

Physiologically relevant Multi-Omics Flux Analysis Reveals Metabolic Mechanisms of drug-Induced Liver Toxicity

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Physiologically relevant Multi-Omics Flux Analysis Reveals Metabolic Mechanisms of drug-Induced Liver Toxicity

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Abstract

Adverse drug reactions remain a major challenge in drug development and clinical practice. Biomarkers for drug-induced liver injury reflect evolving injury rather than enabling early detection or mechanistic insight before leakage occurs. Physiologically relevant assays that also capture dynamic human drug exposure may close that gap. Here, we present a systems pharmacology framework integrating physiologically based pharmacokinetic modeling with multi-omics profiling of primary human liver spheroids exposed to clinically relevant drug concentration trajectories.

Using the anti-tuberculosis drug isoniazid, we combined time-resolved transcriptomics, proteomics, metabolite exchange, and viability measurements with context-specific genome-scale metabolic modeling to resolve pathway-level metabolic function. Despite limited transcriptional changes, proteomic alterations more closely reflected metabolic flux adaptations, particularly in lipid metabolism.

Flux analysis revealed early increases in amino acid utilization preceding apoptosis, indicating stress-induced metabolic reprogramming prior to cell death. Toxic drug exposure induced a lipid-dominated metabolic state characterized by increased lipid turnover, elevated redox demand, and enhanced amino acid utilization, despite limited lipid availability from cell death. In contrast, therapeutic exposure led to a more balanced metabolic state, including bile acid synthesis.

Together, this PBPK-informed, multi-omics framework enables mechanistic interpretation of drug-induced metabolic stress and provides a causality-driven strategy for improving early detection and safety assessment.

Keywords: liver toxicity, metabolic flux analysis, drugs, proteomics, transcriptomics

Introduction

Drug-induced liver injury (DILI) remains a major challenge in pharmaceutical development and clinical practice, contributing to rare but sometimes severe patient injury and substantial healthcare costs [1]. The liver is particularly vulnerable due to its central role in both xenobiotic and endogenous metabolism. Most drugs are recognized as xenobiotics and undergo sequential activation, modification, and conjugation reactions to facilitate clearance, largely catalyzed by cytochrome P450 (CYP) enzymes [2]. These biotransformation processes have a high energy demand and require cofactors, processes that may compete with endogenous metabolic demand, potentially disrupting metabolic homeostasis and thus contributing to toxicity [3, 4].

Current clinical biomarkers for DILI, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and bilirubin, primarily reflect already established or ongoing liver injury and have limited ability to distinguish transient adaptive responses from progressive toxicity. Consequently, there is a critical need for approaches that enable earlier detection and mechanistic characterization of drug-induced hepatotoxicity before irreversible damage occurs [5].

Preclinical DILI models have limited ability to reliably predict clinical DILI. Conventional in vitro assays often rely on non-physiological single-dose exposure conditions. On the other hand, animal models, despite reliably detecting acute hepatotoxicity, fail to detect rare and idiosyncratic forms of human DILI [6]. Recent advances in in vitro systems and large-scale initiatives [7-10] have generated extensive multi-omics datasets, but linking these data to human-relevant pharmacokinetics and functional metabolic outcomes remains challenging.

To address this limitation, we use a systems pharmacology framework integrating physiologically based pharmacokinetic (PBPK) modeling with primary human liver spheroids exposed to clinically relevant time-concentration profiles [11]. In this setting, multi-omics measurements - including transcriptomics, proteomics [12], targeted amino acids, and viability - were collected over 14 days of repeated exposure of either a therapeutic dosing according to the drug label or a toxic concentration profile as identified from in vitro viability measurements. This enabled a time-resolved characterization of drug-induced responses under physiologically relevant conditions.

As a reference drug, we used isoniazid, an anti-tuberculosis drug, which targets the bacterial cell wall of *Mycobacterium tuberculosis* and is known to cause idiosyncratic DILI [13]. Despite extensive research, the mechanisms underlying isoniazid-induced hepatotoxicity remain incompletely understood. Inter-individual susceptibility is influenced by factors such as dose, metabolism, and

genetic variability, including acetylation status [13-16], highlighting the opportunity for mechanistic frameworks that involve context-dependent metabolic responses to help understand the mode of toxicity.

While multi-omics approaches provide comprehensive molecular profiling, changes in individual gene or protein abundance do not necessarily translate into functional metabolic activity. In metabolic systems, cellular function emerges from coordinated fluxes through interconnected networks, and drug-induced responses are often distributed and compensated. As a result, functionally relevant adaptations may occur without pronounced changes at the expression level, necessitating pathway- and flux-level analysis.

Genome-scale metabolic models (GSMMs) provide a mechanistic framework to integrate multi-omics data with biochemical knowledge, enabling the inference of feasible intracellular flux states consistent with mass balance and experimental constraints. In the liver, where xenobiotic metabolism is tightly coupled to energy production, redox balance, and biosynthesis, GSMMs are particularly well suited to capture system-level metabolic adaptations that are not apparent from expression data alone.

To translate molecular changes into pathway-level insights, we reconstruct context-specific GSMMs based on the Human1 reconstruction [17], integrating transcriptomic, proteomic, and metabolite exchange data. To account for the underdetermined nature of these models, we systematically explored alternative feasible flux states rather than relying on a single optimal solution, using DEXOM [18] combined with flux variability analysis (FVA) [19]. This approach enables robust characterization of metabolic rewiring under both therapeutic and toxic isoniazid exposure.

Previous studies have integrated transcriptomic data into context-specific models, for example using TG-GATEs datasets [20], but often lacked flux-level resolution or relied on earlier reconstructions with incomplete liver-specific metabolism [21, 22]. In contrast, our approach integrates multiple omics layers with quantitative exchange measurements to improve flux estimation and mechanistic interpretability.

Metabolic alterations observed in acute Hepatotoxicity and DILI frequently involve lipid metabolism, glycerophospholipid pathways, and bile acid synthesis [5]. Given the central role of hepatocytes in lipid and amino acid metabolism, cellular adaptations or cell death-associated processes may contribute to observed metabolite changes. Little is known about the underlying mechanisms leading to changes in phospholipids in drug-induced hepatotoxicity. To capture these effects, we incorporate lipid availability arising from cell death into the Human1 model, enabling quantitative assessment of its contribution to metabolic rewiring.

Together, this PBPK-informed, multi-omics framework links physiologically relevant drug exposure to network-level metabolic responses. This approach provides a mechanistic basis for understanding

drug-induced metabolic stress and offers a generalizable strategy to distinguish adaptive from toxic responses in human liver systems.

Results

Distinct time-resolved physiology responses to therapeutic and toxic exposure profiles

Using our integrated workflow (Figure 1), we tracked metabolic reprogramming of human liver spheroids exposed to PBPK-informed isoniazid regimens (figure 2A and supplementary figure 1). Therapeutic exposure preserved spheroid size over 14 days, whereas toxic dosing led to apoptosis and ~70% reduction in spheroid volume by day 10 (Figure 2B). Despite biomass loss, toxic-exposed spheroids exhibited increased ATP luminescence per unit spheroid size, indicating elevated metabolic activity prior to cell death (Figure 2C). At day 14, most cells were dead, precluding further omics analyses. These phenotypic outcomes provided quantitative constraints to assess whether metabolic changes preceded or followed cell death.

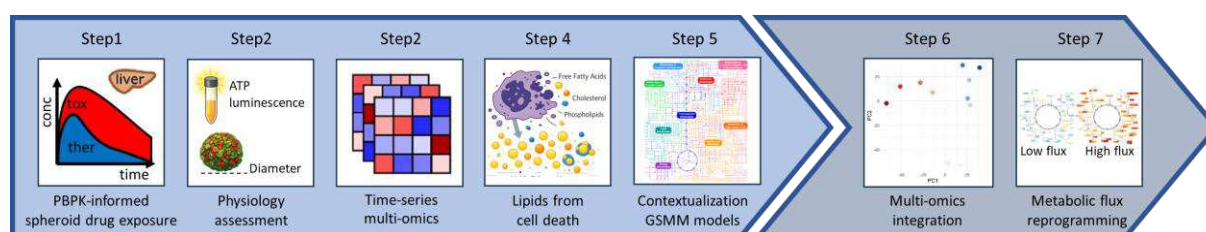


Figure 1. Workflow and assay design.

Integrated experimental–computational workflow for assessing repeated therapeutic and toxic drug exposure. The study design and analysis are organized into seven sequential steps, spanning PBPK-informed assay design, physiology assessment, multi-omics measurements, incorporation lipid availability from cell-death, context-specific constraint-based metabolic modeling, and integrated multi-omics analysis and pathway-level interpretation of metabolic flux reprogramming.

