatly increased, as we saw during the event at the 72 hour time point. Elevated methionine concentrations seem to be a sign of recovery and anabolism after stress. The reduction in glutamate, which we saw during MC activation, is also partially reflected in the fasting data.

The alterations in the carnitine (C0) and acyl-carnitine (C2) during MC activation with a 400% increase in the C2/C0 ratio were also seen during fasting in healthy subjects but to a lesser extent (Fig E2). The C2/C0 ratio increased 212% after 36 hours of no food intake (data not shown).

In Fig E3 the changes between overnight and 36 hours fasting of 69 phosphatidylcholine (PC) subclass variants are shown. There is an overall 2% increase using all PC variants or a 6% increase using only the highest abundant PCs with >50 μ M. We did not see any relevant influence on PC concentrations, similar to fasting, during the acute phase of the MC activation event (Fig 4 main manuscript).

Using the described 8 lysophosphatidylcholine (LPC) variants the weighted average LPC decrease was 22% (Fig E4). Only the LPCs 18:0, 18:1 and 18:2 are statistically significantly different ($p < 0.05$) between baseline and fasting.

The mean increase of 15 sphingomyelins (SMs) was 7%, whereas triacylglycerides (TAGs) decreased by 33% (data not shown). The influence of fasting on diacylglyceride (DAG) variant concentrations was not described.

Comparable data about amino acids and lipids have been published by Krug et al. (2012) who enrolled 15 healthy male subjects⁶. These are available via the following link:

<http://metabolomics.helmholtz-muenchen.de/humet/>. The Krug data show a time course using 10 time points after a 12 hour overnight fast. Gabi Kastenmueller (Institute for Bioinformatics and Systems Biology; Helmholtz Zentrum Munich) provided us with additional data and relevant metabolites and lipids concentrations after 4, 12 and 36 hours of fasting and these data are presented in Fig E5.

The general patterns of metabolomic and lipidomic changes during fasting are consistent with increases in valine, leucine and isoleucine concentrations and minimal to moderate changes in other amino acids and lipids.

Fasting under SIRS with 39°C body temperature might be different, because the metabolic rate is likely 20 to 25% increased⁷, but fever is induced by cytokines and other metabolites and they could specifically influence metabolite concentrations. We are not aware of a publication with metabolomics data inducing elevated temperature under non-inflammatory conditions to possibly circumvent this “chicken-egg” problem.

Ornithine and citrulline concentrations are not reduced during fasting comparable to levels we have seen in the severe MC activation event after 18 hours of fasting. The branched chain amino acids moved in the opposite directions. Phenylalanine did not change at all during fasting, but was statistically significantly increased ($p = 0.01$ compared to tyrosine concentrations) during the MC activation event. In the two fasting studies the LPC levels decreased by a mean of about 20%, but we saw on average a 6.7-fold or 85% reduction. Nevertheless, certain SIRS conditions might induce the autotaxin (ATX) pathway and fever, and therefore show reduced LPC concentrations. Only limited data about LPC/LPA concentration alterations during different inflammatory conditions have been published.

Effect of steroids on the metabolome and lipidome

The mastocytosis patient was treated with a 250 mg prednisolone or 2.8 mg/kg bodyweight bolus immediately after admission, but human and animal data after steroid treatment show

different alterations and are unlikely causative of the perturbations seen in the metabolome and lipidome in this MC activation event for reasons discussed here. Loeffberg et al. (2002) treated 9 healthy volunteers with 60 mg prednisolone per day (0.76 mg/kg/day) for 3 days⁸ and Fig E6 shows the amino acid concentration changes as percent of baseline. Most amino acids including ornithine, arginine and lysine increased with no delta for citrulline and glutamate. Although these subjects were treated for 3 days, the dose was 3.7-fold lower. Nevertheless, an important mechanism of action of prednisolone or steroids in general is the modification of transcription programs and they take many hours to respond. Most deranged parameters in our patient were seen between 4 and 10 hours after administration of prednisolone.

