Published in final edited form as:

Radiat Res. 2018 December ; 190(6): 576–583. doi:10.1667/RR15167.1.

Nonhuman Primates with Acute Radiation Syndrome: Results from a Global Serum Metabolomics Study after 7.2 Gy Total-Body Irradiation

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Abstract

Threats of nuclear terrorism coupled with potential unintentional ionizing radiation exposures have necessitated the need for large-scale response efforts of such events, including high-throughput biodosimetry for medical triage. Global metabolomics utilizing mass spectrometry (MS) platforms has proven an ideal tool for generating large compound databases with relative quantification and structural information in a short amount of time. Determining metabolite panels for biodosimetry requires experimentation to evaluate the many factors associated with compound concentrations in biofluids after radiation exposures, including temporal changes, pre-existing conditions, dietary intake, partial- vs. total-body irradiation (TBI), among others. Here, we utilize a nonhuman primate (NHP) model and identify metabolites perturbed in serum after 7.2 Gy TBI without supportive care [LD_{70/60}, hematologic (hematopoietic) acute radiation syndrome (HARS) level H3] at 24, 36, 48 and 96 h compared to preirradiation samples with an ultra-performance liquid chromatography quadrupole time-of-flight (UPLC-QTOF) MS platform. Additionally, we document changes in cytokine levels. Temporal changes observed in serum carnitine, acylcarnitines, amino acids, lipids, deaminated purines and increases in pro-inflammatory cytokines indicate clear metabolic dysfunction after radiation exposure. Multivariate data analysis shows distinct separation from preirradiation groups and receiver operator characteristic curve analysis indicates high specificity and sensitivity based on area under the curve at all time points after 7.2 Gy irradiation. Finally, a comparison to a 6.5 Gy (LD_{50/60}, HARS level H2) cohort after 24 h postirradiation revealed distinctly increased separations from the 7.2 Gy cohort based on multivariate data models and higher compound fold changes. These results highlight the utility of MS platforms to differentiate time and absorbed dose after a potential radiation exposure that may aid in assigning specific medical interventions and contribute as additional biodosimetry tools.

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INTRODUCTION

In the event of a large-scale mass casualty radiation event, response efforts must be established, including methods for high-throughput biodosimetry for determining required medical triage. Radiation emergencies include intentional exposures from an improvised nuclear device (IND), a radiological dispersal device (RDD) or a terrorist assault on nuclear infrastructure, among others. In addition, risk of unintentional exposures exists from nuclear power plants or occupational accidents. To achieve satisfactory methods to be used in biodosimetry, quantifiable biomarkers indicating both the presence and level of exposure must be determined. Obstacles impeding development of robust biomarker panels for biodosimetry include temporal changes in biomarker concentrations after radiation exposure (1), dose-dependent differences (2), pre-existing conditions (3), combined injury (4), genetic predisposition (5), dietary intake (6) and drug usage, among others. Furthermore, absorbed dose would correlate with severity of acute radiation syndrome (ARS) and dictate medical triage (in this study, cytokine therapy alone versus additional hematopoietic stem cell transplantation), requiring differentiation from biodosimetry methods.

To address these many facets of biomarker development for radiation exposure, several omic techniques have been used including genomics (7), transcriptomics (8), proteomics (9), and metabolomics and lipidomics (10). Of these, metabolomics is the collective analysis of molecules <1 kDa in a tissue or biofluid that directly reflects an organism's phenotypic state. Global metabolomics is a discovery type untargeted analysis ideal for relative quantification of thousands of perturbed compounds that can elucidate the multiple confounding factors associated with determination of absorbed radiation dose in a population. As ethical research prohibits intentional use of radiation experimentation on humans [thus limiting to radiotherapy (3, 11)], our group has concentrated on nonhuman primate (NHP) models, since they are the ideal available comparisons to humans for basic research and warranted under the Food and Drug Administration (FDA) Animal Rule (12). Network analyses on past NHP metabolomics studies have indicated perturbation of multiple metabolic pathways after irradiation including metabolism of steroids, amino acids, fatty acids, lipids, purine and pyrimidine catabolism, which show dose and temporal dependence. Continued studies to determine these effects after irradiation are paramount for metabolomics-based biodosimetry.

