Published in final edited form as:

J Proteome Res. 2017 May 05; 16(5): 2091–2100. doi:10.1021/acs.jproteome.7b00064.

Gas Chromatography/Mass Spectrometry Metabolomics of Urine and Serum from Nonhuman Primates Exposed to Ionizing Radiation: Impacts on the Tricarboxylic Acid Cycle and Protein Metabolism

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Abstract

Ionizing radiation (IR) directly damages cells and tissues or indirectly damages them through reactive free radicals that may lead to longer term adverse sequelae such as cancers, persistent inflammation, or possible death. Potential exposures include nuclear reactor accidents, improper disposal of equipment containing radioactive materials or medical errors, and terrorist attacks. Metabolomics (comprehensive analysis of compounds <1 kDa) by mass spectrometry (MS) has been proposed as a tool for high-throughput biodosimetry and rapid assessment of exposed dose and triage needed. While multiple studies have been dedicated to radiation biomarker discovery, many have utilized liquid chromatography (LC) MS platforms that may not detect particular compounds (e.g., small carboxylic acids or isomers) that complementary analytical tools, such as gas chromatography (GC) time-of-flight (TOF) MS, are ideal for. The current study uses global GC-TOF-MS metabolomics to complement previous LC-MS analyses on nonhuman primate biofluids (urine and serum) 7 days after exposure to 2, 4, 6, 7, and 10 Gy IR. Multivariate data analysis was used to visualize differences between control and IR exposed groups. Univariate analysis was used to determine a combined 26 biomarkers in urine and serum that significantly changed after exposure to IR. We found several metabolites involved in tricarboxylic acid cycle function, amino acid metabolism, and host microbiota that were not previously detected by global and targeted LC-MS studies.

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Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jproteo-me.7b00064. Table S1. Log2 fold-changes after different exposure levels to γ radiation. Figure S1. Multivariate data analysis of serum and urine from NHPs exposed to IR. (PDF)

Notes

The authors declare no competing financial interest.

Graphical Abstract

Keywords

gas chromatography; mass spectrometry; ionizing radiation; nonhuman primate; metabolomics

1. INTRODUCTION

In addition to direct effects from ionizing radiation (IR) interacting with biomolecules, the indirect effects include hydrolysis of water creating reactive oxygen species (ROS) capable of damaging DNA, destruction of cellular bilayers, and molecular peroxidation. Potential exposures to IR include nuclear accidents and terrorist attacks toward civilian populations, such as a radioactive dispersal device (RDD) also referred to as dirty bomb, an improvised nuclear device (IND), or malicious acts on nuclear plants. Biodosimetry is required following exposure to radiation exposure to determine an individual's dose. A recent paper compared five common biodosimetry methods (cytokinesis blocked micronucleus, dicentric chromosome analysis, lymphocyte numbers or depletion, γ -H2AX levels, and in vivo tooth electron paramagnetic resonance) among civilian and military populations, concluding that no methods were capable of processing samples for a civilian population of 50 000 within 2 days, and a combination of two methods was capable within 6 days.¹ However, these results assumed higher time periods for sample transportation, lab processing, and reporting results that may be reduced by specialized robotic platforms, such as the rapid automated biodosimetry tool (RABiT).² Thus high-throughput biodosimetry that would reduce time to determine an individual's level of exposure would aid in reducing mortality through triage, physiological turmoil, and economic burden.³ A promising avenue for a "biologically based" dosimetry method is metabolomics (comprehensive analysis of compounds <1 kDa).^{4–11} Ultimately a select metabolite panel selected should differentiate doses that may cause hematopoietic syndrome (~2–6 Gy in humans), gastrointestinal syndrome (~6–12 Gy in humans), or negligible effects from acute radiation syndrome. Continued metabolomic experiments to determine a robust panel of biomarkers combined with continued instrumentation development are critical for biodosimetry after accidental radiation exposures.

