

Research Article

Plasma lipidomics reveal systemic changes persistent throughout early life following a childhood burn injury

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Abstract

Background: Non-severe paediatric burns can result in poor long-term health outcomes. This occurs even in cases with good acute burn-related outcomes, including minimal scarring. The mechanisms that underpin the transition from non-severe burn to sustained negative long-term health impacts are currently unknown. However, sustained metabolic and immune changes have been observed in paediatric burn studies, suggesting these changes may be important. The plasma lipidome consists of a rich pool of bioactive metabolites that play critical roles in systemic processes including molecular signalling and inflammation. We hypothesised that changes in the plasma lipidome may reflect underlying changes in health status and be linked to long-term health after burn trauma.

Methods: This study analysed the lipidome in children who had previously experienced a non-severe burn, compared to non-injured controls. Thirty-three participants were recruited between the ages of 5 and 8 years who had experienced a non-severe burn between the ages of 1 and 3 years. Plasma samples were also collected from a non-injured, healthy, age and gender matched control group (n = 21). Plasma lipids were measured using reversed-phase liquid chromatography-mass spectrometry (LC-MS).

Results: In total 838 reproducible lipid species from 19 sub-classes passed quality control procedures and progressed to statistical analysis. Analysis of individual lipid metabolites showed significantly higher concentrations of lysophosphatidylethanolamines and phosphatidylethanolamines,

and significantly lower concentrations in myristic, palmitic and palmitoleic acids in the plasma of those who had experienced burn injury compared to controls.

Conclusion: Long-term changes in the lipid profile may give insight into the mechanisms underlying poor long-term health subsequent to non-severe burn injury. Further work to investigate the relationship between long-term pathology and lipidomic changes may lead to a better understanding of the causes of secondary morbidity post-burn and to clinical intervention to reduce the long-term health burden of burn trauma.

Key words: Lipidomics, Lipids, Burns, Trauma, Paediatric, Children, Mass spectrometry

Highlights: Residual and long-lasting differences have been observed in the plasma lipidome in paediatric patients who have previously experienced non-severe burn injury compared to non-injured controls.

This study takes the first steps towards understanding the effects of non-severe burn injury early in life on long-term lipid metabolism.

Background

Burn injury is associated with an inflammatory response that is more extreme than that seen in equivalent non-burn trauma [1,2]. Although the inflammatory response is essential for healing, persistent inflammation and immune responses have been implicated in driving poor health outcomes [3]. It has been established that children who sustain non-severe burns (<10% total body surface area (TBSA)) will suffer poorer health outcomes over their lifetime relative to an uninjured cohort [4]. Non-severe burn injuries are the most common injuries sustained by paediatric patients in Australia, in an analysis of the Burn Registry of Australia and New Zealand from 2009–2018, 86.8% of children sustained non-severe burn injuries [5]. Long-term healthcare data linkage studies have shown that children who suffer even non-severe burn injuries have an increased admission rate and length of stay in hospital for a range of pathologies including diseases of the circulatory system [6], musculoskeletal system [7], diabetes mellitus [8], gastrointestinal system [9] and infectious disease [10] for many years after discharge for their burn. Increased age-adjusted mortality after recovery from the burn has also been observed in children with burn injuries [11], suggesting paediatric burn injury induces sustained health changes that lead to increased long-term morbidity.

The presence of long-term physiological changes in paediatric burn patients have been hypothesised to be the result of chronic systemic inflammation [12]. Such inflammation is associated with the immune response. In the case of burn injury, this can be triggered by thermal injury, toxic compounds and both acute and subsequent cell damage [13]. Long-term immune dysfunction has been described in paediatric burn survivors [11,14], with significant changes seen in levels of TNF- α , IL-2, IL-7 and IFN- γ as well as differences in proportions of B-cells, T-cells and myeloid cells [14]. Systemic inflammatory responses also have an impact on an individual's metabolism, with multiple metabolic pathways

known to be associated with inflamed states, including following burn injury [15].

Metabolic phenotyping is a technique that can be used to measure perturbations in metabolites within a biofluid or tissue, with the aim of assessing physiological variation and molecular abnormalities associated with disease. Indeed, previous work by our group has identified significant perturbations in small molecule metabolic phenotypes following a burn injury in infants, and indicate residual systemic inflammation [15]. Lipidomics is an additional sub-discipline of metabolic phenotyping that aims to specifically target and measure lipid species present in biological samples and provide additional biochemical information that can help detail the systemic response to traumas such as burn injuries. Lipids are omnipresent across biological systems, performing many key biological roles including being an essential constituent of cell membranes, acting as signal molecules and providing a store of energy. Lipids are increasingly recognised as being important in regulating cell phenotype and activity [16] and changes in lipids have recently been reported to be associated with a range of disease conditions [17]. Thus, lipidomics is now widely utilised to study lipid metabolism in inflammation-related disease states [18]. However, although the occurrence of acute dyslipidaemia subsequent to burn injury has been well established, chronic dyslipidaemia in burns has not been thoroughly investigated [19]. Despite altered plasma lipid profiles reported in many chronic inflammation-related conditions including type 1 and 2 diabetes [20] and cardiovascular disease [21], the physiological and pathological functions that lipids have in inflammatory responses in injury have not been determined. Furthermore, specific lipidomic changes associated with systemic inflammation post non-severe burn-injury have not yet been described in the literature.

Therefore, this study aimed to investigate and describe the sustained variance in the lipidome that occurs in children

after a non-severe burn injury in order to gain insight into the mechanisms that underpin the long-term physiological outcomes associated with such injuries.

Methods

Sample collection

All samples were obtained with informed consent of a parent or guardian and the collection was conducted with ethical approval from the Child and Adolescent Health Service WA (approval numbers: 2015219EP; 1111EP; 768EP).

Recruitment was limited to participants that had sustained non-severe (<10% TBSA) burn injury before 3 years of age with subsequent presentation to Princess Margaret Hospital, Western Australia (n = 33). All participants had a scar outcome of <2 on the Vancouver Scar Scale (VSS). All participants were a minimum of three years after their burn injury at the time of blood draw and were not on any modified nutritional regime or any other treatment related to the burn injury. Control samples (n = 21) were selected from a Western Australian biobank of healthy donors and were balanced for age and sex to the burn cohort. Both the burn patients and controls were healthy at the time of collection, and thus not receiving treatment or taking medication for any other illness or condition. All participant samples were from the same geographic area and collected over the same period of time, therefore lifestyle factors are likely to be similar. BMI was not measured for either group. Samples were collected when patients presented to the clinic at Princess Margaret Hospital for routine follow-up appointments, and as such diet was not controlled throughout the study.

