RAPID METABOLIC RESPONSES TO IONIZING RADIATION IN BANK VOLES INTRODUCED TO THE DISTURBED ENVIRONMENT OF THE EXCLUSION ZONE: PRELIMINARY DATA

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Background. Understanding the biological effects of ionizing radiation is essential for evaluating risks to human health, protecting ecosystems, and developing effective radiation safety standards. While controlled laboratory studies have provided important insights, they often fail to capture the complexity of low-dose exposure under natural environmental conditions. Therefore, complementary approaches using ecologically relevant models are critically important. The aim of the study was to evaluate preliminary data on liver metabolic responses to ionizing radiation exposure in bank voles following short-term exposure in a radioactively contaminated environment.

Methods. Experimental design: adult male bank voles (*Clethrionomys glareolus*) originating from uncontaminated control areas were marked with subcutaneous RFID-tags and introduced into the disturbed environment of the Red Forest in the Exclusion Zone near to the Chornobyl NPP. After two and a half weeks, animals were sacrificed, and liver samples were collected immediately, frozen and stored on dry ice until processing. Averages of the estimated absorbed doses during the experiment were $6.74\times10^{-2}\pm0.7\times10^{-2}$ mGy and 58.68±11.5 mGy in control and exposed bank voles, respectively. Metabolomic sample processing was performed at Bioplatforms Australia facilitiy using LC-MS approach. In total, 204 metabolites in each sample were identified. The current dataset includes nine animals (five controls and four irradiated individuals). Due to the limited sample size, the results should be interpreted as preliminary evidence of robust trends rather than definitive biomarkers.

Results and discussion. Generally, the metabolomic profile of exposed animals indicates coordinated alterations in energy metabolism, gut microbiota-derived compounds, and stress-related endocrine signaling. T-tests analysis identified 20 metabolites differing between exposed and control animals (see Table 1). Despite the methodological limitations (only rhamnose remained significant after multiple testing correction, FDR < 0.05), the consistency in direction and functional grouping of altered metabolites suggests coordinated metabolic changes rather than random variation. Most metabolites were increased in irradiated animals. These included microbial-associated metabolites such as phenylacetylglycine, 3-furoic acid, and gamma-butyrolactone, as well as amino acid degradation products including ketoleucine, and 2-hydroxy-3-methylbutyric acid (also associated with inflamation). In addition, stress-related hormones cortisol and cortisone were elevated, pointing to activation of the hypothalamic-pituitary-adrenal axis. The elevated levels of gulonolactone and threonic acid (metabolites related to ascorbate) were found also. In contrast, 4-hydroxyhippuric acid and hydrocinnamic acid, both associated with aromatic compound metabolism and gut microbiome activity, were decreased in exposed animals, suggesting selective disruption of microbial metabolic pathways rather than a uniform increase in microbiome activity.

To further assess the relationship between metabolic changes and the absorbed dose of radiation exposure, Spearman rank correlation analysis was performed. Multiple metabolites showed significant correlations with absorbed dose, indicating dose-dependent effects. The strongest positive correlation was observed for phenylacetylglycine ($r = 0.89$, $p < 0.05$), followed by rhamnose ($r = 0.75$, $p < 0.05$) which is a microbial-derived sugar, and its elevation may reflect altered microbiome composition or increased fermentation activity. Several additional metabolites associated with microbial metabolism and amino acid catabolism (lysine, ketoleucine, gamma-butyrolactone, 3-furoic acid) also demonstrated consistent positive correlations with dose ($r = 0.69$-0.73, $p < 0.05$). Increased ketoleucine and 2-hydroxy-3-methylbutyric acid indicate enhanced branched-chain amino acid degradation which is typically associated with increased energy demand or metabolic stress and inflammatory metabolic pathways. Elevated lysine levels further suggest perturbations in nitrogen balance and protein turnover. The increase in cortisol and its positive correlation ($r = 0.75$, $p < 0.05$) with absorbed dose further supports activation of systemic stress-response

pathways. Glucocorticoids are known to stimulate gluconeogenesis, lipolysis, and protein catabolism, promoting rapid energy availability from substrates, which provides a link between environmental stress exposure and the observed metabolic phenotype. Gulonolactone, a precursor of ascorbic acid synthesis, as well threonic acid, a degradation product of ascorbic acid, were increased that correlated to exposure ($r = 0.68$ for both, $p < 0.05$), as which may reflect enhanced oxidative stress and accelerated turnover of ascorbate due to radiation-induced reactive oxygen species generation.

