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UV and visible light exposure to hair leads to widespread changes in the hair lipidome

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Abstract

Objective: Scalp hair is among the most exposed parts of the human body, yet the impact of visible and UV light on hair lipids, an important structural component of hair, is poorly researched. We have used lipidomics, a broad-based approach to measure lipids in samples, which has hitherto not been applied to UV-exposed hair in the published literature, and could allow for a wider understanding of how UV light impacts on specific hair lipids.

Methods: Mixed blonde Caucasian hair switches were divided into two groups of five, with half of the hair switches exposed to UV and visible light mimicking normal daytime exposure and half left unexposed. LC–MS lipidomics was used to profile the lipids in the hair samples.

Results: A total of 791 lipids and 32 lipid classes with tentative identifications were detected in the hair samples. Nineteen lipid classes and 397 lipids differed between UV-treated and non-treated hair. The main lipid classes that differed were vitamin A fatty acid esters, sterol esters, several ceramides, mono-, di- and triglycerides, phosphatidylethanolamines (all decreased in UV-exposed hair) and bismonoacylglycerolphosphates, acylcarnitines and acylglycines (all increased in UV-exposed hair). Most detected lipids were decreased in UV-exposed hair, supporting earlier work that has found that UV exposure causes oxidation of lipids which would result in a decrease in most lipid classes.

Conclusion: Light exposure to hair has a widespread impact on the hair lipidome. This study also adds to the emerging literature on the hair lipidome, broadening the range of lipid classes reported in hair.

K E Y W O R D S
chemical analysis, hair treatment, Lipidomics, mass spectrometry, UV light exposure

Résumé

Objectif: Le cuir chevelu est l'une des parties les plus exposées de l'organisme. Cependant, l'impact de la lumière visible et des UV sur les lipides capillaires,

un composant structurel important des cheveux, reste mal étudié. Nous avons utilisé la lipidomique, une approche large pour mesurer les lipides présents dans les échantillons de cheveux, qui n'a jusqu'ici pas été appliquée aux cheveux exposés aux UV dans la littérature publiée. Cette approche pourrait permettre de mieux comprendre l'impact de la lumière UV sur des lipides spécifiques des cheveux.

Méthodes: Les mèches de cheveux caucasiens blonds mélangés ont été divisées en deux groupes de cinq, la moitié des mèches de cheveux étant exposées aux UV et à une lumière visible imitant l'exposition diurne normale tandis que l'autre moitié est restée non exposée. Le profil lipidique des échantillons de cheveux a été établi grâce à la lipidomique de la LC-MS.

Résultats: Au total, 791 lipides et 32 classes de lipides avec des identifications provisoires ont été détectés dans les échantillons de cheveux. Entre les cheveux traités par UV et les cheveux non traités, dix-neuf classes de lipides et 397 lipides se sont avérés différents. Les principales classes de lipides qui différaient étaient les esters d'acides gras de la vitamine A, les esters de stérols, plusieurs céramides, les monoglycérides, diglycérides et triglycérides, les phosphatidyléthanolamines (tous diminués dans les cheveux exposés aux UV) et les bismonoacylglycérolphosphates, acylcarnitines et acylglycines (tous augmentés dans les cheveux exposés aux UV). La plupart des lipides détectés dans les cheveux exposés aux UV n'étaient présents qu'à taux réduit, soit un résultat cohérent avec une étude antérieure ayant montré que l'exposition aux UV provoque l'oxydation des lipides, ce qui entraînerait une diminution de la plupart des classes de lipides.

Conclusion: L'exposition des cheveux à la lumière entraîne un impact généralisé sur leur lipidome. Cette étude vient également compléter la littérature émergente sur le lipidome capillaire, élargissant ainsi la gamme de classes lipidiques rapportées dans les cheveux.

INTRODUCTION

Scalp hair is one of the most exposed parts of the human body, with UV radiation from sunlight having a major impact on hair quality, including colour and brittleness. Hair is a complex biological structure, largely made up of keratinized proteins within three major substructures: the cortex, medulla and cuticle. Between the cortex and cuticle, cells are cell membrane complexes, which make up 4%–7% of hair weight and are lipid-rich, hydrophobic structures within structures largely composed of cross-linked protein (Robbins, 2009). Cell membrane lipids are sometimes referred to as internal lipids and contribute to physiomechanical properties of hair, contributing to hair integrity by acting as a 'glue' between protein structures via interaction between lipids and the hydrophobic domains of globular proteins within the cuticle and cortex cells (Robbins, 2009; Marsh, Whitaker, et al., 2018b). In addition to cell membrane complex lipids, sebaceous lipids, derived from sebum and sweat, contribute to hair waterproofing, lubrication and sheen, while lipids from cytoplasmic and nuclear membranes are also present. Composition of lipids in different hair substructures has been found to vary, with the type of lipids being important for function (Takahashi & Yoshida, 2014). Overall, lipids make up 1%–9% of total hair weight. Advanced analytical technologies are helping to further our understanding of hair lipids, including the presence of calcium C16:0 and C18:0 fatty acid deposits along the hair shaft (Marsh, Mamak, et al., 2018a), the specific location of externally applied lipophilic bioactive compounds to the cuticle and medulla (Marsh et al., 2019) and loss of lipids in the cuticle with hair colour loss (Oliver et al., 2019), all which may inform greater understanding of how different lipids contribute to hair structure.

Lipids in the cell membrane complexes are degraded by visible light and UVA and UVB, with the structural lipid 18-methyl eicosanoic acid (18-MEA), cholesterol,

cholesterol sulphate and oleic and palmitoleic acids noted as being vulnerable to photo-oxidative reactions (Hoting & Zimmermann, 1997). Other treatments also impact on lipid stability and oxidation, with hair that has been oxidized by hair dye being more vulnerable to lipid oxidation than chemically unaltered hair (Hoting & Zimmermann, 1997; Grosvenor et al., 2016). Based on ToF-SIMS (a mass spectral imaging method), it was determined that more than 90% of 18-MEA was removed by UV exposure equivalent to 3 months of summer sun (Habe et al., 2011). Lipids may also explain ethnic differences in susceptibility of hair to UV-oxidation (Ji et al., 2013).