The field of metabolomics contributes to the broader field of system biology (e.g., incorporation of genomics, proteomics, etc.) by attempting to obtain a comprehensive view of small molecules present in cells, tissues, or biofluids that may explain perturbations due

to disease states or other phenomena.¹² To obtain this comprehensive view of the metabolome, multiple analytical platforms are required due to the wide chemical diversity of compounds being detected. Within the field of radiation metabolomics, past studies have primarily utilized liquid chromatography (LC) mass spectrometry (MS),^{5,8,11,13–18} with less attention given to gas chromatography (GC) MS^{7,19–21} and nuclear magnetic resonance (NMR) platforms.²² GC–MS is a well-established bioanalytical platform ideal for detecting myriad molecules including volatile small metabolites, isomeric compounds, derivatized organic acids, and carbohydrates.²³ Many of these compounds are vital in energy metabolism (e.g., Warburg effect) and are difficult to analyze by LC–MS because of their inherent chemical properties (e.g., chromatographic separation of isomers).²⁴ In addition to the specificity and sensitivity for identifying specific compounds, multiple databases (e.g., NIST 14 Mass Spectral Library, FiehnLib, MassBank, Golm repository) are dedicated to the identification of compounds based on retention indices or highly reproducible electron impact spectra.^{25–27} The combination of sensitivity, specificity, identification from large user libraries, and powerful deconvolution software places GC–MS as an excellent analytical platform within the “toolbag” for metabolomic studies.

In the current study, we applied a GC time-of-flight (TOF) MS platform to obtain global metabolomic signatures of biofluids (urine and serum) from nonhuman primates (NHPs) 7 days after total body irradiation (TBI). As previously mentioned,¹⁶ 7 day biomarkers are needed because individuals may need extended time for sample processing due to ensuing alarm and panic following potential mass radiation exposures. Additionally, NHP systems have the advantage of (1) clinical signs and mitigator effects from IR exposure being well documented,^{28–31} (2) continually expanding knowledge on metabolomic signatures from IR exposure,^{9,10,13,16,17,32,33} and (3) genetically closer to human studies than murine models.¹⁸ We found several small carboxylic acids, amino acids, and tricarboxylic acid (TCA) cycle intermediates not previously identified by targeted and global metabolomic studies performed on LC–MS platforms. Identification of these compounds through global GC–MS analysis can be further developed for faster targeted LC methodology. These studies combined contribute to the National Institute of Allergy and Infectious Diseases (NIAID) goals of developing high-throughput field deployable instruments for biodosimetry.

2. MATERIALS AND METHODS

2.1. Nonhuman Primate System

The NHPs used included 12 animals (1/2 female, 1/2 male) per treatment that were single-housed and received 0, 2, 4, 6, 7, or 10 Gy TBI as previously described (for urine 2 and 10 Gy $n = 10$, 4 Gy $n = 11$ due to fecal contamination).^{10,16,17} Urine and serum were collected on day 7, stored at -70°C , and shipped on dry ice to Georgetown University Medical Center. Urine was collected by cystocentesis for the treatment group and overnight for the control group. (Urine contaminated by fecal material was discarded.) Veterinarians monitored animals for 24 h/day during the experiment, and strict protocols reviewed and approved by the Institutional Animal Care and Use Committee were followed.

2.2. Chemicals

All reagents were Fisher Optima grade (Fisher Scientific, Hanover Park, IL). Authentic chemical standards were obtained from Sigma-Aldrich (St. Louis, MO) (citric acid, lactic acid, *cis*-aconitic acid, isocitric acid, malic acid, phenylacetic acid, hippuric acid, hexanedioic acid, threonine, palmitic acid, methylsuccinic acid, succinic acid, glycine, leucine, asparagine, isoleucine, serine, lysine, pyroglutamic acid, pipecolic acid, 3-hydroxybutyric acid, glyceric acid, alanine, and aspartic acid), Santa Cruz Biotechnology (Dallas, TX) (propionylglycine), or MP Biomedicals (Santa Ana, CA) (4-hydroxyproline). Internal standards were obtained from Sigma-Aldrich (4-nitrobenzoic acid) or Cambridge Isotope Laboratories (Andover, MA) (citric acid- d_4 , D-sorbitol- $^{13}\text{C}_1$, D,L-glutamic acid- d_5 , and L-leucine- d_3). Chemicals for derivatization and analysis were obtained from Sigma-Aldrich (urease type III, anhydrous sodium sulfate, C4-C24 FAMES, methyl nonanoate, and alkane standard mix [C₁₀-C₄₀]) or Thermo Scientific (Waltham, MA) (*N*-methyl -*N*-[trimethylsilyl] -trifluoroacetamide (MSTFA) with 1% [vol/vol] trimethyl-chlorosilane (TMCS)).

