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Irradiation causes alterations of polyamine, purine and sulfur metabolism in red blood cells and multiple organs

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Abstract

Investigating the metabolic effects of radiation is critical to understand the impact of radiotherapy, space travel, and exposure to environmental radiation. In patients undergoing hemopoietic stem cell transplantation, iron overload is a common risk factor for poor outcomes. However, no studies have interrogated the multi-organ effects of these treatments concurrently. Herein, we use a model that recapitulates transfusional iron overload, a condition often observed in chronically transfused patients. We applied an omics approach to investigate the impact of both iron load and irradiation on the host metabolome. Results revealed dose-dependent effects of irradiation in red blood cells, plasma, spleen, and liver energy and redox metabolism. Increases in polyamines and purine salvage metabolites were observed in organs with high oxygen consumption including the heart, kidney, and brain. Irradiation also impacted the metabolism of the duodenum, colon, and stool, suggesting a potential effect on the microbiome. Iron infusion affected the response to radiation in the organs and blood, especially in erythrocyte polyamines and spleen antioxidant metabolism, and affected glucose, methionine and glutathione systems and tryptophan metabolism in the liver, stool, and brain. Together, the results suggest that radiation impacts metabolism on a multi-organ level with a significant interaction of host iron status.

Graphical Abstract

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AUTHOR INFORMATION

Author contributions FLC, SF, CM, BSW, LW, EAH performed animal experiments; MKR, FC, TN, AD performed metabolomics analyses. MKR, FLC, AD analyzed data and prepared figures. AD wrote the first draft of the manuscript and all the authors contributed to its finalization.

CONFLICT OF INTEREST

Though unrelated to the contents of this manuscripts, the authors declare that AD is a founder of Omix Technologies Inc.. AD is also a consultant for Altis Biosciences LLC., Rubius Inc. and Forma Inc. AD is a consultant for Hemanext Inc. All the other authors disclose no conflicts of interest relevant to this study.

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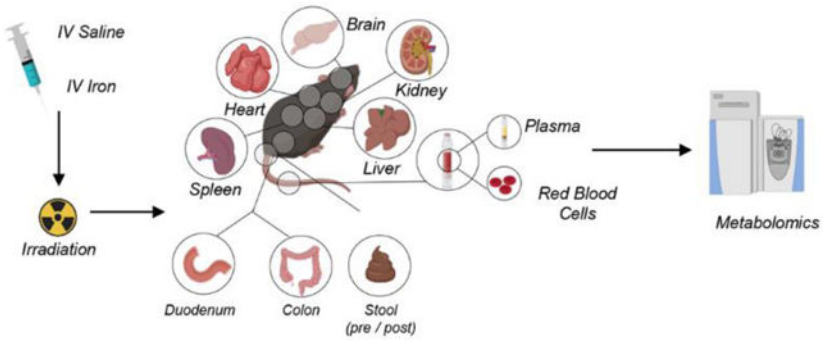

Keywords

Radiation; Iron; Metabolism; Organs; Mass Spectrometry; Polyamine; Methionine

INTRODUCTION

Understanding the metabolic impact of irradiation holds several clinically relevant implications. For example, γ-emitting irradiators (^{137}Cs and ^{60}Co sources) have been used to deliver myeloablative doses of radiation prior to bone marrow transplantation in patients with both hematological disorders and malignancies.[1] Over the past decades, radiotherapy has been a mainstay in the treatment of several cancers, including breast cancer, where treatment with ^{137}Cs at doses of >10 Gy in multiple fractions per day (e.g., 4) have been reported both as external whole breast radiotherapy or interstitial irradiation to the tumor bed.[2] Preclinical animal models of cell and gene therapy, as well as studies on hematopoietic stem cell function rely heavily on mouse models of radiation, prior to adoptive hematopoietic stem cell (HSC) transfer and/or bone marrow transplant. This is the case of studies on aging,[3] (healthy and stress) hematopoiesis,[4] and hematological malignancies.[5] Similarly, common are the studies where genetic ablation of specific genes of interest results in a non-viable mouse in adult age, requiring bone marrow transplant of hematopoietic stem cells from knock out mice to irradiated wild type mice with the goal to investigate the role of a specific gene in adult/old age (e.g., protein L-isoaspartyl o-methyltransferase).[6] Bone marrow irradiation techniques often require a lethal dose of irradiation to ensure adequate engraftment with donor cells. While mice are used extensively in transplantation studies involving bone marrow ablation, limited information is known about the impact of irradiation on systems metabolism.

Such information is relevant for example to determine the metabolic cost of chronic or acute irradiation exposure following space travel or terrorist attacks. Despite these security measures, limited information is available about the systemic impact of radiation from a diagnostic standpoint (e.g., for the rapid assessment of terrorist threats), which could be relevant to inform intervention strategies to mitigate the health toll on victims of such attacks.

In the near future, the National Aeronautics and Space Administration (NASA) and the recently established US Army Space Force are planning long duration and deep space

travel for space exploration and research missions. Similar public and private efforts to explore and perhaps colonize proximal satellites and planets (e.g., missions to the Moon, the Lunar Gateway, or to Mars) will challenge astronauts with a prolonged exposure to microgravity[7] and space radiation, such as high energy particles from galactic cosmic rays and potential particles from solar events.[8] Manned missions to Mars are estimated to last between 650 and 920 days, exposing the astronauts to a cumulative dose equivalent of 870–1200 mSv (3–4.5 Gy).[9] On the other hand, low Earth orbit missions – such as those in the International Space Station (ISS) – partially shield astronauts from radiations otherwise experienced in deep space, owing to the protective effect of Earth's magnetosphere. Yet, ISS astronauts returning to Earth and animal experiments performed onboard have confirmed that spaceflight significantly impacts the cardiovascular and central nervous system, promotes muscle atrophy, cataract formation, and triggers immune system effects – in part also explained by the effects of microgravity. Results from the NASA twin study revealed that genetically identical twins – one on Earth and one on the ISS for 340 days – had differential metabolism, with differential levels of metabolic markers of genotoxic stress, inflammation, and altered amino acid metabolism, Krebs cycle metabolites and tryptophan metabolites (indoles), which all increased in twin inflight.[10]

Similar alterations to the microbiome (especially members of the bacterial taxa *Lachnospiraceae* and *Enterococcaceae*) and metabolites of potential microbial origin (including tryptophan-derived indoles) have been reported in plasma of humans surviving ionizing radiation exposure.[11,12] Studies have also confirmed alterations in tryptophan/indole metabolism in low-dose uranium radiation exposure studies.[13] Studies on serum and plasma have been reported at low dose of radiation (e.g., 4 Gy)[14] or urine (with a total dose of radiation between 5–10 Gy over a 30 day period).[15] Metabolomics studies examining the impact of bone marrow failure-inducing doses of radiation have also been published to document the effect on mice exposed to a higher radiation dose range (5–11 Gy). These identified a significant enrichment in purine metabolism, tricarboxylic acid cycle, fatty acids, acyl-carnitines, and amino acids in the spleen of animals upon early perturbations with γ irradiation.[14] Despite several studies focusing on plasma, to the best of our knowledge, dose response studies focusing on plasma, blood and multiple organs have not been published yet, making it difficult to appreciate system-wide responses to radiation.