Differential information content in time-resolved transcriptomics and proteomics

To assess how regulatory information is distributed across molecular layers, we first performed time-resolved transcriptomic and proteomic profiling under therapeutic and toxic exposure conditions (n=3 biological replicates) [12].

Time-resolved proteomics revealed substantial biological variability across conditions (mean sample correlation 0.84 ± 0.04 ; Figure 2D), indicating robust, condition-specific protein regulation. In contrast, transcriptomes were globally stable (mean correlation 0.998 ± 0.001 , Figure 2E), with modest differential expression. Correlation between transcript and protein abundances was moderate ($r =$

0.32, supplementary Figure 2), consistent with post-transcriptional regulation and protein turnover, while changes in z-scored expression values were weakly correlated ($r = 0.066$).

Hierarchical clustering separated drug exposure from untreated samples in the proteomics data (Figure 2F) and toxic from therapeutic exposure in the transcriptomics data (Figure 2G). Within the toxic condition, early (days 1-3) and late (days 7-10) time points formed distinct clusters, indicating temporal adaptation. Differentially expressed proteins (DEPs) showed substantially greater overlap between dosing conditions than differentially expressed genes (DEGs) (43% vs. 12%, respectively). Functional enrichment of DEPs highlighted cholesterol, amino acid, and glutathione metabolism, alongside apoptotic processes under toxic dosing from day 7 onward, whereas therapeutic dosing induced apoptotic enrichment only at day 14 without detectable biomass loss. DEGs additionally showed enrichment of inflammatory response pathways (supplementary figure 3).

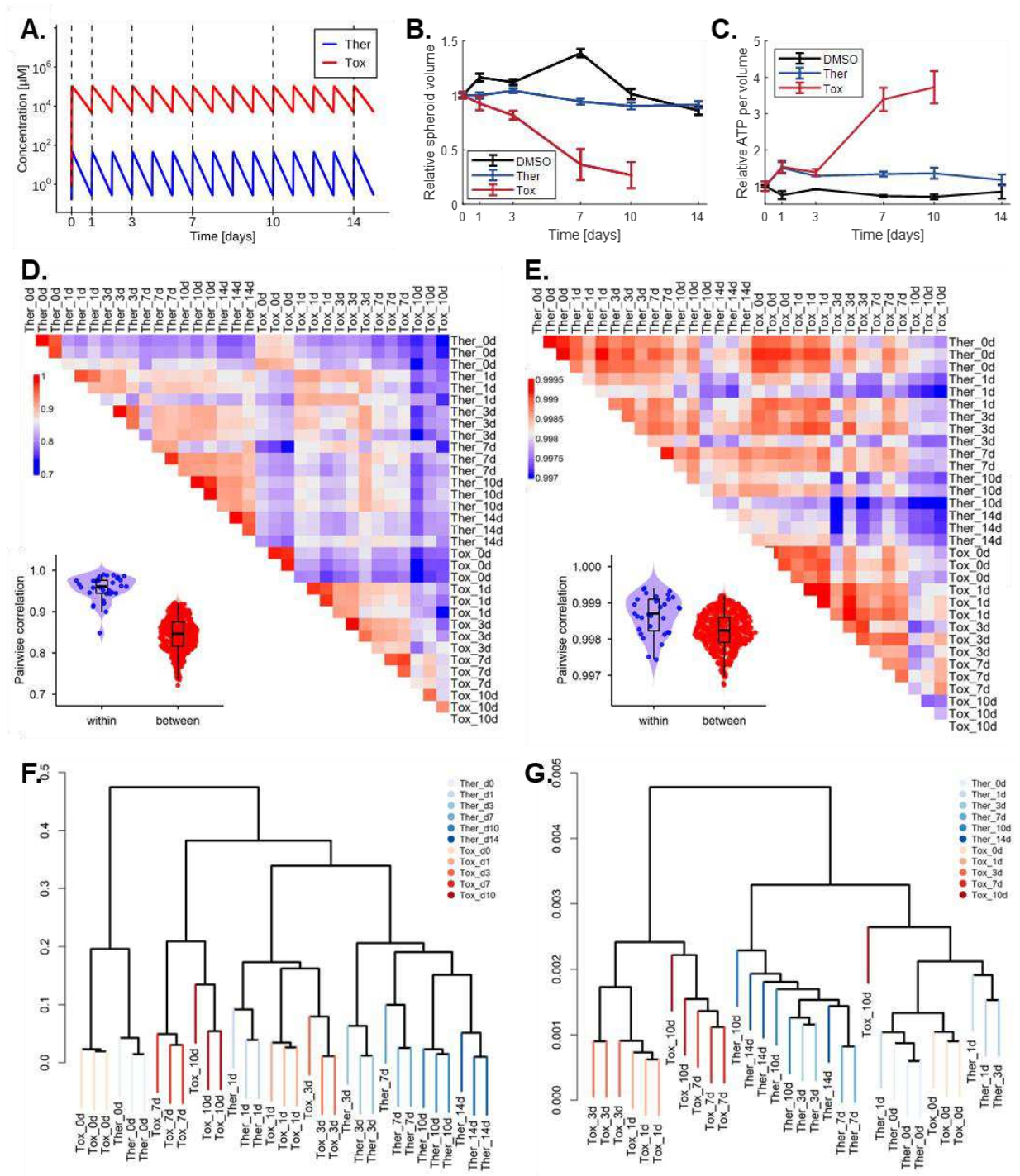


Figure 2. PBPK modelling and data for integration into Human1.

Blue indicates therapeutic conditions and red toxic conditions. **A.** PBPK-derived hepatic exposure profiles for therapeutic and toxic dosing regimens. Black dots and lines indicate sampling time points for multi-omics measurements. **B.** Spheroid size over time, expressed as volume relative to T0. **C.** Cell viability assessed by intracellular ATP luminescence relative to T0. **D-E.** Pairwise correlations between all samples. Each square represents the correlation between ~2,000 quantified proteins (**D**) or ~27,000 transcripts (**E**) from one sample versus another. The color denotes the Pearson correlation coefficient from blue ($r = 0.75$) to red ($r = 1$) for proteomics and from blue ($r = 0.997$) to red ($r = 1$) for

transcriptomics. Note the excellent reproducibility of biological triplicates on the diagonal (red). The inserts show the distribution of the pairwise correlations within replicates and between conditions. The width of each violin reflects the density of observations. Individual data points are overlaid to illustrate the spread and concentration of values. Boxplots are superimposed to indicate the median and interquartile range (IQR), with whiskers representing $1.5 \times$ IQR. **F.** Hierarchical clustering of proteomics **G.** Hierarchical clustering of transcriptomics.

Context-specific models reveal adaptive network expansion

While differential expression analysis highlighted distinct responses at the transcript and protein levels, they do not directly translate to system-level fluxes and aimed to capture exposure-induced metabolic changes by performing uncertainty-aware flux analysis taking into account variability at the network structure as well at the flux level. Using DEXOM with the Human1 reference model, we reconstructed condition-specific metabolic networks. Transcriptomics covered 2884/2887 Human1 genes, representing 62% of the Human1 reactions via gene–protein–reaction relations, whereas proteomics captured 568 proteins covering 23% of reactions. Models reduced from 12,971 reactions in Human1 to 281 (Dimethyl sulfoxide (DMSO) control) and 4298 (therapeutic dosing). Network expansion was observed progressively under therapeutic exposure, with an initial reduction, whereas toxic dosing induced immediate and pronounced network shifts (Figure 3A for proteomics-based flux capacities, supplementary figure 4 for transcriptomics). Expansion was particularly pronounced in lipid metabolism, amino acid catabolism, redox metabolism, and mitochondrial pathways. Model similarity and pairwise flux capacity correlations mirrored these patterns (supplementary Figure 5 and Figure 3B) with relatively narrow flux ranges driven by alternative optima from contextualization rather than wide flux ranges (Figure 3C). Control models remained smaller and more flexible.

