The FASEB Journal

FASEB
Federation of American Societies for Experimental Biology

RESEARCH ARTICLE OPEN ACCESS

High Relevance of Fatty Acid Oxidation in a Migrating Mammal, the Nathusius' Pipistrelle (*Pipistrellus nathusii*)

Alesia Walker[1] | Thuy Thanh Truong[1] | Martin Klingenspor[2] | Anders Hedenström[3] | Gunars Pētersons[4] | Philippe Schmitt-Kopplin[1,5] | Shannon E. Currie[6,7] | Christian C. Voigt[6,8]

[1]Research Unit Analytical BioGeoChemistry, Helmholtz Zentrum München, Neuherberg, Germany | [2]Chair of Molecular Nutritional Medicine, School of Life Sciences, Technical University of Munich, Freising, Germany | [3]Department of Biology, Lund University, Lund, Sweden | [4]Faculty of Veterinary Medicine, Latvia University of Life Sciences and Technologies, Jelgava, Latvia | [5]Analytical Food Chemistry, Technical University of Munich, Freising, Germany | [6]Department Evolutionary Ecology, Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany | [7]School of BioSciences, University of Melbourne, Parkville, Australia | [8]Institute of Biochemistry and Biology, University of Potsdam, Potsdam, Germany

Correspondence: Alesia Walker (alesia.walker@helmholtz-munich.de) | Christian C. Voigt (voigt@izw-berlin.de)

Received: 23 February 2026 | **Revised:** 14 April 2026 | **Accepted:** 8 May 2026

Keywords: acyl carnitines | exercise | metabolomics | migration | Nathusius' pipistrelle | phospholipids

ABSTRACT

Bats are the only mammals capable of powered flight, allowing them to cover relatively long distances in a short time. However, the general inability of mammals to fuel endurance exercise solely by oxidizing fatty acids may prevent bats from undertaking long-distance intercontinental migrations—like birds do. Here, we conducted untargeted metabolomics to reveal the oxidative fuels used by wild caught Nathusius' pipistrelles. We investigated polar metabolites and lipids in whole blood from bats flying under controlled wind tunnel or field conditions and how metabolites respond to the physiological challenge. Around 70% of detected acyl carnitines were significantly elevated after flight in the wind tunnel compared to resting bats. The phospholipid levels varied; some increased while others decreased significantly after flight, and most did not return to resting levels within 1 h of recovery. During migration season, we observed a significant increase of phosphatidylethanolamines with unsaturated fatty acids and a bulk increase of several phosphatidylcholines and their lyso-derivatives. While migration had a clear effect on phospholipids, recovering after flight in both seasons was less pronounced and only 24% of acyl carnitines were increased after 1 h of rest. We conclude that endurance exercise such as migration has a greater influence on lipid composition and their abundance than short flights, which indicates a relatively high relevance of fatty acid oxidation to fuel migration in bats.

1 | Introduction

Long-distance migration requires animals to travel immense distances, sometimes over very short time frames. Even though the cost of transport is relatively low in flying migrants [1], morphological and physiological adaptations are necessary to support high-intensity endurance exercise [2, 3]. Migratory birds are especially well adapted to endurance flights, with some

Abbreviations: ACN, acetonitrile; ATP, adenosine triphosphate; AUC, area under the curve; CAR, acyl carnitine; DAG, diacylglycerol; ESI(+) and ESI(−), positive and negative electrospray ionization, respectively; FA, fatty acid; GNPS, Global Natural Products Social Molecular Networking; HILIC LC–MS/MS, hydrophilic interaction liquid chromatography tandem mass spectrometry; LC–MS, liquid chromatography mass spectrometry; LPC, lysophosphatidylcholine; m/z, mass-to-charge ratio; MGF, MASCOT generic files; MS, mass spectrometry; MS/MS, tandem mass spectrometry; mzd, mass-to-charge ratio difference; NAT, N-acyl taurines; PBRS, Pape Bird Ringing Station; PC, phosphatidylcholine; PCA, principal component analysis; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PLS-DA, partial least squares—discriminant analysis; PS, phosphatidylserine; ROC, receiver operator characteristic; TG, triacylglycerol; TOF MS, time-of-flight mass spectrometry; TOF MS/MS, time-of-flight tandem mass spectrometry; TopK, top knots; UHPLC, ultrahigh-pressure liquid chromatography mass spectrometry; VIP, variables important for the projection.

Shannon E. Currie and Christian C. Voigt should be considered joint senior authors.

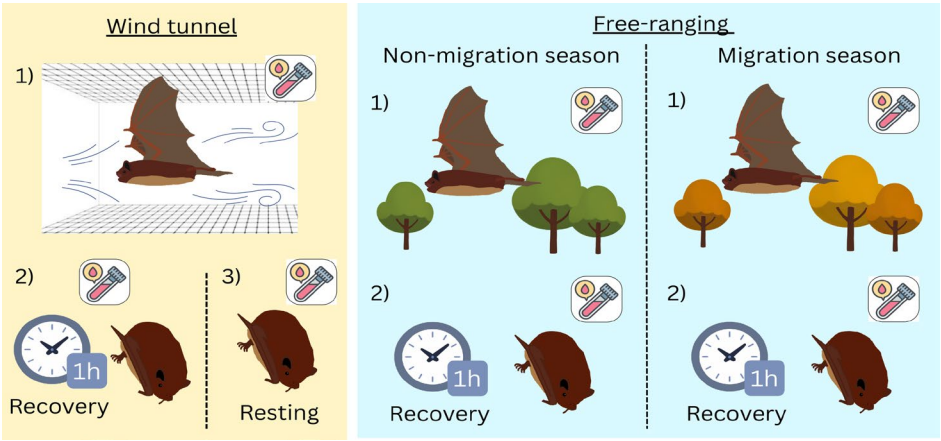

FIGURE 1 | Study design and sample collection scheme of *Pipistrellus nathusii* flying under controlled conditions (wind tunnel) and field conditions (free-ranging) before and during migration season.

species capable of flying thousands of kilometers without showing signs of exhaustion [4, 5]. Physiological adaptations to long-distance migration are less well studied in bats, yet single flights of hundreds of kilometers have been reported in a few species [6–8], and some bats can cover thousands of kilometers between breeding and wintering grounds [9–12]. While migratory birds are known to fuel endurance flights primarily through fatty acid oxidation [2, 3, 13], fuel use in migratory bats is less well understood [14, 15]. Evidence suggests that bats hunt insects while migrating and upregulate fatty acid transport proteins during the migration season [16–18]. However, like most mammals, bats may be constrained in their ability to use fat as a fuel for high-intensity aerobic exercise, which may limit their capacity to cover similar distances as birds during migration [19, 20].

Many metabolic pathways are altered during exercise with the primary function of providing adenosine triphosphate (ATP) for muscle contraction. Dependent upon duration and intensity, creatine phosphate, glycolysis, oxidative phosphorylation, glycogenolysis, lipolysis, fatty acid, and ketone body oxidation are major pathways delivering ATP to fuel exercise [21]. Metabolomics can be used to study the metabolism of exercising mammals under natural conditions or controlled laboratory conditions for short or long endurance training [22–25]. Untargeted metabolite profiling can be performed using a holistic strategy by applying mass spectrometry-based or nuclear magnetic resonance spectroscopy-based approaches [26]. Untargeted metabolomics cover a wider range of compounds and thus can be used to better explore rare metabolite classes or unexpected metabolic pathways [26].

While a number of metabolomic studies have been carried out on migratory birds, to our knowledge, none have been done on migratory bats. Past studies on birds have mostly been conducted at migratory stop-overs [2], or in controlled laboratory conditions [27]. In many cases, individuals were sampled while at rest and not during exercise. Here, we conducted a comparative untargeted metabolomic study in a bat species, the long-distance migrant *Pipistrellus nathusii*. We included both individuals exercising in captivity under controlled conditions and in the wild. We compare exercise (flight)-recovery profiles for whole blood sampled from bats under migratory and non-migratory conditions. Our study provides novel insights into polar metabolites and lipids during endurance exercise in mammals, specifically, the potential convergent evolution of metabolic pathways to support high-intensity exercise in migratory bats.

2 | Materials and Methods

2.1 | Study Design

Blood sampling of bats took place under two collection protocols: (1) under controlled conditions in captive bats and (2) under field conditions in wild bats, illustrated in Figure 1. Under controlled conditions, bats were trained to fly in a wind tunnel for 30 min and whole blood samples were taken to compare metabolomes at rest, immediately after flight, and after 1 h of rest recovery. To compare similar exercise (flight)-recovery profiles in bats prior to and during the migration season, we also collected blood samples from free-ranging individuals immediately after flight (within 3 min of capture) and after 1 h of recovery. For both protocols, we collected blood in the same manner by puncturing the vein of the uropatagium (near the tail) with a sterile 27 G needle. Prior to any procedure, the site of puncture was sterilized with ethanol. We then collected approximately 60 μL of whole blood in a heparinized capillary tube. We used a capillary pipette to expel the blood into a 2 mL cryovial, which was sealed and immediately placed within a dry shipper and cooled with liquid nitrogen. Samples were shipped in the dry shipper from the site of collection to the laboratory and then stored at −80°C until metabolite extraction. We chose blood as the preferred sample type because metabolites from muscles, bones, and liver are released into the bloodstream as a result of aerobic respiration and lipolysis [21]. Moreover, blood can be collected in a minimally invasive way.

2.1.1 | Sample Collection in Bats During Controlled Wind Tunnel Experiments (Controlled Conditions)

We captured 16 adult *P. nathusii* (8 male, 8 female, body mass = 8.7 ± 1.2 g; mean ± standard deviation) at the Pape Bird Ringing Station, Latvia (56°09′57″′ N 21°01′02″ E, Rucava municipality), during the migration season in September

TABLE 1 | Details of sampling conditions and number of samples used in analyses. All post-exercise samples were taken within 3 min of capturing bats mid-flight. All recovery samples were taken from individuals captured mid-flight and left to rest for 1 h.

Controlled conditions			Field conditions			
			Prior migration season		During migration season	
Resting	Post-exercise	Recovery	Post-exercise	Recovery	Post-exercise	Recovery
13 (8 female, 5 male)	16 (8 female, 8 male)	11 (6 female, 5 male)	7 (5 female, 2 male)	9 (6 female, 3 male)	17 (11 female, 6 male)	14 (5 female, 9 male)

2020 under permit number 184/2020 of the Latvian Nature Conservation Agency. After 5 days of acclimatization, bats were transferred to Lund University, where they were kept in indoor cages in groups of four under the permit number 5.8.18-1012472018 of the Lund-Malmö board for ethics in animal research. While in captivity bats were hand fed a diet of *Tenebrio molitor* larvae (mealworms) supplemented with juvenile hand-feeding formula (Harrison's Bird Foods, Tennessee, USA), and were provided water ad libitum in cages. In Lund, bats were trained to fly in a low-turbulence wind tunnel in pairs at wind speeds between 6 and 8 m/s [28], which is close to the flight speeds of migratory bats in the field [29]. An individual was considered trained when it flew for more than 10 min without landing, which took between three and seven training days. Following this training, we began experimental flights with individuals flying in pairs for at least 30 min. All flight training and experimental flights took place during the active phase of the bats between sunset and 2 am. Bats were fasted for at least 16 h prior to flight and were fed and given water after each experimental flight. In nature Nathusius' pipistrelles occasionally forage until dawn and will not become active again until ~1 h after sunset; therefore, inactive (or fasting periods) are at least 11–18 h/day in their summer/autumn range. Each individual underwent two experimental flights, which were conducted on separate days and in a randomized order to ensure that blood volume was replenished between flights. Whole blood samples were collected immediately after flight (post-exercise) or 1 h after the flight (recovery) (Table 1). At the end of the entire experimental period, after 1 week of no flight experiments, a third blood sample was taken during the rest phase between 10 am and 2 pm from each individual in a fasted state (resting) (Table 1).

