The Science of Nature (2019) 106: 33
https://doi.org/10.1007/s00114-019-1627-8

ORIGINAL ARTICLE

Fatty acid profiles of the European migratory common noctule bat (*Nyctalus noctula*)

Christian C. Voigt[1] · Elisabeth Rosner[1,2] · Christopher G. Guglielmo[3] · Shannon E. Currie[1]

Received: 21 February 2019 / Revised: 17 May 2019 / Accepted: 21 May 2019 / Published online: 14 June 2019

Abstract

In animals, fatty acids (FA) are essential as structural components in membranes and for energy storage in adipocytes. Here, we studied the relative proportions of FA in a mammal with extreme changes in metabolic rates. Common noctule bats (*Nyctalus noctula*) switch from energetically demanding long-distance migration at high metabolic rates to regular torpor with extremely low metabolic rates. We found that composition of FA categories differed between adipose tissue types (white adipose tissue (WAT) vs brown adipose tissue (BAT)) and muscle tissue types (skeletal vs heart), but not between sexes. We found oleic acid to be the most abundant FA in all studied tissues. Concentrations of polyunsaturated FA (PUFA) were not always higher in muscular tissue compared with adipocyte tissue, even though high concentrations of PUFA are considered beneficial for low body temperatures in torpor. In all tissues, we observed a high content in monounsaturated fatty acids (MUFA), possibly to compensate for a low PUFA content in the diet. Ratios of ω6/ω3 were lower in the heart than in skeletal muscles of common noctules. Three FA (palmitic, oleic, and linoleic acid) accounted for about 70% of the FA in adipose tissue, which is similar to proportions observed in migrating birds, yet migrating birds generally have a higher PUFA content in muscle and adipose tissues than bats. Bats seem to contrast with other mammals in having a high MUFA content in all tissues. We conclude that FA profiles of bats differ largely from those of most cursorial mammals and instead are—with the exception of MUFA—similar to those of migrating birds.

Keywords Chiroptera · Exercise · Torpor · Migration · Adipose tissue

Introduction

Fatty acids play key roles in structuring cells as phospholipids and in providing energy for metabolism as triacylglycerols. The specific relevance of FA varies according to their length and their level of saturation. FA with at least two double bonds, so-called PUFA, seem to be particularly versatile since they are considered important for both high metabolic rates (e.g., in running mammals: Ayre and Hulbert 1997; Ruf et al. 2006, and migrating birds: Pierce et al. 2005; Maillet and Weber 2007; Klaiman et al. 2009; Price and Guglielmo 2009; Weber 2009; Price 2010; Weber 2011; Pierce and McWilliams 2014) and low metabolic rates (e.g., in hibernators: Florant 1998; Munro and Thomas 2004; Gerson et al. 2008; Ruf and Arnold 2008; Arnold et al. 2015). For high metabolic rates, PUFA are beneficial because they can be more rapidly mobilized than the corresponding saturated aliphatic chains, although PUFA may yield less energy than the saturated variant (Price 2010; Pierce and McWilliams 2014). Yet, based on the same reason, PUFA may also serve as a better oxidative fuel at low body temperatures than saturated fatty acids (SFA), e.g., during hibernation (Rosner and Voigt 2018). For low metabolic rates at low body temperatures, i.e., in torpid and hibernating mammals, it was argued that the location of the double bond defines the specific function of PUFA. Usually PUFA come as two forms in animals. When the first double bond is located at the third carbon position—counting from the methyl (ω) end of the aliphatic chain—

Communicated by: Matthias Waltert

✉ Christian C. Voigt
voigt@izw-berlin.de

[1] Department of Evolutionary Ecology, Leibniz Institute for Zoo and Wildlife Research, 10315 Berlin, Germany

[2] Zoological Institute and Museum, Ernst-Moritz-Arndt-University Greifswald, 17487 Greifswald, Germany

[3] Department of Biology, Advanced Facility for Avian Research, University of Western Ontario, London, ON, Canada

PUFA are called ω3, whereas PUFA are called ω6 when the first double bond is located at the sixth carbon position. Specifically, it was argued that the ratio between ω6/ω3 is essential to ensure activity of cardiomyocytes, heart muscle cells (Florant 1998; Gerson et al. 2008; Ruf and Arnold 2008). This expectation is based on the observation that high ω6/ω3 ratios in cardiomyocytes enable torpid mammals to lower their body temperature without compromising important membrane functions such as the activity of the sarcoplasmic reticulum Ca^{+2} ATPase (SERCA; Ruf and Arnold 2008). Overly high cytosolic concentrations of Ca^{2+} are thought to increase the risk for arrhythmia and cardiac arrest at low body temperatures (Ruf and Arnold 2008). Contrary to ω6, ω3 seems to suppress SERCA activity, yet high ω3 concentrations in phospholipids support pathways that promote ATP delivery. In mammals, high ω6 and low ω3 contents of skeletal myocytes, skeletal muscle cells, are usually related to the maximum running speed (Ruf et al. 2006). Yet, previous studies have mostly been conducted in species with terrestrial locomotion, i.e., cursorial mammals, and only a few about FA content and exercise performance in bats (McGuire et al. 2013), the only mammalian taxon capable of powered flight. Bats experience rapid changes in metabolic rates when switching between flight and torpor (Neuweiler 2000). Such drastic changes in metabolic rates are particularly relevant for migratory bats that switch frequently between torpor and endurance flight (McGuire et al. 2014; Troxell et al. 2019). FA content of heart and skeletal muscles must comply with two extreme conditions in migrating bats. On the one hand, the FA composition needs to match with the needs associated with fluctuating body temperatures during both normothermic and torpid conditions. On the other hand, the FA composition needs to fulfill the requirements of endurance exercise when bats travel long distances. A previous study in hoary bats (*Lasiurus cinereus*) showed that adipose neutral lipids and muscle phospholipids differed in FA composition and that migration and sex affected FA composition and the ratio of ω6 to ω3 PUFA (McGuire et al. 2013).

