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Ambient temperature and nutritional stress influence fatty acid composition of structural and fuel lipids in Japanese quail (*Coturnix japonica*) tissues

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ABSTRACT

In birds, fatty acids (FA) serve as the primary metabolic fuel during exercise and fasting, and their composition affects metabolic rate and thus energy requirements. To ascertain the relationship between FAs and metabolic rate, a distinction should be made between structural and fuel lipids. Indeed, increased unsaturation of structural lipid FAs brings about increased cell metabolism, and changes in the FA composition of fuel lipids affects metabolic rate through selective mobilization and increasing availability of specific FAs. We examined the effects of acclimation to a low ambient temperature (T_a: 12.7 ± 3.0 °C) and nutritional status (fed or unfed) on the FA composition of four tissues in Japanese quail, *Coturnix japonica*. Differentiating between neutral (triglycerides) and polar (phospholipids) lipids, we tested the hypothesis that both acclimation to low T_a and nutritional status modify FA composition of triglycerides and phospholipids. We found that both factors affect FA composition of triglycerides, but not the composition of phospholipids. We also found changes in liver triacylglyceride FA composition in the low-T_a acclimated quail, namely, the two FAs that differed, oleic acid (18:1) and arachidonic acid (20:4), were associated with thermoregulation. In addition, the FAs that changed with nutritional status were all reported to be involved in regulation of glucose metabolism, and thus we suggest that they also play a role in the response to fasting.

1. Introduction

To survive changing environmental conditions, animals must balance their metabolic fuel supply with their rates of energy expenditure. Endotherms that maintain body temperature (T_b) and metabolic rate (MR) at constant high levels face an even greater challenge in regulating energy balance. In birds, fatty acids (FA) are the major metabolic fuel for exercise and in times of reduced food availability (Lindstrom and Piersma, 1993; McWilliams et al., 2004; Guglielmo, 2010). Fatty acids, as components of complex lipids, serve three main functions: as metabolic fuel, as structural components of cell membranes and as signaling molecules. In addition, there is accumulating evidence that the FA composition of the body tissues can affect these different functions (Hulbert and Else, 1999; Larsson et al., 2004; McWilliams et al., 2004; Guglielmo, 2010; Price, 2010). Therefore, the composition of FAs in tissues plays a significant role in supporting metabolism to meet the bird's energetic requirements.

A distinction should be made between the effects of FAs in structural lipids such as those found in the phospholipids of cell membranes and FAs in fuel lipids, such as the triglycerides that are stored in adipose tissue, intramuscular fat droplets, and in the liver of birds (Ramenofsky, 1990; Jenni-Eiermann and Jenni, 1992). Structural lipids are thought to affect the molecular activity of many membrane-bound proteins altering the leakiness of the membranes and ultimately cellular metabolism (Hulbert and Else, 1999). The presence of more unsaturated FAs in cell membranes was shown to result in higher molecular activity, leading to an increase in cellular metabolism and consequently in whole animal MR (e.g., Brookes et al., 1998; Wone et al., 2013). Fuel lipids can also affect MR due to differential mobilization and oxidation of shorter and more unsaturated FAs (Raclot and Groscolas, 1993, 1995; Raclot, 2003). The premise being that birds manipulate the FA composition of their fat depots to facilitate the rapid mobilization of FAs and their transport into myocytes (McWilliams et al., 2004; Maillet and Weber, 2007; Price et al., 2008; Guglielmo, 2010; Price et al., 2011). When birds modulate their MR in response to environmental cues, the relative proportion of triglycerides and phospholipids may change (Price and Valencak, 2012). Many investigators who studied the effect of FA composition of body lipids on MR did not separate these two types of lipids (e.g., Parker and

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Holm, 1990; Chen and Chiang, 2005; McCue, 2008), and therefore the changes that they observed in total FA composition may have been a consequence of changes in the proportions of triglycerides and phospholipids in a particular tissue.

A cue that commonly brings about increased MR in birds is a decrease in ambient temperature (T_a) to below the animal's lower critical temperature (T_{lc}). Several researchers found that birds make seasonal adjustments in the FA composition of structural lipids, typically seen as an increase in unsaturated FAs during fall and winter (Barnett, 1970; Yom-Tov and Tietz, 1978; Conway et al., 1994). According to the homeoviscous hypothesis (Sinesky, 1974; Hazel, 1995), increased FA unsaturation widens the range of temperatures over which membrane fluidity can be sustained, thereby maintaining cellular function at cooler temperatures. These changes in FA composition are thought to occur within hours (Seebacher et al., 2010). Because many avian species were shown to use facultative hypothermia in different ecological contexts (McKechnie and Lovegrove, 2002), it may be adaptive for these birds to regulate cell membrane FA composition to maintain the fluidity of cell membranes in such a way that energy can be mobilized even when T_b changes.