Blood was collected into tubes containing preservative-free heparin, then centrifuged to separate and collect the plasma. Plasma samples were stored at -80°C until analysis. Sample collection protocols were consistent between control and burn injury groups.

Lipid analysis

Lipidomic analysis was performed using an established targeted liquid-chromatography mass spectrometry (LC-MS) assay that has been described in detail previously [22]. In brief, 10 μL of plasma underwent extraction by the addition of 90 μL of isopropanol (Fisher Scientific, Malaga, Western Australia) containing stable isotope-labelled internal standards (Lipidizer™ Internal Standards Kit from Sciex, Framingham, MA, USA, and SPLASH LipidoMIX™, Lyso PI 17:1, Lyso PG 17:1, and Lyso PS 17:1, Avanti Polar Lipids, Alabaster, AL, USA). Samples were then centrifuged ($3500 \times g$ for 10 min at 4°C). A total of 70 μL of supernatant was then transferred into Eppendorf 350 μL 96-well plates for LC-MS analysis. Replicate quality control (QC) samples were prepared from a commercial pooled human plasma sample (BioIVT, New York, NY, USA) and underwent extraction (n = 8) using the method described. These replicate samples were periodically analysed throughout the analytical sequence.

Semi-quantitative lipid analysis of the extracts was performed using a targeted tandem mass spectrometry (MS/MS) approach using pre-defined multiple reaction monitoring (MRM) transitions (Sciex sMRM Pro Builder, Framingham, MA, USA) and in-house defined chromatographic retention time windows [22]. The analytical system consisted of a Sciex ExionLC™ coupled to a Triple Quadrupole Linear Ion Trap (QTRAP 6500+) (SCIEX, Concord, ON, Canada). Reversed-phase separation was performed using a Waters Acquity BEH C18 1.7 μm , 2.1×100 mm column (Waters Corp., MA, USA) at 60°C . Mobile phase A was water/acetonitrile/propan-2-ol (50/30/20, v/v/v) containing 10 mM ammonium acetate and mobile phase B was propan-2-ol/acetonitrile/water (90/9/1, v/v/v) containing 10 mM ammonium acetate. The flow rate was 0.4 mL/min with gradient elution starting at 10% B, increasing to 45% B at 2.7 min, 53% at 2.8 min, 60% B at 8.0 min, 80% B at 8.1 min and holding at 80% B until 11.5 min, 100% B at 12.0 min for 1 min before returning to 10% B for a 2 min re-equilibration for a total cycle time of 15 min. The injection volume was 5 μL . Water, acetonitrile and isopropanol used were all Optima grade, and purchased from Thermo Fisher Scientific (Malaga, Western Australia).

Mass detection was performed using a SCIEX QTRAP 6500+ (SCIEX, Concord, CA) with electrospray ionisation, operated in low mass mode with polarity switching. The following mass spectrometer settings were used: capillary voltage 5500 V (positive ion mode) and -4500 V (negative ion mode), temperature 300°C ; curtain gas 20 psi; ion source gas 1, 40 psi; ion source gas 2, 60 psi. Time-scheduled multiple reaction monitoring (MRM) was used for data acquisition of 1231 transitions (including 1163 lipid species and 68 internal standards). Data were acquired using SCIEX Analyst® 1.7.1 (SCIEX, MA, USA).

Data pre-processing

Raw data were processed using SkylineMS [23] (Version 21.1, MacCoss Lab Software, University of Washington, Seattle, WA, USA). Data pre-processing and feature filtering was performed using R (version 4.4.1; R Foundation, Vienna, Austria) in R Studio version 1.4.1; R Studio, Boston, MA, USA) using in-house scripts. Feature filtering lipid targets were required to pass a noise threshold and have a relative standard deviation of <30%. Signal drift correction was then performed using the statTarget package [24].

Following data pre-processing and filtering, two datasets were produced, the first consisted of individual lipid species targeted by the LC-MS assay, and the second consisted of class level lipid data whereby all individual lipid targets in a specific parent class (e.g. ceramides) were summed and condensed into a single class level value.

Statistics

Data for individual lipid concentrations and total lipid concentration summed by lipid class were analysed.

Table 1. Demographics of study cohort. There is no significant difference in age at time of sampling or sex between the burn and uninjured cohorts

	Burn		Non-burn control		P value (burn compared to control proportion test)
	Male	Female	Male	Female	
Number of samples, n(%)	15 (45.5%)	18 (54.5%)	8 (38.1%)	13 (61.9%)	0.6
Average age at time of collection (years)	6.2	5.7	6.1	6.0	0.8
Standard deviation of age at time of collection (years)	1.0	0.9	0.9	1.0	NA
Average age at time of burn (years)	2.0	1.8	NA	NA	NA
Standard deviation of age at time of burn (years)	0.9	0.7	NA	NA	NA

Multivariate statistical models were run using SIMCA software (V.15.0., Sartorius AG, Göttingen, Germany). Principal Component Analysis (PCA) was used to analyse the quality of the lipidomic dataset and to look for outlier samples. Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA) was then used to stratify the patient groups and to report discriminant lipids from each sample class.

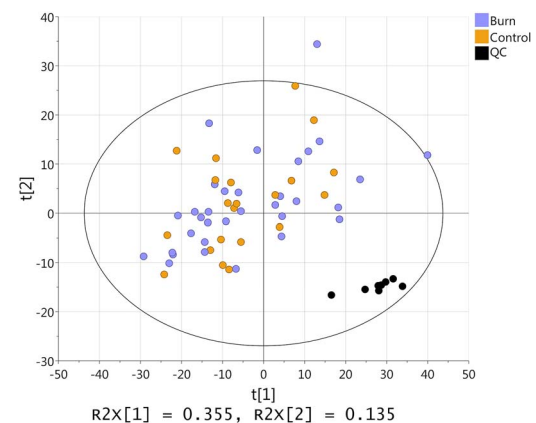
Prism 9.1.12 (GraphPad, 2021, San Diego, California, USA) was used for Mann–Whitney U testing and construction of boxplots. The significance interval used for Mann–Whitney testing was $p < 0.05$. As the study was discovery in its design, corrections for multiple testing were not applied to the univariate statistics.

Pathway analysis was completed with BioPAN (lipidmaps.org) [25] and was used to visualise the key pathways responsible for the lipid differences observed in the data. Processing with BioPAN produced the list of most active and suppressed pathways by Z-score, BioPAN determines a reaction to be significantly active or suppressed at a level of $p < 0.05$, corresponding to $Z > 1.645$.

