

Comprehensive metabolomic analysis of peanut-induced anaphylaxis in a murine model

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Abstract Acute anaphylaxis caused by food allergy is a potentially life-threatening allergic reaction that results in thousands of hospitalizations in North America every year. There are currently no effective means of treatment for anaphylaxis beyond the administration of epinephrine which is only given after a reaction has already started. Furthermore, knowledge regarding the underlying physico-chemical basis of anaphylaxis is largely incomplete. Arguably, the study of anaphylaxis by comprehensive metabolomics offers a unique opportunity to study this phenomenon due to its ability to provide a snap-shot of the chemical state of a subject experiencing anaphylaxis encompassing both endogenous and exogenous factors including dietary and environmental effects. In this study, we sought to apply a

comprehensive metabolomics approach using liquid chromatography–mass spectrometry to a murine model of peanut-induced anaphylaxis. The results of this study revealed that the metabolomic profiles of mice experiencing peanut-induced anaphylaxis followed a distinct pattern that mirrors the time-stage of anaphylaxis being experienced. Direct comparison of anaphylactic mice to time-matched control mice showed that the metabolomic profiles changed considerably as the anaphylactic reaction progressed. This comparison also revealed changes in both known biomarkers of anaphylaxis such as histamine and methylhistamine as well as a suite of metabolites chemically similar to known anaphylaxis mediators but whose biological functions are not well understood. These metabolites include a number of phospholipids such as a variety of phosphatidylcholines and lyso-phosphatidylcholines that may be of interest for further investigation regarding their potential as anaphylaxis mediators or chemical precursors to mediators.

This work is dedicated to the memory of Brian E. McCarry. Chalcraft and Kong contributed equally to this work.

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Keywords Comprehensive metabolomics · Peanut allergy · Anaphylaxis · Liquid chromatography–mass spectrometry · Orthogonal partial least squares-discriminate analysis · Blood serum analysis

Abbreviations

CT	Cholera toxin
GC–MS	Gas chromatography–mass spectrometry
i.p.	Intraperitoneal
LC–ESI–TOF–MS	Liquid chromatography–electrospray ionization–time-of-flight–mass spectrometry
OPLS–DA	Orthogonal partial least squares-discriminate analysis
PIA	Peanut-induced anaphylaxis
PAF	Platelet activating factor

1 Introduction

Anaphylaxis is an acute, often severe and potentially lethal allergic reaction that occurs in sensitized individuals when they are re-exposed to the sensitizing agent or allergen. Anaphylactic reactions can be precipitated by exposure to drugs, venoms and foods. However, foods are the leading cause of anaphylaxis (Brown et al. 2001; Sampson 2003; Smit et al. 2005) as, indeed, they account for one-third to one-half of anaphylaxis cases in emergency departments in developed countries. It has been estimated that approximately 30,000 food-related anaphylactic reactions are treated in US emergency departments resulting in ~2000 hospitalizations (Sampson 2003; Yocum et al. 1987). In the paediatric population, food reactions are responsible for an even larger proportion of anaphylactic reactions (Mehl et al. 2005) with nuts being the most prominent culprits. Of particular concern, there are no treatments for anaphylaxis with the exception of epinephrine, which is only given after a reaction has started. While the term anaphylaxis was formally coined over 100 years ago, the biological and chemical basis of anaphylactic reactions remains largely to be elucidated. This represents a considerable knowledge gap.

The aim of this study was to apply a comprehensive metabolomics approach by liquid chromatography–electrospray ionization–time-of-flight–mass spectrometry (LC–ESI–TOF–MS). This platform was used as a discovery tool to identify metabolomic changes in mice undergoing peanut-induced anaphylaxis (PIA). Unlike other -omics approaches, such as genomics and proteomics, metabolomics provides a biochemical snapshot, reflective of the health status and phenotype of the subject at the time of sampling (Patti et al. 2012). This incorporates not only genetic and enzymatic effects but also the impact of exogenous nutritional and environmental factors, an especially attractive advantage to anaphylaxis and allergy research (Johnson and Gonzalez 2012; McNiven et al. 2011; Patti et al. 2012; Zhang et al. 2011). Furthermore, a metabolomics approach is capable of detecting changes that occur within minutes (the time-frame of anaphylaxis) as opposed to proteomics/genomic changes that generally take longer to be expressed.

The use of an animal model for the study of anaphylaxis affords significant advantages over studies in humans. The first advantage is the ability to precisely control dietary and environmental conditions as well as minimizing genetic variability. Secondly, the opportunity to carry out detailed kinetic studies, ranging from immediately after exposure to the elaboration of a robust clinical and physiological response. Finally, the use of an animal model allows the study of full-blown anaphylaxis; For ethical reasons studies involving humans must be terminated before full anaphylaxis occurs. The murine model used in this study has been

previously characterized and shown to mimic many features of the human condition (Arias et al. 2009; Sun et al. 2007). PIA is exquisitely dependent on peanut-specific immunoglobulins, in particular peanut-specific IgE which, along mast cells, play a most prominent role.

The only reported metabolomics study investigating anaphylaxis to date utilized gas chromatography–mass spectrometry (GC–MS) in a guinea pig model sensitized to ovalbumin and/or cattle albumin (Hu et al. 2012). While this study detected some metabolomic differences between anaphylactic and non-anaphylactic subjects, only one time point was studied, 1 h after challenge. Consequently, the identified metabolite profile was, as acknowledged by the authors, largely associated with down-stream metabolism such as glucose depletion rather than metabolism involved with the development of the anaphylactic reaction. In our study, we monitored the blood serum metabolome of sensitized mice challenged with peanut at various time points starting at 1 min after challenge and up to 40 min post-challenge. We also investigated non-sensitized mice exposed to peanut which, as expected, did not experience anaphylaxis. By comparing anaphylactic mice to time-matched, non-anaphylactic control mice (time-series comparison), and sensitized mice at various stages of the reaction (time-progression comparison), we demonstrated substantial and significant changes in the serum metabolome reflecting the different stages of PIA. Another limitation to the study performed by Hu was that GC–MS is typically not capable of measuring intact lipids, as these compounds are typically lysed during derivatization and only the total fatty acid profile can be measured regardless of the lipid origin. Since at least one known mediator of anaphylaxis, platelet-activating factor (PAF), is an intact phosphatidylcholine, the ability of LC–ESI–MS to measure intact lipids is a considerable analytical advantage.

2 Materials and methods

2.1 Animal model, sample collection and preparation

All animal experimentation was approved by the Animal Research Ethics Board at McMaster University. As previously described (Arias et al. 2009; Sun et al. 2007), female C57BL/6 mice (6–8-weeks-old) were sensitized to peanut by administering on four occasions, 1 week apart, 3.75 mg of peanut butter from KraftTM, which contains 1 mg peanut protein and 10 µg of the adjuvant cholera toxin (CT) (List Biological Laboratories) in 100 µL of sterile phosphate buffered solution by oral gavage. Full details on the preparation of the extract may be found in the supplementary materials. 2 weeks after the last gavage, mice were challenged via intraperitoneal (i.p.) injection with

crude peanut extract. To account for potential metabolomic changes due to the feeding of peanut, additional groups of control mice were treated with peanut butter only without CT; under these conditions, mice are unable to become sensitized (allergic). Separate groups of mice were sacrificed pre-challenge and at 1, 10, 20, 30 and 40 min post-challenge. Each group consisted of 7 mice and, thus, a total of 84 mice were used in this study. Just prior to sacrifice by cervical dislocation, approximately 20–50 μ L of whole blood was collected by retro-orbital bleeding of anesthetized mice with a glass capillary tube. To prevent any bias in metabolite levels due to the effects of bleeding, mice were bled only once at the specified time point. Anaphylaxis was assessed by measuring core body temperature with a rectal probe just prior to blood collection as well as blood hematocrit levels. Peanut-specific IgE levels were also measured and may be found in the supplementary materials. Serum was prepared by allowing the blood to clot whereupon the clear fluid was separated by centrifugation at 4 °C. Precipitation of serum protein and metabolite extraction was performed by the addition of a 1:1 solution of methanol:ethanol containing internal standards (listed in supplemental materials) at 4 °C in a ratio of 4:1 solvent to serum ratio as previously described (Bruce et al. 2009). Precipitated protein was pelleted by centrifugation at 4 °C and the fluid supernatant was removed for analysis by LC-ESI-TOF-MS.

2.2 LC-MS analysis

After thawing, 5 μ L aliquots of each serum extract were pooled and used for quality assurance purposes. For LC separation a SeQuant ZIC HILIC column (2.1 $\times$ 50 mm, 3 μ m) and a HALO RP-Amide column (2.1 $\times$ 50 mm, 2.7 μ m) were connected in series on an Agilent 1200 “RR” series LC operated with a flow rate of 200 μ L/min at 50 °C. Gradient elution was used where solvent “A” was acetonitrile and “B” was 10 mM ammonium acetate brought to a pH of 3.0 by the addition of formic acid (Sigma-Aldrich; St. Louis MO). Gradient conditions began at 95 % A and holding at 95 % A for 0.5 min followed by a linear decrease over 9.5 min to 30 % A, a hold at 30 % A for 5 min, then a linear increase to 95 % A over 2 min. Between runs the columns were re-equilibrated at 95 % A for 13 min. MS detection was done on a Bruker micrOTOF II time-of-flight mass spectrometer carried out over a mass range of 75–1,000 m/z in both positive and negative ionization modes with a scan rate of 2 Hz. Drying gas (N_2) was set at 6 L/min, nebulizing gas pressure was set at 3.0 bar and a capillary potential of 4.5 kV for positive ionization mode and –3.8 kV for negative ionization mode. All injections were 2 μ L (0.4 μ L serum equivalent injected on column). Injections of the pooled sample were carried out

in pentuplicate prior to injections of individual samples, and after every tenth sample throughout the sample set then again in pentuplicate after the last sample to track instrumental performance throughout the analysis of the sample set. MS/MS experiments for metabolite identification of interesting features were carried out on a Bruker Maxis 4D ultra-high resolution QTOF coupled to a Dionex 3000 RS UPLC under similar elution and MS conditions as used with the MicrOTOF II. Collision energies were chosen to provide parent ion intensities of 30–70 % of the most intense fragment ($\sim$ 10–40 eV).

2.3 Data processing

After data acquisition, raw chromatograms were converted to mzXML format by Bruker CompassXport. These chromatograms were then aligned, extracted and integrated by the open source program XCMS (Scripps Institute for Metabolomics). The XCMS companion software package CAMERA (Scripps) was also used to identify isotope and adduct peaks. The areas of the integrated peaks were compared to the peak area of an internal standard for both ionization modes (glycyl-phenylalanine; Sigma-Aldrich, St. Louis MO). Full XCMS parameters used for data extraction and peak alignment may be found in supplementary materials.

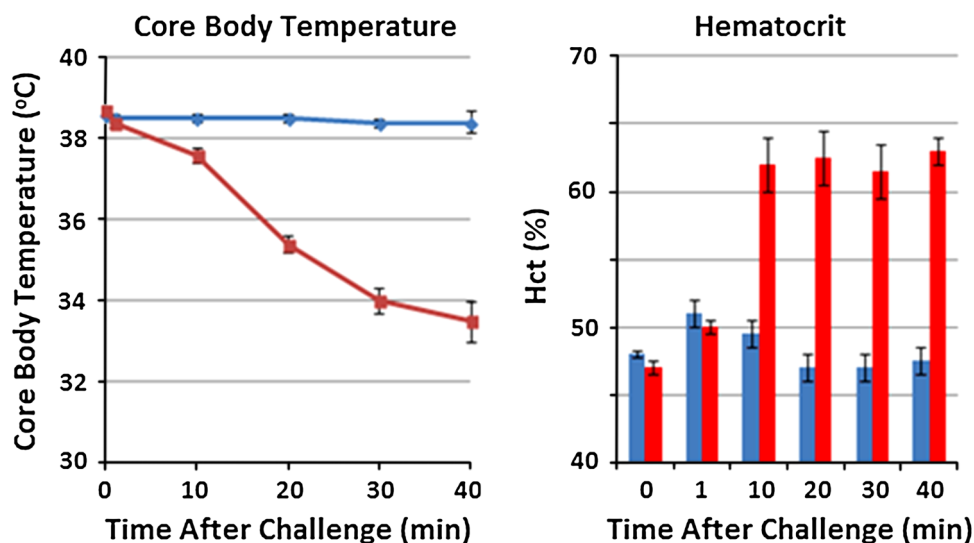
2.4 Statistical analysis

Orthogonal partial least squares-discriminate analysis (OPLS-DA) was performed using the data analysis software package SIMCA P+ (version 12.0.1.0; Umetrics, Umeå Sweden). For all analyses unit variance scaling with a 1/square root block weight was applied. Student’s *t* test and analysis of variance (ANOVA) statistical analyses were performed using Microsoft Excel. Student’s *t* test calculations assumed a 2-tail distribution with heteroscedastic scattering. ANOVA was done with two-factor (2-way) with replication.