2.3. Sample Preparation and Analysis

Urine samples were processed with modification to previously described methods.³⁴ Urine or serum was aliquoted (50 μL per sample, 1 μL per sample pooled for quality control (QC)), internal standards were added (final concentration 20 $\mu\text{g}/\text{mL}$), and urine was treated with 5 μL (160 mg/mL) of urease for 1 h at 37 °C with gentle agitation. Methanol (1 mL) was added and samples were centrifuged at 13 000 rpm for 10 min at 4 °C. The supernatant was transferred to silylated glass tubes, evaporated under N₂, and transferred to a GC vial. Toluene (100 μL , dried with anhydrous sodium sulfate) was added and evaporated before derivatization to reduce H₂O contamination. MSTFA with 1% TCMS (100 μL) was used for derivatization for 1 h at 60 °C with rigorous shaking, and samples were injected (1.5 μL) into the GC-TOF-MS.

Samples were analyzed using a Leco Pegasus HT GC-TOF-MS (St. Joseph, MI) equipped with Gerstel (Linthicum, MD) multipurpose sampler (with cooled injection system and automated liner exchange) and an Rtx-5 (G27) column (5% diphenyl/95% dimethyl polysiloxane, 30 m $\times$ 0.25 mm $\times$ 0.25 μm , length $\times$ internal diameter $\times$ film thickness) with a 5 m Integra-Guard column. The inlet and transfer line temperatures were set to 220 and 270 °C, respectively. Oven temperature program was 70 °C for 0.2 min, then 70 to 270 °C at 10 °C/min (held 4 min), then 270 to 320 °C at 20 °C/min (held 2 min). The injection conditions were 1.5 μL sample volume, 1:10 split, carrier gas helium, 1.2 mL/min constant gas flow rate, and 220 s solvent delay. Electron ionization (EI) mass spectra were collected with scan range from m/z 40 to 600 with the ion source 200 °C and 30 spectra/s acquisition rate. Samples were randomized and 10 samples were injected, followed by a QC sample, alkane reference mix, pyridine, and MSTFA. The inlet was changed after every 10 injections. Data acquisition and analysis was performed with ChromaTOF software (Leco, v. 4.51.6.0).

2.4. Data Processing, Statistical Analysis, and Marker Validation

The collected spectra were processed and deconvoluted with the software ChromaTOF v. 4.51.6.0 (Leco, St. Joseph, MI) with a baseline offset of 2. Spectra were aligned with the statistical compare function with mass threshold of 10, minimum similarity match of 600 to the NIST 14 Mass Spectral Library, max retention time (rt) difference of 2 s, signal-to-noise ratio of 20, and minimum 50% of samples in a class containing the analyte. The deconvoluted and aligned spectra were normalized to the appropriate internal standard (amino acid, carbohydrate, or carboxylic acid). Deconvoluted data were analyzed in the programming language R (v.2.15.2) with the machine-learning algorithm Random Forests (RF), generating a multidimensional scaling plot (MDS) of the top-100 ranked ions for urine or serum and a heatmap (top-40 ranked ions for urine, top-30 ranked ions for serum). Volcano plots (of analytes present in 70% of samples in a group, false discovery rates determined by Benjamini–Hochberg step-up procedure, significant ions determined with Welch's *t* test) were generated with the in-house statistical software MetaboLyzer, and unsupervised standard singular value decomposition-based principal component analysis (PCA) plots were generated.³⁵ Statistically significant ions were determined with a Kruskal–Wallis test and a posthoc Duncan test in SAS 9.4 (Cary, NC). Significant ions were validated by matching their retention indices and EI spectra to authentic standards or the LECO/Fiehn Metabolomics Library and comparison with the NIST mass spectral library. Validated ions were graphed without imputing missing values using GraphPad Prism 6 (GraphPad Software, La Jolla, CA) (means $\pm$ standard error of the mean (SEM), * indicating significantly different from control [$P < 0.05$]). Significant ions for both urine and serum were saved in .csv format and imported into MetaboAnalyst (<http://www.metaboanalyst.ca>³⁶).