Results

Plasma lipids were assessed in the 54 samples, of which there were 21 controls with an average age of 6.04 years (8 male, 13 female) and 33 participants who had previously experienced burn injury with an average age at collection of 5.97 years (18 male, 15 female). The average age at time of injury was 1.94 years, the minimum age was 1 year and maximum was 3 years. The average time between injury and collection was 4.45 years, the minimum time was 3.62 years and maximum was 5.99 years. Of the 33 burn patients included in the study, 29 had a VSS of zero (no visible scarring). No significant differences between the control and burn cohort for sex or age at time of sampling were identified. Demographic profiling of the study and control cohort is outlined in Table 1.

From the raw data, 838 reproducible lipid species from 19 lipid classes passed quality control procedures and progressed to statistical analysis. First, unsupervised principal component analysis (PCA) was performed to assess sample distribution. Figure 1 shows the PCA scores plot of individual

**Figure 1.** Principal component analysis (PCA) scores plot using mass spectrometry data from individual lipid species. R2X[1] and R2X[2] indicate proportion of variance for explained by first and second principal component, respectively. The scores plot is coloured by sample class: burn (purple); control (orange); replicate quality control (QC) (black))

lipid species. The pooled quality control (QC) data points clustered together, indicating consistency in measurement over the course of the run (Figure 1). No distinct patterns or outliers were observed within burn samples (purple) and control samples (orange). (See online supplementary material for a colour version of this figure.)

Supervised Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA) was then performed to identify multivariate differences that existed between the burn and control groups. Separate OPLS-DA models were generated using the individual lipid data (Figure 2) and the class summed data (Figure S1). Loadings from the OPLS-DA models were used to determine differential lipids and lipid classes between the burn and control groups. Permutation testing of the cross validated OPLS-DA scores plots based on 100 random permutations indicated that there was a robust distinction between the burn and control groups (Figure S2).

Univariate analysis (Mann–Whitney U-test) was then performed to confirm the result. Concentration of lipids with the greatest influence on the distinction between the burn and control groups are listed in Table 2. Table 3 lists the average concentration of lipids when summed by lipid

Table 2. Lipids with greatest variance between burn and controls in ascending order of Mann–Whitney *p* value. Lysophosphatidylethanolamine (LPE), phosphatidylethanolamine (PE), and free fatty acids (FFA) with specific sidechain lengths (in parentheses) are significantly different between groups

Principal discriminant phospholipid	Average concentration in burn (uM)	Average concentration in control (uM)	Mann–Whitney <i>p</i> value
LPE (18:1)	0.92	0.54	0.0002
PE (18:1_18:2)	0.09	0.03	0.0003
PE (18:0_16:0)	0.05	0.03	0.0014
PE (18:0_18:0)	0.97	0.61	0.0018
PE (18:1_18:1)	2.47	1.31	0.0022
PE (18:0_22:6)	0.15	0.09	0.0025
LPE 18:0	1.60	1.10	0.0039
FFA (14:0)	2.85	5.06	0.0049
PE (18:2_16:1)	1.72	0.82	0.0059
PE (18:0_18:1)	4.27	3.00	0.0066
FFA (16:0)	30.5	50.5	0.0192
PE (18:1_18:3)	0.07	0.04	0.0282
FFA (16:1)	0.61	0.83	0.0339
PE (P-18:1_16:0)	0.26	0.82	0.0526

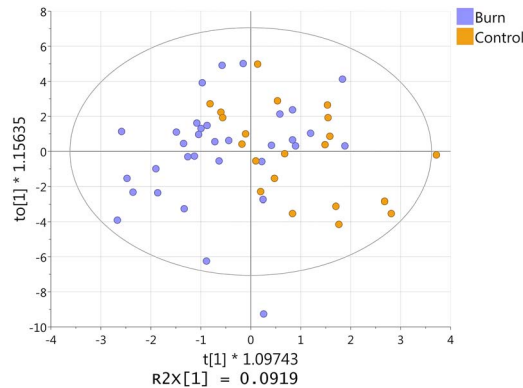


Figure 2. Cross validated orthogonal projections to latent structures discriminant analysis (OPLS-DA) scores plot of mass spectrometry data for individual Lipid Species. The scores plot is coloured by sample class: burn (purple); control (orange). The OPLS-DA model partially separates the sample classes ($R^2=0.043$)

class in burns and controls. [Figure 3](#) enables visualisation of the lipid classes (x-axis) and sidechains (y-axis) with the most significant variance between burn and control groups, whilst boxplots displaying the concentration of specific lipids that are significant in the Mann–Whitney tests are shown in [Figure 4](#).

Pathway analysis

Only 133 of the 838 reproducible lipid species were present in the BioPAN database and underwent processing. BioPAN determines a reaction to be significantly active or suppressed at a level of $p < 0.05$, corresponding to a Z score > 1.645 . Significant pathways are highlighted with bold lines in [Figure 5](#). BioPAN reported four significant reactions across three pathways:

- 1. PS (36:0) to PE (36:0) (Z score of 3.89, [Figure 5a](#));
- 2. DG (34:3) to PE (34:3) (Z score 3.819, [Figure 5b](#));

- 3. FA (16:1) to FA (16:2) (Z score 1.919), [Figure 5c](#);
- 4. FA (16:0) to FA (18:0) to FA (18:1) (Z score 1.785, [Figure 5c](#)).

It should be noted that BioPAN uses summed lipid nomenclature, thus PE (36:0) is the same phospholipid as PE (18:0_18:0) and PE (34:3) is the same as PE (18:2_16:1). At a class level, the BioPAN analysis reported that the conversion of DCERs to CERs to SMs contained significant reactions (Z score 2.283, [Figure 5d](#)).

Discussion

The multivariate and univariate analysis indicated significant variation in individual phospholipids when comparing the burn and non-burn control groups. Lipids are bioactive molecules involved in many cellular processes. They are involved in the transition from acute to chronic inflammation through both the excessive and prolonged action of proinflammatory species and impairment of the function of their anti-inflammatory counterparts [26]. Oxidised phospholipids have also been shown to exert both pro- and anti-inflammatory effects and their presence has been associated with chronic conditions including infection, degenerative disease and metabolic disorders [27].

When observing the phospholipids that were significantly different in the analysis there was commonality in lipid species and sidechain length. Individual PEs, LPEs and FFAs were shown to be significantly different between groups, with total summed LPEs also reporting significant differences. Common sidechains included long chain fatty acids with 16 and 18 carbons in length; most notably the stearic acid (18:0) and oleic acid (18:1) as well as linoleic acid (18:2) and linolenic acid (18:3).

