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Metabolomic insights into the response strategies of bats to high-glucose stimulation

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ABSTRACT

Existing therapeutic options for diabetes remain limited, underscoring the urgent need to explore novel mechanisms for blood glucose regulation. Bats despite diverse dietary habits, making them ideal models for studying hypoglycemic mechanisms. In this study, we performed intraperitoneal glucose tolerance tests (IPGTTs) on five bat species three frugivorous or nectarivorous species (hereafter collectively referred to as frugivorous bats, given the high sugar content of both fruits and nectar) and two insectivorous species. A total of 704 known serum metabolites were analyzed quantitatively using liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS). Compared with insectivorous bats, frugivorous bats exhibited significantly higher levels of amino acid-related metabolites under high-glucose stimulation. These included N-acyl amino acids, aromatic amino acid derivatives, and branched-chain amino acid metabolites, which were enriched significantly in pathways, such as alanine, arginine, and D-amino acid metabolism. Additionally, several known gut-brain axis (GBA) metabolites—such as neurotransmitters, bile acids, and short-chain fatty acids—exhibited strong host dietary and species specificity. Frugivorous bats were enriched in neurotransmitters and short-chain fatty acids associated with insulin regulation, whereas insectivorous bats exhibited relatively higher levels of bile acid metabolites. Overall, this study provides theoretical insights into the metabolic adaptations of frugivorous bats and the glucose-response strategies of insectivorous bats, offering new perspectives on metabolite-based therapeutic approaches to diabetes.

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Keywords: Metabolomics; Bats; Frugivorous; Gut-brain axis; Amino acid compounds

INTRODUCTION

Diabetes is an increasingly prevalent metabolic disorder. Persistently elevated blood glucose levels can lead to severe complications, such as kidney failure, stroke, and vision impairment (Kitabchi et al., 2009; Rolo & Palmeira, 2006). According to 2021 statistics, approximately 537 million adults worldwide were living with diabetes, and this number is projected to rise to 783 million by 2045—an increase of about 46%, which far exceeds the estimated global population growth of 20% over the same period (International Diabetes Federation, 2025). Current therapeutic approaches to diabetes remain limited and no definitive cure is available. Therefore, expanding our understanding of blood glucose-lowering mechanisms is critically important.

Studies of wild animals have provided important insights for human health, suggesting that novel and effective strategies for blood glucose regulation may be discovered in nature. Chiroptera (bats), a group of mammals occupying a unique nocturnal aerial ecological niche, exhibit a broad range of dietary adaptations, including the consumption of insects, fruits, nectar, pollen, and, in some species, blood and vertebrate prey (Neuweiler, 2000; Norberg & Rayner, 1987). Among these, insectivory is regarded as primitive dietary habit, and become a predominant feeding strategy in bats (Weru et al., 2021). Insectivorous bats primarily feed on Lepidoptera, Diptera, and Coleoptera, deriving a diet characterized by high protein and low carbohydrate content (Razgour et al., 2011; Wilman et al., 2014). To facilitate nocturnal foraging, they have evolved highly sophisticated echolocation systems along with corresponding sensory and locomotor adaptations, and at the digestive level, they also exhibit adaptations for processing chitin-rich prey (Kunz &

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Fenton, 2005; Strobel et al., 2013). In contrast, frugivorous bats primarily feed on fruits, nectar, and pollen (González-Gutiérrez et al., 2022; Wilman et al., 2014). Over long evolutionary timescales, they have adapted to high-sugar diets, and exhibited remarkable longevity despite consuming large amounts of simple carbohydrates but (Gorbunova et al., 2020). Most Old World frugivorous bats rely primarily on well-developed visual and olfactory senses for navigation, as only a few species are capable of echolocation via tongue clicking (Elangovan et al., 2006; Hodgkison et al., 2013). Frugivorous bats of the New World family Phyllostomidae utilize a combination of echolocation and olfaction to locate ripe fruits, enabling efficient nocturnal foraging (Korine & Kalko, 2005). Together, these sensory adaptations facilitate the detection of ripe fruits and floral resources and underpin their important ecological roles in pollination and seed dispersal (Kunz et al., 2011; Muscarella & Fleming, 2007). Thus, insectivorous and frugivorous bats differ systematically in dietary composition, sensory modalities, physiological traits, and metabolic pressures, making them an ideal comparative model for investigating diet-associated metabolic regulatory mechanisms.

Previous research on the efficient hypoglycemic mechanisms of frugivorous bats has primarily focused on behavioral, physiological, and genetic aspects. Intense flight activity was observed to prevent the long-tongued fruit bat from experiencing sustained elevations in blood glucose after consuming large amounts of carbohydrates (Kelm et al., 2011). Studies on *Cynopterus sphinx*, *Rousettus aegyptiacus* and *Artibeus lituratus* had demonstrated that the pancreas of frugivorous bats underwent physiological adaptations that enabled more efficient blood glucose reduction (Michelsmore et al., 1998; Protzek et al., 2010). Additionally, research on the SLC2A/GLUT glucose transporter gene family had provided molecular and genetic insights into hypoglycemic mechanisms in these bats (Camacho et al., 2024; Meng et al., 2016). However, research on frugivorous bats—and more broadly, on wild animals—in this context remains largely limited (Camacho et al., 2024).

A growing body of evidence suggests that various metabolites—such as organic acids and their derivatives, nucleotides and their derivatives, and free fatty acids—may regulate glucose metabolism by modulating insulin secretion or pancreatic function (Antonoli et al., 2015; Jeppesen et al., 2013; Mahendran et al., 2013; Trufelli et al., 2011). Notably, certain amino acid-related compounds serve not only as neurotransmitters or intermediates of the tricarboxylic acid (TCA) cycle, but also can be transported into pancreatic β -cells to stimulate insulin secretion. Under specific conditions, certain combinations of amino acids may mimic the stimulatory effects of glucose (Gegelashvili & Bjerrum, 2019; Lavorato et al., 2024; Liu et al., 2008).

Fluctuations in blood glucose levels directly reflect glucose clearance capacity, and serum metabolomic profiling provides a rapid and objective means to capture metabolic changes (Fujisaka et al., 2018; Karasov & Douglas, 2013; Takahashi et al., 2018). In this study, we hypothesized that frugivorous bats has evolved a set of mature hypoglycemic mechanism in long-term high-sugar diets, where certain serum metabolites act important role in regulating blood glucose levels after food intake. Hence, we performed intraperitoneal glucose tolerance tests on five bat species—three frugivorous bats (*Eonycteris spelaea*, *C. sphinx*, and *R. leschenaultii*) and two

insectivorous bats (*Myotis pilosus* and *Hipposideros armiger*)—to investigate changes in serum metabolites under high-glucose stimulation. Using a targeted liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) approach, we quantitatively analyzed serum samples collected from different species and multiple time points, using a custom database comprising 704 known metabolites. By integrating host dietary traits and sampling times after glucose stimulation, we conducted differential metabolite analysis to better understand the metabolic responses of frugivorous bats and the adaptive responses of insectivorous bats to hyperglycemic conditions. This study also provides a valuable reference for identifying naturally active candidate compounds for diabetes treatment.

MATERIALS AND METHODS

Animals

All animal procedures in this study were approved by the Institute of Zoology, Guangdong Academy of Sciences (Approval No. GIZ20220822) and were conducted in accordance with established ethical guidelines. From June to October 2024, five bat species were collected by the research team in southern China. *Myotis pilosus* and *H. armiger* were collected in Guangdong Province, whereas *E. spelaea*, *C. sphinx*, and *R. leschenaultii* were captured in Yunnan Province. During the daytime, we searched for caves and trees where bats roost and captured individuals using hand nets. At dusk and during the evening, mist nets were set up near cave exits along bat flight paths. Approximately 25 to 30 individuals of each species were collected. After capture, each bat was placed individually in a cloth bag and allowed to rest adequately prior to experimentation.

Each bat was taxonomically identified based on morphological characteristics, and individual records were compiled for body weight, age, and sex. Bats were then housed individually in cages (one bat per cage) for acclimation. To simulate natural living conditions, the housing environment was maintained at a constant temperature of 26°C, kept in complete darkness throughout the day, and sprayed regularly with water to maintain humidity. Based on the dietary classification summarized by Wilman et al. (2014) (Figure 1A), the five bat species in this study exhibited distinct dietary profiles. *Myotis pilosus* and *H. armiger* are strictly insectivorous [*M. pilosus* is also known to feed on fish, as reported by Wilson & Mittermeier (2019).]; *E. spelaea* is strictly nectarivorous; *C. sphinx* is strictly frugivorous; and *R. leschenaultii* has a mixed diet composed of approximately 60% fruit and 40% nectar. Considering the high-sugar content in fruits and nectar, in the subsequent discussion we collectively refer to the three frugivorous species as “frugivorous bats”, while the other two species are categorized as “insectivorous bats” unless otherwise specified. The insectivorous bats were fed mealworms and water supplemented with an appropriate multivitamin, whereas the frugivorous bats were provided with bananas, pears, honey water, and clean water.

Intraperitoneal glucose tolerance test (IPGTT)

Once the captive bats had stabilized (as indicated by normal feeding and defecation), approximately 20 individuals from each species were selected for fasting treatment, with a fasting duration of 12 h. The bats were then transferred to the

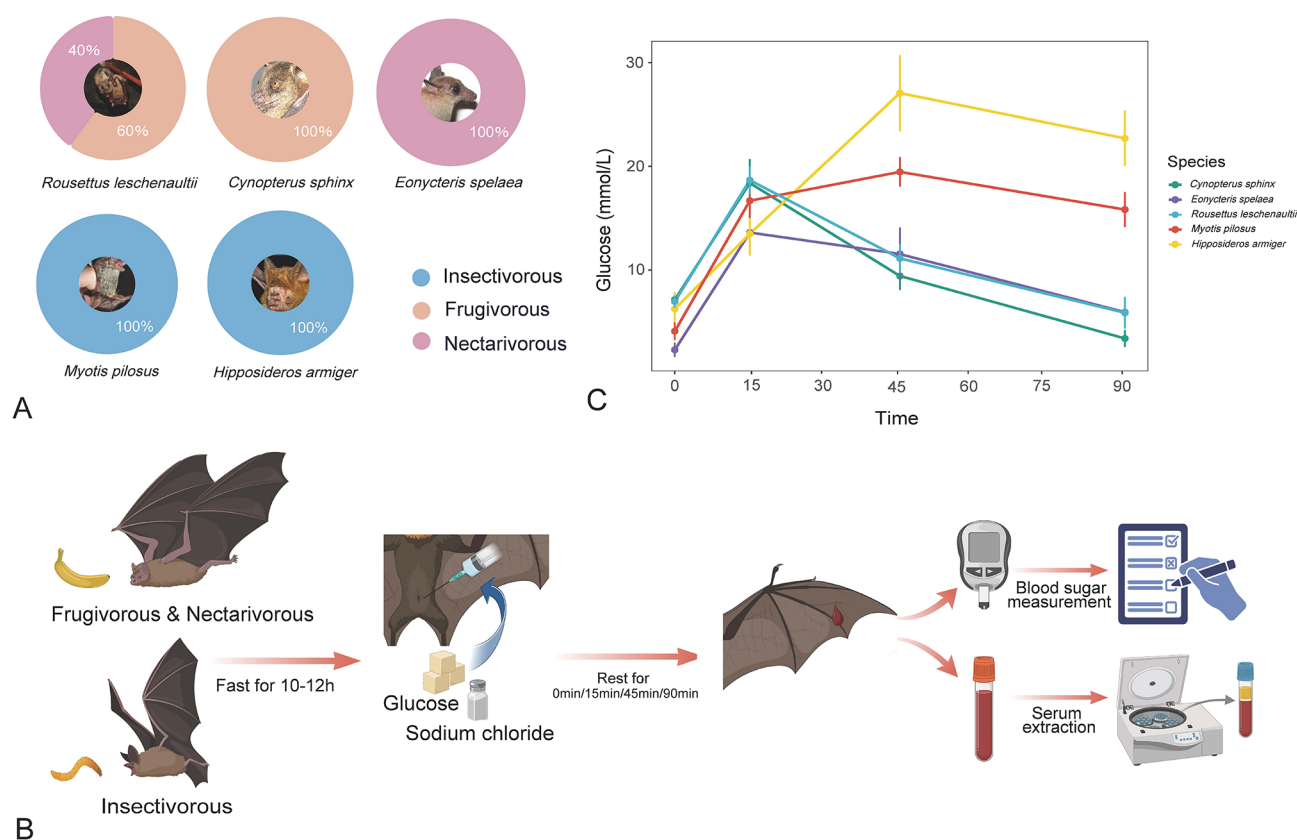


Figure 1 Intra-peritoneal glucose tolerance test (IPGTT)

A: Feeding habits of the five bat species in this study, based on the species-level foraging attributes summarized by Wilman et al. (2014) for world bird and mammal species. B: Flowchart of the glucose tolerance test procedure. C: Blood glucose changes under the IPGTT, presented as a line graph. The five bat species involved are represented by the individuals from which serum was collected. Error bars represent the mean $\pm$ standard error of the mean (SEM). (Due to unavoidable sampling limitations, the M0 and C0 groups each contain 4 individuals, the E45 group contains 6 individuals, and all other groups have $n=5$ by default.)

laboratory for weight measurement. A 20% (w/v) glucose solution in 0.9% saline was prepared by dissolving 20 g of glucose in a portion of 0.9% saline and adjusting the final volume to 100 mL. The solution was sterilized by filtration and prepared fresh for immediate use, and administered intraperitoneally at a dosage of 2 g/kg based on each bat's body weight (Peng et al., 2019). During the injection procedure, the bats were gently wrapped in cloth bags to restrict movement and expose the abdomen, and the injection was performed slowly using a sterile syringe. After the injection, each bat was placed individually in a cloth bag to rest, and the timing was initiated.