Furthermore, radiation can be used in HSC transplantation (HSCT). Although HSCT is the only cure for many malignant and nonmalignant conditions, its use is limited by significant morbidity and mortality.[16–18] Transfusion-induced *iron overload* is common in patients undergoing HSCT.[19] It is also a risk factor for poor HSCT outcomes.[20,21] In mouse models that modify iron status (e.g., by dietary manipulation, chronic transfusion, or intravenous iron administration), we identified specific changes in the gut microbiota and in circulating microbial metabolites.[22] In particular, *Lactobacilli* spp., which are notable for being less dependent on iron for growth,[23] are decreased with increasing iron load. In tandem, fecal and plasma indole metabolites, which have anti-oxidative and anti-inflammatory properties that can modulate cytokine production and host immune responses,[24–26] were reduced. Additionally, murine studies showed that irradiation exposure reduces the level of indoles which is associated with increased risk of graft versus host disease (GVHD), one of the most

severe side effects of HSCT. [27] Thus, determining the impact and interaction of both gamma irradiation and iron levels on the host metabolome is the main focus of this study.

METHODS

Irradiation

Female wild-type C57Bl6 mice were purchased from Charles River Laboratories (Stone Ridge, NY) and used at 8–10 weeks of age. After one week of acclimatization in a pathogen-free facility, cohorts of mice were retro-orbitally infused with phosphate buffer saline (PBS) or 12.5 mg of iron dextran (Henry Shein Animal Health, Dublin, OH), twice a week for 2 weeks for a total of 50 mg of iron. After 2 days of rest, mice were then divided in groups and irradiated with 7, 8, 9, 10, 11 Gy of C-137 (n=5 per group) going from a non-lethal to a lethal dose for this mouse strain. Total dose was split in 2 doses 3 hours apart. Stools were freshly collected after the last dose of PBS/iron (pre-stool) and after irradiation (post-stool). At day +4 post irradiation, mice were euthanized and blood was collected by cardiac puncture in heparinized syringe. Plasma obtained after centrifugation at 4500 g for 10 minutes at +4°C. Tissues, including brain, colon, duodenum, heart, kidney, liver, spleen and stool (pre- or post-irradiation/iron infusion) were weighted and all the biological samples were stored at −80°C until further processing.

Iron-related measurements:

Spleen and liver were collected at necropsy and non-heme iron determined using a wet ashing procedure. In brief, the wet weight of samples was quantified and portions of liver and spleen were placed in 2 ml micro-tubes. Following desiccation at 65 °C for 24 h, 1 ml of acid digestion mixture (3 M hydrochloric acid, 10% trichloracetic acid) was added and samples were heated at 65 °C for an additional 24 h. The acidified sample (50 μl) was then incubated for 30 min with 200 μl of chromogen (1.6 M bathophenanthroline, 2 M sodium acetate, 11.5 M thioglycolic acid). Absorbance at 535 nm of samples and iron standards was measured in duplicate and mean values used for calculating total iron. Iron transferrin saturation in plasma was calculated using Iron/TIBC Reagent Set (Pointe Scientific, Canton, MI).

Metabolomics:

Metabolomics analyses were performed as extensively described in previous studies.[28] A volume of 20μl of frozen plasma or RBC aliquots or 15 mg of ground tissue (GenoGrinder) was extracted in either 480μl or 1 ml, respectively of methanol:acetonitrile:water (5:3:2, *v/v/v*).[29] After vortexing at 4°C for 30 min, extracts were separated from the protein pellet by centrifugation for 10 min at 10,000g at 4°C and stored at −80°C until analysis. Ultra-High-Pressure Liquid Chromatography-Mass Spectrometry analyses were performed using a Vanquish UHPLC coupled online to a Q Exactive mass spectrometer (Thermo Fisher, Bremen, Germany).[30] Samples were analyzed using a 5 minute gradient as described.[30,31] Solvents were supplemented with 0.1% formic acid for positive mode runs and 1 mM ammonium acetate for negative mode runs. MS acquisition, data analysis and elaboration was performed as described.

Statistical methods and data analysis.

Graphs and statistical analyses (unpaired T-test) were prepared with GraphPad Prism 8.0 (GraphPad Software, Inc, La Jolla, CA). Heat maps, hierarchical clustering analyses, partial least square-discriminant analyses, two-way ANOVA were calculated and plotted with MetaboAnalyst 5.0.[32] Bar graphs were plotted with the free online tool Metabolite Autoplotter.[33] Graphics that provide an overview of the experimental design were generated through the software Biorender.

RESULTS

Multi-organ characterization of the metabolic impact of irradiation

Cohorts of iron overloaded or control mice were irradiated with doses ranging from 7 to 11 Gy and metabolomics analyses were performed on RBCs, plasma, and the following organs: brain, colon, duodenum, heart, kidney, liver, spleen and stool (Supplementary Table 1). Results are reported in tabulated form in Supplementary Table 1, which includes metabolite names, KEGG IDs, retention times, accurate intact masses, along with all raw data. For each matrix tested in this study, results were graphed in Supplementary Figure 1, including unsupervised analyses, hierarchical clustering analysis of the significant metabolites by ANOVA and principal component analyses (PCAs), as well as bar plots for significant metabolites as a function of irradiation doses.

Irradiation impacts glycolysis, polyamine, sulfur metabolism and acyl-carnitines in RBCs, plasma, spleen, and liver

Metabolomics data suggest that irradiation causes metabolic damage to RBC energy and redox systems in a dose-dependent fashion (heat map in Figure 1.B), a phenomenon that was accompanied by changes in spleen and liver metabolomes. This is relevant in that spleen and liver are the organs where extravascular hemolysis, i.e., resident macrophage-dependent erythrophagocytosis is observed (Figure 1.C). In Figure 1.D we show a representative PCA of RBCs exposed to increasing doses of irradiation, with PC1 explaining 17% of the total variance across sample groups from mice irradiated with different doses vs untreated mice. Similar patterns were noted in the plasma, spleen and liver metabolomes, with the latter showing a subset of metabolites spiking at the lowest dose of radiation (7 Gy), or peaking at 10–11 Gy. Glycolysis (Figure 1.E), polyamines (especially putrescine), amino acids (especially arginine, glutamine, sulfur metabolites – methionine, taurine – Figure 1.F–G) ranked amongst the top metabolites impacted by irradiation in a dose-response fashion in the spleen. Similar effects were observed in RBCs with respect to glycolysis (decreases of fructose bisphosphate and pyruvate) and sulfur metabolites (taurine – Figure 1.H), purines (inosine and hypoxanthine – Figure 1.I) and polyamines (spermine, spermidine, putrescine – Figure 1.J). Notably, irradiation induced significant decreases in the levels of free carnitine, as well as several acyl-carnitines in RBCs from irradiated mice, suggestive of potential activation of the Lands cycle in red blood cells from irradiated mice even at low doses (Figure 2.A). Irradiation was also found to have an impact on the Krebs cycle in the liver – with peak levels of multiple carboxylic acids (citrate, fumarate and malate at Gy 8), polyamines (putrescine) and poly-unsaturated fatty acids (eicosapentaenoic acid) in the liver, the latter in an irradiation dose-dependent manner (Figure 2.B).