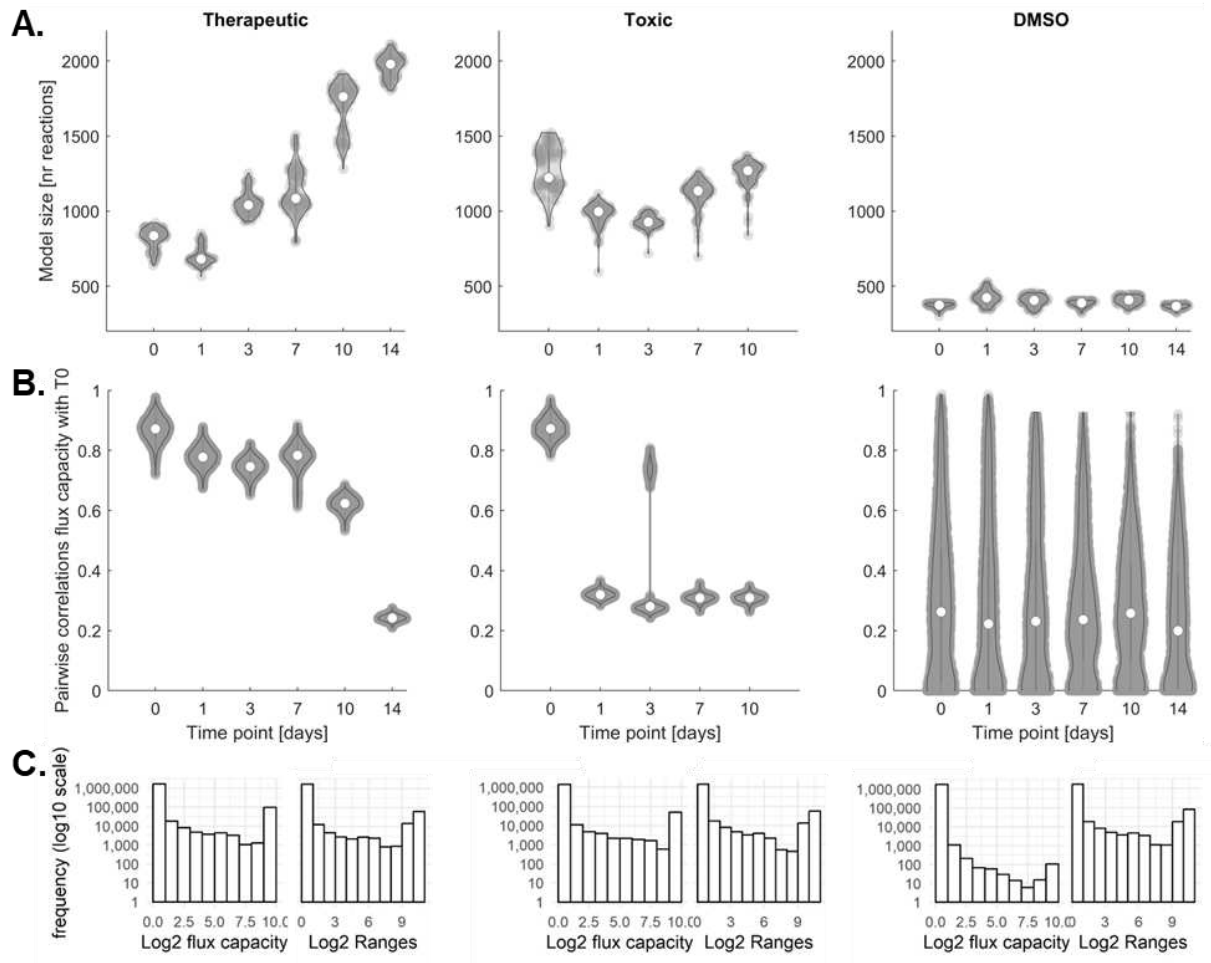


Figure 3. Dynamics of context-specific metabolic models

Context-specific models inferred from proteomics and metabolic constraints for therapeutic, toxic, and DMSO control conditions. Each dot represents one of 100 alternative optimal model reconstructions. The width of each violin reflects the density of observations. **A.** Model size (number of active reactions). **B.** Pairwise Pearson correlation coefficients of flux capacities relative to T0. **C.** Distributions of flux ranges and flux capacities across conditions.

Multi-omics PCA reveals coordinated regulation and proteome-flux coupling

To examine how transcriptomic, proteomic, and predicted flux capacity changes jointly organize across conditions, we performed integrated principal component analysis (PCA). PCA showed that PC1 separated toxic from therapeutic exposure, while PC2 distinguished treated from untreated samples and partly time-dependent variation (Figure 4A). Despite their global stability, transcripts contributed 47% to PC1 (Figure 4B). This indicates coordinated transcriptional regulation underlying the primary exposure response, whereas proteomics and fluxes dominated downstream and temporal effects. Key contributors included carnitine shuttle, arginine/proline metabolism, and bile acid/fatty acid pathways. Therapeutic exposure enriched anabolic pathways such as bile acid synthesis, whereas toxic

dosing favored catabolism, such as keratan sulfate degradation (Supplementary Figure 6). Farnesoid X receptor (FXR) targets were enriched among top PC1 contributors under therapeutic exposure (odds ratio = 7.0, $p = 9.8 \times 10^{-7}$), implicating transcriptional control of lipid and bile acid homeostasis. Proteomic variation dominated PC2 (71%), reflecting mitochondrial stress and metabolic adaptation. Thus, transcriptomic and proteomic layers were both structured, but along distinct and partially orthogonal axes of variation. Proteomic changes correlated more strongly with predicted fluxes ($r = 0.19$) than transcriptomic changes ($r = 0.016$), particularly for fatty acid oxidation, amino acid catabolism, and cholesterol metabolism (Figure 3C). Among the pathways driving this separation, amino acid metabolism emerged as a central adaptation, prompting a closer examination of its functional behavior at the flux level.

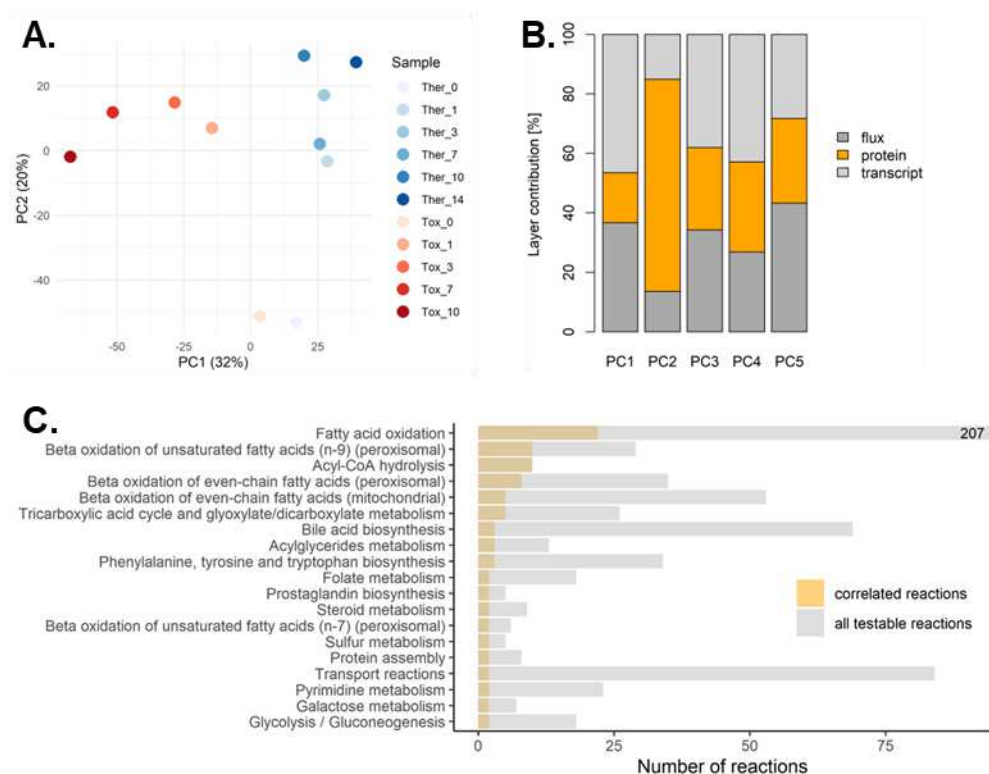


Figure 4. Integrated multi-omics analysis.

A. PCA score plots colored by condition and time point. **B.** Relative contribution of each data layer (transcriptomics, proteomics, fluxomics) to the principal components (PCs). **C.** Number of significant flux–protein correlations per pathway. Orange indicates significant Pearson correlations; grey indicates all tested reactions. Fatty acid oxidation is off-scale and annotated with its corresponding value.

Early amino acid catabolism precedes apoptosis

Consistent with its strong contribution to the multi-omics separation, amino acid metabolism showed pronounced changes at the flux level. Additional measurement of extracellular amino acid exchange rates (see materials and methods), combined with flux simulations, revealed elevated uptake and catabolism of aromatic and essential amino acids following isoniazid exposure (Figure 5). Glutamine and alanine were consumed most in both therapeutic and toxic conditions. Under therapeutic dosing, this response occurred prior to apoptosis or biomass loss, indicating an adaptive stress response not driven by cell death-associated degradation. Toxic exposure further increased essential amino acid uptake such as tryptophan, lysine, methionine, and threonine, whereas uptake decreased under therapeutic exposure. Glutamate was consistently excreted.

Notably at the final therapeutic time point, fluxes shifted from glycolysis to amino-acid-fueled gluconeogenesis, suggesting a late-stage energy adaptation absent under toxic conditions.

Incorporation of measured amino acid exchange rates improved the concordance correlation coefficient with predicted fluxes (CCC = 0.49 vs. 0.034 in unconstrained simulations), indicating that post-translational regulation and mass balance constraints strongly shape metabolic outcomes. When transcriptomics alone was used to infer fluxes, concordance was lower (0.11).