Recently, van Zwol et al. (2017) compared serum amino acid changes in children with and without 30 mg/kg methylprednisolone administration prior to cardiac surgery⁹. The potency of methylprednisolone is about 1.25 compared to prednisolone. They collected samples at 0, 5, 17 and 29 hours and Fig E7 shows the changes in citrulline, arginine and glutamate concentrations normalized to baseline with or without steroid treatment. The zero time point is equivalent to the time of anesthesia induction, which was labeled as -5 hours. The reduced citrulline concentrations after 29 hours might reflect some hypoperfusion and enterocyte dysfunction of the gastrointestinal tract, but was only seen in the group without glucocorticoid treatment. Arginine did not change at all. Although the steroid dose was high, the caveat with this study is the age of the participants.

Six beagle dogs were treated with a high dose of 7 mg/kg/day prednisolone for 3 days and the lipidome was analyzed¹⁰. We extracted the log(2)-fold changes listed in Figures 3, 4 and 5 and converted them to mean-fold changes. The data are presented in Fig E8. The variability was rather high with the exception of sphingomyelins (SMs) and phosphatidylinositols (PIs). Neither PCs and LPCs nor PEs and LPEs were consistently decreased but showed a trend towards higher concentrations.

Finally, we would like to include metabolome and lipidome data from Bordag et al. (2015), who treated 20 healthy male volunteers with 30 mg prednisolone². Although this is a rather low dose, they collected day-time and fasting corrected samples. We present the ratios of steroid treatment 18 hours morning with no-treatment morning for selected amino acids (Fig E9) and lipids (Fig E10). For the PC and LPC lipids we included also the 6, 24 and 30 hour time points.

The 250 mg bolus dose of prednisolone in the MCAS patient was unlikely to be responsible either for the decreases in ornithine, citrulline and arginine or for the massive depletion of

LPCs from the circulation during the severe MC activation event. Although the published studies used different concentrations of steroids, different time courses and different populations including beagle dogs, we did not see patterns that can explain the observations during the MC activation event.

Estimation of the number of mast cells and of the amount of total tissue histamine in various organ systems

The average histamine content per MCs was estimated using the references listed in Table E3. Only lung, skin and gastrointestinal tract estimations were used to calculate an average of 3.2 pg histamine per human MC.

TABLE E3: Average histamine content in human MCs

Tissue	Histamine pg/MC	Reference
Lung	2,03	Metcalfe 1979
Lung	3,25	Paterson 1976
GI tract	2,8	Fox 1985
Skin	4,6	Benyon 1987
Mean	3,2	
(Bladder	7,6	Enerbaeck 1989)
(Nasal mucosa	11,8	Enerbaeck 1989)

The following Table E4 shows estimates of the histamine content in various human organ systems using published histamine concentrations and 3.2 pg histamine per MC. The human average organ weights and the measured histamine content in $\mu\text{g/g}$ wet weight were used to estimate the total histamine content and number of MCs. The total length of the jejunum/ileum was assumed to be 635 cm and the colon 160 cm¹¹. The lengths were used to estimate the weight of jejunum/ileum/colon based on a total weight of 2000 g described in Eve 1966¹².

TABLE E4: Estimation of histamine content and number of MCs in human tissues.

	Organ weight (g)	HIST ($\mu\text{g/g}$) $\$$	HIST (mg)	Number of MCs $\$$	Percent of HIST
Lung $\$$	840	11,3	9,5	3,0E+09	10%
Heart $\#$	279	1,8	0,5	1,6E+08	1%
Jejunum/Ileum $\ast$	1597	30,5	48,6	1,5E+10	50%
Colon $\ast$	403	14,1	5,7	1,8E+09	6%
Pancreas $\#\#$	87,3	11,4	1,0	3,1E+08	1%
Liver $\$$	1561	1,7	2,7	8,3E+08	3%
Spleen $\$$	139	6,3	0,9	2,7E+08	1%

Skin**	3860	7,5	29,0	9,0E+09	30%
Total HIST in mg			97,8	100%	
Total number of MCs				3,1E+10	

#Mubbunu 2018¹³; \$Molina 2012¹⁴; *Eve 1966¹² and Hounnou 2002¹¹; **Goldsmith 1990¹⁵; ##Caglar 2012¹⁶; \$\$Hesterberg 1984¹⁷; Average histamine (HIST) content 3.2 pg/MC.

The lungs, small intestine and the skin are clearly the target organs for local tissue histamine effects.