Here, we studied the relative composition of FA in muscle and adipose tissues obtained from common noctule bats (*Nyctalus noctula*) during summer migration (note that "fall" migration begins in summer months for this species). Common noctules are known to exhibit partial and directed migration throughout Europe (Lehnert et al. 2018). Specifically, we compared the FA profile of common noctules across brown and white fat tissues and cardiac and skeletal muscle tissues. Since previous studies indicated sex- and tissue-specific differences for migratory bats (McGuire et al. 2013), we expected to find differences in FA composition between males and females and also across tissues. We predicted to find higher PUFA concentrations in skeletal and heart muscles than in adipose tissue, because a high PUFA content in myocytes should be beneficial for low body temperature conditions during torpor. Further, we hypothesized that ω6/ω3 ratios should differ between muscular tissues (Ruf and Arnold 2008). In particular, we predicted for migrating bats that heart muscles should have higher ω6/ω3 ratios compared with skeletal muscles in order to prevent arrhythmia and cardiac arrest during torpor (Ruf and Arnold 2008).

Material and methods

Study animals

Since all European bat species are legally protected because of their critical population status, we refrained from sacrificing animals for our study. Instead, we used tissue samples from fresh carcasses of *Nyctalus noctula* found below wind turbines between July and September 2009–2012 in Northeastern Germany. We assumed that these bats were killed in the night before they were found; i.e., no more than 6 h passed between dawn and the time carcasses were deposited in a − 20 °C freezer at the Vogelschutzwarte Buckow. A recent study demonstrated that FA composition in the heart of small bats remained stable for at least 12 h post-mortem (Currie et al. 2019). Thus, the FA composition of carcasses used in our study is likely to remain representative of the FA composition observed in a living animal. In order to justify this and assess whether FA composition was different in carcasses to freshly euthanized animals, we measured the FA composition in one animal that had to be euthanized (Rosner and Voigt 2018; animal care and ethics permit, C 113-0340/12). Tissues of this animal were immediately frozen and later analyzed as with the other samples. Furthermore, during carcass collection and tissue dissection, carcasses were visually assessed for signs of decomposition and discarded in case of any obvious degradation.

We obtained tissue samples of cardiac and skeletal flight muscles (*musculus pectoralis*) from 38 bats and 40 bats, respectively. Further, we obtained 33 white adipose tissue (WAT) samples, the body's main fat store, and 38 brown adipose tissue (BAT) samples, specialized fatty tissue for heat production, as these tissues were not present in all bats. WAT was sampled from both sides of the bats' body (thorax and abdomen) and BAT from the interscapular region of the thorax. Isolated samples were stored at − 80 °C for up to 8 months until analysis.

Sample preparation

Sample preparation and analysis were performed in summer 2013. For each tissue sample, we first rinsed a 16-ml glass vial (IVA Analysentechnik e.K., Meerbusch, Germany) with 0.5 ml 2:1 chloroform to methanol (Carl Roth GmbH & Co.

KG, Karlsruhe, Germany), containing 0.01% butylated hydroxyl toluene (BHT; Merck KGaA, Darmstadt, Germany). Then, we weighed 20 mg of WAT or BAT and 100 mg of heart or skeletal flight muscle, respectively (model: Microbalance ME5, Sartorius AG, Göttingen, Germany), thoroughly minced the tissue, and put it into prepared vials. We added 2 ml of 2:1 chloroform to methanol (0.01% BHT), closed the vial with a PFTE-lined cap, and shook the vial to mix tissue and solvent. Then, we incubated the samples for 5 min at room temperature. Afterwards, 1 ml of 0.25% KCl (Carl Roth GmbH & Co. KG, Karlsruhe, Germany) was added, and the vials were placed in a water bath at 70 °C for 5 min (model: Sonorex Super RK 103H, Bandelin electronic GmbH & Co. KG, Berlin, Germany), after which two layers formed in the vial. The lower layer, containing the FA dissolved in chloroform, was transferred to a pre-weighed 4-ml glass vial (IVA Analysentechnik e.K., Meerbusch, Germany) and dried under nitrogen atmosphere (The Linde Group, Munich, Germany; model: DB-3A, Bibby Scientific Limited Group (Group Q), Staffordshire, UK). Dried total lipid samples were weighed and stored at − 80 °C until gas chromatography.

Total lipid samples were resuspended in 2:1 chloroform to methanol (0.01% BHT; Caledon Laboratories Ltd., Georgetown, ON, Canada) to a concentration of 10 mg/ml. Then, we transferred 150 μl of this solution into a 2-ml glass vial (VWR International, Mississauga, ON, Canada), dried it under nitrogen atmosphere, added 200 μl 0.5 N methanolic HCl (Sigma-Aldrich Co. LLC, Oakville, ON, Canada), and incubated the samples at 90 °C for 30 min to transesterify the lipids to fatty acid methyl esters (FAMEs). Then, we added 800-μl ultrapure water and 500 μl hexane (Caledon Laboratories Ltd., Georgetown, ON, Canada) and vortexed the solution for 15 s (model: lab dancer, VWR International, Mississauga, ON Canada) to dissolve the FAMEs in hexane. This resulted in the formation of two layers with the upper layer, containing the dissolved FAMEs, being transferred into a new 2-ml glass vial. This FA dissolving step was repeated three times, before samples were dried under nitrogen atmosphere. Then, the purified FAMEs were resuspended in 150 μl hexane and transferred into a gas chromatography vial with a Hamilton syringe (VWR International, Mississauga, ON Canada).