Lipidomics, a branch of metabolomics, uses analytical tools to measure a wide range of lipids (Wei et al., 2019). Lipidomic analyses measure lipid classes such as triglycerides, cholesterol esters and different phospholipids, along with individual lipids within these classes. Lipid classes are based on chemical structures that form 'backbones' for fatty acid attachment, which can provide important biological information beyond fatty acids alone. These backbones often indicate different stages of lipid transport or biofunction, including structural roles. Depending on the definition and classification depth, there are six major lipid categories and more than 50 lipid classes and subclasses found in human plasma (Quehenberger et al., 2010). The number of lipids detected in a sample is dependent on not just the sample, but also the extraction method, analytical instrumentation, data analysis and reference database. Surprisingly, there has been little reported application of lipidomics to understanding hair lipids in the published literature, with two studies using liquid chromatography–mass spectrometry to profile hair in relation to greying and drug use (Kim et al., 2020; Wang et al., 2020), and two studies using fingerprinting mass spectrometry methods to study the impact of UV exposure (Cornellison et al., 2011; Habe et al., 2011). Other studies have used metabolomic methods that measure both polar and non-polar metabolites from the same extraction to measure hair composition (Jang et al., 2019). While providing information about polar molecules, the extraction methods (often a mixture of water with either acetonitrile or methanol) usually do not give the same depth of information about lipids as protocols designed to extract and measure lipids. Given the rapid uptake in the use of lipidomics in other areas of biological science, lipidomics could be useful for building on the existing literature on how UV light influences hair lipids.

In this pilot study, we have used LC–MS lipidomics to detect changes to the hair lipidome on exposure to UV light and to test how lipidomics might be useful for advancing understanding of hair biology.

METHODS

Hair samples

Two gram, 6-inch long, tresses of blonde, untreated hair (i.e. no chemical treatment) was purchased from International Hair Importers & Products Inc. (Glendale, NY, US). Five replicate tresses were used for the non-UV- and UV-treated groups. All tresses were washed with a non-conditioning shampoo (0.1 ml/g hair) before UV treatment, rinsed in water and dried before UV exposure.

UV treatment

Tresses in the UV treatment group were placed in UV chambers (Atlas Xenotest solar simulator Ci3000+) under the following condition: $0.37\,\mathrm{W/m^2}$ at 340 nm and $0.78\,\mathrm{W/m^2}$ at 420 nm, 36°C, 85% relative humidity. Tresses were irradiated for 10 h and physically rotated so that each side of the tress received half of the visible light and UV exposure. This exposure procedure was repeated six times. These exposure settings were chosen to simulate $6\times10\,\mathrm{h}$ of average light exposure in Miami, Florida, USA, during summer sunlight hours. Hair tresses were stored in the dark at room temperature.

Sample preparation

Hair tresses were rinsed in water, dried at room temperature and snipped into small (<1 mm length) pieces with care taken to avoid cross contamination due to static electricity and gloves worn to avoid cross contamination with skin lipids. The lipid extraction method was adapted from a method for plasma (Huynh et al., 2019) with an overnight and additional extraction step to allow hair swelling and better penetration of solvent into the hair matrix. 10 mg of the finely cut hair was weighed into a 2 ml microcentrifuge tube, and 500 μl of butanol: methanol (1:1 v/v) and 5 μl of Splash Lipidomix internal standard mixture (Avanti Polar Lipids, Alabaster, AL, USA) were added. The sample was shaken in a bead shaker (Qiagen TissueLyser II, Hilden, Germany) at 30 Hz for 5 min and then sonicated for 1 h (Elmasonic 60 H, Elma, Singen, Germany). The samples were then kept in the dark at room temperature for 16 h. The samples were then centrifuged at $12,000\times g$ for 10 min at 20°C, and the supernatant removed into a 1.5 ml amber chromatography vial. A further 500 μl of butanol: methanol was added to the hair sample, and the shaking, sonication

and centrifugation steps were repeated as above, without the 16 h wait time. The supernatant was pooled with the first extract and the vial capped. Extraction blanks were included alongside the sample extractions, and pooled quality control (QC) samples were made from aliquoting a portion of each sample extract. QC samples were run to prime the column and every 10 injections for normalization. Samples were injected in randomized order.

## LC–MS lipidomics analysis

Detection of lipids was carried out using a Shimadzu LCMS 9030 LC-qTOF mass spectrometer. Separation was carried out Waters Acquity CSH C18 column (1.7 µm particle size, 2.1 ×100 mm ID) at 65°C and eluted over a 17 min gradient with a flow rate of 400 µl min^{-1}. Mobile phase A was water:acetonitrile:isopropanol (50:30:20) with 20 mM ammonium formate, and mobile phase B was water:acetonitrile:isopropanol (1:9:90) with 20 mM ammonium formate. All solvents were LC–MS grade (Optima LCMS grade, Thermo Fisher Scientific, Auckland, New Zealand). The gradient elution programme was as follows: 10%–45% B (0–2.7 min), 45%–53% B (2.7–2.8 min), 53%–65% B (2.8–9 min), 65%–89% B (9–9.1 min), 89%–92% B (9.1–11 min), 92%–100% B (11–11.1 min), held at 100% B (11.1–13.9 min), 100%–10% B (13.9–14 min), held at 10% B (14–17 min) (Su, et al. 2019). The autosampler was held at 20°C, and 2 µl of extract was injected onto the column. Positive electrospray ionization mode was used, and mass spectral data acquired in 'full scan' mode between m/z 50 and 1200, and in data independent acquisition MS/MS mode across the same range in 20 m/z windows to acquire fragmentation data on all lipids, with a collision energy ramp from 6 to 23 eV. Loop time for the MS method was 0.5 s.

