

RESEARCH ARTICLE

Diet and ambient temperature interact to shape plasma fatty acid composition, basal metabolic rate and oxidative stress in great tits

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ABSTRACT

Diet and ambient temperature affect animal physiology, survival and reproductive success. However, knowledge of how these environmental factors interact to shape physiological processes and life-history traits of birds and other animals is largely lacking. By exposing adult great tits (*Parus major*) to two contrasting diets (saturated or unsaturated fatty acids; SFAs and UFAs, respectively) and ambient temperatures (3°C versus 20°C) that the birds encounter in nature, we investigated the effects of these two factors on several physiological parameters. Our results show that diet and ambient temperature interact to affect the composition of plasma fatty acids, basal metabolic rate (BMR) and oxidative stress, which are thought to affect the life-history and survival of individuals. Specifically, birds provided the SFA-rich diet had higher mass-specific BMR and oxidative stress (levels of lipid peroxidation) after exposure to low compared with high ambient temperature, whereas the opposite pattern was evident for birds with a UFA-rich diet. Surprisingly, birds on the SFA diet had higher relative levels of monounsaturated fatty acids compared with the UFA-fed birds at low ambient temperature, whereas the opposite, and expected, pattern was found at the high temperature. Although the present study focuses on the physiological implications of the diet×temperature interaction, our results might also be important for the leading theories of ageing, which currently do not take interactions between environmental factors into account. In addition, the present results are important for wildlife management, especially with regards to anthropogenic feeding of wild animals across variable and changing climatic conditions.

KEY WORDS: Climate, Fatty acids, Lipid peroxidation, Metabolic rate, Oxidative stress, *Parus major*

INTRODUCTION

Nutrition is of paramount importance for animal physiology. Yet, surprisingly little is known about its effects on wild birds, not to mention its potential interaction effects with other environmental factors such as ambient temperature. These interactions can be more important than previously appreciated, especially considering their effects on physiological processes linked to animal fitness. Metabolic rate and oxidative stress are two such processes that respond to changes in both diet and ambient temperature (Broggi et al., 2004; Isaksson et al., 2011; Lin et al., 2006; McKechnie and Swanson, 2010; Stier et al., 2014; Swanson and Vézina, 2015), and are connected to longevity (Buttemer et al., 2010; Careau et al., 2010).

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In light of these physiological processes, the fatty acid (FA) composition of the diet is especially interesting. First, both FA intake and ambient temperature influence the FA composition of animal tissues (Balnave, 1973; Ben-Hamo et al., 2011; McCue et al., 2009; Pierce et al., 2005), with extended effects on metabolic rate and physiological performance (Ben-Hamo et al., 2013; Guglielmo, 2010; Pierce et al., 2005; Price and Guglielmo, 2009; Price et al., 2018; Twining et al., 2016). For instance, changes in ambient temperature might induce alterations in the levels of saturated fatty acids (SFAs) relative to unsaturated fatty acids (UFAs) to maintain cell membrane fluidity (Hazel, 1995; homeoviscous adaptation hypothesis; Sinensky, 1974), and consequently, metabolic rate (Montgomery et al., 2012). Such plastic regulation of membrane composition has been shown in ectothermic vertebrates and hibernating mammals, but it has also been suggested as a possible mechanism for cold acclimation and rest-phase hypothermia in birds (Ben-Hamo et al., 2011, 2013, and references therein).

In addition, the FA composition of the diet can also affect metabolic rate through differences between FAs in their rate of oxidation (Leyton et al., 1987; Price et al., 2011), through FA-specific effects on cell membrane leakiness (Hulbert, 2008) and possibly through lipid phase behaviour (Calhoon et al., 2015; Epand et al., 1991). For instance, chickens feeding from diets rich in polyunsaturated fatty acids (PUFAs) tended to have higher resting metabolic rates (RMRs) than those feeding from SFA-enriched diets (Newman et al., 2002). Moreover, work-induced peak metabolic rate was higher in vireos (*Vireo olivaceus*) feeding from a diet abundant in the PUFA linoleic acid than it was in birds feeding from diets abundant in the MUFA oleic acid (Pierce et al., 2005) but with higher total UFA content. This result suggests that the degree of FA unsaturation affects the metabolic rate of birds, with increased unsaturation being associated with a higher rate of FA oxidation (Price et al., 2011). However, whether or how the FA composition of the diet interacts with ambient temperature to affect the metabolic rate in birds is largely unknown (but see Price et al., 2018). The FA composition of the diet may well have implications for the established negative relationship between metabolic rate and ambient temperature (Broggi et al., 2004, and references therein).

Furthermore, the FA composition of an animal and its metabolic rate – thus, diet and ambient temperature – can affect oxidative stress. Oxidative stress is a state of increased exposure to reactive oxygen species (ROS) that overwhelms the antioxidant system, resulting in oxidative damages, which can lead to cellular dysfunction and ageing (Hulbert, 2005, 2008; Isaksson, 2015; Kirkwood, 2002). In fact, prevailing theories of ageing build on the existence of a positive association between metabolic rate and oxidative stress, i.e. the faster pace of life theory. A higher metabolic rate increases the escape rate of ROS from the mitochondrial membrane, and consequently increases the oxidative damage (Finkel and Holbrook, 2000; Harman, 1956). However, empirical

evidence to support the ‘oxidative stress theory of ageing’ is not conclusive (Finkel and Holbrook, 2000; Isaksson et al., 2011; Kirkwood, 2002; Speakman and Selman, 2011).

An extended version of the oxidative stress theory of ageing is the ‘membrane pacemaker theory of ageing’ (Calhoun et al., 2015; Hulbert, 2005, 2008). This theory emphasizes that the FA composition of membranes influences their sensitivity to oxidative stress, with a higher degree of unsaturation increasing lipid peroxidation and consequently the rate of ageing (Hulbert, 2005). In addition, an increased proportion of polyunsaturated fatty acids [PUFAs, especially docosahexaenoic acid (DHA)] in phospholipid bilayers is thought to result in leakier membranes, increasing metabolic rate through upregulated activity of membrane pumps to maintain electrochemical gradients (Hulbert, 2008). Hence, diet and ambient temperatures are likely to affect the links between metabolic rate and oxidative stress, both directly and indirectly, with potential consequences for longevity, although support for the membrane pacemaker theory is mixed (Calhoun et al., 2015; Price et al., 2018).

As a step towards a better understanding of how diet and temperature may interact to affect bird physiology, we exposed great tits (*Parus major* Linnaeus 1758) to two ambient temperatures (20°C and 3°C), and two contrasting FA diets (SFA-rich vs UFA-rich) that the birds frequently encounter at feeding tables, and which may affect their performance and fitness. We investigated the effects of these factors and their interaction on blood plasma FA composition, basal metabolic rate (BMR) and oxidative stress. We hypothesized that diet and ambient temperature interact to affect the plasma composition of SFA and UFA. This hypothesis is based on the contrasting effects of these two FA groups on membrane fluidity (homeoviscous adaptation) and their different rates of oxidation (Price et al., 2011), hence ATP generation, in relation to differing temperature-dependent energetic demands. On the basis of this, we made three specific predictions. (1) Birds ingesting the SFA-rich diet would show evidence of conversion of SFAs into monounsaturated FAs (MUFAs) at the low ambient temperature as predicted from the homeoviscous adaptation hypothesis, but also to increase the rate of ATP generation (i.e. BMR). This FA conversion in response to cold was not expected for the UFA-fed birds as they were predicted to already have a high proportion of UFA in their membranes. Additionally, due to the predicted diet × temperature interaction for plasma FA composition, we also hypothesized that this interaction would lead to physiological adjustments and costs, by affecting mass-corrected BMR, and oxidative stress, respectively. (2) With regards to BMR, we predicted a stronger effect of the exposure to the low ambient temperature among the SFA-fed birds, because of the predicted alteration in FA biosynthesis (prediction 1) and FA composition affecting the rate of FA oxidation. (3) Owing to the previously shown positive association between BMR and oxidative stress, and the links between FAs and oxidative stress (UFAs are more peroxidation prone than SFAs), we predicted that the SFA-fed birds would suffer from more oxidative damage at the low ambient temperature than at the high temperature (due to increased BMR and biosynthesis of more UFAs), unless oxidative damage was mitigated by boosted antioxidant capacity (AOX). Increased oxidative damage after exposure to low temperature was not expected among the UFA-fed birds, since we predicted that their FA profiles would be less affected by ambient temperature. Hence, to assess the oxidative state, we measured the total antioxidant capacity (AOX) and malondialdehyde (MDA) levels, a frequently used biomarker of oxidative damage (lipid peroxidation) to UFAs. We

focused the present study on measurements from plasma samples to get a reflection of whole body physiology, rather than physiology of specific cell or tissue types.