Sample analysis

FA composition was analyzed on an Agilent Technologies 6890N gas chromatograph with a J&W Scientific DB-23 high-resolution column (30 m, 0. 25 mm, 0.25 μm; Agilent Technologies) and flame ionization detector. The carrier gas was He, flowing at 1.9 ml min^{-1}, and 1-μl samples were injected in a splitless mode at 250 °C. The oven program was 80 °C for 2 min, ramp 5 °C min^{-1} to 180 °C, hold 5 min 180 °C, ramp 1 °C min^{-1} to 200 °C, ramp 10 °C min^{-1} to 240 °C, and hold 3 min 240 °C. FAMEs were identified by comparing their retention times to those of standards included in each run (Supelco 37 component FAME mix, PUFA No. 3 from menhaden oil, FAME mix C8-C24; Sigma-Aldrich Co. LLC, Oakville, ON, Canada). We also added a positive and negative control to each run which confirmed that the protocol was functional and showed no contamination between samples. Relative mass percent was calculated from peak areas, and only fatty acids comprising > 1% of total peak area were quantified. Because lipids were not separated into neutral lipid, polar lipid, and non-esterified fatty acid fractions before analysis, the fatty acid compositions we report are mixtures of all fatty acids in the tissues. However, the compositions of muscles should be dominated by sarcolemmal and mitochondrial phospholipids, WAT by triacylglycerol, and BAT by triacylglycerol and mitochondrial phospholipids.

Statistics

For comparison of FA compositions, we neglected FA with a relative contribution of less than 1% to the total fatty acid profile. We performed a permutational multivariate analysis of variance using distance matrices, which is appropriate for comparing relative proportions (adonis2, package vegan; Oksanen et al. 2013), to assess general differences in the FA profile of animals between fat tissues (BAT vs WAT) and muscle tissues (heart vs skeletal muscle). To assess whether sex had an effect on the fatty acid profile, we added the interaction between sex and tissue as a factor as well as individual to account for repeated measures. Following this, we performed Wilcoxon paired-sign test for pairwise comparisons of different FA categories (SFA, MUFA, PUFA, ω3, ω6, and ω6/ω3 ratios) between tissues (SYSTAT, vs 11, SYSTAT Software GmbH, Erkrath, Germany) and accounted for repeated testing by adjusting p values using a Bonferroni correction. All parameters are presented as means ± standard deviation if not stated otherwise. The level of significance was set to 0.05.

Results

We compared the FA composition across four tissues of common noctule bats, specifically heart and skeletal muscles and brown and white adipose tissues. All bats died at wind turbines in late summer, during the time of migration. Overall, we observed that oleic acid (18:1 ω9) was the most abundant FA in common noctule bats, contributing between 45 to 57% of all FA in the studied tissues, followed by linoleic acid (18:2 ω6) or palmitic acid (16:0; Fig. 1). The average FA profile of bat carcasses did not deviate largely from FA proportions obtained from a single euthanized noctule bat (Fig. 1), except for

