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High content of polyunsaturated fatty acids in muscle phospholipids of a fast runner, the European brown hare (*Lepus europaeus*)

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Abstract To investigate both seasonal changes and possible intracorporal gradients of phospholipid fatty acid composition, skeletal muscles ($n = 124$), hearts ($n = 27$), and livers ($n = 34$) from free-living brown hares (*Lepus europaeus*) were analyzed. Phospholipids from both skeletal muscles and heart had a high degree of unsaturation with $66.8 \pm 0.63\%$ and $65.7 \pm 0.5\%$ polyunsaturated fatty acids, respectively. This is the highest proportion of polyunsaturated fatty acids reported in any mammalian tissue. Polyunsaturated fatty acid content in skeletal muscles was 2.3% greater in winter compared to summer ($F_{1,106} = 17.7$; $P = 0.0001$), which may reflect thermoregulatory adjustments. Arachidonate (C20:4n-6) showed the greatest seasonal increase ($+2.5\%$; $F = 7.95$; $P = 0.0057$). However, there were no pronounced differences in polyunsaturated fatty acid content between skeletal muscles from different locations in the body (*m. iliopsoas*, *m. longissimus dorsi* and *m. vastus*). Total muscle phospholipid polyunsaturated fatty acid content was correlated with polyunsaturated fatty acid content in triacyglycerols from perirenal white adipose tissue depots ($r^2 = 0.61$; $P = 0.004$). Polyunsaturated fatty acids were enriched in muscle phospholipids (56.8–73.6%), compared to white adipose tissue lipids (20.9–61.2%), and liver phospholipids (25.1–54.2%). We suggest that the high degree of muscle membrane unsaturation is related to hare-specific traits, such as a high maximum running speed.

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Abbreviations *BMR*: basal metabolic rate · *DPA*: docosapentaenoic acid · *DHA*: docosahexaenoic acid · *FA*: fatty acid · *MUFA*: monounsaturated fatty acid · *PC*: principal component · *PUFA*: polyunsaturated fatty acid · *SFA*: saturated fatty acid · *UI*: unsaturation index · *WAT*: white adipose tissue

Introduction

Polyunsaturated fatty acids (PUFAs) are essential to biological membranes, both as major constituents of phospholipids, and as regulators of membrane-associated enzymes. Additionally, they affect intercellular transport systems (Stubbs and Smith 1984) and are precursors for eicosanoids, which are involved in complex cellular processes such as immune responses and reproduction (Geiser 1990; Florant 1998; Pond and Mattacks 1998).

Mammals must obtain certain PUFAs, namely linoleic acid (C18:2n-6) and alpha-linolenic acid (C18:3n-3) from their diet because they lack the enzymes necessary for their synthesis, although they can convert essential dietary fatty acids to longer-chain fatty acids (FAs). Thus, diet strongly influences phospholipid composition of muscle membranes (Pan et al. 1994; Ayre and Hulbert 1996a). Membrane fluidity is greatly impacted by PUFA content and increases with increasing PUFA concentration (reviewed by Hazel 1995). Not surprisingly then, mammals that hibernate or undergo daily torpor increase PUFA content both in depot lipids and in membrane phospholipids. These adjustments probably

serve to maintain functionality of membranes even at low body temperatures. Such changes, and beneficial effects of PUFA-enriched diets on hibernation have been shown in ground squirrels, hamsters and marmots (Geiser and Kenagy 1993; Frank and Storey 1995; Geiser and Heldmaier 1995; Bruns et al. 2000). High PUFA contents in depot fats of torpid mammals permit deeper core body temperatures and longer torpor bouts, which may increase winter survival rates (reviewed by Florant 1998). All these observations emphasize the importance of PUFAs for heterothermic mammals.