In this study, we performed global metabolomics on NHP serum at 24, 36, 48 and 96 h after 7.2 Gy TBI, and compared results to a previous study at 24 h after 6.5 Gy irradiation. After 7.2 Gy TBI, NHPs without supportive care will experience approximately 70% mortality within 60 days ($LD_{70/60}$), while 6.5 Gy exposure will elicit 50% mortality within 60 days ($LD_{50/60}$) ($\pm 10\%$). In addition to differences in overall mortality, severe hematologic (hematopoietic) acute radiation syndrome (HARS) is present in the 7.2 Gy cohort (H3) and a hematopoietic stem cell transplantation would be needed for human subjects (~ 4.2 Gy compared to NHPs at 7.2 Gy) while cytokine therapy would be recommended in the 6.5 Gy irradiated cohort (H2) (13). We found temporal fluctuations of compounds within 96 h postirradiation at 7.2 Gy exposure and higher fold changes of perturbed metabolites at 7.2 Gy compared to 6.5 Gy at 24 h postirradiation, which highlights the importance of biofluid

collection timing for successful interpretation of serum metabolic profiles in assigning levels of absorbed radiation dose.

MATERIALS AND METHODS

Animals and Animal Care

Nine naïve male rhesus macaques (*Macaca mulatta*, Chinese sub strain), 4.3–4.6 years of age and weighing 4.3–6.2 kg, were obtained from Primate Products, Inc. (Miami, FL) and maintained in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC). Animals were quarantined for six weeks prior to initiation of the experiment. Animal housing, health monitoring, care and enrichment during the experimental period have been described elsewhere (14). Due to study-specific reasons, paired housing was not possible during the experiment. The animals were housed individually, but they were able to see and touch conspecifics through the cage divider. This also eliminated the chance of conflict injuries that could have been caused by pair-housing. All procedures involving animals were approved by the Armed Forces Radiobiology Research Institute (AFRRI) Institutional Animal Care and Use Committee (IACUC) and Department of Defense Animal Care and Use Review Office (ACURO). This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (15).

Irradiations

NHPs received a 7.2 Gy dose of (LD_{70/60} without supportive care) cobalt-60 gamma radiation at a dose rate of 0.6 Gy/min from both sides of the core of the abdomen (bilateral, simultaneous exposure). The radiation field in the area of the NHP location was uniform within $\pm 5\%$.

AFRRI's dosimetry for photons is based on the alanine/EPR (electron paramagnetic resonance) dosimetry system. Currently, this is one of the most precise dosimetry techniques, which is used, in particular, by national standards laboratories for the most critical measurements and calibrations. Thus, it is one of the very few methods that are used in regular worldwide inter-comparisons of the national standards of Gray. All other details for NHP irradiations are described elsewhere (14).

Serum Sample Collection and Cytokine Analysis by Luminex

Blood was collected by venipuncture from the saphenous vein on the caudal aspect of the lower leg, placed in serum separating tubes, allowed to clot for 30 min and centrifuged (10 min, 400g, 4°C). Serum samples were stored at -70°C until use. Serum samples were stored at -70°C for cytokine analysis or shipped on dry ice to the Georgetown University Medical Center (Washington, DC) for metabolomic analysis.

A Luminex® 200 (Luminex Inc., Austin, TX) was used to simultaneously detect interleukin-8 (IL-8), IL-6, IL-18 and tumor necrosis factor- β (TNF- β) from NHP serum samples and were analyzed as described elsewhere (14). Cytokine analysis was performed using multiplex custom-ordered Bio-Plex® human cytokine assay kits (Bio-Rad® Inc.,

Hercules, CA). Cytokine quantification was performed using Bio-Plex Manager software version 6.1 (Bio-Rad Inc.).

Metabolite Extraction and LC-MS Analysis

Reagents (Optima™ grade; Thermo Fisher Scientific™ Inc., Hanover Park, IL) and chemicals (debrisoquine sulfate, 4-nitrobenzoic acid, carnitine, acetylcarnitine, propionylcarnitine, creatine, hypoxanthine, arginine, betaine) (Sigma-Aldrich® LLC, St. Louis, MO) were the highest purity available.