2.1.2 | Sample Collection in Free-Ranging Bats (Field Conditions)

To compare with bats under field conditions, we collected blood samples from free-ranging bats prior to and during the migration season in Latvia under permit number 114 issued by the Food and Veterinary Service to the Faculty of Veterinary Medicine of the Latvia University of Agriculture and permit number 184/2020 of the Latvian Nature Conservation Agency. In July 2021, prior to the migration season, we collected samples from a total of 23 bats (7 male, 16 female, body mass = 7.5 ± 1.2 g) at Engure Ornithological Station Latvia (57°15′34″ N 23°08′08″ E, Engure municipality), of which 16 samples with sufficient volume could be included in the final analysis (Table 1). During the migration season in the same year (August 2021), we collected samples from a total of 42 (18 males, 24 females, body mass = 8.0 ± 0.7 g) bats at Pape Bird Ringing Station, of which 31 samples had sufficient volume to be included in final metabolomics analysis. All bats were captured in mid-flight using mist nets or a hand net. Similar to the controlled wind tunnel experiments, whole blood was collected either immediately post-exercise (within 3 min of capture in flight) or after 1 h of recovery (Table 1).

2.2 | Untargeted Metabolomics Using HILIC UHPLC–MS/MS Analysis

2.2.1 | Materials

Milli-Q water was obtained from a Milli-Q Integral Water Purification System (Billerica, MA, USA); acetonitrile (ACN) and methanol (MeOH) were purchased from Merck (LiChrosolv, hypergrade for LC–MS; Merck KGaA, Darmstadt, Germany). Ammonium acetate (NH4Ac; LiChropur eluent additive for LC–MS; Merck KGaA) at 0.5 mol/L was adjusted to pH 4.6 with glacial acetic acid (Honeywell; Fluka, Seelze, Germany).

2.2.2 | Sample Collection and Preparation

Whole blood was collected from a total of 87 wild bats captured in Latvia as described above. Blank samples were prepared by using three heparinized capillary tubes and dipping them into 50 μL milli-Q water, and 25 μL was added to 75 μL ACN and treated in the same way as blood samples for metabolite extraction. Each whole blood sample was extracted with three times the volume of ice-cold ACN, vortexed for 10 s, and centrifuged for 10 min (15 300 × g, 4°C). Supernatants were transferred into tubes and stored at −20°C until analysis.

2.2.3 | HILIC LC–MS/MS

Whole blood samples were analyzed by using a UHPLC system (ExionLC; AB Sciex LLC, Framingham, MA, USA) coupled to a quadrupole time-of-flight (TOF) mass spectrometer (X500 QTOF MS; AB Sciex LLC). The mass spectrometer was operated in negative and positive electrospray ionization mode (ESI(−/+)), by using a TurboIonSpray (AB Sciex LLC). The MS was operated in TOF MS and TOF MS/MS scan mode. The TOF MS analyzed mass-to-charge ratios (m/z) between 65 and 1000 Da in profile mode, and MS/MS were generated in data dependent acquisition mode (information-dependent acquisition). Mass spectrometer specific parameters are summarized in Table S1.

Separation of polar metabolites was carried out by an iHILIC-Fusion UHPLC column SS (100 × 2.1 mm, 1.8 μm, 100 Å; HILICON AB, Umeå, Sweden), as described previously [30]. Eluent A consisted of 5 mmol/L NH_4Ac (pH 4.6) in 95% ACN (pH 4.6), and eluent B consisted of 25 mmol/L NH_4Ac (pH 4.6) in 30% ACN. The run started with 0.1% B, keeping it constant for 2 min, then increased B to 99.9% over 7.5 min. 99.9% B was kept for 2 min and reversed to 0.1% B within 0.1 min and held for an additional 0.1 min. The run was completed after 12.1 min, and the column was equilibrated for 5 min before the next injection. The flow rate was set to 0.5 mL/min, the column temperature to 40°C, and the sample manager was cooled to 4°C; 5 μL of the sample was injected into the column. The weak and strong washes consisted of 95% and 10% ACN, respectively. Samples, blanks (extraction controls), pooled sample and 75% ACN were measured within the LC–MS/MS batch. Pooled sample was used to condition the HILIC column and was injected after every tenth sample. Samples were run in a randomized order.

2.2.4 | Data Processing and Metabolite Identification

The raw LC–MS data were post-processed in GeneData Expressionist Refiner MS (version 15.0.7; GeneData GmbH, Basel, Switzerland), including chemical noise subtraction, chromatogram retention time alignment (Pairwise Alignment Based Tree with maximum retention time shift of 0.2 min), chromatographic peak picking, blank peak filter (minimum sample to blank ratio of 10, geometric mean), chromatogram isotope clustering, valid feature filter (cut-off of 50 ESI(+) or 1000 ESI(−)) maximum intensity and presence of features in at least 10% of samples for ESI(−/+), retention time range restriction (0.4–9.0 min), annotation of known peaks (mass-to-charge tolerance of 0.005 Da and retention time tolerance of 0.3), and MS/MS consolidation (keep the MS/MS with highest total ion count per peak within one sample) and export to single MASCOT generic files (MGFs). Data processing resulted in a matrix containing features with mass-to-charge ratios (m/z), retention times, and observed maximum intensities for each sample. MS/MS of features across samples were combined with the *consensusSpectrum* function of the *MSnbase* package [31], using mzd of 0.005, minProp of 0.1 and sum for the *intensityFun* argument. Ions of MS/MS were matched with 0.005 Da, and intensities of aggregated peaks were summed, if ions were present in minimum 10% of matched spectra. Ions with intensities ≤ 500 and m/z values ≥ precursor $m/z + 2$ were discarded from consensus spectra. Identification was done by matching experimental MS/MS spectra against spectral libraries, downloaded from MassBank of North America and MS-DIAL LipidBlast (version 68) by using MSPepSearch release: 02/22/2019; 0.01 Da mass tolerance for precursor and fragment searches. We removed matches with dot product < 500 and multiple matches were filtered by highest dot product. Furthermore, GNPS molecular networking, GNPS Library Search, Sirius 4.9, and MS2Query were used to facilitate metabolite identification or classification of experimental MS/MS [32–34]. The GNPS Library Search was conducted with the following settings: The precursor ion mass tolerance and fragment ion mass tolerance were set to 0.01 Da, minimum matched peaks were set to 1, and the score threshold was set to 0.5. GNPS Molecular Networking was performed with the following settings: the precursor ion mass tolerance was set to 0.01 Da, the MS/MS fragment ion tolerance was set to 0.01 Da, and the cosine score was set to > 0.5 with a minimum matched peak of 1, TopK was set to 10, the maximum component size was set to 100, the maximum shift was set to 200 Da, the minimum cluster size was set to 1, and the maximum analog search mass difference was set to 500. MS2Query hits were kept with ms2query_model_prediction values > 0.5. The cf_direct_parent of the MS2Query classification was used for visualization and the identities of individual features were picked from spectral library or in silico matches of MS/MS, as described above. Data and metabolite identities are summarized in Table S2a–c. For selected lipids, shorthand notation was applied, mainly using species level and molecular species level annotation [35]. Targeted peak picking of amino acids was conducted using Sciex OS Analytics 3.0 (AB Sciex LLC), summarized in Table S3.

2.3 | Statistical Analysis

Statistical analyses were performed in R version 4.5.1 and RStudio 2025.09.1 [36, 37]. Unsupervised and supervised multivariate statistical analyses such as Principal Component Analysis (PCA) and Partial Least Squares—Discriminant Analysis (PLS-DA) were applied to account for the high-dimensional structure of metabolomics data, to reduce dimensionality and to obtain a global view of the data. PLS-DA was used to select the most predictive metabolites, responsible for the classification of interest. The performance of the PLS-DA model was confirmed by area under the curve values and p values, by evaluating them in the first and second components. Furthermore, variables important for the projection (VIP) were extracted from the first and second components of the PLS-DA and VIP > 1 were considered to contribute to the discrimination. Multivariate statistical analysis was performed using the mixOmics package [38]. Maximum intensity values were unit-variance scaled prior to multivariate statistical analysis, and missing values were zero-imputed. Additionally, linear models were used to assess the influence of physiological state, migration, and sex on differences in metabolites with VIP > 1. We considered metabolites with VIP > 1 as most discriminative and p values ≤ 0.05 as statistically significant.

3 | Results

3.1 | Profiling of Whole Blood Metabolome by HILIC LC–MS/MS

First, we explored the polar metabolome of whole blood samples collected from Nathusius' pipistrelles and analyzed by HILIC LC–MS/MS in positive ESI(+) with $n = 87$ and negative ESI(−) ionization mode with $n = 84$. The samples originate from two studies, collected under controlled ($n = 40$) and field conditions ($n = 47$) (Table 1). In ESI(+), we detected 2465 features with 1153 MS/MS spectra. In ESI(−), we detected 1533 features with 816 MS/MS spectra that were present in at least 10% of the data (Figure 2A). Around 25% of features were shared among all bats, and around 37% of features were identified by matching MS/MS against spectral libraries and usage of in silico tools. For further analysis, we categorized metabolites into major classes

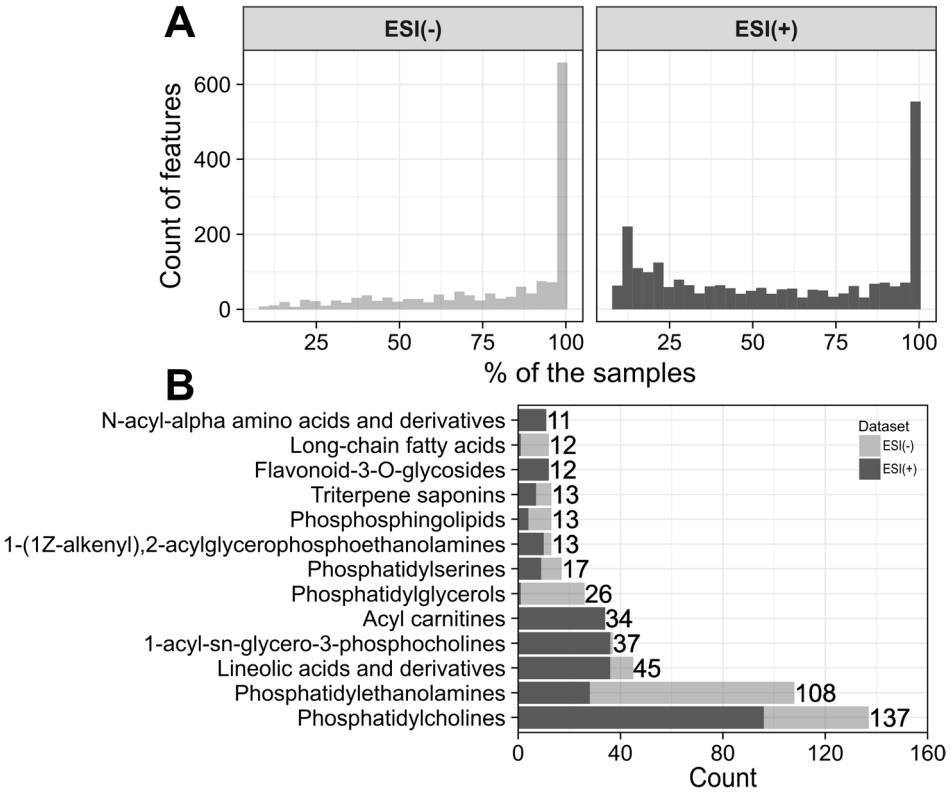

FIGURE 2 | Global overview of whole blood metabolome of Nathusius' pipistrelles. (A) Histogram of metabolite features showing their distribution and number present in at least 10% of whole blood samples collected from Nathusius' pipistrelles, analyzed with HILIC LC–MS/MS in negative and positive electrospray ionization mode (ESI(−/+)), with $n = 84$ for ESI(−) and $n = 87$ for ESI(+). (B) Diversity and abundance of metabolite classes detected in whole blood samples, whereby metabolite features were identified and categorized by the in silico tool MS2Query and only classes with scores > 0.5 and total count > 10 were visualized.