Conversely, strong negative correlations with absorbed dose were detected for 4-hydroxyhippuric acid ($r = -0.82$, $p < 0.05$), arachidic acid ($r = -0.75$, $p < 0.05$), histidine ($r = -0.77$, $p < 0.05$), fructose-1,6-bisphosphate ($r = -0.75$, $p < 0.05$), and a key intermediate in glycogen synthesis and glycosylation pathways – uridine diphosphate glucose ($r = -0.68$, $p < 0.05$), suggesting alteration of specific metabolic pathways at exposured animals. Notably, negatively correlated metabolites include intermediates of carbohydrate and aromatic compound processing, pointing to alterations in glucose metabolism and host-microbiome interactions. These metabolomic findings are consistent with our own unpublished physiological data showing slightly elevated blood glucose levels in exposed small rodents combined with faster glucose clearance during glucose tolerance tests. Elevated glucose may result from glucocorticoid-driven gluconeogenesis, while enhanced glucose utilization is supported by increased metabolic turnover. The negative correlation of fructose-1,6-bisphosphate with dose further suggests increased glycolytic flux and rapid consumption of intermediates. Together, this suggests a state of increased metabolic flexibility, where organisms maintain higher circulating glucose but are able to rapidly uptake and utilize it when needed. Such a phenotype can be interpreted as an adaptive response to environmental stress, allowing organisms to balance energy supply and demand under fluctuating conditions. However, prolonged activation of these pathways may also carry physiological costs, including increased oxidative stress and altered microbiome functions.

Table 1. Important features identified by t-tests

Metabolite	t.stat	p.value	-log10(p)	FDR
Rhamnose	-8.99	5.37×10^{-5}	4.27	0.01096
Ketoleucine	-4.7518	0.00281	2.5518	0.2863
Cortisol	-3.9442	0.00620	2.2077	0.3222
Threonic acid	-3.8000	0.00717	2.1445	0.3222
Gamma-butyrolactone	-3.5349	0.00965	2.0156	0.3222
Phenylacetylglycine	-3.5028	0.01219	1.9141	0.3222
Thiamine pyrophosphate	-3.3696	0.01475	1.8312	0.3222
4-Hydroxyhippuric acid	3.2296	0.01500	1.8239	0.3222
3-Furoic acid	-3.2686	0.01502	1.8235	0.3222
Hydrocinnamic acid	3.3534	0.01579	1.8015	0.3222
2-Hydroxy-3-methylbutyric acid	-2.9594	0.02303	1.6378	0.3980
Pelargonic acid	-3.2556	0.02372	1.6249	0.3980
Pyruvic acid	-2.8197	0.02689	1.5704	0.3980
Cyclic AMP	-2.8533	0.02731	1.5636	0.3980
Ethylmethylacetic acid	-3.0032	0.03612	1.4423	0.4215
Gulonolactone	-2.7387	0.03629	1.4403	0.4215
Cortisone	-2.5534	0.03792	1.4212	0.4215
Capric acid	-2.6752	0.03936	1.4050	0.4215
Lysine	-2.5909	0.04071	1.3903	0.4215
Thymine	-2.6121	0.04944	1.3059	0.4215

Taken together, these preliminary data suggest that radiation exposure induces a metabolic shift toward a high-turnover, stress-adapted state with oxidative component, characterized by increased substrate mobilization, enhanced amino acid catabolism, and altered host–microbiome interactions. This metabolic profile is consistent with increased metabolic flexibility, allowing maintenance of energy homeostasis under environmental stress. Despite the small sample size and limited number of FDR-significant metabolites, the

convergence of independent analytical approaches (Mann-Whitney and t-tests and dose correlation analysis) supports the robustness of the observed trends. Overall, these results suggest that exposed animals undergo adaptive metabolic reprogramming, which may improve short-term physiological performance but could have long-term consequences for metabolic stability and organismal health.