Samples were prepared and analyzed as previously described elsewhere (2). Briefly, serum (5 µl) was deproteinized with 195 µl 66% acetonitrile with internal standards [$2\text{ }\mu\text{M}$ debrisoquine ($M\text{ }p\text{ }H$)⁺=176.1188; $30\text{ }\mu\text{M}$ 4-nitrobenzoic acid ($M - H$)⁻=66.0141], vortexed, incubated on ice (10 min) and centrifuged for 10 min (max speed, 4°C). Samples were injected (2 µl) into a Waterst® Acquity Ultra Performance Liquid Chromatography (UPLC) with a BEH C18 1.7 µm, 2.1 × 50 mm column and coupled to a Xevot® G2 quadrupole time-of-flight (QTOF) mass spectrometer (MS) (Waters Corp., Milford, MA). Samples were acquired in both negative and positive electrospray ionization (ESI) data-independent modes with leucine enkephalin [$(M\text{ }p\text{ }H)$ ⁺=556.2771, ($M - H$)⁻=554.2615] used for LockSpray®.

Data Processing, Statistical Analysis and Compound Validation

Raw data files were manually inspected using MassLynx™ software, version 4.1 (Waters Corp.) and pre-processed through deconvolution and peak alignment in Progenesis® QI (Nonlinear Dynamics, Newcastle, UK) as previously described with preirradiation samples (–4 days) used as controls (2). The preprocessed data matrix was normalized to internal standards [debrisoquine (positive mode) or 4-nitrobenzoic acid (negative mode)]. A multidimensional scaling (MDS) plot for all ions and heatmap for the top 100 ranked ions in ESI⁺ were generated using the machine-learning algorithm Random Forests programmed in R version 2.15.2, and variable importance of validated compounds was visualized with the software MetaboAnalyst (16, 17). Univariate analysis was performed with a Welch's *t* test (*P*, 0.05) for spectral features present 70% in both groups using the software MetaboLyzer (18). Features 70% in a single group was analyzed categorically with a Barnard's test. Putative identification of the spectral features was determined by comparing monoisotopic mass (± 0 ppm error) to the human metabolome database (HMDB) (19) and chemical entities of biological interest database (ChEBI) (20). For analytical validation statistically significant ions must be examined through tandem MS (5–50 V ramping collision energy), which obtains fragmentation patterns that are compared to pure standards and compound databases (e.g., METLIN tandem MS database) for unambiguous identification (21, 22). Validated compounds were checked for outliers and plotted in GraphPad Prism 6 (La Jolla, CA), and receiver operator characteristic (ROC) curves were generated to assess the area under the curve (AUC) and classification performance using a principal component analysis (PCA) class model for dose comparisons or a partial least squares (PLS) model for time comparison in SIMCA-P⁺15 (Umeå, Sweden).

RESULTS AND DISCUSSION

LC-MS Global Metabolomics

A total of 1,516 spectral features were detected in positive mode and 1,016 in negative mode. A MDS plot was constructed for the first two dimensions, which are representative of the greatest contribution of ion variability to the Random Forests model. Multivariate data analysis revealed a slight separation at 24 h from the preirradiation group along dimension 1 of a MDS plot, with greater separation at 36 and 48 h time points (Fig. 1A). The 48 and 96 h groups separated along dimension 1 from preirradiation with increasing separation along dimension 2. Heatmap visualization of the top 100 ranked ions from Random Forests showed higher concentrations of some spectral features at 36 and 48 h with a return to baseline levels at 96 h and another group of spectral features found in higher concentration at 96 h (Fig. 1B). A subset of significant ions were validated through tandem MS (Table 1, Fig. 2) and compared to other NHP biofluids and time points, thus increasing metabolite coverage through 96 h (Table 2). Similar trends are observed in previously reported studies, however, shifts in propionylcarnitine are observed between 12 and 24 h and may be dose specific, as previously reported (23). Furthermore, hypoxanthine and arginine increase at 24 and 36 h, but decrease at day 7 postirradiation. In another study, rapid increases (i.e., 4 h) were found in MG (18:2) levels and then a drop below preirradiation levels after 24 h (1), which was also observed here. The variable importance of validated compounds showed carnitine contributing most to a Random Forests model in MetaboAnalyst with the other acylcarnitines in the top five compounds (Fig. 3A). ROC curves generated in SIMCA-P⁺ 15 from a PLS model showed excellent (AUC = 0.90) to moderate (AUC = 0.75) classification performance for 24 (AUC = 0.91), 36 (AUC = 0.91), 48 (AUC = 0.87) and 96 h (AUC = 0.80) time points (Fig. 3B) (24).