Blood glucose levels were measured at four time points: fasting (0 min) and 15 min, 45 min, and 90 min after injection, using an ACCU-CHEK® Integra glucometer (Roche ACCU-CHEK® Integra, Switzerland). During glucose measurement, bats were gently wrapped in cloth bags with one wing membrane exposed. A disposable lancet was used to puncture the marginal vein of the forearm wing membrane to collect a blood sample, and glucose levels were measured and recorded. To observe glucose dynamics, each bat underwent glucose measurement at all specified time points, including during serum collection.

Serum extraction

Following blood glucose measurements, blood samples were collected from the carotid artery of each experimental bat and transferred to plain sterile 1.5 mL microcentrifuge tubes without anticoagulant to allow natural coagulation, as

anticoagulants or clot activators may interfere with subsequent metabolite analysis. The samples were left at 26°C for 1 h to ensure complete clot formation and then transferred to a 4°C refrigerator for an additional 1 h to facilitate serum separation prior to centrifugation. Subsequently, the samples were centrifuged at 4°C for 10 min at 4000 r/min using an Eppendorf® Centrifuge 5424R (Eppendorf, Germany) to separate the serum. Under a biosafety cabinet, the upper serum layer was transferred to new sterile 1.5 mL centrifuge tubes, rapidly frozen in liquid nitrogen for 5 min, and then stored at -80°C until further analysis.

LC-ESI-MS/MS

Sample extracts were analyzed using an LC-ESI-MS/MS system (UPLC: ExionLC AD, <https://sciex.com.cn/>; MS: QTRAP® 6500+ System, <https://sciex.com/>). The detailed procedure was as follows:

Sample preparation and extraction: After thawing, the sample was vortexed for 10 s. A 50 μ L aliquot of the sample was transferred to a centrifuge tube and mixed with 250 μ L of 20% acetonitrile/methanol (CNW, China; Merck, Germany). The mixture was vortexed for 3 min and then centrifuged at 12000 r/min for 10 min at 4°C. Following centrifugation, 250 μ L of the supernatant was transferred to a new centrifuge tube and stored at -20°C for 30 min. The sample was then centrifuged again at 12000 r/min for 10 min at 4°C. Subsequently, 180 μ L of the supernatant was passed through a Protein Precipitation Plate for further LC-ESI-MS/MS analysis.

To detect derivatized substances, the samples were subjected to derivatization treatment. A 30 μ L aliquot of supernatant extract was mixed with 5 μ L of 200 mmol/L 3-NPH (75% MeOH) (Sigma-Aldrich, USA) and 5 μ L of 150 mmol/L EDC solution containing 4.5% pyridine in methanol (RHAWN, China; Bidepharm, China) in 1.5 mL centrifuge tubes. The mixture was incubated at 30°C for 60 min. After the reaction, the mixture was diluted with 260 μ L of acetonitrile and stored at –20°C for 30 min to terminate the reaction.

UPLC conditions: (1) T3 method: UPLC conditions were as follows: column, Waters ACQUITY UPLC HSS T3 C18 (100 mm $\times$ 2.1 mm i.d., 1.8 μ m); solvent system: water with 0.05% formic acid (A) and acetonitrile with 0.05% formic acid (B). The gradient started at 5% B (0 min), increased to 95% B (8–12.5 min), and then returned to 5% B (12.6–15 min). The flow rate was 0.35 mL/min, the column temperature was maintained at 40°C, and the injection volume was 2 μ L.

(2) Amide method: UPLC conditions were as follows: column, ACQUITY UPLC BEH Amide (2.1 $\times$ 100 mm, 1.7 μ m); solvent system: water with 10 mmol/L ammonium acetate and 0.3% ammonium hydroxide (A), and 90% acetonitrile/water (V/V) (B). The gradient started at 95% B (0–1.2 min), decreased to 70% B (8 min), then to 50% B (9–11 min), and was finally returned to 95% B (11.1–15 min). The flow rate was 0.4 mL/min, the column temperature was set to 40°C, and the injection volume was 2 μ L.

(3) SCFA derivatization method: UPLC conditions were as follows: column, Waters ACQUITY UPLC HSS T3 C18 (100 mm $\times$ 2.1 mm i.d., 1.8 μ m); solvent system: water with 0.05% formic acid (A), and acetonitrile with 0.05% formic acid (B). The gradient started at 30% B (0–0.5 min), increased to 95% B (3.5–4.9 min), and was finally returned to 30% B (5.0–6 min). The flow rate was 0.35 mL/min, the column temperature was maintained at 40°C, and the injection volume was 2 μ L.

ESI-MS/MS conditions: Linear ion trap (LIT) and triple quadrupole (QQQ) scans were performed on a triple quadrupole-linear ion trap mass spectrometer (QTRAP[®] 6500+ LC-MS/MS System), equipped with an ESI Turbo IonSpray interface, operating in both positive and negative ion modes, and controlled by Analyst v.1.6.3 software (Sciex). The ESI source operation parameters were as follows: ion source, ESI $\pm$; source temperature, 550°C; ion spray voltage (IS), 5500 V (positive mode) and –4500 V (negative mode); curtain gas (CUR) was set at 35 psi. Metabolites were analyzed using scheduled multiple reaction monitoring (MRM). Data acquisition was carried out using Analyst v.1.6.3 software (Sciex), whereas MultiQuant v.3.0.3 software (Sciex) was used for metabolite quantification. Mass spectrometer parameters, including declustering potentials (DP) and collision energies (CE) for individual MRM transitions, were further optimized.

Qualitative and quantitative metabolite analyses: If standards were available (Sigma-Aldrich, USA), we compared the accurate precursor ions (Q1), product ions (Q3), retention times, and fragmentation patterns with those of the standards to analyze the primary and secondary MS data. In the absence of standards, we utilized a self-compiled database (MWDB, MetWare Biological Science and Technology Co., Ltd., China), along with publicly available metabolite databases, for data analysis. During the identification process, we excluded repeated signals corresponding to K⁺, Na⁺, NH₄⁺, and other substances with large molecular weights.

Quantitative analysis was performed in multiple reaction

monitoring (MRM) mode. The characteristic ions for each metabolite were selected using the QQQ MS to obtain signal intensities. Chromatographic peak integration and correction were carried out using MultiQuant v.3.0.2 (AB Sciex, Canada). The relative metabolite contents were expressed as the integrals of chromatographic peak areas. The peak area integrals of all samples were input into the standard curve equation, and further calculations were performed to determine the final concentration of each substance. In total, 704 known metabolites were monitored, most of which have been previously reported to be associated with glucose metabolism.

Multivariate statistical analysis: All figures in this study were created using Adobe Illustrator (2023) and BioRender (<https://www.biorender.com/>).

Linear mixed-effects models (LMMs) were constructed in R using the lme4 and lmerTest packages, with blood glucose levels as the dependent variable. Time points, species groups (frugivorous vs. insectivorous), and their interaction were included as fixed effects, while species identity was modeled as a random effect to account for interspecies variation. Degrees of freedom were estimated using the Satterthwaite method, and Type III analysis of variance (ANOVA) was performed to assess the significance of fixed effects. Post hoc comparisons of estimated marginal means were performed using the emmeans package to evaluate pairwise differences between species groups at each time point. A *P*-value under the IPGTT was calculated using the trapezoidal rule and compared between frugivorous and insectivorous bats with independent *t*-tests (*P*<0.05).

Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) was performed using the OPLSR package. OPLS-DA is a supervised extension of PCA that focuses on maximizing separation between predefined groups, making it suitable for identifying metabolites contributing to intergroup differences. Model validity was assessed by cross-validation and permutation testing. Anal function from the *MetaboAnalystR* package to evaluate group differences among samples. Variable Importance in Projection (VIP) scores were calculated from the model. Differential metabolites were identified based on VIP>1 and *P*-value<0.05 (criteria applied uniformly across all comparison groups), with *P*-values calculated using ANOVA (Trygg & Wold, 2002). Two-way ANOVA in R was used to test species, time, and interaction effects on metabolite classes. UpSet plots depicting shared and unique metabolites across sample groups were generated using the UpSetR package. Heatmaps of amino acid metabolites across groups were generated with the ComplexHeatmap package. Metabolite levels were normalized at the sample level using Z-score transformation and subjected to hierarchical cluster analysis (HCA) at the column level, presented as dendrograms.

Pearson correlations among differential amino acid metabolites were calculated using the psych package, and visualized as a heatmap. K-means clustering analysis was performed using the stats package and the optimal number of clusters was determined using the Calinski-Harabasz criterion. Violin plots were created using the ggplot2 package to display the top eight metabolites with the lowest *P*-values, based on multi-omic differential analysis. Serum metabolites were annotated using the Kyoto Encyclopedia of Genes and Genomes (KEGG) compound database (<http://www.kegg.jp/kegg/compound/>), and the annotated metabolites were subsequently mapped to the KEGG pathway database

RESULTS

Changes of blood glucose in bats under high glucose stimulation

We conducted IPGTTs on five bat species (Figure 1B). For each species, serum samples were collected at four time points (0, 15, 45, and 90 min) relative to glucose administration. The 0 min sample was collected immediately before glucose injection (baseline), and the 15–90 min samples were collected after injection. As shown in the line graph (Figure 1C), under baseline conditions (following a 12 h fast and prior to glucose injection), blood glucose levels across the five species were approximately 5.0 mmol/L. The highest baseline glucose level was observed in *C. sphinx* (7.1 mmol/L), whereas the lowest was in *E. spelaea* (2.3 mmol/L). Camacho et al. (2024) found that among New World bats, insectivorous species (e.g., *Myotis*, *Saccopteryx*, *Molossus*, *Peropteryx*) had an average blood glucose concentration of 4.04 ± 1.33 mmol/L, nectarivorous species (e.g., *Glossophaga*, *Choeroniscus*) had 2.46 ± 1.53 mmol/L, and frugivorous species (e.g., *Artibeus*, *Sturnira*, *Dermanura*, *Uroderma*) had 2.04 ± 0.52 mmol/L. Widmaier further reported that Old World fruit bats (*Pteropus vampyrus*, *Pteropus hypomelanus*, *R. aegyptiacus*) exhibited glucose levels ranging from 4.44 to 5.56 mmol/L, which fall within the normal mammalian range. The fasting ($t=0$) results of this study showed that among insectivorous bats, *M. pilosus* and *H. armiger* had blood glucose levels of 4.08 mmol/L and 6.24 mmol/L, respectively; similarly, among frugivorous bats, *E. spelaea* had only 2.28 mmol/L; *R. leschenaultii* and *C. sphinx* had 6.94 mmol/L and 7.13 mmol/L, respectively. These values indicate that the baseline glucose levels prior to the experiment were consistent with the natural fasting ranges reported for each dietary type of bat, providing a valuable reference for subsequent comparisons of glucose absorption and metabolic efficiency under.

