International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 17, Issue 4, 2025

Original Article

INVESTIGATION OF URINARY METABOLOMIC PROFILING FOLLOWING EXPOSURE TO ULTRAVIOLET RADIATION-A BY USING LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY (LC-MS)

ALI MUHSEN ALI[1*], AHMED M. ZHEOAT[2], HUSSEIN ALI KAREEM[1], MUSTAFA KAREEM HASSAN[1]

[1]Pathological Analysis Department, Faculty of Applied Medical Science, Al-Shatrah University, Iraq. [2]Al-Manara College for Medical Sciences, Iraq

*Corresponding author: Ali Muhsen Ali; *Email ali.muhsen@shu.edu.iq

Received: 06 Feb 2025, Revised and Accepted: 02 Jun 2025

ABSTRACT

Objective: This study is designed to explore the impact of Ultraviolet-A (UVA) radiation on the urinary metabolome. The key novelty of this research lies in its focus on recording and analysing UVA-induced modifications, a less explored aspect in human metabolomic studies. Thus, study's aim is identification specific metabolomic changes associated with UVA exposure, emphasizing its potential implications in health and disease biomarker discovery.

Methods: Twenty-Eight urine samples have been collected from fourteen adult healthy individual sat pre-UV exposure (UVS1) and post-UV exposure (UVS2) conditions. Urinary metabolomic profiling was detected by using Liquid Chromatography – High Resolution Mass Spectrometry (LC-HRMS) Data modelling by Principal Components Analysis (PCA) and Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA) were applied after data extraction and normalizing.

Results: Multivariate data analysis by PCA and OPLS-DA models confirm clear separation of the observations as a marked difference based on R2Xcum 0.795, Q2cum 0.590 and R2Xcum: 0.69, R2Ycum: 0.98 and Q2cum: 0.83, respectively, in metabolite profile among study groups, non-exposure and UV-exposure. According for these effects, our results identified significant metabolic changes for important seventeen urinary metabolites. The pathway of significantly metabolites were marked in amino acids (Histidine, Tyrosine, Lysine, Arginine and proline metabolism), carbohydrate and lipids and their derivatives.

Conclusion: Current study concluded that these metabolites may be a potential biomarkers for predictive the impacts of UV sourced by sunlight exposure on some metabolic pathways, including: amino acid, carbohydrates, lipids, peptides, xenobiotics, and Co-factors and vitamins metabolism, which may be influenced by UV sunlight exposure.

Keywords: LC–MS, Ultraviolet radiation (UV), Urine metabolomic profiling, Principal components analysis (PCA), Orthogonal partial least squares discriminant analysis (OPLS-DA)

DOI: https://dx.doi.org/10.22159/ijap.2025v17i4.54103 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Sunlight is the main natural source of Ultraviolet (UV) radiation, especially UV-A, which has beneficial and harmful effects that lead to improvement or damage to a person's health. Skin cancer can be caused by the exposure to sunlight and is listed as one of the causes of death worldwide, killing 66,000 people annually, according to World Health Organization (WHO) statistics [1]. Ultraviolet radiation has been divined as non-ionizing radiation, which mainly emitted by sunlight as well as other artificial light sources such as tanning beds. There are three regions from UV radiation including UVA, UVB and UVC with wavelengths 315–400 nm, 280–315 nm and 100–280 nm, respectively. Unlike UVC radiation which be filtered by atmosphere to prevent its reach to earth's surface, most UVA and few amount of UVB radiation (10%) can pass to reach earth's surface and impact on organisms [2]. Two-way impacts of UV radiation, beneficial and harmful, were noted to be the cause or treatment many health problems. Many researchers focused in their studies on the positive effects of UV radiation and confirmed that UV can be used to enhance levels of vitamin D [3], rosuvastatin calcium [4] and treat jaundice, psoriasis, and vitiligo [5-7]. On the other hand, the UV radiation risks, harmful impacts, such as impaired of immune system, eye problems, skin aging, and cancer were verified in several studies, which have confirmed these negative effects [8-11].

Over the past few decades, metabolomics became one of a powerful techniques used in academic research to improve the knowledge needed for understanding of biological systems based on their metabolic signatures. It can also advance medical research and development by providing more insight into the identification of potential biomarkers for several diseases such as cancer, infectious diseases, and neurodegeneration [12]. The term "metabolites" refers to "a small molecule that are either end products of gene expression or a substrates of a metabolic pathways" [13].

Several scientific studies used different metabolomic techniques such as 1H Nuclear Magnetic Resonance Spectroscopy (^{1}H NMR) and Mass Spectrometry (MS) to shed light on identifying the relationship between the impacts of UV radiation on the metabolomic changes. These studies reported that the impacts of UV radiation exposure were observed on metabolic pathways in non-human subjects such as animals [14-18] and plants [19-26]. Conversely, although there were many research studied the effects of UV radiation on metabolite profiles in non-human subjects, still the studies which investigated these effects on human metabolism were almost non-existent and have not find the desired attention. Two previous studies were conducted by Pearce, 1983 and Yanshole, 2019 have been focused on UV impacts-related human metabolomic changes. A study by Pearse and his team in 1983 directly correlated UV radiation damage and metabolic changes in humans by measuring the response of human skin to ultraviolet light with and without sunscreen. This study showed that metabolic differences are evident when comparing protected and unprotected skin [27]. While, Yanshole's metabolomic study compared aqueous humour and lens metabolites from patients with and without cataracts [28]. Thus, lack of metabolic studies that addressed UV radiation effects on human metabolomic profiling prompted researchers to focus on such studies. Despite the well-known effects of UVA radiation on skin and overall health, there is a significant lack of human-focused metabolic studies. Current research focuses primarily on the biological mechanisms of UV damage, but the overall metabolic profile remains unknown. Therefore, there is an urgent need for human-focused metabolic research to better understand the systemic effects of UV exposure.

More recently, we used Liquid Chromatography–Mass Spectrometry (LC–MS) to detect the impacts of UVA radiation on human metabolic pathways. Our pilot study, which established on LC/MS-based data modelling on human urine and plasma samples to investigate the best scientific tool, can be used to identify metabolomic profiling under UV radiation exposure condition. In comparison with plasma samples, our results concluded that LC/MS-based human urinary metabolomic profiling is best way can be used to identify metabolomic changes and potential metabolites as a predictive biomarkers for UVA radiation effects [29]. Despite all this, questions continue to arise about clarification how the human metabolome responds to UVA exposure and what are metabolic pathways effected. Therefore, there is warranted the study of urinary metabolic changes for understanding of human metabolome following the exposure to UV radiation.

In order to achieve the objectives of current study, LC-MS-based urinary metabolomic profiling was used to perform a broad profile analysis. The first aim is looking for indicates on metabolic changes that may contribute to a better understanding of the physiological metabolite response to low-dose UVA. Second objective focuses on uncovering potential metabolites which may be significantly influenced as a result of the human metabolome responds to UVA exposure.