3. RESULTS

3.1. Multivariate Data Analysis

Multivariate data analysis was utilized to identify global differences between the groups. An unsupervised PCA scores plot for urine indicated increasing separation along principal component (PC) 1 between the control, 6 Gy (a dose leading to hematopoietic syndrome), and 10 Gy (a dose leading to gastrointestinal syndrome) (PC1–29.0%, PC2–19.9%, PC3–8.3%) (Figure 1A). For urine, complete separation occurs between the control and 10 Gy groups. Similar results are observed in the MDS plot generated by RF with the top-100 ranked ions, where the greatest separation between control, 2 Gy, and 4 Gy occurred primarily along dimension 2 in urine (Figure 1B). At IR doses causing hematopoietic syndrome (6 and 7 Gy), separation occurs along dimension 1 and complete separation from control and 10 Gy (gastrointestinal syndrome). A total of 63 significant ions were detected by a volcano plot between control and 10 Gy groups and indicate decreased concentration of metabolites in IR-exposed NHPs (significant analytes detected on left of plot). For serum, experimental groups separated completely from the control along dimension 1, with separation along dimension 2 occurring from 2 to 10 Gy (Figure S1A). Two distinct groups are apparent, where one group is composed of overlapping 2 and 4 Gy and the other group is composed of overlapping 7 and 10 Gy. These two groups almost completely separate along dimension 2. The 6 Gy group overlaps with all other experimental groups. The heatmap

shows lowering concentration of most significant metabolites with some increasing at 10 Gy (Figure S1B).

3.2. Biomarker Identification and Validation

3.2.1. Urine—A total of 277 compounds were aligned and putatively identified by the statistical compare function in ChromaTOF. Compounds were mined for significance by utilizing a Kruskal–Wallis test and validated to an authentic standard by comparison of the retention index and similarity of the EI spectra. Highest log₂ fold-change by exposed dose is reported with all log₂ fold-changes supplied in Table S1. Six intermediates of the TCA cycle were affected by IR exposure (Figure 2, Table 1, Table S1). Citric acid (−4.19 log₂ fold-change, 10 Gy) and isocitric acid (−1.84 log₂ fold-change, 10 Gy) significantly decreased at all IR exposure levels, while *cis*-aconitic acid (−1.29 log₂ fold-change, 10 Gy) and succinic acid (−2.52 log₂ fold-change, 10 Gy) decreased at 6, 7, and 10 Gy and malic acid (−5.02 log₂ fold-change, 10 Gy) at 7 and 10 Gy, which NHPs would experience hematopoietic (6–10 Gy) and gastrointestinal syndrome (10 Gy) (Table 1). Fumaric acid decreased slightly at IR doses causing hematopoietic and gastrointestinal syndrome (6–10 Gy), although not significantly ($P = 0.329$, −1.79 log₂ fold-change, 10 Gy) (Figure 2). Phenylacetic acid (−2.23 log₂ fold-change, 10 Gy), palmitic acid (−1.51 log₂ fold-change, 10 Gy), methylsuccinic acid (−3.75 log₂ fold-change, 10 Gy), and glycine (−3.86 log₂ fold-change, 10 Gy) all significantly decreased at all IR doses (Figure 3, Table 1). Lactic acid (−1.69 log₂ fold-change, 10 Gy) and propionylglycine (−0.60 log₂ fold-change, 10 Gy) decreased at the highest doses, and hippuric acid (−1.47 log₂ fold-change, 10 Gy) decreased at 10 Gy. Adipic acid decreased at 6, 7, and 10 Gy but not significantly ($P = 0.129$, −1.25 log₂ fold-change, 10 Gy).