Distinct patterns of blood glucose increase and decrease were observed among bat species with different dietary habits. The insectivorous bats exhibited a continuous increase in blood glucose levels within the first 45 min post-injection. Specifically, *H. armiger* reached a mean glucose concentration of 27.1 mmol/L at 45 min, whereas *M. pilosus* reached approximately 20 mmol/L at the same time point. Conversely, frugivorous bats showed a more rapid glycemic response, reaching peak glucose levels around 15 min post-injection. Among them, *C. sphinx* and *R. leschenaultii* exhibited glucose concentrations of approximately 18.0 mmol/L, and *E. spelaea* had a mean glucose level of 13.6 mmol/L. At 90 min after intraperitoneal glucose injection, blood glucose levels in *M. pilosus* and *H. armiger* remained elevated at 15.8 mmol/L and 22.7 mmol/L, respectively, which were substantially higher than their baseline levels. Conversely, glucose levels in the frugivorous bats had largely returned to baseline: *E. spelaea* exhibited a mean glucose level of 5.9 mmol/L, slightly above baseline, while *C. sphinx* and *R. leschenaultii* had glucose levels lower than their initial values. Furthermore, individual glucose measurements at each sampling time point generally followed the overall trend illustrated in the line graph, with the exception of a few *H. armiger* individuals whose glucose levels continued to rise at

45 min post-injection (Supplementary Table S1).

ANOVA on glucose responses under high-glucose stimulation revealed a significant effect of species group ($F=6.01$, $P=2.5 \times 10^{-4}$) and a highly significant time $\times$ species group interaction ($F=9.12$, $P=3.18 \times 10^{-6}$). Post hoc pairwise comparisons showed that frugivorous bats exhibited significantly lower glucose levels than insectivorous bats at 45 min (estimate = -12.47 , $P=1.6 \times 10^{-4}$) and 90 min (estimate = -14.21 , $P=5.0 \times 10^{-5}$), whereas no significant differences were observed at 0 min or 15 min. These results indicate that the temporal dynamics of glucose metabolism differ significantly among species. Accordingly, the post-stimulation glucose trends suggest that frugivorous bats have a markedly greater capacity for glucose clearance compared to insectivorous bats.

To further quantify this difference, we calculated the area under the glucose curve (AUC) for each individual and compared it between dietary guilds. Boxplot analysis revealed that frugivorous bats exhibited significantly lower AUC values (946.18 ± 240.12) than insectivorous bats (1698.92 ± 346.52 ; $P=7.46 \times 10^{-5}$), confirming that fruit-eating species clear glucose more efficiently following glucose injection (Supplementary Figure S1).

Detection of metabolites

Based on available reference standards and publicly accessible metabolite data, we compiled a resource database comprising 704 metabolites across 37 chemical classes (Figure 2A; Supplementary Table S2). The five most abundant classes in the database were amino acid derivatives (112 compounds, accounting for 15.91%), small peptides (87 compounds, 12.36%), nucleotides and their derivatives (72 compounds, 10.23%), organic acids and their derivatives (53 compounds, 7.53%), and bile acids (46 compounds, 6.53%).

Following the IPGTT experiment, a total of 20 experimental groups were established, representing five bat species each sampled at four distinct time points (0, 15, 45, and 90 min) before and after glucose administration. Group names were designated using the first letter of the genus name combined with the corresponding time point. Typically, each group consisted of serum samples from five individual bats; however, due to unavoidable sampling difficulties, the M0 and C0 groups included four samples each, while the E45 group included six samples. A total of 99 serum samples were subjected to targeted quantitative metabolite profiling using LC-ESI-MS/MS, based on the previously established metabolite reference database.

Across all groups, the number of detected metabolites ranged from 387 (in group H45) to 440 (in group C15). The five most abundant metabolite classes consistently detected in individual groups were amino acid derivatives (approximately 68 compounds), small peptides (approximately 59 compounds), organic acids and their derivatives (approximately 38 compounds), nucleotides and their derivatives (approximately 34 compounds), and amino acids (approximately 29 compounds) (Figure 2B; Supplementary Tables S3, S4). Two-way ANOVA revealed that species identity significantly affected most metabolite classes (e.g., amino acid derivatives, bile acids, short-chain fatty acids (SCFAs), sugars; $P<0.050$ – 0.001), whereas time points had a significant effect on fewer classes (e.g., azoles, fatty amides, sugar acids, sugars; $P<0.050$ – 0.001). Interactions between species and time were significant for selected classes (e.g.,

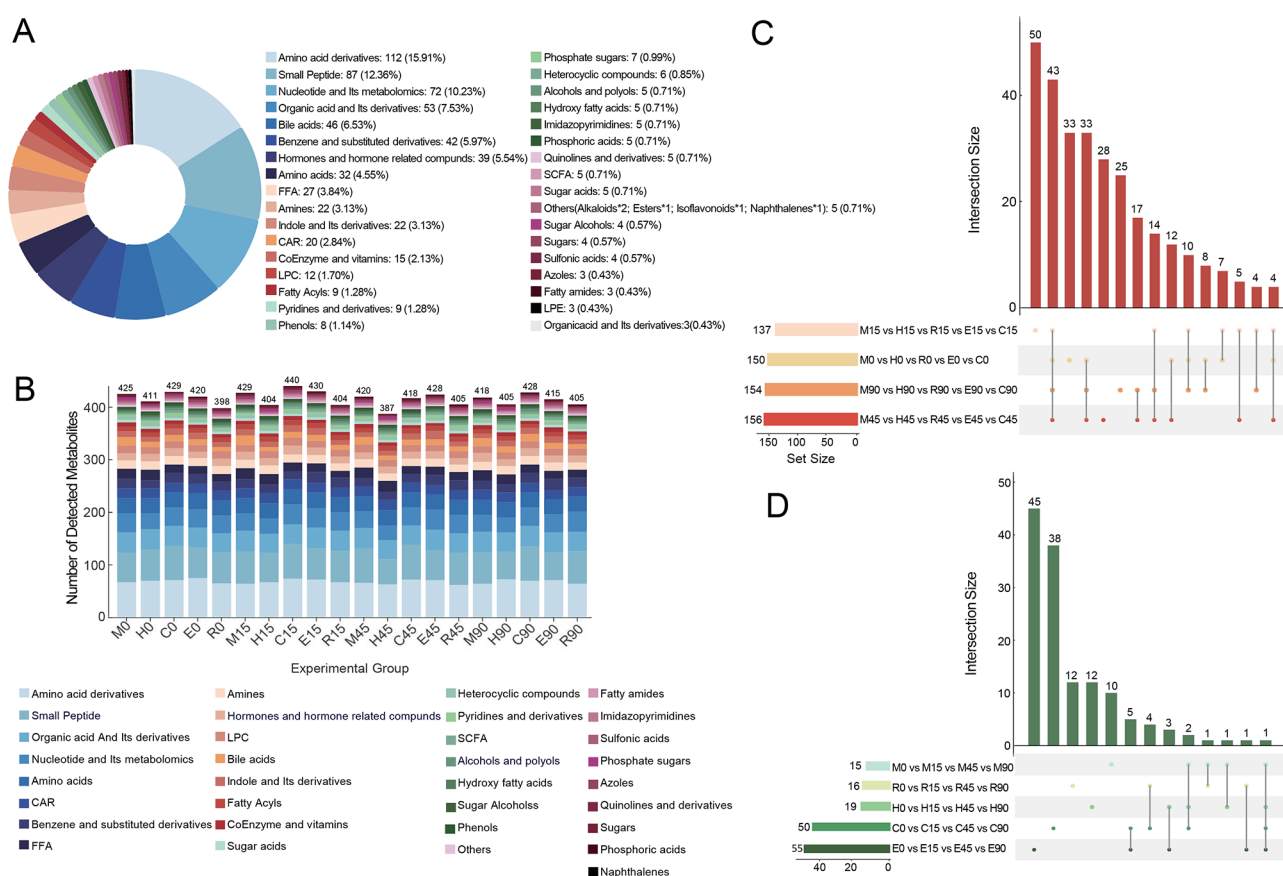


Figure 2 Targeted serum metabolite detection based on liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS)

A: Composition of the 704-metabolite resource database. The pie chart shows the proportion of metabolites in each category. **B:** Metabolite detection for the 20 groups in the IPGTT. A metabolite was recorded if it was present in at least one individual sample at each time point. **C:** UpSetR plot of different groups in IPGTT, categorized by time. The bar chart on the left shows the number of raw data points for each time point, while the dot matrix on the right represents the intersection of differential metabolites between sets. Connecting lines indicate shared metabolites, and individual dots represent metabolites unique to each set. **D:** UpSetR plot of different groups in the IPGTT, categorized by species.

amines, azoles, hormones, SCFAs, sugar alcohols; $P < 0.050 \sim 0.001$) (Supplementary Table S5).

Overlap and differences of metabolites across groups
Group analysis based on identical time points in IPGTT: To investigate interspecies differences in serum metabolites at specific time points before and after the IPGTT, we performed OPLS-DA analysis combined with ANOVA. Differential metabolites across the four time-point groups were visualized using an UpSet plot (Figure 2C). Although the five bat species exhibited distinct trends in blood glucose dynamics, all species were in a glycemic elevation phase at 15 min post-injection, while by 90 min, they had entered a glycemic decline phase.

A total of 43 metabolites exhibited significant interspecies differences across all four time points. A large proportion of these metabolites were nucleotides and their metabolic derivatives, mainly including extensively modified nucleosides and intermediates of purine and pyrimidine metabolism. In addition, many of the remaining metabolites were amino acid-related, particularly branched-chain amino acids and various acetylated amino acids. Additionally, metabolites exhibiting significant differences exclusively within each time point were identified as follows: 33, 50, 28, and 25 metabolites for the respective groups. At 0 min, amino acid derivatives dominated, particularly acetylated amino acids and nitrogen-

containing intermediates involved in amino acid nitrogen metabolism. At 15 min, the major enriched metabolites were organic acids and their derivatives, mainly aromatic amino acid degradation products and nitrogen-containing organic acids, along with benzenoids largely derived from aromatic amino acid catabolism. At 45 min, amino acid derivatives were again predominant, especially metabolites related to sulfur-containing amino acid metabolism and indole-ring-containing compounds. At 90 min, the most enriched category shifted to amino acids and small peptides, reflecting increases in protein degradation products and sulfur-containing amino acids (Supplementary Table S6).

Group analysis based on intraspecific grouping: To investigate species-specific metabolic responses at different time points following high-glucose stimulation, we regrouped the samples and re-screened the serum metabolites. The overlap and distribution of differential metabolites among the five species-specific comparisons were visualized using an UpSet plot (Figure 2D; Supplementary Table S7). Notably, no metabolite showed significant changes across all five species. The number of unique differential metabolites identified within each species was 10 for *M. pilosus*, 12 for *H. armiger*, 38 for *C. sphinx*, 12 for *R. leschenaultii*, and 45 for *E. spelaea*. The two frugivorous species (*C. sphinx* and *E. spelaea*) each exhibited a strong capacity for serum metabolite regulation under IPGTT conditions. In *C. sphinx*, the differential organic

acids were largely associated with energy metabolism pathways, and the nucleotide metabolites were mostly methylated on the base. In *E. spelaea*, the differential organic acids generally contained multiple carboxylic acid groups, the nucleotide metabolites were similar to those in *C. sphinx*, and a greater number of amino acid-related metabolites were enriched, many of which contained aliphatic carbon chains or aromatic rings.

Based on dietary classification, we further examined the overlap of serum metabolites among bats sharing the same diet. Only one metabolite, *N,N*-dimethylglycine, was differentially expressed in both insectivorous species, a compound previously reported to be elevated in the blood and urine of diabetic patients compared to healthy individuals (Lever & Slow, 2010; McEntyre et al., 2015). No single differential metabolite was shared among all three frugivorous species; however, ten metabolites were shared in pairwise comparisons: ribonic acid, N-glycolylneuraminic acid (GcNeu), L-glutamic acid, L-cysteinesulfinic acid, and myo-inositol were common to *C. sphinx* and *E. spelaea*; L-palmitoylcarnitine, palmitoyl ethanolamide, gamma-glutamyl-methionine, and 3-hydroxytyramine (dopamine) were shared between *C. sphinx* and *R. leschenaultii*; and eicosapentaenoic acid was shared between *E. spelaea* and *R. leschenaultii*. These shared differential metabolites mainly belong to three categories: highly polar small molecules (containing multiple hydroxyl, carboxyl, and amino groups), oxidative derivatives, and fatty-acid-derived metabolites, and are largely mapped to pathways related to carbohydrate metabolism, oxidative stress, and lipid transport.