MATERIALS AND METHODS

Chemicals and solvents

HPLC grade Acetonitrile (ACN) and Anala R-grade formic acid (98%) were purchased from Fisher Scientific (Loughborough, UK) and from BDH-Merck (Poole, UK), respectively. HPLC grade water was produced by using A Direct-Q 3 Ultrapure Water System (Millipore, Watford, UK). Ammonium Carbonate $(NH_4)_2CO_3$ and Methanol (MeOH) were from Sigma-Aldrich (Poole, UK). Authentic stock standards obtained from Sigma–Aldrich, UK and diluted four times with ACN before LC-MS analysis. Authentic stock metabolite standards were used to prepare Metabolite Standard Solutions (MSS) by taking 1 mg from authentic stock metabolite standard and dissolve with 1 ml HPLC grade methanol and water (1:1, v/v) than stored at −20 °C. 100ul was taken from each MSS of 60 metabolites were mixed and complete the volume to 10 ml with acetonitrile. The final concentration of each MSS was 10µg/ml. based on this procedure, four mixtures of metabolites standard solutions (MSS1 – MSS4) collected from 240 metabolite will be available as reference data.

Subjects and experimental design

This study is a pre-post study, thus, experimental measurements were collected before and after the exposure to UVA light. Many conditions was required to be applied on all participants, including: non-smoking, apparently healthy, no use of medication, and fasting at least 12 h, to avoid the metabolomics changes caused by the effect of diet. Experimental was performed in Thi-Qar Province during five months (October, 2023-February, 2024). On experiment day, after an overnight fast except the consumption of ~500 ml of water upon waking, the first urine pass was neglected to avoid the changes which may effect on urinary metabolic profiles. The participants were underwent to start the trail with control conditions in the morning (before 10 am). Then, they were exposed to a dose of UVA light by 20 J/cm^{2,} which is approximately equivalent to 30 min in the sunshine in summer. The selected UVA exposure, justified by alignment with realistic environmental conditions, aims to enhance relevance to everyday human exposure.

Twenty human urine samples were collected from ten adult healthy individuals gave their informed consent to participate in this study as volunteers. All participants visited the laboratory to collected urine samples at two times in same day, including: first time point was control condition samples, pre-UV exposure (UVS1), and second time point was UVA condition samples, post-UV exposure (UVS2), as shown in fig. 1.

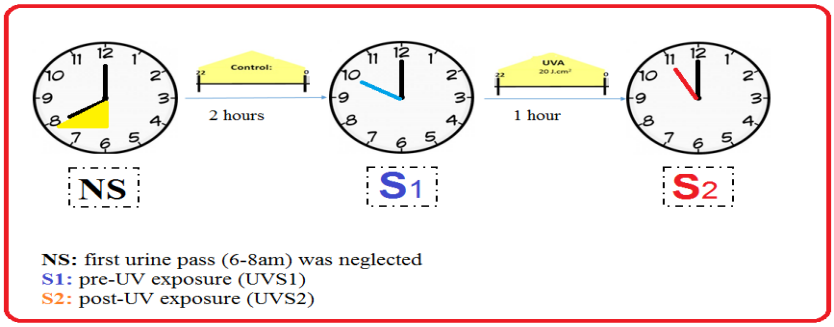

Fig. 1: Indicative representation of urine collection schematic at two consecutive conditions, control conditions, without a dose of UVA light, (UVS1) and Ultraviolet-A conditions, with a dose of UVA light, (UVS2). The first urine sample on experiment day was discharged (typically 6 am–8 am)

Preparation and profiling of sample

Preparation of samples in metabolomics profiling is carried out based on type of specimen and methods used for analysis [30]. Twenty urine samples, which collected during test day were stored at-80 C for its preparation day. After thawed of specimens at room temperature, 200ul of each sample was transferred into micro centrifuge tube contains 800ul of ACN and was shaken by a vortex machine for 3 min. this prepared mixture (1 ml) was centrifuged for 15 min with 15000 *rpm* at 4 °C by refrigerated centrifuge. Finally, the supernatants, for each specimen, produced from centrifugation, were transferred to respective marked HPLC vials to be analysed by LC-MS. Then again, 100 ml urine from each sample were mixed together in same tube to prepare pooled samples which were used as quality controls in order to exclude technical and extraneous biological variations.

LC–MS analysis conditions

LC-MS settings are carefully selected and optimized to achieve effective separation (via chromatography) and accurate, sensitive detection (via mass spectrometry) of analytes. The goal is to extract as much useful information as possible while minimizing interference and maximizing sensitivity for quantitative or qualitative analysis. For LC–MS analysis conditions and data processing, the methods of Ali Muhsen Ali *et al.* 2023 and Ali Muhsen Ali *et al.* 2022 [29, 31] were followed. "Dionex 3000 HPLC" (Thermo Fisher Scientific, Hemel Hempstead, UK) combined with a mass spectrometry (Thermo Fisher Scientific) was used to analyse urine samples in both positive and negative mode set at 50,000 resolution (controlled by Xcalibur version 2.1.0; Thermo Fisher Scientific, Hemel Hempstead, UK). LC-MS method conditions included the scanning of mass range (m/z), capillary temperature and the flow rates of auxiliary gas/sheath, which were at 75–1200, 320 C and 17/50 arbitrary units, respectively. The stationary phase, which is used in this method was zwitterionic-hydrophilic interaction chromatography column, ZIC-pHILIC column, (150 mm x 4.6 mm; 5µm from HiChrom, Reading, UK). While, mobile phase was prepared as following; (A): 20 mmol ammonium carbonate in HPLC grade water (pH 9.2), and (B): HPLC grade acetonitrile (CAN). 10µl of each sample solution was injected on a stationary phase along

with a flow rate of mobile phase at 300 µl/min in binary gradient mode. Binary mode was gradient as follows: 80% of B at 0 min, 20% B at 30 min, 20% B at 36 min, 80% B at 37 min and 80% B 46 min".

LC-MS data processing and statistical analysis

M/Z Mine 2.14 was applied for addressing of raw LC-MS data, obtained from Xcalibur software, to identify the metabolites through matching of the retention time, the accurate masses and alignment of ion chromatograms based on MSS [32]. According to the quality of the peaks, the accurate filtered masses were queried through house metabolite database, had been taken from the Human Metabolome database (HMDB) [33], Lipid Maps (LMSD), www. lipidmaps. org, [34] and the Kyoto Encyclopedia of Genes and Genomes (KEGG) database [35].

After the identification of metabolites, univariate analysis for gotten data were performed. Extracted ion chromatograms peaks were normalised with the pooled sample peak list to filter the data and remove all features are not matched. The normalisation was carried out by dividing the average of areas for each metabolite in one time point across the sum of all the time points for UVS1, UVS2 and pooled samples. Then, the lists of obtained metabolites were exported into Excel (Microsoft Office 2019) and were carefully manually addressed to conduct statistical analysis, including Paired T-Test (p-value generation), Fold Changes (ratio) and Variable Importance Projection (VIP). The critical threshold for the regarding of p-value as being significant and more significant are *P<0.05 and **P<0.01, respectively. VIP is the sum over all model dimensions of the contributions in variable influence. However, a variable with a VIP Score close to or greater than 1 (one) can be considered important in given model [36].