Within nonhibernators, membrane FA composition has rarely been investigated. Studies are largely restricted to bone-marrow fats in ungulates, where PUFA content increases towards peripheral body parts (reviewed by Pond et al. 1993). This high degree of unsaturation in the periphery was related to thermoregulation in the extremities in cold-exposed ungulates (Irving et al. 1957). Similarly, high PUFA levels were found in peripheral tissues of semiaquatic mammals, i.e., beavers (*Castor canadensis*) and muskrats (*Ondatra zibethicus;* Käkelä and Hyvärinen 1996a).

The role of PUFAs for small, terrestrial, nonhibernating mammals is not well understood however. We hypothesized that long chain PUFAs play an important role in maintaining tissue functionality in this group of mammals, particularly in species that use peripheral cooling to save energy in the cold. For example, the European brown hare (*Lepus europaeus*) is relatively small (3–5 kg) and inhabits north-temperate areas, but does not build insulating burrows and thus is exposed to severe climatic conditions. There is indirect evidence (from measurements of core temperature and oxygen consumption) for peripheral cooling during cold exposure in the brown hare (Hackländer et al. 2002). Therefore, we investigated PUFA contents in skeletal muscles, heart, liver, and fat depots of hares collected in their natural habitat during both the summer and winter season.

Our focus on muscle phospholipids was due to several reports pointing to an important role of FA composition in muscle performance (Ayre and Hulbert 1996a; Gorski et al. 1999; Andersson et al. 2000; Helge et al. 2001). This aspect should be particularly important for hares which are specialized on evading predators by rapid flight, and can reach peak velocities of up to 80 km h^{-1} (Zörner 1996). Therefore, we hypothesized that if high phospholipid PUFA proportions enhance muscle activity, they should be particularly enriched in hares compared to other species of similar size. Additionally, we addressed site-specific differences in the PUFA content of muscles. In particular, we wanted to know if hares adjust higher PUFA levels in peripheral than in central muscles. Finally, we hypothesized that phospholipid PUFA amounts in hare muscles undergo seasonal changes. We expected that hares may show seasonal adjustments, with an increased phospholipid PUFA content during winter.

Material and methods

Animals and tissue sampling

One hundred and twenty-four European brown hares were collected from a 64-km^2 area, located approximately 40 km east of Vienna, Austria, (48° 30' N; 15° 45' E, elevation 145 m). Tissue samples were collected either in early winter (November and December, 2000–2002) or in summer (2001), from hares shot by local hunters (winter samples), and from fresh road kills (summer). During the summer, we surveyed roads in the study area from 4:00 a.m. to 8:00 a.m. and collected warm and fresh carcasses that had intact tissue material at those sites where muscles were dissected. We only used fresh tissues without dried blood, gunshot wounds (winter samples), or discolorations.

We analyzed skeletal muscle samples from 101 winter and 23 summer individuals. In addition, in cases when complete carcasses were available, heart ($n = 27$), liver ($n = 34$), and white adipose tissue ($n = 16$) FA composition was determined (winter samples only). Hare mean body mass was $4{,}191 \pm 51.0$ g in adults and $3{,}063 \pm 114.5$ g in juveniles. Age classes were discriminated by dry eye lens weights (Suchentrunk et al. 1991) or, when the skull was severely damaged, by the ossification of the ulna that disappears at approximately 6–8 months of age (Stroh 1931).

In 53 carcasses, three different muscles were dissected: *musculus vastus* from the hind leg, *m. longissimus dorsi* from the back and *m. iliopsoas* from the body core. In 5 of these hares, we also dissected and analyzed the *m. adductor, m. biceps femoris, m. tensor fasciae latae, m. gluteus, m. gracilis, m. vastus, m. sartorius, m. semimembranosus,* and *m. rectus femoris* from the hind leg. In 57 additional animals, only samples from the *m. iliopsoas* were collected. Immediately after sampling, all muscle tissues were placed in plastic bags and stored at –18°C for 6–8 weeks.