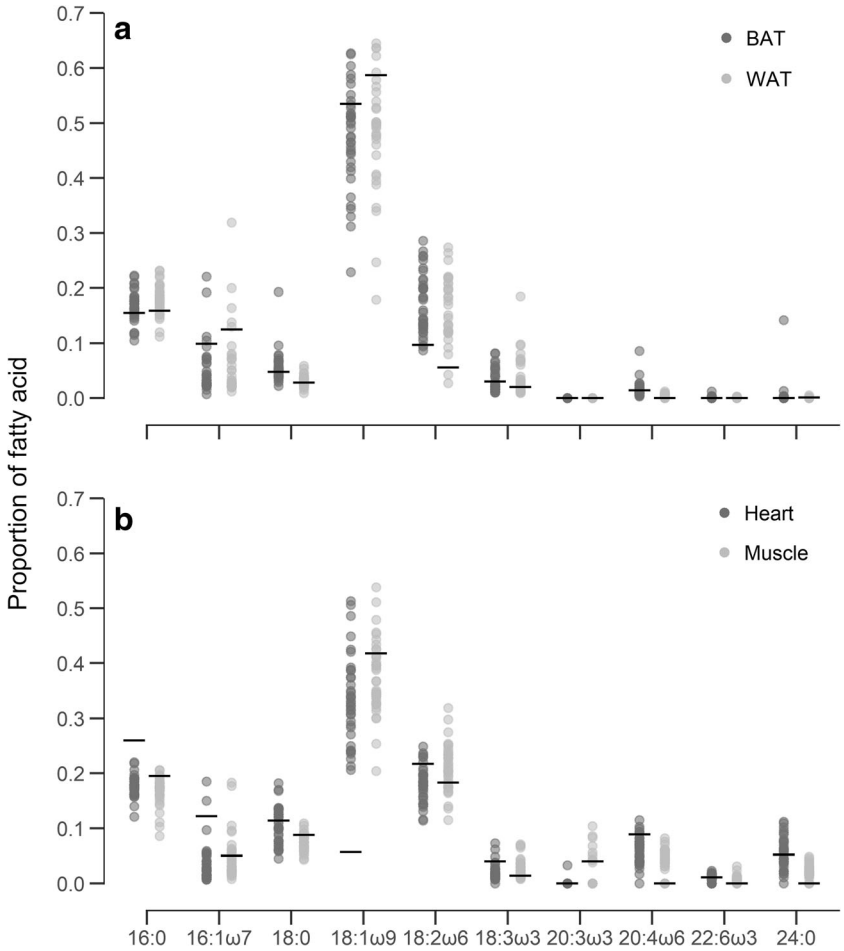

Fig. 1 Proportion of fatty acids (FA) in relation to total FA in four tissues ((A) WAT = white adipose tissue, BAT = brown adipose tissue; (B) skeletal and heart muscle) of *N. noctula* from summer. The solid lines indicate the proportion of fatty acids collected from a single euthanized *N. noctula* for comparison. The polyunsaturated FA (PUFA) linoleic acid, for example, is depicted as 18:2ω6, stating that the carbon chain counts 18 atoms and that there are two double bonds with the first being at the sixth position of the FA chain counted from the methyl end of the FA chain

in the heart tissue, where oleic acid was lower and palmitic acid slightly higher than the carcasses. Permutational multivariate analysis of variance revealed that the proportion of individual FA differed with both tissue type and sex (tissue, $F_{(3,95)} = 54.93$; $p = 0.001$; sex, $F_{(1,95)} = 5.88$; $p = 0.002$), likely related to the greater dispersion of values for females in a number of individual FA (Fig. 2). However, the effect of sex was no longer evident when individual FA were combined into FA categories (SFA, MUFA, PUFA, ω3, ω6) ($F_{1,148} = 1.25$, $p = 0.276$).

After identifying tissue as the parameter explaining the variation in FA profiles, we compared different FA categories across all four tissues to determine where these differences lay (Table 1). We found differences in FA categories between both the two fat tissues (BAT and WAT; corrected $p = 0.006$) and the two muscle tissues (heart and skeletal muscles; corrected $p = 0.006$). This was also true for skeletal muscle when compared with BAT (corrected $p = 0.006$) and heart muscle compared with WAT (corrected $p = 0.042$). Surprisingly however, the FA profiles of skeletal muscle and WAT did not differ significantly (corrected $p = 0.438$), nor did that of BAT when compared with heart muscle (corrected $p = 0.99$).

As PUFA are particularly limiting for these animals, we specifically focused on the concentration of PUFA and found a significant difference in this FA category across the muscle and adipocyte tissues (Friedman, $W = 26.6$; Kendall coefficient = 0.444; corrected $p = 0.006$). PUFA content of heart and skeletal muscles ranged between about 27 and 30%, whereas PUFA content of adipose tissue was slightly lower, ranging between 22 and 24% (Table 1). ω3 PUFA was consistently represented across tissues in a range of 3–5%. The ratio of ω6/ω3 PUFA was variable but high across all tissue types, with the greatest proportional difference in the skeletal muscle tissue.

Discussion

Common noctules (*Nyctalus noctula*), a European migratory bat (Lehnert et al. 2018), face severe physiological challenges in summer when migrating from northern latitudes to southern wintering areas for hibernation. We expected that the FA composition of muscle and adipocyte tissues would adjust to both the need for high endurance exercise during migratory flights and for variable body temperatures during torpor. We found