Cold acclimation has also been associated with increased lipid oxidation (Freeman, 1967) and changes in the FA composition of stored fat (Kodama and Pace, 1964; Chen and Chiang, 2005). However, these results are equivocal, with some studies reporting an increase in the proportion of unsaturated FAs in adipose tissue (Irving et al., 1957; Williams and Platner, 1967), and others reporting increased saturated FAs (Chen and Chiang, 2005). Fasting is a common physiological state in which significant changes in metabolism occur (Castellini and Rea, 1992). Most birds rely almost exclusively on lipid metabolism when they fast, and therefore the selective mobilization and subsequent oxidation of specific FAs was predicted by Raclot (2003) to result in changes in the FA composition of stored fat. In this regard, it has been reported that the FA composition of different body tissues following a fast becomes enriched in saturated FAs (Groscolas and Herzberg, 1997; Mustonen et al., 2009), probably as a result of the preferential metabolism of unsaturated FAs (Raclot and Groscolas, 1995). In addition to changes in stored lipids, there is evidence for selective retention of specific FAs in cell membranes in response to fasting (Groscolas and Herzberg, 1997). Unlike the selective mobilization of shorter chain, unsaturated FAs from fat stores cell membrane lipids appear to follow a different pattern whereby n-6 FAs, such as arachidonic acid (20:4) and linoleic acid (18:2), are preferentially retained in the plasma membrane and may even increase in quantity during fasting (Groscolas and Herzberg, 1997; Nieminen et al., 2006a; Nieminen et al., 2006b; Mustonen et al., 2007). While the significance of such changes in FA composition in tissues remains unclear, most appear to be primarily attributable either to selective mobilization and transport or to gross changes in the proportions of triglycerides and phospholipids in the tissue (Price and Valencak, 2012).

In light of the above, we examined the effects of cold acclimation and fasting on the FA composition of four tissues: pectoral muscle, liver, heart, and claviculocoracoid fat in Japanese quail, *Coturnix japonica*. We differentiated between neutral (i.e., triglycerides) and polar (i.e., phospholipids) lipids to facilitate our understanding of changes we might observe. We hypothesized (H_1) that cold acclimation affects the FA composition of both triglycerides and phospholipids in tissues, but not necessarily in the same manner. We tested the specific predictions that in quail, acclimated to a temperature below their T_{lc}, (1) the unsaturation level of their tissue phospholipids increases to maintain cellular function and (2) unsaturated triglycerides are selectively oxidized to support increased rates of heat production. We further hypothesized (H_2) that fasting affects the FA composition of triglycerides and phospholipids in tissues. We tested the predictions that the tissues of quail subjected to a period of fasting have (1) reduced unsaturation levels and increased chain length of tissue triglycerides due to selective mobilization of more unsaturated FA of shorter chain length and (2) increased levels of n-6 FAs in their tissue phospholipids.

2. Materials and methods

2.1. Experimental design

Sixteen Japanese quail chicks, eight of each sex and of similar age, were purchased from a commercial breeder (Joseph Yanai, Mata, Israel) and 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) until the experiments began. Fully grown quail (6 weeks old) were divided into two body mass (m_b)-matched experimental groups (173.7 ± 12.44 g and 171.4 ± 7.14 g, respectively) containing equal numbers of each sex. The quail were housed individually in cages (40 × 30 × 30 cm) inside one of two controlled temperature chambers. One chamber was maintained within the birds' thermal neutral zone (TNZ; 32.6 ± 0.2 °C), and the other was maintained below the quails' T_{lc} (12.7 ± 3.0 °C). Photoperiods were identical in both treatment groups and followed the natural cycle. During the thermal acclimation period, tap water and feed, consisting of cracked corn, wheat, soy, plus a mixture of vitamins and minerals (Hemed Lachay, Hemed, Israel), were available *ad libitum*. The FA composition of the diet is found elsewhere (Table 1; Ben-Hamo et al., 2011). Body mass (m_b) was measured (±0.1 g) at sunrise each morning, and T_a was continually recorded using calibrated (against an Hg-in glass thermometer with accuracy traceable to the US-NIST), temperature-sensitive data loggers (iButton, Maxim Integrated Products, Dallas Semiconductor) placed in shaded, well-ventilated areas next to each quail cage.

After 7 days, four quail from each group were deprived of food for 4 days. Food was removed from the cages at sunset, and the following photophase and the subsequent scotophase were considered the first day of food deprivation. Tap water was always available to fasting birds. Birds were weighed and T_b was measured daily, as described above.