Metabolic impact of irradiation on mouse brain, liver and kidneys

Given the role of RBCs on oxygen transport and the damaging impact of radiation on RBC metabolism, we thus focused on metabolomics analyses of organs with high oxygen consumption, such as brain, heart and kidney (Figure 3.A). Hierarchical clustering analyses of the metabolites significant by ANOVA revealed radiation dose response patterns in the brain, heart and kidneys (Figure 3.B). This dependency on irradiation dose of metabolic phenotypes was mostly evident in the kidney, as shown by the PCA (Figure 3.C). Significant metabolic alterations were observed following irradiation in heart, kidney and brain (Figure 3.D–G). Across these organs, we observed increases in the levels of polyamines (putrescine in heart and kidney), purines and purine salvage metabolites (inosine, hypoxanthine, S-adenosylmethionine – decreasing in kidney and brain), amino acids (glutamine in kidney) and carboxylic acids (especially decreases in fumarate and malate). Interestingly, succinate was observed to increase in the brain and decrease in the heart following irradiation (Figure 3.D and F), suggesting organ-specific responses to irradiation.

Metabolic impact of irradiation on mouse colon, duodenum and stool

Recent studies have suggested an impact of irradiation on the gut microbiome and associated metabolomes.[11] To test whether these observations were recapitulated in our model, we performed metabolomics analysis was performed in colon, duodenum and stool in intravenous vehicle-treated mice, before and after irradiation i(pre- and post-stool – Figure 4.A). Statistical analyses via ANOVA informed the clustering of metabolites in the heat maps in Figure 4.B, which show a significant impact of irradiation on the metabolomes of these four tissues. Overall, results suggest that radiation induced alterations to metabolites in colon and stool compatible with a potential effect on the gut microbiome (Figure 4.C). Specifically, in the colon, irradiation promoted decreases in the levels of glycolytic metabolites (glucose, pyruvate, lactate), increases in polyamines (putrescine and spermidine) and altered levels of acyl-carnitines, with increases in C4- and C5-carnitine (intermediates of branched chain amino acid catabolism), and decreases in many other acyl-carnitines, especially hydroxylated forms (markers of fatty acid catabolism, with several odd chain fatty acyl-conjugated moieties of potential bacterial origin – Figure 4.D).

Impact of iron infusion on the metabolic responses to irradiation in organs and blood

After appreciating the impact of irradiation on systems metabolism, we wondered whether these effects would be exacerbated by prior infusion of iron, a model that recapitulates transfusional iron overload, a condition often observed in chronically transfused patients with thalassemia or sickle cell disease, or myelodysplastic syndrome.[34] Since some of these patients receiving transfusion may also undergo bone-marrow irradiation as part of HSCT procedures, we investigated whether iron overload would compound the metabolic impact of irradiation on systems metabolism (Figure 5.A).

Results are reported extensively in supplementary table 2 and all unsupervised and more targeted analyses are reported for each matrix in Supplementary Figure 2. Pathway analyses indicated that the top metabolic pathways affected by iron infusion and irradiation included arginine/proline metabolism (also including polyamines as downstream product of arginine metabolism), amino acid metabolism, especially taurine metabolism (Figure 5.B). Principal

component analysis shows a clear effect of iron (red vs blue for saline) and irradiation on the metabolic phenotypes (RBCs are presented as an example in Figure 5.C). In RBCs, the highest effects of iron and irradiation were noted on arginine metabolism and polyamine synthesis While the levels of these metabolites decreased overall with radiation, iron infusion prior to radiation resulted in further decreases in arginine along with increased levels of putrescine and unchanged or decreased levels of the downstream polyamines spermidine and spermine, respectively. (Figure 5.D). Similar effects were noted in the levels of purines (hypoxanthine and urate), with iron inducing decreases in hypoxanthine and increases in urate, despite a trend towards decrease in irradiated mice for both metabolites (Figure 5.E). Iron infusion was accompanied by decreases in methionine and arginine, and increases in taurine levels, with a relatively negligible effect of irradiation dosage (Figure 5.E).

Spleen was the organ showing the highest metabolic change after iron infusion and irradiation, as shown by the PCA in (Figure 5.F), where sample grouping based on irradiation phenotypes was evident across PC1, explaining 22% of the total variance, and iron infusion on PC2, which explained ~14% of the total variance. The effect of intravenous iron infusion on the spleen metabolome was even more evident in the heat map of the metabolites significant by two-way ANOVA (Figure 5.G). The top hits from this analysis are reported as bar plots in Figure 5.H, and include several metabolites in the antioxidant responses (pentose phosphate isomers, reduced and oxidized glutathione), decreasing as a function of iron infusion. Similar trends were observed for pyruvate and citrate – both decreasing in the spleen following iron infusion. Increases in polyamines, especially putrescine, following irradiation in a dose-dependent fashion were further exacerbated by iron infusion (Figure 5.H), which also corresponded to increased glutamine and AMP consumption and hypoxanthine generation in the spleen.

Iron infusion and irradiation significantly impact glucose, sulfur and tryptophan metabolism in the liver, stool and brain

Iron infusion had a significant effect on liver metabolism (Figure 6.A), especially on glycolysis (glucose, fructose bisphosphate, glyceraldehyde 3-phosphate, phosphoglycerate), pentose phosphate pathway (pentose phosphate isomers) and reduced glutathione – all decreasing in iron infused mice (Figure 6.B). On the other hand, iron infusion boosted methionine and taurine metabolism, with several intermediates of this pathway increasing in the liver after iron infusion, including taurine and methionine, methionine sulfoxide, acetyl-choline, with significant decreases in hypotaurine (Figure 6.C).