Data were converted into mzML format and processed using MS DIAL. Data were normalized based on the LOWESS algorithm in MS DIAL and exported for manual curation (checking for duplicate identifications, merging adducts with the same feature ID, data quality checking) before multivariate statistical analyses using SIMCA 16 (Umetrics, Umeå, Sweden) and hierarchical cluster analysis and visualization using Metaboanalyst (Chong et al., 2019) and SIMCA. Matching against the Lipidmaps database (Sud et al., 2007) was done with both processing tools, and lipid classes and individual lipids were analysed separately. Statistical analyses were principal components analysis (PCA) and orthogonal projection to latent structures discriminant analysis (OPLS-DA). These multivariate data analysis tools allow visualization of the data variation where each sample data are made from hundreds of individual lipids or many lipid classes. Differences between UV-treated and non-treated groups for individual lipids and lipid classes were determined using two-tailed t-tests. Statistical analyses were done only for lipids with MS2 level identification, where identification is based on high resolution mass of the intact lipid adduct ion, and its fragmentation pattern. These lipids were grouped and analysed based on lipid classes, individual lipids and on lipids grouped by chain length and degree of unsaturation. Potential false positive findings were corrected for by removing 5% of the variables with $p < 0.05$ with the highest p-values. For example, with 791 lipids detected with MS2 level identification, of the 437 variables that differed with $p < 0.05$, the 40 with the highest p-value below 0.05 were removed.
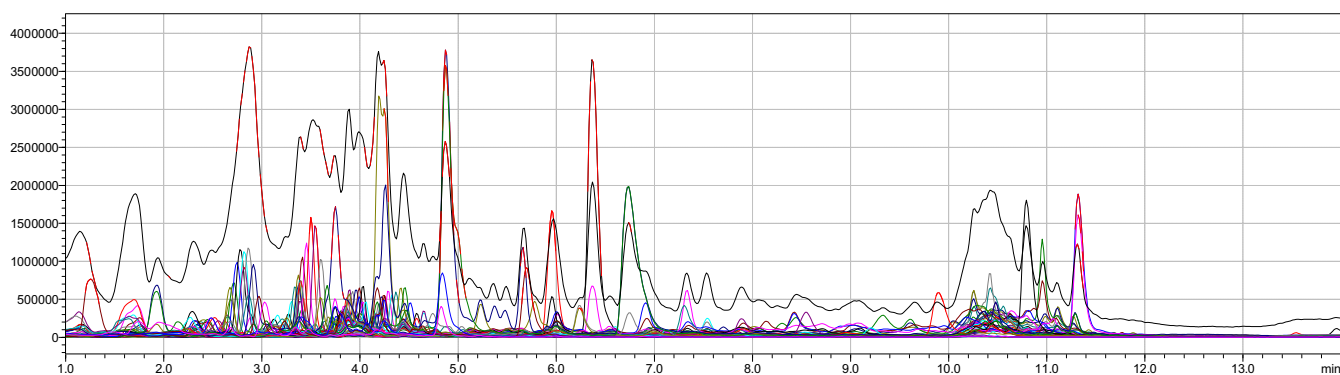
FIGURE 1 Representative LC–MS/MS chromatogram of a hair lipid extract. Peaks eluting during the first 7 min of the chromatogram are generally polar lipids including phospholipids, while those eluting towards the end of the chromatograms are di- and triglycerides. Most of the lipids extracted from hair are polar lipids. The y-axis is mass spectrometer intensity, and x-axis is chromatographic retention time (min). The different peak traces represent the total ion count and fragmentation intensities at different molecular mass windows. [Colour figure can be viewed at wileyonlinelibrary.com]
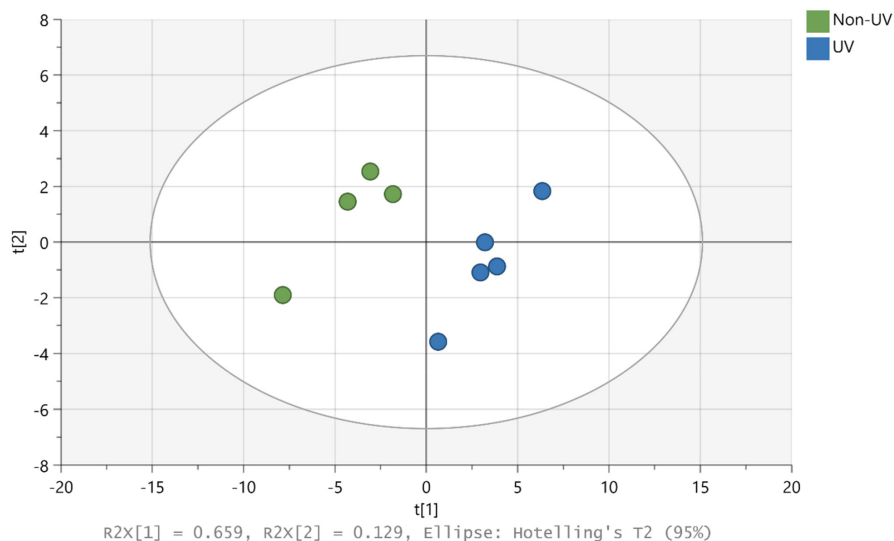
FIGURE 2 Principal components analysis (PCA) scores plot of lipid class data. PCA analysis is unsupervised and the clear difference between UV-exposed and non-UV-exposed hair is indicative of the substantial impact of UV exposure on hair. PC1 accounts for 66% of overall variation, PC2 accounts for 13%. [Colour figure can be viewed at wileyonlinelibrary. com]
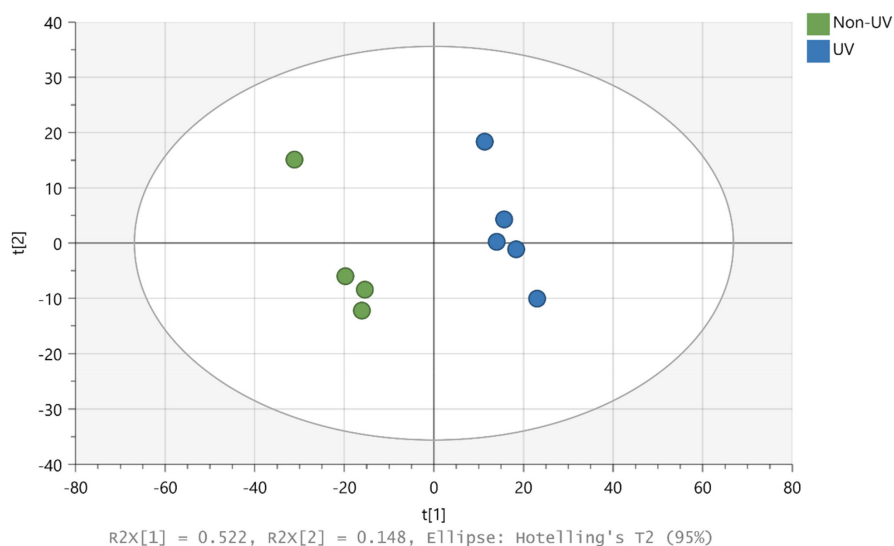
FIGURE 3 Principal components analysis (PCA) scores plot of individual identified lipid data. As for the lipid class data, modelling using the individual lipid data demonstrates the impact of UV exposure on hair lipids. PC1 accounts for 52% of overall variation while PC2 accounts for 15%. [Colour figure can be viewed at wileyonlinelibrary.com]