Eighteen carbon fatty acids have previously been associated with chronic inflammatory conditions. For example,

Table 3. Mean concentration of lipids in burns and controls when summed by lipid class, listed by ascending Mann–Whitney *p* value. Lipid class is listed in full and also with the abbreviation for each class of lipid

Lipid class	Abbreviation	Mean concentration in burn (uM)	Mean concentration in control (uM)	Mann–Whitney <i>p</i> value
Lysophosphatidylethanolamine	LPE	6.02	4.81	0.0201
Free Fatty Acids	FFA	735	857	0.0759
Phosphatidylglycerol	PG	1.80	1.72	0.0956
Dihydroceramides	DCER	25.0	35.5	0.1278
Hexosylceramides	HCER	8.35	7.68	0.2230
Ceramides	CER	11.1	12.0	0.3147
Phosphatidylethanolamine	PE	180	159	0.3234
Diacylglycerides	DAG	37.6	33.3	0.3976
Phosphatidylserine	PS	36.3	38.2	0.4076
Phosphatidylinositol	PI	38.1	36.5	0.4279
Lysophosphatidylinositol	LPI	1.27	1.19	0.5733
Phosphatidylcholines	PC	1352	1314	0.5853
Lysophosphatidylcholine	LPC	431	439	0.6728
Sphingomyelin	SM	134	133	0.6857
Lysophosphatidylglycerol	LPG	0.16	0.15	0.7515
Triacylglycerol	TAG	737	719	0.8055
Cholesterol Esters	CE	1746	1707	0.8466
Monoacylglycerols	MAG	6.40	6.07	0.8742
Lactosylceramides	LCER	10.3	10.1	0.9299

increased plasma levels of stearic acid have been observed in chronic inflammation associated with obesity, along with an increase in proinflammatory cytokine production [28], whilst increased levels of oleic and linoleic acid have been reported in patients with chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF) [29].

Lipids containing docosaheptaenoic acid (22:6) sidechains were also observed at higher concentrations in the burn injured group. However, docosaheptaenoic acid derivatives are known to have anti-inflammatory properties and have been reported to be neuroprotective against degenerative diseases such as Alzheimer's and Parkinson's disease [30]. Whilst this increase would appear to be in contrast to an overall increased proinflammatory milieu post-burn, its presence as a significant feature in our data indicate it may play a role in metabolic and/or immune changes post-burn and requires further investigation.

Palmitic acid (FFA 16:0) has been reported to induce inflammatory responses through various pathways including proinflammatory cytokine production, and high levels of palmitic acid in plasma results in insulin resistance [31]. Palmitoleic acid (FFA 16:1) has been observed to increase in chronic inflammatory conditions such as Crohn's disease, ulcerative colitis, COPD and IPF [29,32]. It is interesting that our data report lower concentrations of palmitic acid and palmitoleic acid in the burn injured group, again contrasting with the observed increase in susceptibility to diseases related to inflammation later in life [8].

Interestingly, there was a decrease in myristic acid in the burn cohort when compared to controls. Oral administration of myristic acid (FFA 14:0) has been shown to decrease skin

inflammation and nociception in a murine model [33]. Understanding whether a decrease in myristic acid contributes to chronic inflammation and decreased nociception following a burn injury requires further investigation.

Interestingly, the boxplot visualisations indicated more variation of the lipids that were significantly higher in the injured group within the burns group. Burn injury is a heterogeneous trauma, and as such may lead to a heterogeneous phenotype in the underlying biochemistry of the response. This may indicate that the response to injury was not consistent between individuals, more granular stratification of poorer responders to burn injury in the future may reveal biochemical drivers of poorer long-term health outcomes. Whilst beyond the scope of this study to achieve this, the identification of significant group mean differences provides a foundation for future longitudinal studies that may enhance the stratification of individual patient response to burn injury.

The pathways highlighted by the BioPAN analysis have not previously been linked to burn injury or inflammation. Of perhaps most relevance to burn injury is the DCERs to CERs to SM pathway, which is known to be mediated by the enzyme Delta 4-Desaturase (DEGS2), a bi-functional enzyme that is involved in the biosynthesis of skin [34]. This pathway is also mediated by Delta 4-Desaturase, Sphingolipid 1 (DEGS1) and Sphingomyelin Synthase 2 (SGMS2). When DEGS1 is overexpressed, as is predicted in this burns cohort, it inhibits biosynthesis of the Epidermal Growth Factor (EGF) receptor, whilst it is believed that SGMS1 may maintain the balance between cell survival and apoptosis by regulating the formation of apoptotic mediators [35]. These factors