Iron infusion also impacted stool (before and after irradiation – Figure 6.D) and the brain. In the brain, several tryptophan metabolites of potential bacterial origin and/or with a role in neurotransmission (indole-acetaldehyde, serotonin, anthranilate) were significantly impacted by iron infusion and irradiation (Figure 6.D), along with carboxylic acids like malate (Figure 6.E), which increased upon iron infusion.

Metabolic correlates to iron levels in the liver and the spleen

Finally, direct measurements of iron in the liver and the spleen highlighted an increase in organ levels of iron as a function of irradiation (~1.5 fold increase) and iron infusion (~15–20 fold increase – Figure 7.A–B). Correlation analyses of metabolomics data from all matrices tested in this study were performed against iron levels in the liver or spleen (Figure 7.C and D). Results confirm the pathway analyses described in the previous paragraphs, showing a significant impact of iron infusion on liver and spleen metabolites (top 25 correlates ranked by Spearman r; $p<0.001$ are reported in Figure 7.E), especially amino acids (asparagine, cysteine, tryptophan, phenylalanine, methionine, proline, threonine) indoles (e.g., indolepyruvate, hydroxyindoleacetate), but also heart carnosine levels– with some organ-specific trends (e.g., indolepyruvate increasing in liver and decreasing in the stool after iron infusion) (Figure 7.F–G).

DISCUSSION

In the present study we report a comprehensive multi-organ characterization of systems responses to radiation doses from 7–11 Gy, in presence and absence of prior iron overload. As a result, we report a significant impact of radiation on arginine metabolism towards the increased synthesis of polyamines in all matrices/organs tested in this study, except for RBCs, where polyamine levels significantly decreased following radiation. These phenomena of polyamines – especially putrescine - increasing in all organs and decreasing in RBCs were further exacerbated by prior iron overload. Though the specific mechanisms underlying this observation are unclear, our findings recapitulate previous observations in cancer cells were c-Myc or p53-dependent signaling regulate some of the rate-limiting enzymes in the polyamine synthesis pathway, at the crossroads of methionine metabolism and purine salvage.[35]

Our results are also consistent with the radiation-induced activation of the rate-limiting enzyme of the polyamine synthesis pathway, ornithine decarboxylase, a phenomenon that had been described in the early 1990's following exposure to ultraviolet radiation[36] or in patients prone to developing skin cancer following prolonged sun exposure.[37] Similar radiation-induced activation of ornithine decarboxylase was associated with poorer outcomes in rodent models of colon cancer, whereby activation of polyamine synthesis pathway abrogated the beneficial anti-radiation effects of the dietary sulfur compound diallyl sulfide.[38] Of note, activation of the polyamine synthesis pathway was here associated with alterations in purine oxidation, decreasing in most organs, especially RBCs, upon radiation. This is an unexpected finding since we would have predicted that radiation induced decreases in energy metabolism (here observed in multiple organs at the glycolytic and mitochondrial level) would have corresponded to increased breakdown of high-energy phosphate purines (ATP, AMP – which we indeed observed) and increases in oxidant stress. Declines in glycolysis were noted upstream to pyruvate kinase, suggesting an effect of irradiation and iron levels (perhaps through Fenton chemistry) on redox sensitive reactive thiols in rate-limiting steps of glycolysis (especially glyceraldehyde 3-phosphate dehydrogenase[39] and pyruvate kinase[40]). With respect to oxidant stress, the main signature identified here – as a function of radiation dose and exacerbated by iron infusion – was the

activation of the pentose phosphate pathway, the decline in glutathione pools and oxidation of methionine and taurine in plasma and RBCs. Since methionine metabolism is linked to polyamine synthesis through purine salvage reaction and methylation processes, future studies should aim at understanding the impact of radiation and iron infusion on epigenetic regulation through methylation of proteins (e.g., to repair deamidation-induced isoaspartyl-damage, which could also target glycolytic enzymes mostly in the solvent accessible areas of their active sites[41]), RNA, histones and DNA.[42] Of note, accumulation of taurine, methionine, methionine sulfoxide and acetyl-choline in the liver following iron infusion and irradiation is suggestive of a significant impact of these treatments on sulfur and one-carbon metabolism. These findings confirm decreases in circulating levels of taurine (e.g., in RBCs) reported in the past as a consequence of radiation,[43] which is relevant in light of the protective role of this metabolite on boosting energy metabolism and antioxidant responses in RBCs.[44] Of note, acute[45] and prolonged[46] stress on RBC elicit increased levels of acyl-carnitine levels accompanied by decreased deformability and increased vesiculation rate,[45] as a function of alterations to the membranes and activation of the Lands cycle pathway to repair (oxidant) damage to (lyso)phospholipids.[47] On the other hand, lower levels of acyl-carnitines are associated with increased susceptibility to osmotic and oxidant stress in healthy blood donors as a function of sex dimorphisms, especially RBCs from hypogonadic males undergoing testosterone-replacement therapy.[48] Radiation in this study resulted in decreased levels of RBC acyl-carnitines. As such, it is possible that radiation itself may interfere with Lands cycle repair of membranes. Since the energy-less, oxidized and less deformable RBC rapidly undergoes extravascular hemolysis in the liver and spleen,[49,50] it is also possible that some of the metabolic signatures observed in these organs following irradiation and iron infusion are not the result of a direct effect of these treatments and at least in part influenced by increased erythrophagocytosis selectively of the most damaged cells. In support, RBC exposed to the highest levels of radiation in this study (11 Gy) had higher levels of acyl-carnitines, suggesting more comprehensive damage to the RBC population as a whole.

Our data also expand on prior findings showing liver increases in the levels of taurine and methionine, suggestive that sulfur metabolism dysregulation follows organ-specific trends which could be at least in part explained by microbial dysbiosis. Indeed, deconjugation of taurine-conjugated bile acids is catalyzed by enzymes expressed by bacteria in the gut microbiome, whereby alterations of bile acid metabolism has been mechanistically implicated in inflammatory complications, such as macrophage activation, in the context of cholestasis,[51] trauma-induced microbiome alterations following decreased enteric perfusion,[52] bacterial infiltration in precancerous intraductal papillary mucinous neoplasms,[53] iron overload[54] and non-alcoholic fatty liver disease[55]. Similar considerations can be made for several short and odd chain (carnitine-conjugated) fatty acids, increasing in the colon and stool following irradiation, especially when preceded by intravenous iron infusion. These findings are consistent with prior reports on alterations of these metabolites as a function of the interactions between host genetics and the gut microbiome on the development of central nervous system autoimmune conditions.[56] Interestingly, decline in highly-unsaturated fatty acids in the context of inflammation-induced neurodegenerative diseases such as Alzheimer's[57] or aging have been recently reported, suggesting that

irradiation could phenocopy at least in part a model of accelerated (metabolic) aging[4] and shortened healthspan in humans.[58] In this view, it is worthwhile to note that alterations of one-carbon metabolism (especially methionine and choline) have been associated with impaired erythropoiesis in aging,[59] neurocognitive deficit in infants[60] and individuals with Down syndrome,[61] which represents a model of accelerated aging,[62] and overall shortened lifespan through regulation of the mechanistic Target of Rapamycin (mTOR).[63]