Multivariate analysis was applied to data modelling by SIMCA-P version 14.0 (Umetrics, Sweden), which is the multivariate data analysis software was used in metabolomics studies. Data modelling was performed based on an unsupervised and a supervised analysis method to construct two common modelling, including Principal Component Analysis (PCA) and Orthogonal Discriminant Analysis Partial Least Squares (OPLS-DA), respectively. These Analysis was designed to evaluate the quality and reliable of models as evidence on acute changes in metabolomic patterns and investigation significantly influential components in the dataset.

RESULTS AND DISCUSSION

UV radiation has a large positive and negative impact on human health. Therefore, there is a necessary need to investigate the human metabolic changes as a result for UV impacts. In such studies to investigate the metabolic changes, LC/MS-based untargeted metabolomic approach is widely relied upon in revealing unique metabolomic fingerprints in order to understand the complex evolutions of metabolic pathways.

Data modelling and the validation

Raw urine data obtained from LC-MS were directly analysed by Xcalibur software and were transferred to M/Zmin2.14 software to extract and aligned the peak values and identify the metabolites. Excel file was organised to arrange and normalise the data to be ready for multivariate statistical analysis. Then, this data was modelled by SIMCA 14-1 software to determine distinct metabolite profiles between non-and UV-exposed (S1UV and S2UV) samples. Purpose of data modelling was to provide easy-to-understand graphical information about process status and relationships between sets of important process variables as well as the validation of model's efficacy in separation between study's groups. In this step, multivariate data analysis was applied through building unsupervised, PCA, and supervised, OPLS-DA, models for pre-and post-exposure to UV radiation samples to quantify how each process step contributes to the development of critical issues.

Fig. 2 illustrates successfully distinguishes between urine groups based on a map of the observations, score plots of PCA model. PCA's score plots indicate that distinct separation of subjects was based on metabolite profile differences before and after UV exposure, S1Us and S2UV. Successfully differentiates obtained from the performing of PCA model was valued by the internal validation tools including: model overview and the value of variance explained [R2X (Cum)], variance explained [R2Y (Cum)] and the variance predicted [Q2 (Cum)] which should be closed to one, not less than 0.5, to make the model is useful and acceptable in data analysis [37].

The overview of PCA model, fig. 2, confirms clear separation of the observations as a marked difference in metabolite profile among these samples. Also, the internal validation method with R2X (cum) (0.795) and Q2(cum) (0.590) values, as listed in table 1, statistically supports goodness and reliable of PCA model as evidence on this differentiate between study's subjects as a result of UV effects.

Typically, PCA's score plot compresses single components and pseudo variables, aiding in grouping original data linearly and addressing dimensionality issues. Despite, the additional objective to confirm the modelling and interpretation of variation between study's groups is required to support results of PCA model. Therefore, the application of OPLS-DA model as supervised model becomes necessary to achieve the best possible prediction and interpretation for further differentiate between the work sets.

The overview of OPLS-DA model illustrates its successful classification through clear separation of variables to highlight significant metabolite differences in study's samples. The quality of OPLS-DA's classification was supported by strong internal validation metrics for this model included (R2X(cum): 0.69, R2Y(cum): 0.98, Q2Y(cum): 0.83), as show on fig. 3 and table 1.

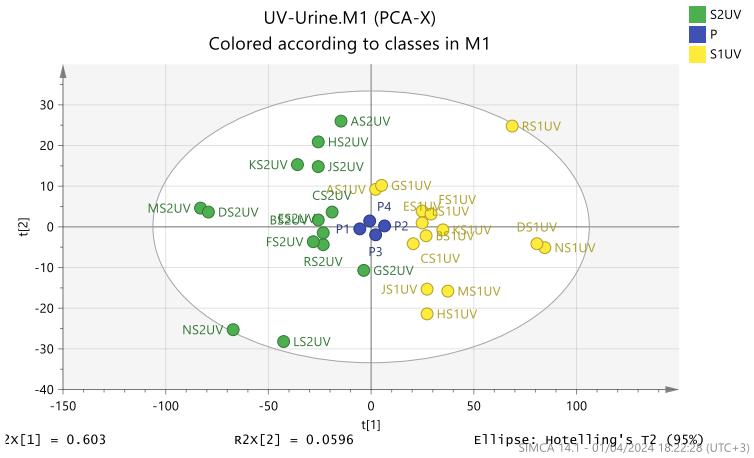

Fig. 2: PCA score plot of non-exposure (S1UV) and exposure UV (S2UV) samples with (R2X (cum) 0.795, Q2 (cum) 0.590 and five components)

Table 1: The statistic description of PCA and OPLSDA models used to describe metabolomic changes in urine of non-exposure and exposure-UV groups

S. No.	Model	Type	Component No. (A)	No. of subjects	R2X(cum)	R2Y(cum)	Q2(cum)
1	M1	PCA-X	5	32	0.795		0.59
2	M2	OPLS-DA	1+2+0	28	0.689	0.981	0.83

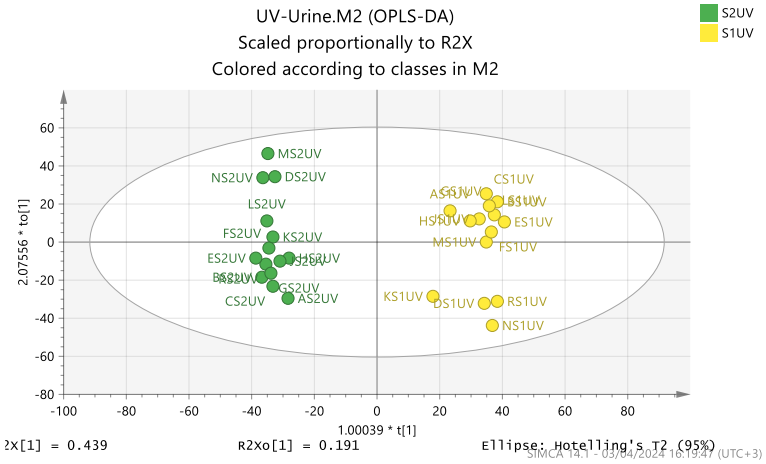

Fig. 3: OPLS-DA score plot of non-exposure (S1UV) and exposure UV (S2UV) urine samples with (R2X (cum)=0.69, R2Y (cum)=0.98 and Q2 (cum)=0.83)

Due to internal validation methods are not sufficient to confirm model validity [38], external validation via permutation tests and cross-validation ANOVA (CV-ANOVA) were performed as a diagnostic tool for assessing the reliability of OPLS-DA model and evaluation its statistical significance [37]. In current study, stratigraphy of blue Q2-values and green R2-values which achieve the conditions of the acceptability of Permutations test, fig. 4, strongly indicates to the quality and goodness of original OPLS-DA model. Furthermore, the significantly value of CV-ANOVA with P-Value = 4.35772e-007 indicates that OPLS-DA is strongly valid to obtain the reliable results. The outputs of the CV-ANOVA are given in a tabulated format as shone in table 2.