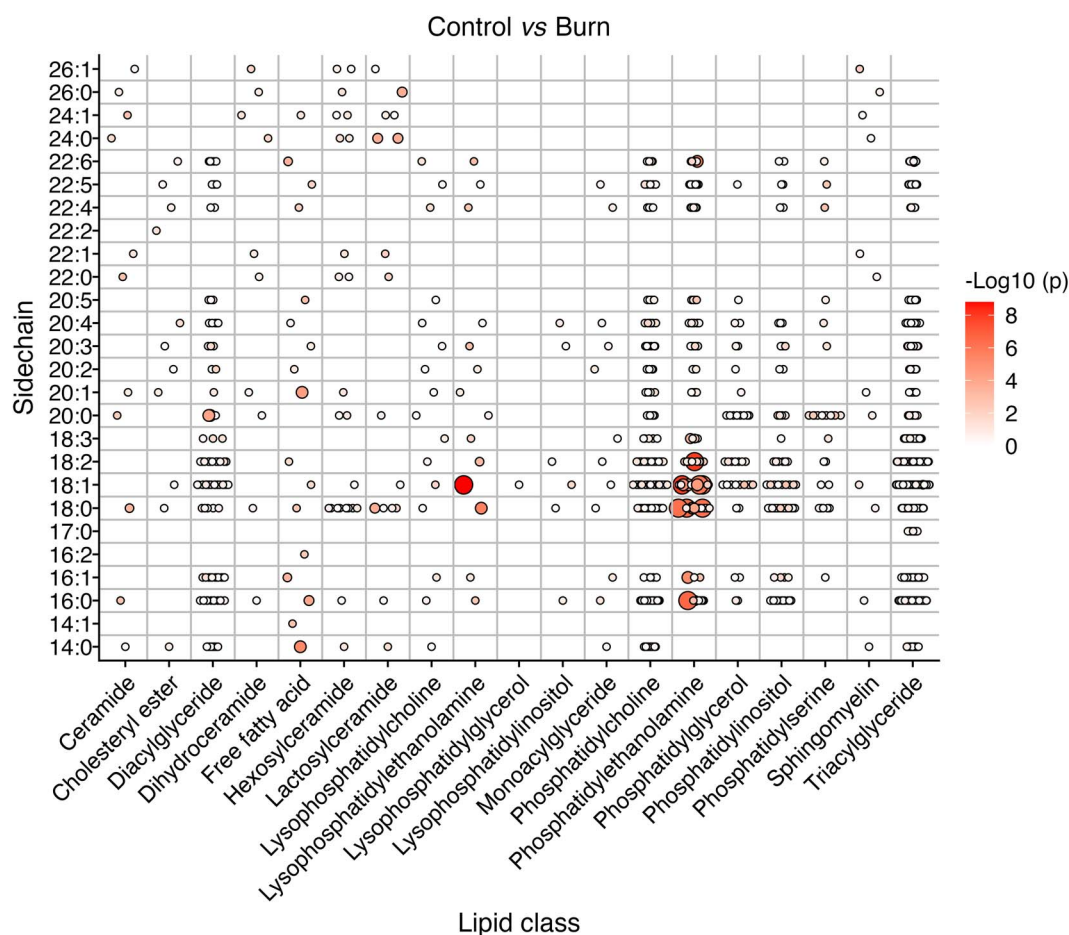


Figure 3. Plot highlighting lipids with greatest variance in concentration between the burn and control groups highlighted by log p -value. Larger and darker points indicate the smallest p -value (Mann–Whitney test) and therefore contribute the greatest variance between the two groups. Replicate points for a particular sidechain length (e.g. 18:1) in each column are representative of each occurrence of the sidechain in a lipid species. For example, PE (18:1_18:1) has two 18:1 sidechains and is represented by two points, the 18:1 sidechain of PE (18:1_18:2) is represented by another point in the same field. The classes with greatest variation are LPE, PE and FFA. Lipid classes that are different between groups include lysophosphatidylethanolamine (LPE), free fatty acids (FFA), phosphatidylglycerol (PG), dihydroceramides (DCER), hexosylceramides (HCER), ceramides (CER), phosphatidylethanolamine (PE), diacylglycerides (DAG), phosphatidylserine (PS), phosphatidylinositol (PI), lysophosphatidylinositol (LPI), phosphatidylcholines (PC), lysophosphatidylcholine (LPC), sphingomyelin (SM), lysophosphatidylglycerol (LPG), triacylglycerol (TAG), cholesterol esters (CE), monoacylglycerols (MAG) and lactosylceramides (LCER)

all appear to indicate an impairment of skin turnover and potential involvement in chronic inflammation.

Furthermore, CER and SM are intimately involved in vascular endothelial function. CERs contribute to endothelial cell dysfunction which increases vascular permeability, whereas SMs, specifically sphingosine-1-phosphate, promote vascular integrity [36]. Although there were no significant changes in CER or SM concentrations observed in the paediatric burn cohort samples, the increase in pathway activity highlighted by the BioPAN analysis indicated that burn injury may be associated with increased vascular permeability that is sustained in the long-term [37]. This may have implications for the increased cardiovascular disease risk and other long-term health effects of paediatric burn injury that have been reported [4,6–10].

The pathway highlighted in Figure 5b (DG (34:3) to PE (34:3)) is reported to be mediated by the enzyme

choline/ethanolamine phosphotransferase 1 (CEPT1). Increased levels of CEPT1 expression have been seen in the arterial intima of lower extremities of individuals with peripheral arterial disease and type 2 diabetes [38]. This residual increase in activity of CEPT1 may be subsequent to burn injury and associated with the increased incidence of cardiovascular disease and diabetes seen subsequent to non-severe burn injury in paediatric patients [6,8].

The conversion of PS (36:0) to PE (36:0) reported to be mediated by Phosphatidylserine Decarboxylase (PISD), an enzyme present in the inner mitochondrial membrane [39], whilst the pathways converting FA (16:1) to FA (16:2); FA (16:0) to FA (18:0) and FA (18:0) to FA (18:1) are reported to be mediated by the enzymes Fatty Acid Desaturase 2 (FADS2); Elongase of Very Long Chain Fatty Acids 6 (ELOVL6) and Stearoyl coenzyme A desaturase 1 (SCD1), respectively. There is limited literature on the increase in activity of these enzymes

