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ORIGINAL PAPER

Dietary fatty acid composition influences tissue lipid profiles and regulation of body temperature in Japanese quail

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Abstract Many avian species reduce their body temperature (T_b) to conserve energy during periods of inactivity, and we recently characterized how ambient temperature (T_a) and nutritional stress interact with one another to influence physiologically controlled hypothermic responses in Japanese quail (*Coturnix japonica*). In the present study, we examined how the fatty acid (FA) composition of the diet influences the FA composition of phospholipids in major organs and how these affect controlled hypothermic responses and metabolic rates in fasted birds. For 5 weeks prior to fasting, quail were fed a standard diet and gavaged each morning with 0.7 ml of water (control), or a vegetable oil comprising saturated fatty acids (SFA; coconut oil), or unsaturated fatty acids (UFA; canola oil). Birds were then fasted for 4 days at a T_a of 15°C. We found that, while fasting, both photophase and scotophase T_b decreased significantly more in the SFA treatment group than in the control group; apparently the former down-regulated their T_b set point. This deeper hypothermic response was correlated with changes in the phospholipid composition of the skeletal muscle and liver, which contained significantly more oleic acid (18:1) and less arachidonic acid (20:4), respectively. Our data imply that these two FAs may be associated with thermoregulation.

Keywords Birds · Heterothermy · Oxygen consumption · Fasting · Temperature regulation

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Introduction

Facultative, physiologically controlled hypothermia, entailing reduced metabolism, and body temperature (T_b), is an adaptive strategy used by many birds to conserve energy during periods of cold stress, food limitation, or both (reviewed by McKechnie and Lovegrove 2002; Schleucher 2004). A puzzling aspect of the facultative controlled hypothermic response is that similar environmental cues can bring about different patterns of controlled hypothermia with regard to its depth and duration, and different rates of cooling upon entry, and re-warming during arousal (McKechnie and Lovegrove 2002; Schleucher 2004). For example, T_b and duration of torpor bouts differed between three species of hummingbirds that were housed together in an outdoor aviary (Bech et al. 1997). Moreover, Brigham et al. (2000) found substantial differences in the torpor patterns of individual Australian owlet-nightjars (*Aegotheles cristatus*) observed under the same environmental conditions. These observations suggest that facultative hypothermia may be induced by certain

environmental cues such as cold temperatures, but that other physiological mechanisms are also involved in modulating the hypothermic response.

The fatty acid (FA) composition of the diet, and ultimately of the body tissues, could affect the controlled hypothermic response and thus serve as a possible physiological mechanism determining it. Beynen and Katan (1985) proposed that saturated fatty acids (SFA) have a greater tendency to be deposited in the body, while unsaturated fatty acids (UFA) are more readily oxidized to yield energy. This notion has been supported by studies on laboratory mice (*Mus musculus*), humans, and chickens (*Gallus gallus domesticus*), where subjects fed diets rich in UFAs had lower energy retention and higher heat production than those fed diets rich in SFAs (Chen and Chiang 2005; Jones and Schoeller 1988; Mercer and Trayhurn 1987; Shimomura et al. 1990; Takeuchi et al. 1995). Chen and Chiang (2005) concluded that increased heat production resulting from a higher unsaturation degree of dietary lipids, can either exacerbate heat stress in chickens kept at high ambient temperature (T_a), or, alternatively, provide a heat source for maintaining constant T_b when kept at low T_a. An assumption underlying these studies is that animals feeding on diets with different FA saturation levels undergo alterations in their membrane phospholipids, and these alterations are associated with major changes in the physical properties of the membranes, such as rotational fluidity and biochemical properties of membrane bound enzymes (Ginsberg et al. 1982; Houslay et al. 1976; Houslay 1985). However, others have suggested an alternative explanation, namely that UFAs lead to a stronger stimulation of the sympathetic nervous system than their saturated counterparts (Chen and Chiang 2005; Mercer and Trayhurn 1987). Since there is uncertainty regarding the mechanism by which peripheral membrane FA composition affects thermoregulation, we addressed this by determining the membrane FA composition of the tissues of Japanese quail (*Coturnix japonica*) fed on different diets, while simultaneously characterizing the birds' responses by measuring T_b and metabolic rate (MR). Although quail evince only shallow hypothermia, the relationship between the physical properties of membranes (e.g., rotational fluidity) and biochemical properties (e.g., membrane bound enzymes activity), and temperature are described by curvilinear functions (e.g., Houslay et al. 1976; Houslay and Gordon 1983). Therefore, even a small change in temperature may lead to a relatively large change in the membrane properties if the temperature change occurs in the exponential phase.

Even though the FA compositions of body lipids generally reflect those of the diet, they do not do so precisely (reviewed in: McWilliams et al. 2004; Pierce and McWilliams 2005). To gain additional insights into the mechanisms underlying regulated hypothermia, the ancillary goals of this study were to identify how closely dietary FAs and tissue FAs are correlated, and to identify specific FAs that may be responsible for influencing the hypothermic response and overall energy expenditure. We analyzed the FA composition of phospholipids in selected quail tissues, namely pectoral muscles and liver of Japanese quail (*Coturnix japonica*), because these organs expend a large proportion of the total energy requirement in birds (Piersma et al. 1996; Schmidt-Nielsen 1997; Weber and Piersma 1996) and because FAs in phospholipids play an important role in regulating metabolism (Else and Wu 1999; Hulbert 2008).