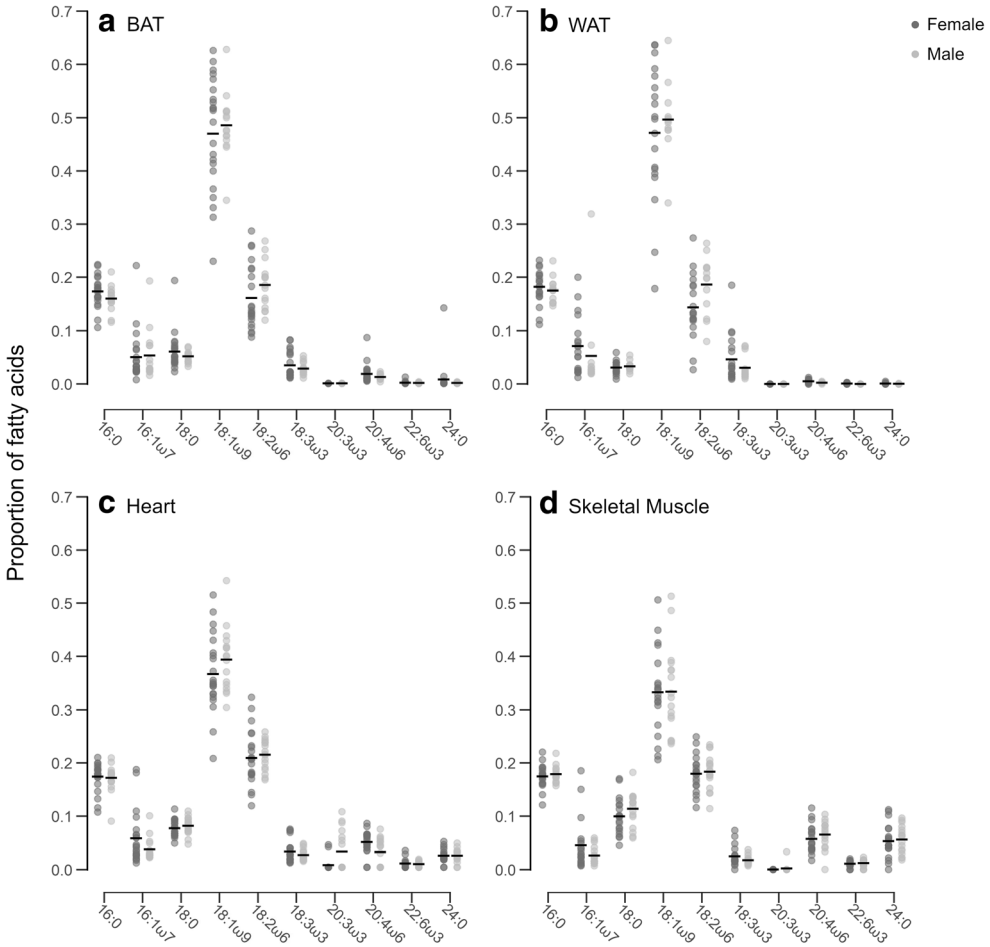

Fig. 2 Raw concentrations of fatty acids as a proportion of total FA in four tissues for *N. noctula* females (dark gray circles) and males (light gray circles) with mean values represented by solid black bars. There was a significant effect of sex across both muscle and adipose tissues, likely related to dispersion of data for females, which is unlikely to be biologically relevant as this effect disappeared when FA were grouped into corresponding categories based on saturation

that there was no broad effect of sex on the composition of FA categories but that many tissues differed significantly in their FA profile. This contrasts with an earlier study in a North American migratory bat, *L. cinereus*, where males had higher ω6/ω3 ratios in skeletal muscle than females during spring migration (McGuire et al. 2013), a pattern that matched with the expectation that females used torpor less often than males owing to the pregnancy of females in spring. This likely cause for a sex-specific difference in ω6/ω3 ratios is absent in summer when we collected carcasses for this study. Overall, ω6/ω3 ratios were about tenfold higher in the flight muscle of *N. noctula* than in *L. cinereus*, which may suggest that common *N. noctula* use torpor more often in late summer than *L. cinereus* in spring. Alternatively, ω6/ω3 ratios of *N. noctula* migrating in late summer were possibly elevated because bats prepared for hibernation or they consumed insects that were more enriched in ω6 and depleted in ω3 than *L. cinereus* migrating in spring.

We found differences in FA composition across all four studied tissues. The most abundant FA was oleic acid, a MUFA, followed by either a PUFA (linoleic acid, 18:2) or a SFA (palmitic acid, 16:0). The higher PUFA content in muscular tissue was partly consistent with our expectation for higher PUFA enrichments in the membrane-rich heart and skeletal muscles compared with adipose tissue (WAT and BAT). Assuming that the PUFA content of adipose tissue generally reflects the dietary PUFA content (Abbott et al. 2012), we infer that the PUFA content of dietary insects should be about 23%. Possibly, common noctules may have obtained PUFA from terrestrial Coleoptera such as scaraboid beetles (*Geotrupes*, among others), a prey item that has been

Table 1 Percentage composition of FA categories and ratios in skeletal and heart muscle and in BAT and WAT. There was a significant difference in FA composition between the fats and muscles and between heart muscle and WAT and skeletal muscle and BAT (superscript letters indicate statistical significance). This was not true for heart tissue and BAT or for skeletal tissue and WAT

	Heart muscle[a,c]	Skeletal muscle[b,d]	BAT[a,e]	WAT[b,f]
SFA	35.6 ± 6.4	28.4 ± 3.4	25.0 ± 4.7	23.7 ± 2.4
MUFA	36.5 ± 9.2	42.1 ± 5.7	53.0 ± 7.1	55.3 ± 8.3
PUFA	27.9 ± 4.4	29.5 ± 4.6	22.0 ± 5.4	21.0 ± 8.0
ω3	3.3 ± 1.7	3.3 ± 2.1	3.3 ± 2.1	4.2 ± 4.2
ω6	24.4 ± 4.5	26.0 ± 4.6	18.5 ± 5.7	16.3 ± 6.3
ω6/ω3	9.2 ± 4.6	11.2 ± 6.4	8.2 ± 5.3	6.7 ± 4.9

confirmed to dominate the diet of common noctule bats during summer and autumn (Poulton 1929; Kolb 1958; Gloor et al. 1995; Jones 1995). Further, we found low concentrations of docosahexaenoic acid (22:6n3) and a low ratio of docosahexaenoic to linoleic acids in adipose tissue, indicative of a diet consisting of terrestrial insects (Koussoroplis et al. 2008; Lam et al. 2013). This contrasts with the diet of North American *L. cinereus*, where docosahexaenoic acid was among the most abundant FA in flight muscle phospholipids, making up about 18% of all FA during spring migration (McGuire et al. 2013). While this could indicate differences in the diet of these two migratory species, it could also be reflective of the differences in methodology between the two studies. McGuire et al. (2013) isolated phospholipids while our study is looking at whole tissue values and therefore includes neutral lipid droplets in muscle tissue.

We observed relatively high concentrations of MUFA, specifically oleic acid, in samples collected from *N. noctula* during summer migration, for example, ~53% in BAT, ~55% WAT, ~37% in heart muscle, and ~42% in skeletal muscle. Apart from one exception, the pattern was consistent with high MUFA concentrations in muscular tissue and adipocytes of other bat species (Table 2). The high MUFA concentrations in muscular tissue of bats contrasted with low values reported for the muscle phospholipids of cursorial mammals, particularly in those with high running ability (13.8 ± 1.5% in: Ruf et al. 2006; Valencak and Ruf 2007). We reject the hypothesis that the low PUFA and high MUFA contents in tissues of common noctules were caused by rapid oxidation of PUFA during decomposition of carcasses, because a recent validation study demonstrated only minor degeneration of FA over several hours of decomposition (Currie et al. 2019). Additionally, the FA profile of a single euthanized noctule bat did not deviate largely from that of all other animals of the carcass collection. Instead, we argue that the diet of most bat species may be PUFA depleted or have low fat content, and thus consumption of MUFA-enriched dietary items or de novo synthesis of MUFA from dietary protein and carbohydrates may be the only way for bats to incorporate FA with at least one double bond in phospholipids and triacylglycerols. A compensatory inclusion of MUFA instead of PUFA has also been suggested for hibernating echidnas (Tachyglossidae; Falkenstein et al. 2001). In contrast to the suggested PUFA limitation, at least one bat species, *R. microphyllum*, seems to have PUFA-enriched WAT and particularly heart muscle in relation to their diet, underlining that *R. microphyllum* may be able to reach relatively high PUFA contents in their tissues, even in the presence of a low PUFA diet (~23% PUFA in the heart muscle of *R. microphyllum*; Levin et al. 2013).