Finally, radiation and iron infusion-induced alterations of indole metabolites, which study recapitulate previous findings in the literature.[8,11,12,64] Expanding on the literature, here we report that the brain metabolome was significantly impacted by iron and radiation, with tryptophan metabolites – including neuroactive serotonin – being the most impacted pathway in the brain. Impacts of iron infusion on brain metabolism were expected, in that iron is a cofactor in several neuron-specific functions, such as myelin and neurotransmitter (e.g., dopamine) synthesis.[65] While no significant alterations of dopamine metabolism were observed in the brain, liver and other organs, phenylalanine levels positively correlated with hepatic iron levels upon intravenous infusion, an observation in keeping with the dependency of the phenylalanine to tyrosine conversion reaction to iron levels.[66] Of note, alterations of indole/serotonin metabolism in the colon/stool and the brain provides further correlative evidence for the systemic impact of iron and radiation through alteration on the gut-brain axis[67] and outcomes of HSCT.[27,68,69]

Limitations of this study are noted, in that only a single omics approach has been used to characterize the impact of iron and irradiation on systems metabolism. Future studies could involve other omics (e.g., transcriptomics, proteomics, lipidomics) to complement metabolomics data. Given the clear signatures in polyamine and kynurenine metabolism, steady state metabolomics data will need to be confirmed through metabolic tracing experiments, for example through the use of stable isotope-labeled arginine and tryptophan to further test the impact of irradiation and iron supplementation on the metabolism of these two amino acids in vivo.

In summary, this study provides for the first time a comprehensive, system-wide look at the impact of radiation and intravenous iron infusion on the metabolism of multiple organs in mice. The results revealed that irradiation impacted the metabolome of the spleen, RBC, plasma, brain, liver, heart, kidney, colon, duodenum and stool, and that these impacts were modulated by iron infusion. In the organs, radiation impacted arginine metabolism as reflected by increased levels of polyamines. In RBCs, the opposite trend was observed, consistent with the previously described radiation-induced activation of ornithine decarboxylase in nucleated cells. Decreases in levels of RBC antioxidants including taurine and glutathione, and polyamines, the apparent activation of the pentose phosphate pathway, as well as decreases in acyl-carnitines, may indicate increased oxidant damage and membrane lipid remodeling (activation of the Lands cycle) in RBCs after irradiation. Our results also suggest that radiation and iron infusion modulate tryptophan metabolism. Moreover, the brain metabolome was significantly impacted by radiation and iron, which has not been described until now. Finally, sulfur metabolism appeared to be dysregulated in an organ-specific manner, potentially owing to microbial dysbiosis. Increases in colon and stool fatty acids and indole/tryptophan metabolites after radiation, especially in the iron treated

groups, may also reflect interactions between the gut microbiome and host genome. These findings further detail the impacts of irradiation across multiple organs and shed light on the effect of iron availability on radiosensitivity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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DATA AVAILABILITY

Raw mass spectrometry data for this study are available for free download at the Metabolomics Workbench website, with the following IDs: ST002031-ST002041.

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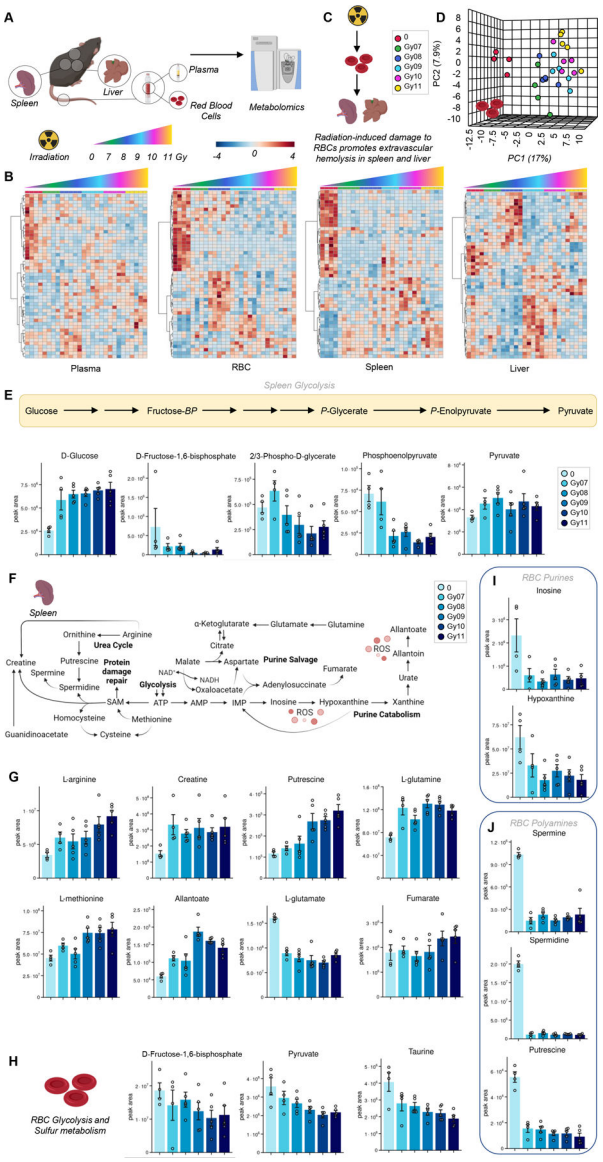

Figure 1 –. Metabolomics analyses of red blood cells, plasma, liver and spleen following irradiation.

We performed metabolomics analyses of red blood cells (RBCs), plasma and organs where erythrophagocytosis occurs, spleen and liver as a function of irradiation (untreated: 0; or irradiated: from 7 to 11 Gy – A). Heat maps of metabolites significant by ANOVA are shown in B. Data suggest progressive damage to RBC energy and redox systems, which corresponds to increased metabolic changes at higher irradiation doses in spleen and liver (C). A representative principal component analysis of RBCs exposed to increasing doses of irradiation (D). Results point at a significant impact of irradiation on glycolytic (E), polyamine (overview of the pathway in F, bar plots in G) and sulfur/amino acid metabolism in the spleen. We report alteration of glycolysis, taurine (H) and purine (J) metabolism in RBCs of irradiated mice.