2.5 Metabolite identification

Metabolite identification was confirmed when possible by comparison to authentic chemical standards encompassing a minimum of two orthogonal measurements as *per* Sumner. When an authentic standard was not available, metabolite identification level was assigned also as *per* Sumner (Sumner et al. 2007). For level 2 identification (comparison to database mass spectra) the METLIN database (Scripps Institute for Metabolomics) or the Human Metabolomic Database (Wishart et al. 2013) were used as the primary resources.

Fig. 1 Core body temperature and hematocrit levels in mice undergoing peanut-induced anaphylaxis as a function of time post i.p. challenge with peanut extract. Data for sensitized mice shown in red; data for non-sensitized mice in blue (Color figure online)



3 Results

3.1 Clinical manifestation of anaphylaxis

In order to assess whether PIA did indeed occur in the subject mice, two measurements indicative of anaphylaxis, core body temperature and blood hematocrit were measured (Arias et al. 2011). As shown in Fig. 1, the core body temperature of the sensitized mice started to decrease almost immediately after challenge and reached a nadir (nearly a 5 °C decrease) at 40 min post-challenge. Likewise, the hematocrit increase (an indication of vascular leakage) observed in sensitized mice was clearly substantial at 10 min post-challenge and remained at that level for the duration of the experiment. These data confirmed that, as expected, all sensitized mice experienced a robust anaphylactic response upon peanut challenge whereas non-sensitized mice did not.

3.2 Total metabolite feature detection and data quality control

After data alignment, feature extraction and isotope/adduct identification by XCMS/CAMERA, ~3,500 metabolomic features were found with a unique retention time and m/z value (excluding redundant isotope/adduct signals) eluting in a chromatographic range showing no ion-suppression as determined by post-column addition (data not shown). Analysis of the pooled sample replicates by OPLS-DA showed a tight clustering indicating good instrumental reproducibility throughout the period of analysis (supplementary material). Metabolite features that showed poor reproducibility in the pooled samples (RSD > 20 %) were not considered in the data set upon the recommendation of Dunn who determined that features with reproducibility

worse than this cut-off coupled with normal biological variability would be too unreliable for analysis (Dunn et al. 2011). Approximately 5 % of the 3,500 unique metabolomic features fit criteria for non-consideration.

3.3 Metabolomic pattern of anaphylaxis progression

While body temperature and blood hematocrit levels provide an indication that a state of anaphylaxis is being experienced, these measurements alone are often insufficient to describe the stage of anaphylaxis (early, middle or late stage). The analysis of the 3,500 metabolomic features found in mice experiencing PIA by OPLS-DA showed a distinct pattern of progression from pre-challenge and very early stage anaphylaxis to mid and late stage as defined by time (time progression comparison; Fig. 2.) At the 10 min mark, the metabolomic profiles begin to partially separate from the pre-challenge cluster and, by 20 min, the profiles are undoubtedly separated. This corresponds well with the clinical and physiological observations made in these mice. Due to the overlap of some clusters, the R^2 (explained variance) and Q^2 (goodness-of-fit/predictive accuracy) scores for this analysis are low; however a qualitative trend is still apparent. In direct contrast, the OPLS-DA analysis of all non-sensitized groups of mice challenged with peanut, which did not experience anaphylaxis (Fig. 2b) show profiles that are almost entirely overlapped with no identifiable or systematic trends being observed. Although only qualitative, these analyses provide evidence that the observed metabolomic changes are due to anaphylaxis and not to the i.p. injection or the administration of peanut. Furthermore, these data reveal distinct metabolomic profiles related to the stage of anaphylaxis development.

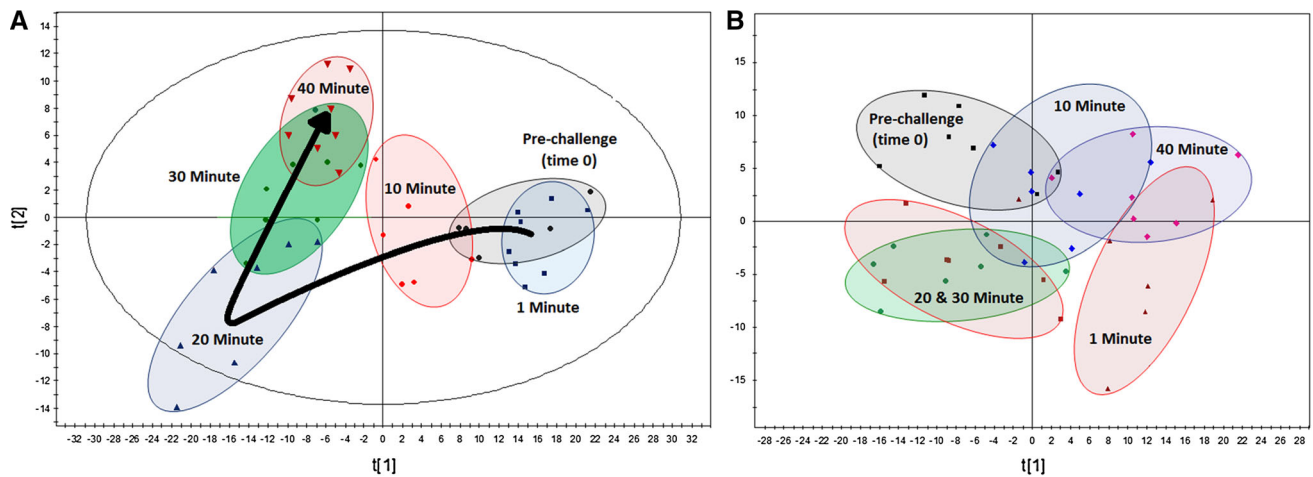
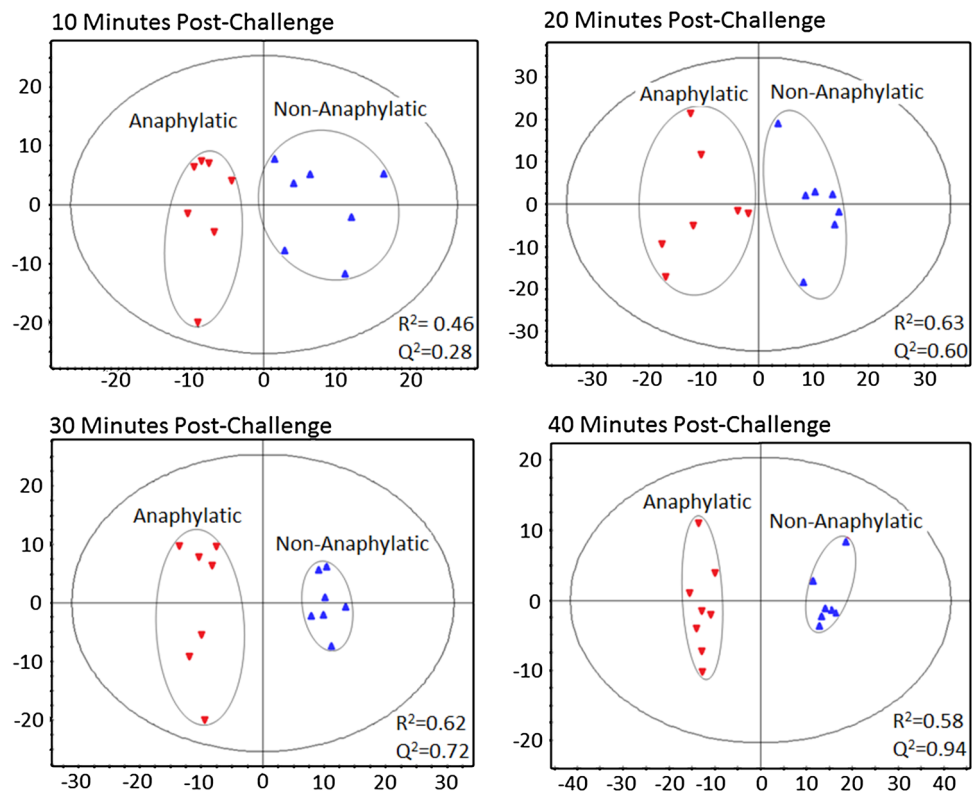


Fig. 2 OPLS-DA score plot of metabolomic features for sensitized mice (a) and non-sensitized mice (b) showing the time progression changes after i.p. challenge. Each time point includes 7 mice per group and each sample encompasses ~3,500 metabolomic features

Fig. 3 OPLS-DA score plot comparing sensitized to non-sensitized mice at various times post-challenge



3.4 Direct comparison of sensitized to non-sensitized mice at defined time-points post-challenge

OPLS-DA analysis of sensitized and non-sensitized mice matched by time point indicates that even before anaphylaxis can be measured (prior to 10 min post-challenge), the metabolomic profiles of these mice show a slight degree of separation (supplemental material). These initial profiles are indicative of the sensitization process rather than a representation of the anaphylactic event itself since

anaphylaxis has not yet occurred. By 10 min post-challenge, the total degree of separation between anaphylactic mice from non-anaphylactic mice reaches levels of moderate predictive quality (R^2 and Q^2 of 0.46 and 0.26 respectively; Fig. 3a) and increases significantly by the 20-min time point (R^2 and Q^2 of 0.63 and 0.60 respectively; Fig. 3b). Not surprisingly, this separation roughly mirrors that of the time-progression analysis where by the 10 min post-challenge time point only slight overlap is observed with mice which are sensitized but not yet

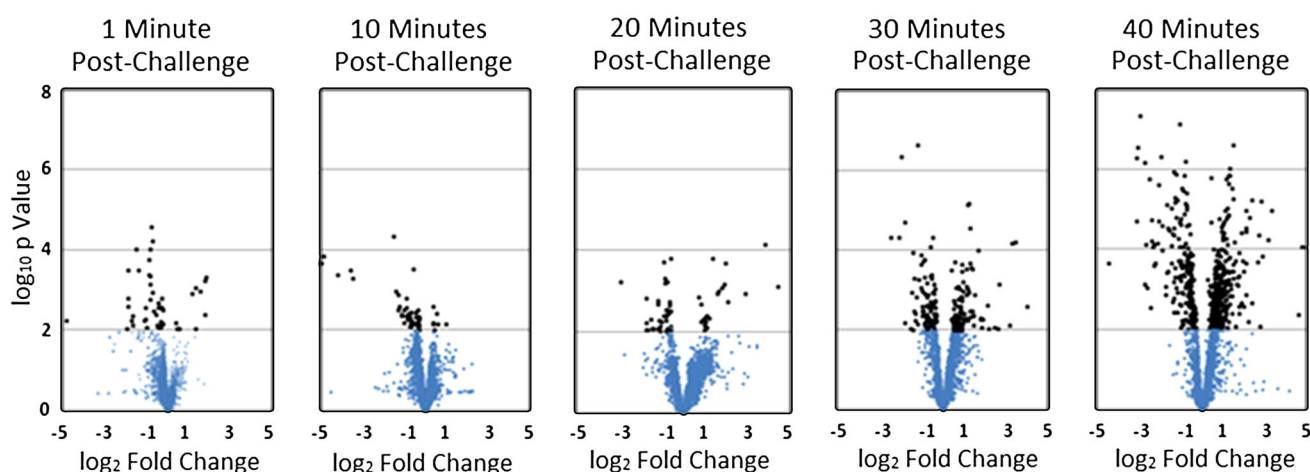


Fig. 4 Volcano plots of metabolite features at various time points post-challenge comparing sensitized to non-sensitized mice. Each plot encompasses ~3,500 metabolomic features. Dots appearing in black represent features with p -values < 0.01

Table 1 Numbers of statistically significant mass spectral features with p -values less than 0.01 and 0.001 which became significantly different at various time points after i.p. peanut challenge

Time after i.p. challenge (min)	Number of significant mass spectral features with fold changes $> \pm 1.5$	
	ANOVA and t test p -value < 0.01	ANOVA and t test p -value < 0.001
1	20	6
10	28 (Total of 48)	5 (Total of 11)
20	59 (Total of 107)	11 (Total of 22)
30	185 (Total of 292)	31 (Total of 53)
40	501 (Total of 793)	149 (Total of 202)

anaphylactic (pre-challenge and 1 min post-challenge) and the cluster representing the 20 min post-challenge mice are clearly separated. By the time late stage anaphylaxis has set in (≥ 30 min post-challenge) the predictive capability of the OPLS-DA model suggests that the observed separation is readily apparent.