RESULTS

Method development

Repeated extractions using two different extraction mixes commonly used for lipidomics (butanol: methanol (1:1), chloroform: methanol (1:1) suggested an initial yield of <30% of all lipids from a single extraction. By including the overnight step to allow greater swelling of the hair fibres, and a second extraction, lipid recovery based on overall signal intensity was >80%.

Hair lipidomics

A total of 3521 features were detected after subtraction of the extraction blank samples. Of these, 791 lipids were identified based on their molecular ion and subsequent MS2 fragmentation matching against the Lipidmaps database. These compounds represented 32 different lipid classes (Table S1). Lipid classes were distributed throughout the chromatogram (Figure 1), with the large majority of detected lipids eluting during the first half of the chromatogram, indicating that there is a high proportion of polar lipids relative to non-polar lipids including triglycerides (eluting around 11 min). It is important to note that in mass spectrometry, different types of molecules will have different ionization efficiencies and therefore very different detector responses. In practice, this means that it is not possible to state that a particular lipid or lipid class is quantitatively higher than another lipid class without the use of a standard curve made from appropriate reference compounds. Relative quantitation within lipid classes and between samples for the same lipid class is valid. One sample was removed (non-UV-treated) as an outlier during initial data evaluation using PCA, due to substantially lower signal intensity compared with the other samples.
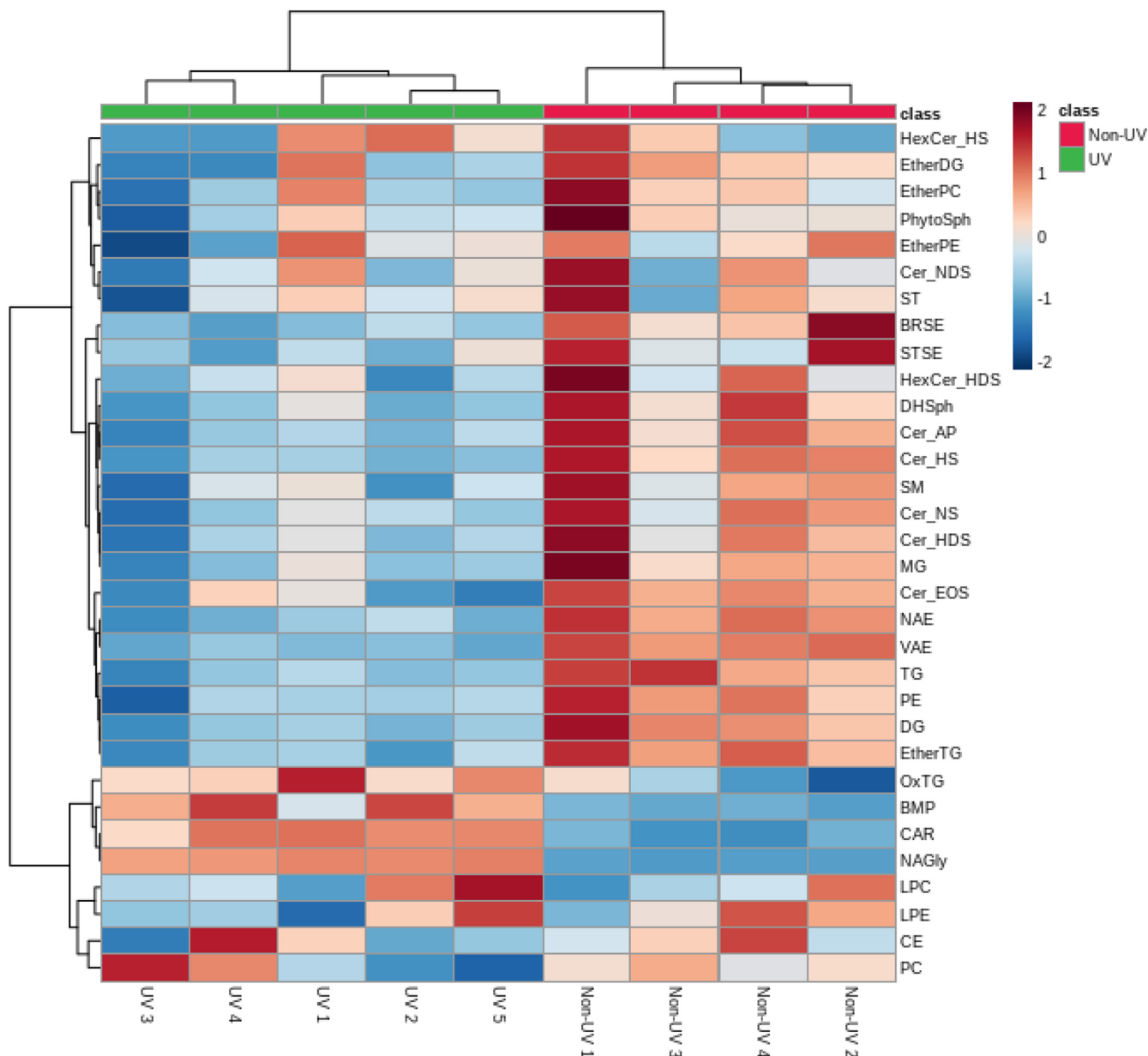
FIGURE 4 Hierarchical clustering heatmap of all detected lipid classes for UV-exposed and non-UV-exposed hair. Data have been scaled by mean centring. Refer to Table S1 for full lipid class names. [Colour figure can be viewed at wileyonlinelibrary.com]