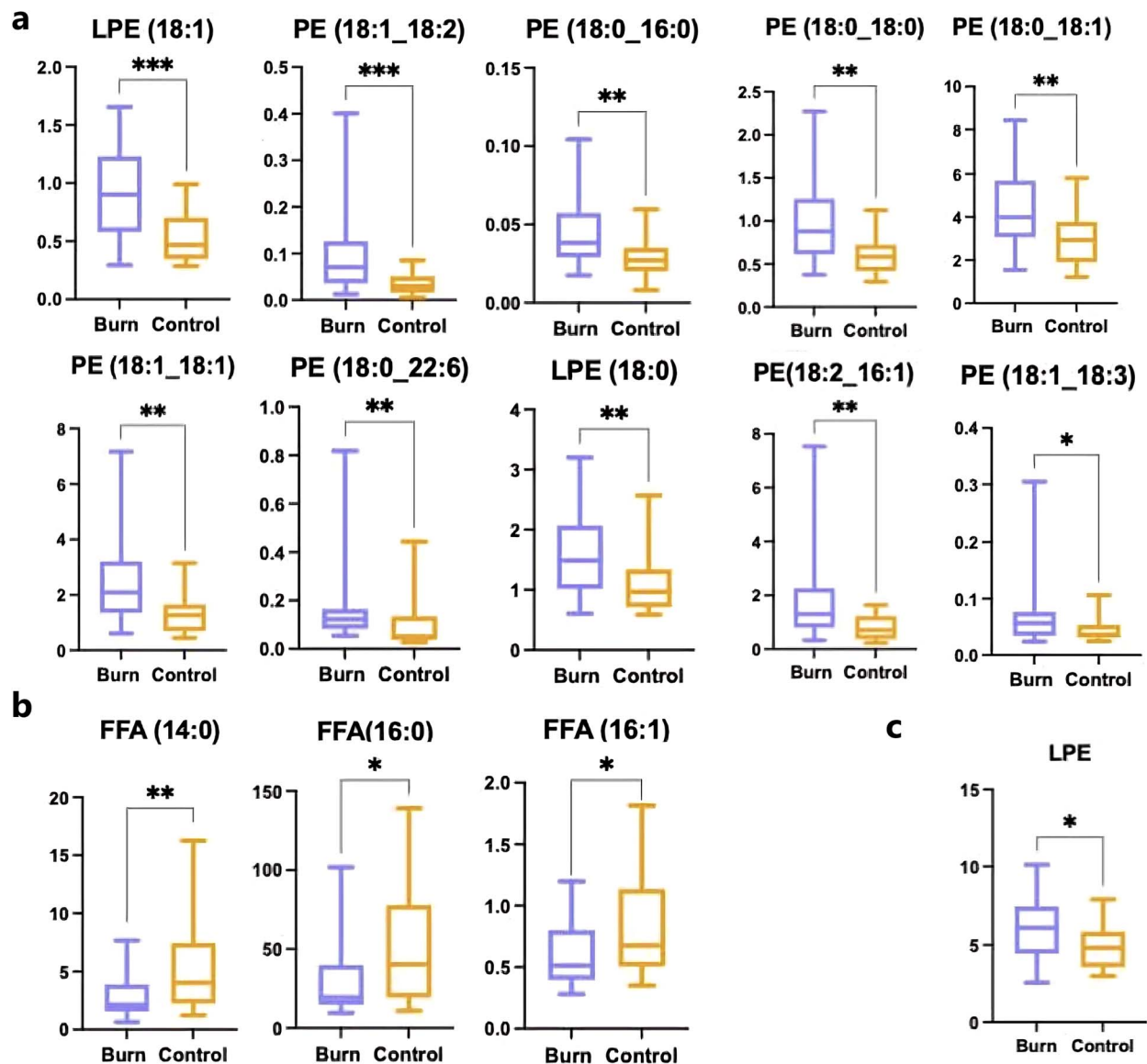


Figure 4. Boxplots of quantified metabolite concentrations, found to be significantly different between burn (purple) and controls (orange) (Mann–Whitney p -values * < 0.05 , ** < 0.01 , *** < 0.001). Lysophosphatidylethanolamine (LPE) and phosphatidylethanolamine (PE) lipid classes are significantly higher in samples from the burn cohort (a); free fatty acids (FFA) are significantly lower in the burn cohort compared to controls (b); Lysophosphatidylethanolamines (LPE) are significantly higher in the burn cohort compared to controls (c)

and pathways in response to acute injury or chronic inflammation and this requires further investigation to understand how this may be linked to burn trauma.

Our understanding of the involvement of lipids in chronic inflammation are continually improving and it may be that the changes in FFA and FA sidechain plasma concentrations observed in the burn injury group result from the non-resolution of inflammation. Previously, it has been suggested that chronic diseases such as CVD, obesity, cancer, IBD, RA are caused by the non-resolution of inflammation [40], and this may also be the reason individuals who experience a burn injury are more at risk of developing these diseases later in life.

It should be noted that this study was performed on a limited cohort, which consisted of a single timepoint collected following non-severe burn injury. We did not measure BMI in either cohort, and this may influence the findings. Importantly, a subset of the burn patients underwent surgical intervention for treatment of their burn. Therefore, the differences we observe between the two cohorts may reflect the impact of surgery and/or anaesthesia, rather than the burn injury itself. To delineate whether it is the injury or the treatment that is significant will require a further study using a surgical intervention cohort as a comparator. Furthermore, dietary factors were not recorded or controlled for in this study, although no participants in either group

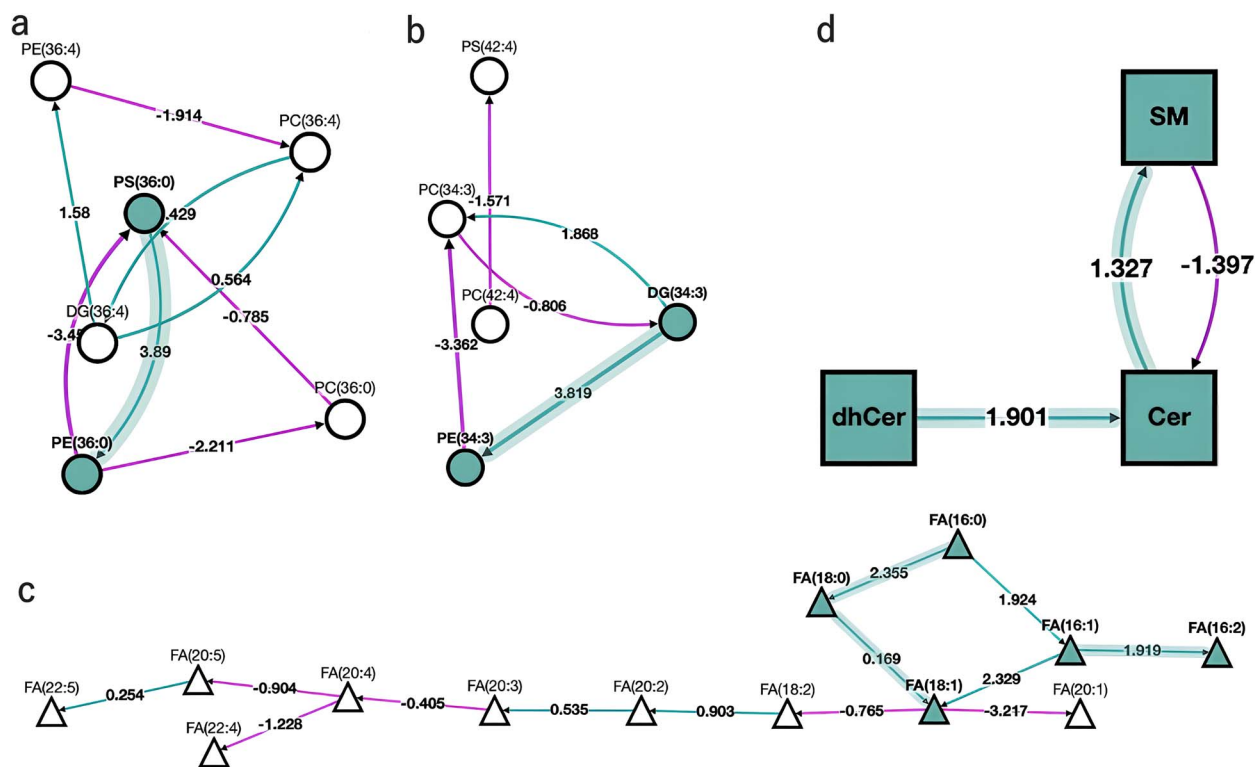


Figure 5. Visualisation of lipid interactions highlighting increased activity of (a) phosphatidylserine (PS (36:0)) to phosphatidylethanolamine (PE (36:0)) pathway, (b) diacylglyceride (DG (34:3)) to PE (34:3) pathway, and (c) fatty acid (FA (16:1)) to FA (16:2) pathway (d) Visualisation of lipid class interactions highlighting increased conversion of dihydroceramides (dhCer) to ceramides (CERs) to sphingomyelin (SM). Affected Lipid Class Pathways: green lines indicate increased activity, purple indicate decreased activity. Bold lines indicate a significant Z score