To determine the individual metabolites that were significantly altered at each time point, both Student's t test and ANOVA were performed with significance cut-offs of $p < 0.01$ and $p < 0.001$ and a total fold change greater than ± 1.5 and these data were plotted as a volcano plot for easy visualization (Fig. 4). While the overall number of significant metabolite changes in the early stages of anaphylaxis is low, the numbers of metabolites that are altered increase substantially by the mid to late stage of anaphylaxis. Table 1 summarizes the total number of metabolite features that change significantly from their pre-challenge levels by time-point and Table 2 lists the metabolites found to be altered up to 10 min post-challenge. A full list of all metabolite features found to change during PIA is shown in the supplementary materials section.

4 Discussion

4.1 Metabolomic changes in mice reflective of early stage anaphylaxis

Intuitively, molecules detected in the early stages of anaphylaxis are likely to play a key initiating role in this biological process (Berni Canani et al. 2011). Almost immediately (1-min) after challenge with peanut, 6 features with t test and ANOVA p -values less than 0.001 and fold changes greater than 1.5 emerge with an additional 20 features being observed with p -values < 0.01 compared to pre-challenge levels. By the 10-min time point, an additional 28 features (for a total of 48) reached statistical significance with p -values less than 0.01, 5 of which were below 0.001. While the low abundance and chemical complexity of some of these metabolite features did not allow for full chemical identification by MS/MS, many were identified by comparison to authentic chemical standards or matching to database spectra. Of the identified metabolites found to be altered in the early stages of PIA two biogenic amines, histamine and methylhistamine, are well known markers of mast cell degranulation (Amin 2012). Unfortunately the specific isomer of methylhistamine (1, 3, or 4) could not be determined due to their similar retention times and mass spectra; however all three common forms are known to be bioactive. While mast cells are not solely responsible for causing PIA, the degranulation of these cells during anaphylaxis is a well established event that, no doubt, plays an important role in eliciting an anaphylactic response (Sun et al. 2007). Interestingly, a number of other metabolites that were identified such as epinephrine, taurine, and ascorbic acid are known to possess vaso-active properties (Abe et al. 1988; Grossmann et al. 2001). Whether these molecules are involved in the

Table 2 48 metabolite found to be significantly altered in early stage PIA (1–10 min post-challenge)

Retention time (min)	m/z	Metabolite	Identification confidence level	p-value (ANOVA)	Fold change
8.2	(−)99.010	Succinic anhydride	2	9.86E − 05	0.6
8.2	(+)104.107	Choline	1	1.40E − 07	0.7
4.5	(−)110.025	Cytosine	1	7.84E − 05	0.8
13.1	(+)112.093	Histamine	1	2.42E − 14	29.5
8.1	(−)115.005	Maleic acid	1	2.58E − 04	0.7
8.9	(+)116.071	Proline	1	6.00E − 02	0.8
9.2	(+)126.022	Taurine	1	3.87E − 05	0.8
12.6	(+)126.102	Methylhistamine*	2	1.40E − 13	2.9
9.5	(+)132.077	Creatine	1	4.04E − 02	0.7
3.9	(+)137.047	Hypoxanthine	1	2.56E − 05	0.2
8.2	(−)175.027	Ascorbic acid	1	1.16E − 04	0.5
3.6	(−)181.052	Homovanilic acid	1	2.63E − 04	0.04
8.2	(−)191.021	Citric acid	1	4.59E − 05	0.6
8.7	(+)218.139	Propionylcarnitine	2	4.38E − 02	0.6
7.8	(+)246.170	2-Methyl-butrylcaritine	2	1.27E − 02	0.7
6.6	(+)269.102	Inosine	1	2.02E − 05	0.4
6.6	(−)274.074	C ₁₄ H ₁₂ NO ₅	3	5.40E − 04	0.3
7.9	(−)282.085	Guanosine	1	3.01E − 04	0.5
7.1	(−)283.075	Xanthosine	1	1.23E − 06	0.7
3.8	(−)309.281	Eicosenoic acid	2	8.16E − 06	2.6
5.9	(−)311.294	Arachidic acid	1	9.41E − 04	1.5
6.6	(−)357.110	C ₁₈ H ₁₈ N ₂ O ₆	3	7.67E − 05	0.5
2.9	(−)367.284	C ₂₀ H ₃₇ N ₃ O ₄	3	1.44E − 06	2.3
2.0	(+)377.257	C ₂₂ H ₃₅ NO ₄	3	1.38E − 03	1.5
6.6	(+)382.148	Met–Met–Thr	2	1.66E − 05	0.4
2.0	(+)399.271	C ₁₈ H ₃₅ N ₆ O ₄	3	8.34E − 04	1.5
3.8	(−)405.268	C ₂₄ H ₃₈ O ₅	3	2.53E − 06	3.4
7.3	(+)414.595	Unknown	4	9.26E − 04	1.5
8.9	(+)441.215	C ₂₀ H ₃₂ N ₄ O ₅ S	3	6.21E − 07	0.7
8.7	(+)452.132	C ₂₂ H ₂₀ N ₄ O ₇	3	6.77E − 12	0.5
8.7	(+)458.275	C ₂₃ H ₄₀ NO ₈	3	7.11E − 12	0.5
6.6	(+)496.181	lysoPC(16:0)	2	4.42E − 05	1.6
7.0	(+)502.298	lysoPE(20:4)	2	7.42E − 06	1.7
7.0	(+)526.337	C ₂₂ H ₄₉ N ₅ O ₇ P	3	1.23E − 04	0.6
3.0	(+)531.342	C ₂₄ H ₄₈ N ₆ O ₅ P	3	1.85E − 04	1.5
6.4	(+)658.544	Unknown	4	2.54E − 04	1.8
4.0	(+)658.549	C ₄₀ H ₇₃ N ₃ O ₄	3	5.44E − 07	2.2
3.3	(+)665.049	Unknown	4	2.74E − 05	1.5
4.4	(+)670.426	PE(29:1)	2	1.22E − 03	1.6
2.0	(+)671.368	Unknown	3	9.51E − 05	1.5
5.2	(+)682.543	Unknown	3	7.91E − 04	0.4
6.4	(−)685.491	Unknown	3	1.78E − 03	0.3
5.2	(−)709.426	Unknown	4	2.80E − 03	2.4
4.0	(−) 717.447	PG (32:2)	2	2.24E − 07	2.9
8.3	(+)738.565	lysoPC(23:5)	2	2.98E − 02	0.7
8.3	(+)740.462	lysoPC(23:4)	2	1.56E − 03	0.7
2.0	(+)757.396	C ₃₃ H ₆₁ N ₂ O ₁₇	3	1.09E − 02	1.6

Table 2 continued

Retention time (min)	m/z	Metabolite	Identification confidence level	p-value (ANOVA)	Fold change
2.1	(+)982.032	Unknown	4	8.83E – 04	1.5

Metabolite identification confidence level based on the criteria proposed by Sumner et al. Full metabolite lists including those altered in late stage PIA may be found in supplementary materials

* The specific isomer of methylhistamine (1, 3, or 4) could not be determined by MS² or by retention time

initiation of the reaction or released in response to the changes in vascular leakage and blood pressure observed remains to be studied.

Of the remaining molecules that were significantly changed during the early stage of PIA, a number are of special interest since they are directly related to either known anaphylaxis mediators or known cellular events occurring during anaphylaxis. One such molecule is choline, which not only has known neuro-active properties, but is an essential component of phosphatidylcholine lipids including the potent anaphylactic mediator commonly known as platelet-activating factor (PAF; 1-*O*-Hexadecyl-2-acetyl-sn-glycero-3-phosphocholine) (Finkelman 2007; Hanahan 1986; Strait et al. 2002). Although PAF could not be detected in this study (likely due to very low abundance), a number of other phosphatidylcholines and lysophosphatidylcholines were altered. The potential mediating properties of these molecules are largely unknown; however, due to their chemical similarity to PAF, these compounds hold value for further investigation either as direct mediators or as chemical precursors to PAF and/or other yet to be discovered phosphatidylcholine-based mediators. These compounds are also known to comprise a large portion of cellular membranes; since a number of known biomarkers and mediators of anaphylaxis including PAF and the leukotrienes are known to originate from scavenged cell membrane remnants, the release of these compounds from degranulated mast cells and basophils may be an important signaling event to trigger an anaphylactic attack (Weller and Dvorak 1994).

4.2 Metabolomic changes found in mid to late stage PIA

By 20 min post-challenge, the number of newly emerging features, i.e., not previously observed in early stage anaphylaxis, nearly doubles. This is followed by an almost exponential increase in emerging differences observed at the 30–40 min time points when the clinical severity of anaphylaxis is at its maximum. Indeed, by the 40-min time point just under 800 of the 3,500 metabolites or ~23 % of the total observable metabolome were significantly changed compared to pre-challenge levels.

Included in the metabolites with *p*-values lower than 0.001 is leukotriene C₄ which is an arachidonic acid metabolite known to be a powerful vaso-dilator; in fact, its potency has been estimated to be up to 100× that of histamine (Laidlaw and Boyce 2012). Also found was a profound increase in the number of phosphatidylcholines, lysophosphatidylcholines and phosphatidylethanolamines. As stated previously, it is unknown if these lipids are actual mediators of anaphylaxis, if they are essential chemical precursors to PAF and other mediators, or if they are remnants of mast cell degranulation. However, we suggest that these molecules warrant further study regarding their role in the mechanism of anaphylaxis or as potential biomarker candidates.

A vast number of potentially interesting metabolites were identified in late-stage anaphylaxis. While these metabolites are surely not significant initiating mediators, they reflect the patho-physiological complexity of a full-blown anaphylactic reaction. For example, a number of molecules including glucose (as its Na⁺ adduct) were found to be dysregulated during late stage PIA. This change was also observed by Hu et al. Changes in metabolites such as glucose are likely the result of downstream effects arising from profound changes in metabolic activity at this life-threatening stage of the process. Further investigation on the role of these metabolites may help inform the pathophysiological basis of the anaphylactic reaction.

5 Concluding remarks

In this study we applied a comprehensive metabolomics approach to study the changes occurring in the blood serum metabolome of mice undergoing peanut induced anaphylaxis. This study revealed that the metabolomic profiles of mice undergoing anaphylaxis changed both dramatically and systematically as anaphylaxis progressed as evidenced by multivariate OPLS-DA analysis. Statistical comparisons revealed that as many as 800 serum metabolites representing approximately 23 % of the observable metabolome significantly change during the time period studied. Of the identified metabolites observed to change during

anaphylaxis, compounds with known links to anaphylaxis such as histamine and leukotriene C₄ were observed, in addition to a number of other compounds with known vaso-active and neuro-active properties. Furthermore, a large number of phospholipids including phosphatidylcholines and lyso-phosphatidylcholines were significantly altered during anaphylaxis. The chemical similarity of these compounds to PAF suggests that these compounds warrant further study to elucidate their role/mechanism in anaphylaxis and their potential as diagnostic markers of anaphylaxis.

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Conflict of interest The authors declare no conflicts of interest.