2.2. Metabolic rates

We measured MR of quail that were fasting or feeding *ad libitum* on the first and fourth nights of the experiment, from sunset and until sunrise. The quail that were feeding were not postabsorptive, but since they were measured throughout the night, we excluded the first 6 h of data, assuming that the birds were post-absorptive after that. Metabolic rates were determined by indirect calorimetry using an eight-channel open-flow gas analysis system as described by Ben-Hamo et al. (2010). Rates of oxygen consumption ($\dot{V}O_2$) were measured at a T_a of either 12 ± 1 °C or 33 ± 1 °C in a temperature controlled cabinet. The fractional concentrations of oxygen in the incurrent and excurrent gas streams (i.e., F_IO_2 and F_EO_2) were measured with a FoxBox O_2 analyzer (Sable Systems International, Las Vegas, NV, USA). $\dot{V}O_2$ was calculated using Eq. 2 of Hill (1972) and was converted to units of power (W), assuming 20.08 J/ml O_2 (Schmidt-Nielsen, 1997). For consistency, we used the minimum MR, averaged over 6-min periods.

2.3. Fatty acid analysis

Quail were weighed and killed by decapitation in the morning following the last day of the experiment. Pectoral muscle, liver, heart and claviculocoracoid adipose tissue were harvested, placed in individually labeled glass vials and frozen. Tissue samples were later freeze-dried and then homogenized using pestle and mortar. Total lipids were extracted from bird tissues 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

Table 1
Mean fatty acid composition (%) ± 1SD of neutral and polar lipids from four tissues of Japanese quail (*Coturnix japonica*).

	Neutral lipids				Polar lipids			
Fatty acids	Adipose	Heart	Liver	Pectoralis	Adipose	Heart	Liver	Pectoralis
14:0	1.2 ± 0.5	1.7 ± 0.9	1.5 ± 0.6	1.0 ± 0.4	1.0 ± 0.6	2.2 ± 0.6	0.4 ± 0.1	1.3 ± 0.7
14:1	0.5 ± 0.7	1.2 ± 0.6	0.0 ± 0.0	1.5 ± 0.7	0.6 ± 0.5	1.7 ± 0.8	0.1 ± 0.1	1.1 ± 0.8
16:0	18.1 ± 3	16.1 ± 5.6	20.9 ± 11	20.7 ± 2.5	16.7 ± 8.4	9.5 ± 7.5	21.2 ± 2.1	15.1 ± 2
16:1	7.0 ± 2.8	6.9 ± 2.9	7.1 ± 4.1	9.2 ± 4.0	5.5 ± 2.7	1.6 ± 0.6	1.9 ± 1.1	1.9 ± 0.9
18:0	6.6 ± 2.3	6.1 ± 2.1	12.9 ± 6	6.2 ± 2.7	10.9 ± 2.5	22.1 ± 2.5	22.8 ± 2.1	24.0 ± 2.5
18:1	44.8 ± 2.9	30.4 ± 11.5	28.3 ± 9.6	42.0 ± 7.1	34.9 ± 10.3	12.8 ± 4.4	17.5 ± 6.5	12.0 ± 4.2
18:2 n-6	15.3 ± 4.4	13 ± 5.2	8.7 ± 4.0	11.9 ± 2.9	15.5 ± 4.5	15.7 ± 2.5	11.2 ± 1.9	12.9 ± 1.8
18:3 n-3	0.4 ± 0.3	1.5 ± 2.0	0.8 ± 2.2	0.3 ± 0.4	0.7 ± 0.7	0.1 ± 0.1	0.3 ± 0.2	0.1 ± 0.2
20:4 n-6	0.5 ± 0.6	2.8 ± 2.9	4 0 ± 3.2	1.8 ± 3.0	3.6 ± 2.3	0.5 ± 0.2	15.8 ± 3.3	0.4 ± 0.2
20:3 n-6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.5	27.7 ± 3.6	0.1 ± 0.1	22.4 ± 3.3
20:3 n-3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.7 ± 0.9	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.2
20:5 n-3	0.1 ± 0.2	1.9 ± 2.2	0.1 ± 0.3	0.0 ± 0.0	0.4 ± 0.6	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
22:6 n-3	0.0 ± 0.1	1.1 ± 0.9	0.8 ± 1.1	0.2 ± 0.3	0.5 ± 0.7	1.3 ± 0.3	3.5 ± 2.2	3.6 ± 1.2