Comparison between UV- and non-UV-treated hair

Lipidomics data were analysed separately as lipid classes (32 variables) and individual lipids (791 variables). UV-compared with non-UV-treated hair could be separated using principal components analysis along PC1 (Figures 2 and 3) for both lipid class and individual lipid data. For lipid classes, PC1 explained 66% of total variation, and for individual identified lipids, PC1 explained 52%. This suggests that the impact of UV treatment on hair lipids is substantial as the difference is clear without the need for supervised analysis. Cluster analysis of lipid classes sorted UV- and non-UV-treated samples correctly, with

some heterogeneity in the impact on lipid classes in individual samples (Figure 4), although effects on the most strongly impacted lipids was consistent between samples (Figure 5). Both PCA and hierarchical cluster analysis are unsupervised multivariate analysis methods, meaning that they model variation based on the data irrespective of any predefined groups. These results show that the major source of variation in hair lipids is the UV treatment. OPLS-DA, in contrast to PCA and hierarchical clustering, is a supervised multivariate modelling method, which models the difference between predefined groups, and in this study enables identification of the most important lipids for defining a difference between UV- and non-UV-treated hair from the large number of lipids detected.
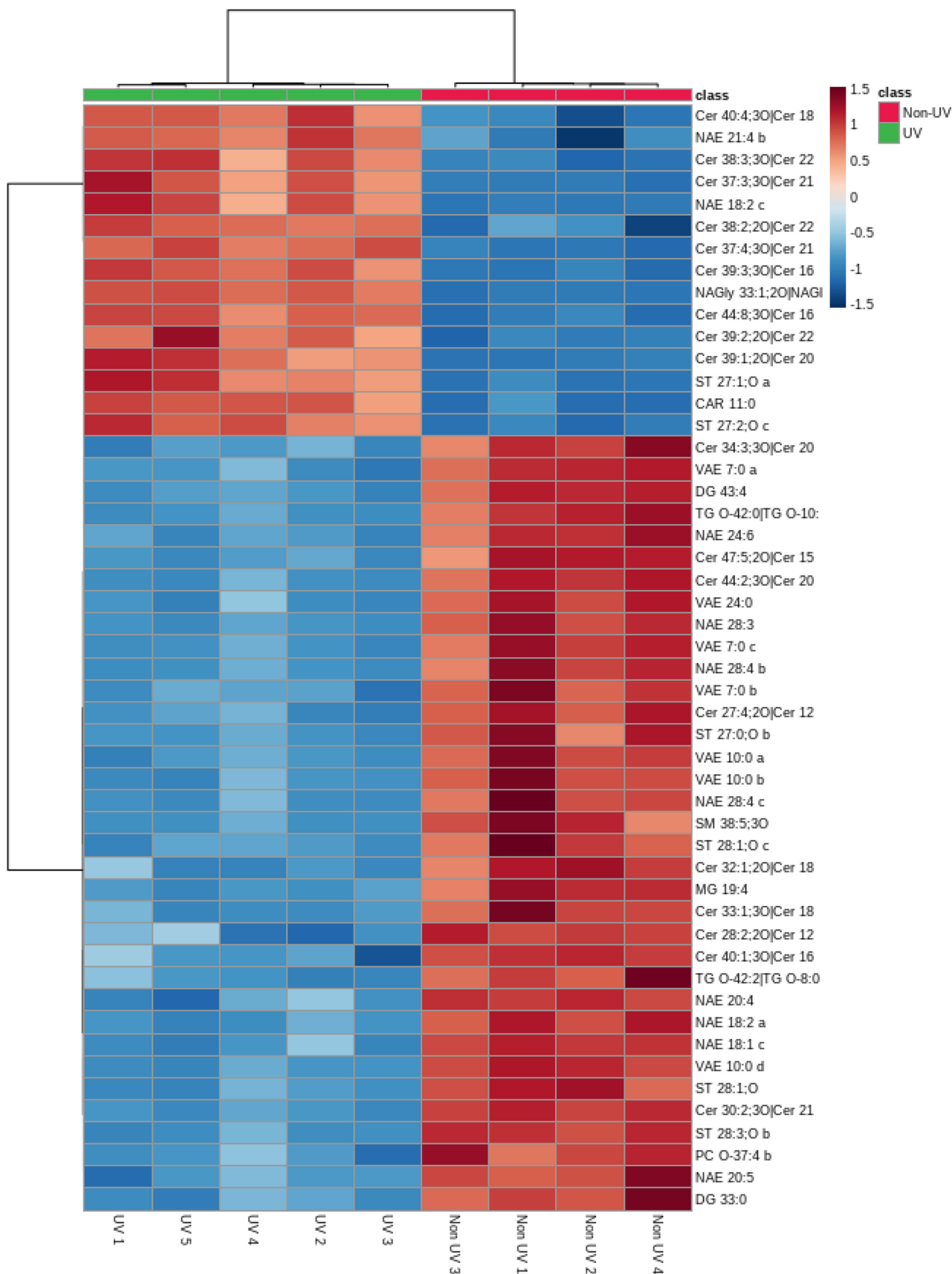
FIGURE 5 Hierarchical clustering heat map of the 50 most significantly different lipids between hair exposed to UV light or not. Data have been scaled by mean centring. Refer to Table S1 for full lipid names. [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Lipid classes that differed between UV-exposed and non-UV-treated hair