Of the validated compounds, carnitine was found in highest levels at 24 h ($P < .001$, 3.76 fold change) but was also significantly higher from preirradiation levels at 36 ($P < .001$, 1.81 fold change), 48 ($P < .001$, 1.99 fold change) and 96 h ($P < .001$, 2.05 fold change). Similarly, levels of acetylcarnitine ($P < .001$, 1.96 fold change) and propionylcarnitine ($P < .001$, 4.56 fold change) were higher at 24 h, as well as 96 h ($P = 0.022$, 1.65 fold change) for acetylcarnitine and 36 ($P < .001$, 4.62 fold change) and 96 h ($P < 0.036$, 1.60 fold change) for propionylcarnitine. Carnitine and its corresponding acyl ester compounds have been identified and quantified in numerous studies and can show drastic dose-dependent changes due to radiation exposure (10, 25). These compounds are integral for fatty acid transportation across mitochondrial membranes for subsequent lipid metabolism by enzymatic β oxidation. The intricate linkages between levels of carnitine and acylcarnitines play several roles in carbohydrate and lipid metabolism. Thus, perturbed levels have been linked to many metabolic disorders and disease states. While increased levels of carnitine have been documented at 7 days (~4 Gy and higher in urine and serum), here we show it remains elevated throughout 24–96 h after irradiation and may be a marker for more immediate biodosimetry (2, 23, 26). Acetylcarnitine also increased in biofluids at 7 days (>.4 Gy in urine, >.7 Gy in serum) (2, 25, 26). While carnitine and acetylcarnitine consistently show increases in NHP biofluids at all doses in previously published studies (>.2 Gy), other acylcarnitines, such as propionylcarnitine, butyrylcarnitine and

valeryl carnitine, were previously found to be decreased at day 7 after 2 Gy irradiation (23). Given the ubiquity of carnitine and acylcarnitine elevation in multiple animal models and time points, they are valuable compounds to include in a potential radiation marker panel, however, false positives due to previously mentioned metabolic disorders, dietary intake and temporal patterns of perturbation must be considered as well as large differences between sexes (26).

Creatine was only moderately higher at 24 h ($P = 0.001$, 2.22 fold change) and, interestingly, two other common metabolites normally found at different concentrations (citrulline and hypoxanthine) were not detected (citrulline) or were not perturbed to a high degree (hypoxanthine only significantly higher at 36 h, $P = 0.009$, 1.38 fold change). Creatine can be synthesized in the liver or kidney through arginine amidino group transfer to glycine by enzymatic action of glycine amidinotransferase forming guanidinoacetate and subsequent methylation by guanidinoacetate N-methyltransferase. Changes in creatine and resulting muscular weakness have long been observed from radiation exposure (27). Creatine increased in serum at 24 h and returned to basal levels at 36–96 h, which is consistent with patterns observed in urine (28), however, it increased again at day 7 after >7 Gy doses (26). Hypoxanthine is an indicator of DNA damage as it is formed from adenine deamination or enzymatic transformation of inosine. At day 7, hypoxanthine slightly increased in urine and decreased in serum, however, the levels did not appear to be dose dependent (26, 29). It is also increased in urine at 24 h after 8.5 Gy and 36 h after 6.5 Gy irradiation (28) and in human males at 6 h after TBI (11). In this study, we saw a slight increase at 36 h after 7.2 Gy irradiation, indicating that hypoxanthine may be a better biomarker to determine more pronounced stages of ARS.

Two amino acids, arginine and betaine, were also found perturbed in this study. Arginine increased at 24 h ($P = 0.005$, 1.33 fold change) and 36 h ($P < 0.001$, 1.45 fold change) and betaine decreased at 48 h ($P = 0.002$, 0.65 fold change) and 96 h ($P < 0.022$, 0.71 fold change). Arginine is obtained from dietary sources (essential amino acid) or can be synthesized from citrulline by argininosuccinate synthase to form argininosuccinic acid, then cleaved by argininosuccinate lyase to arginine and fumarate. It has been used in combination with glutamine and 3-hydroxyisovaleric acid as a mitigator for radiation-induced gastrointestinal injury (30) and has a myriad of other functions, including serving as a precursor building block for other amino acids, growth hormone stimulation and functions in the urea cycle. While decreases have been observed at day 7, here levels increased at 24 and 36 h postirradiation. Betaine is an alpha amino acid (amino group attached to alpha carbon) that is an oxidized form of choline and is involved in osmoregulation, detoxification, reduction of nitric oxide release and, similar to arginine, may alleviate gastrointestinal injury (31). While previously detected by a ^1H NMR platform and found to increase in mouse urine at 72 h after 3 Gy and day 5 after 3, 5 and 8 Gy irradiation, it has not been detected in previously reported NHP models (32). The reduction seen at 48 and 96 h postirradiation here may indicate a higher incidence of oxidation during this period.