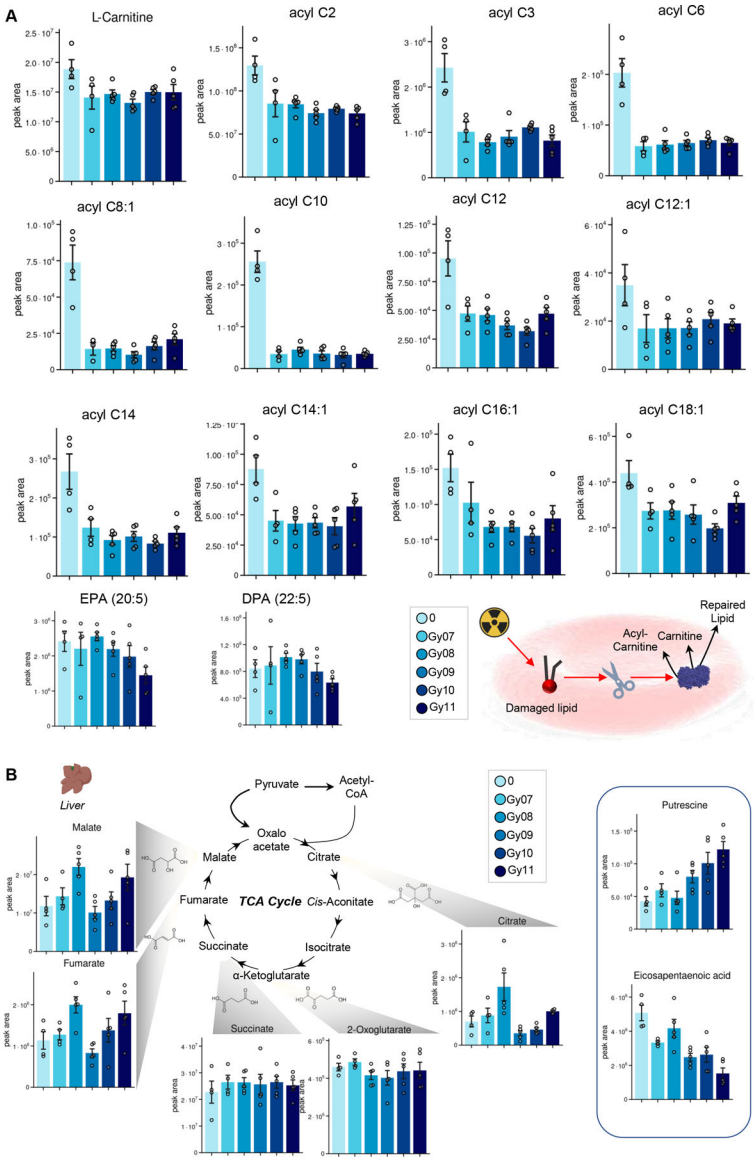

Figure 2 –. Alterations of acyl-carnitine metabolism in RBCs from irradiated mice

(A). Irradiation was also found to have an impact on the Krebs cycle, polyamines and poly-unsaturated fatty acids in the liver (B).

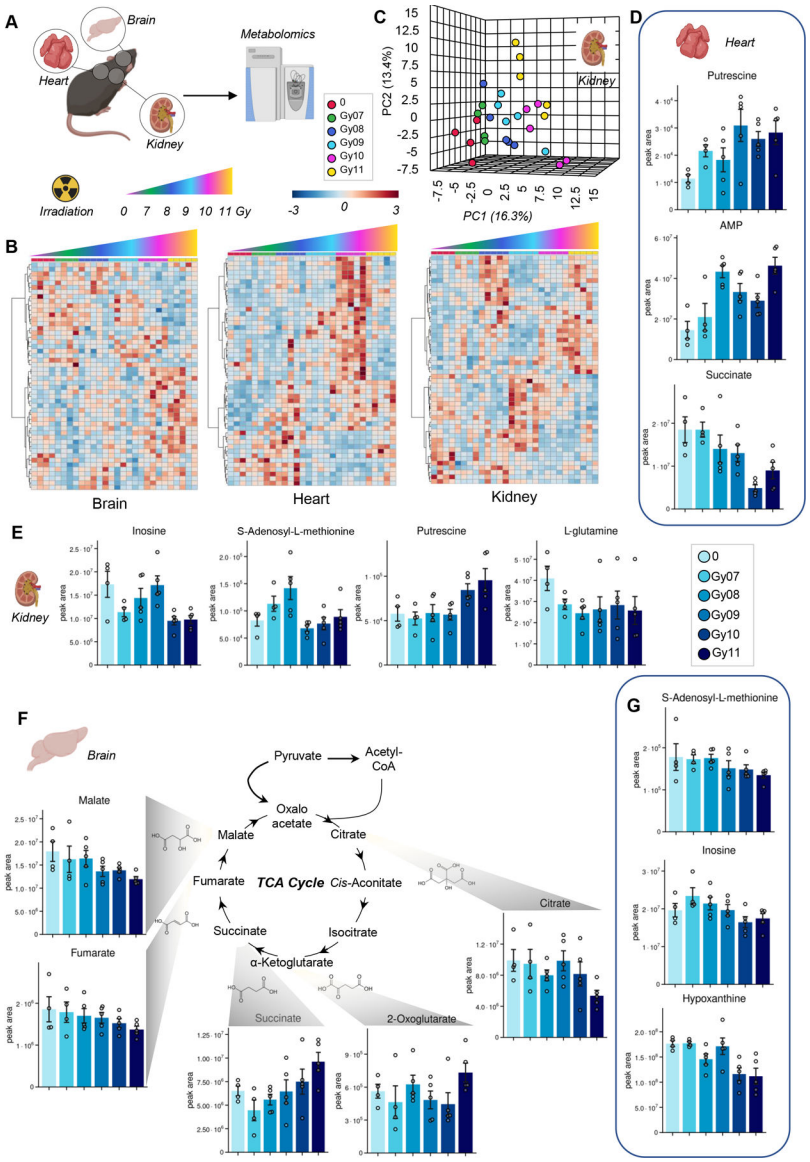

Figure 3 –. Metabolic impact of irradiation on mouse brain, liver and kidneys

(A), as determined by hierarchical clustering of the metabolites significant by ANOVA (B) and principal component analysis (representative PCA for kidneys in C). In D-G, bar plots of significantly altered polyamines, purines, amino acids and carboxylic acids in heart, kidney and brain.