3.2.2. Serum—A total of 208 compounds were aligned and putatively identified. No TCA intermediates were identified, but a high number of amino acids were affected by IR exposure (Figure 4, Table 2). Highest log₂ fold-change by exposed dose is reported with all log₂ fold-changes supplied in Table S1. Amino acids showed three patterns after IR exposure compared with control that were to increase in concentration (branched chain amino acids- leucine and isoleucine), decrease at low doses and gradually increase in concentration at 10 Gy (e.g., lysine and aspartic acid), or decrease in concentration (remaining amino acids) (Figure 4). Branched chain amino acids (isoleucine, 0.98 log₂ fold-change, 10 Gy) increased after IR exposure, although not significantly in leucine ($P = 0.102$, 0.48 log₂ fold-change, 10 Gy). Lysine (−1.50 log₂ fold-change, 4 Gy), threonine (−0.75 log₂ fold-change, 4 Gy), and aspartic acid (−2.49 log₂ fold-change, 4 Gy) decreased at lower exposure doses and levels increased at 10 Gy; however, concentrations were lower at all IR exposures. Serine (−0.86 log₂ fold-change, 2 Gy), alanine (−0.2 log₂ fold-change), glycine (−0.59 log₂ fold-change, 7 Gy), and asparagine (−0.96 log₂ fold-change, 2 Gy) all reduced significantly after IR exposure (Figure 4). Pyroglutamic acid (0.49 log₂ fold-change, 7 Gy) increased slightly but not significantly ($P = 0.054$). and 4-hydroxyproline (−1.37 log₂ fold-change, 10 Gy) exhibited a dose-dependent decrease (Figure 5). Glyceric acid (−0.83 log₂ fold-change, 10 Gy), pipecolic acid (−3.20 log₂ fold-change, 10 Gy), and lactic acid (−1.39 log₂ fold-change, 4 Gy) all decreased after IR exposure. 3-Hydroxybutyric acid (1.60 log₂ fold-change, 10 Gy) was significantly higher.

4. DISCUSSION

In this study, we conducted global untargeted metabolomic profiling of urine and serum from NHPs 7 days after exposure to IR using a GC-TOF-MS platform. In urine, several TCA cycle intermediates were perturbed after IR. Together with previous LC-MS studies, these results indicate altered energy metabolism (e.g., glycolysis and fatty acid β -oxidation), mitochondrial dysfunction, with a shift toward possible ketogenesis. In serum, several amino acids and their derivatives were identified, suggesting possible decreases in substrates for TCA cycle function or tissue damage.^{10,37} In both biofluids, other novel small carboxylic acids (e.g., phenylacetic acid) and methyl-branched fatty acid (e.g., methylsuccinic acid) biomarkers previously not detected by LC platforms were altered after IR exposure, highlighting the importance of using multiple analytical platforms to obtain a comprehensive view of changes in metabolic pathway biomarkers. After identification of these smaller volatile compounds through global GC-MS metabolomics, further efforts may be expended for faster targeted LC methodology useful for rapid biodosimetry.

Past studies have implicated IR exposure to affect cellular energy metabolism and ATP production, such as altered fatty acid β -oxidation, TCA cycle, glycolysis, and ketogenesis.^{9,16,18,20} Carnitine and acylcarnitines are commonly affected after IR exposure in mice,¹⁴ NHPs,^{10,16} and humans,¹⁸ suggesting inhibition of free fatty acids (FFAs) entering mitochondria for ATP production through fatty acid β -oxidation or a dysregulated pathway of complete fatty acid metabolism. In the current study, decreases are also observed in acylglycines in urine possibly from altered fatty acid metabolism, specifically propionylglycine. In addition to altered metabolism of FFAs for acetyl-CoA production, TCA cycle intermediate levels are eliminated from the body in urine after IR exposure. While TCA cycle intermediates have been identified in past studies, their chemical nature (e.g., separation of citric acid and isocitric acid) makes detection by LC-MS difficult. By using GC-MS we readily detected all TCA intermediates, except succinyl-CoA and oxaloacetic acid (possibly due to thermal degradation), in NHP urine. Citric acid and isocitric acid were decreased in samples from all doses of IR exposure, while succinic acid, *cis*-aconitic acid, and malic acid were decreased at higher doses compared with controls. Decreases in urinary citric acid have also been observed due to increases in 17-hydroxycorticosteroids, which were seen to increase in our previous study.^{16,38} Alterations in TCA cycle function may push a shift toward other energy production pathways such as ketogenesis. Increase in ketogenesis may contribute to imbalance of blood pH levels, reduced efficiency of ATP production, and increase in fatty acid oxidation and toxic products. Lactic acid was identified as one of the most abundant compounds present in serum and can be utilized as an energy source during abnormal glucose/fatty acid metabolism. Its lower concentration in IR-exposed NHPs may be due to it being utilized as an energy source for ketogenesis, as 3-hydroxybutyric acid (a product of ketogenesis) increased in serum after IR exposure.