Elevated expression of amino acid compounds in frugivorous bats under the IPGTT, especially in *E. spelaea*
Comparative analysis of *E. spelaea* and other bats at the same treatment time: Targeted metabolomic analysis revealed that amino acid-related metabolites—including small peptides, amino acids, and their derivatives—consistently exhibited strong discriminative power across multiple experimental groups (Figure 2; Supplementary Table S7). Among the five species, *E. spelaea* exhibited the highest number of differential metabolites across the four time points under high-glucose stimulation, with relatively elevated levels of amino acid-related compounds. Therefore, *E. spelaea* was designated as a separate group (Espe), while the remaining four bat species were combined into a single group (Bats) for the subsequent screening of differential amino acid-related metabolites across different time points before and after IPGTT (one sample from the E0 group was excluded following quality control because it was identified as an outlier).

Our metabolite database contains a total of 231 amino acid-related compounds. The numbers of differential amino acid-related metabolites identified across the four time groups were 29, 48, 23, and 30, respectively, with 10 metabolites consistently selected as differential candidates in all four groups. In the OPLS-DA two-dimensional score plots for each time point, a clear separation was observed between the Espe and Bats groups along the first principal component, which explained 45.1% to 51.3% of the total variance in the predictive component (Figure 3A, D, G, J). The OPLS-DA model validation plots showed R^2X values ranging from 0.684 to 0.805 and R^2Y values from 0.951 to 0.976, indicating a strong ability to explain variance in both input and response variables. Additionally, Q^2 values ranged from 0.892 to 0.915,

reflecting robust predictive performance and strong generalizability of the models (Figure 3B, E, H, K). Collectively, these results support the scientific validity of this grouping strategy.

To investigate changes in the relative abundance of significantly different amino acid-related metabolites, we performed Z-score normalization on their expression data across the four time points. This normalization helped eliminate the influence of absolute abundance differences among metabolites and highlighted relative variation trends across different samples. The heatmap visualized expression levels through color intensity (Figure 3C, F, I, L). The results showed that the overall expression levels of differential amino acid-related metabolites in the Espe group were significantly higher than those in the Bats group at all four time points. In addition, HCA at the column level revealed clear clustering within both the Espe and Bats groups. These findings indicate that *E. spelaea* exhibits highly specific expression patterns of amino acid-related metabolites across all time points.

Based on the IPGTT glucose measurements described above, we focused on two critical time points (15 min and 45 min) reflecting *E. spelaea* response to high-glucose stimulation. Compared to the fasting state (0 min), 25 amino acid-related metabolites showed differential expression during the rapid blood glucose increase phase (15 min), primarily including acylated amino acid derivatives, aromatic amino acid derivatives, branched-chain amino acid derivatives, and small peptides. Among these, nine metabolites remained differentially expressed during the blood glucose decline phase (45 min).

To investigate whether the elevated expression of amino acid-related metabolites is associated with dietary habits in bats and to avoid the potential confounding effect of the extreme metabolic profile of *E. spelaea* on the other two high-sugar diet species, we excluded the Espe group and regrouped the remaining four species into two categories: a High-sugar group and an Insectivorous group. Following the same analytical approach, we performed OPLS-DA and generated hierarchical clustering heatmaps. The results consistently showed that the High-sugar group exhibited significantly higher expression levels of differential amino acid-related metabolites across all four time points compared to the Insectivorous group (Supplementary Figure S2).

Intraspecific comparison of *E. spelaea* under IPGTT: Given the marked changes in amino acid-related metabolites in *E. spelaea*, we performed pairwise comparisons among the four time points within the Espe group to further investigate amino acid metabolism under high-glucose stimulation. A total of 40 amino acid-related metabolites were identified as significantly different across the four time points. Pairwise correlations among these differential metabolites were calculated using PCC, and a correlation heatmap was generated (Figure 4A). Overall, most amino acid metabolites exhibited positive correlations ($R \approx 0.36$), forming highly correlated modules, suggesting that they may function in the same or similar metabolic pathways (Supplementary Table S8). However, alpha-methyl-DL-phenylalanine ($R = -0.54$, $P = 0.04$) and ALA-PRO ($R = -0.45$, $P = 0.09$) showed relatively strong negative correlations with other amino acid-related metabolites.

K-means clustering analysis was used to characterize the temporal trends of amino acid-related metabolites under high-glucose stimulation (Figure 4B). This method iteratively assigns each data point to the nearest of a predefined number

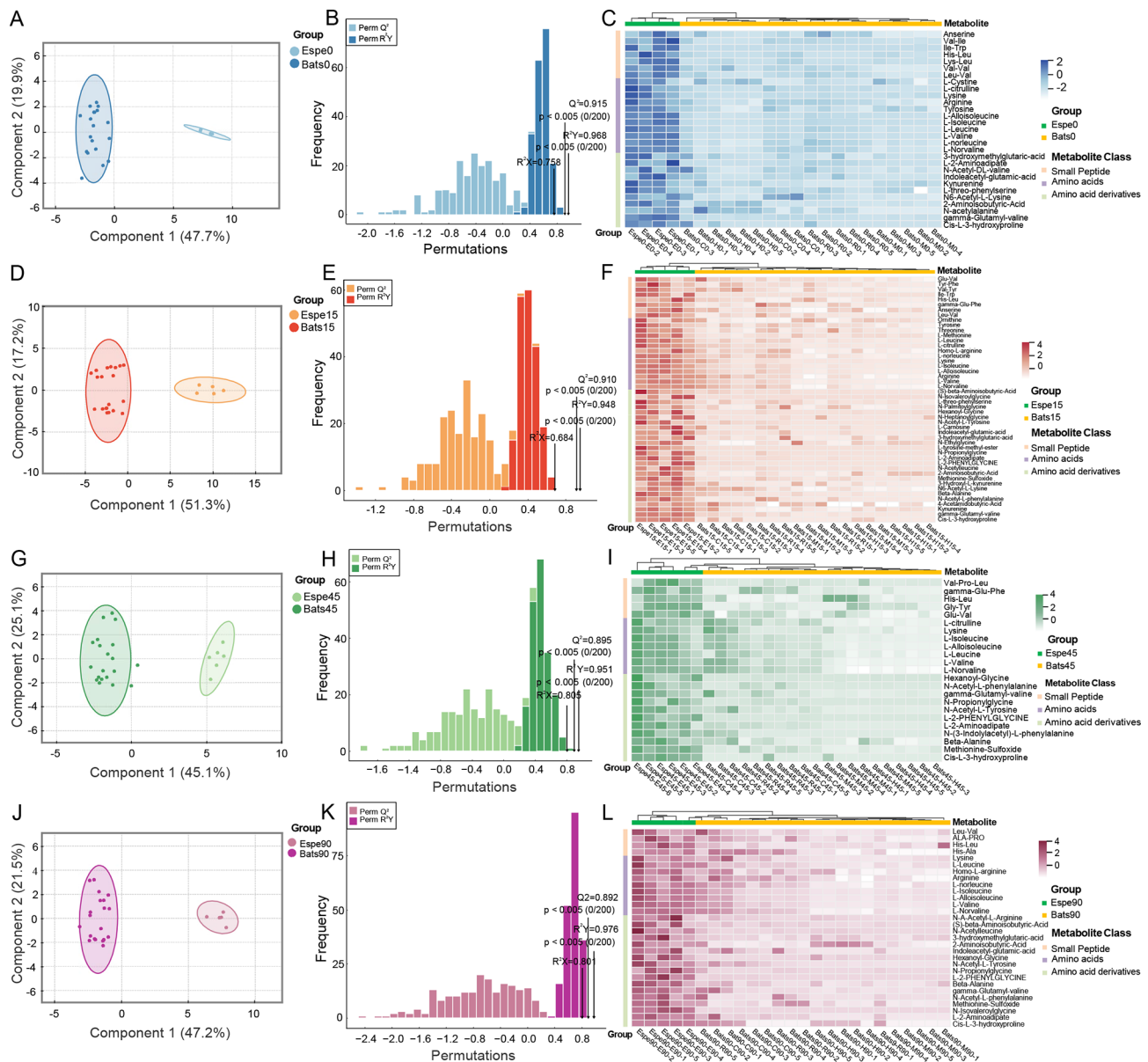


Figure 3 Differences in serum amino acid-related metabolites between *Eonycteris spelaea* and the other four bat species under high-glucose stimulation

A, D, G, J: Two-dimensional Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) score plots. Each point represents a sample, with different colors indicating the group. Ellipses represent the 95% confidence interval for each group. The X- and Y-axes show the scores of the predictive and orthogonal components, respectively, representing between-group and within-group variation. The percentages indicate the proportion of variance explained by the model. B, E, H, K: OPLS-DA model validation plots. The model parameters include R^2X , R^2Y , and Q^2 , where R^2X and R^2Y indicate the explained variance of the X and Y matrices, respectively, and Q^2 represents the model's predictive performance. The closer these values are to 1, the more stable and reliable the model. The X-axis represents the R^2Y and Q^2 values, whereas the Y-axis indicates the frequency of classification performance obtained from 200 random permutation tests. C, F, I, L: Heatmaps. Z-score normalization was applied to all metabolites across samples, followed by hierarchical clustering at the column (sample) level. Panels A–C represents the analysis at 0 min, D–F at 15 min, G–I at 45 min, and J–L at 90 min.

(K) of centroids, updating the centroids at each step to partition the dataset into distinct subclusters. Among the resulting clusters, subclusters 2, 4, and 5 included 30 amino acid-related metabolites that exhibited similar temporal patterns. These metabolites showed an overall trend of an initial increase followed by a decrease, with a turning point at 15 min post-injection, and their levels did not return to baseline by 90 min. This decline below the baseline level may reflect their extensive utilization during the glucose-lowering response. The overall pattern showed a general resemblance

to the blood glucose dynamics observed in *E. spelaea* (Figure 1C) and was consistent with the positive correlations among amino acid metabolites shown in Figure 4A. Notably, subcluster 3 exhibited a sustained increase relative to baseline, comprising mainly amino acid-related metabolites, which indirectly reflects enhanced protein hydrolysis and the accumulation of modified amino acids under high-glucose stimulation (Supplementary Table S9). Violin plots highlighted the eight most significantly altered metabolites across the four time points in *E. spelaea*: kynurenine, gamma-glutamyl-valine,