MATERIALS AND METHODS

Birds and experimental design

All procedures were conducted in accordance with Swedish legislation and approved by the Malmö–Lund animal ethics committee (M175-14). Female great tits (*Parus major*) were captured from nest boxes during the evenings of 15–18 January 2014 ($n=29$). Two field sites were used: three parks within the city of Malmö (55°35′N, 12°59′E, $n=14$), as well as the rural forest of Vomb (approximately 42 km NE of Malmö, 55°40′N, 13°31′E, $n=15$), both in Southern Sweden (see Andersson et al., 2015 for detailed habitat characteristics). The average daily ambient temperature during the 2 weeks before capture and the last day of capture (i.e. 1–18 Jan 2014) was 4°C in both environments. Females were brought into the laboratory where they were kept individually in cloth bags overnight (at 10°C), after which a first blood sample (110 µl) was collected with a heparinized syringe from the jugular vein of the post-absorptive females (i.e. 12–14 h after they entered their nest box to roost), and tarsus length and body mass were measured. Blood samples were kept on ice for 0–1 h, after which they were centrifuged at 170 g for 10 min, the plasma supernatant was stored at –80°C until analysed.

After the first sampling bout, females were individually housed (in ~1 m³ cages) inside a climate-controlled chamber where they were held at 10°C (8 h:16 h light:dark; lights on at 09:00 h) for 2–5 days before the experiment started (the number of days depended on when they were caught). Caged females always had *ad libitum* access to their diet and water. Females from the two populations were equally split between the diet rich in SFAs, and the diet rich in UFAs (Table 1). Likewise, birds caught on different days were equally split into the two diet treatments. The number of days in captivity prior to the start of the experiment did not influence any of our physiological measurements (all $P>0.10$). The SFA diet consisted of coconut fat mixed with boiled wheat berry at a 1:5 ratio (w/w). The UFA diet was a mix of sunflower oil and sunflower seeds (10 seeds per day) with boiled wheat berry at the same w/w ratio. To compensate for initial differences in the antioxidant α -tocopherol (vitamin E) between the two diets, 50 mg α -tocopherol was added per 100 g coconut fat, ensuring that both diets contained 60.5 mg α -tocopherol per 100 g fat. Birds on both diets were also provided with 10 mealworms per day.

At the start of the experiment, the ambient temperature was gradually increased over 1 day to 20°C (henceforth referred to as ‘high’ ambient temperature) and then kept constant for 6 days. In the morning of day 8 of the experiment, a second blood sample was taken (see above) and birds were weighed. After sampling, birds were retained on their diets and the temperature was gradually reduced over 2 days to 3°C (henceforth referred to as ‘low’ ambient temperature), which was then kept constant for 6 days. In the morning of the last day of the cold treatment (day 16 of the experiment), a third blood sample was taken, and birds were weighed again. Birds were then put back into their cages with the same diets, and the temperature was increased over 2 days to 20°C and then kept constant for 6 days. This second high temperature exposure was included to be able to verify that the physiological effects observed were *de facto* due to changes in ambient temperature, and not simply due to time in captivity or time-dependent physiological adjustments to uniform but contrasting diets. During the last morning of this high ambient

Table 1. Relative levels of fatty acids (percentage of total fatty acid content) in the SFA-rich and UFA-rich fats, mealworms and boiled wheat berry (data are the means of 3 measurements)

Fatty acid	Formula	Fatty acid group	Coconut fat (SFA-rich)	Sunflower oil (UFA-rich)	Mealworm	Boiled wheat berry*
Caprylic acid	8:0	SFA	3.2	–	–	–
Capric acid	10:0	SFA	4.2	–	–	–
Lauric acid	12:0	SFA	43.8	–	0.2	–
Myristic acid	14:0	SFA	21.4	–	4.4	–
Palmitic acid	16:0	SFA	13.0	7.9	14.3	20.3
Stearic acid	18:0	SFA	13.7	4.2	3.1	0.5
Palmitoleic acid	16:1 n -7	MUFA	–	0.1	2.8	0.1
Oleic acid	18:1 n -9	MUFA	0.6	32.7	46.7	13.5
<i>cis</i> -Vaccenic acid	18:1 n -7	MUFA	–	0.5	–	1.7
Linoleic acid	18:2 n -6	ω -6 PUFA	0.1	54.5	27.8	60.3
α -Linolenic acid	18:3 n -3	ω -3 PUFA	–	<0.1	0.7	3.6

SFA, saturated fatty acid; UFA, unsaturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

*The total fat content of boiled wheat berry is <1% (w/w) and thus is likely to have only minor effects on bird plasma fatty acid composition.

temperature treatment (day 24 of the experiment), a fourth blood sample was taken, birds were weighed a final time and then released where they were captured. All blood samples were taken before lights were turned on, assuring that all females were in a post-absorptive state. BMR was measured on three occasions (see below) prior to each of the three experimental bleeding sessions.

Fatty acid extraction and gas chromatography/mass spectrometry (GC/MS)

The protocol for FA extraction and analysis followed Andersson et al. (2015). A total lipid extraction of 5 μ l plasma was performed for 1 h at room temperature (RT) using 50 μ l chloroform:methanol (2:1 v/v) containing 1.67 μ g of the internal standard methyl *cis*-10-heptadecenoate (purity >99%, Aldrich). The solvent was then evaporated under a stream of N₂, and the concentrated lipid extracts were subjected to base methanolysis using 100 μ l KOH/methanol (0.5 mol l⁻¹) for 1 h at 40°C to convert the fatty-acyl moieties into corresponding methyl esters (FAME). The reaction was terminated with 100 μ l HCl/methanol (0.5 mol l⁻¹), and the resulting FAMEs were extracted in 300 μ l re-distilled *n*-hexane. The samples were washed twice with deionized H₂O, and then dried using anhydrous sodium sulfate. The extracts were analysed on an Agilent 5975 mass spectrometer coupled to an Agilent 6890 GC, equipped with an HP-88 capillary column [(88%-Cyanopropyl)aryl-polysiloxane; 30 m, 0.25 mm inner diameter, film thickness (d.f.) 0.20 μ m; Agilent]. Helium was used as carrier gas (flow: 1 ml min⁻¹). The following temperature programme was used: 80°C for 1 min, then increased by 10°C min⁻¹ to 230°C which was held for 20 min.

Twenty-one FAs (Tables S1 and S2) could be identified by comparing mass spectra and retention times with those of synthetic standards. Two of the (very) minor FAs (arachidic acid and γ -linolenic acid) co-eluted, thus they could only be quantified as a combined peak. Concentrations of plasma FAs were calculated based on the amount of the internal standard (Tables S1 and S2). The FAs of the two dietary fats (i.e. coconut fat and sunflower oil), wheat berry and the mealworm supplement (excluding their heads, which are not eaten by the tits) were extracted, analysed by GC/MS, and quantified using exactly the same methods as for the plasma FAs, but with the chloroform:methanol extraction step run overnight (n =3 per dietary item). The FA composition of these foods is shown in Table 1.

Markers of oxidative stress

Total non-enzymatic antioxidant capacity (AOX) and lipid peroxidation were measured in blood plasma. The total

AOX was measured using the ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996). 5 μ l plasma was used for the analysis, which proceeded according to Eikenaar et al. (2017).

Lipid peroxidation was measured as the amount of malondialdehyde (MDA) in plasma (analysed using 20 μ l plasma) using gas chromatography/mass spectrometry (GC/MS). The extraction and identification of the MDA proceeded according to Eikenaar et al. (2017). The plasma was mixed with 50 μ l O-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine hydrochloride (PFBHA HCl; 1 mmol l⁻¹ in sodium acetate buffer; pH 5.0) for derivatization. This reaction proceeded for 1 h at room temperature. The resulting MDA-bis-(PFB-oxime) derivatives were then extracted in 300 μ l *n*-hexane containing 1.65 pg μ l⁻¹ 3-bromofluorobenzene as internal standard. The samples were washed twice with deionized H₂O and then dried using anhydrous sodium sulfate. After condensation under N₂, the samples were analysed using an Agilent 5975 mass-selective detector coupled to an Agilent 6890 GC with a non-polar capillary column (HP-5MS; 30 m, 0.25 mm inner diameter, d.f. 0.25 μ m; J&W Scientific). The GC oven was programmed to 60°C for 1 min, and then increased by 15°C min⁻¹ to 150°C followed by 10°C min⁻¹ to 270°C, which was held for 5 min. The characteristic ion at m/z 250 was measured under selected ion monitoring mode and used for MDA quantification. All chemicals used were purchased from Sigma-Aldrich (Stockholm, Sweden).