Lipid class	UV-exposed hair	Non-UV-exposed hair	Fold change	*p*-Value
Sterol ester	3356 ± 256	5052 ± 820	1.51	0.0030
Acylcarnitine	2158 ± 324	393 ± 188	0.18	0.0000
Ceramide alpha-hydroxy fatty acid-phytospingosine	139 251 ± 10 811	186 681 ± 19 416	1.34	0.0023
Ceramide esterified omega-hydroxy fatty acid–sphingosine	17 975 ± 1773	21 533 ± 805	1.20	0.0078
Ceramide hydroxy fatty acid–dihydrosphingosine	650 344 ± 42 348	769 940 ± 65 757	1.18	0.0127
Ceramide hydroxy fatty acid–sphingosine	389 184 ± 20 463	532 982 ± 47 901	1.37	0.0005
Ceramide non-hydroxyfatty acid–sphingosine	1 720 000 ± 56 041	1 870 000 ± 78 099	1.09	0.0112
Diacylglycerol	230 279 ± 13 289	316 483 ± 26 952	1.37	0.0004
Sphinganine	2447 ± 480	4227 ± 902	1.73	0.0065
Ether-linked triacylglycerol	191 416 ± 14 180	254 088 ± 16 319	1.33	0.0005
Monoacylglycerol	56 313 ± 3406	67 270 ± 5554	1.19	0.0080
N-acyl ethanolamine	712 440 ± 21 574	830 945 ± 23 795	1.17	0.0001
N-acyl glycine	14 695 ± 473	5408 ± 165	0.37	0.0000
Oxidized triglyceride	66 890 ± 5534	53 674 ± 7379	0.80	0.0177
Phosphatidylethanolamine	23 805 ± 1330	27 965 ± 1305	1.17	0.0022
Sphingomyelin	408 777 ± 14 667	437 932 ± 15 983	1.07	0.0247
Triacylglycerol	449 221 ± 29 568	608 821 ± 47 135	1.36	0.0004
Vitamin A fatty acid ester	119 978 ± 5190	186 925 ± 8708	1.56	0.0000

Note: Data are means ± standard deviation of normalised LC-MS peak areas. Fold change is the non-UV exposed mean / UV-exposed mean, with values <1 indicating higher concentrations in UV-exposed, and values >1 indicating higher concentrations in non-UV exposed hair.

Supervised modelling methods are prone to 'overfitting' where an apparently good separation between groups is obtained, but is not robust. To interpret OPLS-DA models, two model metrics are used: R2X, which describes whether the groups can be differentiated based on the data on a scale of 0–1; and Q2, which describes the robustness of the model cross-validation on a scale of −1 to 1. While interpretation of these metrics depends on the context of the study and data, generally an R2X > 0.5 is considered good for describing the difference between two groups, and a Q2 > 0.5 indicates reasonable model robustness. OPLS-DA modelling of lipid classes resulted in an R2X of 0.85 with a Q2 of 0.76. Lipid classes that differed between UV exposures included N-acyl glycines, vitamin A fatty acid esters, acylcarnitines, N-acyl ethanolamines and triglycerides (Table 1), with 19 of the 32 lipid classes detected differing between exposures. For individual lipids, the OPLS-DA model resulted in an R2X of 0.96 and a Q2 of 0.91, indicating a both a strongly predictive and robust model. 397 lipids differed between UV- and non-UV exposures. Of these, 325 were higher in non-UV-exposed hair and 71 higher in UV-exposed hair. To simplify data interpretation, further analysis was only based on the 100 most important lipids for the OPLS-DA model, based on the variable importance for the projection (VIP) score (Table S2). Based on a comparison of average lipid chain length and number of double bonds, UV exposure did not lead to a difference due to lipid chain length or double bonds for these 100 lipids.

Identification of oxidized lipids is complex due to the wide variety of potential redox products from each individual lipid, especially from polyunsaturated lipids (Ni et al., 2019). Oxidized lipids generally have a shorter retention time in reversed-phase chromatography compared with their parent lipid (Ni et al., 2019). Using this change in chemistry, we analysed all detected peaks (identified and unidentified) and found that there was a lower proportion of features that were higher in non-UV-exposed hair between 1–2 min (0.39) compared with lipids that eluted after 2 min (Table S2). This further supports that UV exposure caused lipid oxidation even if the exact identification of the oxidation products was not possible.

DISCUSSION

Lipidomics is a broad-based analytical approach that aims to detect as wide a range of lipids as possible. The untargeted method used here profiles the lipid

composition without any specific focus on particular lipids or lipid classes. This approach to lipidomics is not quantitative but does allow relative quantification of the same lipids between samples. This limits direct comparison with earlier literature but has the advantage that understanding the impact of UV exposure on hair lipids is not limited to predefined lipids. Broadly, hair lipids have been classified into eight groups: fatty acids including 18-MEA, wax esters, hydrocarbons, squalene, cholesterol, cholesterol sulphate, ceramides and triglycerides (Robbins, 2009). In our lipidomics method, we do not detect free fatty acids (including 18-MEA) nor wax esters and free-hydrocarbons as they are poorly resolved with LC–MS, and we did not detect squalene. Although detecting 32 lipid classes appears to suggest we have detected many more lipids than earlier reported, most of the detected lipid classes fall within the broad categories previously reported—for example, sphingolipids are building blocks of ceramides, and we have detected several classes of ceramide. Similarly, mono-, di- and oxidized triglycerides can be considered to fall under the earlier reported broad category of 'triglycerides'. Of lipid classes not usually reported in hair, acylcarnitines have been detected in hair using metabolomics (Eisenbeiss et al., 2019) and phospholipids using lipidomics (Kim et al., 2020), while we did not find any earlier reports of vitamin A fatty acid esters in hair.

UV exposure had a major impact on the lipidome of hair, with more than half of the lipid classes detected changing after UV exposure. This supports earlier work that has found that UV exposure has an important impact on hair lipids in general (Trüeb, 2015a, 2015b). Due to the large number of individual lipids that differed between UV-exposed and non-UV-exposed, it is not feasible to discuss all differences detected. We hypothesized that there may be a difference between lipids between exposures related to substituted fatty acid chain length and degree of unsaturation. However, comparing these two factors in the 100 features that differed the most between light exposures suggests that fatty acid chain length and unsaturation did not explain the difference. The major difference between light exposed and non-exposed was due to difference in lipid classes. The main lipid classes that differed were vitamin A fatty acid esters, sterol esters, several ceramides, mono-, di- and triglycerides, phosphatidylethanolamines (all decreased in UV-exposed hair) and bismonoacylglycerolphosphates, acylcarnitines and acylglycines (all increased in UV-exposed hair). These represent a broad range of lipid classes and give a window into some of the lipids present in the hair follicle and incorporated into the hair shaft that are susceptible to UV exposure. Hair samples had been washed with shampoo and thoroughly rinsed with water prior to UV exposure

and after UV exposure before preparation for lipidomics analysis, so while the shampoo treatment should have removed sebaceous lipids, some of the detected lipids may be of sebaceous origin.