Two lipid compounds also decreased, including LysoPC (14:0) at 24 h ($P = 0.003$, 0.80 fold change) and 96 h ($P = 0.008$, 0.75 fold change) and MG (18:2) at 36 h ($P = 0.009$, 0.94 fold change) and 48 h ($P = 0.018$, 0.95 fold change). The pattern of MG (18:2) is similar to an

observed lipidomic serum analysis of NHPs after 6.5 Gy irradiation, where MG (18:2) showed a high increase at 4 h, returned to basal levels at 8 h, followed by a steady decrease up to 72 h. LysoPC (14:0) was not identified in previous NHP studies. In addition to acylcarnitines, purines and amino acid like compounds, lipids are prone to oxidation due to structural patterns of unsaturation (one or more double bonds in acyl chains) and have been the topic of previously published radiation lipidomic studies (1, 2, 33–35). Individual lipid levels are highly temporally dynamic within a month postirradiation (1), and some may be used to determine delayed effects of acute radiation exposure, such as fibrosis (33). Others, such as free fatty acids, can be hydrolyzed from larger inert compounds, such as triacylglycerides and phosphatidylcholines. Arachidonic acid and docosahexaenoic acid are fatty acids that can be enzymatically converted to pro-inflammatory compounds through the cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 pathways that can accompany cytokine production. At high radiation doses (i.e., 10 Gy), these are found in higher concentration as acyl components on larger inert lipids, which may be used for transportation to wound site (2), and exposure to radiation increases their inflammatory eicosanoid products (3, 35). While inflammatory eicosanoids were not tested in the current study, pro-inflammatory cytokine levels showed significant differences between pre- and postirradiated samples [TNF- β (96 h), IL-6 (48 h) and IL-18 (36, 48, 96 h)], as well as cytokines involved in neutrophil chemotaxis [IL-8 (48 h)] (Supplementary Fig. S1; <http://dx.doi.org/10.1667/RR15167.1.S1>).

Comparison to 6.5 Gy Dose at 24 h

In a previously published study, our group examined global serum metabolomic profiles of NHPs at 12 and 24 h after 6.5 Gy irradiation as part of a gamma-tocotrienol (GT3) efficacy study (36). Given the importance of these treatments, we compared differences in our validated compounds at 24 h postirradiation (Supplementary Figs. S2–4, Table 3). Hypoxanthine was present in minimal concentrations in the 6.5 Gy irradiated cohort and is not included in the current comparison. Higher fold changes were observed for all compounds in the 7.2 Gy irradiated cohort (Table 3). Interestingly, an opposite trend was observed for propionylcarnitine between the 6.5 and 7.2 Gy irradiated cohorts, but differing levels have been observed at day 7 postirradiation where 2 Gy is lower from a control group but increased at 4–10 Gy (23). PCA and ROC curve analysis of the validated compounds show better separation and predictability at 7.2 Gy (PCA $R^2 = .97$, $Q^2 = 0.63$; AUC = 0.95) than 6.5 Gy (PCA $R^2 = 0.70$, $Q^2 = -0.03$; AUC = 0.80) (Supplementary Fig. S4).

A 6.5 Gy dose of radiation is the LD_{50/60} value for this animal model in the absence of supportive care, which elicits hematopoietic syndrome and would be appropriate for cytokine therapy in humans (1, 14). At the current dose of 7.2 Gy (LD_{70/60}), additional mortality and minor gastrointestinal damage would occur and medical interventions would be drastically different, as nearly all bone marrow would be damaged and stem cell transplantation would be required. Hematopoietic stem cells for transplantation can be collected from a different part of the patient's body (autologous) or from matching donors (allogeneic). In terms of allogeneic transplantation, the lack of human leukocyte antigen (HLA)-matched siblings and the urgency related to nuclear disaster would require a higher rate of using haploidentical donors, thus increasing chances of primary graft failure (37).