Generally, the quality of PCA and OPLS-DA models in separation and distinguishing between study groups, non-exposure and UV-exposure, were validated and confirmed by internal and external validation tools. The goodness and robust of these models are the evidence on the reliability of distinct metabolic patterns, which can be used to determinate the metabolic changes related to the impacts of the exposure of UV radiation. These metabolic patterns of urine metabolites indicate that the exposure of UV may be the reason for significantly changes in many metabolic pathways. These results match with previous studies [29, 39]. To determine urine metabolites which affected by UV exposure, the metabolic pathways and metabolite profiling were the main aim of current study.

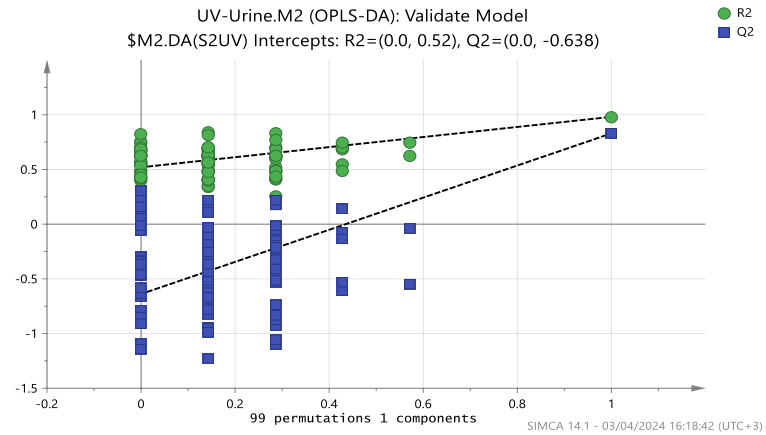

Fig. 4: Cross validation of OPLSDA model for the classification of study's groups (S1UV and S2UV) in urine samples by the permutations test

Table 2: The outputs of the CV-ANOVA validation of OPLSDA model validation for the classification of study's groups (S1UV and S2UV) in urine samples. SS: the sum of squares, DF: degrees of freedom, MS: mean squares, F: F-test, SD: Standard deviation

M2 (Untitled)	SS	DF	MS	F	p-Value	SD
Total corr.	27	27	1			1
Regression	22.4002	6	3.73337	17.0445	4.3577E-07	1.93219
Residual	4.59978	21	0.219037			0.468014

Metabolic pathway and metabolite profiling

Multivariate analysis of LC/MS-obtained urine data revealed significant differences in metabolite profiles between samples exposed to UV light and those that were not exposed.

Analyses results detected 2733 metabolic features which were generated from LC/MS analysis within 20 min and divided to 1398 features in the positive mode and 1335 features in the negative mode. These metabolic features were identified accordingthe mass characteristics, including exact mass and retention time, less than 3 ppm deviation, with MS^1 and MS^2 levels in house metabolite database [36].

The data filtering by normalization and the univariate analysis were carried out to unveiled significant metabolites in urine samples. In this step, Eighty-eight significant metabolites in both +Ve and -Ve, ion modes were detected based on the critical threshold for the regarding of p-value as being significant is<0.05 and fold change, ratio S2UV – S1UV, as are detailed in Supplementary Data (DS1). Identification of metabolic pathways to detect metabolic pathways disrupted by UV radiation were analysed. Of all pathways analysed, metabolic pathway of only 49 metabolite were identified as significantly altered pathways between the radiation and non-radiation exposure groups as are described in table 3.

Table 3: The significantly metabolites and their metabolic pathways obtained from the OPLSDA model separating between non-exposure and exposure-UV in urine samples based on the critical threshold for the regarding of p-value as being significant is<0.05

S. No.	Row M/Z	Retention time	Molecular formula	Name	P-value S1UV-S2UV	Ratio S2UV/S1UV	Vip
Amino acid metabolism							
1	139.05	7.43	C6H6N2O2	Urocanate	0.0001	1.766	1.7
3	137.04	7.43	C6H6N2O2	Urocanate	0.0004	1.611	1.5
4	139.05	9.85	C6H8N2O2	Methylimidazoleacetic acid	0.0013	1.288	0.9
5	152.11	19.83	C9H13NO	N-Methyltyramine	0.0126	1.330	1.0
6	160.06	9.94	C6H11NO4	L-2-Aminoadipate	0.0128	1.241	1.0
7	121.03	7.96	C7H6O2	Benzoate	0.0136	1.249	0.9
8	174.09	16.13	C6H13N3O3	L-Citrulline	0.0141	1.193	0.8
9	160.06	9.94	C6H11NO4	L-2-Aminoadipate	0.0141	1.240	0.9
10	141.07	9.69	C6H8N2O2	Methylimidazoleacetic acid	0.0176	1.143	0.7
11	162.08	9.62	C6H11NO4	L-2-Aminoadipate	0.0201	1.786	1.2
12	141.07	9.69	C6H8N2O2	Methylimidazoleacetic acid	0.0209	1.153	0.7
13	181.05	10.34	C6H12O4S	5-Methylthio-D-ribose	0.0233	1.132	0.7
14	116.04	9.98	C4H7NO3	L-2-Amino-3-oxobutanoic acid	0.0263	1.191	0.9
15	137.04	11.28	C6H6N2O2	Nicotinamide N-oxide	0.0276	1.278	1.0
16	121.03	9.00	C7H6O2	Benzoate	0.0282	1.161	0.7
17	104.07	14.50	C4H9NO2	4-Aminobutanoate	0.0283	1.123	0.7
18	160.10	12.58	C7H13NO3	5-Acetamidopentanoate	0.0288	1.413	1.0
19	161.09	13.83	C6H12N2O3	D-Alanyl-D-alanine	0.0341	1.331	0.9
20	130.09	11.12	C6H13NO2	L-Leucine	0.0365	1.250	0.8
21	130.05	5.54	C5H7NO3	L-1-Pyrroline-3-hydroxy-5-carboxylate	0.0391	1.241	1.0
22	158.04	12.12	C6H7NO4	2-Aminomuconate	0.0406	1.105	0.6
23	144.08	15.87	C5H11N3O2	4-Guanidinobutanoate	0.0406	1.403	1.1
24	84.08	14.82	C5H9N	Piperideine	0.0468	1.191	0.8
Carbohydrate metabolism							
25	165.08	9.55	C6H12O5	3-Deoxyglucosone	0.0000	0.437	1.0
26	177.04	14.12	C6H10O6	D-Glucono-1,5-lactone	0.0048	0.726	0.9
27	153.00	11.87	C3H7O5P	Propanoyl phosphate	0.0299	1.447	1.2
28	351.06	17.39	C12H16O12	4-(4-Deoxy-alpha-D-gluc-4-enuronosyl)-D-galacturonate	0.0485	1.219	0.9
Lipid metabolism							
29	103.04	15.52	C4H8O3	(R)-3-Hydroxybutanoate	0.0474	0.740	1.0
30	203.13	5.87	C10H18O4	[FA (10:0/2:0)] Decanedioic acid	0.0100	1.346	1.1
32	151.04	8.72	C8H8O3	[PK] 6-Methylsalicylic acid	0.0376	0.817	0.9
33	167.11	6.59	C10H16O2	[PR] (1S,4R)-1-Hydroxy-2-oxolimonene	0.0316	1.557	1.1
Metabolism of cofactors and vitamins							
34	145.05	11.49	C6H8O4	2,3-Dimethylmaleate	0.0285	0.745	1.2
35	215.14	10.28	C10H18N2O3	Dethiobiotin	0.0401	1.186	0.9
Purine Metabolism							
36	167.02	12.98	C5H4N4O3	Urate	0.0325	1.173	0.8
Peptide(di-)							
38	187.11	13.40	C8H16N2O3	Glycyl-leucine	0.0290	1.212	0.8
39	189.12	15.52	C8H16N2O3	Glycyl-leucine	0.0300	1.234	0.9
40	203.14	14.27	C9H18N2O3	Leu-Ala	0.0465	1.173	0.8
41	264.12	15.77	C9H17N3O6	Ser-Gly-Thr	0.0195	1.204	0.9
42	232.13	10.50	C9H17N3O4	Asparaginyl-Valine	0.0288	1.207	0.9
43	314.17	7.24	C14H23N3O5	Thr-Pro-Pro	0.0371	1.329	1.0
Xenobiotics drugs etc							
44	187.10	11.62	C9H16O4	Azelaic acid	0.0033	1.540	1.2
45	254.12	9.75	C10H15N5O3	Penciclovir	0.0122	1.230	0.9
46	212.10	8.61	C9H13N3O3	Zalcitabine	0.0205	1.204	0.9
48	210.09	8.78	C9H13N3O3	Zalcitabine	0.0425	1.196	0.9
49	95.06	9.63	C5H6N2	4-Aminopyridine	0.0453	1.101	0.6