separate glass vial, and dried under N_2. Samples were stored at -20 °C for up to 2 weeks (Christie, 2003) before analysis. Polar and neutral lipid fractions were separated by passing samples through a 500-mg silica column (Bond ElutTM; Varian USA) with chloroform for neutral lipids, followed by methanol for polar lipids (McCue et al., 2009). Each lipid fraction was transmethylated for one hour at 85 °C in anhydrous methanol (Sigma-Aldrich, St. Louis, MO, USA) containing 2% H_2SO_4 (v/v). The reaction was terminated by adding water, and 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, Torrance CA, USA), programmed temperature vaporization injector, and flame ionization detector, using helium as a carrier gas. The temperature program began with a 1-min hold at 130 °C followed by a linear increase of 2 °C min^{-1} to 240 °C and final hold of 10 min at the high temperature. FAMEs, ranging in length from 14 to 24 carbons, were identified by comparing chromatograms with retention times of FAMEs of a fish oil standard (Avanti Polar Lipids, Alabaster AL, USA) resolved in parallel with the unknown samples. All FA analyses were done within 2 months of tissue preparation (Christie, 2003). Unfortunately, we lost all the samples derived from heart tissue of fed quail of the low T_a group, and therefore we could not test for the effect of temperature on changes in the FA composition of the heart tissue.

2.4. Statistical analysis

Proportions of fatty acids are reported as percentages of total FA by mass. In most cases, it was impossible to resolve between 18:1n-9 and the less common 18:1n-7 FAs; therefore, proportions of these were combined into a single value referred to as 18:1. Results of the FA profiles of tissues from male and female quail were combined within respective treatment groups once it was determined that there were no detectable differences between them. Unsaturation indices (UI) were calculated as the sum of the fraction of each unsaturated FA (Ni) multiplied by its respective degree of unsaturation (i) and by 100 (e.g., Pan and Storlien, 1993; Stuart et al., 1998) following McCue (2008):

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

We used multivariate analysis of variance (MANOVA) with T_a and nutritional state (fed or unfed) as fixed factors and the various FAs as dependent variables for both polar and neutral lipids. Roy's greatest root was the statistic we used for comparison in all MANOVA tests. We considered only FAs that accounted for >1% of the total fatty acids in each tissue. We used a two-way ANOVA to test the effect of T_a and nutritional state on MR and a mixed-effects linear model to examine the relationships among these variables and m_b on individuals, with individual as a random factor. Finally, we used a linear model to compare differences in UI between the different lipid classes and treatments. We used multiple comparisons to compare between groups that we found significantly different using Bonferroni corrected *p*-values. Means are presented ± 1 SE, and $\alpha = 0.05$ was chosen as the lowest acceptable level of significance. All statistical analyses were done with "R" 3.0.0.

3. Results

3.1. Body mass

Quail in both temperature treatment groups had similar initial m_b (173.7 ± 12.44 g and 171.4 ± 7.14 g), but following 4 days of fasting, the group kept at a T_a below their T_{lc} lost significantly more m_b than those kept within their TNZ (Fig. 1; mixed-effects linear model: $t_{46} = 2.99$, $p = 0.004$).

3.2. Metabolic rates

The quail kept below their T_{lc} had significantly higher mass-specific MR (MR/m_b) than the quail kept within their TNZ (11.84 ± 1.53 mW/g vs. 8.48 ± 0.92 mW/g; two-way ANOVA: $F_{1,8} = 12.15$, $p = 0.008$;

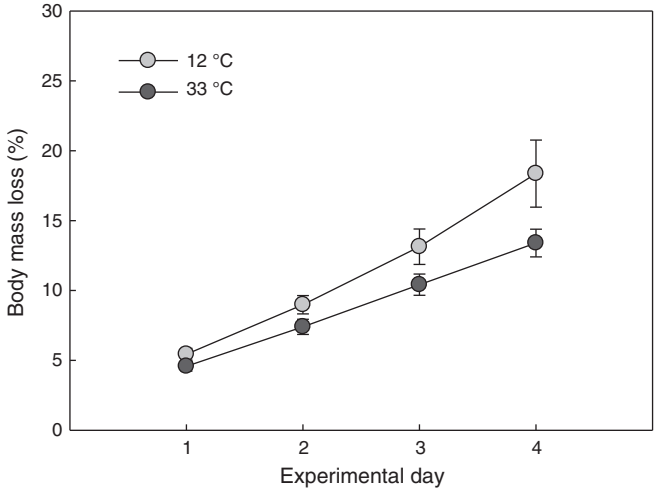

Fig. 1. The fraction of initial body mass (in %) lost during 4 days of fasting in Japanese quail. Light grey symbols denote the means for 8 quail that were kept at a temperature below their lower critical temperature (12.7 ± 3.0 °C), and dark grey symbols denote means for 8 quail that were kept within their thermoneutral zone (32.6 ± 0.2 °C). Error bars are ± SE.

Fig. 2). MR/m_b decreased significantly in both groups during the 4-day fast (Fig. 2; two-way ANOVA: $F_{1,8} = 23.68$, $p = 0.001$).