We investigated the following hypotheses: (H_1) differences in dietary FA composition affects tissue FA composition in Japanese quail, and tissue composition is correlated with modulation in their hypothermic responses; and (H_2) that the degree of unsaturation of the diet affects the depth of hypothermia, by testing the following predictions: (1) that FA composition of tissue membranes is more unsaturated in quail feeding on a UFA-supplemented diet, and that the degree of unsaturation is correlated with decreases in both T_b and MR during controlled hypothermia; and (2) that quail fed a UFA-supplemented diet have higher T_b and MR than those fed a SFA-supplemented diet.

Methods

Animals

Twenty-four Japanese quail chicks, hatched within the same 24-h period, were purchased from a commercial breeder (Joseph Yanai, Mata, Israel). All quail were maintained in outdoor aviaries (2.5 × 2.5 × 3 m) on the Sede Boqer Campus of Ben-Gurion University (30°52′N, 34°46′E) and habituated to a diet of commercial poultry feed (Hemed Lachay, Hemed, Israel; see Table 1) ad libitum until the start of experiments. At 5 weeks of age (day 0 of experiment), quail were assigned to one of three experimental groups of four males and four females each; the groups were closely matched in mean body mass ($\bar{m}_b$) and variation thereof. The UFA group, with a $\bar{m}_b$ of 208.6 ± 14.2 g (SD), was fed 0.7 ml of canola oil (Table 1) by gavage with a feeding tube (FTP 18–30, Instech Solomon, Plymouth Meeting, PA) at sunrise each morning. At the same time the SFA group ($\bar{m}_b$ = 208.0 ± 17.4 g) was gavaged with 0.7 ml of coconut oil (Table 1), and a control group (m_b = 208.2 ± 12.3 g) with 0.7 ml of water (Nagahuedi et al. 2009). These feeding trials were done for 4 weeks, during which quail were kept in four adjacent aviaries

Table 1 Fatty acid profiles of the basic experimental diet and the two vegetable oils that were fed to Japanese quail (*Coturnix japonica*)

Fatty acids	Basic diet (% total FAs)	Canola oil (% total FAs)	Coconut oil (% total FAs)
8:0	–	–	4.90
10:0	–	–	5.64
12:0	–	–	38.27
14:0	–	–	16.51
16:0	9.13	5.86	9.26
18:0	4.75	1.76	9.26
18:1	13.88	42.14	5.79
18:2	25.33	12.07	9.75
18:3	2.42	4.04	–
20:1	–	1.19	–
22:1	1.31	2.07	–

The basic diet was poultry feed consisting of corn, wheat, soy, plus a mixture of vitamins and minerals, and fed to all experimental quail. The canola and coconut oils were fed to the UFA and SFA groups, respectively. Data presented only for the major fatty acids (i.e., >1%)

(1.5 × 1.5 × 2.2 m). The sexes were separated and six males or six females were kept in each aviary. Birds were offered food and water ad libitum between sunrise and sunset, and were weighed to ±0.1 g each morning before gavage. Although we did not measure food intake, $\bar{m}_b$ of the different treatment groups did not differ throughout the experiment, and we were able to detect differences in FA composition of their tissues (see results).

During the fifth week of the feeding trials, we equipped the birds with calibrated temperature sensitive data loggers (iButton DS1921H-F50, Maxim Integrated Products, Dallas Semiconductor) that recorded T_b with a resolution of 0.125°C ± 1°C every 15 min. The iButtons were individually calibrated between 37°C and 42°C in a controlled-temperature water bath (Thermo Haake model V26), against a mercury-in-glass thermometer with accuracy ±0.1°C traceable to the US NIST (Taylor #0205 0198, ASTM 64C Precision). The steady-temperature values of each iButton were regressed against the corresponding stable calibration thermometer temperature. Upon retrieval of the iButtons at the end of the experiment, T_b for each animal was corrected with its logger's equation.

On the first day of the sixth week, quail were transferred to individual cages (40 × 30 × 30 cm) and housed in a temperature controlled room at 14.9 ± 1.4°C (mean ± SD), and allowed 10 days to habituate to the new experimental conditions; then all of their body masses had stabilized. Following this period, four quail from each group were fasted for 4 days to induce hypothermia (Ben-Hamo et al. 2010). Food was removed from the cages at sunset, and the following photophase and the subsequent scotophase were considered as the first day of fasting.

Metabolic rates

We measured MR of quail during their rest-phase in three nutritional states: (1) postprandial birds; (2) after 1 day of fasting; and (3) after 4 days of fasting. Metabolic rates were determined by indirect calorimetry with an eight-channel open-flow gas analysis system. $\dot{V}O_2$ was calculated from measurements made while six quail rested in individual 1.9 L plastic chambers (Lock and Lock, Seoul, Korea) with constant flow rate around 800 ml/min. Each chamber was equipped with a hardware-cloth floor above a paraffin oil excreta trap. Chamber temperatures were maintained by placing them together in a controlled temperature cabinet (Refritherm-5, Struers, Denmark) at T_a of 15 ± 1°C.