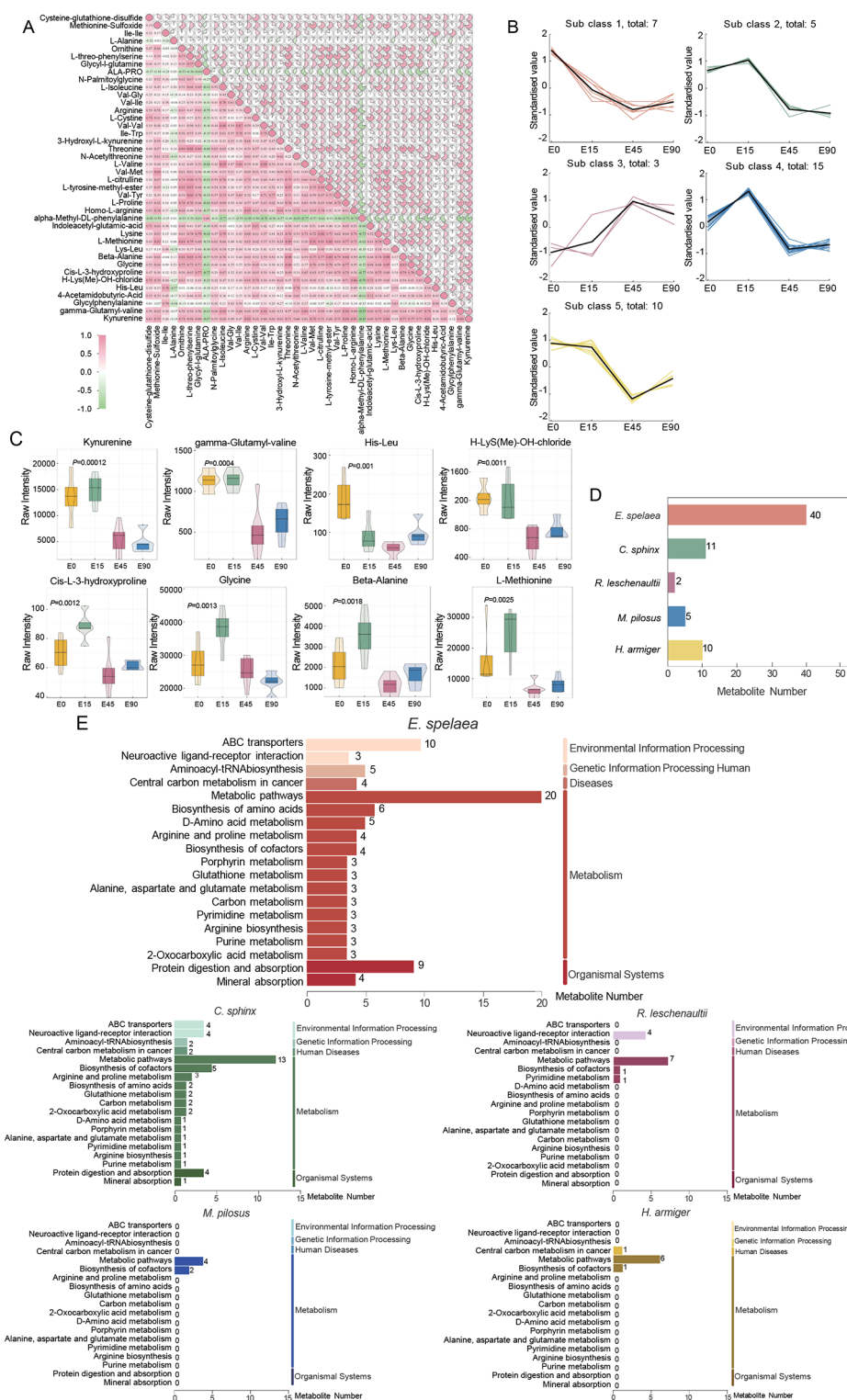


Figure 4 Correlation analysis of serum metabolites under high-glucose stimulation in the *Eonycteris spelaea* group

A: Correlation heatmap (corplot) of amino acid-related metabolites. Correlation coefficients between metabolites are shown using different formats in the lower-left and upper-right triangles. B: K-means clustering analysis. The optimal number of clusters ($K=5$) was determined using the Calinski-Harabasz algorithm. Each line plot represents metabolites grouped into the same cluster. The x-axis indicates the sample group, and the y-axis shows the normalized metabolite abundance. C: Violin plots of the eight most significantly altered metabolites. The inner box represents the boxplot, and the outer shape shows the kernel density estimate. Small circles denote outliers. Wider regions indicate a higher probability of values occurring in that range. Metabolites are ranked by P -value. D: Bar plot showing the number of differential amino acid metabolites identified in the five bat species under IPGTT. E: KEGG pathway enrichment analysis of differential amino acid metabolites. Pathways with ≥ 3 enriched differential metabolites were selected for display. The x-axis represents the number of enriched metabolites per pathway, and the right side indicates the primary classification according to the KEGG database. Comparative analyses of the four time points in the other four bat species were also performed, and the corresponding enrichment results for 19 pathways are presented.

His-Leu, H-Lys(Me)-OH-chloride, cis-L-3-hydroxyproline, glycine, beta-alanine, and L-methionine (Figure 4C). Among these, His-Leu belonged to subcluster 1, whereas the remaining seven metabolites were classified into subclusters 2, 4, and 5 (Supplementary Table S9). However, given the complexity of the relationship between metabolic and phenotypic data, further mechanistic studies are needed to validate these findings. Applying the same screening approach to the other four bat species revealed substantially fewer differential amino acid-related metabolites compared to *E. spelaea* (Figure 4D).

Pathway enrichment of high glucose-responsive metabolites based on KEGG database: Differential metabolites identified in *E. spelaea* before and after IPGTT were annotated using the KEGG compound database (Figure 4E). A total of 19 KEGG pathways were enriched, spanning five major first-level pathway categories. Pathways enriched with a substantial number of significantly altered metabolites (defined as those containing five or more compounds) included ABC transporters, aminoacyl-tRNA biosynthesis, D-amino acid metabolism, and protein digestion and absorption. Additional enriched pathways were related to arginine and proline metabolism, as well as purine and pyrimidine metabolism. These findings further support the hypothesis that *E. spelaea* actively mobilizes amino acid-related metabolites in response to glucose stimulation to rapidly lower blood glucose levels.

Subsequently, we analyzed the differential metabolites enriched in these 19 KEGG pathways for the other four bat species, which revealed that the number of enriched metabolites in these species was significantly lower than that in *E. spelaea*. Meanwhile, the other two frugivorous bats also exhibited slightly stronger enrichment than the insectivorous bats (Figure 4E). These results suggest that amino acid metabolites may play an important role in the efficient glucose-lowering process of frugivorous bats.

Gut-brain axis (GBA)-associated metabolic changes induced under high-glucose stimulation

The GBA plays a crucial role in the bidirectional communication between the central nervous system and the gastrointestinal tract, primarily mediated by metabolites and other signaling molecules. In light of previous studies highlighting its importance in glucose metabolism and insulin regulation, we further examined metabolic differences among the 20 sample groups from the perspective of the GBA to explore potential gut-derived metabolic signals associated with the host glucose response. Differential metabolites were subsequently screened for further analysis. Based on chemical classification, the identified metabolites were grouped into five major categories: neurotransmitters, hormones and hormone-related compounds, SCFAs, bile acids, and amino acids. Boxplots illustrating representative metabolites that exhibited distinct species-specific patterns are presented (Figure 5).

Frugivorous bats generally exhibited higher levels of multiple neurotransmitter-related metabolites following IPGTT. Notably, *R. leschenaultii* showed elevated levels of histamine, whereas *C. sphinx* displayed higher levels of indole-3-propionic acid. In *E. spelaea*, dopamine (3-hydroxytyramine) was particularly abundant. In contrast, serotonin was detected at higher levels in insectivorous bats. Regarding hormones and hormone-related compounds, *E. spelaea* had higher

levels of deoxycorticosterone, whereas *M. pilosus* exhibited elevated levels of thyroxine. For SCFAs, *E. spelaea* showed strong expression of propionic acid (PA) and valeric acid (VA); both *E. spelaea* and *C. sphinx* had increased levels of butyric acid (BA), while *H. armiger* exhibited elevated levels of acetic acid (AA). In terms of bile acids, chenodeoxycholic acid and isolithocholic acid were more abundantly expressed in certain frugivorous bats; however, most bile acid metabolites were more highly expressed in the insectivorous species. For instance, *M. pilosus* showed higher serum levels of glycocholic acid, glycodehydrocholic acid, and tauro-beta-muricholic acid, while both *H. armiger* and *M. pilosus* exhibited elevated expression of taurocholic acid. Among amino acid-related metabolites, glycine, L-aspartate, serine, and tyrosine were expressed at higher levels in certain frugivorous bats, whereas L-glutamic acid was more prominently expressed in *M. pilosus*.

DISCUSSION

Frugivorous bats exhibit enhanced glucose clearance under high glucose stimulation

Due to their nocturnal flight niche, bats have high energy demands and exhibit remarkable interspecific dietary diversity, making them ideal models for studying metabolic adaptation. This study focused on five Old World bat species with distinct dietary specializations, including obligate frugivores (*C. sphinx*), obligate nectarivores (*E. spelaea*), mixed fruit- and nectar-feeders (*R. leschenaultii*), and obligate insectivores (*M. pilosus* and *H. armiger*) (Figure 1A). To assess glucose clearance capacity in frugivorous bats, IPGTT was performed on all five species. This method bypasses the gastrointestinal tract and delivers glucose directly into the bloodstream, thereby eliminating the influence of digestion (White et al., 2025). Previous studies using IPGTT in mouse models have shown that this approach significantly elevates blood glucose levels, far exceeding those observed after a standard meal, and effectively simulates the hyperglycemia seen in diabetic patients (Andrikopoulos et al., 2008; Kelm et al., 2011; Small et al., 2022). In our study, the three frugivorous species exhibited rapid glucose elevation and clearance, with peak glucose levels lower than those of the insectivorous bats (Figure 1B, C; Supplementary Figure S1). These findings are consistent with those reported by Camacho et al. (2024) in New World Phyllostomid frugivores.

Serum metabolite changes provide new insights into glucose metabolism under high glucose stimulation

In recent years, studies on metabolic alterations in patients with type 2 diabetes (T2D) have become increasingly comprehensive, revealing strong associations between the disease and metabolites such as amino acids, lipids, and carbohydrates (Gao et al., 2023; Manoharan et al., 2023; Wang et al., 2023). In our multi-omic comparisons across four time points before and after IPGTT in five bat species (Figure 2C; Supplementary Table S6), nucleotides and their derivatives accounted for a substantial proportion of the differentially abundant metabolites. At 15 min post-injection, when blood glucose levels were elevated across all species, organic acids and their derivatives, as well as benzenoids, were more prominent among the differentially abundant metabolites. Comparative analyses between dietary groups (frugivorous vs. insectivorous) revealed significant differences

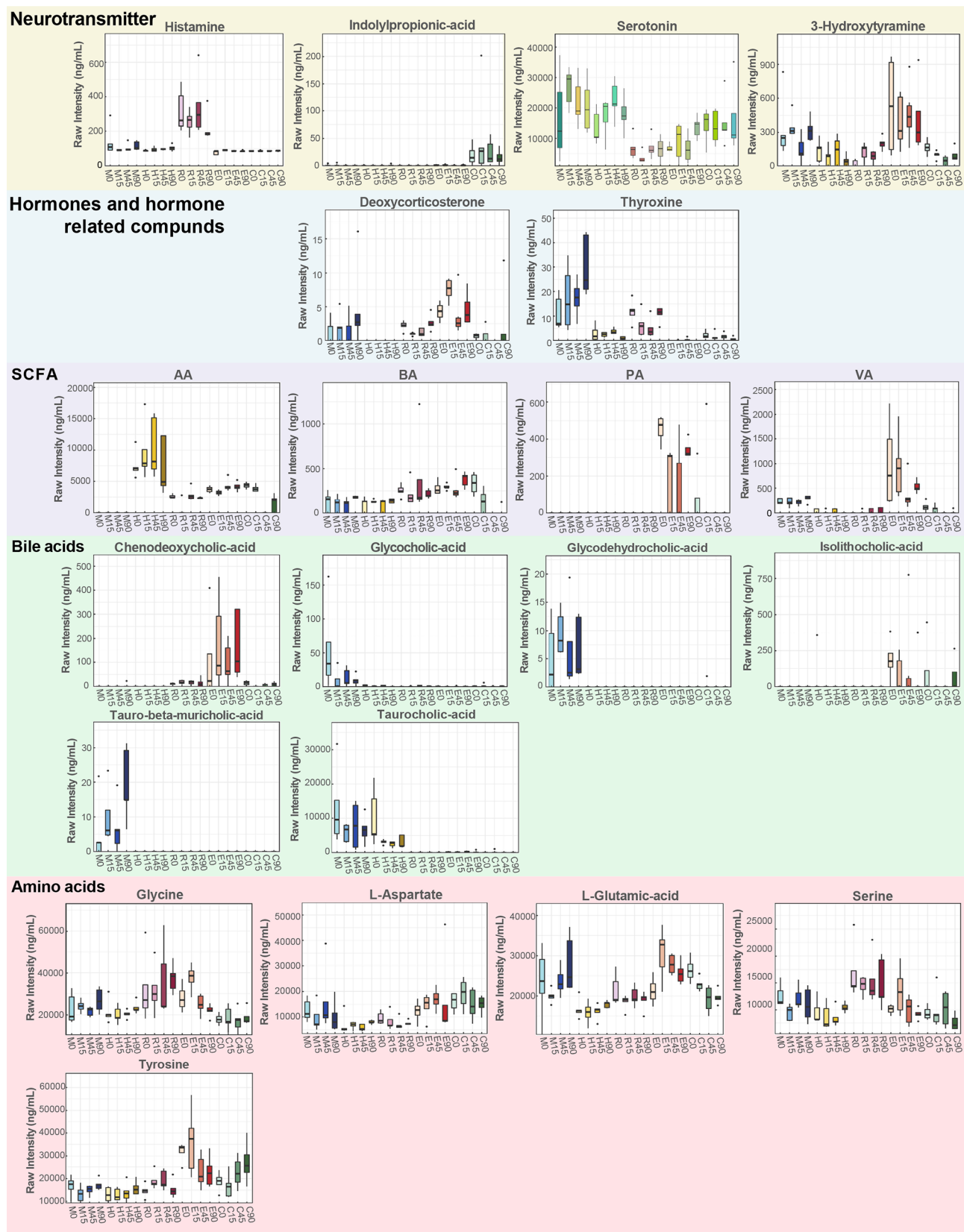


Figure 5 Comparative boxplot of gut-brain axis (GBA)- related metabolites

Based on previous GBA studies, the selected metabolites were grouped into five categories: neurotransmitters, hormones and hormone-related compounds, SCFAs, bile acids, and amino acids. The boxplots display metabolites showing clear species-level patterns. The x-axis represents individual samples from different groups (≥ 4 samples per group), and the y-axis indicates the absolute concentration of each metabolite (ng/mL), calculated by converting peak areas to concentrations using linear regression equations derived from standard curves.

in organic acids, their derivatives, and free fatty acids at multiple time points (Figure 2D; Supplementary Table S7).