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Appendix 1. XCMS and CAMERA Operational Parameters

```
sleep=0.0001
```

```
family="s", plotype="m"
```

```
bw = 30, minfrac = 0.5, minsamp= 1, mzwid = 0.25, max = 5, sleep = 0
```

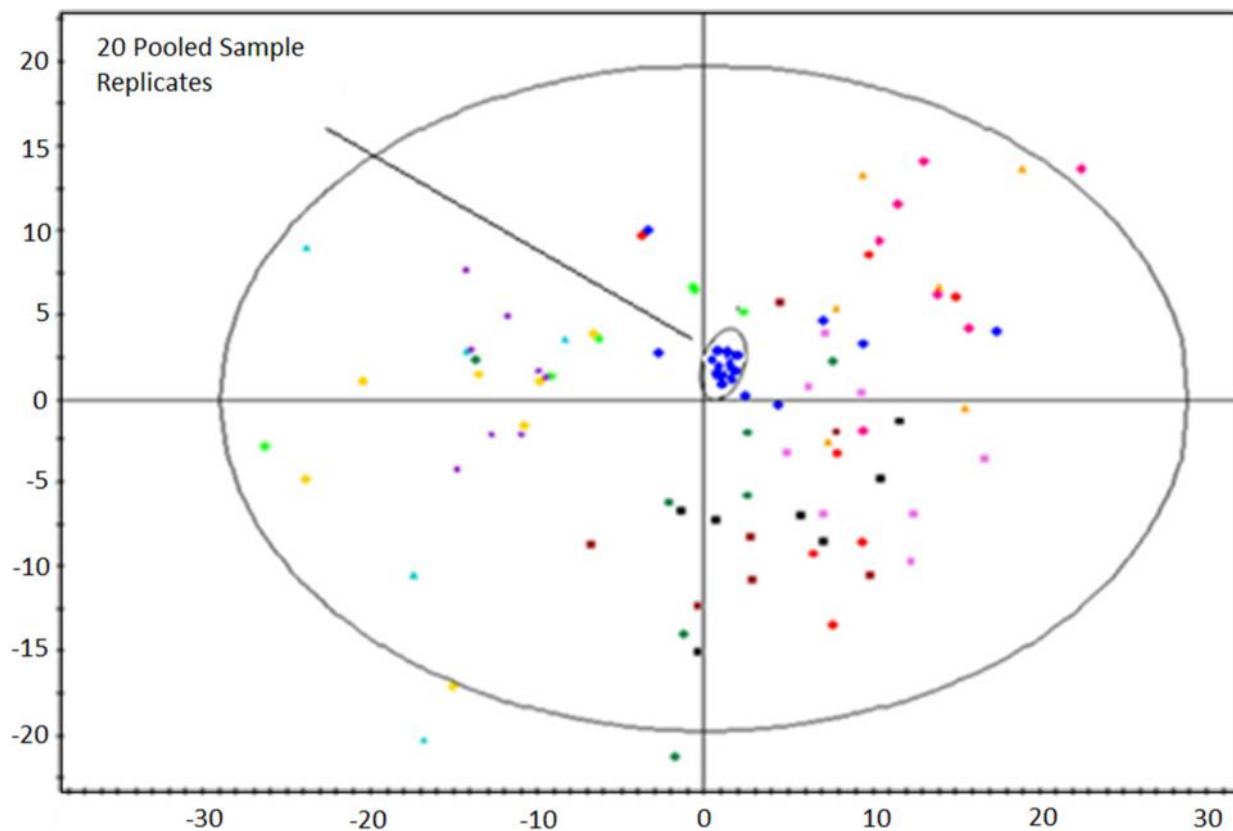
```
xsa <- xsAnnotate(xset3)
```

```
groupFWHM(xsa, perfwlm=0.6)
```

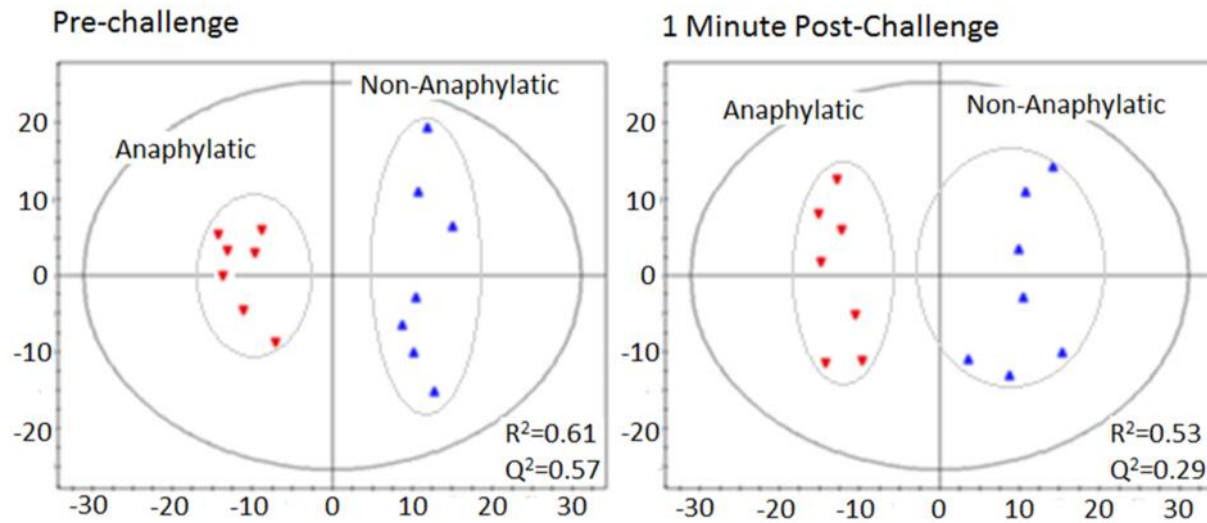
```
xsaFl <- findIsotopes(xsaF, mzabs=0.01, maxcharge=3, maxiso=3, ppm=5)
```

```
xsaC <- groupCorr(xsaFl, cor_eic_th=0.75, polarity="")
```

Appendix 2. OPLS-DA analysis with pooled sample replicates



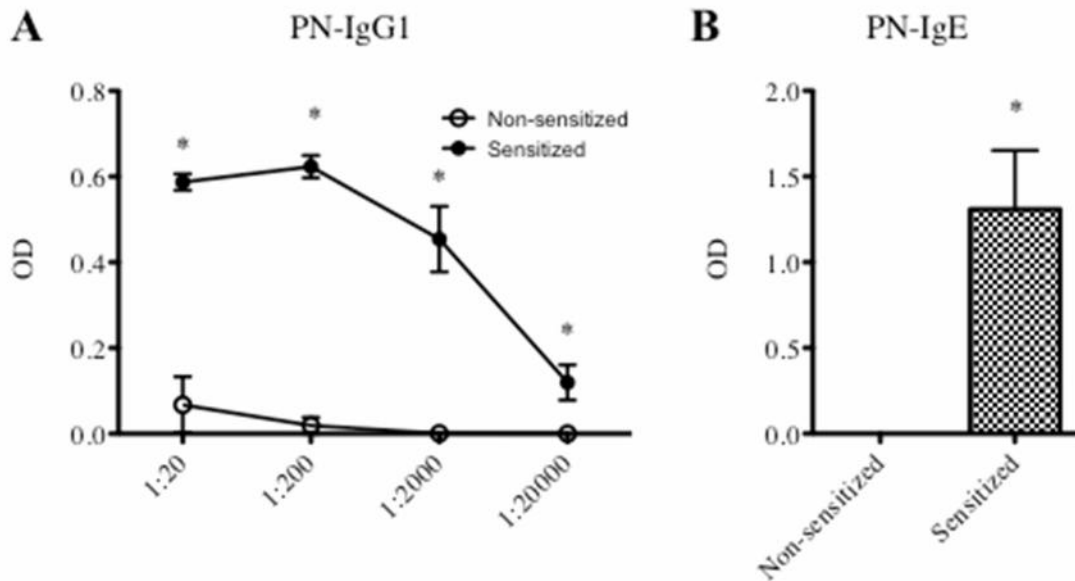
Appendix 3. OPLS-DA analysis of Sensitized and Non-sensitized mice pre-challenge and 1-minute post-challenge mice



Appendix 5. Preparation of peanut extract

For each gavage, 3.75 mg of Kraft All Natural™ peanut butter containing roughly 1 mg of peanut protein (determined and labeled on the product by the manufacturer) was resuspended in 500 μ L of phosphate buffer solution (PBS). For the challenge, each mouse was given crude peanut extract (Greer laboratories, Lenoir, North Carolina) containing 1 mg of peanut protein (determined by Greer laboratories) in 500 μ L PBS.

Appendix 6. Levels of peanut specific IgE levels in sensitized and non-sensitized mice



Appendix 7. Metabolites Exhibiting statistically significant changes 20 minutes post challenge and beyond in mouse serum. Metabolites identified unambiguously by comparison to authentic chemical standards are listed as 1°; Metabolites identified based on matches between their mass spectra and mass spectra found in databases are listed as 2°; metabolites where chemical formulae were assigned based on accurate mass and isotope ratio measurements are listed as 3°; True unknown metabolites are listed as 4°.

m/z	Identification	Retention time (min)	p Value	Identification Confidence
(-)92.024	Aniline	3.9	<0.001	1°
(-)102.054	GABA	3.1	0.007	1°
(-)108.021	Hypotaurine	3.9	<0.001	1°
(-)110.025	Cytosine	4.1	<0.001	1°
(+)110.044	Hypotaurine	3.9	<0.001	1°
(-)111.018	Uracil	4	0.001	1°
(+)112.051	Cytosine	5.2	0.004	1°
(-)112.053	Creatinine	6.7	0.001	1°
(+)112.087	Histamine	13.1	0.004	1°
(+)113.035	Uracil	4	<0.001	1°
(+)132.102	Isoleucine	7.9	0.001	1°
(-)134.069	Methylcysteine	2.9	0.009	1°
(+)136.051	Methylcysteine	8.4	0.001	1°

(+)136.064	Adenine	3.3	0.008	1°
(+)150.059	Methionine	8.3	0.008	1°
(+)153.042	Xanthine	3.9	0.003	1°
(-)155.011	Orotic acid	7.6	0.003	1°
(+)156.077	Histidine	11.8	<0.001	1°
(-)167.022	Uric acid	8	<0.001	1°
(+)170.093	1-Methylhistidine	11.9	0.007	1°
(-)174.041	Acetyl-asp	7.6	0.005	1°
(-)174.080	Argininic acid	3.7	0.005	1°
(+)175.002	Aconitic acid	3.9	0.004	1°
(-)175.025	Ascorbic acid	8.2	0.001	1°
(-)266.091	Adenosine	3.4	0.006	1°
(-)267.073	Inosine	6.6	0.003	1°
(+)268.108	Adenosine	3.4	0.009	1°
(+)283.082	Xanthosine	5.7	0.006	1°
(+)487.179	Cytidine (2M+H)	7.5	0.01	1°
(+)86.096	Pyrrolidinone	7.9	0.001	2°
(+)108.008	Diketogulonic acid	9.2	0.006	2°
(+)126.102	Methylhistamine	12.6	0.001	2°
(+)139.052	Urocanic acid	3.3	0.009	2°
(+)168.035	Thioguanine	6.2	0.003	2°
(-)176.040	Formyl-met	0	0.006	2°
(+)177.103	Allantoic acid	8.2	0.001	2°
(+)188.175	N-acetylspermidine	13.3	<0.001	2°
(-)191.996	5,6-Dihydroxyindole-2-carboxylic acid	9.3	0.006	2°
(+)202.181	Capryloylglycine	7.6	0.008	2°
(+)226.084	Murrayanine	5.2	0.005	2°
(+)232.059	N2-Succinyl-L-glutamic acid 5-semialdehyde	13.2	<0.001	2°
(-)232.059	Deoxyribose phosphate (NH ₄ ⁺ adduct)	13.2	<0.001	2°
(-)243.064	Uridine	4	0.005	2°
(+)244.093	C ₁₀ H ₁₀ N ₇ O	7.5	0.006	2°
(+)298.351	Isoprostane	4.5	0.001	2°
(+)329.270	MG(16:0)	4	0.005	2°
(+)418.156	Met-met-his	8.5	0.002	2°
(+)423.278	DHAP(18:0)	4	0.009	2°
(+)441.217	Tyr-lys-met	8.9	0.007	2°
(-)452.279	lysoPE(16:0)	7.3	0.009	2°
(-)476.278	lysoPE(18:2)	7.1	0.003	2°
(+)478.294	lysoPE(18:2)	7.1	0.001	2°
(+)478.294	lysoPE(18:2)	7.3	0.006	2°
(-)480.309	lysoPE(18:0)	7.9	0.002	2°
(+)502.296	lysoPE (20:4)	7	0.002	2°
(-)506.319	PE (20:1)	8.1	0.003	2°