3.3. Fatty acid composition of neutral lipids

The FA composition of neutral lipids from all four tissues is presented in Table 1. There was a significant change in FA composition of the claviculocoracoid adipose tissue during the 4-day fast (Fig. 3A; MANOVA: $Roy_{(1,6)} = 7674$, $p = 0.02$); namely, we observed a decrease in the fractions of palmitic acid (16:0) and palmitoleic acid (16:1). The liver FA composition of fasting quail differed from that of quail feeding *ad libitum* (Fig. 3B; MANOVA: $Roy_{(1,7)} = 23$, $p = 0.04$); arachidonic acid (20:4 n-6) increased and oleic acid (18:1) decreased in fasted animals. Similarly, we found that liver FA composition differed between quail acclimated to different T_as (Fig. 3C; MANOVA: $Roy_{(1,7)} = 32$, $p = 0.03$); arachidonic acid (20:4 n-6) increased and oleic acid (18:1) decreased in the quail maintained below their T_{lc}.

The pectoral muscle FA composition differed between fasting quail and those feeding *ad libitum* (Fig. 3C; MANOVA: $Roy_{(1,7)} = 8$, $p = 0.04$); palmitic acid (16:0) and palmitoleic acid (16:1) decreased and linoleic acid (18:2 n-6) increased during the 4-day fast. There was no difference in pectoral muscle FA composition between quail acclimated to different T_a (MANOVA: $Roy_{(1,7)} = 1$, $p = 0.6$). In the heart tissue, there were no differences between quail either from the different nutritional status groups, or between the groups acclimated to different T_as (Fig. 3D; MANOVA: $Roy_{(1,6)} = 24$, $p = 0.4$, $Roy = 41$, $p = 0.3$).

3.4. Fatty acid composition of polar lipids

The FA composition of polar lipids from all four tissues is presented in Table 1. There was no change in the FA composition of polar lipids in the claviculocoracoid adipose tissue (MANOVA: temperature-$Roy_{(1,6)} = 18$, $p = 0.4$, nutritional status-$Roy_{(1,6)} = 55$, $p = 0.2$), the heart (MANOVA: temperature-$Roy_{(1,9)} = 7$, $p = 0.5$, nutritional status-$Roy_{(1,9)} = 7$, $p = 0.4$), the liver (MANOVA: temperature-$Roy_{(1,7)} = 41$, $p = 0.3$, nutritional status-$Roy_{(1,7)} = 48$, $p = 0.3$), or the pectoral muscle (MANOVA: temperature-$Roy_{(1,11)} = 20$, $p = 0.08$, nutritional status-$Roy_{(1,11)} = 13$, $p = 0.1$).

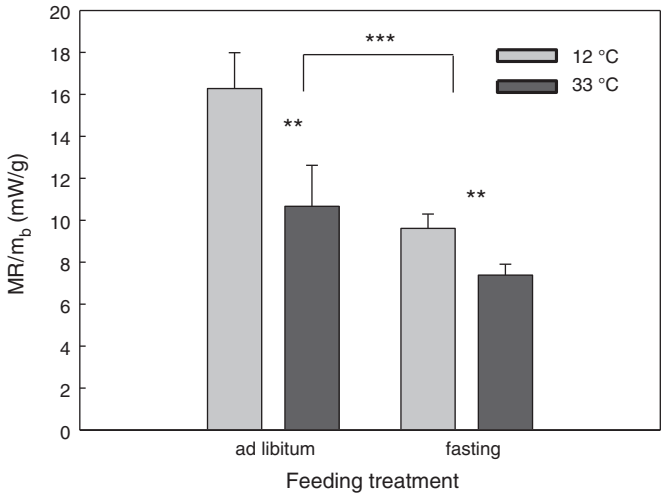

Fig. 2. Mass-specific metabolic rate (MR/m_b) of Japanese quail as a function of their nutritional status (fed or unfed). Light grey bars denote the means of 4 quail kept at a temperature below their lower critical temperature (12.7 ± 3.0 °C), and dark grey bars denote the means of 4 quail kept within their thermoneutral zone (32.6 ± 0.2 °C). Error bars are ± SE. ***$p < 0.001$ and **$p < 0.01$.

3.5. Unsaturation index

Polar lipids were significantly more unsaturated ($UI_{(polar)} = 134.6$) than neutral lipids (Fig. 4; ANOVA: $F_{1,65} = 181.2$, $p < 0.001$, $UI_{(neutral)} = 96.2$), and following the 4-day fast, there was a significant increase in the unsaturation index of all tissues (Fig. 4; ANOVA: $F_{1,65} = 21.8$, $p < 0.001$). In contrast, there was no relation between T_a and UI (ANOVA: $F_{1,65} = 3.6$, $p = 0.06$).