(Figure 2B). MS/MS spectra of ESI(−/+) were mostly identified as lipids with polar headgroups, such as phosphatidylcholines ($n = 137$), phosphatidylethanolamines ($n = 108$), linoleic acids and derivatives ($n = 45$), 1-acyl-sn-glycero-3-phosphocholines ($n = 37$), acyl carnitines ($n = 34$), phosphatidylglycerols ($n = 26$), and phosphatidylserines ($n = 17$) (Figure 2B).

3.2 | Whole Blood Metabolome of Captive Bats Under Controlled Conditions

To understand the effect of exercise on the whole blood metabolome of bats, we collected blood samples from bats ($n = 40$) either immediately post-exercise ($n = 16$) or after 1 h of recovery ($n = 11$) from flight in a wind tunnel (controlled conditions), compared to a fasted resting state ($n = 13$). A PCA accounted for approximately 32% of the total variance in metabolites, but without any exercise-related clustering (Figure S1A,B). Supervised PLS-DA analyses revealed some clear clustering associated with exercise versus rest (Figure 3A,B). We observed that 12% of the total variance was explained in the first component of the PLS-DA (Figure 3A,B). Samples collected from resting bats could be distinguished from samples collected from bats after flight (including samples collected immediately post-flight and after 1 h of recovery) based on significant area under the curve (AUC)-values of the PLS-DA model (Table S4). In the second component of the PLS-DA, we also found significant AUC values for samples collected from bats after 1 h of recovery, compared to other groups. In total, 1065 features in ESI(+) and 446 features in ESI(−) had variables important for the projection (VIP) values > 1 for the first component of the PLS-DA. Most of the features were elevated immediately post-exercise in ESI(−/+), compared to resting and recovery phase (Figure 3C). Phosphatidylethanolamines (PE; $n = 45$), phosphatidylcholines (PC; $n = 42$), and lineolic acid and derivatives ($n = 14$) showed varied responses to exercise, with the majority increasing but some also decreasing (Figure 3C). All acyl carnitines (CAR; $n = 27$) increased immediately post-exercise and many remained elevated during recovery, for example CAR 12:0, but dropped significantly compared to post-exercise, without reaching completely baseline levels (Figure 3D). Lysophosphatidylcholine (LPC O-13:0) was increased at exercise and remained elevated during recovery, while PE 36:3 (PE 18:1_18:2) showed the opposite pattern, decreased with exercise and did not recover 1 h after flight (Figure 3D).

We used linear models to test whether exercise and sex explained some variation in the metabolite data. Most of the metabolites were only influenced by flight ($n = 685$), with less metabolites affected by sex, mostly observed in ESI(+) ($n = 33$) (Figure 4A). We found that the short flights of bats in the wind tunnel were associated with high counts of phosphatidylethanolamines ($n = 36$), phosphatidylcholines ($n = 32$), acyl carnitines ($n = 24$), and N-acyl-alpha amino acids and derivatives ($n = 9$), mainly increased directly after flight compared to the resting state

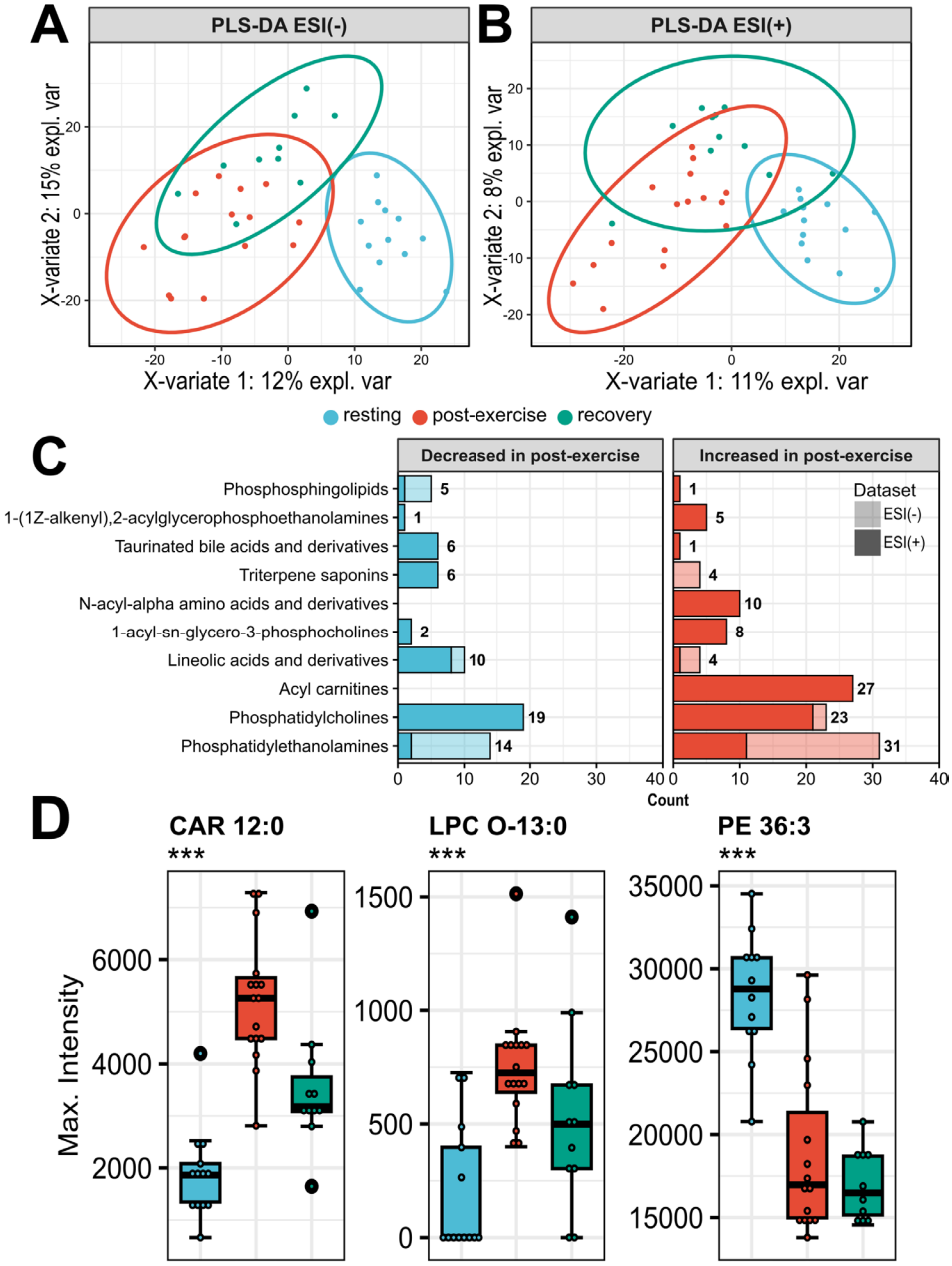

FIGURE 3 | Blood metabolome profiles, classes and individual metabolites of Nathusius' pipistrelles in response to flights under controlled conditions. (A, B) Partial least squares-discriminant analysis scores (PLS-DA) score plots visualizing metabolite data derived from negative and positive electrospray ionization mode ESI(−/+) of whole blood samples, collected from bats under controlled conditions, at resting, post-exercise and recovery. (C) Bar plot illustrates the number of metabolite classes affected by flights under controlled conditions, analyzed in ESI(−/+), including only features with variables important for the project (VIP) > 1 and counts > 5. (D) Boxplots of CAR 12:0, LPC O-13:0, and PE 36:3 displaying their maximum intensities based on physiological state (colors as for panels A and B). Significance was calculated using linear models for metabolites with VIP > 1: $p < 0.05$ (*); $p < 0.01$ (**); $p < 0.001$ (***). Color coding and number of samples ($n = 40$): resting (light blue): $n = 13$, post-exercise (red): $n = 16$, and recovery (green): $n = 11$. CAR, acyl carnitine; expl. var., explained variance; LPC, lysophosphatidylcholine; PE, phosphatidylethanolamine.

(Figure 4B). In many cases, the effects of exercise continued to be evident after 1 h of recovery. For example, the fatty acid FA 18:3 called linolenic acid, PE 37:5 (PE 20:4_17:1; 20:5_17:0), and PE O-36:6 (PE P-16:0/20:5) were similarly elevated immediately post-exercise and after 1 h of recovery when compared with the resting state (Figure 4C). Two medium-chain acyl carnitines, CAR 10:0 and CAR 11:1, showed a slightly different pattern of elevation, declining after recovery but not returning to a resting level (Figure 4C). Whereas choline, a precursor of phosphatidylcholines, was lower in blood samples collected immediately after post-exercise and returned to a resting level after recovery (Figure 4C). We also found several other short-, medium-, and long-chain acyl carnitines affected by exercise (Table S5). Interestingly, four metabolites from the class of N-acyl taurines were elevated immediately after flight and returned to resting levels after 1 h of recovery (Figure S2A). Furthermore, two phosphatidylcholines (PC 34:1 [PC 16:0_18:1] and PC-DAG 34:5 [PC-DAG 16:0_18:5]) showed elevated intensities in both post-flight groups compared to rest, while two other phosphatidylcholines (PC 34:0 [PC 16:0_18:0] and PC 36:2 [PC 18:0_18:2]) showed the