(-)508.341	PE (20:0)	8.5	<0.001	2°
(-)520.410	lysoPC(18:1)	26.5	0.001	2°
(-)524.278	lysoPE(22:6)	7	<0.001	2°
(-)526.313	PE (22:5)	8	0.008	2°
(+)538.386	PE (22:0)	8.7	<0.001	2°
(+)540.331	PC (20:5)	8.1	0.002	2°
(+)542.324	LysoPC (20:5)	7.9	0.003	2°
(-)544.317	PC (20:3)	0	0.001	2°
(-)553.411	TG (30:0)	4.6	0.009	2°
(+)717.448	PG (32:2)	4	0.007	2°
(+)744.547	PC(33:2)	28.6	0.007	2°
(-)762.510	PC(36:4)	22.9	0.001	2°
(-)766.542	PC(35:4)	29	0.007	2°
(+)768.097	Coenzyme A	7	0.002	2°
(+)770.561	PE (38:3)	28.4	0.008	2°
(-)788.567	PE (38 :5)	4.5	0.01	2°
(+)801.431	PGP (32:1)	2.3	0.002	2°
(+)804.548	PC(38:7)	23.7	<0.001	2°
(+)190.053	Acetyl-met	2.3	0.001	2°
(-)85.029	C ₄ H ₅ O ₂	5.4	<0.001	3°
(-)88.040	C ₃ H ₆ NO ₂	5	0.001	3°
(-)88.041	C ₃ H ₆ NO ₂	9.5	0.007	3°
(+)88.076	C ₄ H ₁₀ NO	8.2	0.007	3°
(-)99.009	C ₄ H ₃ O ₃	8.2	0.009	3°
(-)99.045	C ₅ H ₇ O ₂	4.2	0.006	3°
(-)101.024	C ₄ H ₅ O ₃	2.2	0.009	3°
(+)104.107	C ₄ H ₉ NO ₂	8.3	0.008	3°
(-)107.037	C ₆ H ₅ NO	8.6	0.001	3°
(+)109.076	C ₆ H ₉ N ₂	12.6	0.009	3°
(-)110.072	C ₅ H ₈ N ₃	11.9	0.001	3°
(+)112.051	C ₄ H ₆ N ₃ O	7.5	0.005	3°
(-)114.032	C ₅ H ₆ O ₃	7.6	0.001	3°
(+)114.067	C ₆ H ₈ N ₃ O	6.6	0.001	3°
(-)114.093	C ₆ H ₁₂ NO	2.1	0.002	3°
(-)115.040	C ₅ H ₇ O ₃	3.5	0.002	3°
(-)116.035	C ₄ H ₆ NO ₃	6	<0.001	3°
(-)116.048	C ₅ H ₈ O ₃	7.9	<0.001	3°
(+)116.071	C ₅ H ₁₀ NO ₂	8.9	0.004	3°
(-)119.034	C ₄ H ₇ O ₄	4.1	<0.001	3°
(+)121.013	C ₃ H ₅ O ₅	2.1	<0.001	3°
(+)122.020	C ₃ H ₆ O ₅	2.7	0.002	3°
(+)126.069	C ₇ H ₁₂ O ₂	5.7	<0.001	3°
(+)128.019	C ₂ H ₂ N ₅ O ₂	9.2	0.002	3°
(-)128.036	C ₅ H ₆ NO ₃	8	0.006	3°
(+)128.131	C ₇ H ₁₆ N ₂	7.6	0.003	3°
(+)130.088	C ₆ H ₁₂ NO ₂	12	0.001	3°

(-)130.088	$C_6H_{13}NO_2$	7.8	0.003	3°
(-)131.047	$C_4H_7N_2O_3$	5	0.001	3°
(+)132.077	$C_4H_{10}N_3O_2$	9.5	<0.001	3°
(+)133.033	$C_2H_5N_4O_3$	8.3	0.006	3°
(+)133.062	$C_4H_9N_2O_3$	5	<0.001	3°
(-)136.016	$C_7H_4O_3$	20.7	0.001	3°
(+)136.048	$C_3H_8N_2O_4$	9.6	0.003	3°
(+)139.039	$C_5H_5N_3O_2$	20.7	<0.001	3°
(+)140.069	$C_5H_8N_4O$	8.9	0.001	3°
(-)145.063	$C_5H_9N_2O_3$	9.7	0.004	3°
(-)145.099	$C_6H_{13}N_2O_2$	12.1	<0.001	3°
(+)146.061	C_9H_8NO	7.9	0.003	3°
(-)147.053	$C_5H_9NO_4$	8.5	<0.001	3°
(+)147.113	$C_6H_{15}N_2O_2$	12.1	0.002	3°
(+)149.023	$C_8H_5O_3$	21.2	0.001	3°
(+)150.013	$C_2H_4N_3O_5$	9.8	0.001	3°
(-)153.029	$C_6H_5N_2O_3$	7.1	<0.001	3°
(+)154.059	$C_4H_6N_6O$	9.6	<0.001	3°
(+)155.044	$C_4H_5N_5O_2$	5	<0.001	3°
(-)156.067	$C_7H_{10}NO_3$	2.2	<0.001	3°
(-)157.038	$C_6H_7NO_4$	7.6	<0.001	3°
(+)159.026	$C_4H_5N_3O_4$	3.9	<0.001	3°
(-)160.062	$C_6H_{10}NO_4$	3.6	<0.001	3°
(+)160.134	$C_8H_{18}NO_2$	9.5	0.001	3°
(+)162.113	$C_7H_{16}NO_3$	10	0.001	3°
(+)163.063	$C_9H_9NO_2$	2	<0.001	3°
(-)164.073	$C_9H_{10}NO_2$	7.6	<0.001	3°
(+)167.000	$C_4H_7O_5S$	9.5	0.001	3°
(-)168.014	$C_3H_6NO_7$	6.2	<0.001	3°
(-)170.082	$C_8H_{12}NO_3$	2	0.001	3°
(+)173.131	$C_8H_{17}N_2O_2$	7.6	<0.001	3°
(+)174.006	$C_8H_2N_2O_3$	13.2	<0.001	3°
(+)174.137	$C_8H_{18}N_2O_2$	7.6	<0.001	3°
(-)178.051	$C_9H_8NO_3$	2.3	0.001	3°
(+)181.161	$C_{12}H_{21}O$	2.1	<0.001	3°
(+)187.059	$C_6H_9N_3O_4$	6.1	0.002	3°
(-)187.073	$C_7H_{11}N_2O_4$	8.1	0.004	3°
(-)188.058	$C_{10}H_8N_2O_2$	8	<0.001	3°
(+)189.160	$C_9H_{21}N_2O_2$	12.6	<0.001	3°
(+)191.056	$C_8H_7N_4O_2$	2.3	0.001	3°
(+)191.091	$C_6H_{13}N_3O_4$	7.9	<0.001	3°
(+)192.096	$C_4H_{12}N_6O_3$	7.9	<0.001	3°
(+)192.159	$C_9H_{22}NO_3$	6.8	<0.001	3°
(-)193.014	$C_9H_5O_5$	7.6	0.001	3°
(-)195.010	$C_6H_{11}O_7$	7.6	0.001	3°
(-)201.039	$C_6H_7N_3O_5$	9.4	<0.001	3°

(-)201.089	$C_8H_{13}N_2O_4$	8.4	0.01	3°
(+)203.053	$C_6H_9N_3O_5$	8.6	0.008	3°
(-)204.067	$C_{11}H_{10}NO_3$	3.6	0.002	3°
(+)204.124	$C_9H_{18}NO_4$	9.2	0.009	3°
(-)206.081	$C_{11}H_{12}NO_3$	2.1	0.004	3°
(+)209.105	$C_{11}H_{15}NO_3$	3.6	0.001	3°
(-)210.042	$C_{10}H_4N_5O$	6.6	<0.001	3°
(-)213.087	$C_7H_{11}N_5O_3$	12.1	0.006	3°
(+)215.081	$C_{12}H_{11}N_2O_2$	8	0.003	3°
(-)222.077	$C_{11}H_{12}NO_4$	3.4	0.001	3°
(-)225.063	$C_8H_9N_4O_4$	8.6	0.009	3°
(-)226.085	$C_{11}H_{14}O_5$	6.6	0.003	3°
(+)226.105	$C_7H_{12}N_7O_2$	9.6	0.005	3°
(+)228.099	$C_9H_{14}N_3O_4$	6.6	0.002	3°
(+)229.159	$C_{16}H_{21}O$	9.6	0.002	3°
(-)230.103	$C_{10}H_{16}NO_5$	2.7	<0.001	3°
(+)231.087	$C_{11}H_{11}N_4O_2$	3.6	0.001	3°
(+)231.097	$C_7H_{13}N_5O_4$	8.1	0.001	3°
(+)231.137	$C_{13}H_{17}N_3O$	5.4	0.001	3°
(+)231.142	$C_5H_{15}N_{10}O$	6.8	<0.001	3°
(+)232.155	$C_{11}H_{22}NO_4$	8.2	0.001	3°
(+)233.106	$C_9H_{16}N_2O_5$	2.9	<0.001	3°
(+)235.084	$C_{12}H_{13}NO_4$	7.3	0.009	3°
(+)235.166	$C_{10}H_{23}N_2O_4$	8.8	0.001	3°
(-)242.080	$C_{12}H_{10}N_4O_2$	7.5	0.004	3°
(+)243.048	$C_8H_9N_3O_6$	8	0.002	3°
(-)246.154	$C_7H_{18}N_8O_2$	6.8	0.001	3°
(+)246.171	$C_{12}H_{24}NO_4$	7.9	<0.001	3°
(+)250.040	$C_3H_6N_8O_6$	4.1	0.002	3°
(-)252.030	$C_{12}H_4N_4O_3$	11.8	0.005	3°
(+)259.028	$C_4H_9N_3O_{10}$	9.6	0.007	3°
(+)260.186	$C_{13}H_{26}NO_4$	7.6	<0.001	3°
(+)262.165	$C_{12}H_{24}NO_5$	9	<0.001	3°
(-)268.054	$C_7H_{12}N_2O_9$	2.9	<0.001	3°
(-)269.055	$C_{12}H_7N_5O_3$	7.6	<0.001	3°
(-)274.085	$C_{15}H_{14}O_5$	6.6	<0.001	3°
(+)275.112	$C_{11}H_{13}N_7O_2$	3.6	<0.001	3°
(-)276.123	$C_{13}H_{16}N_4O_3$	7.8	0.001	3°
(+)282.121	$C_{13}H_{18}N_2O_5$	8.6	0.002	3°
(-)284.133	$C_8H_{20}N_4O_7$	7.6	<0.001	3°
(+)288.214	$C_{10}H_{30}N_3O_6$	7.4	<0.001	3°
(+)288.292	$C_{17}H_{38}NO_2$	3.8	<0.001	3°
(-)293.092	$C_{15}H_{11}N_5O_2$	4.7	0.008	3°
(+)296.066	$C_{15}H_{10}N_3O_4$	10.5	<0.001	3°
(+)297.157	$C_{15}H_{23}NO_5$	3.9	0.003	3°
(+)304.043	$C_{12}H_8N_4O_6$	6.7	<0.001	3°