In cursorial mammals, maximum running speed decreased with increasing MUFA content of muscle phospholipids (Ruf et al. 2006), which contrasts with the high MUFA content of bat myocytes and the ability of bats for extended high exercise performance when foraging and migrating on the wing. PUFA content of skeletal myocytes in common noctules was typical for an average cursorial mammal, yet only about half of that recorded for similar-sized Insectivora (range of PUFA 53–62%; range of MUFA 11–14%), and higher than the PUFA content of skeletal myocytes in most similar-sized rodents (range of PUFA 15–20; range of MUFA 27–33%, Ruf et al. 2006) with the exception of deer mice (*Peromyscus maniculatus*) that also reach high PUFA contents in skeletal muscles (Geiser et al. 2007). In common noctules, ω6 PUFA content of skeletal myocytes was at the lower end of those values typical for cursorial mammals, but higher than that of closely related and similar-sized Insectivora. Considering the high metabolic rates of flying bats, particularly during prolonged migratory flights, we expected that ω6 concentrations in skeletal myocytes of *N. noctula* would reach values similar to those of cursorial mammals with high maximum running speeds (Ruf et al. 2006). The contrasting results lead us conclude that the high exercise physiology of bats seems

Table 2 Comparative percentages of MUFA reported for muscle and adipose tissues in other insectivorous bat species compared with one frugivorous bat (*). *na* not available

Species	WAT	Skeletal muscle	Heart muscle	Total body fat	BAT	
Lasiurus cinereus	> 60%	> 20%	na	na	na	(McGuire et al. 2013)
Myotis lucifugus	na	na	na	~54%	62–76%	(Wells et al. 1965; Ewing et al. 1970; Warner and Zar 1982)
Myotis thysanodes	na	na	na	~58%	na	(Ewing et al. 1970)
Myotis yumanensis	na	an	na	~60%	na	(Ewing et al. 1970)
*Neoromicia nana**	na	na	na	na	3%	(Hill et al. 2016)
Pipistrellus pipistrellus	48–56%	na	na	na	42–54%	(Arévalo et al. 1990)
Pipistrellus kuhli	~50%	na	~28%	na	na	(Levin et al. 2013)
Rhinopoma microphyllum	~45%	na	~32%	na	na	(Levin et al. 2013)

not to be impaired by the high MUFA and relatively low PUFA contents of skeletal or cardiac muscles.

The convergent evolution of powered flight imposed similar selective forces on bats and birds, yet the physiology of migration differs in various ways between these two taxa, with so far unknown consequences for the FA profiles of their tissues. For example, bats, but not birds, routinely enter torpor during migration when stopping between flights of consecutive nights (Wikelski et al. 2003; McGuire et al. 2014). In addition, bats might forage during migratory flights, a behavior that has been called aerial refueling (Voigt et al. 2012). Thus, they do not seem to power migration exclusively by oxidizing lipids, which are the main oxidative fuel for most migratory birds (Pierce et al. 2005; Maillet and Weber 2007; Klaiman et al. 2009; Price and Guglielmo 2009; Weber 2009; Price 2010; Weber 2011). A meta-analysis conducted for the FA profiles of migrating birds revealed several patterns that can partly be observed in migratory bats as well. For example, migratory birds store mostly 16- and 18-carbon FA in adipocytes (McWilliams et al. 2004). Specifically, palmitic acid (16:0), oleic acid (18:1 ω9c), and linoleic acid (18:2 ω6c) accounted for at least 75% of the FA in adipocytes of migrating birds (McWilliams et al. 2004). These three FA comprised also ~ 70% of all FA in the adipose tissue of *N. noctula* (this study) and ~ 66% of all FA in adipocytes of *L. cinereus* (McGuire et al. 2013). Yet, hibernating mammals have also similar cumulative enrichments of these FA (Carneheim et al. 1989; Arnold et al. 2011). Thus, it is unclear if a high concentration of these FA is caused by the physiological needs imposed by low or high metabolic rates.

Conclusions

Similar to most other bats that have been studied so far with respect to FA profiles, we found high MUFA contents in all studied tissues of common noctule bats. This contrasts with previous inferences that high ω6 content, but not high MUFA content, is beneficial for mammals with high exercise physiology. Thus, we conclude that FA profiles of bats differ largely from those of most cursorial mammals. About 70% of all FA comprised one SFA (palmitic acid), one MUFA (oleic acid), and one PUFA (linoleic acid). With the exception of the high MUFA content, FA profiles of migrating bats appear to be similar to some extent to those of migrating birds. Further detailed studies comparing seasonal changes in fatty acid compositions of triacylglycerol and phospholipid fractions of muscles and adipose tissues of migratory and non-migratory bats are needed to better understand how fatty acids relate to migration and hibernation.