Various metabolite classes have been implicated in the regulation of glucose homeostasis through distinct yet interconnected mechanisms. For instance, adenosine compounds can stimulate insulin secretion, and adenosine signaling plays a central role in maintaining glucose balance and in the pathophysiology of T2D (Andersson, 2014; Antonioli et al., 2015; Koupenova & Ravid, 2013). In this study, nucleotide-related metabolites that consistently showed differences across the four time-point groups were mainly involved in purine and pyrimidine metabolism. Previous studies have indicated that adenosine nucleotides and their metabolic products act as mediators in the crosstalk between lipid and glucose metabolism (Kim et al., 2012; Kodama et al., 2009). Meanwhile, in the interspecies comparison groups, frugivorous bats exhibited more differential metabolites belonging to polycarboxylic acids. Correspondingly, studies in animals and patients with T2D have shown that oral administration of sebacic acid can improve glycemic control, likely by enhancing insulin sensitivity and reducing hepatic gluconeogenesis and glucose output (Mingrone et al., 2013). Similarly, ketone-body-related organic acids reflect the dynamic balance between ketogenesis and ketolysis—processes that are often disrupted in diabetes and associated with impaired insulin secretion (Jin & Ma, 2021; Mahendran et al., 2013). In addition, benzoate administration in mice has been shown to alleviate glucose intolerance while enhancing glucose-stimulated insulin secretion and β -cell proliferation (Brial et al., 2022). Lipid metabolism also contributes to glucose dysregulation: diabetic patients typically exhibit elevated levels of circulating fatty acids, particularly saturated fatty acids, which are linked to increased endogenous glucose production (Liu et al., 2010; Trufelli et al., 2011). However, the effects of polyunsaturated fatty acids remain inconsistent across studies (Jeppesen et al., 2013; Kaushik et al., 2009; van Woudenberg et al., 2009). Moreover, amino acid-related changes were evident in our data, consistent with previous reports highlighting their role in modulating glucose metabolism and insulin dynamics (White et al., 2025).

Strategies of frugivorous bats in response to high-glucose stimulation: emphasis on amino acid metabolism

Amino acids, as essential nutrients, primarily participate in protein synthesis. However, growing evidence suggests that amino acids are also closely involved in the regulation of blood glucose and associated hormonal pathways (Kolic et al., 2023). For example, some amino acids act directly as neurotransmitters (Gegelashvili & Bjerrum, 2019) or are converted into α -ketoglutarate to enter the TCA cycle (Lavorato et al., 2024; Newsholme et al., 2007). Others are transported into pancreatic β -cells via Na^+ -dependent co-transporters or specific amino acid transport channels, leading to cellular depolarization and subsequent stimulation of insulin secretion (Henquin & Meissner, 1981; Hermans et al., 1987; Liu et al., 2008). In some cases, certain amino acids can even directly induce insulin release (Han et al., 2021; Pedersen et al., 2019). Under specific conditions, stimulation of pancreatic islets with selected amino acids has been shown to promote insulin secretion in a manner similar to glucose (Kolic et al., 2023; White et al., 2025).

In this study, we found that bats with high-sugar diets,

particularly *E. spelaea*, exhibited pronounced enrichment of amino acid-related metabolites (Figure 3; Supplementary Table S2). Compared to the fasting state, 25 amino acid-related metabolites showed distinct alterations during the rapid increase in blood glucose levels (15 min), including N-acyl amino acids, aromatic amino acid derivatives, branched-chain amino acid (BCAA) derivatives, and small peptides. Meanwhile, acetylated amino acid derivatives, as well as aromatic amino acids and their metabolites, were among the more abundant differential metabolites identified in the time- and species-based comparative analyses across the five bat species (Figures 2C, D; Supplementary Tables S6, S7). Among them, nine metabolites remained differentially expressed during the glucose-lowering phase (45 min). N-acyl amino acids are typically secreted by brown adipose tissue, and some members of this class that enhance mitochondrial respiration have been shown to improve glucose clearance in diet-induced obese mouse models (Li et al., 2020; Lin et al., 2018). Aromatic and branched-chain amino acids are common components of food and pharmaceuticals and are known to regulate glucose and lipid metabolism (Manoharan et al., 2023; Wang et al., 2023). Compared with healthy individuals, the levels of these amino acids are elevated in patients with T2D (Ling et al., 2023). However, Natividad et al. (2018) reported that certain aromatic amino acid metabolites can be transformed by the gut microbiota via the aryl hydrocarbon receptor pathway to produce glucagon-like peptide-1 (GLP-1), thereby stimulating insulin secretion. In addition, studies have shown that acetylation of lysine residues in malate can enhance tricarboxylic acid (TCA) cycle activity, thereby alleviating insulin resistance and delaying the onset and progression of T2D (Zhou et al., 2019). These findings collectively suggest that the expression of these amino acids may play important biological roles in glucose homeostasis.

Furthermore, the pronounced expression of amino acid-related metabolites appears to be closely associated with the high-glucose challenge (Figures 4B–D; Supplementary Table S9). Under high-glucose stimulation, the dynamic changes in amino acid-related metabolites were largely positively correlated with one another (Figure 4A; Supplementary Table S8), and their overall trends closely mirrored the blood glucose dynamics observed under IPGTT. This may indirectly support previous findings that combinations of amino acids stimulate insulin secretion more effectively than individual amino acids alone (Bolea et al., 1997; McClenaghan et al., 1996). Several amino acids and their derivatives identified in our dataset have previously been implicated in glucose metabolism (Figure 4C; Supplementary Table S2). For instance, long-term dietary studies have shown a positive association between methionine intake and diabetes risk, regardless of dietary source (Sun et al., 2023). Kynurenine, β -alanine, and glycine are known to influence systemic metabolism through their roles in neurotransmitter pathways (Davidoff et al., 1967; Mithaiwala et al., 2021; Wu et al., 1993; Yan-Do et al., 2016). BCAA, such as valine and leucine—known to be insulin-sensitive—also appeared in our data as dipeptide derivatives, including γ -glutamyl-valine and histidine-leucine (His-Leu) (Anderson et al., 2017; Floyd et al., 1966, 1970; Pozefsky et al., 1969). Moreover, some amino acid metabolites exhibited high expression levels, such as H-Lys (Me)-OH-chloride, a lysine derivative, and 3-hydroxyproline, a derivative of proline. All of the aforementioned amino acid-related metabolites responded to

IPGTT. Notably, these metabolites exhibited a general trend of gradual decline following high-glucose stimulation, although some showed an initial increase (Figure 4B, C; Supplementary Table S9). Regardless of whether levels subsequently rebounded, this pattern suggests that these metabolites may be mobilized as part of the physiological response to reduce elevated blood glucose levels.

Under high-glucose stimulation, *E. spelaea* exhibited extensive enrichment of metabolic pathways, many of which were associated with amino acid mobilization (Figure 4E). In addition to core pathways, such as amino acid biosynthesis, metabolism, and transport, several specific pathways stood out, including those involved in alanine and arginine metabolism. Previous studies have suggested that alanine and arginine may mediate communication between pancreatic α -cells and β -cells (Capozzi et al., 2019; El et al., 2021). D-amino acids—such as D-serine and D-aspartate—have been identified as novel neurotransmitters with potential roles in metabolic regulation (Roychaudhuri & Snyder, 2022; Wolosker et al., 1999). Purine metabolism, which produces adenosine, has been shown to promote insulin secretion from pancreatic islets (Andersson, 2014; Antonioli et al., 2015), whereas pyrimidine nucleotides, such as UTP, support mitochondrial pyruvate oxidation in murine cells (Sahu et al., 2024). Certain aliphatic 2-oxo acids have also been shown to induce insulin release in perfused mouse pancreas models (Lenzen & Panten, 1980). Additionally, our results revealed enrichment in cofactor metabolism pathways, which have rarely been associated with glucose metabolism in previous studies (García et al., 2023; Moon, 2024; Vilas-Boas et al., 2021). Notably, García et al. (2023) and Vilas-Boas et al. (2021) reported elevated expression of NADPH-producing enzymes in adipocytes of diabetic individuals, although the underlying signaling pathways remain poorly understood. Consistent with these findings, *C. sphinx* showed less pronounced but still notable enrichment in these pathways compared to *E. spelaea*, whereas other bat species exhibited even lower levels of enrichment. These pathways may offer valuable insights for future research on glucose metabolism.

Metabolomic landscape of bat GBA response to hyperglycemic stimulation: reflecting species and dietary preferences

Gut microbiota may initiate the dramatic increase in serum amino acids in frugivorous bats. The GBA is a bidirectional communication system between the central nervous system and the gastrointestinal tract that regulates gut function and influences brain activity, mood, and cognition (Agirman et al., 2021). Recent studies have highlighted the critical physiological and pathological roles of the GBA in metabolic disorders, particularly in the development and progression of T2D. Among the key mediators of this interaction are gut microbiota-derived metabolites (de Lartigue & Diepenbroek, 2016; Lal et al., 2001; Longo et al., 2023), which can enter systemic circulation and influence brain function.

During feeding, the gut senses nutrient intake and transmits signals to the central nervous system, particularly the hypothalamus, to coordinate food intake, energy metabolism, and glucose homeostasis (de Lartigue & Diepenbroek, 2016; Lal et al., 2001; Lam et al., 2009; Schwartz et al., 2000). Recent studies have shown that the gut microbiota can influence GBA signaling by synthesizing key neurotransmitters and branched-chain amino acids, thereby facilitating communication between the brain and the gut (Duca &

Covasa, 2012; Wachsmuth et al., 2022). Several microbial metabolites, including short-chain fatty acids, choline, tryptophan metabolites, and branched-chain amino acids, have been implicated in insulin secretion (Gérard & Vidal, 2019; Keskitalo et al., 2018; Plovier et al., 2017). We conducted a statistical analysis of substances that may participate in or regulate GBA function (Figure 5).

Autonomic nerves surrounding pancreatic islets contain neurotransmitters and their corresponding synthetic enzymes, which can interact with G protein-coupled receptors (GPCRs) on β -cells to exert regulatory effects (Gautam et al., 2006; Rosengren et al., 2010). In this study, certain frugivorous bats exhibited higher levels of multiple neurotransmitter-related metabolites than insectivorous bats, with the exception of serotonin, which was present at higher levels in insectivorous bats. Notably, *M. pilosus* showed elevated levels of thyroxine. Yan et al. (2022) reported that thyroid hormones influence glucose homeostasis through GLP-1 signaling, and that activation of hepatic thyroid hormone pathways promoted insulin secretion and lowered blood glucose levels. Bile acids modulate systemic lipid, cholesterol, and glucose metabolism by activating the farnesoid X receptor (FXR) and Takeda G protein-coupled receptor 5 (TGR5) (Cryan et al., 2019; Longo et al., 2023). SCFAs, primarily produced through gut microbial fermentation, serve as energy substrates, affect microglial maturation in the central nervous system, and stimulate insulin secretion (Doroszkiwicz et al., 2021; Heiss & Olofsson, 2019). Both bile acids and SCFAs exhibited species-specific expression patterns in our study. GLP-1 secretion from intestinal L-cells is often co-induced by these compounds (Richards et al., 2021; Sam et al., 2012). These findings suggest that, given the complexity of the GBA, future research should consider the combined influence of multiple metabolites and regulatory factors.

Aside from diet, the insectivorous and frugivorous bats involved in this study have distinct physiology and life histories. Most prominently, frugivorous bats have better vision and sense of smell than insectivorous bats, while insectivorous bats have stronger echolocation abilities than frugivorous bats. These physiological discrepancies may contribute to the serum metabolic differences between frugivorous and insectivorous bats.