Air from outside the building was pumped through a purge gas generator (Pure Gas, Broomfield, CO, USA, model #PCDA-1-12-m-32-C) that removed CO_2 and water vapor to less than 1 ppm. The fractional concentrations of oxygen in the incurrent and excurrent gas streams were measured with a FoxBox oxygen analyzer (Sable Systems International, Las Vegas, NV, USA). The O_2 analyzer was zeroed using pure N_2 (Maxima Co., Mitzpeh Ramon, Israel) and spanned using dry CO_2 free air at the same flow rates. $\dot{V}O_2$ was calculated using equation 2 of Hill (1972), and was converted to power units (W) assuming 20.08 J/ml O_2 (Schmidt-Nielsen 1997). For consistency, we considered resting metabolic rate to be the minimum MR averaged for 6-min periods.

Fatty acid analysis

After 4 days of food deprivation, quail were weighed to ±0.1 g, and killed by decapitation. Pectoral muscle and liver were harvested, placed in individually labeled glass vials, and frozen. Tissue samples were later freeze-dried, and then homogenized using mortar and pestle. Total lipids were extracted from the homogenate over ice in a 2:1:0.8 mixture of methanol:chloroform:water, following Bligh and Dyer (1959). Lipids were recovered in the chloroform phase, transferred to a separate glass vial, and dried under a stream of N_2. Samples were stored at −20°C for up to 2 weeks before analysis. Polar fractions were separated by passing samples through a 500 mg silica column (Bond Elut^{TM}; Varian USA) with chloroform followed by methanol (McCue et al. 2009). The polar lipid fraction was recovered and dried under vacuum. Transmethylation took place in anhydrous methanol containing 2% H_2SO_4 (v/v) at 85°C, and after 1 h was terminated by addition of water. Fatty acid methyl esters (FAMEs) were extracted in hexane and transferred to 300 µl autosampler vials. FAMEs were separated in a gas chromatograph (GC Ultra, Thermo, Italy) with a capillary column (ZBwax, Phenomonex, USA), programmed temperature vaporization (PTV)

injector, and flame ionization detector, using helium as a carrier gas. The temperature program was a 1-min hold at 80°C followed by a linear increase of 6°C min^{-1} to 240°C, and final hold of 10 min at the high temperature. FAMEs ranging in length from 10 to 24 carbons were identified by comparing chromatograms with retention times of FAMEs from standards resolved in parallel with the unknown samples.

Major FAs were considered those that accounted for an average of >1% of the total in respective tissue-lipid fractions (McCue 2007, 2008b). In most cases, it was impossible to resolve 18:1n9 and 18:1n7, therefore, proportions of these two isomers were combined into a single value referred to as 18:1. Unsaturation indices (UI) were calculated as the sum of the percent concentration of each unsaturated FA (N_i) multiplied by its respective degree of unsaturation (i) and by 100 (Geiser et al. 1992; Jezierska et al. 1982; Pan and Storlien 1993; Stuart et al. 1998) following McCue (2008b):

$$\mathrm{UI} = \left[\sum_{i=1}^{6} N_i \cdot i\right] 100.$$

Statistical analysis

We used repeated measures analysis of variance (RM-ANOVA) to investigate the relationships among dietary treatments, T_b, m_b, and MR over the 4 days of fasting. When the assumption of sphericity was violated, we used the Greenhouse-Geisser correction to modify the degrees of freedom of both treatment and error. In addition, we used a split-plot ANOVA model to test differences in the composition of fatty acids in muscle and liver. We tested data for normality of distribution with the Shapiro–Wilk test, and for homoscedasticity with Levene's test. When data were significantly different, we further examined the relationship using either Dunnett's or Tukey's post-hoc tests according to the question of interest. Mass specific metabolic rates (MR/m_b) are presented. Means are presented ±1 SD and $\alpha = 0.05$ was chosen as the highest acceptable level of significance. Statistical analyses were done using STATISTICA 9.0 (StatSoft, Tulsa, OK, USA) and graphs were produced with SigmaPlot 11.0 (Systat Software Inc., Chicago, IL, USA).