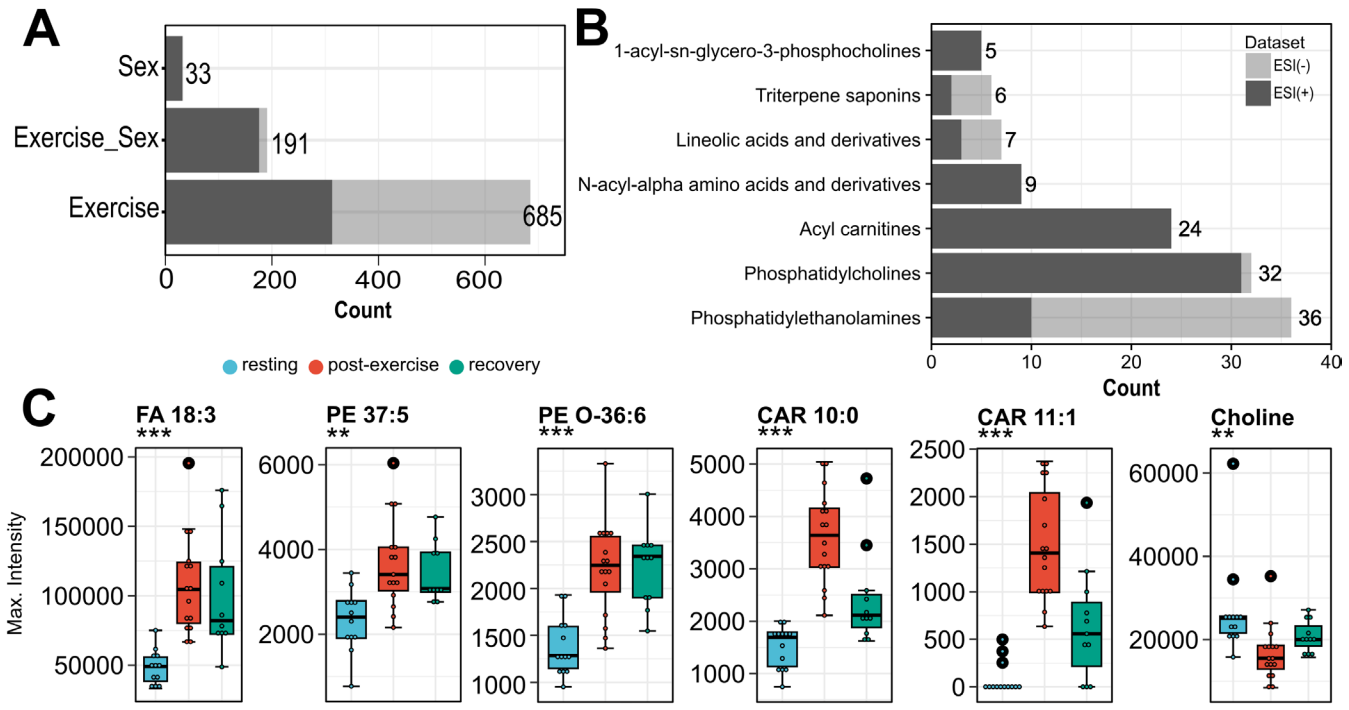

FIGURE 4 | Exercise- or sex-driven response of blood metabolite classes and metabolites of Nathusius' pipistrelles under controlled conditions. (A) Bar plots representing the count of metabolites significantly altered by one or two conditions in negative and positive electrospray ionization mode ESI(−/+). (B) Distribution of metabolite classes which were significantly altered by exercise under controlled conditions in ESI(−/+) with counts > 5. (C) Boxplots of one fatty acid FA 18:3, two phosphatidylethanolamines (PE 37:5 and PE O-36:6), two acyl carnitines (CAR 10:0 and CAR 11:1) and choline, displaying their maximum intensities as a function of physiological state. Significance was calculated using linear models accounting for exercise and sex for metabolites with VIP > 1: $p < 0.05$ (*); $p < 0.01$ (**); $p < 0.001$ (***). Color coding and number of samples ($n = 40$): resting (light blue): $n = 13$, post-exercise (red): $n = 16$, and recovery (green): $n = 11$. CAR, acyl carnitine; FA, fatty acid; PE, phosphatidylethanolamine.

opposite pattern (Figure S2B). Five amino acids out of 18 were significantly altered under controlled flying conditions, shown in Figure S3A–C.

Alanine (Ala) was increased directly after flight (post-exercise) compared to recovery and resting values. Glutamic acid (Glu) and glycine (Gly) were significantly reduced in the recovery group compared to post-exercise levels, whereas valine (Val) was reduced in bats directly after flights (post-exercise) compared to resting values (Figure S3A). Tyrosine (Tyr) was significantly increased in bats immediately after post-exercise and returned to resting levels after recovery (Figure S3B).

3.3 | Whole Blood Metabolome in Free-Ranging Bats Across Seasons

Principal component analysis (PCA) for ESI(−/+) revealed a high variability and no specific clustering of samples or metabolites according to season (premigration vs. migration) or physiological state (post-exercise vs. recovery; Figure S4A,B). We visualized the first two components of metabolite data obtained from migrating bats ($n = 47$), which explained around 23% of the total variance for ESI(−/+). Partial least square discriminant analysis (PLS-DA) model separated visually the two seasons in the first dimension, with around 11% of the total variance explained (Figure 5A,B). Physiological state emerged as a discriminating factor in the second dimension of the PLS-DA, covering 7% of the total variance. AUC values and p values were calculated for the first and second component of the PLS-DA and revealed significant differences for all four comparisons with all AUC values above 0.9, while three reached significance already in the first component (Table S4). To identify those metabolites that discriminate between groups in pairwise comparisons (one group vs. others) in the PLS-DA score plots, we extracted the VIP values of the first and second dimension and defined features with VIP values > 1 as most promising candidates. In the first dimension of the PLS-DA, 942 features in ESI(+) and 536 features in ESI(−) distinguished at least one group from the others, while in the second dimension, 1065 features in ESI(+) and 588 features in ESI(−) distinguished one group from the others. A substantial number of phosphatidylcholines, phosphatidylethanolamines, and acyl carnitines were affected by season and physiological state (Figure 5C). Most of the classes were increased during migration season and in recovery state with phosphatidylcholines ($n = 141$), phosphatidylethanolamines ($n = 68$), and acyl carnitines ($n = 9$) (Figure 5C,V). Lower counts of classes were observed for phosphatidylcholines ($n = 45$) and phosphatidylethanolamines ($n = 60$), which were increased during migration season and post-exercise (Figure 5C, II). Some of the phosphatidylethanolamines ($n = 72$) were only affected by migration (Figure 5C, VII + VIII). For example, PE 38:7 (PE 20:4_18:3; 20:5_18:2), PC 35:1 (PC 17:0_18:1), and CAR 20:4 increased in blood in bats during the migratory season compared to prior migratory season and only CAR 16:4 was significantly different due to physiological state (Figure 5D).

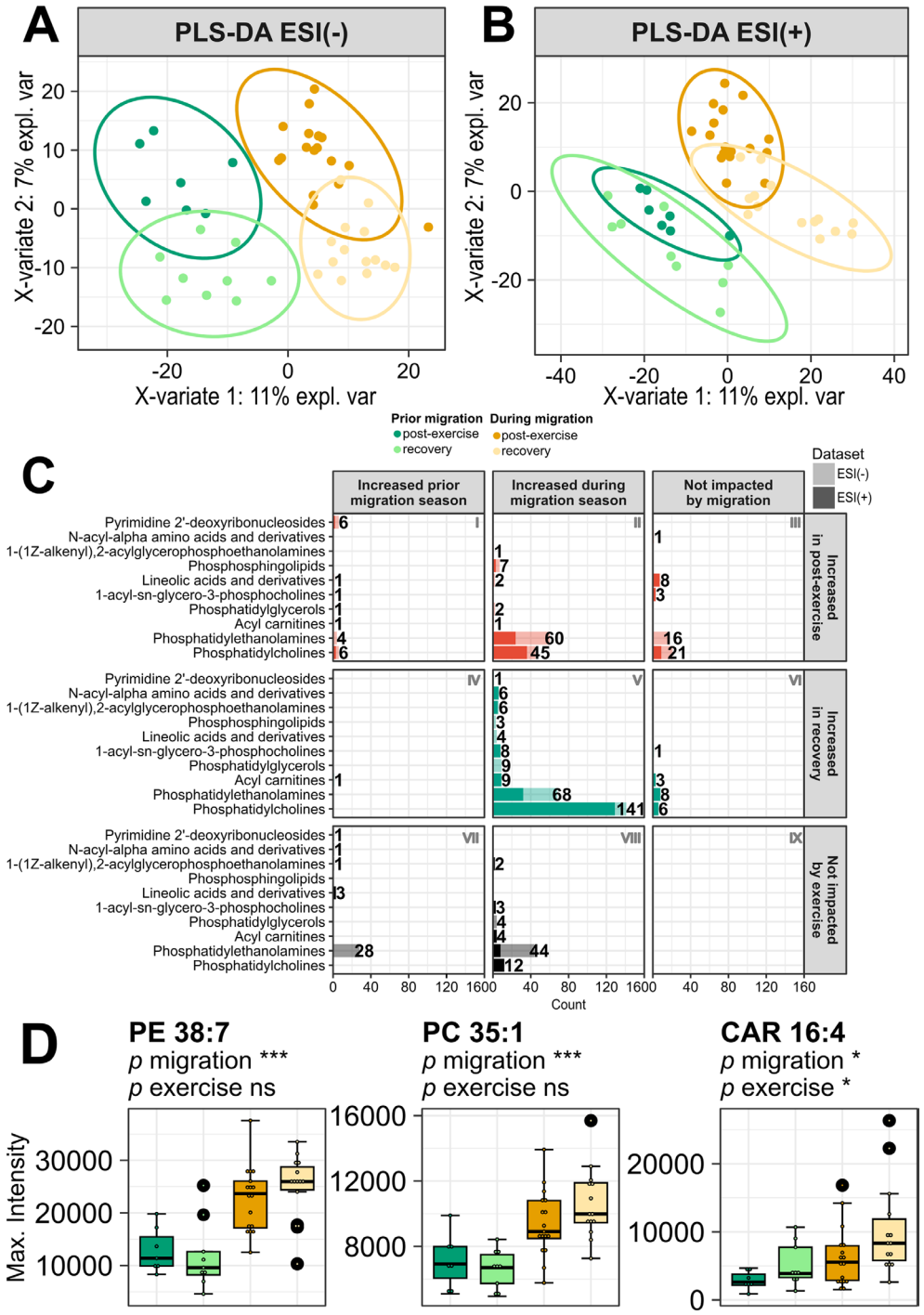

FIGURE 5 | Blood metabolome profiles, classes and selected metabolites collected from free-ranging Nathusius' pipistrelles prior to and during the migratory season and two physiological states. (A, B) Partial least squares-discriminant analysis (PLS-DA) scores plot visualizing metabolite data derived from negative and positive electrospray ionization mode ESI(−/+) of whole blood samples analyzed from free-ranging bats under field conditions collected from two seasons. Samples were taken immediately post-exercise or after 1 h of rest as recovery in both seasons. (C) Bar plots illustrating metabolite classes affected by season and physiological state in ESI(−/+), specifically metabolite features with variables important for the projection (VIP) > 1 and counts > 5. (D) Boxplots of two phospholipids (PE 38:7 and PC 35:1) and one long-chain acyl carnitine (CAR 20:4), displaying their maximum intensities, divided into four groups by season and physiological state. Significance was calculated using linear models accounting for migration, exercise and sex for metabolites with VIP > 1: $p < 0.05$ (*); $p < 0.01$ (**); $p < 0.001$ (***). Color coding and number of samples ($n = 47$): dark green = prior migration post-exercise ($n = 7$), light green = prior migration recovery ($n = 9$), orange = during migration post-exercise ($n = 17$), yellow = during migration recovery ($n = 14$). CAR, acyl carnitine; expl. var., explained variance; PC, phosphatidylcholine; PE, phosphatidylethanolamine.