Lipid extraction and analysis

We sampled 0.5 g of each muscle, 0.2 g liver and 0.05 g white adipose tissue (WAT). For lipid extraction from muscle and liver we used chloroform and methanol (2:1 v/v) according to Folch et al. (1957). All solvents contained butylhydroxytoluol in order to avoid oxidative modification of PUFAs. The lipid classes were separated on silica gel thin layer chromatography plates (Kieselgel 60, F254, 0.5 mm, Merck) with a mobile phase of *n*-hexane, diethylether, and formic acid (80:20:1, v/v/v). The lipid bands were made visible under ultraviolet light and the phospholipid fraction was isolated.

Muscle, heart, and liver phospholipid extracts were transesterified by heating (100°C) under nitrogen for 30 min (Eder 1995). Phospholipids were transesterified in sealed vials that contained 1 ml of 20% borontrifluoride in methanol. WAT tissue lipids were treated according to same method but without prior lipid extraction. The esters were then extracted into hexane and analyzed by GLC (Perkin Elmer Autosystem XL with Autosampler and FID; Norwalk, USA) using a capillary column (HP INNOWax, 30 m×0.25 mm; Hewlett Packard, USA). The temperature program was sufficient to elute all FA methyl esters.

FA methyl esters were identified by comparing retention times with those of FA methyl ester standards (Sigma-Aldrich, St. Louis, USA). Peaks were integrated using the Turbochrom 4.1 Software (Perkin Elmer, Norwalk, USA).

Data analysis

Data are given as mean ± SE. For all tissue samples an unsaturation index (UI) was computed (Couture and Hulbert 1995).

Statistical tests were performed using S-Plus 6.1 for Windows (Insightful Corporation, Seattle, USA). Differences between skeletal muscles were examined with linear mixed effects models (procedure "lme"; Pinheiro and Bates 2000), which incorporate the

interdependence of data obtained from different muscles collected from the same individual. Alpha levels were assessed based on type III sum of squares. Sample sizes vary because we analyzed only fresh and undamaged tissue material, and because we could not always obtain complete carcasses. Percentages were normalized using the arcsine-transformation (Sokal and Rohlf 1995). After initial comparisons among muscle types, all further analyses were restricted to data obtained from *m. iliopsoas,* which provided the largest sample ($n = 110$).

To see whether a statistical analysis supports combining FAs into certain classes, such as saturated fatty acids (SFAs) and PUFAs, we employed a principle component (PC) analysis. Consistent with this analysis, FA types were combined according to their degree of saturation and location of double bonds: SFAs: C14:0, C15:0, C16:0, C17:0, C18:0; MUFAs: C16:1, C18:1; n-6 PUFAs: C18:2, C20:4 and n-3 PUFAs: C18:3, C20:5, C22:5, and C22:6. These classes (as well as the ratio of n-3 to n-6 PUFAs) were used as response variables in subsequent tests.

In brown hares, FA composition is not significantly different between males and females, or between age classes (T.G. Valencak, unpublished data). Therefore, data from all animals were pooled for further analysis. In cases of variance heteroscedascity, we used a weighting procedure to correct for unequal variances between groups (using the "Identity" variance structure in procedure GLS in S-Plus 6.1). Pearson's coefficients were computed to test for correlations between FA compositions in skeletal muscles, heart, liver, and WAT.

Results

Differences between muscles

We found only slight differences in the phospholipid FA composition of the three skeletal muscles analyzed. Phospholipids of the dorsal muscles (*m. longissimus dorsi, m. iliopsoas*) contained 67.1 ± 0.2% PUFAs, while the *m. vastus* from the hind leg contained 65.7 ± 0.3% PUFAs ($F_{2,67} = 4.71$, $P = 0.012$). The mean SFA proportion (28.2 ± 0.3%) was highest in the *m. vastus,* whereas the dorsal muscles contained only 26.9 ± 0.2% SFAs ($F_{2,67} = 7.46$, $P = 0.0012$). MUFAs were found in equal proportions in phospholipids from all muscles (overall mean: 5.3 ± 0.1%). An additional comparison of ten locomotory muscles from the hind leg showed no significant differences in phospholipid FA composition. Mean PUFA content was not significantly different between muscles of different location within the hind leg ($F_{10,36} = 0.57$, $P = 0.829$).