Comparison of measured metabolites in the mastocytosis patient with the NIST SRM1950 comparator

Most metabolites between the MCAS patient and the pooled NIST SRM1950 standard are quite similar (Fig E11). It is expected that histamine concentrations are elevated in the mastocytosis patient. Sarcosine (SARC) is 4.6 fold higher. Although spermine appears significantly higher, the limit of detection (LOD) for spermine is 3.3 μ M (1.65 μ M used for presentation) and the MCAS sample was measured at 5.1 μ M.

Comparison of measured lipid classes in the mastocytosis patient with the NIST SRM1950 comparator

The lipidome of the mastocytosis patient is very similar to the NIST standard plasma (Fig E12). The largest difference is seen with the 7 cholesterol esters of 29 mM versus 65 mM for the NIST standard. Importantly, mean LPC concentrations are almost identical.

Metabolites and lipid class subvariants are stable during 4 years of storage (Fig E13)

Gastrointestinal blood flow is severely compromised in a dog anaphylaxis model (Fig E14)

Additional metabolomics and lipidomics data (Fig E15 and E16)

Additional discussion topics

What are possible trigger mechanisms?

The patient describes upper respiratory infections as the main trigger for MC activation events but how an infection might lead to massive MC degranulation or activation is unknown. Based on the time course, depletion of LPC may have preceded severe MC activation, which started about 3 hours after admission. Degradation of LPCs likely started during the night. Reduced LPC concentrations have been described in sepsis, malaria and trypanosomiasis, but the mechanisms were never defined²⁰⁻²³. A reasonable hypothesis would be that SIRS conditions activate integrin receptors on endothelial cells leading to increased local ATX activity with LPA release activating MCs. This ultimately starts a positive feedback loop driven by histamine release itself activating endothelial cells (Fig 6 main manuscript). If histamine release is exhausted, because MCs are degranulated or liberated histamine rapidly counterbalanced by the release of diamine oxidase (DAO), the positive feedback loop is interrupted and conditions improve. Depletion of LPC could also provide a stop signal. It is tempting to speculate that MCs per se are involved in the development of SIRS. Heparan sulfate and heparin are considered to be damage-associated molecular pattern molecules (DAMPs) activating TLR4 receptors on endothelial cells like lipopolysaccharides²⁴. Because mastocytosis patients have a significantly increased MC burden, minor perturbations in the propensity to induce more MC degranulation via a viral infection, medications or food ingredients might tip the balance. The general immunological concept of cellular priming could be involved. In human MCs LPA-mediated chemokine release is increased manifold after IL-4 priming²⁵.

What are possible therapeutic consequences?

The most significantly affected organ during the described MCAS was clearly the gastrointestinal tract. Constriction of the splanchnic circulation occurs before manifest arterial hypotension²⁶. This central pressure preservation mechanism could have contributed to enterocyte dysfunction. Infusion of citrulline, which is converted to arginine in the kidneys, alone or in combination with arginine, might have increased arginine concentrations and reduced the endothelial nitric oxide synthase (eNOS) uncoupling and release of damaging free radicals^{27,28}. Nevertheless, increased nitric oxide synthesis could have increased not only hypotension but also vascular leakage, tissue pressure and therefore hypoxia with further deterioration of the enterocyte function. Arginase inhibitors could also be beneficial²⁹. Supplementation with tetrahydrobiopterin (THB4) might be also considered as a new

therapeutic option. Positive effects in sepsis and other conditions with endothelial cell dysfunction have been described³⁰. Based on the time course of plasma histamine concentrations rapid, efficient and continuous degradation of histamine at admission or early during protracted MCAS might have prevented the life-threatening symptoms which occurred 4 to 8 hours later. The usage of recombinant human DAO at the onset of symptoms should be tested. This enzyme is able to rapidly remove histamine from human plasma³¹. Diamine oxidase will readily diffuse into the interstitial space considering that capillaries are leaky. The histamine gradient will be disrupted by high local DAO concentrations with reduced transendothelial cell migration of inflammatory cells and translocation of bacteria. In healthy pregnant women the steady state concentration of DAO in skin blister fluid, whose composition is considered to be equivalent to the composition of interstitial fluid, was 12% of plasma values without excess histamine or increased vascular leakage³². During inflammation this rate likely increases to 50% or more as has been shown for other proteins³³. Plasma and interstitial fluid equilibration might happen rapidly under SIRS conditions with collaborative induction of vascular leakage by potent mediators like histamine and LPA³⁴. The attack clearly started hours before admission and it is tempting to speculate that ATX inhibition might have reduced depletion of LPC, inhibited the release of LPA and possibly prevented full development of the attack. Only clinical studies will elucidate the therapeutic potential of ATX inhibitors in severe MCAS. Several ATX inhibitors are in clinical development³⁵. Suitable animal models of MCAS do not exist. One could also argue that the depletion of LPC is involved in the development of symptoms. As described above LPC is reduced in sepsis and other inflammatory conditions. In several animal sepsis models LPC administration demonstrated beneficial effects including reduction in mortality³⁶⁻³⁸. It is not clear how LPC caused these effects, but uncompetitive inhibition of phospholipase A₂ activity and suppression of the release of HMGB1, an intracellular DAMP, might be involved^{36,39}. If depletion of LPC causes increased inflammation, administration of LPC might dampen an excessive inflammatory response. Nevertheless, if LPA plays an important role via increased ATX activity, administration of LPC might increase LPA concentrations with potential negative consequences. Combined inhibition of ATX and/or LPA receptor signaling with LPC administration might demonstrate clinical benefits.