Lipid availability and metabolite excretion under stress

The increased reliance on amino acid catabolism was paralleled by adjustments in lipid metabolism, suggesting coordinated use of multiple energy and redox sources under isoniazid exposure. Total Lipid availability from cell death was low ($<0.16 \text{ mmol} \cdot \text{gDW}^{-1} \cdot \text{h}^{-1}$ corresponding to $<6.6 \times 10^{-4} \text{ mmol} \cdot \text{gDW}^{-1} \cdot \text{h}^{-1}$ per lipid species in figure 5B), indicating minimal contribution to energy. Despite this, inclusion of lipid availability reshaped predicted metabolism. Toxic exposure increased fatty acid uptake, β -oxidation, cholesterol turnover, phospholipid remodeling, carnitine shuttle activity, and pantothenate/CoA metabolism (Figure 6C). Lipid-derived reducing equivalents likely facilitated redox balancing for β -oxidation and glutathione-dependent detoxification, enabling higher amino acid catabolism under toxic stress improving concordance between predicted and measured amino acid fluxes from CCC = 0.26 without to 0.49 including lipid availability from cell death. Proteomic enrichment reinforced these changes, with cholesterol efflux most enriched (supplementary figure 3A) alongside triglyceride homeostasis, phospholipid efflux, and intermembrane lipid transfer. Therapeutic dosing primarily enriched lysosomal lipid catabolism and glycolipid metabolism (supplementary figure 3B).

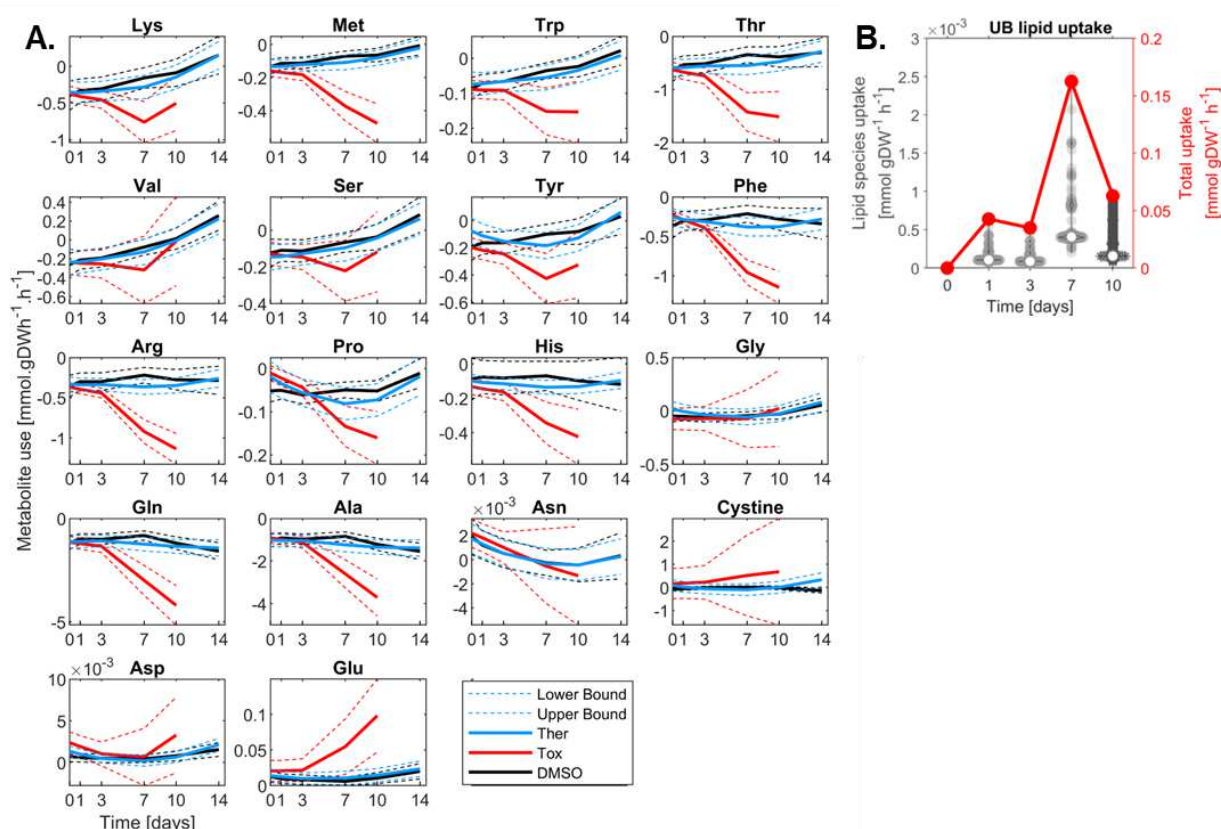


Figure 5. Time-resolved metabolite exchange rates

A. Amino acid exchange rates. Positive values indicate secretion, negative values uptake from the medium. Solid lines represent fitted exchange rates, while dotted lines indicate standard deviations used as upper and lower bounds for metabolic modeling. **B.** Upper bound (UB) on lipid uptake from cell death derived lipid degradation: total lipid availability is shown on the right axis, the availability of individual species as a violin plot on the left axis.

Differential pathway-level metabolic reprogramming

Consistent with increased amino acid uptake and catabolism, toxic exposure was accompanied by enhanced nitrogen disposal and widespread metabolite excretion. In total, 178 metabolites were increased at one or more time points (Figure 6A), including CO_2 , $\text{NH}_3/\text{NH}_4^+$, urea, and proton excretion, reflecting elevated oxidative metabolism and nitrogen turnover. In parallel, increased secretion of medium-chain fatty acids, bile acid conjugates, eicosanoids, and sphingolipid intermediates indicated extensive lipid remodeling. Increased secretion of pyridoxal linked these changes to altered cofactor turnover.

Markers of extracellular matrix and membrane turnover were also increased, including products of chondroitin sulfate degradation and sphingolipid metabolism, consistent with structural remodeling under toxic stress. Lipid peroxidation was indicated by elevated oxidized linoleate-containing species,

while increased glutathione and (conjugated) bilirubin excretion supported a state of heightened detoxification demand.

In contrast, therapeutic dosing resulted in a more balanced excretion profile, with 130 metabolites increased (Figure 6B), including bile acids, long-chain fatty acids, and acylcarnitines.

Pathway enrichment analysis confirmed distinct metabolic programs. Toxic exposure induced a lipid-centered, redox-coupled catabolic state (Figure 6C), characterized by increased lipid turnover and suppression of de novo bile acid synthesis, alongside enrichment of transporter-mediated bile acid handling (Human1 “recycling” subsystem). In vitro, this reflects increased transporter activity rather than enterohepatic circulation. FXR target enrichment was absent (odds ratio = 0, $p = 1$), consistent with a stress-adapted metabolic regime.

In contrast, therapeutic exposure showed a more temporally restricted and balanced response (Figure 6D), involving fewer pathways and preserving anabolic functions, including bile acid biosynthesis and amino acid metabolism, as well as spheroid biomass. Consistent with this adaptive state, FXR targets were strongly enriched (odds ratio = 63, $p = 2.0 \times 10^{-9}$), indicating maintained regulatory control and metabolic flexibility.

Together, these results indicate that toxic exposure induces a coordinated metabolic reprogramming characterized by increased lipid turnover, enhanced amino acid utilization, and elevated redox and cofactor demand.