(+)305.264	C ₁₁ H ₃₁ N ₉ O	21.2	<0.001	3°
(-)307.264	C ₂₀ H ₃₅ O ₂	2.8	<0.001	3°
(+)314.234	C ₁₃ H ₃₅ N ₂ O ₄ P	7.3	0.001	3°
(-)332.240	C ₁₂ H ₃₄ N ₃ O ₇	2.1	<0.001	3°
(-)333.094	C ₁₂ H ₁₇ N ₂ O ₉	4.1	0.002	3°
(-)334.031	C ₈ H ₁₅ O ₁₂ P	9.6	0.009	3°
(+)336.307	C ₁₅ H ₄₄ O ₇	21.5	0.001	3°
(+)338.051	C ₉ H ₉ N ₉ O ₄ P	8.3	0.001	3°
(+)340.043	C ₁₀ H ₁₅ NO ₁₀ P	8.3	<0.001	3°
(+)359.092	C ₈ H ₁₇ N ₅ O ₁₁	9.6	0.001	3°
(+)362.226	C ₁₃ H ₃₄ N ₂ O ₉	3.2	0.002	3°
(+)364.054	C ₁₀ H ₉ N ₁₀ O ₄ P	10.5	<0.001	3°
(-)367.284	C ₁₆ H ₄₀ N ₄ O ₃ P	2.9	0.002	3°
(+)368.327	C ₂₁ H ₄₂ N ₃ O ₂	22.9	0.004	3°
(-)369.069	C ₂₃ H ₁₄ O ₃ P	11.9	0.007	3°
(-)375.173	C ₉ H ₂₅ N ₇ O ₉	8.9	<0.001	3°
(+)377.082	C ₁₁ H ₁₅ N ₅ O ₁₀	9.2	0.001	3°
(-)378.036	C ₁₃ H ₁₅ O ₁₁ P	7.6	0.001	3°
(-)381.173	C ₁₁ H ₂₉ N ₂ O ₁₂	2.7	<0.001	3°
(-)383.189	C ₁₅ H ₂₈ O ₇ P ₂	4	0.005	3°
(+)394.296	C ₁₉ H ₄₃ N ₂ O ₄ P	7.2	0.009	3°
(+)396.312	C ₁₉ H ₄₅ N ₂ O ₄ P	7.2	0.002	3°
(+)397.084	C ₁₁ H ₁₇ N ₄ O ₁₂	9	<0.001	3°
(-)401.157	C ₁₃ H ₂₈ N ₃ O ₉ P	8.5	0.002	3°
(+)416.073	C ₁₈ H ₁₄ N ₃ O ₉	10.4	<0.001	3°
(-)417.014	C ₉ H ₁₁ N ₃ O ₁₆	9.6	0.003	3°
(-)418.004	C ₁₁ H ₉ N ₅ O ₁₁ P	9.2	0.003	3°
(+)424.343	C ₂₅ H ₄₆ NO ₄	7.3	<0.001	3°
(+)430.377	C ₂₄ H ₅₀ N ₂ O ₄	24.1	<0.001	3°
(+)438.306	C ₂₁ H ₄₄ NO ₈	7.3	0.001	3°
(+)442.356	C ₂₈ H ₄₆ N ₂ O ₂	7.6	0.009	3°
(-)445.330	C ₂₄ H ₄₁ N ₆ O ₂	2.3	<0.001	3°
(+)447.059	C ₂₃ H ₁₃ NO ₉	11.3	0.005	3°
(+)448.325	C ₁₈ H ₄₆ N ₃ O ₉	22.3	<0.001	3°
(-)449.363	C ₂₇ H ₄₆ O ₅	3.6	0.004	3°
(-)658.542	C ₄₀ H ₇₃ N ₃ O ₄	4	0.002	3°
(-)688.352	C ₃₆ H ₅₁ NO ₁₂	8	0.002	3°
(+)94.221	Unknown	9.7	0.001	4°
(-)97.005	Unknown	7.6	<0.001	4°
(-)101.029	Unknown	4.2	0.001	4°
(-)118.654	Unknown	3.1	0.006	4°
(+)120.003	Unknown	9.7	0.007	4°
(+)123.448	Unknown	7.7	0.01	4°
(+)124.034	Unknown	14.9	<0.001	4°
(+)125.019	Unknown	10	<0.001	4°
(-)125.074	Unknown	8.2	0.009	4°

(+)127.125	Unknown	7.7	0.005	4°
(-)131.839	Unknown	8	0.007	4°
(+)140.002	Unknown	15.6	<0.001	4°
(+)143.998	Unknown	15.6	0.005	4°
(+)151.215	Unknown	12.1	0.001	4°
(+)153.069	Unknown	2.1	<0.001	4°
(-)155.238	Unknown	4	0.001	4°
(+)158.097	Unknown	2.2	0.01	4°
(+)158.997	Unknown	15.6	<0.001	4°
(+)179.614	Unknown	12	0.006	4°
(-)180.975	Unknown	9.6	<0.001	4°
(+)184.075	Unknown	8	0.003	4°
(-)187.043	Unknown	3.8	<0.001	4°
(-)188.950	Unknown	3.6	<0.001	4°
(-)194.084	Unknown	2.2	0.002	4°
(-)194.928	Unknown	11.7	<0.001	4°
(-)196.030	Unknown	6.4	0.001	4°
(+)197.009	Unknown	7.6	0.001	4°
(+)200.073	Unknown	10	0.004	4°
(-)203.084	Unknown	7.9	0.006	4°
(-)209.095	Unknown	5.6	<0.001	4°
(-)221.015	Unknown	9.3	0.007	4°
(+)226.125	Unknown	2.6	0.002	4°
(+)233.106	Unknown	2.9	0.005	4°
(-)236.022	Unknown	9.3	<0.001	4°
(-)241.000	Unknown	11.9	0.001	4°
(+)244.265	Unknown	4.2	0.009	4°
(+)246.176	Unknown	12.3	<0.001	4°
(-)248.073	Unknown	2.7	0.005	4°
(-)248.962	Unknown	9.6	<0.001	4°
(+)251.669	Unknown	2	<0.001	4°
(-)260.400	Unknown	3.2	0.005	4°
(+)261.004	Unknown	4.2	0.008	4°
(+)262.886	Unknown	11.7	<0.001	4°
(-)269.253	Unknown	6.4	0.009	4°
(+)269.995	Unknown	9.7	0.002	4°
(+)270.317	Unknown	4	0.001	4°
(-)270.317	Unknown	4	0.006	4°
(-)271.607	Unknown	3.5	0.002	4°
(-)271.607	Unknown	3.5	<0.001	4°
(+)271.683	Unknown	2.1	0.003	4°
(+)273.665	Unknown	3.3	0.001	4°
(+)273.682	Unknown	2	<0.001	4°
(+)279.039	Unknown	4	<0.001	4°
(+)282.283	Unknown	2.1	0.001	4°
(-)292.988	Unknown	9.7	0.004	4°

(-)294.091	Unknown	9.6	0.003	4°
(+)295.695	Unknown	2	0.002	4°
(+)296.694	Unknown	2	<0.001	4°
(+)303.307	Unknown	3.4	0.003	4°
(+)312.548	Unknown	8.3	0.002	4°
(+)316.323	Unknown	3.8	0.009	4°
(+)324.330	Unknown	2.9	0.009	4°
(+)352.524	Unknown	6.8	<0.001	4°
(+)355.579	Unknown	21.1	0.005	4°
(-)360.975	Unknown	9.7	0.006	4°
(+)366.663	Unknown	8.9	<0.001	4°
(+)379.084	Unknown	9.2	0.004	4°
(-)381.074	Unknown	9.3	0.002	4°
(-)381.486	Unknown	8.3	<0.001	4°
(+)386.967	Unknown	21.4	0.006	4°
(-)388.684	Unknown	8.9	<0.001	4°
(+)403.860	Unknown	8.9	<0.001	4°
(+)404.973	Unknown	9.6	0.009	4°
(+)410.690	Unknown	9	<0.001	4°
(+)411.094	Unknown	8.1	0.003	4°
(+)419.203	Unknown	9	<0.001	4°
(+)419.203	Unknown	9	<0.001	4°
(-)419.554	Unknown	8.3	0.007	4°
(+)425.079	Unknown	11.3	0.003	4°
(+)425.378	Unknown	4	0.007	4°
(+)426.771	Unknown	2.4	<0.001	4°
(-)427.786	Unknown	9.7	0.005	4°
(-)432.708	Unknown	9	<0.001	4°
(+)435.788	Unknown	2.4	0.006	4°
(+)438.783	Unknown	3.8	0.008	4°
(+)441.217	Unknown	8.9	0.001	4°
(-)447.059	Unknown	11.3	0.009	4°
(+)451.361	Unknown	21.3	0.002	4°
(+)452.132	Unknown	8.7	0.003	4°
(-)452.923	Unknown	9.6	<0.001	4°
(+)454.200	Unknown	6.4	0.003	4°
(-)458.260	Unknown	8.7	0.001	4°
(+)458.638	Unknown	7.5	0.006	4°
(-)459.811	Unknown	2.6	0.002	4°
(-)461.786	Unknown	9.7	0.001	4°
(+)462.172	Unknown	4	0.004	4°
(+)463.229	Unknown	9	0.001	4°
(+)466.532	Unknown	4.5	0.003	4°
(-)466.938	Unknown	9.6	0.004	4°
(-)467.984	Unknown	4.1	0.008	4°
(+)468.309	Unknown	8	0.002	4°

(-)468.897	Unknown	9.6	<0.001	4°
(+)468.973	Unknown	7.1	0.005	4°
(-)470.343	Unknown	7.4	<0.001	4°
(+)470.568	Unknown	8.3	<0.001	4°
(+)471.054	Unknown	11.3	0.001	4°
(+)471.343	Unknown	2.4	0.007	4°
(+)471.562	Unknown	8.3	0.004	4°
(-)472.556	Unknown	8.3	0.004	4°
(-)473.280	Unknown	2	<0.001	4°
(+)473.362	Unknown	2.9	0.005	4°
(+)475.391	Unknown	6.8	<0.001	4°
(-)475.380	Unknown	3.6	0.004	4°
(+)475.380	Unknown	3.6	0.006	4°
(-)476.721	Unknown	9	0.003	4°
(-)477.395	Unknown	5.2	0.005	4°
(+)478.976	Unknown	7.8	0.002	4°
(+)480.310	Unknown	7.2	0.003	4°
(-)482.314	Unknown	4.7	0.007	4°
(+)482.324	Unknown	8.1	0.002	4°
(+)482.653	Unknown	5.6	0.009	4°
(-)485.001	Unknown	9.6	0.001	4°
(+)485.243	Unknown	9	0.004	4°
(-)485.619	Unknown	7.2	0.002	4°
(+)487.648	Unknown	6.5	0.009	4°
(+)488.428	Unknown	24.1	0.002	4°
(+)488.981	Unknown	7.8	0.006	4°
(-)492.359	Unknown	9.6	0.004	4°
(+)495.773	Unknown	9.7	<0.001	4°
(+)496.195	Unknown	6.6	<0.001	4°
(-)496.272	Unknown	2	0.006	4°
(+)496.341	Unknown	8.1	0.002	4°
(+)496.830	Unknown	3.5	<0.001	4°
(-)498.262	Unknown	7	0.006	4°
(+)498.324	Unknown	7.4	0.004	4°
(+)498.901	Unknown	9.6	<0.001	4°
(+)501.056	Unknown	8.3	<0.001	4°
(-)501.394	Unknown	3.6	0.004	4°
(+)501.394	Unknown	3.6	0.007	4°
(+)502.830	Unknown	2.6	0.009	4°
(+)504.825	Unknown	5.4	0.003	4°
(-)505.392	Unknown	2.3	0.001	4°
(+)506.375	Unknown	22.3	0.002	4°
(+)507.256	Unknown	9	0.008	4°
(-)507.273	Unknown	4.5	0.001	4°
(+)508.354	unknown	8	0.002	4°
(+)508.341	Unknown	8.5	0.004	4°

(+)510.357	Unknown	8.3	<0.001	4°
(+)512.337	Unknown	8	0.005	4°
(+)512.918	Unknown	9.6	<0.001	4°
(-)512.967	Unknown	3	0.003	4°
(+)513.635	Unknown	3	0.005	4°
(+)514.389	Unknown	20.6	0.004	4°
(-)516.282	Unknown	8.1	0.001	4°
(+)516.999	Unknown	7	0.006	4°
(+)517.673	Unknown	6.9	0.005	4°
(+)518.342	Unknown	3.9	0.002	4°
(-)519.852	Unknown	2.1	<0.001	4°
(+)520.341	Unknown	8	0.001	4°
(-)521.040	Unknown	9.2	<0.001	4°
(+)521.344	Unknown	8	0.001	4°
(+)521.662	Unknown	8.5	0.002	4°
(+)524.340	Unknown	8.1	0.006	4°
(+)524.370	Unknown	2.6	0.005	4°
(+)524.372	Unknown	8.5	0.001	4°
(+)524.829	Unknown	6.5	<0.001	4°
(-)527.332	Unknown	4	0.006	4°
(+)527.332	Unknown	4	<0.001	4°
(-)527.407	Unknown	2.7	0.002	4°
(+)527.676	Unknown	7.6	0.005	4°
(+)528.381	Unknown	20.7	0.001	4°
(+)529.057	Unknown	2.4	0.001	4°
(-)529.423	Unknown	4	0.001	4°
(+)529.426	Unknown	5.8	0.009	4°
(-)530.302	Unknown	8.1	0.008	4°
(-)530.839	Unknown	8.5	<0.001	4°
(+)531.349	Unknown	3	0.006	4°
(+)531.474	Unknown	29.7	<0.001	4°
(-)531.681	Unknown	7.1	0.007	4°
(+)533.638	Unknown	2.5	0.003	4°
(+)534.297	Unknown	9.4	0.001	4°
(-)536.118	Unknown	2.7	<0.001	4°
(-)537.487	Unknown	2.8	0.003	4°
(+)537.642	Unknown	2.7	0.007	4°
(-)538.315	Unknown	7.9	<0.001	4°
(+)538.357	Unknown	2.2	<0.001	4°
(+)538.386	Unknown	8.7	<0.001	4°
(+)538.161	Unknown	27.6	0.006	4°
(+)540.367	Unknown	8.2	0.006	4°
(+)540.949	Unknown	9.6	<0.001	4°
(+)541.016	Unknown	6.5	0.005	4°
(+)543.574	unknown	7.3	0.001	4°
(-)544.149	Unknown	2	<0.001	4°