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537

Figure legends

FIG E1. Amino acid changes after 36 hours of fasting.

The means of μM concentrations of the different metabolites after overnight fasting were divided by the means after additional 24 hours of fasting and the ratios subtracted from 1. Data are from the supplement of Rubio-Aliaga et al. (2011)¹ and comprise 10 healthy subjects.

FIG E2. Carnitine (C0) and acyl-carnitine changes during fasting.

Data are from the supplement of Rubio-Aliaga et al. (2011)¹.

FIG E3. Phosphatidylcholine lipids increase by 2% on average.

Data are from the supplement of Rubio-Aliaga et al. (2011)¹.

FIG E4. Lysophosphatidylcholine (LPC) lipids decrease 22% after 36 hours of fasting using weighted average calculation.

Dark bars are data during fasting; White bars represent the MC activation data of the same LPC variants from the mastocytosis patient for comparison. Fasting data are from the supplement of Rubio-Aliaga et al. (2011)¹. Only the LPCs 18:0, 18:1 and 18:2 are statistically significantly different ($p < 0.05$) between baseline and fasting.

FIG E5. Metabolite and lipid concentration changes comparing 4, 12 and 36 hours of fasting

(A) Carnitine (C0); Acetylcarnitine (C2), various amino acids; xLeu includes leucine and isoleucine; (B) 61 Phosphatidylcholine variants; (C) 8 Lysophosphatidylcholine (LPC) variants; (D) 12 Sphingomyelin (SM) variants; Each curve represents the mean of 15 healthy individuals after 4, 12 and 36 hours of fasting as described in Krug et al. (2012)⁶. Data were generously provided by Gabi Kastenmueller.

FIG E6. Prednisolone at 60 mg/day increased most amino acids after three days of treatment in healthy volunteers.

Data are from Table 3 in Loeffberg et al. (2002)⁸.

FIG E7. High dose methylprednisolone did not reduce ARG and GLN but CIT concentrations after 29 hours.

Data are from Table 2 in van Zwol et al. (2017)⁹. G+ (black symbols) means with glucocorticoid treatment including 33 children. Sixteen children were not treated with glucocorticoids (G-; white symbols).

FIG E8. Mean lipid class changes after high dose prednisolone treatment for 3 days in 6 beagle dogs.

Data are from Sieber-Ruckstuhl et al. (2019)¹⁰. The numbers after the lipid classes represent the included subvariants. Of the 37 PCs lipids only 27% (10/37) fold-changes were statistically significant. For the PIs all changes were statistically significant. The error bars represent the standard deviation of the mean-fold changes from the 6 dogs.

FIG E9. Low dose prednisolone increased the amino acids PHE, GLN, ARG, ORN and PRO after 18 hours.

Data are from the supplement of Bordag et al. (2015)². The white bars represent changes with a p-value of <0.001. CIT did not change at all. BL = baseline; Tx = Prednisolone treatment for 18 hours.

FIG E10. Low dose prednisolone did not change PCs and LPCs after 18 hours.

The mean ratios with standard deviations of 6 LPCs, 1 LPE, 11 PCs, 5 SMs, 7 TAGs and 1 DAG (18:1 and 18:2 combined) are presented. Data are from the supplement of Bordag et al. (2015) and included 20 healthy volunteers²; BL = baseline; Tx = Prednisolone treatment.