Of the lipid classes that were impacted by UV exposure, vitamin A fatty acid esters is notable due to the well described role of retinoids (vitamin A and vitamin A derivatives) in the hair follicle for development and maintenance of hair and sebaceous glands (Everts, 2012, Suo, VanBuren et al. 2021). The majority of retinol that enters is primarily converted to retinyl esters, as too much free retinol is potentially toxic to the hair follicle (Everts, 2012). Retinyl esters are the main dietary source of retinoids, although they can also be derived from β-carotene, α-carotene and β-cryptoxanthin (Everts and Akuailou 2021).

The role of retinol or retinyl esters in the hair shaft does not appear to have been investigated, although the role of retinoids in animal models of UV-induced skin cancers has been investigated, with mixed results showing both protection against and induction of cutaneous squamous cell carcinomas (Everts and Akuailou 2021), suggested to be due to differences in timing of topical application and differences in animal models. Retinoic acid synthesis appears to occur widely within the hair follicle (Everts, 2012), explaining why many retinyl esters were detected in our study. Other work has found that retinyl esters were dose dependently depleted in hairless mice exposed to UVB radiation, in contrast to retinol, which was partially protected from oxidation, possibly by retinol-binding protein (CRBP-1) (Sorg, Tran et al. 1999). Studies on retinyl-palmitate, the main retinyl ester in skin, found that it was readily decomposed by exposure to both UVA and UVB radiation in cosmetic formulations (Tolleson, Cherng et al. 2005).

Sterol esters were identified as being higher in non-UV-treated hair. Matching against the LipidMaps database suggests that these were plant sterol esters, which is supported by the low signal to noise ratio of these peaks (around 3–10). Although not necessarily expected in hair, and with low bioavailability, plant sterols from the diet can be incorporated into human tissues and biofluids (Vanmierlo, Popp et al. 2011).

Concentrations of several ceramide-related lipid species (ceramide alpha-hydroxy fatty acid phytosphingosines, ceramide esterified omega-hydroxy fatty acid sphingosine, ceramide hydroxy fatty acid–sphingosine, ceramide hydroxy fatty acid-dihydrosphingosine, and ceramide non-hydroxy fatty acid sphingosine, sphinganine and sphingomyelin) were decreased after UV exposure. Ceramides are a common lipid in hair and have been associated with improved skin and hair health through their function in barrier and water holding functions (Borodzicz, Rudnicka et al. 2016). They have been suggested to play a

role in signal transduction and cell regulation during keratinization of hair (Masukawa, Tsujimura et al. 2006). Some studies have suggested that ceramides may protect against UV damage in skin and their binding in skin is impacted by UV exposure (Takagi, Nakagawa et al. 2004), although whether this is the case in hair has not been determined. Given the structural function in hair, a reduction with UV exposure could be interpreted as a mechanism behind increased hair brittleness with UV exposure.

Mono-, di- and triglycerides are commonly found in human tissues and their presence is not unexpected in hair. There were reduced concentrations in UV-exposed hair, likely through oxidation. This is supported by detection of higher concentrations of oxidized triglycerides in UV-exposed hair. Kim et al found that triglycerides and free fatty acid concentrations were reduced in the UV-exposed skin of humans, with molecular evidence for reduced synthesis of these lipids (Kim, Jin et al. 2010), although it is likely that triglycerides were also lost through oxidation, something that was not measured in that study. In a small study by Cornellison, et al. (2011), di- and triglycerides were among the lipids found to be reduced by peroxide oxidation of hair. Photooxidation by UV exposure of unsaturated fatty acids within glycerol–fatty acid complexes would be expected as this has been demonstrated in model systems (Shahidi and Zhong 2010).

Phosphatidylethanolamines (PE) and N-acylethanolamines (NAE) were higher in non-UV-exposed hair. PE and NAE are commonly found in nature and are important for many biological processes, although this is beyond the scope of this work. PE have been previously reported in hair (Kim, Jang et al. 2020) and in a study of people with premature canities (greying of the hair) PE were higher in pigmented control hair root follicles compared with those with white hair (Wang, Wang et al. 2020). Ethanolamine derivatives are used in some hair dye formulations (Bailey, Zhang et al. 2014), especially monoethanolamine, and it could be speculated that the presence of long-chain PE in non-UV-exposed hair indicates that UV exposure cleaves PE to shorter ethanolamine derivatives.

Acylcarnitines were also found to increase when hair was exposed to UV light. Carnitines bind long-chain fatty acids for transport into mitochondria for β-oxidation for energy production, and their presence in hair may be an artefact from lipid transport into the hair follicle. Acylcarnitines have been previously reported in hair (Eisenbeiss, Steuer et al. 2019), with differences linked to methamphetamine abuse (Kim, Jang et al. 2020). As there would be no logical mechanism for production of acylcarnitines in the presence of UV light, a mechanism behind the increase in UV-exposed hair is not clear.

UV exposure caused a major increase in N-acylglycines (NAG). As the hair used is not influenced by biological requirements, either UV exposure induced production of NAG or inhibited the natural degradation of NAG. NAGs have been suggested to also have endocannabinoid-like activity as for NAE (Anderson and Merkler 2017), and their detection in hair suggests that they are incorporated in the function of the hair follicle.