(+)544.317	Unknown	8.3	0.006	4°
(+)544.342	Unknown	7.9	0.001	4°
(+)546.015	Unknown	4.3	<0.001	4°
(-)546.353	Unknown	8.7	<0.001	4°
(+)546.355	Unknown	2.9	0.001	4°
(-)547.297	Unknown	2.2	0.001	4°
(+)547.355	Unknown	7.9	0.009	4°
(+)547.526	Unknown	3.8	0.004	4°
(+)547.645	Unknown	2.6	0.004	4°
(+)548.499	Unknown	29.7	0.002	4°
(+)548.851	Unknown	6.3	<0.001	4°
(+)551.537	Unknown	4.1	0.001	4°
(+)552.071	Unknown	8.3	0.004	4°
(-)552.318	Unknown	3	0.006	4°
(-)552.306	Unknown	7.9	<0.001	4°
(+)552.395	Unknown	9.3	0.006	4°
(+)553.361	Unknown	3.3	0.001	4°
(+)553.411	Unknown	4.6	0.004	4°
(-)554.301	Unknown	8	0.002	4°
(+)554.392	Unknown	8.1	0.003	4°
(+)555.356	Unknown	6.8	0.001	4°
(+)555.441	Unknown	6.1	0.008	4°
(-)556.055	Unknown	8.3	0.004	4°
(-)557.458	Unknown	23.9	0.006	4°
(+)557.664	Unknown	3.4	0.003	4°
(-)558.063	Unknown	11.1	<0.001	4°
(+)558.297	Unknown	7.9	0.002	4°
(-)558.331	Unknown	8.4	0.001	4°
(-)559.469	Unknown	2.2	0.002	4°
(+)560.327	Unknown	9.4	0.004	4°
(+)560.703	Unknown	5.2	0.001	4°
(-)562.009	Unknown	3.7	0.001	4°
(+)562.081	Unknown	7.2	0.006	4°
(-)562.315	Unknown	7.9	0.007	4°
(+)562.867	Unknown	5	<0.001	4°
(-)563.501	Unknown	2.7	0.001	4°
(+)564.334	Unknown	8.6	0.005	4°
(-)567.534	Unknown	4	0.002	4°
(-)568.264	Unknown	7.5	<0.001	4°
(+)568.366	Unknown	3.1	0.003	4°
(+)568.301	Unknown	8.6	0.006	4°
(+)569.428	Unknown	29.7	0.004	4°
(+)569.578	Unknown	7	0.009	4°
(+)570.361	Unknown	6.7	0.002	4°
(+)571.030	Unknown	7	0.006	4°
(-)571.580	Unknown	2.7	0.004	4°

(-)571.668	Unknown	4.2	0.006	4°
(+)572.341	Unknown	3.1	0.006	4°
(+)572.364	Unknown	8	0.005	4°
(-)572.481	Unknown	27.6	0.001	4°
(-)573.438	Unknown	3.5	0.005	4°
(+)575.201	Unknown	6.5	0.004	4°
(-)575.462	Unknown	4.1	0.003	4°
(+)575.462	Unknown	4.1	0.004	4°
(+)575.484	Unknown	28.8	0.007	4°
(-)576.352	Unknown	8.7	0.003	4°
(+)576.350	Unknown	4.1	0.003	4°
(-)588.332	Unknown	7.8	0.007	4°
(+)589.525	Unknown	29.7	<0.001	4°
(-)589.717	Unknown	6.2	0.003	4°
(+)590.380	Unknown	3.5	0.009	4°
(+)590.557	Unknown	8.5	0.003	4°
(+)591.024	Unknown	4.6	<0.001	4°
(-)591.319	Unknown	2.3	0.009	4°
(+)591.549	Unknown	8.5	<0.001	4°
(+)591.594	Unknown	7.2	0.002	4°
(+)591.670	Unknown	2.9	0.008	4°
(+)592.367	Unknown	6.9	0.003	4°
(-)593.436	Unknown	23.8	0.009	4°
(+)594.920	Unknown	9.6	0.001	4°
(+)595.349	Unknown	7.4	0.002	4°
(+)595.508	Unknown	2.3	0.001	4°
(+)598.320	Unknown	8	0.003	4°
(+)600.331	Unknown	8	0.006	4°
(+)601.339	Unknown	7.4	0.002	4°
(+)601.352	Unknown	5.4	0.001	4°
(+)601.357	Unknown	3.8	0.007	4°
(+)602.002	Unknown	3	0.002	4°
(+)602.349	Unknown	8.5	0.005	4°
(+)603.067	Unknown	8.3	0.003	4°
(+)604.725	Unknown	6.5	0.008	4°
(+)604.899	Unknown	2.7	0.007	4°
(-)605.404	Unknown	3.5	0.002	4°
(+)606.301	Unknown	7.8	0.004	4°
(+)606.679	Unknown	3	0.006	4°
(+)606.892	Unknown	6.1	0.002	4°
(+)607.325	Unknown	3.8	<0.001	4°
(-)608.357	Unknown	7.9	0.009	4°
(+)608.936	Unknown	9.6	<0.001	4°
(+)610.907	Unknown	9.6	<0.001	4°
(+)611.056	Unknown	8.3	0.003	4°
(+)611.148	Unknown	11.3	0.003	4°

(+)611.688	Unknown	3.3	0.008	4°
(-)612.390	Unknown	3.9	0.009	4°
(+)613.056	Unknown	8.3	0.007	4°
(+)613.605	Unknown	7.3	0.001	4°
(+)614.330	Unknown	2.8	0.003	4°
(+)614.343	Unknown	9.6	<0.001	4°
(-)616.457	Unknown	5	0.002	4°
(-)616.650	Unknown	7.9	0.008	4°
(+)616.680	Unknown	3.1	0.006	4°
(+)617.111	Unknown	7.5	<0.001	4°
(+)619.901	Unknown	4.7	0.002	4°
(+)621.022	Unknown	3.2	0.003	4°
(+)624.613	Unknown	7.3	<0.001	4°
(-)625.320	Unknown	2.4	0.004	4°
(+)626.409	Unknown	3	0.007	4°
(-)627.785	Unknown	7.1	0.002	4°
(+)629.323	Unknown	3.7	0.008	4°
(+)630.322	Unknown	9.5	0.001	4°
(+)630.702	Unknown	6.3	0.005	4°
(+)631.354	Unknown	3.1	<0.001	4°
(-)632.353	Unknown	7.8	0.001	4°
(+)634.537	Unknown	25.2	<0.001	4°
(+)634.536	Unknown	23.9	0.003	4°
(+)634.877	Unknown	9.6	0.006	4°
(-)635.347	Unknown	2.5	0.003	4°
(+)635.621	Unknown	7.4	<0.001	4°
(+)635.698	Unknown	2.9	0.007	4°
(+)636.901	Unknown	7.3	<0.001	4°
(+)638.577	Unknown	3.6	0.007	4°
(-)638.937	Unknown	2.9	0.003	4°
(+)641.353	Unknown	2.7	0.007	4°
(+)642.019	Unknown	2.7	0.004	4°
(+)643.028	Unknown	25.2	0.001	4°
(+)643.018	Unknown	23.9	0.003	4°
(+)644.833	Unknown	5.4	0.002	4°
(+)645.188	Unknown	28.8	0.008	4°
(+)645.377	Unknown	6.5	0.009	4°
(+)646.320	Unknown	7.8	0.003	4°
(+)646.695	Unknown	3.1	0.002	4°
(-)647.373	Unknown	7.9	0.003	4°
(+)648.304	Unknown	7.9	0.005	4°
(-)649.419	Unknown	7.9	0.004	4°
(-)650.278	Unknown	7	0.003	4°
(+)651.040	Unknown	3.1	0.009	4°
(-)652.390	Unknown	5.2	0.003	4°
(+)655.050	Unknown	3.9	<0.001	4°

(-)656.353	Unknown	7.8	0.004	4°
(-)656.523	Unknown	2.8	0.007	4°
(-)656.883	Unknown	9.6	0.004	4°
(+)656.924	Unknown	5.9	0.006	4°
(+)657.881	Unknown	7.5	0.006	4°
(+)658.544	Unknown	6.4	0.001	4°
(+)660.053	Unknown	6.8	0.002	4°
(+)660.066	Unknown	8.3	0.002	4°
(+)660.369	Unknown	2.8	<0.001	4°
(+)661.177	Unknown	8.2	0.008	4°
(-)662.352	Unknown	8	0.004	4°
(-)663.430	Unknown	2.7	0.002	4°
(+)665.049	Unknown	3.3	0.01	4°
(+)665.414	Unknown	7.6	0.006	4°
(+)665.705	Unknown	3.3	0.004	4°
(-)666.030	Unknown	2.4	0.005	4°
(+)668.170	Unknown	7.8	0.01	4°
(-)669.348	Unknown	2.7	0.002	4°
(+)669.726	Unknown	4.3	0.003	4°
(+)670.056	Unknown	6.3	0.002	4°
(+)670.428	Unknown	4.4	<0.001	4°
(+)670.719	Unknown	6.4	0.004	4°
(+)671.036	Unknown	2.8	0.003	4°
(+)672.433	Unknown	7	0.007	4°
(+)672.712	Unknown	8.3	0.007	4°
(+)673.129	Unknown	7	0.008	4°
(+)673.351	Unknown	3.6	0.002	4°
(+)674.730	Unknown	6.9	0.002	4°
(+)677.578	Unknown	28.8	<0.001	4°
(+)678.429	Unknown	5.6	0.003	4°
(-)679.373	Unknown	2.7	<0.001	4°
(-)680.351	Unknown	7.8	0.001	4°
(+)680.705	Unknown	3.2	0.006	4°
(+)682.327	Unknown	9.6	0.004	4°
(+)682.327	Unknown	9.6	0.001	4°
(-)683.399	Unknown	7.9	0.003	4°
(+)683.642	Unknown	7.1	0.004	4°
(+)684.728	Unknown	6.6	0.002	4°
(+)685.048	Unknown	3.7	0.001	4°
(+)685.401	Unknown	6.6	0.004	4°
(-)685.523	Unknown	6.4	0.004	4°
(-)686.354	Unknown	7.9	<0.001	4°
(+)687.205	Unknown	8.4	0.01	4°
(-)687.519	Unknown	26.7	0.002	4°
(+)688.344	Unknown	5.2	0.002	4°
(+)689.406	Unknown	7	0.003	4°

(+)689.720	Unknown	3.2	0.003	4°
(+)690.074	Unknown	7	0.001	4°
(+)690.384	Unknown	3.2	0.009	4°
(+)691.346	Unknown	5.3	0.003	4°
(+)692.447	Unknown	5	0.002	4°
(+)692.953	Unknown	4.1	0.01	4°
(+)694.399	Unknown	2.4	0.001	4°
(+)695.052	Unknown	3.2	0.003	4°
(+)695.864	Unknown	3.5	0.008	4°
(+)696.031	Unknown	8.3	0.003	4°
(-)698.481	Unknown	7.9	0.002	4°
(+)699.403	Unknown	6.7	0.004	4°
(+)699.410	Unknown	5.3	0.009	4°
(+)699.712	Unknown	3.2	0.002	4°
(+)700.053	Unknown	3.1	0.004	4°
(+)700.072	Unknown	6.7	0.003	4°
(+)700.390	Unknown	2	0.006	4°
(-)700.364	Unknown	7.8	<0.001	4°
(+)703.319	Unknown	2	0.004	4°
(+)702.868	Unknown	9.6	0.002	4°
(+)704.082	Unknown	7.2	0.007	4°
(+)708.747	Unknown	6.7	0.006	4°
(+)709.076	Unknown	4.4	0.002	4°
(-)709.500	Unknown	5.3	0.001	4°
(+)709.720	Unknown	3.2	0.003	4°
(+)709.735	Unknown	4.4	0.005	4°
(+)710.368	Unknown	5.1	0.002	4°
(-)710.360	Unknown	8.3	0.003	4°
(+)710.727	Unknown	4.4	0.003	4°
(+)711.048	Unknown	2.4	0.005	4°
(+)711.074	Unknown	8.3	0.007	4°
(+)711.365	Unknown	5.1	0.001	4°
(+)711.366	Unknown	3.2	0.004	4°
(+)712.364	Unknown	5.1	0.003	4°
(+)713.067	Unknown	8.3	0.009	4°
(-)713.363	Unknown	2.8	0.007	4°
(+)714.084	Unknown	6.9	0.002	4°
(+)714.396	Unknown	3.3	0.003	4°
(-)714.457	Unknown	4.6	0.01	4°
(-)714.509	Unknown	25.8	0.01	4°
(+)714.743	Unknown	6.9	0.004	4°
(+)715.062	Unknown	3.3	0.004	4°
(+)715.064	Unknown	8.3	0.008	4°
(+)716.523	Unknown	25.8	0.007	4°
(+)718.757	Unknown	7.3	0.004	4°
(+)720.384	Unknown	2.9	0.001	4°