Since PLS-DA cannot discriminate whether metabolites are altered by multiple biological factors, we applied linear models to all features with VIP > 1 from PLS-DA using season, physiological state, and sex as potentially relevant factors. We observed that most of the metabolites were influenced by season ($n = 677$) and by physiological state ($n = 137$), with only

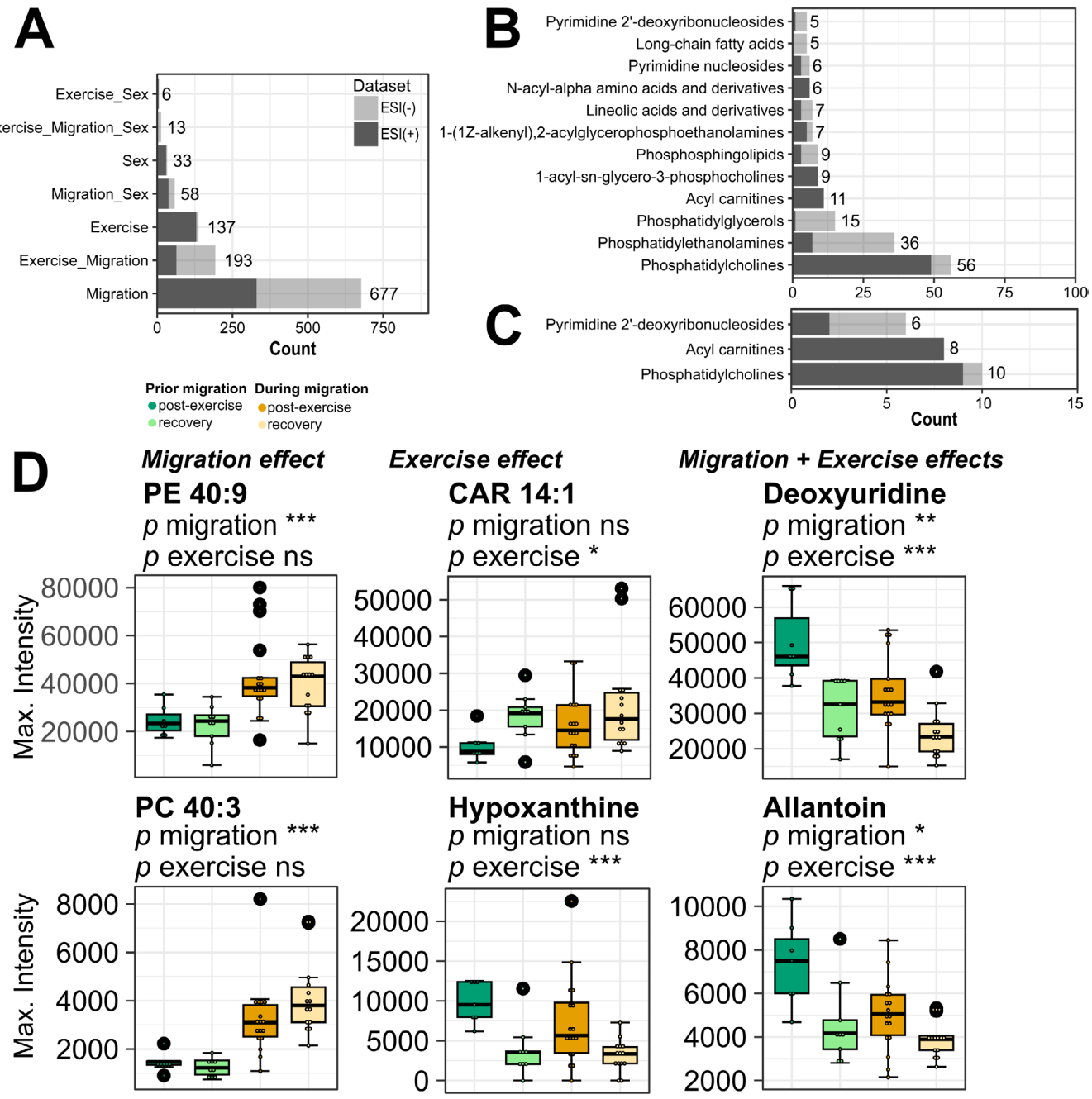

FIGURE 6 | Migration-, exercise-, or sex- driven response of blood metabolite classes and metabolites of Nathusius' pipistrelles under field conditions. (A) Bar plot visualizing the number of significant metabolite features influenced by season, physiological state or sex or by their interactions. (B) Distribution of metabolite classes significantly altered through migration for negative and positive ionization mode ESI(−/+). (C) Distribution of metabolite classes significantly altered through physiological state for negative and positive ionization mode ESI(−/+). (D) Boxplots of six metabolites, displaying the effect of migration (PE 40:9 and PC 40:3), exercise (CAR 14:1 and hypoxanthine), or migration + exercise (deoxyuridine and allantoin). Significance was calculated using linear models accounting for migration, exercise, and sex for metabolites with VIP > 1: $p < 0.05$ (*); $p < 0.01$ (**); $p < 0.001$ (***). Color coding and number of samples ($n = 47$): dark green = prior migration post-exercise ($n = 7$), light green = prior migration recovery ($n = 9$), orange = during migration post-exercise ($n = 17$), yellow = during migration recovery ($n = 14$). CAR, acyl carnitine; PC, phosphatidylcholine; PE, phosphatidylethanolamine.

a few ($n = 33$) being affected by sex (Figure 6A). Exercise and sex-specific differences were most pronounced in ESI(+). A total of 257 features were influenced by two factors in both ionization modes and a small number of features by all three factors ($n = 13$). As outlined by PLS-DA, linear models revealed the most significant differences were related to season. We observed the highest count of features for phosphatidylcholines ($n = 56$) and phosphatidylethanolamines ($n = 36$), which support the results of our multivariate statistics (Figure 6B). Physiological state-related metabolites (post-exercise and recovery) were extracted from the linear model, with 10 phosphatidylcholines summarizing that this class is significantly associated with both season and physiological state of Nathusius' pipistrelles, whereas phosphatidylethanolamines varied only with season (Figure 6C). We

found two further classes such as acyl carnitines ($n = 8$) and pyrimidine 2′-deoxyribonucleosides ($n = 6$) that were significantly influenced by exercise across seasons (Figure 6C). Phospholipids such as PE 40:9 (PE 20:4_20:5) and PC 40:3 (PC 18:0_22:3) were significantly affected by migration and increased during migration season (Figure 6D, "Migration effect"). CAR 14:1 and Hypoxanthine showed a significant exercise effect (Figure 6D, "Exercise effect"). Deoxyuridine and allantoin were both reduced during recovery from exercise compared to immediately post-exercise, regardless of season, but were increased prior to migration (Figure 6D, "Migration + Exercise effects").

Out of 18 amino acids, 10 (Ala, Gln, His, Pro, Phe, Thr, Trp, Tyr, Leu/Ile, and Lys) were influenced by physiological state alone, independent of season (Figure S3A–C). They were only significantly changed due to physiological state, with higher levels immediately post-exercise compared to the recovery phase (Figure S3A–C). We also observed a strong tendency toward higher amino acid levels in non-migratory bats post-exercise compared to the recovery state. Four amino acids (Asn, Gly, Met, and Val) were significantly influenced by both season and physiological state and were lower during recovery from exercise (Figure S3A). Arginine (Arg) and Serine (Ser) were altered by migration but remained unaffected by the physiological state, where Arg was reduced prior and Ser increased prior to migration (Figure S3A).

4 | Discussion

Flying vertebrates possess a number of physiological adaptations that enable them to undertake high-intensity exercise during migration, sometimes even over long distances. While it is clear that migratory birds fuel endurance flights mostly with fatty acids [2, 3, 13], only a handful of studies have been conducted about the oxidative fuel used by migratory bats [17, 18]. To our knowledge, no studies have applied metabolomics to compare post-exercise individuals between the migration and non-migration seasons, or in wind tunnel settings under controlled conditions. Metabolomic studies performed on biological samples of wild mammals are still rare, despite significant progress in metabolomics research on humans and laboratory mammals [39]. Recently, there has been a growing interest in untargeted metabolomics to study avian metabolism during migration, capturing a broader picture of metabolism rather than focusing on bulk lipids and their fatty acid composition or compounds involved in energy metabolism [40–42].

In general, bats flying under controlled conditions respond with comparable counts of phosphatidylethanolamines (PEs) and phosphatidylcholines (PCs). These responses may appear as increases and decreases after exercise compared to the resting state. Most metabolites were altered immediately after exercise and did not return to the level of resting individuals, even after 1 h of recovery. Although lipids are major components of the human exercise metabolome [43], their role as an oxidative fuel remains uncertain. Acyl carnitines, such as CAR 12:0, CAR 10:0, and CAR 11:1, showed a clear pattern in bats, with all of them increasing during exercise and significantly decreasing during recovery. However, they did not reach baseline levels after 1 h of rest. The observed increase of acyl carnitines induced by exercise and their subsequent recovery are highly important, as these metabolites play a pivotal role in the transport and β-oxidation of fatty acids within mitochondria (carnitine shuttle). First, fatty acids are activated to acyl-CoAs by acyl-CoA synthetases. Then, they are converted to acyl carnitines by carnitine palmitoyltransferase I on the outer mitochondrial membrane. Acyl carnitines are subsequently transported into the mitochondrial matrix by carnitine-acylcarnitine translocase, where they are reconverted to acyl-CoAs by carnitine palmitoyltransferase II for entry into the β-oxidation pathway, ultimately generating ATP [44, 45]. Therefore, the transient increase of acyl carnitines during exercise is a strong indicator of active fatty acid transport and oxidation. Our findings align well with several previous studies in exercising laboratory mammals. For instance, studies on rats have demonstrated an increase in acyl carnitines in plasma after 45 min of running [46]. Specifically, short-chain (C2–C5), medium-chain (C6–C12), and long-chain acyl carnitines (C13–C20) increased, reflecting an enhanced import of fatty acids into muscle tissue to meet energetic demands [46]. Ezagouri et al. observed around 300 muscle metabolites altered by moderate-intensity exercise executed in mice. These included several phospholipids such as PCs, PEs, and phosphatidylglycerols (PGs), which decreased after exercise [47]. Of the 38 acyl carnitines examined, 17 were found to be dependent on both exercise and time. Many of these increased after exercise, including CAR 16:0, CAR 10:0, CAR 8:0, and CAR 6:0. This suggests incomplete fatty acid oxidation and mitochondrial overload in muscles. Humans performing moderately intense exercise increased their acyl carnitine levels immediately after running, which then dropped back to baseline levels 3 h post-run [48], which is consistent with our results. A recent multi-omics study in humans identified a substantial quantity of complex lipids, including cholesteryl esters, PCs, diacylglycerols, ceramides, and sphingomyelins, which increased directly after acute exercise [49].