Limitations of this study

It's the first time to report the study of hypoglycemic mechanism of bats. Frugivorous bats exhibited a more positive serum metabolic response to blood glucose increases than insectivorous bats, and the enrichment of amino acids in serum may imply a strategy for adapting to high-sugar diets. However, there are several limitations to this study: (1) We included only five bat species, comprising three frugivores and two insectivores. Given the diversity of bat physiology and life history, these findings may not fully represent the hypoglycemic mechanisms of all frugivorous bats. (2) We administered glucose via intraperitoneal injection rather than oral gavage or feeding, thereby ignoring organic or gut microbial absorption of glucose before it enters the blood. Future work should investigate the roles of organs and gut microbiota in glucose absorption under more physiological conditions. (3) To focus on known hypoglycemic metabolites, the study employed targeted metabolomics with a database of 704 selected metabolites; thus, some novel metabolites that lower blood glucose may have been omitted. This highlights the importance of conducting untargeted metabolomics in

future studies to complement these findings. (4) The hypoglycemic functions of the altered amino acids in frugivorous bats, particularly their influence on insulin secretion, need to be verified in model animals. Such validation would enhance the value of this work and provide possibilities for translating the findings into clinical research.

CONCLUSION

In this study, we performed targeted quantitative analyses of 704 serum metabolites across five bat species to investigate their metabolic adaptation mechanisms under high-glucose stimulation. Our results revealed that serum amino acid-related metabolites were significantly more abundant in frugivorous bats than in insectivorous bats. The dynamic changes observed during the IPGTT are likely closely linked to the regulation of insulin secretion. The differential metabolites primarily included N-acyl amino acids, aromatic amino acid derivatives, and branched-chain amino acid derivatives, which were enriched in metabolic pathways such as alanine, arginine, and D-amino acid metabolism. We therefore speculate that the efficient hypoglycemic metabolic adaptations in frugivorous bats are primarily associated with amino acid-related metabolites. Additionally, we examined metabolites associated with the GBA and found that frugivorous bats exhibited enhanced profiles of neurotransmitter-related compounds compared to insectivorous species. Hormones, bile acids, and short-chain fatty acids also displayed species-specific expression patterns, indicating that these molecules may play important roles in regulating glucose metabolism in bats. This study provides valuable metabolomic data that enhance our understanding of the metabolic adaptations of frugivorous bats and the potential mechanisms employed by insectivorous bats to cope with hyperglycemic conditions. Furthermore, it offers novel insights into the development of metabolite-based therapeutic strategies for diabetes. Future research integrating metabolomics with metagenomics and transcriptomics may help to unravel the comprehensive metabolic regulatory networks underlying glucose metabolism in frugivorous bats.

DATA AVAILABILITY

The raw metabolomics data generated from the LC-ESI-MS/MS analysis were deposited in the Science Data Bank (<https://doi.org/10.57760/sciencedb.36054>).

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

L.B.Z., M.G., and J.H.Q. designed the experiments. J.H.Q. and M.G. drafted the manuscript. L.B.Z., M.G., J.X.L. and H.B.Z. reviewed and edited the manuscript. L.B.Z. and X.Y.H. participated in animal sample collection. J.H.Q., Z.S.H., J.Y.Z., A.R.Y., T.T.S., and M.Z.L. completed the main experiments. J.H.Q. performed the data analysis and visualization. All authors read and approved the final version of the manuscript.

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REFERENCES

- Agirman G, Yu KB, Hsiao EY. 2021. Signaling inflammation across the gut-brain axis. *Science*, **374**(6571): 1087–1092.
- Anderson KA, Huynh FK, Fisher-Wellman K, et al. 2017. SIRT4 is a lysine deacylase that controls leucine metabolism and insulin secretion. *Cell Metabolism*, **25**(4): 838–855. e15.
- Andersson O. 2014. Role of adenosine signalling and metabolism in β -cell regeneration. *Experimental Cell Research*, **321**(1): 3–10.
- Andrikopoulos S, Blair AR, Deluca N, et al. 2008. Evaluating the glucose tolerance test in mice. *American Journal of Physiology: Endocrinology and Metabolism*, **295**(6): E1323–E1332.
- Antonioli L, Blandizzi C, Csóka B, et al. 2015. Adenosine signalling in diabetes mellitus—pathophysiology and therapeutic considerations. *Nature Reviews Endocrinology*, **11**(4): 228–241.
- Bolea S, Pertusa JAG, Martín F, et al. 1997. Regulation of pancreatic β -cell electrical activity and insulin release by physiological amino acid concentrations. *Pflügers Archiv*, **433**(6): 699–704.
- Brial F, Matsuda F, Gauguier D. 2022. Diet dependent impact of benzoate on diabetes and obesity in mice. *Biochimie*, **194**: 35–42.
- Camacho J, Bernal-Rivera A, Peña V, et al. 2024. Sugar assimilation underlying dietary evolution of Neotropical bats. *Nature Ecology & Evolution*, **8**(9): 1735–1750.
- Capozzi ME, Svendsen B, Encisco SE, et al. 2019. β cell tone is defined by proglucagon peptides through cAMP signaling. *JCI Insight*, **4**(5): e126742.
- Cryan JF, O'Riordan KJ, Cowan CSM, et al. 2019. The microbiota-gut-brain axis. *Physiological Reviews*, **99**(4): 1877–2013.
- Davidoff RA, Shank RP, Graham LT, et al. 1967. Is glycine a neurotransmitter?: Association of glycine with spinal interneurons. *Nature*, **214**(5089): 680–681.
- de Lartigue G, Diepenbroek C. 2016. Novel developments in vagal afferent nutrient sensing and its role in energy homeostasis. *Current Opinion in Pharmacology*, **31**: 38–43.
- Doroszkiwicz J, Groblewska M, Mroczko B. 2021. The role of gut microbiota and gut-brain interplay in selected diseases of the central nervous system. *International Journal of Molecular Sciences*, **22**(18): 10028.
- Duca FA, Covasa M. 2012. Current and emerging concepts on the role of peripheral signals in the control of food intake and development of obesity. *British Journal of Nutrition*, **108**(5): 778–793.
- El K, Gray SM, Capozzi ME, et al. 2021. GIP mediates the incretin effect and glucose tolerance by dual actions on α cells and β cells. *Science Advances*, **7**(11): eabf1948.
- Elangovan V, Priya EYS, Marimuthu G. 2006. Olfactory discrimination ability of the short-nosed fruit bat *Cynopterus sphinx*. *Acta Chiropterologica*, **8**(1): 247–253.
- Floyd JC Jr, Fajans SS, Conn JW, et al. 1966. Stimulation of insulin secretion by amino acids. *The Journal of Clinical Investigation*, **45**(9): 1487–1505.
- Floyd JC Jr, Fajans SS, Pek S, et al. 1970. Synergistic effect of certain amino acid pairs upon insulin secretion in man. *Diabetes*, **19**(2): 102–108.
- Fujisaka S, Avila-Pacheco J, Soto M, et al. 2018. Diet, genetics, and the gut microbiome drive dynamic changes in plasma metabolites. *Cell Reports*, **22**(11): 3072–3086.
- Gao JL, Yang T, Song BH, et al. 2023. Abnormal tryptophan catabolism in diabetes mellitus and its complications: opportunities and challenges. *Biomedicine & Pharmacotherapy*, **166**: 115395.
- García JG, Ansorena E, Izal I, et al. 2023. Structure, regulation, and physiological functions of NADPH oxidase 5 (NOX5). *Journal of Physiology and Biochemistry*, **79**(2): 383–395.
- Gautam D, Han SJ, Hamdan FF, et al. 2006. A critical role for β cell M₃ muscarinic acetylcholine receptors in regulating insulin release and blood