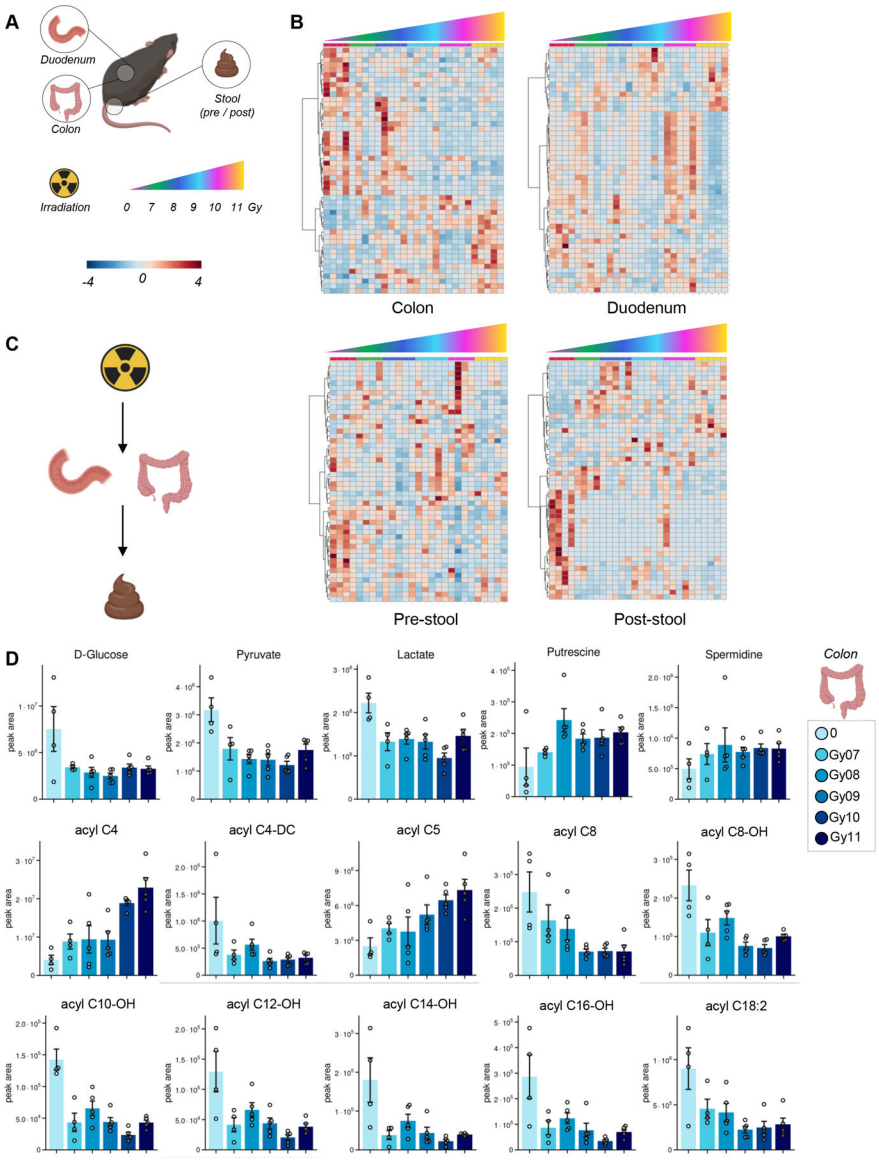

Figure 4 –. Metabolic impact of irradiation on mouse colon, duodenum and stool

(A), as determined by ANOVA (heat maps in B show significant metabolic patterns). Results suggest that radiation induced alterations to metabolites in colon and stool compatible with a potential effect on the gut microbiome (C). In D, representative bar plots for top metabolites affected by irradiation in the colon, including glycolytic metabolites, polyamines and acyl-carnitines (especially hydroxylated forms).

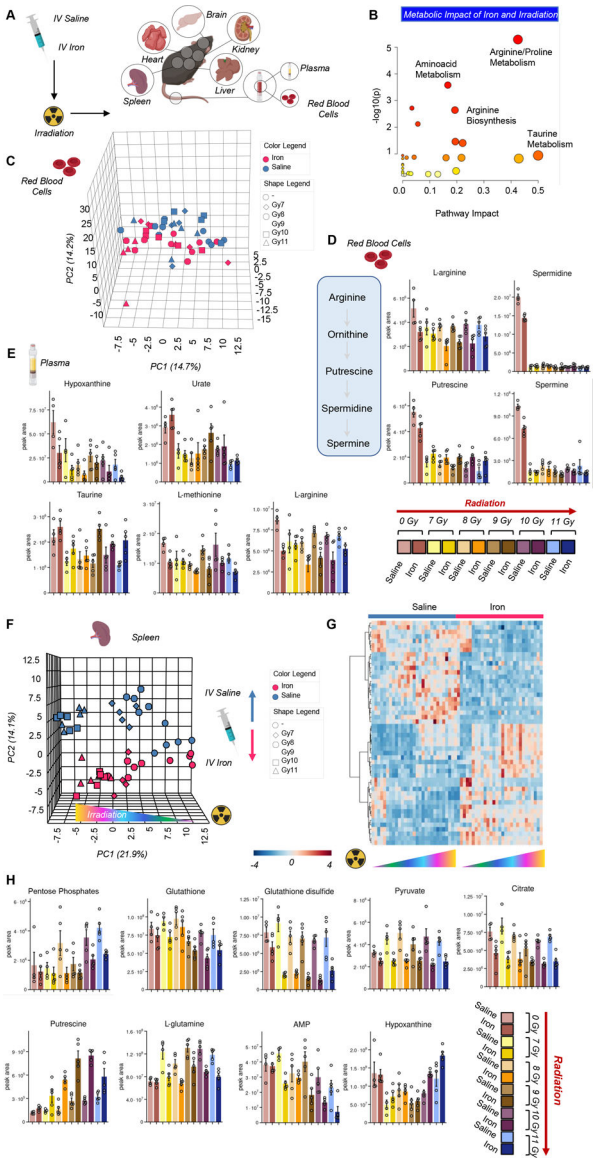

Figure 5 –. Impact of iron infusion on the metabolic responses to irradiation in organs and blood (cells –

A). An overview of the top metabolic pathways affected by iron infusion and irradiation is provided in B. In C, principal component analysis shows a clear effect of iron (red vs blue for saline) and irradiation on the red blood cell metabolic phenotypes, with the highest effects noted on red blood cell arginine metabolism and polyamine synthesis (D), purine oxidation and sulfur metabolism in plasma (E). Spleen was the organ showing the highest metabolic changes after iron infusion and irradiation, as shown by the principal component analysis in F. In G, heat map of the spleen metabolites significant by two-way ANOVA. The top hits from this analysis are reported as bar plots in H.