Results

Body mass

While birds were habituated to the experimental diets there were no differences in $\bar{m}_b$ between the different treatment groups (Fig. 1; RM-ANOVA: Group: $F_{(2,20)} = 0.49$, $p = 618$; Group × Days: $F_{(5.32,53.25)} = 1.18$, $p = 0.330$). However, m_b of all quail increased significantly over the first 30 days, but then stabilized (Fig. 1; RM-ANOVA: Days: $F_{(2.66,53.25)} = 204.23$, $p < 0.001$, Tukey's post-hoc test: MS = 31.30, $p_{5–10\ d} < 0.001$, $p_{10–15\ d} < 0.001$, $p_{15–20\ d} < 0.001$, $p_{20–25\ d} = 0.05$, $p_{25–30\ d} < 0.001$, $p_{30–35\ d} = 0.992$, $p_{35–40\ d} = 0.414$, $p_{40–45\ d} = 0.787$). While fasting, the groups lost m_b at similar rates (Fig. 1; RM-ANOVA: Group: $F_{(2,19)} = 1.75$, $p = 0.201$, Feeding status: $F_{(2.64,50.25)} = 423.75$, $p < 0.001$; $m_{b(postprandial)} = 241.66 \pm 4.27$ g, $m_{b(1\ d\ fasting)} = 224.83 \pm 4.00$ g, $m_{b(2\ d\ fasting)} = 217.15 \pm 4.03$ g, $m_{b(3\ d\ fasting)} = 207.11 \pm 4.38$ g, $m_{b(4\ d\ fasting)} = 198.79 \pm 4.41$ g).

Body temperature

During scotophase, T_b in all quail declined significantly when they were deprived of food, and they entered shallow, controlled nocturnal hypothermia that became progressively deeper each day (RM-ANOVA, Feeding status:

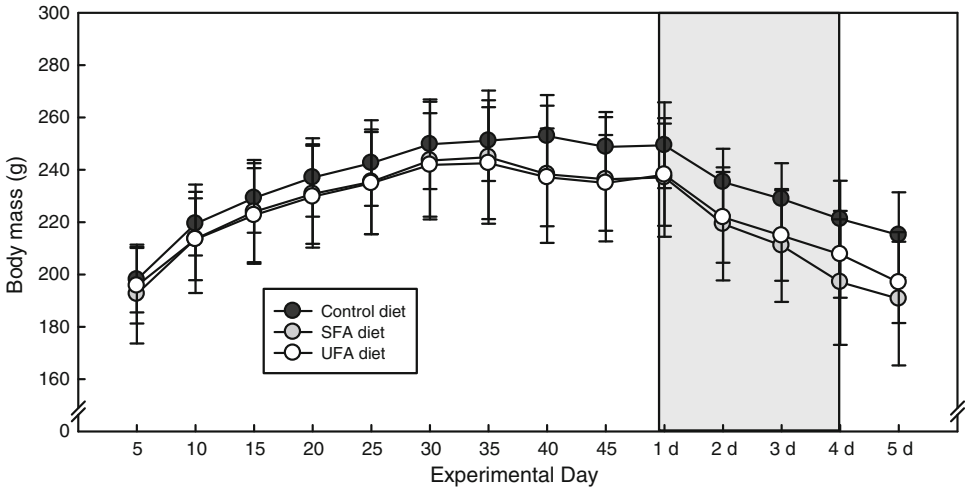

Fig. 1 Body mass of Japanese quail throughout the experiment. Each point represents mean body mass ($\bar{m}_b$) (±SE), of eight quail weighed in the morning. The m_b in the period prior to fasting was averaged for each five sequential days. Since not all quail began fasting at the same time; the m_b in the fasting period is presented for days of fasting for each group. Food was removed on the night before day 1 of fasting. The *shaded area* represents the period in which the quail were deprived of food

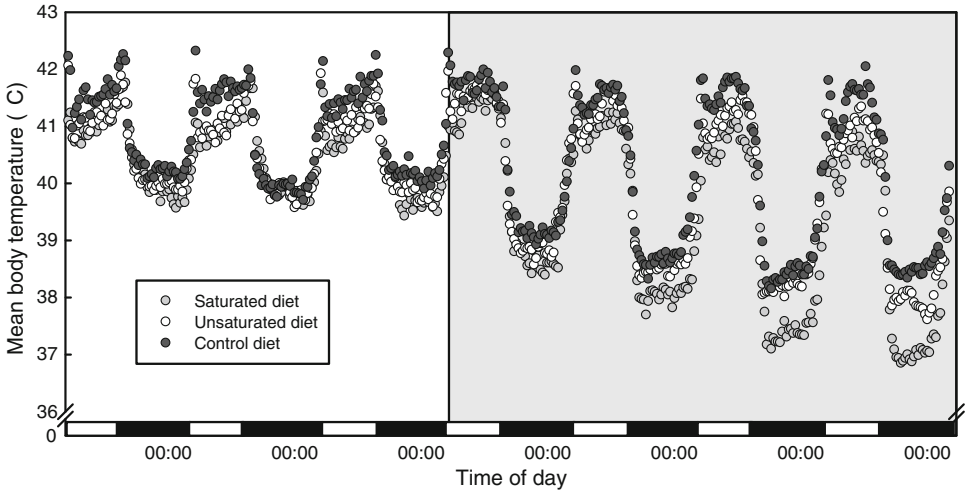

Fig. 2 Body temperature (T_b) as a function of time in fasting Japanese quail. Each point represents the mean T_b of eight quail; error bars (±0.65, maximum SE) are omitted for clarity. *White* and *black bars* correspond to photophase and scotophase, respectively. *Shaded area* represents the period in which the quail were deprived of food