For more data filtration to detect the final significantly important features, another factor from univariate analyses, VIP, "is a score that indicates how "important" a feature is to the model", was assessed to account the relative influence of each variable. Based on the VIP score, which be closed to or greater than 1 (one), the metabolites which have the greatest contribution in given model will be evaluated [40]. Table 4 summarizes seventeen features which have marked difference in its profile according the main statistic value (p-value and VIP). The features with VIP>1.0 and p<0.05 were considered to be the significantly important metabolites and can be the marker of the metabolic characteristics of UV impacts. The pathway of significantly metabolites which marked in amino acids (Histidine, Tyrosine, Lysine, Arginine and proline metabolism), carbohydrate and lipids and their derivatives, table 4. In particular, all-important features, nine metabolites, of amino acids and their derivatives, are elevated (↑), high fold change (Ratio S2UV/S1UV>1.0), as a responding to UV exposure at comparison between study samples S1UV and S2UV, pre-and post-exposure, respectively. Increase of the levels of amino acids metabolites is evidence on the effect of UV radiation in enhancing the metabolism of amino acids, which may be beneficial on human health. Amino acids are used by the body to make proteins, enzymes, hormones and other important molecules to maintain the homeostasis in the body. In addition to its participate in the biosynthesis of proteins, amino acids also serve as precursors of metabolites related to environmental changes and many other vital processes in the organism, such as cellular osmoregulation, protecting membrane integrity and the production and removing Reactive Oxygen Species (ROS) [41-43]. Amino acids such as alanine, which is synthesized from pyruvate by alanine aminotransferase, is closely related to glycolysis and the Tri-Carboxylic Acid (TCA) cycle [39, 44].

Urocanate and Nicotinamide N-oxide are important metabolites derivatised histidine metabolism, one of amino acids metabolism map, have been significantly affected by UV radiation as it is observed in this study, table 4. Urocanate is considered intermediary product of the metabolic pathway of histamine to glutamate and is associated with increased excitatory neurotransmission. The various roles of urocanate in health and disease and its systemic anti-inflammatory and immunosuppressive properties, such as suppressing cell-mediated immunity, attenuating sclerosis, and protecting cells from UV injury, was reported by several studies [40, 45-47].

Table 4: The high significantly important metabolites according the main statistic value with VIP>1.0 and p<0.05. Elevated metabolites ↑ and reduced metabolites ↓

Ion	Name	P-value S1UV-S2UV	Fold change	Vip	Pathway
	Amino acid metabolism				
P	Urocanate↑	0.0001	1.77	1.7	Histidine metabolism
N	Urocanate↑	0.0004	1.61	1.5	Histidine metabolism
P	N-Methyltyramine↑	0.0126	1.33	1.0	Tyrosine metabolism
P	L-Citrulline↑	0.0141	1.19	1.0	Arginine and proline metabolism
P	L-2-Aminoadipate↑	0.0201	1.79	1.2	Lysine metabolism
N	Nicotinamide N-oxide↑	0.0276	1.28	1.0	Histidine metabolism
P	5-Acetamidopentanoate↑	0.0288	1.41	1.0	Lysine degradation
N	L-1-Pyrroline-3-hydroxy-5-carboxylate↑	0.0391	1.24	1.0	Arginine and proline metabolism
P	4-Guanidinobutanoate↑	0.0406	1.40	1.1	Arginine and proline metabolism
	Carbohydrate metabolism				
P	3-Deoxyglucosone↓	0.0000	0.44	1.0	Fructose and mannose metabolism
N	D-Glucono-1,5-lactone↓	0.0048	0.73	1.0	Pentose phosphate pathway
	Lipid metabolism				
P	(R)-3-Hydroxybutanoate↓	0.0474	0.74	1.0	Butanoate metabolism
N	[FA (10:0/2:0)] Decanedioic acid↑	0.0100	1.35	1.1	Fatty Acids and Conjugates
P	[PR] (1S,4R)-1-Hydroxy-2-oxolimonene↑	0.0316	1.56	1.1	Isoprenoids
	Metabolism of cofactors and vitamins				
P	2,3-Dimethylmaleate↓	0.0285	0.74	1.2	Nicotinate and nicotinamide metabolism
	Peptide(di-)				
N	Thr-Pro-Pro↑	0.0371	1.33	1.0	Polar peptide
	Xenobiotics drugs etc				
P	Azelaic acid↑	0.0033	1.54	1.2	Fatty Acids and Conjugates

Other important metabolites derivatised from amino acids metabolism, particularly arginine and purine metabolism, which are also products of cellular amino acid metabolism, such as L-Citrulline and 4-Guanidinobutanoate, it effects in the rodent central nervous system [48], were significantly associated with the impact of UV exposure. Additionally, a focus on developmental programming in non-communicable diseases, the roles of L-arginine synthesis and its metabolism have been considered by many researchers in multiple settings. In their studies, they reported the effects of amino acids such as L-arginine and L-citrulline on blood pressure regulation and can affect nitric oxide. Nitric Oxide (NO) is a physiological compound in the human body that is produced by NO Synthase (NOS) during the oxidation of L-arginine and can lower blood pressure by increasing blood flow. Increased nitric oxide production has been shown to reduce Erectile Dysfunction (ED), Type 2 Diabetes Mellitus (T2DM) and decrease muscle soreness [39, 49-52].

The LC-MS analysis showed the presence of carbohydrate metabolites, including 3-Deoxyglucosone (3-DG) and D-Glucono-1,5-lactone (Gluconolactone).