Unnecessary transplants may also lead to increased long-term morbidity, neurocognitive dysfunction and fatigue (38, 39). A noninvasive method for differentiating between these doses would be beneficial for administering the proper therapy. Comparing these two cohorts, the metabolomic signature is clearly more pronounced in the 7.2 Gy irradiated cohort for carnitine, propionylcarnitine and creatine. Superior separation as evidenced by PCA plots and classification by ROC curve analysis (Supplementary Fig. S4; [http://dx.doi.org/10.1667/RR15167.1.S1](https://doi.org/10.1667/RR15167.1.S1)) demonstrate that proper metabolite panels may be able to discriminate between these two cohorts and predict therapeutic strategies.

CONCLUSIONS

We utilized a LC-MS platform to perform a global metabolomic analysis of NHP serum at 24, 36, 48 and 96 h after 7.2 Gy (LD_{70/60}) TBI, compared levels of validated compounds to other NHP biofluids analyzed in previously published studies by our group and others (1, 2, 23, 25, 26, 28, 29, 36, 40) and finally compared these results to those from our previous work, after a 6.5 Gy (LD_{50/60}) dose (36). We identified serum metabolites and cytokines perturbed 24–96 h after 7.2 Gy TBI, which are relevant time points for identifying exposed individuals and assigning medical triage for ARS after potential radiation accidents. In addition, validated metabolites exhibited increased fold changes, higher separation from multivariate data analysis and higher sensitivity and specificity compared to 6.5 Gy exposure, which could potentially identify individuals requiring hematopoietic stem cell transplantation in addition to cytokine therapy.

To shift MS-based metabolomics from laboratory to clinic requires detailed knowledge of both temporal differences and dose effects on metabolite levels, as in the event of a nuclear emergency from an IND or industrial accident, in which the logistics of collecting biofluids from a population at a set time point is unreasonable. Current efforts are underway to make these metabolomic studies more clinically relevant, such as identifying confounding issues associated with comparison to a human population (3, 11) (e.g., pre-existing conditions and combined injury). Higher throughput from MS platforms may be achieved from additional advances in front-end separations, such as differential mobility spectrometry (29). Also, further experimentation should continue to increase the precision of dose estimates by utilizing a multiple biomarker panel and possibly integrating biomarkers from other studies, such as transcriptomics or proteomics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

This work was funded by the National Institutes of Health (National Institute of Allergy and Infectious Diseases, grant no. 1R01AI101798; PI AJF) and the Defense Threat Reduction Agency (CBM.RAD.01.10.AR.005 to VKS). The authors acknowledge the Lombardi Comprehensive Cancer Metabolomics Shared Resource, which is in part supported by award no. P30CA051008 (PI Louis Weiner) from the National Cancer Institute. Partial support was provided by PHS grant nos. 5 T32 CA 968620 and CMCR 5U19AI067773–13 (PI Evan L. Pannkuk) [subaward no. 20(GG011896–34)]. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute, National Institutes of Health, the Armed Forces Radiobiology Research Institute, the Uniformed Services University of the Health Sciences or the U.S. Department of Defense.

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FIG. 1.

Analysis with the Random Forests algorithm showing (panel A) multidimensional scaling (MDS) plot of all ions in ESI⁺ and (panel B) heatmap of the top 100 ions from ESI⁺. The 36 and 48 h groups separate from preirradiation and 24 h along dimension 1, while the 96 h group is closer to preirradiation but separates along dimension 2. A large group of ions are visibly upregulated in the 36 and 48 h groups from others as visualized by a heatmap (dimension 1 distance -0.2–0.4, dimension 2 distance -0.4–0.4; open circles represent misclassified samples).

FIG. 2.

Validated metabolites with significant concentration fluxes 24, 36, 48 and 96 h after 7.2 Gy irradiation identified by LC-MS global metabolomics. * $P < 0.05$ and ** $P < 0.001$.

FIG. 3.

Panel A: Validated compounds ranked by contribution with Random Forests analysis (lowest to highest contribution to model performance from bottom to top; lower values indicate less importance to each metabolite in the predicting group). Color bar (right) indicates importance of metabolites to each group (high = red, low = green). Note higher importance correlates with fold change intensity represented in Fig. 2. Panel B: Receiver operator characteristic (ROC) curves of validated compounds generated by partial least squares (PLS) regression in SIMCA-P⁺ 15.