4. Discussion

Our data indicate that in pectoral muscle, liver, and claviculocoracoid fat tissues of Japanese quail, the FA composition of phospholipids respond less to changes in MR and fasting than the FA composition of triglycerides. While the FA composition of triglycerides was different between both quail acclimated to different T_as and fed or fasted quail (nutritional status) (Fig. 3), we found no significant changes in the FA composition of phospholipids. Our observations concur with previous reports that the FA composition of phospholipids is more resistant to alteration than that of triglycerides, and although it can change with drastic changes in diet, the variation in composition is often limited to changes in a few specific FAs with no net change in the total fractions of unsaturated FAs (Ayre and Hulbert, 1997; Newman et al., 2002; Thil et al., 2003; Turner et al., 2004; Vaillancourt and Weber, 2007; Guderley et al., 2008; Klaiman et al., 2009; Nagahuedi et al., 2009). The underlying assumption is that phospholipids have a lower turnover rate than triglycerides and that their resistance to change may be due to their function in maintaining membrane fluidity and cellular function (reviewed by Price and Valencak, 2012). It is possible that a longer fasting period would have resulted in changes in the FA composition of phospholipids.

In addition, we found that the UI of phospholipids was significantly higher than that of triglycerides (Fig. 4), which is also in accord with previous studies (Nelson, 1962; Delgado et al., 1994; Price and Guglielmo, 2009). Price and Valencak (2012) suggested this difference may explain the changes observed in tissue FA composition is fasting animals, where total FA composition, and not the composition of purified polar and neutral lipids, was measured. According to these authors, since triacylglyceride oxidation increases in fasting animals, but not necessarily phospholipid oxidation, total FA composition tends towards unsaturation because of the difference in the proportions of phospholipids and triglycerides (Price and Valencak, 2012). Our results emphasize the importance of separating these two classes of lipids in order to understand the importance of the qualitative and quantitative changes that take place in their composition.

4.1. Fatty acid composition and thermoregulation

In the present study, only the triglycerides from the liver differed between quail acclimated to different T_as (Fig. 3), and we did not find support for the homeoviscous hypothesis in our quail. Specifically, the fraction of arachidonic acid (20:4 n-6) in liver increased and that of oleic acid (18:1) decreased in quail maintained below their T_{lc}. Reductions in oleic acid content and increases in arachidonic acid content are routinely documented in the brown adipose tissue of rodents exposed to T_{lc} (Ocloo et al., 2007), but birds do not possess brown adipose tissue. In birds, the liver is the main site of *de novo* synthesis of lipids and therefore plays a key role in lipid metabolism by serving as a hub for fatty acid synthesis and circulation (Nguyen et al., 2008). It is possible that the change in T_a brought about changes in metabolism of arachidonic acid and oleic acid in the liver. We previously reported that these two FAs may be involved in thermoregulation in Japanese quail (Ben-Hamo et al., 2011). We found that an increase in the fraction of oleic acid causes down regulation of thermogenesis (Ben-Hamo et al., 2011), which may explain why quail acclimated to a $T_a < T_{lc}$ in this study had lower levels of this FA.