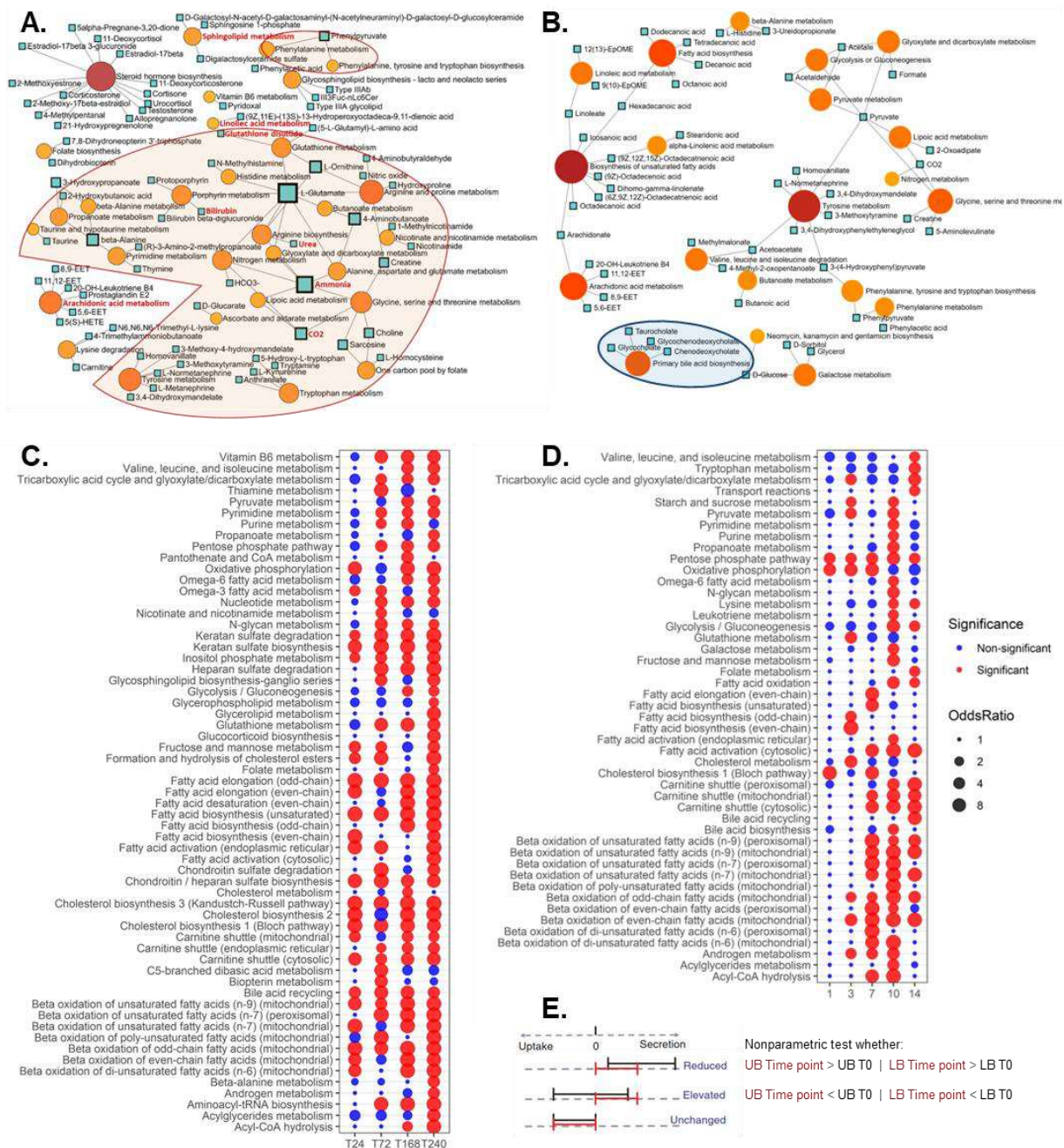


Figure 6. Up-regulated metabolic activities

A-B. Significantly altered excreted metabolites in toxic (A) and therapeutic (B) conditions mapped to pathways using MetaboAnalyst [23]. Amino acids are highlighted in (A) and bile acids in (B). **C-D.** Flux enrichment analysis from proteomics-constrained models upon toxic (C) and therapeutic exposure (D). Pathways that are up-regulated in at least one time point are shown. Dot size indicates odds ratio; color denotes statistical significance (adjusted p-value < 0.05). **E.** Significance thresholding strategy, in which is reaction is considered significantly altered if either the upper or lower bound from FVA is significantly altered across 100 alternative model reconstructions.

TCA cycle as a hub linking lipid, amino acid, and mitochondrial metabolism

To mechanistically interpret how the pathway-level changes are integrated at the network level, we next examined the underlying flux organization of the individual reactions. A conceptual overview of the adaptations is shown in Figure 7A, where toxic isoniazid exposure drives oxidative stress and lipid release from damaged cells, leading to increased lipid uptake and cofactor imbalance. These processes converge to increase redox demand and promote metabolic rewiring in surviving cells.

At the network level, flux enrichment analysis identified the tricarboxylic acid cycle (TCA) cycle as a central metabolic hub integrating inputs from amino acid catabolism, fatty acid oxidation, cholesterol side-chain shortening, and bile acid metabolism (Figure 7B). These pathways converge through anaplerotic reactions that replenish TCA intermediates, sustaining elevated metabolic activity under isoniazid-induced stress. This integration is tightly coupled to cofactor turnover, including NAD(P)H and ATP/ADP, linking carbon flux to cellular energy and redox balance.

Propionate metabolism emerged as a key connecting metabolite within this network. Increased conversion of propionyl-CoA to succinyl-CoA provides a direct entry point into the TCA cycle, linking odd-chain fatty acid oxidation, cholesterol metabolism, and amino acid catabolism to anaplerosis and ATP production. Consistent with this, uptake of amino acids with glucogenic and ketogenic potential - including glutamine, tryptophan, lysine, methionine, and threonine - was elevated under toxic conditions.

In contrast, acetyl-CoA appears to play a more context-dependent role. Rather than serving primarily as a net carbon source for TCA cycle oxidation, acetyl-CoA is preferentially directed toward biosynthetic and regulatory processes, including cholesterol turnover, membrane remodeling, isoniazid acetylation, and redox-coupled energy metabolism.

Clinical relevance of metabolic rewiring

To assess whether the observed metabolic adaptations are reflected in patients, we analyzed clinical data from patients receiving isoniazid using the Explorys database [24] (Figure 7C). In total, 2,713 patients received isoniazid treatment, of whom 15 developed DILI (0.55%), consistent with the 0.5-1% incidence reported in LiverTox [13]. Among patients with DILI, ALT and AST levels reached up to 37-fold and 22-fold above the upper limit of normal (ULN), respectively. Because baseline measurements were unavailable for these patients, they are not shown in Figure 7C. Across the broader cohort, ALT and AST levels increased significantly following isoniazid treatment, independent of DILI status, indicating early liver injury (Figure 7C). Total bilirubin exceeded the ULN in 8 of 163 patients, consistent with impaired hepatic function.

Beyond these standard clinical markers, measurements related to early metabolic adaptation were limited. Circulating amino acids and phospholipids, predicted by our model to be altered under toxic conditions, were not routinely assessed. However, glucose and urea measurements showed increased variability following isoniazid treatment. Urea levels exceeded the ULN in 6 of 71 patients and fell below the lower limit of normal in 7 patients, while glucose exceeded the ULN in 9 of 32 patients and was decreased in 1 patient. Although the number of available measurements was limited, these findings are broadly consistent with model-predicted alterations in central carbon metabolism and nitrogen handling.

Together, these observations support the presence of drug-induced metabolic rewiring in patients and suggest that alterations in lipid, redox, and nitrogen metabolism may precede or complement classical DILI biomarkers.

Discussion

Adaptive metabolism precedes degeneration

Isoniazid exposure induced coordinated metabolic reprogramming, characterized by increased amino acid demand, enhanced lipid utilization, and expanding network complexity. Crucially, these metabolic changes precede apoptosis and cannot be explained by biomass degradation alone. By integrating PBPK-informed exposure, multi-omics data, uncertainty-aware flux analysis, and lipid availability from cell death, we mechanistically disentangled adaptive metabolism from secondary cell death effects. This integrative framework provides new insight into the metabolism of drug-induced liver injury (DILI) and highlights the value of constraint-based modeling for resolving causality in complex toxicological settings.

Clinical studies report elevated circulating amino acid levels in DILI patients, commonly attributed to increased proteolysis [25]. Released amino acids can subsequently fuel catabolic and stress-response

pathways that support energy production, redox balance, and detoxification. Consistent with this paradigm, our flux simulations predicted increased amino acid uptake and catabolism following isoniazid exposure. Notably, increased amino acid metabolism was already observed under therapeutic dosing, preceding measured cell death, and further amplified under toxic exposure. This suggests that enhanced amino acid utilization represents an early adaptive response to metabolic stress rather than a passive consequence of cellular degeneration.

CYP enzymes link xenobiotic clearance and endogenous lipid metabolism

CYP enzymes form a central interface between xenobiotic metabolism and endogenous lipid pathways. In the Human1 model, CYP2E1, one of the primary enzymes involved in isoniazid clearance, is associated with 102 reactions spanning bile acid, cholesterol, steroid, fatty acid, and vitamin D metabolism. This extensive connectivity highlights how increased xenobiotic burden can influence multiple metabolic subsystems through shared enzymatic capacity and dependence on reducing equivalents such as NADPH.

Our flux analysis indicates that toxic exposure shifts this balance. Specifically, we observed increased bile acid transport and conjugation without a corresponding increase in de novo bile acid synthesis. Within the Human1 framework, the “recycling” subsystem primarily represents transporter-mediated redistribution rather than physiological enterohepatic circulation. We therefore interpret this pattern as a shift toward enhanced bile acid export capacity rather than increased synthesis.