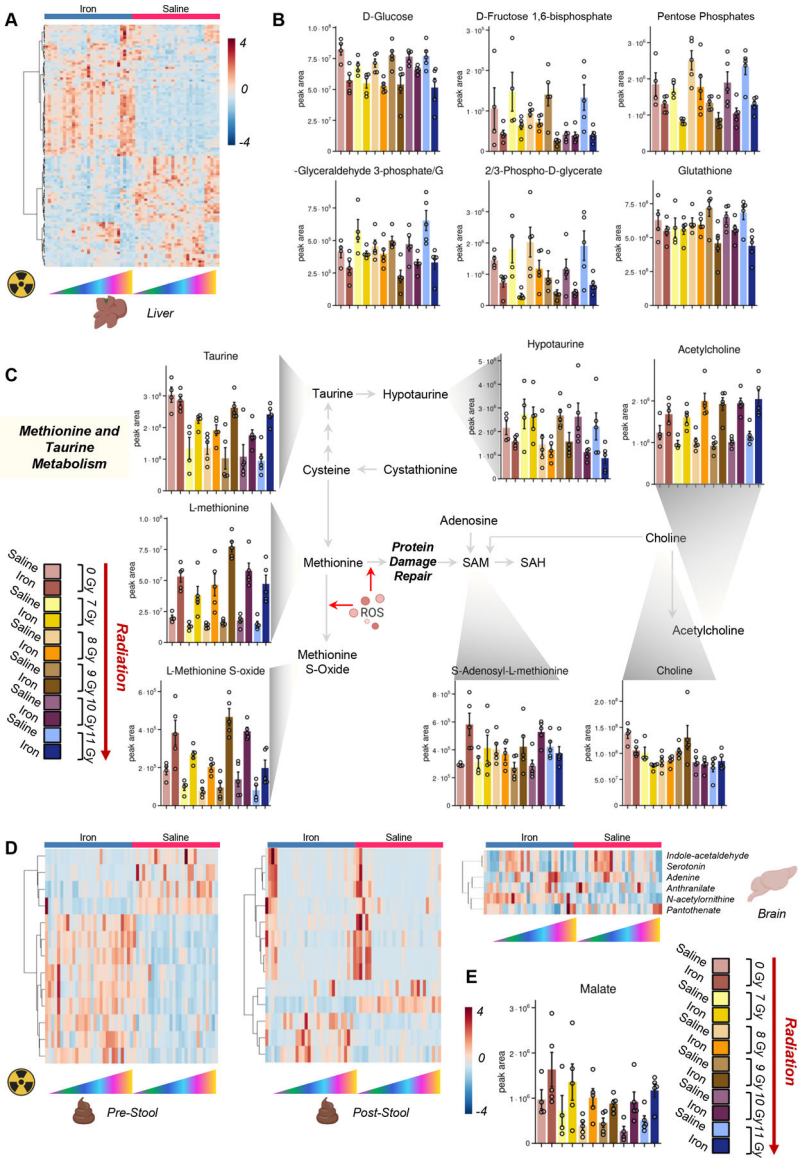

Figure 6 –. Iron infusion and irradiation significantly impact liver, stool and brain metabolism.

Heat map in A shows a significant impact of iron infusion and irradiation on the liver metabolome, with top significant metabolites with differential trends mapping in the glycolytic pathway, glutathione, taurine and sulfur homeostasis (B and C, respectively). Strong metabolic impacts of iron infusion and irradiation were observed in the stool (with iron infusion without irradiation – pre-stool; with iron infusion and after irradiation – post-stool – D) and in the brain, where several tryptophan metabolites (D) and carboxylic acids like malate (E) were altered by iron infusion.

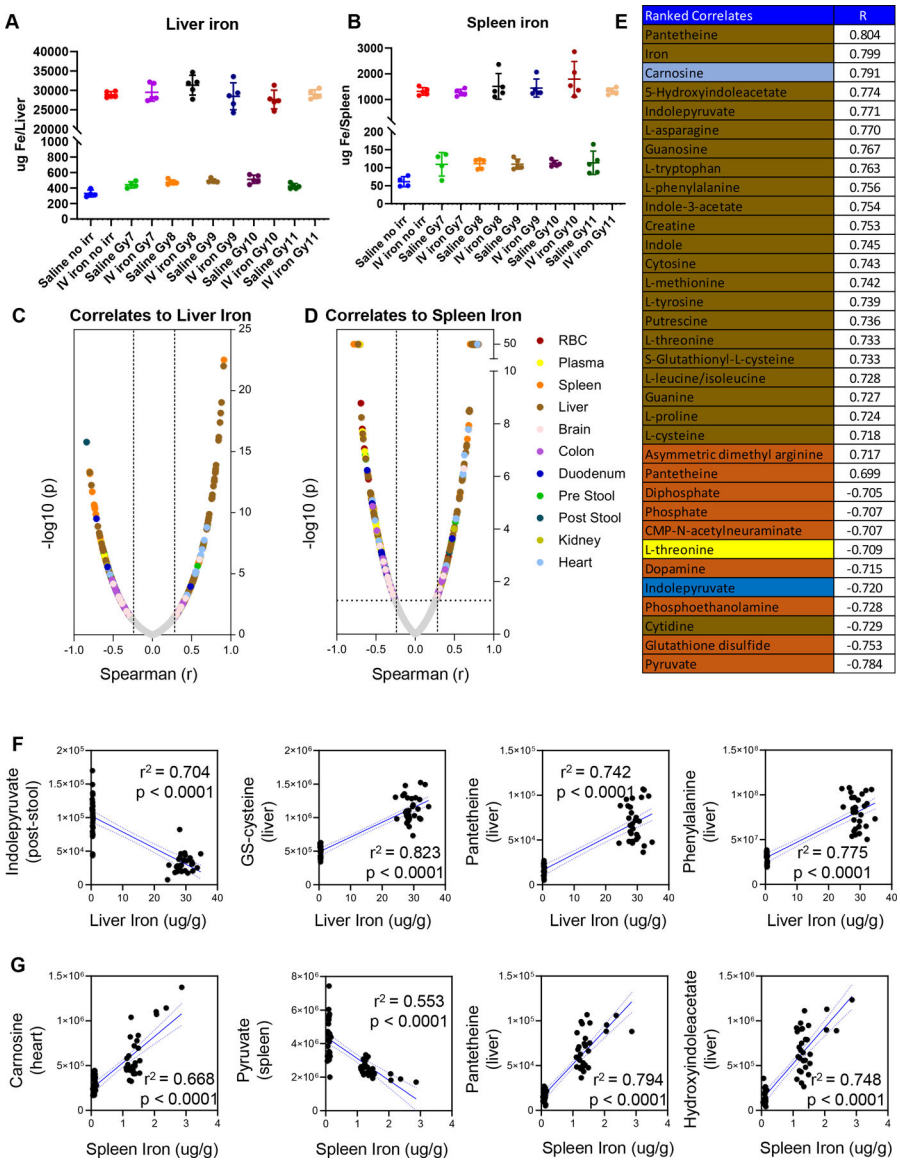

Figure 7 –. Direct measurements of iron in liver (A) and spleen (B) and metabolic correlates across organs (C-D), as color-coded in D.

The same color code is applied to the top 25 significant correlates (Spearman) to spleen iron (strongly correlated to liver iron as well). In F and G, line plots show selected correlates to liver and spleen iron.