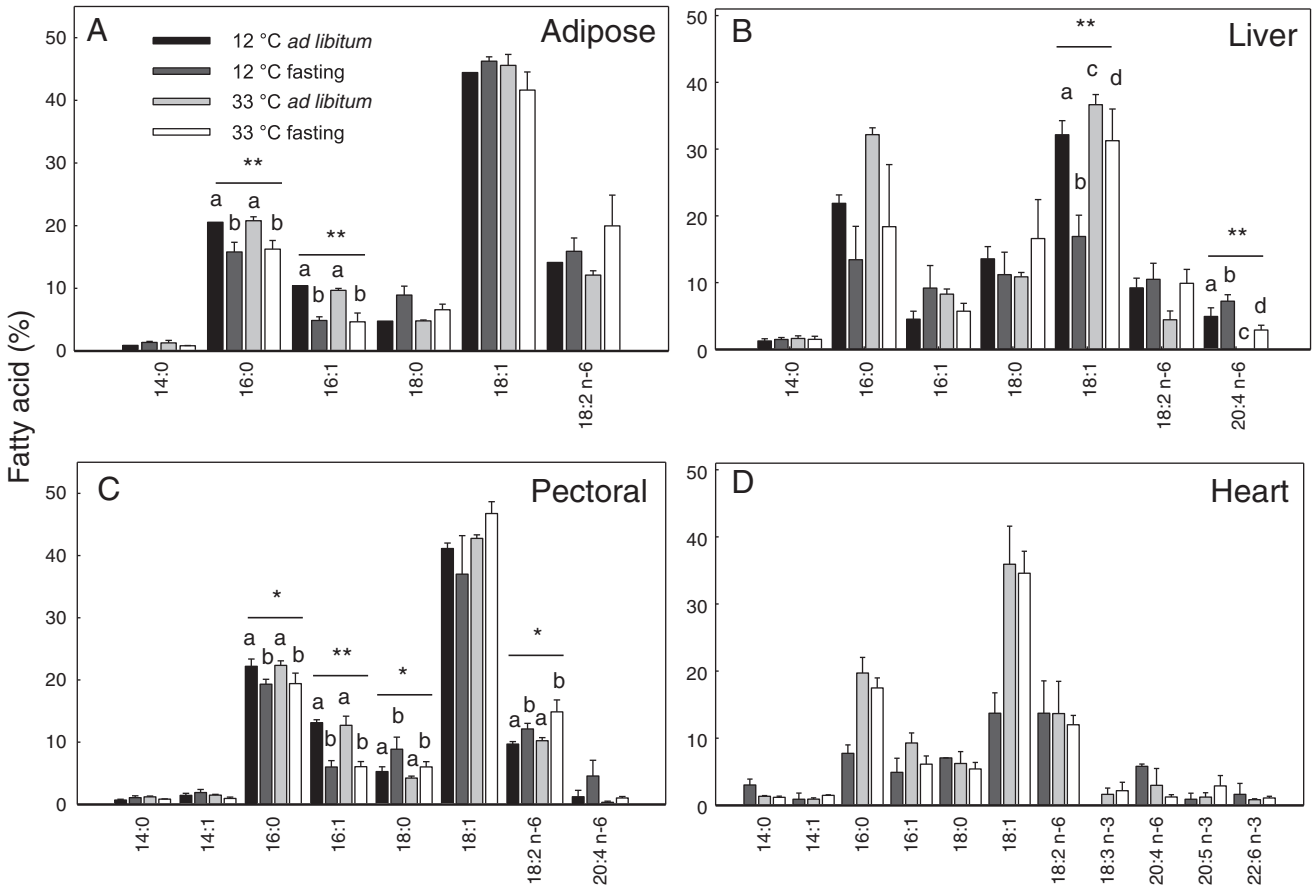

Fig. 3. The fatty acid composition (%) of neutral lipids in the (A) adipose tissue, (B) liver, (C) pectoral muscle and (D) heart muscle. Black bars denote the means of 4 quail that were fed *ad libitum* and kept at a temperature within their thermoneutral zone; dark grey bars denote the means of 4 quail that fasted and were kept below their lower critical temperature (T_{lc}); light grey bars denote the means of 4 quail that were fed *ad libitum* and kept within their thermoneutral zone; and white bars denote the means of 4 quail that fasted and were kept within their thermoneutral zone. Error bars are ± SE. *Fatty acids that were affected by the treatment with $p < 0.05$. **Fatty acids that were affected by the treatment with $p < 0.01$. Different letters denotes statistically significant differences.

We acclimated quail to different T_as, which resulted in significant differences in their MR (Fig. 2), with quail kept below their TNZ having higher MR/m_b. Interestingly, inhibition of eicosanoid production (eicosanoids being biologically active downstream products of arachidonic acid) in rats kept below their T_{lc} impaired their thermoregulatory ability (Bizzi et al., 1965; Satinoff, 1972; Solomonovich and Kaplanski, 1985).

An alternative explanation may be that oleic acid, which is synthesized *de novo*, may be reduced simply due to a systemic decline in fatty acid synthesis because of the increased energy requirements associated with a low T_a, and as a result the relative proportion of other FAs, arachidonic acid in this case, increased.

4.2. The effect of fasting on fatty acid composition

The FA composition of triglycerides in claviculocoracoid adipose tissue, liver and pectoral muscle all changed significantly during the 4-day fast (Fig. 3). The general trend we observed in all tissues was a decrease in the proportions of shorter, long-chain FAs—namely, C16. These results are consistent with observations that birds selectively mobilize and oxidize shorter FAs (Price et al., 2008; Mustonen et al., 2009; Price et al., 2011). Price and Valencak (2012) questioned the effect of fasting on FA composition and suggested that changes observed in FA composition following a fast result from a combination of selective mobilization and oxidation of specific FAs as well as fasting induced differences in the proportions of triglycerides and phospholipids. In the present study, we observed evidence for the selective mobilization of shorter and unsaturated FAs. This is supported by the reductions in the proportion of 16-carbon FAs in the adipose and muscle triglycerides (Fig. 3). Because we separated neutral and polar fractions, it is likely that the changes in the FA composition are not only a result of the cumulative effects of selective mobilization of particular fatty acids, rather there is also selective mobilization and metabolism of FAs that play a role in the birds' response to fasting.