were on specific medically directed dietary interventions at time of sampling. Therefore, dietary factors could influence these findings. Further investigations using larger cohorts will be critical to validate the reported findings and would help to better understand the link between burn trauma and systemic changes in the lipidome. Nevertheless, this data provides additional evidence for sustained systemic impacts of burn trauma in children. There is increasing evidence from epidemiological, patient and animal studies that children suffer from poor long-term health after burn trauma [4,7,11,15]. Understanding how these changes are mediated and therefore how to intervene to improve long-term health increasingly appears to be a challenge facing those treating burns. The data suggests metabolic change, as observed by changes in lipid profiles, may be one aspect of this sustained change that warrants further investigation. It will be critical to investigate the mechanistic links between burn trauma and lipid changes, for example the addition of cellular immune function data and longitudinal sampling, which may provide an opportunity for clinical intervention to improve the health outcomes of infants following burn injury.

Conclusions

This study suggests there are residual and long-lasting differences in the plasma lipidome in paediatric patients who have previously experienced non-severe burn injury compared to non-injured controls. These may be related to the injury or

the treatment, and validation is essential to support these exploratory findings and remove the possibility for other lifestyle factors to be driving the observed differences. In order to further understand the effect of early life burn injury on long-term metabolism further work is needed to establish a greater understanding of pathways and processes, with larger sample sizes and longitudinal studies to validate these findings. Furthermore, as not all patients will develop secondary pathologies post-burn injury, further stratifying the population as they age may reveal mechanistic insights that lead from burn injury to negative health outcomes in later life. Work to understand the lipid profile has the potential to define the underlying mechanisms in play and may facilitate the prediction and identification of those who may be vulnerable to such change, helping to drive a tailored personalised treatment regime and allow the tracking of recovery over time at the physiological level.

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Supplementary material

Supplementary material is available at *Burns & Trauma* online.

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Availability of data and materials

Data is included within the manuscript.

Authors’ contributions

EK: Investigation, data acquisition, data curation, formal analysis, visualisation and manuscript preparation. MR: Methodology. EH: Conceptualisation, funding, formal analysis and manuscript preparation. JKN: Funding acquisition and manuscript preparation. MWF: Conceptualisation, funding acquisition and manuscript preparation. FMW: Conceptualisation, funding acquisition and manuscript preparation. LW: Conceptualisation, methodology, data curation, formal analysis, visualisation and manuscript preparation. NG: Conceptualisation, methodology, data acquisition, data curation, formal analysis, visualisation and manuscript preparation.

Ethics Approval

All samples were obtained with informed consent of a parent or guardian and the collection was conducted with ethical approval from the Child and Adolescent Health Service WA (approval numbers: 2015219EP; 1111EP; 768EP).

Consent for publication

Not applicable.

Conflict of interest

The authors declare no competing interests.

Availability of data and materials

Data for this manuscript is available at 10.6084/m9.figshare.23949921.

References

1. Valvis SM, Waithman J, Wood FM, Fear MW, Fear VS. The immune response to skin trauma is dependent on the Etiology of injury in a mouse model of burn and excision. *J Invest Dermatol*. 2015;135:2119–28.
2. Cassidy JT, Phillips M, Fatovich D, Duke J, Edgar D, Wood F. Developing a burn injury severity score (BISS): adding age and total body surface area burned to the injury severity score (ISS) improves mortality concordance. *Burns*. 2014;40:805–13.
3. Fear VS, Poh W-P, Valvis S, Waithman JC, Foley B, Wood FM, et al. Timing of excision after a non-severe burn has a significant impact on the subsequent immune response in a murine model. *Burns*. 2016;42:815–24.
4. Duke JM, Rea S, Boyd JH, Randall SM, Wood FM. Mortality after burn injury in children: a 33-year population-based study. *Pediatrics*. 2015;135:e903–10. <https://doi.org/10.1542/peds.2014-3140>.
5. Ryder C, Mackean T, Hunter K, Rogers K, Holland AJA, Ivers R. Burn injuries in hospitalized Australian children—an epidemiological profile. *J Burn Care Res*. 2021;42:381–9.
6. Duke JM, Randall SM, Fear MW, Boyd JH, Rea S, Wood FM. Long-term effects of Pediatric burns on the circulatory system. *Pediatrics*. 2015;136:e1323–30. <https://doi.org/10.1542/peds.2015-1945>.
7. Duke JM, Randall SM, Fear MW, Boyd JH, Rea S, Wood FM. Increased admissions for musculoskeletal diseases after burns sustained during childhood and adolescence. *Burns*. 2015;41:1674–82.
8. Duke JM, Randall SM, Fear MW, Boyd JH, O’Halloran E, Rea S, et al. Increased admissions for diabetes mellitus after burn. *Burns*. 2016;42:1734–9.
9. Stevenson AW, Randall SM, Boyd JH, Wood FM, Fear MW, Duke JM. Burn leads to long-term elevated admissions to hospital for gastrointestinal disease in a west Australian population based study. *Burns*. 2017;43:665–73.
10. Duke JM, Randall SM, Wood FM, Boyd JH, Fear MW. Burns and long-term infectious disease morbidity: a population-based study. *Burns*. 2017;43:273–81.
11. Jeschke MG, Gauglitz GG, Kulp GA, Finnerty CC, Williams FN, Kraft R, et al. Long-term Persistence of the pathophysiologic response to severe burn injury. *PLoS One*. 2011;6:e21245. <https://doi.org/10.1371/journal.pone.0021245>.
12. Nielson CB, Duethman NC, Howard JM, Moncure M, Wood JG. Burns: pathophysiology of systemic complications and current management. *J Burn Care Res*. 2017;38:e469–81. <https://doi.org/10.1097/BCR.0000000000000355>.
13. Kuznetsova TA, Andryukov BG, Besednova NN. Modern aspects of burn injury Immunopathogenesis and prognostic Immunobiochemical markers (mini-review). *BioTech (Basel)*. 2022;11:18. <https://doi.org/10.3390/biotech11020018>.
14. Johnson BZ, McAlister S, McGuire HM, Palanivelu V, Stevenson A, Richmond P, et al. Pediatric burn survivors have long-term immune dysfunction with diminished vaccine response. *Front Immunol*. 2020;11:1481. doi.org/10.3389/fimmu.2020.01481.
15. Begum S, Johnson BZ, Morillon A-C, Yang R, Bong SH, Whiley L, et al. Systemic long-term metabolic effects of acute non-severe paediatric burn injury. *Sci Rep*. 2022;12:13043–10. <https://doi.org/10.1038/s41598-022-16886-w>.
16. Capolupo L, Khven I, Lederer AR, Mazzeo L, Glousker G, Ho S, et al. Sphingolipids control dermal fibroblast heterogeneity. *Science*. 2022;376:eabh1623. <https://doi.org/10.1126/science.aabh1623>.