The major component of muscle membrane phospholipids was linoleic acid (32.6 ± 0.3%). The most unsaturated FAs were docosapentaenoic acid (DPA; C22:5n-3) and docosahexaenoic acid (DHA; C22:6n-3), with mean proportions of 8.3 ± 0.2% and 1.9 ± 0.1%, respectively. The *m. longissimus dorsi,* had slightly (1%) lower proportions of these two highly-unsaturated FAs. *M. iliopsoas, m.longissimus dorsi,* and *m. vastus* had UIs of 225.1 ± 1.3, 216.9 ± 1.4 and 218.0 ± 1.6, respectively.

Fatty acid classes

The first three PCs computed (based on the FA composition of *m. iliopsoas*), described 85% of the variance between individuals (Table 1).

Table 1 Component loadings resulting from a principle component (PC) analysis of phospholipid fatty acid proportions in brown hare muscles

	Component loadings		
	PC 1	PC 2	PC 3
SFA	−0.033	–0.381	–0.658
MUFA	0.047	0.034	–0.440
C18:2n-6	–0.650	–0.470	0.427
C18:3n-3	0.403	–0.022	0.357
C20:4n-6	–0.450	0.722	–0.045
C20:5n-3	0.363	–0.178	0.198
C22:5n-3	0.279	0.279	0.146
C22:6n-3	0.035	0.033	0.002
Variance explained by components (%)	56.8	16.9	11.3
Total variance explained (%)	56.8	73.7	85.0

For simplicity, saturates and monounsaturates were combined. This was justified by a prior analysis showing that all loadings had identical signs in these classes for all components (or were negligibly small)
SFA saturated fatty acids, *MUFA* monounsaturated fatty acids

Table 2 Fatty acid composition of membrane phospholipids in the *m. iliopsoas* of brown hares, *Lepus europaeus* in summer and winter

	Summer $n = 23$	Winter $n = 87$	*F*	*P*
C14:0	0.7 ± 0.13	0.3 ± 0.01	7.32	0.008
C15:0	0.3 ± 0.02	0.1 ± 0.01	44.24	< 0.0001
C16:0	13.1 ± 0.40	10.4 ± 0.18	40.20	< 0.0001
C17:0	0.6 ± 0.03	0.5 ± 0.01	13.59	< 0.0001
C18:0	14.0 ± 0.30	15.3 ± 0.12	15.12	0.0002
C16:1n-7	0.4 ± 0.05	0.5 ± 0.02	1.01	0.382
C18:1n-9	5.2 ± 0.29	5.1 ± 0.14	1.96	0.16
C18:2n-6	34.7 ± 0.50	32.0 ± 0.37	9.13	0.003
C18:3n-3	5.2 ± 0.42	6.6 ± 0.25	3.41	0.067
C20:4n-6	9.8 ± 0.55	12.3 ± 0.29	7.95	0.0057
C20:5n-3	5.8 ± 0.39	5.1 ± 0.22	6.11	0.015
C22:5n-3	8.4 ± 0.31	10.1 ± 0.20	17.20	0.0001
C22:6n-3	1.9 ± 0.06	1.9 ± 0.04	0.10	0.764
∑SFA	28.0 ± 0.47	26.1 ± 0.16	25.60	< 0.0001
∑MUFA	5.6 ± 0.30	5.6 ± 0.16	0.10	0.803
∑PUFA	65.8 ± 0.65	68.1 ± 0.22	17.74	0.0001
∑N6	44.5 ± 0.87	44.3 ± 0.59	0.03	0.874
∑N3	21.3 ± 0.90	23.8 ± 0.58	4.20	0.04
UI	213.8 ± 2.38	228.9 ± 1.33	31.71	< 0.0001