Proteins and amino acids (and other biomolecules) react with free radicals and are capable of producing peroxides further damaging tissues in biological systems,³⁹ and lowered levels of select amino acids and their enzymatic cofactors may be a sign of decreased longevity.⁴⁰ Amino acids play important roles in biological oxidations, cell metabolism, and protein

synthesis and, to a lesser extent than glucose and lipids, can be directly oxidized for ATP production.⁴¹ Previous studies have identified changes in levels of glucogenic (valine, proline, glutamate, histidine, alanine, arginine, and asparagine) and ketogenic/glucogenic (tyrosine) amino acids in NHP serum after IR.^{10,17} Here we have expanded our knowledge on the effects of IR on amino acid levels to include serine, glycine, leucine, threonine, isoleucine, asparagine, lysine, and aspartic acid (Table 2). These amino acids may follow specific catabolic pathways to form intermediates (i.e., pyruvic acid, oxaloacetic acid, α -ketoglutaric acid, succinyl-CoA, acetoacetic acid), which are fed into the TCA cycle. Previous studies have reported that the response of amino acids to IR is complicated, as different trends in serum amino acid concentration are observed post-IR at different time points.^{10,37} Concentrations decrease at all doses, decrease/increase only at specific doses, or slightly increase in concentration after IR exposure (e.g., two branched amino acids in current study). Possible explanations for shifts in amino acid serum concentrations have been protein breakdown, altered amino acid metabolism, diet shifts, cell death, and damage to kidneys and intestinal lining.^{10,17,19,22,32}

In addition to finding perturbed levels of essential/nonessential amino acids, modified amino acids were found to be perturbed in NHP serum. Phenylacetic acid is a metabolite of phenylalanine, where phenylalanine is transaminated to phenylpyruvate (EC:1.4.5.1; EC:1.4.3.2), to phenylacetaldehyde (EC:4.1.1.43), and to phenylacetic acid (EC:1.2.1.5). While levels decrease with IR exposure, phenylacetic acid is a biomarker for nephritis and impaired renal function.⁴² Pyroglutamic acid is a cyclic lactam formed from glutamic acid either through enzymatic or nonenzymatic routes and glutathione degradation⁴³ and slightly increased after IR exposure. While pyroglutamic acid may play roles in protein stability and antibody function, it is still an understudied compound, and spontaneous cyclization of glutamic acid during sample preparation and GC analysis should be further studied.⁴⁴ 4-Hydroxyproline (*trans*-OH proline) is a major constituent of collagen and decreased with higher doses as previously reported.^{10,37} However, 4-hydroxyproline may serve as an important biomarker for determining post-mortem lung fibrosis because it is a major constituent of increased collagen deposition.⁴⁵

Changes in metabolites originating from the gut microflora post-IR have been documented in the past.^{7,8,20} The gut microbiota can conjugate glycine and benzoic acid, thus producing hippuric acid.⁴⁶ Pilocolic acid is produced by microbial metabolism of lysine from the diet or may be a biomarker of liver disease.⁴⁷ As past studies have documented, compounds from microbial sources may be important biomarkers of radiation signatures, especially because high doses of radiation cause cell death of intestinal stem cells. Other exogenous metabolites detected include adipic acid; its decreased concentration in urine is likely due to reduced dietary intake. One fatty acid (palmitic acid) and fatty acid conjugate (methylsuccinic acid) decreased in concentration in urine, which may be diet-related. Glyceric acid is an oxidized form of glycerol that was found in lower concentration in serum.