TABLE 1

Validated Compounds in NHP Serum Perturbed after TBI

RT min	Experimental m/z	Calculated m/z	Mass error ppm	Formula	HMDB ID	Metabolite name
0.27	162.1127	162.1130	1.46	$C_7H_{13}NO_3$	0000062	Carnitine
0.28	204.1234	204.1236	1.76	$C_9H_{17}NO_4$	0000201	Acetylcarnitine
0.31	218.1397	218.1392	4.84	$C_{10}H_{19}NO_4$	0000824	Propionylcarnitine
0.27	132.0771	132.0773	2.57	$C_4H_9N_3O_2$	0000064	Creatine
0.29	137.0463	137.0463	3.58	$C_5H_4N_4O$	0000157	Hypoxanthine
6.92	468.3099	468.3070	1.12	$C_{22}H_{46}NO_7P$	10379	LysoPC (14:0) ^a
7.89	355.2830	355.2848	3.55	$C_{21}H_{38}O_4$	11538	MG (18:2) ^a
0.24	175.1195	175.1195	2.88	$C_6H_{14}N_4O_2$	0000517	Arginine
0.27	118.0864	118.0869	1.46	$C_5H_{11}NO_2$	0000043	Betaine

^aThe precise stereochemistry of these could not be determined with the current technique; all H⁺ adducts.

TABLE 2

Time Points and Levels of Metabolites in NHP Biofluids after Irradiation

Metabolite name	4 h	12 h	24 h	36 h	48 h	96 h	Day 7
Carnitine			↑ ^c *	↑*	↑*	↑*	↑ ^{d,f,j}
Acetylcarnitine			↑*			↑*	↑ ^{f,k,o}
Propionylcarnitine		↓ ^c	↑*	↑*			↓↑ ^l
Creatine			↑ ^a *				↑ ^h
Hypoxanthine			↑ ^b	↑*			↓ ^e ↑ ^{g,n}
LysoPC (14:0)			↓*			↓*	
MG (18:2)	↑ ⁱ			↓*	↓*		
Arginine			↑*	↑*			↓ ^m
Betaine					↓*	↓*	

* Current study.

^a urine after 6.5 Gy [11.2 fold change (FC)] irradiation (28).

^b urine after 8.5 Gy (3.4 FC) irradiation (28).

^c serum after 6.5 Gy (1.6 FC) irradiation (36).

^d serum after 2 (1.3 FC), 4 (1.6 FC), 6 (1.6 FC), 7 (1.8 FC) and 10 Gy (2.1 FC) irradiation (2).

^e serum after 4 (0.7 FC), 6 (0.5 FC) and 7 Gy (0.6 FC) irradiation (2).

^f urine after 4 (8.6 FC), 6 (18.6 FC), 7 (42.0 FC) and 10 Gy (137.4 FC) irradiation (26).

^g urine after 2 (1.7 FC), 7 (1.5 FC) and 10 Gy (2.4 FC) irradiation (26).

^h urine after 7 (2.2 FC) and 10 Gy (7.7 FC) irradiation (26).

ⁱ serum after 6.5 Gy (2.5 FC) irradiation (1).

^j serum after 6 (1.5 FC), 7 (1.5 FC), and 10 Gy (2.2 FC) irradiation (23).

^k serum after 7 (1.8 FC) and 10 Gy (2.8 FC) irradiation (23).

^l serum down at 2 Gy (0.4 FC) but up at 10 Gy (1.9 FC) irradiation (23).

^m serum after 2 (0.6 FC), 4 (0.7 FC), 6 (0.7 FC) and 7 Gy (0.7 FC) irradiation (23).

ⁿ also quantified by differential mobility spectrometry-mass spectrometry (40).

^o also quantified by differential mobility spectrometry-mass spectrometry (25).

TABLE 3

Compound Levels in NHP Serum 24 h after 6.5 or 7.2 Gy TBI Compared to Preirradiation Serum Levels

Metabolite	Fold change (6.5 Gy) ^a	<i>P</i> value	Fold change (7.2 Gy)	<i>P</i> value
Carnitine	1.66	0.007	3.76	<0.001
Acetylcarnitine	1.80	0.062	1.96	<0.001
Propionylcarnitine	0.79	0.046	4.56	<0.001
Creatine	1.33	0.184	2.22	0.001
LysoPC (14:0)	0.77	0.247	0.80	0.003
MG (18:2)	0.94	0.110	0.97	0.150
Arginine	1.10	0.420	1.33	0.005
Betaine	0.85	0.398	0.93	0.780

^a Values were obtained from a previously published study (36). *P* value determined by a Welch's *t* test; all H+ adducts.