Values are given in weight%, mean ± SE. The strength of seasonal changes is indicated by *F* statistics and corresponding *P* values
PUFA polyunsturated fatty acids, *UI* unsaturation index

Firstly, individuals differed in the ratio between n-6 and n-3 PUFAs, which explained 56.8% of the variance. Secondly, hares with high linoleic acid (C18:2n-6) content had low levels of arachidonic acid (C20:4n-6), and vice versa (Table 1, PC2). Thirdly, individuals with high PUFA content had low contents of both SFAs and MUFAs (PC3). This analysis showed that the relation between several FA classes described most of the variance in the data set. Thus, we subsequently compared similar groups of FAs in addition to individual FAs (Table 2).

Seasonal changes in muscle phospholipid composition

While the content of all saturates with chain length ≤ 17 decreased during winter, monounsaturates remained stable (Table 2). Proportions of PUFAs significantly increased during winter, with the exceptions of C18:2n-6 and C20:5n-3 which decreased by 2.7% and 0.7%, respectively. The largest increase (+2.5%) in any individual FA towards winter was observed for arachidonic acid, C20:4n-6. The increase in phospholipid PUFA content, mostly at the expense of SFAs (Fig. 1) was statistically significant (Table 2). Seasonal differences in membrane phospholipids were also reflected by the UI. Winter tissue samples had a 7.1% higher UI value than tissues from summer months. This pattern of seasonal change was similar in all three muscles investigated.

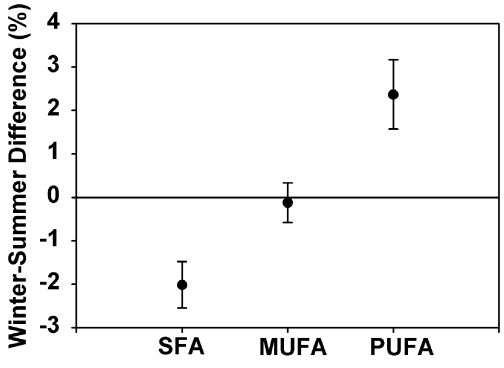

Fig. 1 Seasonal changes in the proportions of saturated fatty acids (SFAs), monounsaturated FAs (MUFAs), and polyunsaturated FAs (PUFAs) in muscle phospholipids of brown hares (mean winter-summer difference ± 95% confidence limits; $n_{Winter} = 87$, $n_{Summer} = 23$)

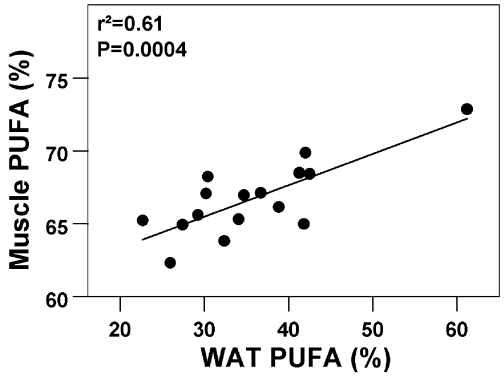

Fig. 2 Correlation between PUFA content (%) in kidney, white adipose tissue (WAT), and muscle phospholipids. *Dots* represent individual hares. The regression line is given by $PUFA_{Muscle} = 59 + 0.97 \times PUFA_{WAT}$

Comparison between skeletal muscle, heart, liver, and WAT lipids

PUFA content in muscle phospholipids was positively correlated ($r^2 = 0.61$, $df = 14$, $P = 0.004$) with the corresponding PUFA content in the perirenal WAT (Fig. 2). However, there were major differences between the range of the PUFA contents in muscle compared to those in depot lipids. In perirenal WAT the total PUFA amount ranged from 22.6% to 61.2% (mean: 35.7 ± 2.3%), whereas in muscle phospholipids PUFA content varied from 62.3% to 72.9% (mean: 66.8 ± 0.6%). Thus, the proportion of PUFAs was always higher in muscle tissues than in depot fats of the same individuals.