$F_{(2.15,43.03)} = 65.93$, $p < 0.001$, Feeding status × Group: $F_{(4.30,43.03)} = 1.53$, $p = 0.21$, $T_{b(postprandial)} = 39.98 \pm 0.70$°C, $T_{b(1\ d\ fasting)} = 38.81 \pm 0.69$°C, $T_{b(2\ d\ fasting)} = 38.39 \pm 0.73$°C, $T_{b(3\ d\ fasting)} = 37.95 \pm 0.87$°C, $T_{b(4\ d\ fasting)} = 37.75 \pm 1.15$°C). The depth of nocturnal hypothermia differed among the groups (Fig. 2, RM-ANOVA, Group: $F_{(2,20)} = 3.44$, $p = 0.05$). Specifically, quail that received the SFA supplement had lower scotophase T_b than quail fed the control diet (Dunnett's test: 37.06 ± 1.11°C vs. 38.48 ± 1.00°C, $p = 0.015$), but not lower than those that received the UFA supplement (Dunnett's test: 37.81 ± 0.99°C, $p = 0.195$).

We also found that photophase T_b increased significantly in the quail on the first day of food deprivation, but it decreased to normal levels on the following days (RM-ANOVA, Feeding status: $F_{(2.13,\ 42.54)} = 6.81$, $p < 0.001$, Feeding status × Group: $F_{(4.25,\ 42.54)} = 1.26$, $p = 0.278$, $T_{b(postprandial)} = 41.65 \pm 0.57$°C, $T_{b(1\ d\ fasting)} = 41.98 \pm 0.61$°C, $T_{b(2\ d\ fasting)} = 41.75 \pm 0.57$°C, $T_{b(3\ d\ fasting)} = 41.71 \pm 0.68$°C, $T_{b(4\ d\ fasting)} = 41.62 \pm 0.75$°C). In addition, we found that photophase T_b differed among the groups (Fig. 2, RM-ANOVA, Group: $F_{(2,20)} = 4.10$, $p = 0.032$). Specifically, quail that received supplementary SFA had a lower photophase T_b than quail fed the control (ctrl) diet (Dunnett's test: 41.46 ± 0.64°C vs. 42.21 ± 0.81°C, $p = 0.012$), but not than those fed a UFA supplement (Dunnett's test: 41.61 ± 0.49°C, $p = 0.423$). Finally, we compared the difference between maximum photophase T_b and minimum scotophase T_b (ΔT_b) and found that it increased to the same extent during fasting in the treatment groups (RM-ANOVA, Group: $F_{(2,20)} = 0.75$, $p = 0.484$, Feeding status: $F_{(2.24,44.70)} = 74.59$, $p < 0.001$, Group × Feeding status: $F_{(4.47,44.70)} = 1.28$, $p = 0.290$, $\Delta T_{b(postprandial)} = 1.66 \pm 0.45$°C, $\Delta T_{b(1\ d\ fasting)} = 3.17 \pm 0.50$°C, $\Delta T_{b(2\ d\ fasting)} = 3.36 \pm 0.34$°C, $\Delta T_{b(3\ d\ fasting)} = 3.76 \pm 0.0.55$°C, $\Delta T_{b(4\ d\ fasting)} = 3.87 \pm 0.90$°C).

Metabolic rate

There were no significant differences in postprandial MR/m_b among the three treatment groups (Fig. 3, RM-ANOVA: Group: $F_{(2,9)} = 0.25$, $p = 0.784$; $MR_{(ctrl)} = 11.61 \pm 1.29$ mW/g, $MR_{(UFA)} = 11.25 \pm 0.89$ mW/g, $MR_{(SFA)} = 11.37 \pm 1.53$ mW/g). Quail from all groups had significantly reduced MRs once fasted, and MR continued to decline as fasting progressed (RM-ANOVA: Feeding status: $F_{(2,18)} = 48.45$, $p < 0.001$, Feeding status × Group: $F_{(4,18)} = 0.54$, $p = 0.710$; Resting MR (RMR) = 11.41 ± 1.15 mW/g, hypothermic MR $(HMR)_{(day\ 1)} = 8.57 \pm 2.26$ mW/g, $HMR_{(day\ 4)} = 4.97 \pm 2.22$ mW/g). Despite the differences in T_b described above,

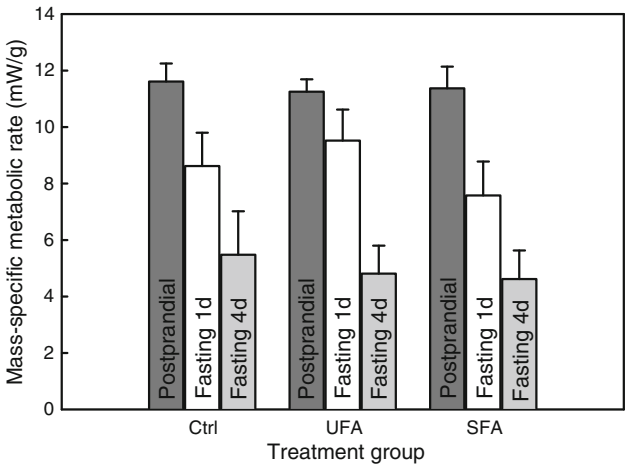

Fig. 3 Mass-specific metabolic (MR/m_b) rates of Japanese quail fed different diets. *Dark grey bars* represent the average MR/m_b of eight quail during a night when they were feeding ad libitum. *White bars* represent the average MR/m_b of eight quail on the night after 1 day food deprivation. *Grey bars* represent the average minimum MR/m_b of eight quail on the night after 4 days of food deprivation. *Error bars* are SE