A plausible explanation is that elevated xenobiotic burden redistributes CYP activity and NADPH usage away from biosynthetic pathways toward detoxification. Under therapeutic exposure, this balance appears preserved, allowing coordinated regulation of drug metabolism and bile acid synthesis. In contrast, under toxic exposure, increased demand for xenobiotic clearance and redox buffering may constrain CYP-dependent bile acid synthesis while favoring conjugation and export processes that reduce intracellular bile acid accumulation.

This interpretation aligns with hepatocellular stress-response paradigms in which bile acid homeostasis is maintained primarily through altered transport and conjugation rather than increased synthesis [26]. Consistently, our model predicts increased bile acid glucuronidation and export (e.g., CDA-24G) under toxic conditions. Given the absence of microbiome-derived secondary bile acids in this system, we interpret these fluxes as proxies for enhanced conjugation and export capacity rather than literal production of specific bile acid species.

Rerouting of tryptophan metabolism reflects stress-induced metabolic adaptation

Our simulations predict a condition-specific rerouting of tryptophan metabolism under toxic exposure, characterized by increased downstream catabolism and altered kynurenine excretion. Notably, these

changes were driven by increased tryptophan uptake constrained by exchange fluxes rather than differential expression of enzymes such as IDO1 or TDO2, indicating that they arise from flux redistribution rather than transcriptional regulation.

The kynurenine pathway is coupled to NAD⁺ metabolism and redox balance, suggesting a role in managing oxidative stress and maintaining mitochondrial function. Given known interactions between bile acid signaling, FXR activity, and tryptophan metabolism [27], this shift likely reflects broader metabolic coordination under hepatocellular stress.

These findings illustrate a key advantage of constraint-based modeling: the ability to detect functional metabolic rerouting that is not apparent at the level of gene or protein expression. In this context, altered tryptophan metabolism may represent a downstream signature of metabolic stress rather than a primary driver of toxicity, consistent with its proposed role (together with phenylalanine and tyrosine) as a biomarker in mice [28].

FXR signaling integrates bile acid, lipid, and amino acid metabolism

FXR target genes were strongly enriched under therapeutic exposure but not under toxic conditions, indicating a loss of coordinated bile acid regulation under stress. FXR acts as a central regulator linking bile acid synthesis, lipid metabolism, and xenobiotic detoxification, primarily by repressing CYP7A1 and promoting bile acid export.

Bile acid conjugation provides an additional layer of regulation, as glycine- and taurine-conjugated bile acids differ in FXR affinity. FXR also influences amino acid metabolism, including urea cycle activity, and gluconeogenesis, coupling nitrogen disposal and redox balance to bile acid homeostasis.

The absence of FXR enrichment under toxic conditions suggests that these regulatory circuits become uncoupled during stress. Additional pathways, including FGF1–FGFR4–HNF4 α signaling, may partially compensate xenobiotic burden [29].

Proteomics-informed fluxes better capture functional metabolic states

The modest correlation between transcriptomic and proteomic data ($r \approx 0.3$) indicates substantial post-transcriptional regulation and highlights that these data layers capture complementary aspects of cellular state. Accordingly, models derived from transcriptomics and proteomics produced distinct flux distributions.

Proteomics-informed fluxes showed higher concordance with experimentally measured amino acid exchange rates than transcriptomics-derived fluxes (0.49 vs. 0.16) and stronger overall agreement with omics data (0.19 vs. 0.03). This indicates that protein abundance more closely reflects catalytic capacity under these conditions. These differences are consistent with the multi-layered regulation of metabolism, where fluxes depend not only on expression but also on factors such as enzyme

saturation, cofactor availability, network dependencies and mass-balance constraints. As already reviewed by Machado et al. [30] central metabolic pathways in particular are heavily regulated beyond transcription, making transcript levels alone unreliable proxies for flux.

At the same time, transcriptomics provided broader coverage and captured coordinated regulatory programs, as reflected in its strong contribution to the primary axis of variation in integrated PCA. The divergence between gene- and protein-level contributions to principal components suggests that regulation operates across partially decoupled layers, with increased convergence at the pathway level.

Importantly, our framework retains reactions lacking direct proteomic evidence when required for mass balance and known physiology. For example, CYP7A1, undetected at the protein level but transcriptionally upregulated, was included due to its central role in bile acid synthesis. Similarly, inferred increases in cholesterol synthesis were supported indirectly by proteomic upregulation of lipoprotein transport proteins (APOA1, APOA2, APOB, APOC2, APOE).

Drug–metabolite interactions and cofactor depletion as hidden regulatory layers

Beyond gene and protein regulation, direct interactions between drugs and endogenous metabolites represent an additional layer of metabolic control. Isoniazid is known to non-enzymatically form adducts with ketone acids, NAD⁺, and vitamin B₆ derivatives, including pyridoxal and pyridoxal-5'-phosphate (PLP) [31]. PLP depletion impairs a wide range of aminotransferases and enzymes involved in sphingolipid metabolism [32].

Such effects are not explicitly captured in current genome-scale models, including Human1, which does not encode PLP as a stoichiometric cofactor for many reactions. This represents an important limitation, as cofactor depletion can substantially alter effective enzyme activity without changes in expression levels. Incorporating explicit enzyme constraints and drug–cofactor interactions into enzyme-constrained genome-scale models [33] holds promise for improving flux prediction accuracy and mechanistic resolution.

Model choices and controls

As expected from stringent cut-offs, the resulting context-specific models were relatively small and not designed to recapitulate all hepatic metabolic functions. Rather, they capture exposure-induced metabolic changes. While the unconstrained Human1 model supported biomass production, most context-specific models did not, in agreement with observed spheroid size stability or decline over time.

While we did not explicitly model amino acid release from proteolysis, most amino acids were supplied in excess, and albumin degradation was not predicted under the applied constraints. Including proteolysis would therefore primarily increase uncertainty without altering key conclusions. Although DMSO is not entirely inert [34], its effects were minor compared to isoniazid, supporting its use as a control.

Early adaptive responses and implications for DILI biomarkers

Notably, therapeutic dosing already induced measurable metabolic changes, consistent with clinical observations that isoniazid can alter liver biomarkers without progressing to overt injury [13]. In vivo, additional systemic buffering mechanisms, including hormonal, immune, cytokine, microbiome including FXR-mediated signaling, are likely to further stabilize hepatic metabolic homeostasis.

Current clinical markers of DILI lack specificity (ALT and AST) or primarily reflect established injury (bilirubin). In addition, a challenge for diagnostics is to be able to assess if early markers ALT and AST will evolve to DILI or not. Several alternative biomarkers have been proposed, including glutathione depletion, bile transporter expression, and ATP levels [5]. Our results point to a broader set of candidate markers associated with early metabolic adaptation, including circulating amino acids, urea, ammonia, bile acids, phospholipids, propionate, and glucose. Notably, increased amino acid catabolism and altered gluconeogenic fluxes were observed prior to detectable cell death, indicating that these changes precede overt toxicity.

This interpretation is supported by clinical observations of elevated blood glucose in specific cases [35, 36] and observed in large-scale datasets of patients taking isoniazid in the Explorys database. Similarly, heterogeneous changes in urea levels were observed clinically, with both increases and decreases across patients. Increased urea is consistent with the model-predicted elevation in amino acid catabolism and nitrogen disposal, whereas decreased urea may reflect impaired hepatic function or altered systemic metabolism. These findings highlight the distinction between intracellular metabolic flux adaptations and circulating biomarker levels. A similar observation applies to bilirubin. While the model predicts increased bilirubin production as part of a stress response, clinical measurements reflect the balance between production and hepatic clearance, resulting in both increased and decreased circulating bilirubin levels across patients.

Together, these findings suggest that DILI is preceded by a metabolically distinct pre-injury phase, characterized by redox imbalance and altered carbon and nitrogen metabolism, offering opportunities for earlier and more specific detection and intervention.

Interpretation of clinical biomarker data in DILI patients was limited by the small number of available measurements after exclusion of confounding conditions, as many patients presented with comorbidities that could influence biomarker levels.

Outlook

Future extensions incorporating enzyme constraints, drug-cofactor interactions, and explicit cofactor dependencies may further improve mechanistic resolution and predictive accuracy. More broadly, this framework provides a foundation for integrating physiologically relevant exposure models with multi-omics data to enable mechanism-based assessment of drug-induced liver injury.

Methods

Spheroid cultivation

The 3D InSight™ Human Liver Microtissue is a commercially available co-culture model consisting of approximately 1,000 primary human hepatocytes and ~1,000 non-parenchymal liver cells, including Kupffer and liver endothelial cells (InSphero; <https://insphero.com/products/liver/toxicology-models/>). For the HeCaToS project [12], microtissues were generated from a multi-donor batch of primary human hepatocytes (5 male and 5 female donors, aged 7–59 years) and non-parenchymal liver cells derived from a single 27-year-old Caucasian donor. Microtissues were maintained in 3D InSight™ Human Liver Microtissue Maintenance Medium-AF (Cat. #CS-07-001a-01) containing galactose.