We found a decrease in the fraction of palmitic acid (16:0) in claviculocoracoid adipose tissue, but not in the liver. The adipocyte triglycerides are hydrolyzed to non-esterified FA and glycerol and released into the plasma, bound by albumin and carried to the muscles (Ramenofsky, 1990; Raclot, 2003). The decrease in the fraction of palmitic acid in pectoral muscle suggests that there was oxidation of this FA from both extra- and intra-muscular sources. In this regard, Blumenthal (1983) found that albumin-bound palmitic acid in plasma increases hepatic gluconeogenesis in the liver of rats, which may suggest that quail released palmitic acid into their blood in response to fasting. Analyses of free FAs in circulation are needed to confirm this.

The fraction of linoleic acid (18:2 n-6) in pectoral muscle triglycerides increased during fasting (Fig. 3), which is in accordance with the reported retention of linoleic acid in adipose tissue of pheasant, *Phasianus colchicus mongolicus* (Mustonen et al., 2009). However, this observation is in contrast to the selective mobilization of linoleic acid found in fasting rats (Herzberg and Farrell, 2003). These differences may be explained by the different diets of quail and rats or, alternatively, by differences in the biochemical pathways of FA

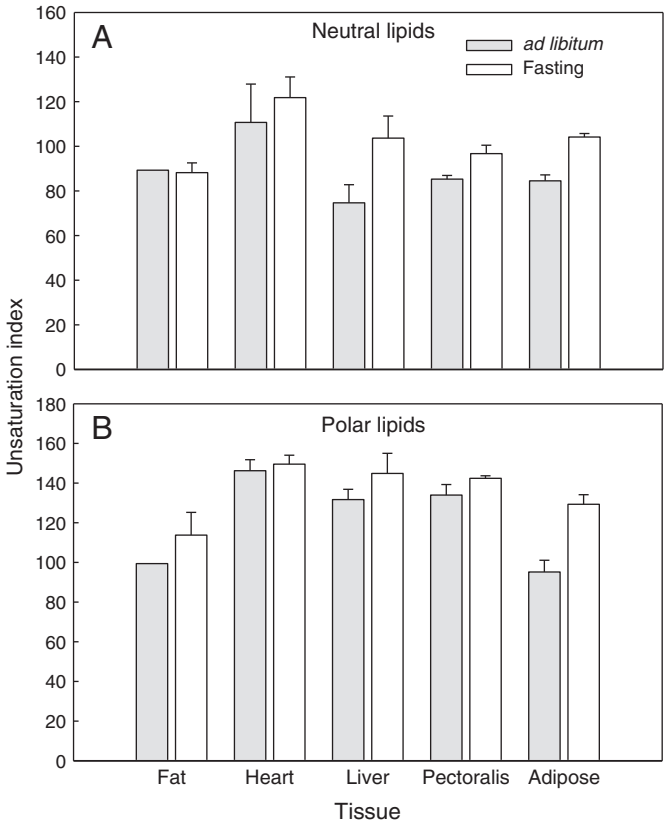

Fig. 4. Unsaturation index of (A) neutral and (B) polar lipids in four tissues of Japanese quail kept below their lower critical temperature (T_{lc}) and within their thermoneutral zone combined. Grey bars denote means of 4 quail that were fed *ad libitum*, and white bars denote means of 4 quail that fasted for 4 days. Error bars are ± SE.

synthesis and mobilization (see Mustonen et al., 2009). Finally, the increase we found in the fraction of liver arachidonic acid is consistent with other studies of fasting animals (Qu et al., 1998). Here, the underlying postulate is that during fasting, arachidonic acid metabolism in the liver is down-regulated (Qu et al., 1998), whereas upon re-feeding, arachidonic acid metabolism increases, and that its metabolites play a role in the hormonal regulation of glucose levels (Groener et al., 1979; Qu et al., 1998).

5. Conclusions

Japanese quail acclimated to different ambient temperatures and with different nutritional statuses (fed or unfed) differed in the FA composition of their tissue triglycerides, whereas we found no such differences in the FA composition of tissue phospholipids. These findings support the idea that triglycerides respond more flexibly in their composition to changes in metabolic rate and food availability than do phospholipids. We found that only liver triglycerides changed in response to acclimation of quail to different T_as, probably because of the important role of the liver in lipid metabolism in birds. Of note is the fact that the two FAs found to be significantly affected by the change in T_a were previously reported to be involved in thermoregulation. In addition, we found that fasting induced a decrease in the fraction of shorter-chain FAs, which is consistent with the idea that they are selectively mobilized and oxidized. Therefore, we conclude that the changes in FA composition in the body of a fasting animal may be a result of the basal selective metabolism of specific triglycerides and their increased metabolism during fasting, and not necessarily an adaptation to fasting itself (Price and Valencak, 2012). Nevertheless, we observed an increase in tissue FA UIs following fasting, and the specific FAs that were changed with the nutritional status of the quail are all involved in regulation of glucose metabolism in the body. We posit that these specific FAs may be involved in the response of the quail to fasting as well.

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