Respirometry

BMR was measured as overnight oxygen consumption (\dot{V}_{O_2}) using the open-circuit respirometer previously described by Nord and Nilsson (2011). Owing to a limited number of test chambers/channels, not all birds could be included in these measurements. In total, 47 recordings were done, one to three nights prior to blood sampling (see Table S3). Fifteen birds were recorded for all three ambient temperature treatment points (n_{UFA} =8, n_{SFA} =7 individuals); two additional UFA-fed birds (of rural origin) died after the first measurement. The birds were of both urban (n =8) and rural (n =9, including the two fatalities) origin. The metabolic measurements lasted for 12 h (19:00 to 07:00 h) to ensure a post-absorptive state. Each bird was placed in a sealed Plexiglas chamber (0.6 litres) and placed in the dark in a climate cabinet (type VEM 03/500, Heraeus Vötsch, Hanau, Germany), maintained at 30°C, i.e. within their thermoneutral zone. One chamber was always left empty, acting as the baseline measurement in the setup.

The flow rate was regulated and measured by a Flowbar8 (Sable Systems) and set to 200 ml min⁻¹. A multiplexer (RM8, Sable Systems) switched between the channels every 13 min and only one channel actively measured O₂ and CO₂ at the time. To allow flushing of the tubing and analysers, no data were collected for 2 min between each 13 min measurement period. The CO₂ analyser was zero-calibrated with pure nitrogen (99.998% N₂). The oxygen analyser was also zero calibrated with pure nitrogen and then calibrated with dry ambient air to 20.95% oxygen before measurements started each evening.

\dot{V}_{O_2} was calculated according to Withers (2001), accounting for the fact that CO₂ was not scrubbed prior to measurements. The lowest 7 min running average of \dot{V}_{O_2} (ml O₂ min⁻¹) was used as a measure of BMR. All BMR results reported in the main document are mass-corrected. The raw BMR data (not corrected for body mass), respiratory quotients and body mass for each individual BMR measurement are presented in Table S3.

Data analysis

The relative plasma levels (percentage of total FA content) of the four FA groups (total SFA, total MUFA, total ω -3 PUFA and total ω -6 PUFA) and all individual FAs were calculated and logit-transformed. MDA concentrations were log₁₀-transformed to obtain a normal distribution. The logit-transformed relative FA levels (not the absolute concentrations) were analysed statistically. Owing to overparameterization of the BMR model when mass was included, BMR was standardized for body mass and for respirometer channel by using the residuals from a general linear mixed model (GLMM) between BMR and body mass, including respirometer channel as a random factor. These residuals were then used to test for experimental effects (see below).

Prior to the experiment, there was no difference between birds from the two populations in the plasma levels of the four FA groups, MDA or body condition (mass/tarsus length³) (all $P > 0.05$). However, there was a difference in AOX between the populations ($F_{1,25} = 5.25$, $P = 0.031$), with birds of urban origin having higher AOX capacity than birds of rural origin before the experiment (see also Salmón et al., 2018). In addition, prior to the experiment there was no difference in any of the FA groups, AOX, MDA or body condition between the birds that were assigned to the different diets (all $P > 0.05$).

The experimental data (i.e. data derived from blood samples 2–4) were analysed by performing GLMMs for all physiological markers, i.e. using the four FA groups, nine individual FAs (those that accounted for >1% of total plasma FA content in at least one sample), mass-corrected BMR, body mass, MDA and AOX as response variables. All initial GLMMs included ‘diet’ (SFA vs UFA diet groups) and ‘temperature’ (high vs low) as main factors along with their interaction. Note that the two high temperature treatments were tested together in the fixed factor ‘temperature’. ‘Individual’ was included as a random factor to account for the repeated measurements. Secondly, in the case of a significant diet × temperature interaction, the diet and temperature groups were analysed separately to investigate their independent effects. Thirdly, because of the limited sample size, we investigated the interaction between population of origin and temperature separately for each of the diet groups. These GLMMs included ‘population’ (urban vs rural), ‘temperature’ (high vs low) as main factors as well as their interaction. Again, ‘individual’ was included as a random factor. From here onwards, only statistically significant results are presented. All statistical analyses were performed in JMP v.11 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Saturated fatty acids

Absolute and relative levels of all FAs are reported in Tables S1 and S2, respectively. A diet × temperature interaction ($F_{1,56} = 28.87$, $P < 0.001$) was found for relative total SFA in plasma (Fig. 1A). Consistent with a high proportion of SFA in coconut fat (Table 1), the SFA-fed birds had higher relative total SFA than the UFA-fed birds at both ambient temperatures (low: $F_{1,27} = 32.52$, $P < 0.001$; high: $F_{1,27} = 204.30$, $P < 0.001$). However, among the SFA birds, plasma relative total SFA was lower at the low ambient temperature than at the high temperature ($F_{1,25} = 12.64$, $P = 0.002$), whereas the UFA birds showed the opposite response to the different ambient temperatures ($F_{1,31} = 30.58$, $P < 0.001$). Consistent with the above results, the proportions of the individual SFAs lauric acid, myristic acid and stearic acid showed similar interactions between diet and temperature (lauric acid: $F_{1,56} = 14.45$, $P < 0.001$; myristic acid: $F_{1,56} = 70.96$, $P < 0.001$; stearic acid: $F_{1,56} = 15.62$, $P < 0.001$; Fig. 2A,B,D) as did total SFA, and were higher in plasma of the SFA birds than in the UFA birds at both temperatures (ranging from $F = 5.89$ to 764.51 , $P = 0.022$ to < 0.001). However, plasma proportions of palmitic acid showed a consistent temperature effect, higher at low compared with high ambient temperature ($F_{1,56} = 118.84$, $P < 0.001$; Fig. 2C). Within the UFA birds, relative total SFA was higher in urban compared with rural birds ($F_{1,14} = 6.62$, $P = 0.022$), independent of ambient temperature.

Monounsaturated fatty acids

Relative total MUFA in plasma also showed a significant diet × temperature interaction ($F_{1,56} = 29.78$, $P < 0.001$, Fig. 1B). Opposite to total SFA, plasma MUFA was significantly higher at the low compared with the high ambient temperature among the SFA birds ($F_{1,25} = 22.08$, $P < 0.001$). The UFA birds showed the opposite pattern ($F_{1,31} = 7.84$, $P = 0.009$). Also, diet had significant effects within each temperature group. Specifically and unexpectedly, MUFA was higher in the SFA birds than in the UFA birds at the low ambient temperature ($F_{1,27} = 4.56$, $P = 0.042$), whereas the opposite, and expected, pattern was evident at the high temperature ($F_{1,27} = 16.77$, $P < 0.001$). The most abundant plasma MUFA (oleic acid; the only MUFA present at >1% of total FA content; Fig. 2E), which is also abundant in sunflower seed oil (Table 1), accordingly showed similar results as total MUFA (diet × temperature interaction: $F_{1,56} = 29.20$, $P < 0.001$).

Polyunsaturated fatty acids

Relative total ω -6 PUFA in plasma differed between diet groups, with the UFA birds having higher proportions ($F_{1,29,64} = 53.31$, $P < 0.001$, Fig. 1C), consistent with the FA profile of sunflower seed oil (i.e. a high proportion of linoleic acid; Table 1). Temperature also affected the proportion of this FA class, with higher ω -6 PUFA proportions at the low compared to high temperature across diets ($F_{1,56} = 6.41$, $P = 0.014$). As expected, we observed a strong dietary effect of the essential FA linoleic acid, the dominating FA in sunflower seed oil, on its relative plasma level (Fig. 2F), consistent with the difference for total ω -6 PUFA (diet; $F_{1,30} = 86.23$, $P < 0.001$; temperature; $F_{1,56} = 4.85$, $P = 0.032$). Although significantly lower proportions of linoleic acid were present in the plasma of the SFA birds than in the UFA birds, it was still retained at levels >16% of the total FA content (Fig. 2F). The most likely source of this dietary FA is the supplemented mealworms, and possibly also the wheat berries, although their total fat content is below 1% after boiling (Table 1). In contrast to linoleic acid, dihomo- γ -linolenic acid (DGLA) was higher in the SFA birds than in the UFA birds