FIG E11. The metabolome of the MCAS patient baseline is similar to NIST SRM1950.

The two MCAS patient baseline samples were drawn 11 months apart. The means (+/- SD) are shown; For the NIST SRM1950 plasma the SDs of the duplicate determinations are included. The duplicates were used as quality controls during the MCAS sample analysis; BA = Beta-alanine; xxx-CA = Acyl-carnitines; CA = Carnitine; CREA = Creatinine; HcySS = Homocystine; GABA = Gamma-amino-butyric acid; HIST = Histamine; AC = Acetyl-carnitine; AABA = Alpha-amino-butyric acid; SARC = Sarcosine (Methylglycine).

FIG E12. The lipidome of the MCAS patient baseline samples is very similar to NIST SRM150.

The two MCAS patient baseline samples were drawn 11 months apart. The sums of the means of the different lipid subclass variants are shown (excluding cholesterol esters); For the NIST SRM1950 samples the means of triplicate determinations are shown. They were used as quality controls. The number of included subclass variants is listed in brackets. CER = Ceramides; DAG = Diacylglycerides; LPC/LPC-O = Lysophosphatidylcholines; LPE = Lysophosphatidylethanolamines; PC/PC-O = Phosphatidylcholines; PE/PE-O = Phosphatidylethanolamines; PI = Phosphatidylinositols; PS = Phosphatidylserines; SM = Sphingomyelins; TAG = Triacylglycerides (TAGs).

FIG E13. Metabolites and lipid class subvariants are stable comparing baseline and follow-up samples after 4 years of storage.

The upper **(A)** and lower **(B)** panels show the ratios of fresh (Follow-up) to 4 year old (Baseline) mean concentrations (n = 1409) of selected metabolites. The raw data are published in supplementary Table 1 of Lacruz et al. (2018)¹⁸. The upper panel shows carnitine, acetyl-carnitine, 15 amino acids and 9 lysophosphatidylcholine (LPC) subvariants. The lower panel shows ratios of 72 phosphatidylcholine (PC) and 10 sphingomyeline (SM) subvariants. Unstable metabolites would be indicated by increased ratios. For example a 50% reduction in the concentration measured in the follow-up samples would generate a ratio of 2.0; The mean (SD) ratios for amino acids, LPCs, PCs and SMs are 1.04 (0.076), 1.02 (0.040), 0.99 (0.014) and 1.01 (0.012) respectively; C0 = carnitine; C2 = acetyl-carnitine; xLeu includes leucine and isoleucine; FU = Follow-up; BL = Baseline.

FIG E14: Gastrointestinal blood flow is severely reduced after induction of mast cell degranulation in a dog anaphylaxis model.

Raw data of blood flow (ml/min/100 g tissue) to various tissues were used from Table 4 published in Kapin and Ferguson (1985)¹⁹. The absolute blood flow values at baseline before induction of anaphylaxis were used to normalize the published data; Blood flow values in the gastrointestinal tract did not return to baseline concomitant with liver blood flow or MAP values. Adrenals show hyperperfusion (negative values) after 20 minutes. Brain blood flow is slightly reduced in the acute phase but approximately 40% starting at 30 and lasting until 180 minutes; MAP = Mean Arterial Pressure.

FIG E15. Additional metabolomics data during the severe MC activation event

(A) Branched chain amino acids valine (VAL), leucine (LEU), isoleucine (ILE), phenylalanine (PHE; p = 0.01), tyrosine (TYR) and the F/Y ratio (p = 0.02); The F/Y ratio was 102% of BL after 72 hours; **(B)** Glutamic acid (GLU; p = 0.03), glutamine (GLN), methionine (MET), threonine (THR), proline (PRO) and 4-hydroxy-PRO (4H-PRO); **(C)** Serine (SER), histidine (HIS), asparagine (ASP), alanine (ALA), 5-hydroxy-lysine (OH-K), homocystine (HcySS; p = 0.02); OH-K and HcySS have overlapping curves; Glycine is similar to HIS (not shown); For a description of how the panels were generated see above under **Additional comments to some Figure legends in the main manuscript.**

FIG E16. Additional lipidomics data during the severe MC activation event

All measured diacylglycerides **(A)**; sphingomyelines **(B)** and triacylglycerides **(C)**. For a description of how the panels were generated see above under **Additional comments to some Figure legends in the main manuscript.**