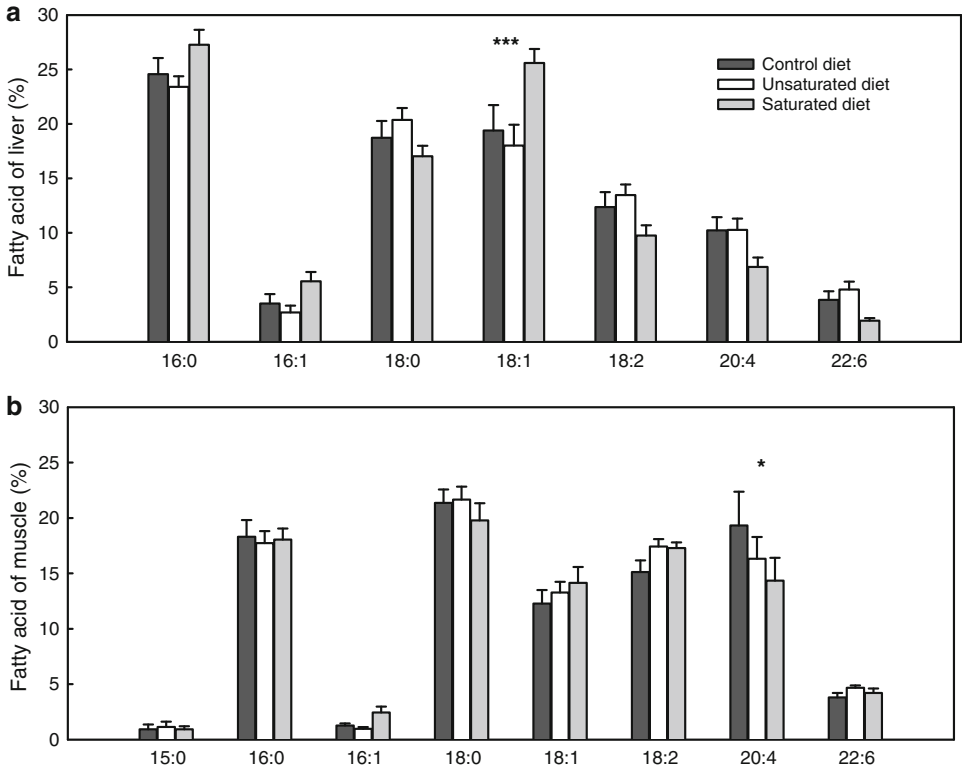

Fig. 4 Percentages of the different major (>1%) fatty acids in tissues of Japanese quail. *Dark grey bars* represent the average percentage of fatty acids in the control group ($n = 8$); *white bars* represent the average percentage of fatty acids in the UFA-supplemented group ($n = 8$); and *light grey bars* represent the average percentage of fatty acids in the SFA-supplemented group ($n = 8$). *Error bars* are SE. **a** Partial fraction of the fatty acids in the liver; **b** partial fraction of the fatty acids in the muscle. $*p < 0.05$, $***p < 0.001$

no significant differences in HMR were observed among the groups on day 1 ($HMR_{(ctrl)} = 8.62 \pm 2.36$ mW/g, $HMR_{(UFA)} = 9.52 \pm 2.21$ mW/g, $HMR_{(SFA)} = 7.58 \pm 2.40$ mW/g) or day 4 of fasting ($HMR_{(ctrl)} = 5.48 \pm 3.08$ mW/g, $HMR_{(UFA)} = 4.81 \pm 1.99$ mW/g, $HMR_{(SFA)} = 4.62 \pm 2.01$ mW/g).

Fatty acid composition

We found an expected effect of diet on the UI of muscle and liver tissues, that is that the SFA group had lower UI than both the UFA group and the control group (Fig. 5; two-way ANOVA: Diet groups: $F_{(2,38)} = 7.40$, $p = 0.002$, Tukey's test: $UI_{(SFA)} = 145.21 \pm 39.24$; $UI_{(UFA)} = 170.4 \pm 29.11$; $UI_{(ctrl)} = 173.01 \pm 39.91$; $p_{(SFA\ vs.\ UFA)} = 0.010$, $p_{(SFA\ vs.\ ctrl)} = 0.005$). In addition, the UI of liver tissue was consistently lower than that of muscle in all three treatment groups (Fig. 5; two-way ANOVA: Tissues: $F_{(1,38)} = 68.85$, $p < 0.001$, $UI_{(liver)} = 137.47 \pm 28.05$, $UI_{(muscle)} = 192.86 \pm 22.40$).