PBPK-informed exposure design

To mimic physiologically relevant drug exposure, spheroids were treated with isoniazid using concentration-time profiles derived from PBPK modeling, as described previously [37]. PBPK simulations were performed using PK-Sim (Open Systems Pharmacology; <http://www.open-systems-pharmacology.org/>) to estimate liver exposure profiles following oral dosing. These profiles were translated into an in vitro exposure scheme with once-daily dosing. Therapeutic exposure was based on clinically relevant dosing (600 mg once daily), while toxic exposure was defined using a PBPK-informed reverse dosimetry approach based on concentration-response experiments.

Concentration-response experiments (for dose definition and amino acid analysis)

To determine toxic exposure levels, concentration-response experiments were performed prior to the multi-omics study [11]. Microtissues were exposed to increasing concentrations of isoniazid (0–10,000 µmol/L) for 14 days. Medium was changed every 2-3 days and collected for subsequent analysis. Cell

viability was monitored to identify exposure conditions corresponding to a 20% reduction in viability (IC_{20}) after 7 days. Based on these experiments, concentrations corresponding to therapeutic (233 $\mu\text{mol/L}$) and toxic (2441 $\mu\text{mol/L}$) exposure were selected.

Medium collected from these experiments was used for targeted amino acid analysis. Samples were obtained at 0, 2, 5, 7, 10, 12, and 14 days, enabling time-resolved characterization of amino acid exchange under defined concentration conditions.

The IC_{20} -derived toxic exposure level was subsequently integrated with PBPK-derived liver concentration profiles to define the toxic dosing regimen applied in the multi-omics experiments.

Multi-omics exposure experiments

For the main study, microtissues were exposed to PBPK-derived therapeutic and toxic concentration–time profiles for up to 14 days [11, 12]. Samples were collected at 0, 1, 3, 7, and 10 days for both exposure conditions, and at 14 days for the therapeutic and DMSO control conditions. Each condition included three biological replicates per time point (Supplementary Fig. 1).

To obtain sufficient material for downstream analyses, each biological replicate consisted of multiple microtissues that were exposed individually and pooled prior to extraction.

Time-resolved measurements included transcriptomics, proteomics, and viability assessments. Protocols for RNA sequencing and proteomics have been described previously [12].

Cell viability was assessed by intracellular ATP content and microtissue size. ATP levels were measured using the CellTiter-Glo® 3D Cell Viability Assay (Promega) according to the manufacturer's instructions. Microtissue size was estimated by measuring spheroid diameter using light microscopy.

Targeted amino acid analysis

Frozen medium samples from spheroid experiments were thawed and filtered to remove potential precipitates before LC-MS/MS analysis using cellulose-acetate syringe filter (0.2 μm , DIA-Nielsen; Düren, Germany). Amino acids were measured and quantified according to the method described by Thiele et al. [38] with the same LC hardware setup and chromatography conditions, except that the triple quadrupole MS/MS system was an API4000 (ABSciex, Darmstadt, Germany). MS/MS analysis was done using TurboV ion source for electrospray ionization in positive mode with scheduled MRM mode with Q1/Q3 in unit resolution, collision gas 5 psi, curtain gas 25 psi, the support gases GS1/GS2 at 45 psi/65 psi, ion spray voltage 5.5 kV, source temperature at 650°C, interface heater on, entrance potential 10 and target scan time of 2 s. The identical compound specific MS/MS fragments of measured amino acids were used as given by

Thiele et al. [38]. Average product ion (PI) spectra for the API4000 device were acquired in a compound specific manner in terms of declustering potential (DP), collision energy (CE) and collision cell exit potential (CXP) by direct infusion of standard solution dissolved in 50% methanol (v/v). Instrument control and data acquisition was performed with ABSciex Analyst software.

Bioinformatics transcriptomics and proteomics

Bioinformatics analyses were performed in R (versions 4.5.2). RNA-seq data were analyzed using DESeq2-normalized read counts [39], while proteomics data were based on mean-centered intensities as described previously [12]; both datasets were $\log_2$ -transformed prior to analysis. For temporal profiling, genes and proteins with at least four observations per time series were retained. Temporal expression profiles were examined using the R package MaSigPro [40], fitting linear and quadratic regression models for each gene and protein. Profiles were considered significantly different from a zero-profile based on an $R^2 \geq 0.7$ and a Benjamini–Hochberg (BH)-adjusted p-value < 0.05 . Hierarchical clustering was performed using Ward’s method with a distance metric of (1 - the pairwise complete Pearson correlation).

Functional enrichment analyses of genes and proteins were conducted using Metascape with default settings [41], using all detected genes or proteins as background. When multiple protein identifiers were available, the first identifier was retained. To derive toxicity timelines for apoptosis, enrichment analyses were performed on MaSigPro results across progressively truncated time windows (0-14 days, 0-10 days, 0-7 days, 0-3 days, and 0-1 day), and the presence of apoptosis-related terms was evaluated.

Constraints for metabolite exchange

Quantitative constraints on metabolite exchange were derived from time-resolved measurements of extracellular amino acids across multiple experimental time points. To convert concentration changes into quantitative uptake and secretion rates, metabolite measurements were normalized by estimated spheroid dry weight. Hepatocyte dry weight was estimated based on reported values of an average hepatocyte volume of $3670 \pm 805 \mu\text{m}^3$ [42] and a dry weight of approximately 431 pg per cell [43]. Spheroid dry weight was inferred from measured spheroid size assuming a constant cell density.

For each measured metabolite, uptake and secretion rates were computed as $\text{mmol} \cdot \text{gDW}^{-1} \cdot \text{h}^{-1}$. To obtain continuous exchange constraints aligned with the multi-omics time points, second-order polynomial fits were applied to the time-course data. These fits were used to interpolate exchange rates at intermediate time points and to extrapolate the rates at time zero.

Genome-scale metabolic modelling was performed using the COBRA Toolbox v3.0 [44] with the Gurobi solver (Gurobi Inc.) in MATLAB 2023b and 2024b (MathWorks). The Human1 genome-scale metabolic network [17] was used as the starting model. Prior to contextualization, the model was checked for stoichiometric consistency and its ability to carry flux under minimal media conditions. An upper bound on biomass production was only used during the preliminary feasibility checks and was set based on the maximal observed spheroid size increase across conditions.

Models were constrained using three complementary sets of exchange constraints. First, only metabolites present in the media were allowed for uptake; seven compounds, mainly vitamins, were added iteratively to permit biomass production as described in Meeson et al. [45]. Second, constraints come from experimentally measured amino acids, and third lipid species released through biomass degradation. In total, 51 medium components, 19 measured metabolites, 7 growth-supporting reactions, and 313 lipid species derived from lipid degradation (supplementary file 1) were allowed for uptake. Upper and lower bounds on uptake and secretion bounds were set according to the fitted exchange rates normalized by dry weight plus or minus one standard deviation.

To ensure mass balance consistency, exchange bounds were clipped to predefined global minimum and maximum flux limits from FVA. Production of essential metabolites was disallowed to prevent artificial generation of nutrients not supplied by the medium. The valine constraint for DMSO at 14 days was relaxed to its 10 days value for feasibility.

Lipid degradation associated with spheroid size loss was incorporated as additional upper bounds on the uptake of lipid-derived metabolites. These bounds were estimated from the measured decrease in spheroid dry weight and the lipid fraction of biomass defined in Human1 as explained below.

Incorporation of lipid degradation-derived uptake constraints

To account for metabolite availability arising from cell death–associated lipid breakdown, we introduced lipid-derived substrates as condition-specific constraints on extracellular metabolite uptake in the genome-scale metabolic models. Lipid availability was implemented as upper bounds on exchange reactions rather than by explicitly modeling intracellular degradation.

The lipid-containing biomass pool in the Human1 reconstruction (MAR10063) was traced through the metabolic network (e.g., via the PG-CL pool) to its terminal metabolites. Terminal metabolites were defined as small lipid-derived species (e.g., free fatty acids, glycerol, cholesterol, and choline-containing fragments) that are not further degraded within the traced subnetwork.

Metabolites present in multiple compartments were collapsed into a compartment-agnostic representation, after which only extracellular forms were considered for uptake constraints.

The total availability of lipid-derived metabolites was estimated from experimentally measured spheroid loss over time. Changes in spheroid size were converted to dry weight loss using an average hepatocyte volume of $3670 \mu\text{m}^3$ [42] and a dry weight of approximately 431 pg per cell [43]. Dry weight loss was then converted to a maximal molar release rate by assuming steady degradation over the measurement interval and a lipid fraction of 22%. The resulting flux was distributed across terminal metabolites according to their relative stoichiometric contributions inferred from the degradation tracing.