5. CONCLUSIONS

Because of ongoing threats to the population from exposure to radiation from possible nuclear terrorist attacks or nuclear accidents, there is a need for rapid diagnosis to determine

an individual's exposed dose for assigning appropriate triage. Ideally, noninvasive biofluids from large civilian populations could be quickly processed within 48 h of collection. However, with the certain panic ensuing such an event, it is important to identify longer term biomarkers (e.g., 7 days) because the response logistics may not permit biofluid collections within the ideal time frame. Because a large majority of radiation metabolomic studies have been performed with LC–MS platforms, we utilized a GC–MS system due to its higher suitability in determining compounds such as small carboxylic acids and amino acids in biological fluids. While some compounds may be affected by decreased food intake or stress, we found several TCA intermediates in urine that were lower in concentration after IR exposure, suggesting mitochondrial energy dysfunction and possible shifts toward ketogenesis. In serum, a number of amino acids were perturbed, which may affect energy metabolism or depict tissue damage. Finally, several novel carboxylic acids were identified, which complements previous LC–MS studies on NHP biofluids 7 days post-IR.^{10,16,17,48} Future studies should compile and validate biomarker panels that show dose dependency and determine tissue sources of metabolites.

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 51R01AI101798-03 (P.I.: A.J.F.). E.L.P. was supported by a PHS Grant no. 5 T32 CA 9686-20. We acknowledge the Lombardi Comprehensive Cancer Metabolomics Shared Resource (MSR), which is supported in part by Award Number P30CA051008 (P.I.: Louis Weiner) from the National Cancer Institute. We thank the Lombardi Comprehensive Cancer MSR for data acquisition. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute or the National Institutes of Health.

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Figure 1.

Multivariate data analysis of urine from NHPs exposed to IR. (A) PCA scores plot comparing the control group, 6 Gy (representative dose leading to hematopoietic syndrome), and 10 Gy (representative dose leading to gastrointestinal syndrome) (PC1–29.0%, PC2–19.9%, PC3–8.3%). Separation along PC1 indicates that most of the variation can be attributed to level of acute radiation syndrome. (B) MDS plot generated from RF analysis. Separation observed in MDS plot of the top 100 ions indicates that absorbed dose increases and so does separation along dimension 1.

Figure 2.

General schematic of the TCA cycle and other metabolic pathways and how exposure to IR affects relative abundance of intermediates in urine. IR exposure reduces TCA cycle intermediates, most notably the levels of citric acid, isocitric acid, *cis*-aconitic acid, malic acid, and succinic acid. The decrease in fumaric acid is less pronounced. A decrease in lactic acid is seen in both urine and serum, which may indicate that it is being shuttled in the ketogenesis pathways. Further evidence of increased ketogenesis is the increase in 3-hydroxybutyric acid. *Increases in carnitine and acylcarnitines in urine and serum suggest perturbation to fatty acid β -oxidation determined in refs 10, 16, and 17 (EC:4.2.1.3, aconitate hydratase; EC:1.1.1.42, isocitrate dehydrogenase; EC:1.2.4.2, oxoglutarate dehydrogenase; EC:6.2.1.4, succinate-CoA ligase (GDP-forming); EC:6.2.1.5, succinate-CoA ligase (ADP-forming); EC:1.3.5.4, fumarate reductase; EC:1.3.5.1, succinate dehydrogenase; EC:4.2.1.2, fumarate hydratase; EC:1.1.5.4, malate dehydrogenase; EC:2.3.3.8, ATP citrate synthase).

Figure 3.

Response of urine biomarkers from NHPs exposed to different IR doses. A significant decrease in relative abundance is observed for all IR doses for phenylacetic acid, palmitic acid, methylsuccinic acid, and glycine. Slight decreases are observed in the hematopoietic syndrome dose region for lactic acid, hippuric acid, adipic acid, and propionylglycine. * indicates significantly different from control as determined by a posthoc Duncan test ($P < 0.05$; mean $\pm$ SEM).