3-Deoxyglucosone (3-DG) is a dicarbonyl sugar's metabolite and is synthesized via the Maillard reaction and the polyol pathway. It is as a glucose degradation product can be used as a marker for hyperglycemia in patients with diabetes mellitus [53].

Stress of dicarbonyl compounds has been listed under the main causes of cell and tissue dysfunction, which leading to a variety of health problems such as aging, cancer and other skin disorders, psoriasis and vitiligo, as well as its principal role in the development of diabetic and uremic complications and Alzheimer's disease. Gas Chromatography-Mass Spectrometry (GC-MS) and LC-MS have been used to detect the significantly increase of 3-DG levels in diabetes and uremia plasma [53, 54]. Finally, it could be a potential target for the development of anticancer drugs against skin cancer related to UV radiation. Gluconolactone, second identified carbohydrate metabolite, is polyhydroxy acid (PHA) containing a gluconolactone molecule and is a product of D-glucose oxidation stimulated by the enzyme glucose oxidase. Gluconolactone is an essential carbohydrate metabolite found in all living organisms, and has metal chelating, moisturizing and antioxidant activities. Gluconolactone's ability in removing the free radicals and its using as a skin exfoliant in cosmetic products to reduce the harmful effects of UV radiation on the skin is attributed to its moisturizing and antioxidant properties [55].

In the current study, 3-Deoxyglucosone and Gluconolactone identified as important carbohydrate metabolites with VIP ≥ and P-value<0.5

and were low-significance metabolites based on the low value of fold change (Ratio S2UV/S1UV= 0.437 and 0.726, respectively).

Based on the minimize literature review in our previous article [29], many articles have been published on metabolic changes in non-human, plants and animals, exposed to UV radiation, in comparison with studies on these effects on human metabolomic profiles, which are almost non-existent. Some of these studies reported that these metabolic changes related to the impact of UV exposure were particularly involved in the differential metabolites of amino acid and carbohydrate in plants and animals. These studies used common analytical technologies, include LC-MS and GC-MS to investigate the role of UV exposure on inhibition and enhancing the sugars, fatty acids and amino acids [56-59].

In considering to our findings, some of the metabolic changes were somewhat compatible with previous observations revealed in last our study with regard to the impact of UV on amino acid, carbohydrate and lipids metabolism [29]. Despite some similarities between previous and present findings, the differences were present and clear in the type and number of metabolites identified. These differences may be due to several reasons, including: samples number and ethnicity of participants as well as identification of metabolic pathway and metabolite profiling in urine was not the main aim of our previous study. Finally, according to our knowledge, there is no metabolomic study has been performed about the relationship between urinary metabolites and UV radiation impacts. Thus, the current study is the first LC/MS-based metabolomics study to identify the metabolites in urine samples that can be used as potential biomarkers to recognize the changes in metabolic pathway and metabolite profiling in healthy human as a result of the exposure of UV radiation exposure.

CONCLUSION

In summary, current study clearly demonstrated disruptions in key metabolic pathways, including amino acids, carbohydrate and lipids and their derivatives. Fourteen metabolites, particularly amino acids metabolites were significantly elevated under UV exposure conditions. While, the significantly reducing in carbohydrate metabolites were observed at same conditions.

Thus, overall, the significant metabolites which were identified from the variations in urine metabolomics as a result of the exposure of UV radiation sourced by sunlight can be used a potential biomarkers for predictive the changes in some metabolic pathways and evaluation the beneficial and harmful effects of this exposure on human health.

DATA AVAILABILITY

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

ACKNOWLEDGEMENT

The authors would like to thank the Strathclyde Institute of Pharmacy and Biomedical Sciences (SIPBS), University of Strathclyde as awarding institution of the doctoral thesis submitted by Ali Muhsen Ali, which included the description of most of the instrumentations, materials and methods used in this paper [https://stax.strath.ac.uk/concern/theses/kk91fk54w]

AUTHORS CONTRIBUTIONS

Ali A. and Ahmed M. organized the study, prepared and analysed samples and extracted data. Ali A., Majeed A. and Hussein A. modeled data. Ali A. wrote the manuscript. Ali A., Ahmed M., Majeed A. and Hussein A. collected samples.

SUPPLEMENTARY DESCRIPTION

The significantly metabolites, in both +Ve and -Ve ion modes, and their metabolic maps obtained from the OPLSDA model separating between non-exposure and exposure-UV in urine samples based on the critical threshold for the regarding of p-value as being significant is<0.05 and fold change, ratio S2UV – S1UV. *Supplementary Data-SD1.pdf*

CONFLICTS OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

1. WHO. Intersun, the Global UV Project: a Guide and Compendium. Geneva, Switzerland: World Health Organization; 2003. Available from: http://www.who.int/uv/publications/intersunguide/en/. [Last accessed on 06 Jun 2025].
2. Reddy PS, Suresh Kumar AS, Jain V. Sunscreens: developments and challenges. Int J Appl Pharm. 2018;10(6):54-9. doi: 10.22159/ijap.2018v10i6.27379.
3. Weller RB. Sunlight has cardiovascular benefits independently of vitamin D. Blood Purif. 2016;41(1-3):130-4. doi: 10.1159/000441266, PMID 26766556.
4. Funde A, J Kokat. A validated stability indicating UV-spectrophotometric simultaneous estimation of rosuvastatin calcium and fenofibrate in bulk and pharmaceutical formulation. Int J Chem Res. 2021;5(1):1-8. doi: 10.22159/ijcr.2021v5i1.144.
5. Bataille V, Bykov VJ, Sasieni P, Harulow S, Cuzick J, Hemminki K. Photoadaptation to ultraviolet (UV) radiation *in vivo*: photoproducts in epidermal cells following UVB therapy for psoriasis. Br J Dermatol. 2000;143(3):477-83. doi: 10.1111/j.1365-2133.2000.03698.x, PMID 10971317.
6. El Zawahry BM, Bassiouny DA, Sobhi RM, Abdel Aziz E, Zaki NS, Habib DF. A comparative study on efficacy of UVA1 vs. Narrow band UVB phototherapy in the treatment of vitiligo. Photodermatol Photoimmunol Photomed. 2012;28(2):84-90. doi: 10.1111/j.1600-0781.2011.00643.x, PMID 22409711.
7. Piltingsrud HV, Odland LT, Fong CW. An evaluation of fluorescent light sources for use in phototherapy of neonatal jaundice. Am Ind Hyg Assoc J. 1976;37(7):437-44. doi: 10.1080/0002889768507489, PMID 961603.
8. Cejka C, Platenik J, Sirc J, Ardan T, Michalek J, Brunova B. Changes of corneal optical properties after UVB irradiation investigated spectrophotometrically. Physiol Res. 2010;59(4):591-7. doi: 10.33549/physiolres.931867, PMID 19929139.
9. Liu D, Fernandez BO, Hamilton A, Lang NN, Gallagher JM, Newby DE. UVA irradiation of human skin vasodilates arterial vasculature and lowers blood pressure independently of nitric oxide synthase. J Invest Dermatol. 2014;134(7):1839-46. doi: 10.1038/jid.2014.27, PMID 24445737.
10. Norval M, McLoone P, Lesiak A, Narbutt J. The effect of chronic ultraviolet radiation on the human immune system. Photochem Photobiol. 2008;84(1):19-28. doi: 10.1111/j.1751-1097.2007.00239.x, PMID 18173697.
11. Surdu S, Fitzgerald EF, Bloom MS, Boscoe FP, Carpenter DO, Haase RF. Occupational exposure to ultraviolet radiation and risk of non-melanoma skin cancer in a multinational European study. PLOS One. 2013;8(4):e62359. doi: 10.1371/journal.pone.0062359, PMID 23638051.
12. Simithy J, Sidoli S, Garcia BA. Integrating proteomics and targeted metabolomics to understand global changes in histone modifications. Proteomics. 2018;18(18):e1700309. doi: 10.1002/pmic.201700309, PMID 29512899.
13. Abooshahab R, Ardalani H, Zarkesh M, Hooshmand K, Bakhshi A, Dass CR. Metabolomics a tool to find metabolism of endocrine cancer. Metabolites. 2022;12(11):1154. doi: 10.3390/metabo12111154, PMID 36422294.
14. Park HM, Shin JH, Kim JK, Lee SJ, Hwang GS, Liu KH. MS-based metabolite profiling reveals time-dependent skin biomarkers in UVB-irradiated mice. Metabolomics. 2014;10(4):663-76. doi: 10.1007/s11306-013-0594-x.
15. Park HM, Shon JC, Lee MY, Liu KH, Kim JK, Lee SJ. Mass spectrometry-based metabolite profiling in the mouse liver following exposure to ultraviolet B radiation. PLOS One. 2014;9(10):e109479. doi: 10.1371/journal.pone.0109479, PMID 25275468.
16. Risa O, Saether O, Lofgren S, Soderberg PG, Krane J, Midelfart A. Metabolic changes in rat lens after *in vivo* exposure to ultraviolet irradiation: measurements by high resolution MAS ^{1}H NMR spectroscopy. Invest Ophthalmol Vis Sci. 2004;45(6):1916-21. doi: 10.1167/iovs.03-1292, PMID 15161858.
17. Tessem MB, Bathen TF, Cejkova J, Midelfart A. Effect of UV-A and UV-B irradiation on the metabolic profile of aqueous humor in rabbits analyzed by ^{1}H NMR spectroscopy. Invest Ophthalmol Vis Sci. 2005;46(3):776-81. doi: 10.1167/iovs.04-0787, PMID 15728530.