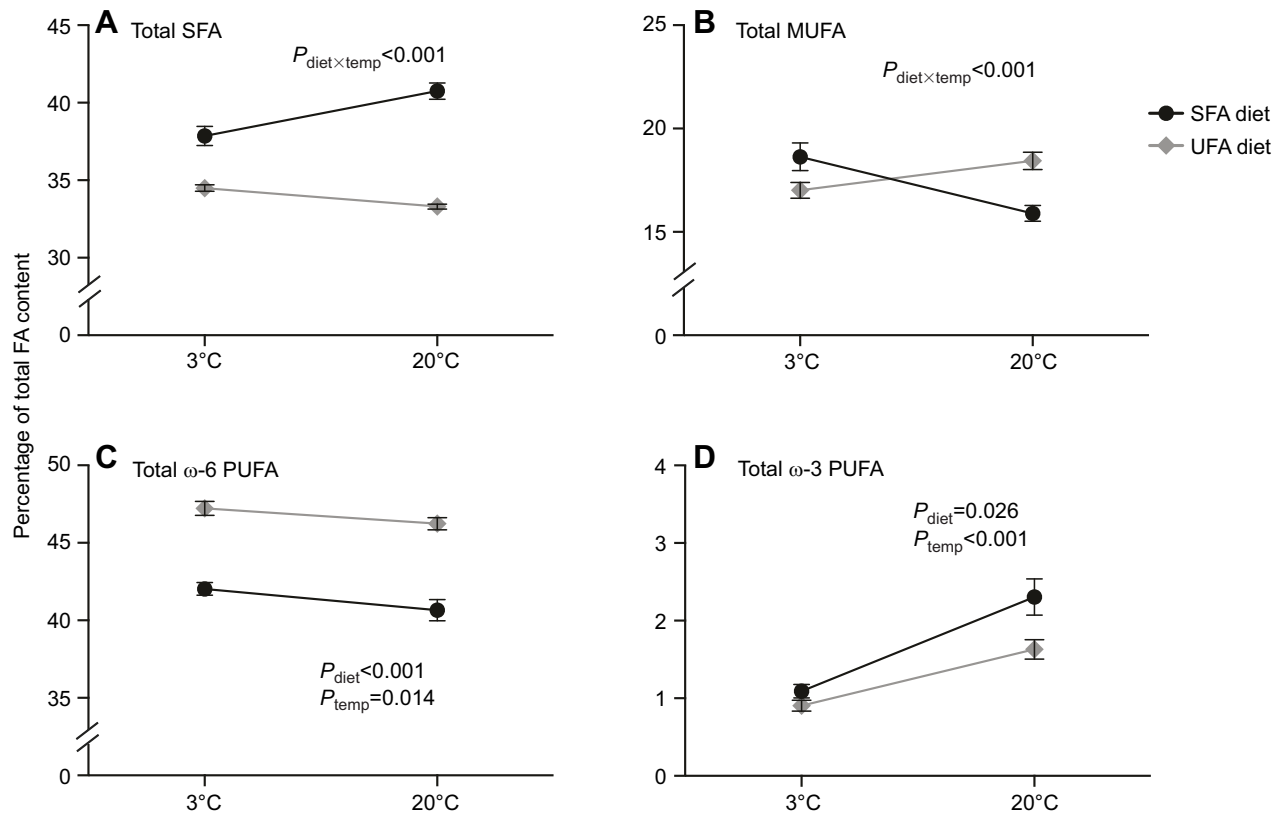


Fig. 1. Proportion of four groups of fatty acids in blood plasma of great tits provided diets rich in saturated fatty acids or unsaturated fatty acids at different ambient temperatures. (A) Total saturated fatty acids (SFAs). (B) Total monounsaturated fatty acids (MUFAs). (C) Total ω -6 polyunsaturated fatty acids (PUFAs). (D) Total ω -3 PUFAs. Significant effects from the models are indicated by the P -values. When the diet \times temperature interaction was significant, the effects of main factors are not reported. In these cases, the fatty acid (FA) proportions of plasma from birds provided the two diets were analysed separately, with statistical outcomes reported in the main text. Note that the y -axis scale differs between figure panels. $N_{\text{total}}=29$ birds. Data are means \pm s.e.m.

($F_{1,29}=17.29$, $P<0.001$). As for linoleic acid, DGLA was present in the plasma at slightly higher proportions at the low compared with the high ambient temperature ($F_{1,56}=4.82$, $P=0.032$). No significant results were revealed for arachidonic acid (Fig. 2H).

Total relative ω -3 PUFA in plasma was low in all experimental birds, consistent with the low abundance of this FA class in the two provided fat diets (Table 1). Nevertheless, total relative ω -3 PUFA still differed between diet groups, with the SFA birds having somewhat higher levels ($F_{1,31.01}=5.44$, $P=0.026$, Fig. 1D). Total ω -3 PUFA was also higher at the high compared with the low ambient temperature across the two diet groups ($F_{1,56}=53.42$, $P<0.001$). The effect of temperature on total ω -3 PUFA was largely driven by the variation in the most abundant FA of this class, DHA (temperature; $F_{1,56}=63.20$, $P<0.001$; Fig. 2I; all other ω -3 PUFAs were well below 1% of total plasma FA content; Table S2).

Metabolic rate and body mass

Mass-corrected BMR (always measured at 30°C) showed a significant diet \times temperature interaction ($F_{1,29.437}=10.90$, $P=0.003$, Fig. 3). Specifically, the SFA-provided birds had higher mass-corrected BMR after exposure to the low compared with the high ambient temperature ($F_{1,13}=26.31$, $P<0.001$), whereas the mass-corrected BMR in the UFA birds did not change significantly with the previous ambient temperature exposure. In addition, mass-corrected BMR was higher in the SFA birds than in the UFA birds after the low ambient temperature exposure, whereas the pattern was reversed after exposure to the high temperature (low: $F_{1,13}=4.89$, $P=0.046$; high: $F_{1,14.9}=6.39$, $P=0.023$). Population of origin

affected mass-corrected BMR, but only in the UFA birds ($F_{1,7.73}=7.13$, $P=0.029$), with rural birds having higher mass-corrected BMR than urban birds. It should also be noted that the diet-specific significant effect on mass-corrected BMR was not driven by changes in body mass, because there was no significant diet \times temperature interaction on body mass ($F_{1,27.91}=0.58$, $P=0.452$).

The effect of diet and ambient temperature was also analysed for the body mass of all birds included in the experiment (i.e. also those not included in the BMR measurements). Only ambient temperature affected body mass, with a higher mass at the low compared with the high temperature across diet treatments ($F_{1,56}=10.86$, $P=0.002$).

Oxidative stress

The diet \times temperature interaction was also significant for MDA ($F_{1,56}=4.63$, $P=0.040$, Fig. 4A). MDA tended to increase with ambient temperature in the UFA birds, but rather tended to decrease with temperature in the SFA birds. However, none of these independent effects were significant between temperatures within a given diet, or between diets within a given temperature (all $P>0.05$). Diet affected the total AOX capacity ($F_{1,28.33}=14.27$, $P<0.001$, Fig. 4B), with the UFA birds having higher AOX capacity than the SFA group across temperatures.

DISCUSSION

This study shows that diet and ambient temperature to a large extent interact to shape physiological processes. Thus, experiments designed to address the effect of one ecological factor in isolation