Although there is limited direct evidence from non-model organisms focusing specifically on acyl carnitine dynamics during exercise, several metabolomics and mechanistic studies in model organisms and humans consistently reinforce the critical role of acyl carnitines as indicators of fatty acid flux and mitochondrial function, and even as potential signaling molecules, during physiological exertion [50]. While the carnitine shuttle is known to mediate fatty acid transport into mitochondria in birds [51], we found no previous studies that measured acyl carnitines and their potential changes in birds or bats following short-duration flights under trained conditions. Studies solely showed that carnitine palmitoyltransferase (CPT) activity did not change significantly with short-term flight or cold training in house sparrows, but both maximal exercise and thermogenic metabolic rates were positively correlated with CPT activity [52]. Some avian studies measure acyl carnitines in blood, for example, in Quaker parrots [53], or in gray and blue tits [54], and this existing data support the feasibility of measuring this metabolite class in birds and bats. Another important but rarely reported class of metabolites is the N-acyl taurines (NATs) [55], which were significantly altered in whole blood of flying bats and showed similar patterns to acyl carnitines, and in our study, we could identify four long-chain NATs. A past study in humans showed that short-chain forms of carnitine and taurine are increased significantly after full marathon, with no impact on

free carnitine and taurine [56]. In our controlled experiments, post-flight bats showed the same response with increased levels of short- and long-acyl carnitines and NATs but not their precursors. Long-chain NATs regulate metabolism, modulate TRP channels, support mitochondrial energy use, reduce inflammation, and contribute to endocannabinoid-related signaling [57, 58]. However, their role in non-model mammals remains largely unexplored.

We also present evidence of increased levels of metabolites associated with lipid metabolism in free-ranging Nathusius' pipistrelles. In our comparison of whole blood profiles of migrating and non-migrating bats, we identified several lipid classes that differed between the two seasons, including PCs, PEs, PGs, and acyl carnitines. This finding challenges the widely held notion that mammals have limited capacity to transport and oxidize fatty acids during exercise [19, 20]. Our results also suggest that the levels of these lipid classes are influenced by physiological state and sex, however to a much lesser degree than season. We observed that PEs consisting of unsaturated fatty acids (e.g., PE 38:7, PE 37:4, and PE 37:5), changed significantly during migration whereas those with saturated fatty acids did not. As mammals, bats are incapable of synthesizing polyunsaturated fatty acids de novo, and must therefore acquire these fatty acids from their diet in order to build this kind of PEs [59]. Nathusius' pipistrelles have been shown to consume more insect species inhabiting aquatic environments, such as *Cyphon phragmiteticola* and *Agonum piceum*, during the migration season, whereas bats from summer colonies fed predominantly on forest-associated species like *Bupalus pinaria* and *Promethes sulcator* [16]. Aquatic and riparian insects are typically richer in long-chain polyunsaturated fatty acids, which could contribute to the higher abundance of unsaturated PEs detected in migratory bats [60]. These findings indicate that seasonal dietary shifts may influence the lipid composition of bat blood, providing a dietary route for metabolic adaptation during migration. Some evidence suggests that migratory birds benefit from dietary polyunsaturated fatty acids, which improve exercise by increasing the capacity for oxidative metabolism [61]. Polyunsaturated fatty acids (n-3) also act as ligands for peroxisome proliferator activator receptors, thereby upregulating genes involved in fatty acid transport, β-oxidation, and mitochondrial biogenesis. Alternatively, they are incorporated into flight muscle membranes, thereby altering the membrane fluidity and the activity of membrane-bound proteins in cellular and organelle membranes such as those in mitochondria. Different bird studies show that phospholipid composition changes with physiological demands, but the direction of change in phospholipids is not always the same [41, 62, 63]. A more recent comparison of chickadees and goldfinches also demonstrated seasonal remodeling: both species increased PC(18:0/20:4) in winter, and only the non-migratory chickadees maintained higher polyunsaturated fatty acids into the cold season [41]. These studies highlight that diet, season, and metabolic strategy shape phospholipid profiles differently across species, and similar factors may explain the phospholipid patterns we see in bats.

As stated above, migration impacts phospholipid metabolism, resulting in increased PC, PEs, and lysoPCs. There were no changes in choline, a PC precursor, and a decrease in serine, but no change in phosphatidylserines (PS), lysoPS, and lysoPEs. The enhanced Kennedy pathway provides complex lipids (PCs and PEs) for membrane synthesis or remodeling. However, there is a preference for unsaturated PEs with a nonselective bulk increase in PC [64]. We hypothesize that fatty acid remodeling, also known as the Lands cycle, occurs specifically in PC/lysoPC but not in PE/lysoPE or PS/lysoPS via lysophosphatidylcholine acyltransferase and Acyl-CoA:lysoPL acyltransferases and phospholipase A2 [65]. In our study, choline remained constant across seasons, indicating that there are mechanisms involved in maintaining or replenishing choline levels. However, serine can flow into other pathways such as sphingolipid biosynthesis or one-carbon metabolism without contributing to PS levels. This pattern indicates a strategic metabolic adjustment that supports the energetic and structural demands of migration. It is also noteworthy that endurance-trained human athletes typically have higher levels of muscle PCs and PEs at baseline than resting or metabolically compromised individuals, with levels decreasing at recovery 2 h post-exercise [66].

Regarding amino acid metabolism, we observed no clear seasonal impact on overall levels of amino acids. However, the levels of several amino acids increased immediately after flight under field conditions compared to recovery levels. This increase in circulating amino acids may be due to protein degradation in active muscles providing substrates for the tricarboxylic acid cycle, or to gluconeogenesis, particularly if carbohydrate stores are limited during sustained flight [20, 67]. Interestingly, this exercise-driven increase in amino acids could not be replicated under controlled wind tunnel conditions. This highlights the potential for differences in metabolic demands or substrate utilization between simulated and natural flight environments. Alanine, a key glycogenic amino acid [67], increased during exercise both before and after migration; however, its baseline level was unaffected by the migratory event itself. Alanine serves as a crucial precursor for gluconeogenesis and is readily converted to pyruvate and glutamate via transamination, thus linking protein catabolism to glucose production [67]. Tyrosine was another amino acid that was influenced in bats in both field and wind tunnel conditions, showing a higher response immediately after flight under field conditions. This could reflect its role in neurotransmitter synthesis or general protein turnover during periods of high physiological stress. Our previous work shows that migration in *Pipistrellus nathusii* imposes acute oxidative stress, as flying individuals exhibit higher oxidative damage and altered antioxidant responses compared to resting bats, with recovery occurring during rest periods [68]. Our data does not inform on the key indicator of intracellular oxidative stress, which is the ratio of reduced to oxidized glutathione [69], because only oxidized glutathione (GSSG) was detected, while reduced glutathione (GSH) was not. However, GSSG did not show significant changes under either controlled or field conditions and GSSG alone is not biologically meaningful to rule out oxidative stress. Although we found that similar metabolite classes were altered by exercise in both laboratory and field conditions, our results were inconsistent when the two conditions were compared. This was most obvious for acyl carnitines. While being significant in both studies, under controlled conditions we observed increased levels immediately post-flight compared to the recovery group. The opposite was true for migratory bats under field conditions where acyl carnitine levels increased in the recovery group compared to post-flight. Unexpectedly, we did not observe a strong

impact on amino acids under controlled conditions. This is most likely due to the difference in the diets and behaviors of the bats in the two studies. In captivity, the bats were fasted for at least 16 h prior to flight and collection of blood, and this could not be controlled for, or verified in wild bats. In addition, captive bats were fed a stable diet of mealworms and given enough food to ensure that they always remained above their capture weight. In the wild, however, bats have the opportunity to forage selectively based on their nutrient requirements. However, they also often face energy deficits due to the high costs of foraging. Consequently, their fuel storage is limited. The ability to enter torpor is an essential mechanism that enables Nathusius' pipistrelles to balance their energy requirements and conserve energy by reducing their metabolic rate while at rest. In captivity, our bats entered torpor for extended periods between experimental flights (Shannon E. Currie; personal observation), potentially for longer than bats in the wild. This likely impacted their fuel storage for flights in the wind tunnel. It is also possible that the observed differences were a function of overall flight time, given that metabolite profiles are known to change with exercise duration in other mammals [46, 70], and we could not control for the duration of flight activity of bats prior to capture in field settings.

5 | Conclusions

Unlike birds, which obtain energy for long-distance migrations through the oxidation of fatty acids, it was previously assumed that mammals had limited endurance capacity due to a lack of the necessary enzymes. We hypothesized that migratory bats had evolved convergently to migratory birds in terms of their metabolic properties and thus derive the energy for their high flight metabolic rate from the oxidation of fatty acids, unlike non-flying mammals. Our observation revealed that fatty acid oxidation plays a significant role in providing energy for flight in Nathusius' pipistrelles. In particular, we demonstrated increased levels of lipids, such as phosphatidylethanolamines containing unsaturated fatty acids in migrating bats. Several acyl carnitines were elevated in the blood of captive bats flying in a wind tunnel. This suggests that fatty acid transport and β-oxidation are important during short flights, as they deliver bound fatty acids to mitochondria. Metabolites of pyrimidine metabolism were impaired, and the glycogenic amino acid alanine was depleted in bats after flight, suggesting some form of protein depletion. Our findings provide valuable information about the physiological adaptations of an endurance exercising mammal, the long-distance migrant *Pipistrellus nathusii*. This species could serve as a future mammal model to elucidate exercise-related adaptations in the enzymes involved in oxidizing fatty acids.

Author Contributions

Christian C. Voigt and Shannon E. Currie conceived the original study idea and designed the study. Alesia Walker, Thuy Thanh Truong, and Philippe Schmitt-Kopplin managed and performed metabolomics analysis. Alesia Walker and Thuy Thanh Truong analyzed and visualized metabolomics data. Martin Klingenspor helped interpret the data, advised students, and provided scientific input on the paper. Anders Hedenström and Gunars Pētersons provided resources for the study, advised students, and provided scientific input on the paper. Alesia Walker, Shannon E. Currie, and Christian C. Voigt interpreted and drafted the original manuscript. All authors were involved in revising the manuscript.

Acknowledgments

We thank the station team at Pape for their support. Open Access funding enabled and organized by Projekt DEAL.

Funding

Funding for this research was provided by the SAW Leibniz Association (grant no. K101/2018).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Processed metabolomics data and sample description are provided in tables of Supporting Information and mzML files are available in MassIVE under MSV000100891 for ESI(−) and under MSV000100893 for ESI(+). This study is available at the NIH Common Fund's National Metabolomics Data Repository (NMDR) website, the Metabolomics Workbench [71], https://www.metabolomicsworkbench.org where it has been assigned Study ID ST004721. The data can be accessed directly via its Project DOI: https://doi.org/10.21228/M8SS1B. This work is supported by NIH grant U2C-DK119886 and OT2-OD030544 grants.

References

1. T. Alerstam, A. Hedenström, and S. Åkesson, "Long-Distance Migration: Evolution and Determinants," *Oikos* 103, no. 2 (2003): 247–260, https://doi.org/10.1034/j.1600-0706.2003.12559.x.