18. Tessem MB, Midelfart A, Cejkova J, Bathen TF. Effect of UVA and UVB irradiation on the metabolic profile of rabbit cornea and lens analysed by HR-MAS ^{1}H NMR spectroscopy. Ophthal Res. 2006;38(2):105-14. doi: 10.1159/000090511, PMID 16374053.
19. Kaling M, Kanawati B, Ghirardo A, Albert A, Winkler JB, Heller W. UV-B mediated metabolic rearrangements in poplar revealed by non-targeted metabolomics. Plant Cell Environ. 2015;38(5):892-904. doi: 10.1111/pce.12348, PMID 24738572.
20. Morales LO, Brosche M, Vainonen JP, Sipari N, Lindfors AV, Strid A. Are solar UV-B- and UV-A-dependent gene expression and metabolite accumulation in arabidopsis mediated by the stress response regulator radical-induced cell DEATH1? Plant Cell Environ. 2015;38(5):878-91. doi: 10.1111/pce.12341.
21. Mudhafar M. Extraction identification of chemical constituents and *in vitro* evaluation against cells and bacterial pathogens of the leaves of polyalthia rumphii. J Med Pharm Chem Res. 2024;6(7):927-43.
22. Pandohee J, Stevenson PG, Conlan XA, Zhou XR, Jones OA. Off line two-dimensional liquid chromatography for metabolomics: an example using agaricus bisporus mushrooms exposed to UV irradiation. Metabolomics. 2015;11(4):939-51. doi: 10.1007/s11306-014-0749-4.
23. Vidovic M, Morina F, Milic S, Zechmann B, Albert A, Winkler JB. Ultraviolet-B component of sunlight stimulates photosynthesis and flavonoid accumulation in variegated plectranthus coleoides leaves depending on background light. Plant Cell Environ. 2015;38(5):968-79. doi: 10.1111/pce.12471, PMID 25311561.
24. Wargent JJ, Nelson BC, McGhie TK, Barnes PW. Acclimation to UV-B radiation and visible light in Lactuca sativa involves up-regulation of photosynthetic performance and orchestration of metabolome-wide responses. Plant Cell Environ. 2015;38(5):929-40. doi: 10.1111/pce.12392, PMID 24945714.
25. Zheoat AM. In silico studies for the antihypertensive effect of hibiscus acid and related compounds from Hibiscus sabdariffa. J Med Pharm Chem Res. 2025;7(3):473-9.
26. Atmakuru, R. Investigations on the photolysis of imazethapyr in aqueous solutions under direct sunlight by HPLC uv and lc-ms/ms-esi-applications of green alga as a potential biomarker. Int J Chem Res. 2012;3(1):45-51.
27. Pearse AD, Marks R. Response of human skin to ultraviolet radiation: dissociation of erythema and metabolic changes following sunscreen protection. J Invest Dermatol. 1983;80(3):191-4. doi: 10.1111/1523-1747.ep12534024, PMID 6827129.
28. Yanshole VV. Quantitative metabolomic analysis of changes in the lens and aqueous humor under development of age-related nuclear cataract. Metabolomics. 2019;15(3):29. doi: 10.1007/s11306-019-1495-4, PMID 30830501.
29. Ali AM, Monaghan C, Muggeridge DJ, Easton C, Watson DG. LC/MS-based discrimination between plasma and urine metabolomic changes following exposure to ultraviolet radiation by using data modelling. Metabolomics. 2023;19(2):13. doi: 10.1007/s11306-023-01977-0, PMID 36781606.
30. Issaq HJ, Van QN, Waybright TJ, Muschik GM, Veenstra TD. Analytical and statistical approaches to metabolomics research. J Sep Sci. 2009;32(13):2183-99. doi: 10.1002/jssc.200900152, PMID 19569098.
31. ALI AM, HUSSEIN AD. Data modelling of LC/MS-based metabolomic profiling to compare between human plasma and urine samples associated with beetroot juice. Int J Appl Sci Technol. 2022;4(4):17.
32. Pluskal T, Castillo S, Villar-Briones A, Oresic M. MZ mine 2: modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data. BMC Bioinformatics. 2010;11:395. doi: 10.1186/1471-2105-11-395, PMID 20650010.
33. Wishart DS, Tzur D, Knox C, Eisner R, Guo AC, Young N. HMDB: the human metabolome database. Nucleic Acids Res. 2007;35:D521-6. doi: 10.1093/nar/gkl923, PMID 17202168.
34. Conroy MJ, Andrews RM, Andrews S, Cockayne L, Dennis EA, Fahy E. LIPID MAPS: update to databases and tools for the lipidomics community. Nucleic Acids Res. 2024;52(D1):D1677-82. doi: 10.1093/nar/gkad896, PMID 37855672.
35. Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 2000;28(1):27-30. doi: 10.1093/nar/28.1.27, PMID 10592173.
36. Muhsen Ali A, Burleigh M, Daskalaki E, Zhang T, Easton C, Watson DG. Metabolomic profiling of submaximal exercise at a standardised relative intensity in healthy adults. Metabolites. 2016;6(1):9. doi: 10.3390/metabo6010009, PMID 26927198.
37. Trivedi DK, Iles RK. The application of SIMCA P+in shotgun metabolomics analysis of ZIC® HILIC-MS spectra of human urine-experience with the Shimadzu IT-TOF and profiling solutions data extraction software. J Chromatogr Sep Tech. 2012;3:1-5.
38. Triba MN, Le Moyec L, Amathieu R, Goossens C, Bouchemal N, Nahon P. PLS/OPLS models in metabolomics: the impact of permutation of dataset rows on the K-fold cross-validation quality parameters. Mol Biosyst. 2015;11(1):13-9. doi: 10.1039/c4mb00414k, PMID 25382277.
39. Monaghan C, McIlvenna LC, Liddle L, Burleigh M, Weller RB, Fernandez BO. The effects of two different doses of ultraviolet-A light exposure on nitric oxide metabolites and cardiorespiratory outcomes. Eur J Appl Physiol. 2018;118(5):1043-52. doi: 10.1007/s00421-018-3835-x, PMID 29516257.
40. Hart PH, Norval M. The multiple roles of urocanic acid in health and disease. J Invest Dermatol. 2021;141(3):496-502. doi: 10.1016/j.jid.2020.07.017, PMID 32919759.
41. Ashraf M, Foolad MR. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ Exp Bot. 2007;59(2):206-16. doi: 10.1016/j.envexpbot.2005.12.006.
42. Hildebrandt TM, Nunes Nesi A, Araujo WL, Braun HP. Amino acid catabolism in plants. Mol Plant. 2015;8(11):1563-79. doi: 10.1016/j.molp.2015.09.005, PMID 26384576.
43. Martinez Luscher J, Torres N, Hilbert G, Richard T, Sanchez Diaz M, Delrot S. Ultraviolet-B radiation modifies the quantitative and qualitative profile of flavonoids and amino acids in grape berries. Phytochemistry. 2014;102:106-14. doi: 10.1016/j.phytochem.2014.03.014, PMID 24713570.
44. Kim S, Lee H, Kim KH. Metabolomic elucidation of recovery of Melissa officinalis from UV-B irradiation stress. Ind Crops Prod. 2018;121:428-33. doi: 10.1016/j.indcrop.2018.05.002.
45. Correale J, Farez MF. Modulation of multiple sclerosis by sunlight exposure: role of cis-urocanic acid. J Neuroimmunol. 2013;261(1-2):134-40. doi: 10.1016/j.jneuroim.2013.05.014, PMID 23800457.
46. Korhonen E, Bisevac J, Hyttinen JM, Piippo N, Hytti M, Kaarniranta K. UV-B-induced inflammasome activation can be prevented by cis-urocanic acid in human corneal epithelial cells. Invest Ophthalmol Vis Sci. 2020;61(4):7. doi: 10.1167/iovs.61.4.7, PMID 32271889.
47. Zhu H, Wang N, Yao L, Chen Q, Zhang R, Qian J. Moderate UV exposure enhances learning and memory by promoting a novel glutamate biosynthetic pathway in the brain. Cell. 2018;173(7):1716-1727.e17. doi: 10.1016/j.cell.2018.04.014, PMID 29779945.
48. Adkins DE, McClay JL, Vunck SA, Batman AM, Vann RE, Clark SL. Behavioral metabolomics analysis identifies novel neurochemical signatures in methamphetamine sensitization. Genes Brain Behav. 2013;12(8):780-91. doi: 10.1111/gbb.12081, PMID 24034544.
49. Khalaf D, Krüger M, Wehland M, Infanger M, Grimm D. The effects of oral l-arginine and l-citrulline supplementation on blood pressure. Nutrients. 2019;11(7):1679. doi: 10.3390/nu11071679, PMID 31336573.
50. Oplander C, Volkmar CM, Paunel Gorgulu A, Van Faassen EE, Heiss C, Kelm M. Whole body UVA irradiation lowers systemic blood pressure by release of nitric oxide from intracutaneous photolabile nitric oxide derivates. Circ Res. 2009;105(10):1031-40. doi: 10.1161/circresaha.109.207019, PMID 19797169.
51. Rose AJ. Amino acid nutrition and metabolism in health and disease. Nutrients. 2019;11(11):2623. doi: 10.3390/nu11112623, PMID 31683948.
52. Stamler JS, Meissner G. Physiology of nitric oxide in skeletal muscle. Physiol Rev. 2001;81(1):209-37. doi: 10.1152/physrev.2001.81.1.209, PMID 11152758.
53. Mittelmaier S, Funfrocken M, Fenn D, Pischetsrieder M. 3-deoxygalactosone, a new glucose degradation product in peritoneal dialysis fluids: identification, quantification by HPLC/DAD/MSMS and its pathway of formation. Anal Bioanal Chem. 2011;399(4):1689-97. doi: 10.1007/s00216-010-4456-3, PMID 21136045.