Figure 4.

Response of serum amino acid biomarkers from NHPs exposed to different IR doses. A decrease in relative abundance is observed for all IR doses for serine, alanine, glycine, threonine, asparagine, lysine, and aspartic acid; however, there are slight increases at 10 Gy for lysine, threonine, and aspartic acid. The branched chain amino acids, leucine and isoleucine, increase with higher absorbed IR doses. * indicates significantly different from control as determined by a posthoc Duncan test ($P < 0.05$; mean $\pm$ SEM).

Figure 5.

Response of serum organic acid biomarkers from NHPs exposed to different IR doses. Lactic acid was found in the highest relative abundance of serum metabolites and significantly decreased for all IR doses. Pyroglutamic acid had a slight increase but not significantly. Pipecolic acid, 4-hydroxyproline, and glyceric acid decreased in concentration. The 3-hydroxybutyric acid increased at 10 Gy, suggesting an increase in ketogenesis. * indicates significantly different from control as determined by a posthoc Duncan test ($P < 0.05$; mean $\pm$ SEM).

Table 1

Urinary Biomarkers Detected by GC-MS of Nonhuman Primates Exposed to γ Radiation^a

metabolite	retention index	unique mass	P value	H value	Log2 fold change control vs 10 Gy	no. TMS	HMDB ID
citric acid	1845	273	<0.001	36.72	-4.19	4	HMDB 00094
lactic acid	1061	117	0.030	12.36	-1.69	2	HMDB 00190
isocitric acid	1846	273	0.003	18.25	-1.84	4	HMDB 00193
cis-aconitic acid	1763	73	0.003	18.34	-1.29	3	HMDB 00072
phenylacetic acid	1306	73	<0.001	25.14	-2.23	1	HMDB 00209
hippuric acid	1824	206	0.015	14.09	-1.47	2	HMDB 00714
palmitic acid	2050	117	0.003	17.82	-1.51	1	HMDB 00220
methylsuccinic acid	1333	73	0.006	16.21	-3.75	2	HMDB 01844
succinic acid	1320	247	0.001	20.07	-2.52	2	HMDB 00254
propionylglycine	1170	144	0.004	17.27	-0.60	1	HMDB 00783
malic acid	1504	233	0.012	14.89	-5.02	3	HMDB 00744
glycine	1315	174	0.012	14.32	-3.86	3	HMDB 00123

^a P value and H value from a Kruskal–Wallis test.

Table 2Serum Biomarkers Detected by GC-MS of Nonhuman Primates Exposed to γ Radiation

metabolite	retention index	unique mass	P value	H value	Log2 fold change	no. TMS	HMDB ID
threonine	1400	219	<0.001	23.08	-0.71 ^b	3	HMDB 00167
lysine	1722	74	0.001	22.21	-1.45 ^b	3	HMDB 00182
aspartic acid	1535	232	0.003	17.83	-1.26 ^b	3	HMDB 06483
serine	1372	204	0.003	17.66	-0.86 ^b	3	HMDB 00187
alanine	1102	116	0.002	18.71	-0.57 ^c	2	HMDB 00161
asparagine	1690	116	0.001	19.81	-0.65 ^c	3	HMDB 00168
glycine	1315	174	0.042	11.65	-0.56 ^c	3	HMDB 00220
isoleucine	1303	158	0.006	16.26	0.98 ^c	2	HMDB 00172
4-hydroxyproline	1542	230	<0.001	27.03	-1.37 ^c	3	HMDB 00725
pipecolic acid	1272	84	0.044	11.38	-3.20 ^c	1	HMDB 00070
glyceric acid	1343	189	0.034	12.09	-0.83 ^c	3	HMDB 00139
3-hydroxybutyric acid	1163	117	<0.001	26.57	1.60 ^c	2	HMDB 00357
lactic acid	1061	117	0.003	18.00	-1.10 ^c	2	HMDB 00190

^b Highest log₂ fold-change control vs 2 Gy.^c Highest log₂ fold-change control vs 10 Gy; P value and H value from a Kruskal–Wallis test.