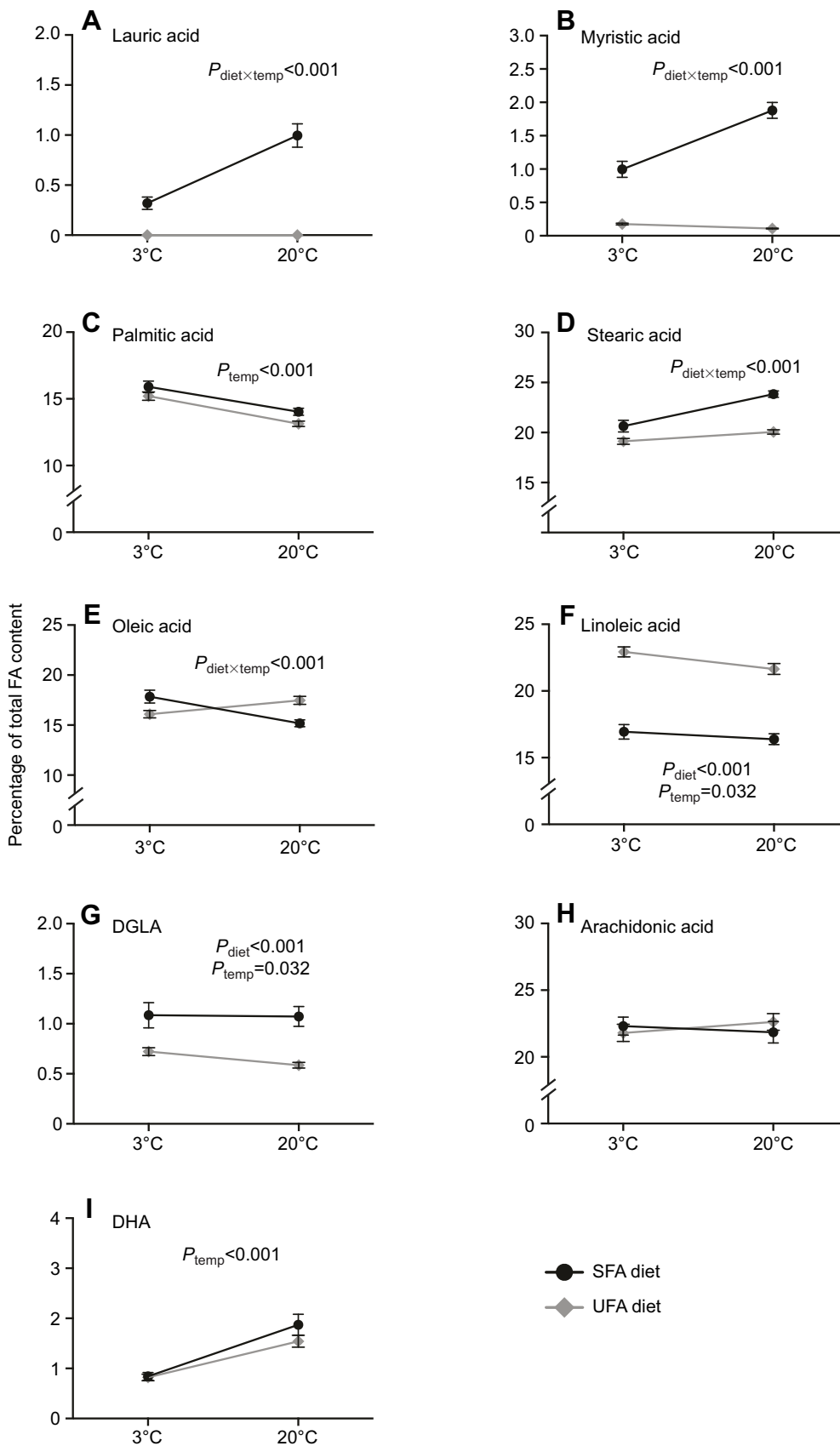


Fig. 2. Proportion of individual fatty acids at $\geq 1\%$ of total fatty acid content in blood plasma of great tits provided diets rich in SFA or UFA at different ambient temperatures. (A–D) Plasma variation in individual SFAs. (E) The monounsaturated FA oleic acid. (F–H) ω -6 Polyunsaturated FAs. (I) The ω -3 polyunsaturated FA DHA. Significant effects from the models are indicated by the P -values. When the diet \times temperature interaction was significant, the effects of main factors are not reported. In these instances, the fatty acid proportions of birds provided the two diets were also analysed separately (see main text). Note that the y -axis scale differs between figure panels. DHA, docosahexaenoic acid, DGLA, dihomo- γ -linolenic acid. $N_{\text{total}} = 29$ birds. Data are means \pm s.e.m.

might not always be relevant in natural contexts where multiple factors act in concert to shape animal physiology. We demonstrate diet \times temperature interactions on plasma FA composition, mass-

corrected BMR and oxidative stress, which are all linked to each other and to animal fitness (reviewed in Hulbert and Abbott, 2012; Swanson, 2010).

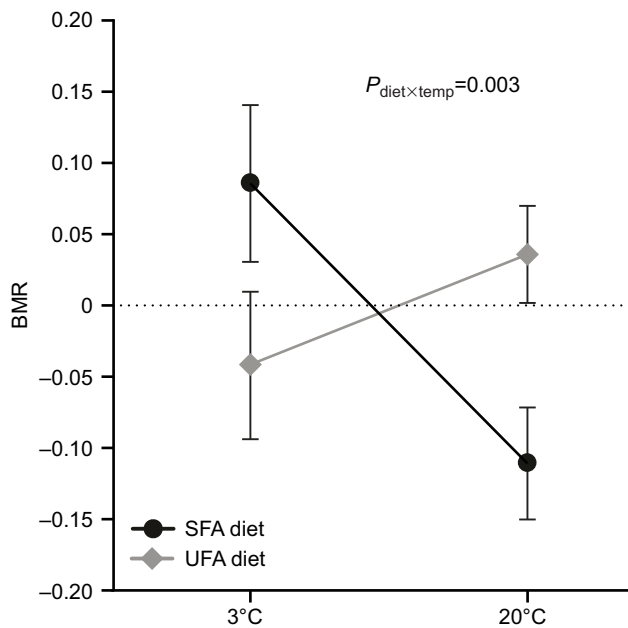


Fig. 3. Residual BMR from a general linear mixed model between BMR ($\text{ml O}_2 \text{ min}^{-1}$) and mass (g) of great tits provided a diet enriched in SFA or UFA after exposure to different ambient temperatures. All BMR measurements were taken within the thermoneutral zone at 30°C . $N_{\text{total}}=17$ birds. Data are residual LSMs \pm s.e.m.

Interaction effects of diet and temperature on plasma fatty acid composition

We provided great tit females with either of two diets that differed markedly in the relative amounts of SFAs vs UFAs, and demonstrate clear effects on plasma FA composition, largely reflecting differences in the fat content between the diets. Both diets are ecologically relevant because oils from seeds comprise a large proportion of the natural and anthropogenic winter nutrition of great tits as well as other passerine birds, and saturated fat is regularly also provided at feeding tables through tallow balls and coconut fat (Andersson et al., 2015; Robb et al., 2008). Although only oil from sunflower seeds was provided to the UFA birds, the fatty acid composition of these seeds largely resembles that of other common seeds and nuts provided at feeding tables, including oat seeds and peanuts (Beare-Rogers et al., 2001; Becker, 2008).

In accordance with our first prediction, ambient temperature modulated the effects of the diet treatments for two of the FA groups, the total MUFA and total SFA. This is interesting because MUFA (especially oleic acid; Table 1) was abundant in the UFA-rich diet but almost absent in the SFA-rich diet. Yet, the UFA-fed birds had higher relative MUFA than the SFA-fed birds only at the high ambient temperature; the opposite was true at the low temperature. Interestingly, an interaction in the opposite direction was revealed for total SFA, and for the individual SFAs stearic acid, myristic acid and lauric acid (Fig. 2). Since the dominant MUFA oleic acid can be biosynthesized from the abundant stearic acid, this finding suggests that birds ingesting a surplus of SFA may convert a significant amount of stearic acid into its monounsaturated counterpart at low ambient temperatures. Moreover, the shorter-chained SFAs lauric acid and myristic acid (12 and 14 carbon SFAs, respectively) do not appear to have been desaturated directly because we did not detect monounsaturated 12 and 14 carbon FAs in the plasma of any of the birds. Instead, these SFAs may have been elongated and then possibly converted to oleic acid, unless they decreased in plasma as a result of the birds' metabolism. In addition to temperature and diet-dependent regulation of FA biosynthesis, it is possible that selective mobilization of FAs from fat stores to the circulating system in face of differing, temperature-dependent, metabolic demands contributes to the observed variation in plasma SFA and MUFA (Guglielmo, 2010; McWilliams et al., 2004; Price et al., 2008, 2013). Although we measured FAs in plasma, the observed variation in relative SFA and MUFA levels is in line with the 'homeoviscous adaptation hypothesis', predicting that a lower proportion of SFA is desirable at low ambient temperatures to maintain membrane fluidity and cell function (Hazel, 1995; Sinensky, 1974). However, our results also suggest that reductions in the proportion of SFAs, at least in the plasma, only occurs in response to cold when SFAs are present above a certain threshold, because we did not observe this temperature-dependent effect on plasma SFA in the UFA-fed birds. Intriguingly, yellow-rumped warblers (*Dendroica coronata*) strongly preferred a diet rich in oleic acid over a stearic acid-rich diet, especially at low ambient temperatures (1°C). In addition, warblers that had no choice but to feed from a diet rich in stearic acid displayed significant body weight loss and mortality (7 of 10 birds died) when ambient temperature was reduced from 21°C to 11°C over 3 days (McWilliams et al., 2002). Taken together, these findings suggest