54. Niwa T. 3-deoxyglucosone: metabolism, analysis, biological activity, and clinical implication. J Chromatogr B Biomed Sci Appl. 1999;731(1):23-36. doi: 10.1016/s0378-4347(99)00113-9, PMID 10491986.
55. Bernstein EF, Brown DB, Schwartz MD, Kaidbey K, Ksenzenko SM. The polyhydroxy acid gluconolactone protects against ultraviolet radiation in an *in vitro* model of cutaneous photoaging. Dermatol Surg. 2004;30:189-96. doi: 10.1111/j.1524-4725.2004.30060.x, PMID 14756648.
56. Liu Y, Liu J, Abozeid A, Wu KX, Guo XR, Mu LQ. UV-B radiation largely promoted the transformation of primary metabolites to phenols in Astragalus mongholicus seedlings. Biomolecules. 2020;10(4):504. doi: 10.3390/biom10040504, PMID 32225015.
57. Lyu J, Wang C, Liang DY, Liu L, Pandey LK, Xu HW. Sensitivity of wild and domesticated Rhododendron chrysanthum to different light regime (UVA, UVB, and PAR). Photosynthetica. 2019;57(3):841-9. doi: 10.32615/ps.2019.098.
58. Sun Q, Liu M, Cao K, Xu H, Zhou X. UV-B irradiation to amino acids and carbohydrate metabolism in rhododendron chrysanthum leaves by coupling deep transcriptome and metabolome analysis. Plants (Basel). 2022;11(20):2730. doi: 10.3390/plants11202730, PMID 36297754.
59. Wang X, Zang Y, Xue S, Shang S, Xin J, Tang L. Integrated analysis of the physiological, transcriptomic and metabolomic responses of Neoporphyra haitanensis after exposure to UV-B radiation: an energy metabolism perspective. Front Mar Sci. 2024;11:1372252. doi: 10.3389/fmars.2024.1372252.