2. L. Jenni and S. Jenni-Eiermann, "Fuel Supply and Metabolic Constraints in Migrating Birds," *Journal of Avian Biology* 29, no. 4 (1998): 521–528, https://doi.org/10.2307/3677171.

3. S. R. McWilliams, C. Guglielmo, B. Pierce, and M. Klaassen, "Flying, Fasting, and Feeding in Birds During Migration: A Nutritional and Physiological Ecology Perspective," *Journal of Avian Biology* 35, no. 5 (2004): 377–393, https://doi.org/10.1111/j.0908-8857.2004.03378.x.

4. P. F. Battley, N. Warnock, T. L. Tibbitts, et al., "Contrasting Extreme Long-Distance Migration Patterns in Bar-Tailed Godwits *Limosa lapponica*," *Journal of Avian Biology* 43, no. 1 (2012): 21–32, https://doi.org/10.1111/j.1600-048X.2011.05473.x.

5. J. Ouwehand and C. Both, "Alternate Non-Stop Migration Strategies of Pied Flycatchers to Cross the Sahara Desert," *Biology Letters* 12, no. 4 (2016): 20151060, https://doi.org/10.1098/rsbl.2015.1060.

6. E. Hurme, I. Lenzi, M. Wikelski, T. A. Wild, and D. K. N. Dechmann, "Bats Surf Storm Fronts During Spring Migration," *Science* 387, no. 6729 (2025): 97–102, https://doi.org/10.1126/science.ade7441.

7. H. V. Richter and G. S. Cumming, "First Application of Satellite Telemetry to Track African Straw-Coloured Fruit Bat Migration," *Journal of Zoology* 275, no. 2 (2008): 172–176, https://doi.org/10.1111/j.1469-7998.2008.00425.x.

8. D. A. Vasenkov, N. S. Vasiliev, N. V. Sidorchuk, and V. V. Rozhnov, "Autumn Migration of Greater Noctule Bat (*Nyctalus lasiopterus*): Through Countries and Over Mountains to a New Migration Flight Record in Bats," *Doklady Biological Sciences* 513, no. 1 (2023): 395–399, https://doi.org/10.1134/S0012496623700746.

9. J. T. Alcalde, M. Jiménez, I. Brila, V. Vintulis, C. C. Voigt, and G. Pētersons, "Transcontinental 2200 km Migration of a Nathusius'

Pipistrelle (*Pipistrellus nathusii*) Across Europe," *Mammalia* 85, no. 2 (2021): 161–163, https://doi.org/10.1515/mammalia-2020-0069.

10. G. Pētersons, "Seasonal Migrations of North-Eastern Populations of Nathusius' Bat *Pipistrellus nathusii* (Chiroptera)," *Myotis* 41 (2004): 29–56.

11. C. C. Voigt, S. E. Currie, and L. P. McGuire, "Chapter 11—Bat Migration and Foraging: Energy-Demanding Journeys on Tight Budgets," in *A Natural History of Bat Foraging*, ed. D. Russo and B. Fenton (Academic Press, 2024), 199–215, https://doi.org/10.1016/B978-0-323-91820-6.00006-1.

12. T. J. Weller, K. T. Castle, F. Liechti, C. D. Hein, M. R. Schirmacher, and P. M. Cryan, "First Direct Evidence of Long-Distance Seasonal Movements and Hibernation in a Migratory Bat," *Scientific Reports* 6, no. 1 (2016): 34585, https://doi.org/10.1038/srep34585.

13. L. Z. Gannes, "Comparative Fuel Use of Migrating Passerines: Effects of Fat Stores, Migration Distance, and Diet," *Auk* 118, no. 3 (2001): 665–677, https://doi.org/10.2307/4089928.

14. C. G. Guglielmo, "Obese Super Athletes: Fat-Fueled Migration in Birds and Bats," *Journal of Experimental Biology* 221, no. S1 (2018): jeb165753, https://doi.org/10.1242/jeb.165753.

15. C. C. Voigt, K. Sörgel, and D. K. N. Dechmann, "Refueling While Flying: Foraging Bats Combust Food Rapidly and Directly to Power Flight," *Ecology* 91, no. 10 (2010): 2908–2917, https://doi.org/10.1890/09-2232.1.

16. F. Krüger, E. L. Clare, W. O. C. Symondson, O. Keišs, and G. Pētersons, "Diet of the Insectivorous Bat *Pipistrellus nathusii* During Autumn Migration and Summer Residence," *Molecular Ecology* 23, no. 15 (2014): 3672–3683, https://doi.org/10.1111/mec.12547.

17. L. P. McGuire, M. B. Fenton, and C. G. Guglielmo, "Seasonal Upregulation of Catabolic Enzymes and Fatty Acid Transporters in the Flight Muscle of Migrating Hoary Bats, *Lasiurus cinereus*," *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 165, no. 2 (2013): 138–143, https://doi.org/10.1016/j.cbpb.2013.03.013.

18. C. C. Voigt, K. Sörgel, J. Šuba, O. Keišs, and G. Pētersons, "The Insectivorous Bat *Pipistrellus nathusii* Uses a Mixed-Fuel Strategy to Power Autumn Migration," *Proceedings of the Royal Society B: Biological Sciences* 279, no. 1743 (2012): 3772–3778, https://doi.org/10.1098/rspb.2012.0902.

19. L. P. McGuire and C. G. Guglielmo, "What Can Birds Tell Us About the Migration Physiology of Bats?," *Journal of Mammalogy* 90, no. 6 (2009): 1290–1297, https://doi.org/10.1644/09-mamm-s-084r.1.

20. J.-M. Weber, "Metabolic Fuels: Regulating Fluxes to Select Mix," *Journal of Experimental Biology* 214, no. 2 (2011): 286–294, https://doi.org/10.1242/jeb.047050.

21. M. Hargreaves and L. L. Spriet, "Skeletal Muscle Energy Metabolism During Exercise," *Nature Metabolism* 2, no. 9 (2020): 817–828, https://doi.org/10.1038/s42255-020-0251-4.

22. J. C. David and C. G. Guglielmo, "Dietary Effects on Prediction of Body Mass Changes in Birds by Plasma Metabolites," *Auk* 123, no. 3 (2006): 836–846, https://doi.org/10.1093/auk/123.3.836.

23. A. S. Dixit, R. Chetri, and N. S. Singh, "Utilization of Biomolecules as Fuel Energy and Their Physiological Mechanism During Migration in Birds—A Review," *Journal of Environmental Biology* 43 (2022): 1–10, https://doi.org/10.22438/jeb/43/1/MRN-1901.

24. S. Sato, A. L. Basse, M. Schönke, et al., "Time of Exercise Specifies the Impact on Muscle Metabolic Pathways and Systemic Energy Homeostasis," *Cell Metabolism* 30, no. 1 (2019): 92–110.e4, https://doi.org/10.1016/j.cmet.2019.03.013.

25. Y. Xiong, Y. Hao, Y. Cheng, et al., "Comparative Transcriptomic and Metabolomic Analysis Reveals Pectoralis Highland Adaptation Across Altitudinal Songbirds," *Integrative Zoology* 17, no. 6 (2022): 1162–1178, https://doi.org/10.1111/1749-4877.12620.

26. A. Alonso, S. Marsal, and A. Julià, "Analytical Methods in Untargeted Metabolomics: State of the Art in 2015," *Frontiers in Bioengineering and Biotechnology* 3 (2015): 23.

27. N. J. Gupta, R. K. Nanda, S. Das, M. K. Das, and R. Arya, "Night Migratory Songbirds Exhibit Metabolic Ability to Support High Aerobic Capacity During Migration," *ACS Omega* 5, no. 43 (2020): 28088–28095, https://doi.org/10.1021/acsomega.0c03691.

28. C. J. Pennycuick, T. Alerstam, and A. Hedenström, "A New Low-Turbulence Wind Tunnel for Bird Flight Experiments at Lund University, Sweden," *Journal of Experimental Biology* 200, no. 10 (1997): 1441–1449, https://doi.org/10.1242/jeb.200.10.1441.

29. S. A. Troxell, M. W. Holderied, G. Pētersons, and C. C. Voigt, "Nathusius' Bats Optimize Long-Distance Migration by Flying at Maximum Range Speed," *Journal of Experimental Biology* 222, no. 4 (2019): jeb176396, https://doi.org/10.1242/jeb.176396.

30. N. Sillner, A. Walker, M. Lucio, et al., "Longitudinal Profiles of Dietary and Microbial Metabolites in Formula- and Breastfed Infants," *Frontiers in Molecular Biosciences* 8 (2021): 660456, https://doi.org/10.3389/fmolb.2021.660456.

31. L. Gatto, S. Gibb, and J. Rainer, "MSnbase, Efficient and Elegant R-Based Processing and Visualization of Raw Mass Spectrometry Data," *Journal of Proteome Research* 20, no. 1 (2021): 1063–1069, https://doi.org/10.1021/acs.jproteome.0c00313.

32. N. F. de Jonge, J. J. R. Louwen, E. Chekmeneva, et al., "MS2Query: Reliable and Scalable MS2 Mass Spectra-Based Analogue Search," *Nature Communications* 14, no. 1 (2023): 1752, https://doi.org/10.1038/s41467-023-37446-4.

33. K. Dührkop, M. Fleischauer, M. Ludwig, et al., "SIRIUS 4: A Rapid Tool for Turning Tandem Mass Spectra Into Metabolite Structure Information," *Nature Methods* 16, no. 4 (2019): 299–302, https://doi.org/10.1038/s41592-019-0344-8.

34. M. Wang, J. J. Carver, V. V. Phelan, et al., "Sharing and Community Curation of Mass Spectrometry Data With Global Natural Products Social Molecular Networking," *Nature Biotechnology* 34, no. 8 (2016): 828–837, https://doi.org/10.1038/nbt.3597.

35. G. Liebisch, E. Fahy, J. Aoki, et al., "Update on LIPID MAPS Classification, Nomenclature, and Shorthand Notation for MS-Derived Lipid Structures," *Journal of Lipid Research* 61, no. 12 (2020): 1539–1555, https://doi.org/10.1194/jlr.S120001025.

36. Posit Team, "RStudio: Integrated Development Environment for R" (2025), http://www.rstudio.com/.

37. R Core Team, "R: A Language and Environment for Statistical Computing" (2025), https://www.R-project.org/.

38. F. Rohart, B. Gautier, A. Singh, and K.-A. Lê Cao, "mixOmics: An R Package for 'Omics Feature Selection and Multiple Data Integration," *PLoS Computational Biology* 13, no. 11 (2017): e1005752, https://doi.org/10.1371/journal.pcbi.1005752.

39. V. V. Yanshole, A. D. Melnikov, L. V. Yanshole, et al., "Animal Metabolite Database: Metabolite Concentrations in Animal Tissues and Convenient Comparison of Quantitative Metabolomic Data," *Metabolites* 13, no. 10 (2023): 1088, https://doi.org/10.3390/metabo13101088.

40. A. Domer, W. Jasinska, L. Rosental, et al., "Comparative Analysis of the Plasma Metabolome of Migrating Passerines: Novel Insights Into Stopover Metabolism," *Journal of Avian Biology* 2025, no. 2 (2025): e03331, https://doi.org/10.1111/jav.03331.