Acknowledgments We thank Doris Fichte, Anja Luckner, Karin Sörgel, Tobias Dürr, Gudrun Wibbelt, Raymond Thomas, and Morag Dick for the help during specific stages of the project. We thank the anonymous reviewers for their constructive comments.

Funding information This project was supported by a travel grant from the DAAD, a Discovery Grant from the Natural Sciences and Engineering Research Council of Canada, the Canada Foundation for Innovation, and the Ontario Research Fund.

References

Abbott SK, Else PL, Atkins TA, Hulbert AJ (2012) Fatty acid composition of membrane bilayers: importance of diet polyunsaturated fat balance. Biochim Biophys Acta Biomembr 1818(5):1309–1317. https://doi.org/10.1016/j.bbamem.2012.01.011

Arévalo F, Burgos MJ, del Hoyo N, López-Luna P (1990) Seasonal variations in the lipid composition of white and brown tissues in the bat Pipistrellus pipistrellus. Comp Biochem Physiol B 95(3): 535–539. https://doi.org/10.1016/0305-0491(90)90016-M

Arnold W, Ruf T, Frey-Roos F, Bruns U (2011) Diet-independent remodeling of cellular membranes precedes seasonally changing body temperature in a hibernator. PLoS One 6(4):e18641. https://doi.org/10.1371/journal.pone.0018641

Arnold W, Giroud S, Valencak TG, Ruf T (2015) Ecophysiology of omega fatty acids: a lid for every jar. Physiology 30:232–240. https://doi.org/10.1152/physiol.00047.2014

Ayre KJ, Hulbert AJ (1997) Dietary fatty acid profile affects endurance in rats. Lipids 32(12):1265–1270. https://doi.org/10.1007/s11745-006-0162-5

Carneheim C, Cannon B, Nedergaard J (1989) Rare fatty acids in brown fat are substrates for thermogenesis during arousal from hibernation. Am J Phys Regul Integr Comp Phys 256(1):R146–R154. https://doi.org/10.1152/ajpregu.1989.256.1.R146

Currie SE, Fasel NJ, Mène-Saffrané L (2019) Valuable carcasses: postmortem preservation of fatty acid composition in heart tissue. Conserv Physiol 7(1). https://doi.org/10.1093/conphys/coz005

Ewing WG, Studier EH, O'Farrell MJ (1970) Autumn fat deposition and gross body composition in three species of Myotis. Comp Biochem Physiol 36(1):119–129. https://doi.org/10.1016/0010-406X(70)90658-4

Falkenstein F, Kortner G, Watson K, Geiser F (2001) Dietary fats and body lipid composition in relation to hibernation in free-ranging echidnas. J Comp Physiol B 171(3):189–194

Florant GL (1998) Lipid metabolism in hibernators: the importance of essential fatty acids. Am Zool 38(2):331–340. https://doi.org/10.1093/icb/38.2.331

Geiser F, McAllan BM, Kenagy GJ, Hiebert SM (2007) Photoperiod affects daily torpor and tissue fatty acid composition in deer mice. Naturwissenschaften 94(4):319–325. https://doi.org/10.1007/s00114-006-0193-z

Gerson AR, Brown JCL, Thomas R, Bernards MA, Staples JF (2008) Effects of dietary polyunsaturated fatty acids on mitochondrial metabolism in mammalian hibernation. J Exp Biol 211(16):2689–2699. https://doi.org/10.1242/jeb.013714

Gloor S, Stutz H-PB, Ziswiler V (1995) Nutritional habits of the noctule bat *Nyctalus noctula* (Schreber, 1774) in Switzerland. Myotis 32(33):231–242

Hill K, van Aswegen S, Schoeman MC, Claassens S, Jansen van Rensburg P, Naidoo S, Vosloo D (2016) Foraging at wastewater treatment works affects brown adipose tissue fatty acid profiles in banana bats. Biol Open 5(2):92–99. https://doi.org/10.1242/bio.013524

Jones G (1995) Flight performance, echolocation and foraging behaviour in noctule bats *Nyctalus noctula*. J Zool 237(2):303–312. https://doi.org/10.1111/j.1469-7998.1995.tb02764.x

Klaiman JM, Price ER, Guglielmo CG (2009) Fatty acid composition of pectoralis muscle membrane, intramuscular fat stores and adipose tissue of migrant and wintering white-throated sparrows (Zonotrichia albicollis). J Exp Biol 212(23):3865–3872. https://doi.org/10.1242/jeb.034967

Kolb A (1958) Nahrung und Nahrungsaufnahme bei Fledermäusen. Z Säugetierk 23:84–95

Koussoroplis A-M, Lemarchand C, Bec A, Desvilettes C, Amblard C, Fournier C, Berny P, Bourdier G (2008) From aquatic to terrestrial food webs: decrease of the docosahexaenoic acid/linoleic acid ratio. Lipids 43(5):461–466. https://doi.org/10.1007/s11745-008-3166-5

Lam MM-Y, Martin-Creuzburg D, Rothhaupt K-O, Safi K, Yohannes E, Salvarina I (2013) Tracking diet preferences of bats using stable isotope and fatty acid signatures of Faeces. PLoS One 8(12): e83452. https://doi.org/10.1371/journal.pone.0083452

Lehnert LS, Kramer-Schadt S, Teige T, Hoffmeister U, Popa-Lisseanu A, Bontadina F, Ciechanowski M, Dechmann DKN, Kravchenko K, Presetnik P, Starrach M, Straube M, Zoephel U, Voigt CC (2018) Variability and repeatability of noctule bat migration in Central Europe: evidence for partial and differential migration. Proc R Soc B Biol Sci 285(1893):20182174. https://doi.org/10.1098/rspb.2018.2174