We found significant differences in the FA composition of liver phospholipids; the SFA group had a greater fraction of oleic acid (18:1) than either the control or the unsaturated groups (Fig. 4a, split-plot ANOVA: Group × FA: $F_{(12,84)} = 3.40$, $p < 0.001$, Tukey's test: $18{:}1_{(SFA)} = 25.60 \pm 3.61\%$, $18{:}1_{(ctrl)} = 19.40 \pm 6.58\%$, $18{:}1_{(UFA)} = 18.02 \pm 5.42\%$, $p_{(SFA\ vs.\ ctrl)} = 0.002$, $p_{(SFA\ vs.\ UFA)} = 0.01$).

In addition, there were significant differences in the FA composition of muscle phospholipids among the treatment groups; the SFA group had a lower proportion of arachidonic acid (20:4) than the control group (Fig. 4b, split-plot ANOVA: Group: $F_{(1,11)} = 7.39$, $p = 0.02$, Tukey's test: $20{:}4_{(ctrl)} = 21.82 \pm 5.10\%$, $20{:}4_{(SFA)} = 15.74 \pm 4.63\%$, $20{:}4_{(UFA)} = 18.06 \pm 2.74\%$; $p_{(SFA\ vs.\ ctrl)} = 0.03$, $p_{(SFA\ vs.\ UFA)} = 0.996$).

Discussion

Dietary fatty acids affect membrane composition

In support of our first hypothesis (H_1), we found that the degree of FA unsaturation in the quail tissues reflected that of the lipids they ate. This result is evident from the lower UI found in both muscle and liver of the SFA group (Fig. 5), and is consistent with other studies where FA composition in body tissues was found to be strongly influenced by the composition of dietary FA (Austin 1993; Crespo and Esteve-Garcia 2001; Pierce et al. 2004; Pierce and McWilliams 2005; Price and Guglielmo 2009). The differences in UI are due to a lower proportion of arachidonic acid in muscle phospholipids (Fig. 4) and a higher proportion of oleic acid in liver phospholipids (Fig. 5) in the SFA group. When animals are fed diets enriched with

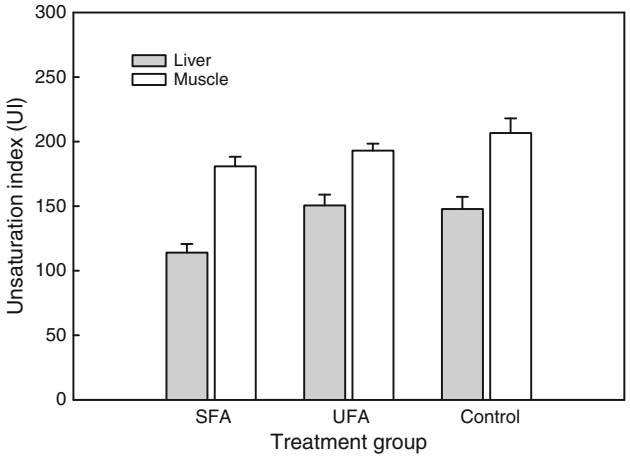

Fig. 5 Unsaturation index of liver and the muscle tissues of Japanese quail. *Each bar* represents the average UI for eight quail (±SE)

specific FAs, they incorporate a substantial quantity of these into their membrane phospholipids, which, in turn, are representative, at least to some extent, of the dietary FA composition (Houslay and Gordon 1983). This effect of dietary nutrient allocation to tissues should be particularly evident in rapidly growing animals (MacAvoy et al. 2005; McCue 2008a; Trueman et al. 2005). The alterations in membrane phospholipids result in changes in the properties of the membranes (e.g., rotational fluidity, membrane bound protein activity etc.), and such changes can be substantial (Houslay and Gordon 1983). For example, when the unsaturation level of membrane phospholipids was increased it was followed by a twofold increase in the number of insulin receptors of Ehrlich cells in mice (Ginsberg et al. 1982).

Dietary fatty acids are associated with thermoregulation

We found that the FA composition of the diet affected T_b during the photophase and the scotophase (Fig. 2), which supports our second hypothesis (H_2). Photophase T_b significantly increased on the first day of fasting. Since we did not quantify 'activity' during fasting, we cannot identify the cause of this difference. Nevertheless, previous studies on rock pigeons (*Columba livia*) and Japanese quail reported similar increases in T_b, correlated with increased motor activity, and posited that this was due to the increased foraging activity (Hohtola et al. 1991; Rashotte et al. 1995). In addition, and in support of our prediction, the SFA group had lower scotophase and photophase T_b than the control group. The difference between scotophase and photophase T_b remained similar to that of the other experimental groups, suggesting that birds in the SFA-supplemented group may have down-regulated their T_b set point in response to food deprivation (Fig. 2). However, and in contrast to our prediction, the changes we observed in T_b were not accompanied by concomitant changes in MR, as was evident from the similar MRs in all three treatment groups (Fig. 3).