41. B. W. M. Wone and D. L. Swanson, "Metabolic Profiling and Integration of Metabolomic and Transcriptomic Data From Pectoralis Muscle Reveal Winter-Adaptive Metabolic Responses of Black-Capped Chickadee and American Goldfinch," *Frontiers in Ecology and Evolution* 10 (2022): 1–16, https://doi.org/10.3389/fevo.2022.866130.

42. S. V. Zimin, A. Zimin, E. Shochat, Y. Brotman, and O. Ovadia, "Fuel Stores and Time of Day Account for Variation in Serum Metabolomes of

Passerine Migrants Stopping Over," *Journal of Avian Biology* 2025, no. 2 (2025): e03311, https://doi.org/10.1111/jav.03311.

43. D. Schranner, G. Kastenmüller, M. Schönfelder, W. Römisch-Margl, and H. Wackerhage, "Metabolite Concentration Changes in Humans After a Bout of Exercise: A Systematic Review of Exercise Metabolomics Studies," *Sports Medicine—Open* 6, no. 1 (2020): 11, https://doi.org/10.1186/s40798-020-0238-4.

44. M. M. Adeva-Andany, I. Calvo-Castro, C. Fernández-Fernández, C. Donapetry-García, and A. M. Pedre-Piñeiro, "Significance of l-Carnitine for Human Health," *IUBMB Life* 69, no. 8 (2017): 578–594, https://doi.org/10.1002/iub.1646.

45. R. R. Ramsay and V. A. Zammit, "Carnitine Acyltransferases and Their Influence on CoA Pools in Health and Disease," *Molecular Aspects of Medicine* 25, no. 5–6 (2004): 475–493, https://doi.org/10.1016/j.mam.2004.06.002.

46. K. A. Overmyer, C. R. Evans, N. R. Qi, et al., "Maximal Oxidative Capacity During Exercise Is Associated With Skeletal Muscle Fuel Selection and Dynamic Changes in Mitochondrial Protein Acetylation," *Cell Metabolism* 21, no. 3 (2015): 468–478, https://doi.org/10.1016/j.cmet.2015.02.007.

47. S. Ezagouri, Z. Zwighaft, J. Sobel, et al., "Physiological and Molecular Dissection of Daily Variance in Exercise Capacity," *Cell Metabolism* 30, no. 1 (2019): 78–91.e74, https://doi.org/10.1016/j.cmet.2019.03.012.

48. R. Lehmann, X. Zhao, C. Weigert, et al., "Medium Chain Acylcarnitines Dominate the Metabolite Pattern in Humans Under Moderate Intensity Exercise and Support Lipid Oxidation," *PLoS One* 5, no. 7 (2010): e11519, https://doi.org/10.1371/journal.pone.0011519.

49. K. Contrepois, S. Wu, K. J. Moneghetti, et al., "Molecular Choreography of Acute Exercise," *Cell* 181, no. 5 (2020): 1112–1130.e16, https://doi.org/10.1016/j.cell.2020.04.043.

50. F. Xiang, Z. Zhang, J. Xie, et al., "Comprehensive Review of the Expanding Roles of the Carnitine Pool in Metabolic Physiology: Beyond Fatty Acid Oxidation," *Journal of Translational Medicine* 23, no. 1 (2025): 324, https://doi.org/10.1186/s12967-025-06341-5.

51. E. R. Price, J. F. Staples, C. L. Milligan, and C. G. Guglielmo, "Carnitine Palmitoyl Transferase Activity and Whole Muscle Oxidation Rates Vary With Fatty Acid Substrate in Avian Flight Muscles," *Journal of Comparative Physiology B* 181, no. 4 (2011): 565–573, https://doi.org/10.1007/s00360-010-0542-2.

52. Y. Zhang, T. Carter, K. Eyster, and D. L. Swanson, "Acute Cold and Exercise Training Up-Regulate Similar Aspects of Fatty Acid Transport and Catabolism in House Sparrows (*Passer domesticus*)," *Journal of Experimental Biology* 218, no. pt. 24 (2015): 3885–3893, https://doi.org/10.1242/jeb.126128.

53. H. Beaufrère, S. M. Gardhouse, R. D. Wood, and K. D. Stark, "The Plasma Lipidome of the Quaker Parrot (*Myiopsitta monachus*)," *PLoS One* 15, no. 12 (2020): e0240449, https://doi.org/10.1371/journal.pone.0240449.

54. H. Watson, J.-Å. Nilsson, E. Smith, et al., "Urbanisation-Associated Shifts in the Avian Metabolome Within the Annual Cycle," *Science of the Total Environment* 944 (2024): 173624, https://doi.org/10.1016/j.scitotenv.2024.173624.

55. A. Saghatelian, M. K. McKinney, M. Bandell, A. Patapoutian, and B. F. Cravatt, "A FAAH-Regulated Class of N-Acyl Taurines That Activates TRP Ion Channels," *Biochemistry* 45, no. 30 (2006): 9007–9015, https://doi.org/10.1021/bi0608008.

56. T. Miyazaki, Y. Nakamura-Shinya, K. Ebina, et al., "N-Acetyltaurine and Acetylcarnitine Production for the Mitochondrial Acetyl-CoA Regulation in Skeletal Muscles During Endurance Exercises," *Metabolites* 11, no. 8 (2021): 522, https://doi.org/10.3390/metabo11080522.

57. T. J. Grevengoed, S. A. J. Trammell, M. K. McKinney, et al., "N-Acyl Taurines Are Endogenous Lipid Messengers That Improve Glucose Homeostasis," *Proceedings of the National Academy of Sciences of the United States of America* 116, no. 49 (2019): 24770–24778, https://doi.org/10.1073/pnas.1916288116.

58. S. A. J. Trammell, L. F. Gamon, K. Gotfryd, et al., "Identification of Bile Acid-CoA:Amino Acid N-Acyltransferase as the Hepatic N-Acyl Taurine Synthase for Polyunsaturated Fatty Acids," *Journal of Lipid Research* 64, no. 9 (2023): 100361, https://doi.org/10.1016/j.jlr.2023.100361.

59. E. R. Price, "Dietary Lipid Composition and Avian Migratory Flight Performance: Development of a Theoretical Framework for Avian Fat Storage," *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 157, no. 4 (2010): 297–309, https://doi.org/10.1016/j.cbpa.2010.05.019.

60. T. P. Parmar, A. L. Kindinger, M. Mathieu-Resuge, et al., "Fatty Acid Composition Differs Between Emergent Aquatic and Terrestrial Insects—A Detailed Single System Approach," *Frontiers in Ecology and Evolution* 10 (2022): 1–13, https://doi.org/10.3389/fevo.2022.952292.

61. D. Maillet and J.-M. Weber, "Performance-Enhancing Role of Dietary Fatty Acids in a Long-Distance Migrant Shorebird: The Semipalmated Sandpiper," *Journal of Experimental Biology* 209, no. 14 (2006): 2686–2695, https://doi.org/10.1242/jeb.02299.

62. C. Guglielmo, T. Williams, G. Zwingelstein, G. Brichon, and J. M. Weber, "Plasma and Muscle Phospholipids Are Involved in the Metabolic Response to Long-Distance Migration in a Shorebird," *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology* 172, no. 5 (2002): 409–417, https://doi.org/10.1007/s00360-002-0266-z.

63. J. M. Klaiman, E. R. Price, and C. G. Guglielmo, "Fatty Acid Composition of Pectoralis Muscle Membrane, Intramuscular Fat Stores and Adipose Tissue of Migrant and Wintering White-Throated Sparrows (*Zonotrichia albicollis*)," *Journal of Experimental Biology* 212, no. 23 (2009): 3865–3872, https://doi.org/10.1242/jeb.034967.

64. F. Gibellini and T. K. Smith, "The Kennedy Pathway—*De Novo* Synthesis of Phosphatidylethanolamine and Phosphatidylcholine," *IUBMB Life* 62, no. 6 (2010): 414–428, https://doi.org/10.1002/iub.337.

65. B. Wang and P. Tontonoz, "Phospholipid Remodeling in Physiology and Disease," *Annual Review of Physiology* 81 (2019): 165–188, https://doi.org/10.1146/annurev-physiol-020518-114444.

66. S. Lee, F. Norheim, H. L. Gulseth, et al., "Skeletal Muscle Phosphatidylcholine and Phosphatidylethanolamine Respond to Exercise and Influence Insulin Sensitivity in Men," *Scientific Reports* 8, no. 1 (2018): 6531, https://doi.org/10.1038/s41598-018-24976-x.

67. M. M. Adeva-Andany, N. Pérez-Felpete, C. Fernández-Fernández, C. Donapetry-García, and C. Pazos-García, "Liver Glucose Metabolism in Humans," *Bioscience Reports* 36, no. 6 (2016): e00416, https://doi.org/10.1042/bsr20160385.

68. D. Costantini, O. Lindecke, G. Pētersons, and C. C. Voigt, "Migratory Flight Imposes Oxidative Stress in Bats," *Current Zoology* 65, no. 2 (2019): 147–153, https://doi.org/10.1093/cz/zoy039.

69. D. P. Jones, "[11] Redox Potential of GSH/GSSG Couple: Assay and Biological Significance," in *Methods in Enzymology*, vol. 348, ed. H. Sies and L. Packer (Academic Press, 2002), 93–112, https://doi.org/10.1016/S0076-6879(02)48630-2.

70. M. Dambrova, M. Makrecka-Kuka, J. Kuka, et al., "Acylcarnitines: Nomenclature, Biomarkers, Therapeutic Potential, Drug Targets, and Clinical Trials," *Pharmacological Reviews* 74, no. 3 (2022): 506–551, https://doi.org/10.1124/pharmrev.121.000408.

71. M. Sud, E. Fahy, D. Cotter, et al., "Metabolomics Workbench: An International Repository for Metabolomics Data and Metadata, Metabolite Standards, Protocols, Tutorials and Training, and Analysis Tools," *Nucleic Acids Research* 44, no. D1 (2016): D463–D470, https://doi.org/10.1093/nar/gkv1042.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Blood metabolome profiles of bats from controlled conditions. **Figure S2:** N-acyl taurines and phosphatidylcholines are affected by flight under controlled conditions. **Figure S3:** Eighteen amino acids, detected in negative electrospray ionization ESI(-) under controlled and field conditions. **Figure S4:** Blood metabolome profiles from free-ranging bats collected over two seasons and for two physiological states. **Table S1:** Mass spectrometer specific parameters for HILIC LC in ESI(−) or ESI(+). **Table S2a:** Metabolite data from whole blood of captive bats under controlled conditions in ESI(+/−). **Table S2b:** Metabolite data from whole blood of free-ranging bats across seasons under field conditions in ESI(+/−). **Table S2c:** Metabolite identities in ESI(+/−). **Table S3:** Amino acid data from whole blood of bats under controlled or field conditions in ESI(−). **Table S4:** A summary of area under the curve (AUC) values and *p* values for component 1 and 2 of PLS-DA, calculated for controlled and field conditions in ESI(−/+). **Table S5:** List of acyl carnitines influenced by exercise including a summary of acyl carnitines, categorized by their acyl chain length. The count is describing the number of significant acyl carnitines under controlled conditions.