17. Meikle TG, Huynh K, Giles C, Meikle PJ. Clinical lipidomics: realizing the potential of lipid profiling. *J Lipid Res.* 2021;62:100127. <https://doi.org/10.1016/j.jlr.2021.100127>.
18. Zhang C, Wang K, Yang L, Liu R, Chu Y, Qin X, *et al.* Lipid metabolism in inflammation-related diseases. *Analyst.* 2018;143:4526–36.
19. Khubchandani A, Shaikh M, Sachde J, Suri M, Sanghani H, Gandhi P. Study of alterations in lipid profile after burn injury. *Indian Journal of Burns.* 2011;19:52–5.
20. Arneth B, Arneth R, Shams M. Metabolomics of type 1 and type 2 diabetes. *Int J Mol Sci.* 2019;20. <https://doi.org/10.3390/ijms20102467>.
21. Tabassum R, Rämö JT, Ripatti P, Koskela JT, Kurki M, Karjalainen J, *et al.* Genetic architecture of human plasma lipidome and its link to cardiovascular disease. *Nat Commun.* 2019;10:4329. <https://doi.org/10.1038/s41467-019-11954-8>.
22. Gray N, Lawler NG, Zeng AX, Ryan M, Bong SH, Boughton BA, *et al.* Diagnostic potential of the plasma Lipidome in infectious disease: application to acute SARS-CoV-2 infection. *Meta.* 2021;11:467. <https://doi.org/10.3390/metabo11070467>.
23. Adams KJ, Pratt B, Bose N, Dubois LG, St John-Williams L, Perrott KM, *et al.* Skyline for small molecules: a unifying software package for quantitative metabolomics. *J Proteome Res.* 2020;19:1447–58.
24. Luan H, Ji F, Chen Y, Cai Z. statTarget: a streamlined tool for signal drift correction and interpretations of quantitative mass spectrometry-based omics data. *Anal Chim Acta.* 2018;1036:66–72.
25. Gaud C, Sousa BC, Nguyen A, Fedorova M, Ni Z, O'Donnell VB, *et al.* BioPAN: a web-based tool to explore mammalian lipidome metabolic pathways on LIPID MAPS. *F1000Res.* 2021;10:4. <https://doi.org/10.12688/f1000research.28022.2>.
26. Chiurchiù V, Leuti A, Maccarrone M. Bioactive lipids and chronic inflammation: managing the fire within. *Front Immunol.* 2018;9. <https://doi.org/10.3389/fimmu.2018.00038>.
27. Freigang S. The regulation of inflammation by oxidized phospholipids. *Eur J Immunol.* 2016;46:1818–25.
28. Miao H, Chen L, Hao L, Zhang X, Chen Y, Zhihua R, *et al.* Stearic acid induces proinflammatory cytokine production partly through activation of lactate-HIF1 α pathway in chondrocytes. *Sci Rep.* 2015;5. <https://doi.org/10.1038/srep13092>.
29. Nambiar S, Tan DBA, Clynick B, Bong SH, Rawlinson C, Gummer J, *et al.* Untargeted metabolomics of human plasma reveal lipid markers unique to chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis. *Proteomics Clin Appl.* 2021;15:e2000039. <https://doi.org/10.1002/prca.202000039>.
30. Chamani S, Bianconi V, Tasbandi A, Pirro M, Barreto GE, Jamialahmadi T, *et al.* Resolution of inflammation in neurodegenerative diseases: the role of Resolvins. *Mediat Inflamm.* 2020;2020:3267172. <https://doi.org/10.1155/2020/3267172>.
31. Korbecki J, Bajdak-Rusinek K. The effect of palmitic acid on inflammatory response in macrophages: an overview of molecular mechanisms. *Inflamm Res.* 2019;68:915–32.
32. Akazawa Y, Morisaki T, Fukuda H, Norimatsu K, Shiota J, Hashiguchi K, *et al.* Significance of serum palmitoleic acid levels in inflammatory bowel disease. *Sci Rep.* 2021;11:16260. <https://doi.org/10.1038/s41598-021-95923-6>.
33. Alonso-Castro A, Serrano Vega R, Gutiérrez S, Isiordia-Espinoza M, Solorio C. Myristic acid reduces skin inflammation and nociception. *J Food Biochem.* 2022;46:e14013. <https://doi.org/10.1111/jfbc.14013>.
34. Ohi K, Ursini G, Li M, Shin JH, Ye T, Chen Q, *et al.* DEGS2 polymorphism associated with cognition in schizophrenia is associated with gene expression in brain. *Transl Psychiatry.* 2015;5:e550.
35. Rozhkova AV, Zinovyeva MV, Sass AV, Zborovskaya IB, Limborska SA, Dergunova LV. Expression of sphingomyelin synthase 1 (SGMS1) gene varies in human lung and esophagus cancer. *Mol Biol.* 2014;48:340–6.
36. Maceyka M, Spiegel S. Sphingolipid metabolites in inflammatory disease. *Nature.* 2014;510:58–67.
37. Farina JA, Rosique MJ, Rosique RG. Curbing inflammation in burn patients. *Int J Inflamm.* 2013;2013:715645. <https://doi.org/10.1155/2013/715645>.
38. Zayed MA, Jin X, Yang C, Belaygorod L, Engel C, Desai K, *et al.* CEPT1-mediated Phospholipogenesis regulates endothelial cell function and ischemia-induced angiogenesis through PPAR α . *Diabetes.* 2021;70:549–61.
39. Schuiki I, Daum G. Phosphatidylserine decarboxylases, key enzymes of lipid metabolism. *IUBMB Life.* 2009;61:151–62.
40. Nathan C, Ding A. Nonresolving inflammation. *Cell.* 2010;140:871–82.