Levin E, Yom-Tov Y, Hefetz A, Kronfeld-Schor N (2013) Changes in diet, body mass and fatty acid composition during pre-hibernation in a subtropical bat in relation to NPY and AgRP expression. J Comp Physiol B 183(1):157–166. https://doi.org/10.1007/s00360-012-0689-0

Maillet D, Weber JM (2007) Relationship between n-3 PUFA content and energy metabolism in the flight muscles of a migrating shorebird: evidence for natural doping. J Exp Biol 210(3):413–420. https://doi.org/10.1242/jeb.02660

McGuire LP, Fenton MB, Guglielmo CG (2013) Phenotypic flexibility in migrating bats: seasonal variation in body composition, organ sizes and fatty acid profiles. J Exp Biol 216(5):800–808. https://doi.org/10.1242/jeb.072868

McGuire LP, Jonasson KA, Guglielmo CG (2014) Bats on a budget: torpor-assisted migration saves time and energy. PLoS One 9(12): e115724. https://doi.org/10.1371/journal.pone.0115724

McWilliams SR, Guglielmo C, Pierce B, Klaassen M (2004) Flying, fasting, and feeding in birds during migration: a nutritional and physiological ecology perspective. J Avian Biol 35(5):377–393. https://doi.org/10.1111/j.0908-8857.2004.03378.x

Munro D, Thomas DW (2004) The role of polyunsaturated fatty acids in the expression of torpor by mammals: a review. Zoology 107(1):29–48. https://doi.org/10.1016/j.zool.2003.12.001

Neuweiler G (2000) The biology of bats. Oxford University Press, Oxford

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin P, O'Hara R, Simpson G, Solymos P, Stevens M, Szoecs E, Wagner H (2013) Vegan: community ecology package R package version 254

Pierce BJ, McWilliams SR (2014) The fat of the matter: how dietary fatty acids can affect exercise performance. Integr Comp Biol 54(5):903–912. https://doi.org/10.1093/icb/icu098

Pierce BJ, McWilliams SR, O'Connor TP, Place AR, Guglielmo CG (2005) Effect of dietary fatty acid composition on depot fat and exercise performance in a migrating songbird, the red-eyed vireo. J Exp Biol 208(7):1277–1285. https://doi.org/10.1242/jeb.01493

Poulton EB (1929) British insectivorous bats and their prey. Proc Zool Soc London 99(2):277–303. https://doi.org/10.1111/j.1469-7998.1929.tb07743.x

Price ER (2010) Dietary lipid composition and avian migratory flight performance: development of a theoretical framework for avian fat storage. Comp Biochem Physiol A Mol Integr Physiol 157(4):297–309. https://doi.org/10.1016/j.cbpa.2010.05.019

Price ER, Guglielmo CG (2009) The effect of muscle phospholipid fatty acid composition on exercise performance: a direct test in the migratory white-throated sparrow (Zonotrichia albicollis). Am J Phys Regul Integr Comp Phys 297(3):R775–R782. https://doi.org/10.1152/ajpregu.00150.2009

Rosner E, Voigt CC (2018) Oxidation of linoleic and palmitic acid in pre-hibernating and hibernating common noctule bats revealed by 13C breath testing. J Exp Biol 221(4):jeb168096

Ruf T, Arnold W (2008) Effects of polyunsaturated fatty acids on hibernation and torpor: a review and hypothesis. Am J Physiol Regul Integr Comp Physiol 294(3):R1044–R1052. https://doi.org/10.1152/ajpregu.00688.2007

Ruf T, Valencak T, Tataruch F, Arnold W (2006) Running speed in mammals increases with muscle n-6 polyunsaturated fatty acid content. PLoS One 1(1):e65. https://doi.org/10.1371/journal.pone.0000065

Troxell SA, Holderied MW, Pētersons G, Voigt CC (2019) Nathusius' bats optimize long-distance migration by flying at maximum range speed. J Exp Biol 222(4):jeb176396. https://doi.org/10.1242/jeb.176396

Valencak TG, Ruf T (2007) N3 polyunsaturated fatty acids impair lifespan but have no role for metabolism. Aging Cell 6(1):15–25. https://doi.org/10.1111/j.1474-9726.2006.00257.x

Voigt CC, Sorgel K, Suba J, Keiss O, Petersons G (2012) The insectivorous bat *Pipistrellus nathusii* uses a mixed-fuel strategy to power autumn migration. Proc R Soc B Biol Sci 279(1743):3772–3778. https://doi.org/10.1098/rspb.2012.0902

Warner T, Zar JH (1982) Fatty acid composition of brown fat and brain fat of the little brown bat, Myotis lucifugus, during hibernation. Comp Biochem Physiol B 73(3):613–615. https://doi.org/10.1016/0305-0491(82)90084-0

Weber JM (2009) The physiology of long-distance migration: extending the limits of endurance metabolism. J Exp Biol 212(5):593–597. https://doi.org/10.1242/jeb.015024

Weber JM (2011) Metabolic fuels: regulating fluxes to select mix. J Exp Biol 214(2):286–294. https://doi.org/10.1242/jeb.047050

Wells HJ, Makita M, Wells WW, Krutzsch PH (1965) A comparison of the lipid composition of brown adipose tissue from male and female bats (Myotis lucifugus) during hibernating and non-hibernating seasons. Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism 98(2):269–277. https://doi.org/10.1016/0005-2760(65)90120-7

Wikelski M, Tarlow EM, Raim A, Diehl RH, Larkin RP, Visser GH (2003) Costs of migration in free-flying songbirds. Nature 423(6941):704–704. https://doi.org/10.1038/423704a