The effect of arachidonic acid on the circadian cycle of T_b

In support of our prediction, we found that T_b in fasting quail was lower in the SFA group than in the control group, but not in the UFA group (Fig. 2). In addition, the amplitude of the circadian cycle of T_b remained similar among all three treatment groups. Another difference between the control and the SFA groups, but not the UFA group, was the lower proportion of arachidonic acid in the SFA group (Fig. 4b). Arachidonic acid is a precursor for synthesis of eicosanoids, molecules known to be involved in signaling fever and hyperthermia in animals (reviewed by Aronoff and Romanovsky 2007; Smith et al. 2000). Several studies have reported that eicosanoid levels are elevated when rats (*Rattus norvegicus*) are kept below their lower critical temperature, and that inhibiting eicosanoid activity resulted in a decline in T_b, suggesting that eicosanoids may participate in the generation of cold defense responses (Bizzi et al. 1965; Satinoff 1972; Solomonovich and Kaplanski 1985). Moreover, several arachidonic acid conjugates have been shown to serve as endogenous receptor-ligands of cannabinoids that have been long known to cause hypothermic effects in mammals (Holtzman et al. 1969). The level of arachidonic acid conjugates is elevated in the central nervous system, their concentrations vary over a diel cycle, and their injection into peripheral, rather than brain, tissue induces hypothermia in mice (Fride and Mechoulam 1993; Sugiura et al. 1995; Valenti et al. 2004).

Do metabolic rates differ among the treatment groups?

We found that MRs were similar among all three treatment groups, despite observed differences in T_b (Fig. 3). A possible explanation for the lack of differences is that thermal conductance in the SFA group was higher than in the UFA and control groups. However, the small sample size used in this study and the high variances among birds could have led to our inability to detect relatively small differences between the groups.

Mass specific metabolic rates are proportional to difference between T_b and T_a (Calder and King 1974). On the fourth day of fasting, the SFA group had a scotophase T_b that was, on average, 1.42°C lower than that of the control group. Assuming that thermal conductance was equal in both groups, such a difference in T_b would result in a 6.05% lower MR in the SFA group. We found that the mean MR of the SFA group on the fourth day of fasting

was 15.69% lower than that of the control group, suggesting that there may be differences in MR among the treatment groups, with the SFA group having the lowest. Such an effect of SFAs was observed by Chen and Chiang (2005) who found that UFAs are more readily used as metabolic fuel than SFAs. This is also supported by other studies showing that UFAs are more readily oxidized, and produce more heat, than SFAs (Beynen and Katan 1985; Jones and Schoeller 1988; Mercer and Trayhurn 1987; Shimomura et al. 1990; Takeuchi et al. 1995).

The role of oleic acid in thermogenesis

Unsaturated fatty acids could increase heat production by uncoupling the electron transport chain (Locke et al. 1982a, b), which results in increased thermogenesis and lowered efficiency of oxidative phosphorylation in reptiles and mammals (Brand et al. 1991; Else and Hulbert 1987). In addition, during extensive oxidation of lipids in the liver (e.g., in hibernating mammals or in other fasted animals), oleic acid esters accumulate resulting in the inhibition of adenine nucleotide translocase (ANT) (Lerner et al. 1972; Shug et al. 1971; Wojtczak and Zaluska 1967); ANT is known to mediate the uncoupling of the mitochondrial electron transport chain from oxidative phosphorylation, and thus ATP production, in birds (Walter and Seebacher 2009). Lerner et al. (1972) concluded that levels of oleic acid esters in the liver act as natural regulators of ANT activity, and the uncoupling of oxidative phosphorylation. In support of this idea, Wojtczak and Zaluska (1967) found that ANT activity in rat liver was inhibited by oleic acid. Such observations are consistent with our finding that the proportion of oleic acid in the liver was higher in quail whose T_b set point was lower in the hypothermic state (Falkenstein et al. 2001; Fietz et al. 2003). We, therefore, propose that high levels of oleic acid in the tissues of quail fed supplementary SFAs lead to down-regulation of thermogenesis. It is, however, important to note that the proportion of oleic acid was higher in the SFA group than both the UFA and the control groups, indicating that the trend of lower MR and T_b in this group may be explained by the uncoupling of oxidative phosphorylation.

Conclusions

The results of the present study support the concept that dietary FAs have thermoregulatory consequences in animals. We have shown how, under the same environmental conditions (i.e., cold stress and fasting), but different dietary FA composition, Japanese quail modulate their thermoregulatory responses. Possibly, SFA-supplemented quail lowered their T_b set point and managed their thermal conductance correspondingly. We also examined FA composition of muscle and liver tissues in order to elucidate possible physiological mechanisms that account for these differences and found that differences in T_b were correlated with different FA composition of the tissues. Quail whose T_b decreased more had a higher proportion of liver oleic acid, which is believed to inhibit ANT activity and thus reduce thermogenesis. The tissues of these quail also had lower proportions of arachidonic acid that is thought to be involved in regulation of T_b through the metabolites it yields. Further study of how specific FAs in different tissues, and how classes of lipids, are influenced by different environmental variables is necessary for a mechanistic understanding of the relationship between environmental variables and physiological response at the FA level.

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