- glucose homeostasis in vivo. *Cell Metabolism*, **3**(6): 449–461.
- Gegelashvili G, Bjerrum OJ. 2019. Glutamate transport system as a key constituent of glutamatergic molecular pathology and pharmacological modulation in chronic pain. *Neuropharmacology*, **161**: 107623.
- Gérard C, Vidal H. 2019. Impact of gut microbiota on host glycemic control. *Frontiers in Endocrinology*, **10**: 29.
- González-Gutiérrez K, Castaño JH, Perez-Torres J, et al. 2022. Structure and roles in pollination networks between Phyllostomid bats and flowers: a systematic review for the Americas. *Mammalian Biology*, **102**(1): 21–49.
- Gorbunova V, Seluanov A, Kennedy BK. 2020. The world goes bats: living longer and tolerating viruses. *Cell Metabolism*, **32**(1): 31–43.
- Han GR, Takahashi H, Murao N, et al. 2021. Glutamate is an essential mediator in glutamine-amplified insulin secretion. *Journal of Diabetes Investigation*, **12**(6): 920–930.
- Heiss CN, Olofsson LE. 2019. The role of the gut microbiota in development, function and disorders of the central nervous system and the enteric nervous system. *Journal of Neuroendocrinology*, **31**(5): e12684.
- Henquin JC, Meissner HP. 1981. Effects of amino acids on membrane potential and 86Rb^+ fluxes in pancreatic beta-cells. *American Journal of Physiology: Endocrinology and Metabolism*, **240**(3): E245–E252.
- Hermans MP, Schmeer W, Henquin JC. 1987. The permissive effect of glucose, tolbutamide and high K^+ on arginine stimulation of insulin release in isolated mouse islets. *Diabetologia*, **30**(8): 659–665.
- Hodgkinson R, Ayasse M, Häberlein C, et al. 2013. Fruit bats and bat fruits: the evolution of fruit scent in relation to the foraging behaviour of bats in the New and Old World tropics. *Functional Ecology*, **27**(4): 1075–1084.
- International Diabetes Federation. 2025. IDF diabetes atlas. <https://diabetesatlas.org>.
- Jeppesen C, Schiller K, Schulze MB. 2013. Omega-3 and omega-6 fatty acids and type 2 diabetes. *Current Diabetes Reports*, **13**(2): 279–288.
- Jin Q, Ma RCW. 2021. Metabolomics in diabetes and diabetic complications: insights from epidemiological studies. *Cells*, **10**(11): 2832.
- Karasov WH, Douglas AE. 2013. Comparative digestive physiology. *Comprehensive Physiology*, **3**(2): 741–783.
- Kaushik M, Mozaffarian D, Spiegelman D, et al. 2009. Long-chain omega-3 fatty acids, fish intake, and the risk of type 2 diabetes mellitus. *The American Journal of Clinical Nutrition*, **90**(3): 613–620.
- Kelm DH, Simon R, Kuhlow D, et al. 2011. High activity enables life on a high-sugar diet: blood glucose regulation in nectar-feeding bats. *Proceedings of the Royal Society B: Biological Sciences*, **278**(1724): 3490–3496.
- Keskitalo A, Munukka E, Toivonen R, et al. 2018. *Enterobacter cloacae* administration induces hepatic damage and subcutaneous fat accumulation in high-fat diet fed mice. *PLoS One*, **13**(5): e0198262.
- Kim TH, Lee SS, Yoo JH, et al. 2012. The relationship between the regional abdominal adipose tissue distribution and the serum uric acid levels in people with type 2 diabetes mellitus. *Diabetology & Metabolic Syndrome*, **4**(1): 3.
- Kitabchi AE, Umpeierrez GE, Miles JM, et al. 2009. Hyperglycemic crises in adult patients with diabetes. *Diabetes Care*, **32**(7): 1335–1343.
- Kodama S, Saito K, Yachi Y, et al. 2009. Association between serum uric acid and development of type 2 diabetes. *Diabetes Care*, **32**(9): 1737–1742.
- Kolic J, Sun WG, Johnson JD, et al. 2023. Amino acid-stimulated insulin secretion: a path forward in type 2 diabetes. *Amino Acids*, **55**(12): 1857–1866.
- Korine C, Kalko EKV. 2005. Fruit detection and discrimination by small fruit-eating bats (Phyllostomidae): echolocation call design and olfaction. *Behavioral Ecology and Sociobiology*, **59**(1): 12–23.
- Koupenova M, Ravid K. 2013. Adenosine, adenosine receptors and their role in glucose homeostasis and lipid metabolism. *Journal of Cellular Physiology*, **228**(8): 1703–1712.
- Kunz TH, de Torrez EB, Bauer D, et al. 2011. Ecosystem services provided by bats. *Annals of the New York Academy of Sciences*, **1223**(1): 1–38.
- Kunz TH, Fenton MB. 2005. Bat Ecology. Chicago: University of Chicago Press.
- Lal S, Kirkup AJ, Brunsden AM, et al. 2001. Vagal afferent responses to fatty acids of different chain length in the rat. *American Journal of Physiology: Gastrointestinal and Liver Physiology*, **281**(4): G907–G915.
- Lam CKL, Chari M, Lam TKT. 2009. CNS regulation of glucose homeostasis. *Physiology*, **24**(3): 159–170.
- Lavorato M, Iadarola D, Remes C, et al. 2024. *dlh^{en3}* zebrafish exhibit altered mitochondrial ultrastructure, morphology, and dysfunction partially rescued by probucol or thiamine. *JCI Insight*, **9**(18): e178973.
- Lenzen S, Panten U. 1980. 2-oxocarboxylic acids and function of pancreatic islets in obese-hyperglycaemic mice. Insulin secretion in relation to ^{45}Ca uptake and metabolism. *The Biochemical Journal*, **186**(1): 135–144.
- Lever M, Slow S. 2010. The clinical significance of betaine, an osmolyte with a key role in methyl group metabolism. *Clinical Biochemistry*, **43**(9): 732–744.
- Li VL, Kim JT, Long JZ. 2020. Adipose tissue lipokines: recent progress and future directions. *Diabetes*, **69**(12): 2541–2548.
- Lin H, Long JZ, Roche AM, et al. 2018. Discovery of hydrolysis-resistant isoindoline *N*-acyl amino acid analogues that stimulate mitochondrial respiration. *Journal of Medicinal Chemistry*, **61**(7): 3224–3230.
- Ling ZN, Jiang YF, Ru JN, et al. 2023. Amino acid metabolism in health and disease. *Signal Transduction and Targeted Therapy*, **8**(1): 345.
- Liu LY, Li Y, Guan CM, et al. 2010. Free fatty acid metabolic profile and biomarkers of isolated post-challenge diabetes and type 2 diabetes mellitus based on GC-MS and multivariate statistical analysis. *Journal of Chromatography B, Analytical Technologies in the Biomedical and Life Sciences*, **878**(28): 2817–2825.
- Liu ZP, Jeppesen PB, Gregersen S, et al. 2008. Dose- and glucose-dependent effects of amino acids on insulin secretion from isolated mouse islets and clonal INS-1E beta-cells. *The Review of Diabetic Studies*, **5**(4): 232–244.
- Longo S, Rizza S, Federici M. 2023. Microbiota-gut-brain axis: relationships among the vagus nerve, gut microbiota, obesity, and diabetes. *Acta Diabetologica*, **60**(8): 1007–1017.
- Mahendran Y, Vangipurapu J, Cederberg H, et al. 2013. Association of ketone body levels with hyperglycemia and type 2 diabetes in 9,398 Finnish men. *Diabetes*, **62**(10): 3618–3626.
- Manoharan L, Roth B, Bang C, et al. 2023. An okinawan-based Nordic diet leads to profound effects on gut microbiota and plasma metabolites linked to glucose and lipid metabolism. *Nutrients*, **15**(14): 3273.
- McClenaghan NH, Barnett CR, O'Harte FPM, et al. 1996. Mechanisms of amino acid-induced insulin secretion from the glucose-responsive BRIN-BD11 pancreatic B-cell line. *Journal of Endocrinology*, **151**(3): 349–357.
- McEntyre CJ, Lever M, Chambers ST, et al. 2015. Variation of betaine, *N*, *N*-dimethylglycine, choline, glycerophosphorylcholine, taurine and trimethylamine-*N*-oxide in the plasma and urine of overweight people with type 2 diabetes over a two-year period. *Annals of Clinical Biochemistry*, **52**(3): 352–360.
- Meng FX, Zhu L, Huang WJ, et al. 2016. Bats: body mass index, forearm mass index, blood glucose levels and *SLC2A2* genes for diabetes. *Scientific Reports*, **6**(1): 29960.
- Michelmores AJ, Keegan DJ, Kramer B. 1998. Immunocytochemical identification of endocrine cells in the pancreas of the fruit bat, *Rousettus aegyptiacus*. *General and Comparative Endocrinology*, **110**(3): 319–325.
- Mingrone G, Castagneto-Gissey L, Macé K. 2013. Use of dicarboxylic acids in type 2 diabetes. *British Journal of Clinical Pharmacology*, **75**(3): 671–676.
- Mithaiwala MN, Santana-Coelho D, Porter GA, et al. 2021. Neuroinflammation and the kynurenine pathway in CNS disease: molecular

- mechanisms and therapeutic implications. *Cells*, **10**(6): 1548.
- Moon DO. 2024. NADPH dynamics: linking insulin resistance and β -cells ferroptosis in diabetes mellitus. *International Journal of Molecular Sciences*, **25**(1): 342.
- Muscarella R, Fleming TH. 2007. The role of frugivorous bats in tropical forest succession. *Biological Reviews*, **82**(4): 573–590.
- Natividad JM, Agus A, Planchais J, et al. 2018. Impaired aryl hydrocarbon receptor ligand production by the gut microbiota is a key factor in metabolic syndrome. *Cell Metabolism*, **28**(5): 737–749. e4.
- Neuweiler G. 2000. The Biology of Bats. Oxford: Oxford University Press.
- Newsholme P, Bender K, Kiely A, et al. 2007. Amino acid metabolism, insulin secretion and diabetes. *Biochemical Society Transactions*, **35**(5): 1180–1186.
- Norberg UM, Rayner JMV. 1987. Ecological morphology and flight in bats (Mammalia; Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **316**(1179): 335–427.
- Pedersen MG, Tagliavini A, Henquin JC. 2019. Calcium signaling and secretory granule pool dynamics underlie biphasic insulin secretion and its amplification by glucose: experiments and modeling. *American Journal of Physiology: Endocrinology and Metabolism*, **316**(3): E475–E486.
- Peng XW, He XY, Sun YX, et al. 2019. Difference in glucose tolerance between phytophagous and insectivorous bats. *Journal of Comparative Physiology B*, **189**(6): 751–756.
- Plovier H, Everard A, Druart C, et al. 2017. A purified membrane protein from *Akkermansia muciniphila* or the pasteurized bacterium improves metabolism in obese and diabetic mice. *Nature Medicine*, **23**(1): 107–113.
- Pozefsky T, Felig P, Tobin JD, et al. 1969. Amino acid balance across tissues of the forearm in postabsorptive man. Effects of insulin at two dose levels. *The Journal of Clinical Investigation*, **48**(12): 2273–2282.
- Protzek AOP, Rafacho A, Viscelli BA, et al. 2010. Insulin and glucose sensitivity, insulin secretion and β -cell distribution in endocrine pancreas of the fruit bat *Artibeus lituratus*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, **157**(2): 142–148.
- Razgour O, Clare EL, Zeale MRK, et al. 2011. High-throughput sequencing offers insight into mechanisms of resource partitioning in cryptic bat species. *Ecology and Evolution*, **1**(4): 556–570.
- Richards P, Thornberry NA, Pinto S. 2021. The gut-brain axis: identifying new therapeutic approaches for type 2 diabetes, obesity, and related disorders. *Molecular Metabolism*, **46**: 101175.
- Rolo AP, Palmeira CM. 2006. Diabetes and mitochondrial function: role of hyperglycemia and oxidative stress. *Toxicology and Applied Pharmacology*, **212**(2): 167–178.
- Rosengren AH, Jokubka R, Tojjar D, et al. 2010. Overexpression of Alpha2A-adrenergic receptors contributes to type 2 diabetes. *Science*, **327**(5962): 217–220.
- Roychoudhuri R, Snyder SH. 2022. Mammalian D-cysteine: a novel regulator of neural progenitor cell proliferation. *BioEssays*, **44**(7): 2200002.
- Sahu U, Villa E, Reczek CR, et al. 2024. Pyrimidines maintain mitochondrial pyruvate oxidation to support de novo lipogenesis. *Science*, **383**(6690): 1484–1492.
- Sam AH, Troke RC, Tan TM, et al. 2012. The role of the gut/brain axis in modulating food intake. *Neuropharmacology*, **63**(1): 46–56.
- Schwartz MW, Woods SC, Porte D Jr, et al. 2000. Central nervous system control of food intake. *Nature*, **404**(6778): 661–671.
- Small L, Ehrlich A, Iversen J, et al. 2022. Comparative analysis of oral and intraperitoneal glucose tolerance tests in mice. *Molecular Metabolism*, **57**: 101440.
- Strobel S, Roswag A, Becker NI, et al. 2013. Insectivorous bats digest chitin in the stomach using acidic mammalian chitinase. *PLoS One*, **8**(9): e72770.
- Sun XM, Chen YX, Shu J, et al. 2023. The association between methionine intake and diabetes in Chinese adults—results from the China health and nutrition survey. *Nutrients*, **15**(1): 116.
- Takahashi M, Ozaki M, Kang MI, et al. 2018. Effects of meal timing on postprandial glucose metabolism and blood metabolites in healthy adults. *Nutrients*, **10**(11): 1763.
- Trufelli H, Famiglini G, Termopoli V, et al. 2011. Profiling of non-esterified fatty acids in human plasma using liquid chromatography-electron ionization mass spectrometry. *Analytical and Bioanalytical Chemistry*, **400**(9): 2933–2941.
- Trygg J, Wold S. 2002. Orthogonal projections to latent structures (O-PLS). *Journal of Chemometrics*, **16**(3): 119–128.
- van Woudenberg GJ, van Ballegooijen AJ, Kuijsten A, et al. 2009. Eating fish and risk of type 2 diabetes: a population-based, prospective follow-up study. *Diabetes Care*, **32**(11): 2021–2026.
- Vilas-Boas EA, Almeida DC, Roma LP, et al. 2021. Lipotoxicity and β -cell failure in type 2 diabetes: oxidative stress linked to NADPH oxidase and ER stress. *Cells*, **10**(12): 3328.
- Wachsmuth HR, Weninger SN, Duca FA. 2022. Role of the gut-brain axis in energy and glucose metabolism. *Experimental & Molecular Medicine*, **54**(4): 377–392.
- Wang ZA, Wang YQ, Li ZY, et al. 2023. Lipid metabolism as a target for cancer drug resistance: progress and prospects. *Frontiers in Pharmacology*, **14**: 1274335.
- Weru J, Chege P, Kinyuru J. 2021. Nutritional potential of edible insects: a systematic review of published data. *International Journal of Tropical Insect Science*, **41**(3): 2015–2037.
- White PJ, Wewer Albrechtsen NJ, Campbell JE. 2025. Islet hormones at the intersection of glucose and amino acid metabolism. *Nature Reviews Endocrinology*, **21**(7): 397–412.
- Wilman H, Belmaker J, Simpson J, et al. 2014. EltonTraits 1.0: species-level foraging attributes of the world's birds and mammals. *Ecology*, **95**(7): 2027.
- Wilson DE, Mittermeier RA. 2019. Handbook of the Mammals of the World: Vol. 9: Bats. Barcelona: Lynx Edicions.
- Wolosker H, Sheth KN, Takahashi M, et al. 1999. Purification of serine racemase: biosynthesis of the neuromodulator D-serine. *Proceedings of the National Academy of Sciences of the United States of America*, **96**(2): 721–725.
- Wu FS, Gibbs TT, Farb DH. 1993. Dual activation of GABA_A and glycine receptors by β -alanine: inverse modulation by progesterone and 5 α -pregnan-3 α -ol-20-one. *European Journal of Pharmacology: Molecular Pharmacology*, **246**(3): 239–246.
- Yan Y, Niu ZM, Sun C, et al. 2022. Hepatic thyroid hormone signalling modulates glucose homeostasis through the regulation of GLP-1 production via bile acid-mediated FXR antagonism. *Nature Communications*, **13**(1): 6408.
- Yan-Do R, Duong E, Manning Fox JE, et al. 2016. A glycine-insulin autocrine feedback loop enhances insulin secretion from human β -cells and is impaired in type 2 diabetes. *Diabetes*, **65**(8): 2311–2321.
- Zhou B, Shen ZL, Liu YS, et al. 2019. Proteomic analysis reveals that lysine acetylation mediates the effect of antemortem stress on postmortem meat quality development. *Food Chemistry*, **293**: 396–407.