For each terminal metabolite, the corresponding exchange reaction was identified using Human1 annotations. Uptake constraints were applied only to metabolites with existing extracellular exchange reactions, and no new transport reactions were introduced. Upper bounds on uptake (negative flux direction) were set proportional to the estimated lipid-derived availability, while original secretion bounds were retained to allow metabolite export.

These lipid-derived uptake constraints were applied in a condition-specific manner and combined with experimentally measured amino acid exchange rates and media constraints. This enabled quantitative integration of cell death-associated lipid availability into flux simulations while maintaining model feasibility. and avoiding spurious recycling

Amino acid availability from proteolysis was not included. Albumin present in the culture medium can serve as a protein source and is already represented in the model; however, its degradation is energetically costly and was not predicted by the model. Moreover, most amino acids were present in excess in the medium, with only transient limitations observed for phenylalanine, tryptophan, and asparagine. Including proteolysis-derived amino acids would therefore primarily increase excess substrate availability and introduce additional uncertainty without improving model predictions.

Contextualization of metabolic models

To ensure feasibility of metabolic activity without imposing artificial growth, the Human1 biomass reaction was not enforced during contextualization. This decision was motivated by experimental observations showing that spheroid size remained constant or decreased over time upon isoniazid exposure. Instead, the ATP maintenance reaction was enforced in all models to represent basal energetic demand.

100 Context-specific genome-scale metabolic models were reconstructed independently for each condition and time point using DEXOM [18], an iMAT-based algorithm. DEXOM identifies networks maximizing consistency between reaction activity and gene and protein expression data and, unlike related algorithms such as iMAT-FVA [46] and iMAT-AO [47], systematically explores

alternative optimal network states. This is critical because even multi-omics–constrained metabolic models remain underdetermined and encompass a space of alternative steady-state flux distributions. Because gene and protein expression values span several orders of magnitude, expression data were standardized by computing z-scores for each gene or protein per condition. Z-scores were mapped to binary activity states using a thresholding scheme: reactions associated with expression above the mean plus one standard deviation were considered active, whereas reactions below the mean minus two standard deviations were considered inactive (Supplementary Figure 7). The lower threshold was intentionally lenient to avoid excluding reactions required for basal metabolism.

Given the high correlation between biological replicates ($r = 0.999$ for transcriptomics and $r = 0.96$ for proteomics), replicate measurements were averaged prior to model reconstruction.

Model size was quantified as the number of reactions carrying nonzero flux, and similarity between models was assessed using the Jaccard index based on active reaction sets.

Flux predictions and enrichments

Although integration of multi-omics data substantially reduces the solution space of context-specific genome-scale metabolic models, the resulting networks remain underdetermined and therefore admit multiple alternative flux distributions. To quantify this residual ambiguity, we characterized the feasible flux space using FVA [19].

For each time point, and condition, and the 100 context-specific models, FVA was performed using the COBRA Toolbox. While the standard FVA implementation is computationally efficient, it failed for a subset of models that nevertheless yielded feasible flux balance analysis solutions. For these cases, a fallback implementation was used in which each reaction flux was sequentially maximized and minimized to obtain valid lower and upper bounds.

A reaction flux was considered upregulated if either the upper bounds or the lower bounds at a given time point exceeded the corresponding bounds at time 0 (T_0), as assessed by a Wilcoxon rank-sum test with Benjamini–Hochberg correction (figure 6E). Conversely, downregulation was defined analogously.

For each reaction R , flux capacity was defined as the maximum absolute value of its FVA bounds, averaged across the 100 models:

$$FluxCapacity_R = \frac{\sum_{n=1}^{100} \log_2(\max(abs(LB_{FVA}), abs(UB_{FVA}))+1)}{n_{100}} \quad (1)$$

Where LB represents the lower bound from FVA and UB the upper bound. To quantify global temporal shifts in metabolic capacity, pairwise Pearson correlation coefficients (PPFC) were computed between flux capacity vectors at each time point (T) and those at (T_0):

$$PPFC_T = \text{corr}(\text{FluxCapacity}_T, \text{FluxCapacity}_{T_0}) \quad (2)$$

Agreement between measured and predicted amino acid exchange fluxes was assessed using the concordance correlation coefficient (CCC):

$$CCC = \frac{2 \cdot r \cdot \sigma_{\text{measured}} \cdot \sigma_{\text{predicted}}}{\sigma_{\text{measured}}^2 + \sigma_{\text{predicted}}^2 + (\mu_{\text{measured}} - \mu_{\text{predicted}})^2} \quad (3)$$

where r denotes the Pearson correlation coefficient, σ the standard deviation, and μ the mean of measured and predicted fluxes.

Pathway-level enrichment analysis was performed by computing a pathway score for each of the 147 Human1 subsystems, defined as the number of reactions exhibiting differential flux spans at each time point. Subsystem enrichment was evaluated using a one-sided Fisher's exact test by comparing each pathway score against the average pathway score across all subsystems. Pathways were considered significantly upregulated for a BH-adjusted p-value < 0.05

Pathway enrichments of excreted metabolites were calculated and plotted using MetaboAnalyst 6.0 using default settings [23].

Integrated principal component analysis

Integrated PCA was performed to assess global differences in metabolic response between therapeutic and toxic exposure conditions across time points. A combined dataset was constructed by integrating predicted flux capacities from the proteome-based context-specific metabolic models with matched transcriptomic and proteomic measurements. Features were filtered to retain reactions with gene-protein relationships for which both transcript and protein measurements were available and that showed non-zero variance across samples. Missing proteomic values were imputed using k-nearest neighbor imputation ($k = 3$) when at least 50% of samples contained observed values. All variables were log2-transformed and standardized using z-score normalization to ensure comparable scaling across omics layers.

PCA was performed using singular value decomposition on the centered and scaled matrix. Principal components explaining the largest variance were used to visualize separation between conditions. Feature loadings were examined to identify molecular measurements and reactions contributing to the principal axes of variation. Relative contributions of each data layer were quantified by aggregating the absolute values of flux, transcript, and protein features.

Data extraction from the Explorys database

A within-patient analysis was performed to assess biomarker changes after isoniazid treatment. Patients with recorded isoniazid exposure, associated laboratory measurements, and diagnoses were extracted from the Explorys database, accessed 15 April 2026 [24]. Observations were excluded if

patients had chronic conditions known to affect the respective biomarker, or if an acute diagnosis occurred within ± 30 days of measurement (see Supplementary Data 2). To minimize confounding by pre-existing conditions, only patients with baseline values within the clinical reference range were included. If a patient had multiple drug episodes, the episode with the greatest number of observations was selected.

For each patient, two time windows were defined: a baseline period covering the 90 days prior to treatment initiation, and a risk period starting 15 days after treatment initiation and extending up to 6 months, or until treatment end plus an additional 10 days, as described in [13]. Within each window, multiple measurements per biomarker were summarized as patient-level medians. Baseline and risk-period medians were compared using the Wilcoxon signed-rank test (minimum $n = 10$ per biomarker), with Benjamini–Hochberg false discovery rate correction applied for multiple testing.

DILI cases were defined using ICD-10 diagnosis codes for toxic liver disease (K71.X) and adverse effects or poisoning by antimycobacterial agents (T37.1XX), linked to isoniazid exposure through patient and encounter identifiers. Patients receiving a concomitant antimycobacterial drug were excluded.

Statistical analysis

Statistical analyses were performed as described in the respective method sections for each data type. Unless otherwise specified, tests were two-sided. p-values were adjusted for multiple testing using the Benjamini–Hochberg procedure across all tested features ($n = 12,971$ for fluxes, $n = 27,924$ for transcripts, $n = 1,162$ proteins for toxic conditions and $n = 1,334$ for therapeutic conditions). Adjusted p-values (false discovery rate) < 0.05 were considered statistically significant.

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Competing interests

The authors declare no competing interests.

Author Contributions

Z.S., H.C., and L.K. designed the study. Z.S. and H.C. wrote the code to perform the model reconstructions. Z.S performed the metabolic flux simulations., analyzed the data, and wrote the original manuscript. M.O., L.M.B., and A.R. coordinated the amino acid and viability measurements. A.Z.S. performed the EXPLORYS analysis. L.K., H.C., and L.M.B. provided the discussion. All authors reviewed and approved the final manuscript.

Data availability

Transcriptomic and proteomics data have been made publicly available [12]. All data generated or analyzed during this study are included in this published article and its Supplementary Information files.

Code availability

The authors declare that the code supporting the findings of this study is available within the article's Supplementary Information files as a MATLAB-files, and R-files, and Python-files.

Supplementary Information

Supplementary Figures

Supplementary Data 1: media constraints

Constraints from metabolites on exchange reactions: media, additions to allow growth, lipid availability from cell death, and exchange of amino acids.

Supplementary Data 2: Explorys

Exclusion criteria

Supplementary Data 3

Matlab scripts

Supplementary Data 4

R scripts

Supplementary Data 5

Python scripts

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Supplementary Files

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