Few papers have included lipid profiling in hair before, and in our analysis, we have found several lipid classes that have hitherto not been reported. Kim, Jang et al. (2020) reported detection of acylcarnitines, phosphatidylcholines (PC) and sphingomyelins (SM) in hair and their difference between heavy methamphetamine users and controls, using methanol as an extraction solvent. Phosphatidylethanolamines, PC, SM, diglyceride, vitamin D and cholesteryl-glucoside were reported as changing cases of premature canities (Wang, Wang et al. 2020). In both cases, the overall detection of lipids in hair was not reported, although Wang et al. reported 2440 features being identified using the LipidMaps database. Other studies, notably by Masukawa et al., have used several methods to get a comprehensive overview of hair lipids, finding cholesterol, ceramides, squalene, wax esters, hydrocarbons, triglycerides and free fatty acids (Masukawa, Narita et al. 2005a, Masukawa, Tsujimura et al. 2005b, 2006, Masukawa and Tsujimura 2007). These characterizations were based on using a combination of thin layer chromatography, gas chromatography–mass spectrometry and liquid chromatography-single quadrupole mass spectrometry, which are all 'targeted' methods in that they are set up to look for specific lipids. A major difference with our approach (and similar to that of Wang et al.) is that we have used a profiling strategy to detect all extracted lipids and then used matching against a database to detect and identify specific lipids. This fundamentally different approach may explain why we have detected many more lipid classes than previously reported. This also means it is difficult to compare directly with quantitative lipid analysis from past papers due to lipidomics profiling being not absolutely quantitative, due to differences in how well different lipids ionize, which is crucial for mass spectrometry detection. Of the lipids that we have detected and have not been previously reported in hair, we note that all are known to be present in human cellular and cytoplasmic membranes. As the follicular cell membrane is incorporated into the hair shaft, it would be expected that there are a number of cell and cytoplasmic membrane lipids present. Whether these have a functional impact on hair at any of its stages including in the follicle bulb, during hair growth or in finished hair remains to be elucidated. We note that due to the washing and handling of the hair used in this study, these are unlikely to be artefacts from the preparation process.

As with all untargeted lipidomics analyses, there is potential for misidentification due to the large number of possible isomers and difficulty of deconvoluting low intensity spectra. We have used a combination of fragmentation-based database matching and manual assessment of identification against retention time to curate the list of identified lipids. Further work using different methods will be useful for confirming these findings, which suggest that the hair lipidome is more complex than commonly reported. A further limitation is that we have used a database-matching methodology to narrow down the number of lipids to focus on in this paper, meaning that there are potentially more lipids present that have not been identified and therefore not reported here. This could include lipid oxidation and other degradation products which are highly heterogeneous. The LipidMaps database is the most comprehensive available, but may be lacking with regards to some lipids important for hair. 18-MEA has been commonly reported in the literature on hair lipids as one of the most common fatty acids present in the internal hair shaft, and future work should aim to determine whether 18-MEA is incorporated into specific lipid classes. LC–MS as used in our lipidomics method is ideal for separating lipid classes, but not individual free fatty acids. Gas chromatography remains the most appropriate method for measuring individual fatty acids, and while not commonly carried out, a combination of LC–MS lipidomics and GC for fatty acid analysis may help further understanding of the relationship between free- and bound fatty acids, and lipid classes that often determine biological relevance and localization of lipids.

Given the complexity of hair as a matrix and the known difficulties in extracting lipids, especially internal lipids, from the hair fibre, it can always be argued that an extraction process was not complete. Other methods have used chemically strong processes for lipid extraction, including hydrolysis or saponification. These are not viable options for lipidomics as the same procedures are likely to cleave some or many of the lipid species. Methanol is among the 'hair swelling' solvents, which enhance extraction of internal lipids (Robbins 2009), and it is likely that with the overnight extraction, giving a demonstrably higher extraction rate, internal lipids are represented among the lipids profiled. The most commonly used extraction solvent for hair in the literature is the Folch method (chloroform: methanol, 7:3 v/v), which has a proven track record for lipid extraction (Robbins 2009), but the use of chloroform creates an additional health and environmental hazard. Butanol:methanol performs similarly to chloroform:methanol (Huynh, Barlow et al. 2019) and is effective at extracting triglycerides which are the most lipophilic lipid species detected in our method. We found that triglycerides in hair were low relative to human plasma (the matrix the starting method was developed for), with most lipids being more polar and well within the extractable range of butanol:methanol. There is certainly scope for further work on hair lipid extraction procedures, especially methods that could allow identification of the impact of UV light on lipids in different structural areas of hair.

There have been many studies on the impact of UV exposure on hair, including on aspects of hair lipids (Fernández, Barba et al. 2012, Ji, Park et al. 2013, Maeda, Yamazaki et al. 2018). This work sheds light on the complexity of lipid changes and suggests that it is not simply a case of lipids being degraded by UV exposure. As UV exposure reduces hair integrity with an increase in the risk for brittleness and split ends, this may be in part due to changes to specific lipids. Further functional studies on hair quality in conjunction with lipidomics may help to identify any role specific lipid changes have on hair function.

Analysis of lipids and metabolites in hair is an important emerging area, but largely focused on physical health (e.g. maternal and foetal health (Sulek, Han et al. 2014) or drug use (Kim, Jang et al. 2020). Hair lipidomics could be a useful adjunct to work on the 'exposome' (what an individual has been exposed to [De Vecchi, da Silveira Carvalho Ripper et al. 2019, Appenzeller, Chadeau-Hyam et al. 2020]), and finding specific markers of UV exposure in hair could enable estimation of overall UV exposure from a non-invasive sample. To date, there have been no published studies that have correlated hair lipidome measurements with hair quality parameters and there is wide scope for more detailed and better understanding of how lipids interact with hair quality. Further work is needed to explore inter- and intra-individual variation in the hair lipidome, in what should be a rewarding emerging area of research.

CONCLUSIONS

In this pilot study, the lipidome of the hair shaft was found to undergo a wide range of changes on exposure to UV light. Many lipids which could be associated with better hair quality, such as ceramides, were at lower concentrations in UV-exposed hair. Applying lipidomics to measuring changes to the hair shaft is a feasible method for deeper understanding of how exposure to UV and other hair treatments and exposures impact on hair composition. Furthermore, this work demonstrates that the lipid composition of hair is more complex than commonly reported.

CONFLICT OF INTEREST

Authors ABR, EM, EJL and IH have no conflict of interest to declare.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.