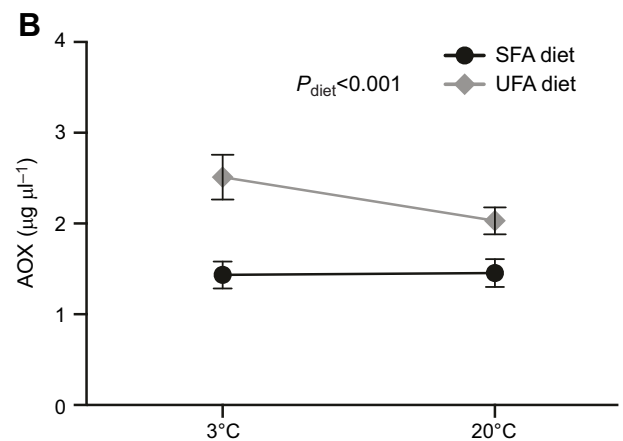
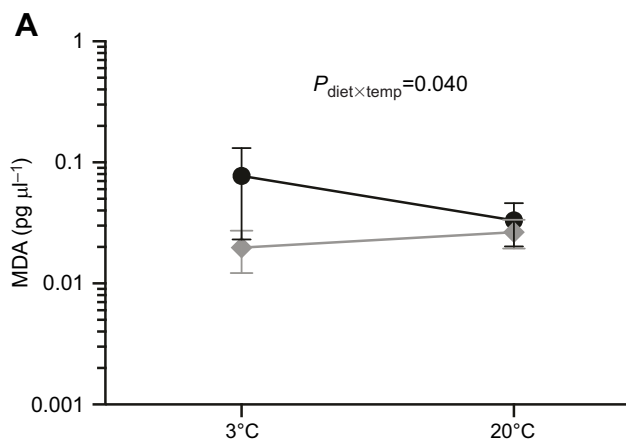


Fig. 4. Measures of oxidative stress in blood plasma of great tits provided diets either rich in SFA or UFA at different ambient temperatures. (A) Lipid peroxidation as measured by the plasma level of malondialdehyde (MDA). (B) Total antioxidant (AOX) capacity. Significant effects from the models are indicated by the P -values. The diet \times temperature interaction was significant for MDA, and the effects of main factors are thus not reported. The MDA level was therefore also analysed separately for birds that were provided the two diets, with statistical outcomes reported in the main text. $N_{\text{total}}=29$ birds. Data are means \pm s.e.m.

that retaining a high proportion of SFA in the diet of birds, or in their tissues, at low ambient temperature could be associated with a fitness cost. For example, retaining too rigid mitochondrial membranes at low ambient temperature when there is also a high energy demand for thermoregulation could be detrimental to bird survival, especially because SFAs are also oxidized by birds at a slower rate than mono- or di-unsaturated FAs of the same chain length (i.e. 18 carbons) (Price et al., 2011).

Interaction effects of diet and temperature on basal metabolic rate

Previous studies on birds and mammals report independent effects of the FA profile of the diet and ambient temperature on metabolic rate (Broggi et al., 2004; Hulbert and Abbott, 2012; McKechnie and Swanson, 2010). In rats (Shimomura et al., 1990; Takeuchi et al., 1995) and birds (Newman et al., 2002), the intake of UFA-enriched diets resulted in higher resting- or work-induced metabolic rates compared to SFA-provided controls when animals were held at constant and relatively high ambient temperatures. The observed higher metabolic rates were proposed to be due to a higher rate of oxidation of UFAs compared with that of SFAs, with especially longer-chain SFAs being more frequently incorporated into fat storages (Leyton et al., 1987; Sanz et al., 2000a,b; Shimomura et al., 1990; Takeuchi et al., 1995). Price and colleagues (2011) showed that muscle tissue of white-throated sparrows (*Zonotrichia albicollis*) oxidized the di-unsaturated linoleic acid (18:2n-6) 40% faster, and the saturated stearic acid (18:0) 75% slower, than the monounsaturated oleic acid (18:1n-9). These results suggest that the degree of unsaturation of FA substrates, and thus the composition of available FAs, is an important modulator of the metabolic rate of birds (see also Pierce et al., 2005). In addition, the higher proportion of PUFAs in cell membranes of birds on a UFA-rich diet is thought to increase ion leakage, resulting in an increased activity of ion pumps to maintain electrochemical gradients across membranes and thus increasing metabolic rate (Hulbert, 2005, 2008; Hulbert and Else, 2005; Pamplona et al., 1998).

In accordance with our second prediction, we found an interaction between diet and previous ambient temperature exposure on mass-corrected BMR. The independent analysis of body mass confirms that this interaction for BMR was not driven by differential changes in mass in relation to diet and ambient temperature. Adjustments in BMR in response to different ambient temperatures have also been shown in other bird species in captivity over similar short time scales (days to weeks) (McKechnie et al., 2007). Our observed differences in mass-corrected BMR between birds of the two diet groups after the high temperature treatment are in accordance with those reported for other species (discussed above), with birds provided with the UFA-rich diet having higher BMR compared with the SFA-fed birds. However, our results are reversed after the birds had adjusted to the low ambient temperature, with higher BMR in the SFA birds than in the UFA birds. Since there was no significant difference in mass-corrected BMR between temperatures in the UFA birds, the effect was largely driven by temperature effects among the SFA birds. Our results on plasma FA compositions suggest that birds feeding from SFA-rich diets convert a significant proportion of their SFAs into MUFAs (i.e. oleic acid) when exposed to low ambient temperature. This likely involves increased activity of FA biosynthesis enzymes requiring additional metabolic energy (Landriscina et al., 1972), which could contribute to the increases in BMR after low ambient temperature exposure.

In addition, the higher level of the MUFA oleic acid in the SFA-fed birds at the low ambient temperature could also contribute to the

increased BMR, due to its higher rate of oxidation compared with its saturated counterpart, stearic acid (Price et al., 2011). Also, linoleic acid, with an even higher oxidation rate than oleic acid (Price et al., 2011), showed higher plasma proportions at the low compared with high temperature (in both diet groups), which may also contribute to higher BMR in the SFA-fed birds after cold exposure. In contrast, the long-chain ω -3 PUFA DHA was lower in plasma at the low compared with high ambient temperature across diets. Interestingly, in a diet manipulation experiment (fish oil with abundant DHA vs seed oil with abundant linoleic acid), red-winged blackbird (*Agelaius phoeniceus*) nestlings achieved higher cold-induced RMR when they had lower DHA and higher linoleic acid in the phospholipids of pectoralis muscle (Price et al., 2018). In addition, a diet \times temperature interaction for RMR in the blackbirds was also observed during the cold challenge (Price et al., 2018). Hence, different proportions of ω -3 and ω -6 PUFAs available for oxidation seem to contribute to the metabolic rate of birds, and different PUFAs might be preferentially oxidized at different ambient temperatures. However, since the linoleic acid and DHA levels in the present study showed similar temperature effects in both diet groups, and linoleic acid was always much higher in the UFA birds, variation in these two FAs cannot alone underlie the diet \times temperature interaction shown for BMR.

An alternative, but mutually non-exclusive, explanation for diet- and temperature-dependent variation in BMR is that the dominating MUFA oleic acid disproportionately affects lipid phase behaviour in membranes (Calhoun et al., 2015; Epanand et al., 1991). In line with this hypothesis, the diet \times temperature interaction for oleic acid follows the same direction as the interaction for BMR. Future studies at the molecular and cellular levels are needed to test the outlined hypotheses and to better understand the mechanistic underpinnings of the variation in BMR observed in the present study.

Interaction effects of diet and temperature on oxidative stress

Diet and ambient temperature are also likely to interact to affect oxidative stress via differential generation of ROS (our third prediction) mediated via fatty acid-induced variation in BMR (Leyton et al., 1987; Price et al., 2018). In addition, the differential susceptibility of SFAs vs UFAs to lipid peroxidation (Pamplona et al., 2000), whereby the higher degree of unsaturation increases susceptibility to ROS (Gaschler and Stockwell, 2017), could increase oxidative damage to birds with higher levels of UFA. As predicted, we found a significant diet \times temperature interaction for MDA, with the SFA birds showing a decreased MDA concentration with increased ambient temperature, while the UFA birds showed the opposite trend. However, the differences in MDA between temperatures were not significant within the respective diet groups. It is possible that the interaction for MDA was partly driven by the interaction shown for BMR. However, after the high temperature treatment, the BMR was higher in the UFA-provided birds than in the SFA-provided birds, yet their MDA levels were similar between the two diet groups, suggesting that processes other than BMR (e.g. differences in enzymatic antioxidant defence, not measured here) contribute to the accumulation of oxidative damage. Because no diet \times temperature interaction was observed for the PUFAs, which are more peroxidation prone than SFAs and MUFAs, it is unlikely that differential lipid peroxidation susceptibility underlies the results observed for MDA.