(-)722.412	Unknown	7.9	0.001	4°
(+)724.085	Unknown	4.8	0.003	4°
(-)724.376	Unknown	7.8	<0.001	4°
(+)724.726	Unknown	3.3	0.001	4°
(+)725.559	Unknown	9.6	0.006	4°
(+)726.855	Unknown	4.5	0.001	4°
(-)727.447	Unknown	7.9	<0.001	4°
(+)728.757	Unknown	7	0.003	4°
(+)729.074	Unknown	3.4	0.004	4°
(+)729.423	Unknown	7	0.007	4°
(+)730.898	Unknown	9.6	0.007	4°
(+)732.409	Unknown	2.3	0.008	4°
(+)732.875	Unknown	4.9	0.002	4°
(+)733.841	Unknown	4.9	0.001	4°
(+)734.084	Unknown	4.1	0.003	4°
(-)734.390	Unknown	8.1	0.006	4°
(+)737.565	Unknown	8.3	0.001	4°
(-)738.379	Unknown	8.5	0.01	4°
(+)738.565	Unknown	8.3	0.006	4°
(+)738.754	Unknown	6.8	0.005	4°
(+)738.759	Unknown	5.4	0.004	4°
(+)739.074	Unknown	3.4	0.002	4°
(+)739.578	Unknown	8.3	0.007	4°
(+)739.738	Unknown	3.4	0.003	4°
(-)740.399	Unknown	8.5	0.005	4°
(+)740.568	Unknown	8.3	0.004	4°
(+)743.107	Unknown	6.5	0.007	4°
(+)743.433	Unknown	7.1	0.006	4°
(+)743.745	Unknown	3.7	<0.001	4°
(+)744.099	Unknown	7.2	0.004	4°
(+)744.907	Unknown	9.6	0.009	4°
(+)745.072	Unknown	3.8	0.003	4°
(+)745.201	Unknown	22.9	0.009	4°
(+)747.871	Unknown	4.4	0.001	4°
(+)748.108	Unknown	7.4	0.005	4°
(-)748.114	Unknown	9.6	0.002	4°
(+)748.753	Unknown	4.5	0.002	4°
(+)750.405	Unknown	2.9	0.005	4°
(-)751.450	Unknown	7.9	0.003	4°
(+)753.098	Unknown	6.9	0.005	4°
(-)754.081	Unknown	3.6	0.008	4°
(+)756.546	Unknown	25.7	0.008	4°
(-)758.377	Unknown	8	0.005	4°
(+)760.215	Unknown	24.2	<0.001	4°
(-)760.529	Unknown	4.3	0.002	4°
(+)760.583	Unknown	23.7	<0.001	4°

(+)761.429	Unknown	2.4	0.004	4°
(+)761.586	Unknown	23.8	<0.001	4°
(+)763.099	Unknown	5	0.003	4°
(+)763.417	Unknown	2.4	0.001	4°
(+)763.747	Unknown	3.8	0.004	4°
(+)763.600	Unknown	5	0.008	4°
(+)764.407	Unknown	2.8	0.003	4°
(+)765.074	Unknown	2.8	0.002	4°
(+)766.538	Unknown	23.7	<0.001	4°
(-)767.425	Unknown	3.3	0.003	4°
(+)767.775	Unknown	6.4	0.01	4°
(+)768.751	Unknown	3.8	0.006	4°
(+)768.920	Unknown	2.5	0.008	4°
(+)769.086	Unknown	3.8	0.005	4°
(+)769.884	Unknown	4.3	0.003	4°
(+)770.855	Unknown	9.6	0.001	4°
(+)772.012	Unknown	22.8	0.003	4°
(+)772.779	Unknown	7.3	0.008	4°
(+)773.099	Unknown	4.4	0.005	4°
(+)773.754	Unknown	4.4	0.002	4°
(+)774.418	Unknown	4.5	0.002	4°
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(+)775.079	Unknown	4.5	0.003	4°
(+)776.910	Unknown	3.1	0.002	4°
(+)779.086	Unknown	3.9	0.004	4°
(+)779.750	Unknown	4	0.004	4°
(+)780.505	Unknown	2	0.005	4°
(+)781.151	Unknown	28.5	0.002	4°
(+)782.567	Unknown	28.5	<0.001	4°
(+)783.760	Unknown	4	0.007	4°
(+)784.524	Unknown	3.5	<0.001	4°
(+)788.105	Unknown	4.8	0.003	4°
(+)789.090	Unknown	4.6	0.002	4°
(+)790.081	Unknown	4.5	0.002	4°
(+)792.777	Unknown	6.1	0.007	4°
(-)792.856	Unknown	9.6	0.003	4°
(+)793.421	Unknown	4.1	0.002	4°
(+)793.427	Unknown	2.7	0.004	4°
(+)794.092	Unknown	4.1	0.002	4°
(+)796.529	Unknown	9.4	0.002	4°
(+)798.104	Unknown	4.3	0.002	4°
(+)798.766	Unknown	4.4	0.001	4°
(+)799.426	Unknown	3	0.002	4°
(+)800.414	Unknown	3	0.002	4°
(+)803.422	Unknown	4.6	0.001	4°
(+)803.559	Unknown	2.5	0.01	4°

(+)804.466	Unknown	2.7	0.006	4°
(+)804.772	Unknown	4.5	0.002	4°
(+)806.560	Unknown	26.6	0.006	4°
(+)808.102	Unknown	4.3	0.002	4°
(+)808.433	Unknown	2.7	0.005	4°
(+)808.765	Unknown	4.3	0.005	4°
(+)809.577	Unknown	26.5	<0.001	4°
(+)811.987	Unknown	3.3	0.008	4°
(+)812.947	Unknown	2.6	0.002	4°
(+)813.110	Unknown	4.7	0.003	4°
(+)813.910	Unknown	4.1	0.003	4°
(-)816.533	Unknown	28.6	0.003	4°
(+)818.430	Unknown	4.7	0.002	4°
(+)818.431	Unknown	3.4	0.009	4°
(+)819.098	Unknown	4.7	0.005	4°
(+)820.439	Unknown	2.8	<0.001	4°
(+)820.929	Unknown	2.9	0.009	4°
(+)820.929	Unknown	2.9	0.004	4°
(+)822.437	Unknown	4.5	0.004	4°
(+)823.445	Unknown	4.6	0.004	4°
(+)823.445	Unknown	4.6	0.002	4°
(+)826.808	Unknown	7.2	0.01	4°
(+)827.119	Unknown	5	0.004	4°
(+)828.434	Unknown	2.4	0.009	4°
(+)830.567	Unknown	24	<0.001	4°
(+)832.581	Unknown	26.4	<0.001	4°
(+)833.109	Unknown	4.8	0.005	4°
(+)834.612	Unknown	2.9	<0.001	4°
(+)835.581	Unknown	2.6	<0.001	4°
(+)835.921	Unknown	4	0.004	4°
(+)836.800	Unknown	7	0.007	4°
(+)837.781	Unknown	4.8	0.007	4°
(+)838.842	Unknown	9.6	0.003	4°
(+)841.149	Unknown	7.3	0.007	4°
(+)841.261	Unknown	24	0.003	4°
(+)841.816	Unknown	7.3	0.005	4°
(+)842.467	Unknown	2.8	0.002	4°
(-)842.532	Unknown	26.6	0.009	4°
(+)843.453	Unknown	2.8	0.004	4°
(+)843.600	Unknown	2.4	0.007	4°
(-)844.558	Unknown	8.2	0.004	4°
(+)847.777	Unknown	5	0.001	4°
(+)848.489	Unknown	3.1	0.006	4°
(+)848.563	Unknown	7.5	0.006	4°
(+)849.958	Unknown	2.2	<0.001	4°
(+)850.421	Unknown	5.6	0.005	4°

(+)850.563	Unknown	7.4	0.009	4°
(+)850.563	Unknown	7.4	<0.001	4°
(+)851.422	Unknown	5.6	0.002	4°
(+)852.858	Unknown	9.6	0.001	4°
(+)853.702	Unknown	23.8	0.003	4°
(+)854.560	Unknown	23.2	0.002	4°
(+)855.520	Unknown	4.1	0.002	4°
(+)864.409	Unknown	23.3	0.001	4°
(+)864.467	Unknown	2.8	0.008	4°
(+)864.467	Unknown	2.8	0.002	4°
(+)864.964	Unknown	3.4	0.002	4°
(+)866.159	Unknown	7.3	0.009	4°
(+)868.555	Unknown	2.2	0.002	4°
(+)871.012	Unknown	3.4	0.009	4°
(+)874.014	Unknown	8.3	0.007	4°
(-)874.561	Unknown	24	0.002	4°
(+)875.711	Unknown	23.7	0.001	4°
(-)876.573	Unknown	26.4	0.003	4°
(+)878.987	Unknown	2.8	0.002	4°
(+)880.954	Unknown	3.8	0.003	4°
(+)881.166	Unknown	7.4	0.008	4°
(+)883.581	Unknown	1.9	0.006	4°
(+)885.503	Unknown	3.1	0.008	4°
(-)886.008	Unknown	3.1	0.009	4°
(+)886.970	Unknown	3.3	0.006	4°
(+)889.410	Unknown	4.5	0.007	4°
(+)889.589	Unknown	7	0.003	4°
(+)892.590	Unknown	3.9	0.006	4°
(+)892.667	Unknown	3	0.01	4°
(-)893.016	Unknown	3.8	0.009	4°
(+)893.016	Unknown	3.8	0.002	4°
(+)893.482	Unknown	2.2	0.001	4°
(+)893.981	Unknown	2.9	0.009	4°
(+)893.981	Unknown	2.9	0.002	4°
(+)894.941	Unknown	5.3	0.002	4°
(+)901.000	Unknown	2.9	0.007	4°
(+)901.000	Unknown	2.9	0.001	4°
(+)901.967	Unknown	3.7	0.002	4°
(+)902.451	Unknown	3	0.005	4°
(+)906.829	Unknown	9.6	<0.001	4°
(+)908.028	Unknown	3.3	0.008	4°
(+)908.989	Unknown	2.7	0.002	4°
(+)911.857	Unknown	9.7	0.005	4°
(+)912.582	Unknown	2.3	0.005	4°
(+)916.001	Unknown	3	0.007	4°
(+)917.957	Unknown	5.3	<0.001	4°

(-)922.612	Unknown	3.7	0.003	4°
(+)924.979	Unknown	3.6	0.002	4°
(+)930.024	Unknown	3.6	0.006	4°
(+)930.990	Unknown	2.7	0.002	4°
(+)932.423	Unknown	4.3	0.002	4°
(+)933.836	Unknown	6.8	<0.001	4°
(+)934.724	Unknown	6.9	0.002	4°
(+)939.008	Unknown	3.1	0.006	4°
(+)938.463	Unknown	5.3	0.002	4°
(+)938.599	Unknown	7.6	0.004	4°
(+)945.030	Unknown	3.3	0.006	4°
(+)945.502	Unknown	3.4	0.007	4°
(+)946.456	Unknown	3	0.003	4°
(+)946.988	Unknown	3.5	0.009	4°
(+)956.605	Unknown	2.5	0.001	4°
(+)960.029	Unknown	3.3	0.002	4°
(+)960.976	Unknown	5.2	0.002	4°
(+)968.552	Unknown	9.6	0.006	4°
(+)969.508	Unknown	3.5	0.008	4°
(+)975.018	Unknown	2.6	0.004	4°
(+)976.448	Unknown	4.2	0.005	4°
(+)981.534	Unknown	3.4	0.005	4°
(+)982.035	Unknown	2.1	0.01	4°
(+)983.999	Unknown	5.2	0.001	4°
(+)986.185	Unknown	2	0.005	4°
(+)987.975	Unknown	4.2	<0.001	4°
(+)988.830	Unknown	9.6	0.004	4°
(+)989.535	Unknown	3.4	0.009	4°
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(+)990.485	Unknown	3	0.01	4°