In contrast to MDA, AOX did not show an interaction between diet and ambient temperature. Instead, there was a clear dietary

effect, with the UFA birds having higher AOX capacity than the SFA birds (see also Ciftci et al., 2018; Jankowski et al., 2016). This high AOX capacity in the UFA birds probably combats accumulation of oxidative damage. Since we experimentally controlled for the intake of the main dietary antioxidant (vitamin E), across the two diet treatments, the results for AOX are unlikely to be directly caused by a difference in dietary intake of antioxidants.

Effects of population origin

The population origin affected the proportion of total SFA in plasma and BMR in our study. Among the UFA-fed birds, the proportion of total SFA was consistently higher, and BMR was consistently lower, in urban compared with rural birds, throughout the experiment. This result may suggest that these populations differ in diet-dependent metabolic conversions of FAs, which was previously suggested based on differential expression of genes involved in FA biosynthesis in birds from these populations (Watson et al., 2017). Further investigation is needed to understand this intriguing variation.

Conclusions

The present study shows that there is an interplay between the fatty acid composition of the diet and ambient temperature that affects the physiological systems of birds. Feeding from a diet rich in SFA at low ambient temperature and a diet rich in UFA at high ambient temperatures appears to be costly in terms of increased energy expenditure (higher BMR), and possibly also oxidative damage to lipids. Thus, the proposed metabolic conversion of SFA to MUFA, and the UFA proportion in the tissues could be determined by a trade-off, balancing the needs of metabolic efficiency, membrane fluidity and protection against oxidative stress, ultimately affecting survival (McWilliams et al., 2002). Although we measured FAs in the circulating system rather than in the membranes, several of our results are in line with this framework and we foresee intriguing research in this area in the future.

Addressing the physiological effects and fitness consequences of the interaction between diet and ambient temperature is important both from a mechanistic perspective but also in an ecological context, with the current intensification of anthropogenic feeding of birds and climate change (Isaksson, 2015; Jokimäki et al., 1996; Jones and Reynolds, 2008; Plummer et al., 2013; Yow, 2007). The present SFA and UFA-rich diets based on coconut and sunflower fats (and other seeds with similar FA contents; Beare-Rogers et al., 2001), respectively, are two common fat sources that humans provide to birds during winter. Despite the massive scale of avian food provisioning in northern Europe and North America, our understanding of its impacts on life-sustaining physiology and its links to survival is still incomplete. This is particularly important given that the climate is changing at both regional (e.g. urban heat islands) and global scales. Hence, a better understanding of temperature-dependent effects of nutrition on animal physiology is needed.

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Competing interests

The authors declare no competing or financial interests

Author contributions

M.N.A., J.N., J.-Å.N. and C.I. designed and performed the experiment. M.N.A., J.N. and C.I. performed fieldwork. M.N.A. and C.I. performed fatty acid analysis and oxidative stress assays, respectively. J.N. and J.-Å.N. performed metabolic

rate measurements. C.I. performed statistical analysis. M.N.A. and C.I. together drafted the manuscript, and all authors contributed to revisions.

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Data availability

All raw data for the fatty acid and BMR analyses are available in Tables S1–S3 of this article.

Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.186759.supplemental>

References

- Andersson, M. N., Wang, H.-L., Nord, A., Salmón, P. and Isaksson, C. (2015). Composition of physiologically important fatty acids in great tits differs between urban and rural populations on a seasonal basis. *Front. Ecol. Evol.* **3**, 93.
- Balnavae, D. (1973). The influence of environmental temperature on the fatty acid composition of liver and carcass lipids in the young male chick fed on equalized and *ad lib.* intakes. *Comp. Biochem. Physiol. A* **44**, 1075-1083.
- Beare-Rogers, J. L., Dieffenbacher, A. and Holm, J. V. (2001). Lexicon of lipid nutrition (IUPAC Technical Report). *Pure Appl. Chem.* **73**, 685-744.
- Becker, R. (2008). Fatty acids in food cereal grains and grain products. In *Fatty Acids in Foods and Their Health Implications* (ed. C. K. Chow), pp. 303-316. Boca Raton: CRC Press, Taylor & Francis Group.
- Ben-Hamo, M., McCue, M. D., McWilliams, S. R. and Pinshow, B. (2011). Dietary fatty acid composition influences tissue lipid profiles and regulation of body temperature in Japanese quail. *J. Comp. Physiol. B* **181**, 807-816.
- Ben-Hamo, M., McCue, M. D., Khozin-Goldberg, I., McWilliams, S. R. and Pinshow, B. (2013). Ambient temperature and nutritional stress influence fatty acid composition of structural and fuel lipids in Japanese quail (*Coturnix japonica*) tissues. *Comp. Biochem. Physiol. A* **166**, 244-250.
- Benzie, I. F. F. and Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal. Biochem.* **239**, 70-76.
- Broggi, J., Orell, M., Hohtola, E. and Nilsson, J.-Å. (2004). Metabolic response to temperature variation in the great tit: an interpopulation comparison. *J. Anim. Ecol.* **73**, 967-972.
- Buttemer, W. A., Abele, D. and Costantini, D. (2010). From bivalves to birds: oxidative stress and longevity. *Funct. Ecol.* **24**, 971-983.
- Calhoun, E. A., Ro, J. and Williams, J. B. (2015). Perspectives on the membrane fatty acid unsaturation/pacemaker hypotheses of metabolism and aging. *Chem. Phys. Lipids* **191**, 48-60.
- Careau, V., Réale, D., Humphries, M. M. and Thomas, D. W. (2010). The pace of life under artificial selection: personality, energy expenditure, and longevity are correlated in domestic dogs. *Am. Nat.* **175**, 753-758.
- Ciftci, M., Simsek, U. G., Dalkilic, B., Erisir, Z., Mutlu, S. I., Azman, M. A., Ozcelik, M., Yilmaz, O., Baykalir, Y. and Tonbak, F. (2018). Effects of essential oil mixture supplementation to basal diet on fattening performance, blood parameters and antioxidant status of tissues in Japanese quails exposed to low ambient temperature. *J. Anim. Plant Sci.* **28**, 421-430.
- Eikenaar, C., Källstig, E., Andersson, M. N., Herrera-Dueñas, A. and Isaksson, C. (2017). Oxidative challenges of avian migration: a comparative field study on a partial migrant. *Physiol. Biochem. Zool.* **90**, 223-229.
- Epand, R. M., Epand, R. F., Ahmed, N. and Chen, R. (1991). Promotion of hexagonal phase formation and lipid mixing by fatty acids with varying degrees of unsaturation. *Chem. Phys. Lipids* **57**, 75-80.
- Finkel, T. and Holbrook, N. J. (2000). Oxidants, oxidative stress and the biology of ageing. *Nature* **408**, 239-247.
- Gaschler, M. M. and Stockwell, B. R. (2017). Lipid peroxidation in cell death. *Biochem. Biophys. Res. Commun.* **482**, 419-425.
- Guglielmo, C. G. (2010). Move that fatty acid: fuel selection and transport in migratory birds and bats. *Integr. Comp. Biol.* **50**, 336-345.
- Harman, D. (1956). Aging: a theory based on free radical and radiation chemistry. *J. Gerontol.* **11**, 298-300.
- Hazel, J. R. (1995). Thermal adaptation in biological membranes: is homeoviscous adaptation the explanation? *Annu. Rev. Physiol.* **57**, 19-42.
- Hulbert, A. J. (2005). On the importance of fatty acid composition of membranes for aging. *J. Theor. Biol.* **234**, 277-288.
- Hulbert, A. J. (2008). The links between membrane composition, metabolic rate and lifespan. *Comp. Biochem. Physiol. A* **150**, 196-203.

- Hulbert, A. J. and Abbott, S. K. (2012). Nutritional ecology of essential fatty acids: an evolutionary perspective. *Aust. J. Zool.* **59**, 369-379.
- Hulbert, A. J. and Else, P. (2005). Membranes and the setting of energy demand. *J. Exp. Biol.* **208**, 1593-1599.
- Isaksson, C. (2015). Urbanization, oxidative stress and inflammation: a question of evolving, acclimatizing or coping with urban environmental stress. *Funct. Ecol.* **29**, 913-923.
- Isaksson, C., Sheldon, B. C. and Uller, T. (2011). The challenges of integrating oxidative stress into life-history biology. *Bioscience* **61**, 194-202.
- Jankowski, J., Juśkiewicz, J., Zduńczyk, P., Kosmala, M., Zieliński, H., Antoszkiewicz, Z. and Zduńczyk, Z. (2016). Antioxidant status of blood and liver of turkeys fed diets enriched with polyunsaturated fatty acids and fruit pomaces as a source of polyphenols. *Pol. J. Vet. Sci.* **19**, 89-98.
- Jokimäki, J., Suhonen, J., Inki, K. and Jokinen, S. (1996). Biogeographical comparison of winter bird assemblages in urban environments in Finland. *J. Biogeogr.* **23**, 379-386.
- Jones, D. N. and Reynolds, J. S. (2008). Feeding birds in our towns and cities: a global research opportunity. *J. Avian Biol.* **39**, 265-271.
- Kirkwood, T. B. L. (2002). Evolution of ageing. *Mech. Ageing Dev.* **123**, 737-745.
- Landriscina, C., Gnoni, G. V. and Quagliariello, E. (1972). Fatty-acid biosynthesis. The physiological role of the elongation system present in microsomes and mitochondria of rat liver. *Eur. J. Biochem.* **29**, 188-196.
- Leyton, J., Drury, P. J. and Crawford, M. A. (1987). Differential oxidation of saturated and unsaturated fatty acids *in vivo* in the rat. *Br. J. Nutr.* **57**, 383-393.
- Lin, H., Decuyper, E. and Buyse, J. (2006). Acute heat stress induces oxidative stress in broiler chickens. *Comp. Biochem. Physiol. A.* **144**, 11-17.
- McCue, M. D., Amitai, O., Khozin-Goldberg, I., McWilliams, S. R. and Pinshow, B. (2009). Effect of dietary fatty acid composition on fatty acid profiles of polar and neutral lipid tissue fractions in zebra finches, *Taeniopygia guttata*. *Comp. Biochem. Physiol. A.* **154**, 165-172.
- McKechnie, A. E. and Swanson, D. L. (2010). Sources and significance of variation in basal, summit and maximal metabolic rates in birds. *Curr. Zool.* **56**, 741-758.
- McKechnie, A. E., Chetty, K. and Lovegrove, B. G. (2007). Phenotypic flexibility in the basal metabolic rate of laughing doves: responses to short-term thermal acclimation. *J. Exp. Biol.* **210**, 97-106.
- McWilliams, S. R., Kearney, S. B. and Karasov, W. H. (2002). Diet preferences of warblers for specific fatty acids in relation to nutritional requirements and digestive capabilities. *J. Avian Biol.* **33**, 167-174.
- McWilliams, S. R., Guglielmo, C., Pierce, B. and Klaassen, M. (2004). Flying, fasting, and feeding in birds during migration: a nutritional and physiological ecology perspective. *J. Avian Biol.* **35**, 377-393.
- Montgomery, M. K., Hulbert, A. J. and Buttemer, W. A. (2012). Metabolic rate and membrane fatty acid composition in birds: a comparison between long-living parrots and short-living fowl. *J. Comp. Physiol. B.* **182**, 127-137.
- Newman, R. E., Bryden, W. L., Fleck, E., Ashes, J. R., Buttemer, W. A., Storlien, L. H. and Downing, J. A. (2002). Dietary n-3 and n-6 fatty acids alter avian metabolism: metabolism and abdominal fat deposition. *Br. J. Nutr.* **88**, 11-18.
- Nord, A. and Nilsson, J.-Å. (2011). Incubation temperature affects growth and energy metabolism in blue tit nestlings. *Am. Nat.* **178**, 639-651.
- Pamplona, R., Portero-Otín, M., Riba, D., Ruiz, C., Prat, J., Bellmunt, M. J. and Barja, G. (1998). Mitochondrial membrane peroxidizability index is inversely related to maximum life span in mammals. *J. Lipid Res.* **39**, 1989-1994.
- Pamplona, R., Portero-Otín, M., Riba, D., Requena, J. R., Thorpe, S. R., López-Torres, M. and Barja, G. (2000). Low fatty acid unsaturation: a mechanism for lowered lipoperoxidative modification of tissue proteins in mammalian species with long life spans. *J. Gerontol. Ser. A Biol. Sci. Med. Sci.* **55**, B286-B291.
- Pierce, B. J., McWilliams, S. R., O'Connor, T. P., Place, A. R. and Guglielmo, C. G. (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**, 1277-1285.
- Plummer, K. E., Bearhop, S., Leech, D. I., Chamberlain, D. E. and Blount, J. D. (2013). Fat provisioning in winter impairs egg production during the following spring: a landscape-scale study of blue tits. *J. Anim. Ecol.* **82**, 673-682.
- Price, E. R. and Guglielmo, C. G. (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. Physiol. Regul. Integr. Comp. Physiol.* **297**, R775-R782.
- Price, E. R., Krokfors, A. and Guglielmo, C. G. (2008). Selective mobilization of fatty acids from adipose tissue in migratory birds. *J. Exp. Biol.* **211**, 29-34.
- Price, E. R., Staples, J. F., Milligan, C. L. and Guglielmo, C. G. (2011). Carnitine palmitoyl transferase activity and whole muscle oxidation rates vary with fatty acid substrate in avian flight muscles. *J. Comp. Physiol. B.* **181**, 565-573.
- Price, E. R., Armstrong, C., Guglielmo, C. G. and Staples, J. F. (2013). Selective mobilization of saturated fatty acids in isolated adipocytes of hibernating 13-lined ground squirrels *Ictidomys tridecemlineatus*. *Physiol. Biochem. Zool.* **86**, 205-212.
- Price, E. R., Sirsat, T. S., Sirsat, S. K. G., Curran, T., Venables, B. J. and Dzialowski, E. M. (2018). The membrane pacemaker hypothesis: novel tests during the ontogeny of endothermy. *J. Exp. Biol.* **221**, jeb174466.
- Robb, G. N., McDonald, R. A., Chamberlain, D. E. and Bearhop, S. (2008). Food for thought: supplementary feeding as a driver of ecological change in avian populations. *Front. Ecol. Environ.* **6**, 476-484.
- Salmón, P., Stroh, E., Herrera-Dueñas, A., von Post, M. and Isaksson, C. (2018). Oxidative stress in birds along a NOx and urbanisation gradient: an interspecific approach. *Sci. Total Environ.* **622**, 635-643.
- Sanz, M., Flores, A. and Lopez-Bote, C. J. (2000a). The metabolic use of energy from dietary fat in broilers is affected by fatty acid saturation. *Br. Poult. Sci.* **41**, 61-68.
- Sanz, M., Lopez-Bote, C. J., Menoyo, D. and Bautista, J. M. (2000b). Abdominal fat deposition and fatty acid synthesis are lower and β -oxidation is higher in broiler chickens fed diets containing unsaturated rather than saturated fat. *J. Nutr.* **130**, 3034-3037.
- Shimomura, Y., Tamura, T. and Suzuki, M. (1990). Less body fat accumulation in rats fed a safflower oil diet than in rats fed a beef tallow diet. *J. Nutr.* **120**, 1291-1296.
- Sinensky, M. (1974). Homeoviscous adaptation—a homeostatic process that regulates the viscosity of membrane lipids in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **71**, 522-525.
- Speakman, J. R. and Selman, C. (2011). The free-radical damage theory: accumulating evidence against a simple link of oxidative stress to ageing and lifespan. *BioEssays* **33**, 255-259.
- Stier, A., Massemin, S. and Criscuolo, F. (2014). Chronic mitochondrial uncoupling treatment prevents acute cold-induced oxidative stress in birds. *J. Comp. Physiol. B* **184**, 1021-1029.
- Swanson, D. L. (2010). Seasonal metabolic variation in birds: functional and mechanistic correlates. *Curr. Ornithol.* **17**, 75-129.
- Swanson, D. L. and Vézina, F. (2015). Environmental, ecological and mechanistic drivers of avian seasonal metabolic flexibility in response to cold winters. *J. Ornithol.* **156**, 377-388.
- Takeuchi, H., Matsuo, T., Tokuyama, K., Shimomura, Y. and Suzuki, M. (1995). Diet-induced thermogenesis is lower in rats fed a lard diet than those fed a high oleic acid safflower oil diet, a safflower oil diet, or a linseed oil diet. *J. Nutr.* **125**, 920-925.
- Twining, C. W., Brenna, J. T., Lawrence, P., Shipley, J. R., Tollefson, T. N. and Winkler, D. W. (2016). Omega-3 long-chain polyunsaturated fatty acids support aerial insectivore performance more than food quantity. *Proc. Natl. Acad. Sci. USA* **113**, 10920-10925.
- Watson, H., Videvall, E., Andersson, M. N. and Isaksson, C. (2017). Transcriptome analysis of a wild bird reveals physiological responses to the urban environment. *Sci. Rep.* **7**, 44180.
- Withers, P. C. (2001). Design, calibration and calculation for flow-through respirometry systems. *Aust. J. Zool.* **49**, 445-461.
- Yow, D. M. (2007). Urban heat islands: observations, impacts, and adaptation. *Geogr. Compass* **1**, 1227-1251.