The PUFA content of skeletal muscle phospholipids was correlated to the PUFA content observed in heart ($r^2 = 0.31$, $df = 25$, $P = 0.0033$) and liver phospholipids ($r^2 = 0.19$, $df = 32$, $P = 0.001$). The range of PUFA content in heart muscle was similar to that of skeletal muscle (57.3–73.1%; mean: 65.7 ± 0.5%) but greater than that of liver (25.1–54.2%; mean: 42.0 ± 0.7%).

Discussion

Differences between muscles

Unexpectedly, we found no intracorporal gradient towards increased PUFA contents in peripheral tissues in brown hares. The most peripheral tissue investigated here, the *m. vastus* in the hind leg, had the lowest PUFA content of the three muscles compared. Similarly, none of the additional hind leg muscles analyzed showed higher PUFA contents than central muscles. Intracorporal gradients of unsaturation have been described in depot lipid FAs in reindeer, beavers and muskrats (Irving et al. 1957; Pond et al. 1993; Käkelä and Hyvärinen 1996a). Brown hares may not show such a gradient in FA unsaturation for several reasons. First, as these gradients are thought to reflect some biochemical adaptation to cooler peripheral tissues, hares may always maintain normothermic temperatures within their extremities. This explanation seems unlikely, however, because juvenile hares showed clear signs of peripheral cooling during cold exposure (Hackländer et al. 2002), and because peripheral cooling is a common mechanism for reducing energy expenditure in the cold. However, the highest proportions of PUFAs typically occur in the most peripheral, coldest body parts such as the lower legs (Irving et al. 1957; Pond et al. 1993), or even in plantar fat depots and body appendages (Käkelä and Hyvärinen 1996a), while total muscle mass in lower legs of hares was insufficient for an accurate determination of FA composition. Second, gradients may be more pronounced within certain muscles than between different muscles. For instance, gradients of increasing unsaturation from central to peripheral tissue parts have been found in

depot fats of reindeer, in otter tails or within seal blubber (Pond et al. 1993; Käkelä and Hyvärinen 1995, 1996b). Third, it is conceivable that in brown hares maintenance of an extremely high mean PUFA content of 67% in muscle phospholipids guarantees that PUFAs are abundant in all body parts, with further increases leading to little additional improvement of biochemical adaptations. In this view, hares could have evolved mechanisms for a strong general, site-independent enrichment of muscle phospholipid PUFAs from dietary FAs.

Seasonal changes

Hares in this study showed a clear seasonal increase of PUFA content and degree of unsaturation, albeit with a moderate absolute change of 2.3% and 7.1%, along with correspondingly decreased SFAs in winter. We expected a general increase of unsaturation during the colder season from well documented effects of cold exposure that increases PUFA content in fish (Cossins et al. 1977; Hazel 1979) and birds (Chainier et al. 2000). It is well known that unsaturated FAs generally increase proton leakiness of phospholipid membranes and thus facilitate metabolic rate and heat production (Brookes et al. 1998; Chainier et. al. 2000). Moreover, it has been shown recently that both the ubiquitous and predominantly muscle-specific mammalian mitochondrial uncoupling proteins (UCP2 and UCP3) show highest affinity to, and activation by, PUFAs (Zackova et al. 2003). Thus, higher PUFA levels should lead to increased uncoupling and nonshivering thermogenesis outside the brown adipose tissue, e.g., in skeletal muscles during times of cold exposure. Interestingly, to our knowledge this study is the first to report a seasonal change of phospholipid FA composition in a mammal.

The relatively low amplitude of these seasonal changes in PUFA concentrations in hares may have a simple explanation: The animals investigated here were all collected in their natural habitat in lower Austria. Even in summer (June–September), mean minimum ambient temperature in lower Austria is 15.3°C (Müller 1993), which is clearly below the thermoneutral zone of hares (Hackländer et al. 2002; and unpublished data). Hence, hares probably were cold acclimatized throughout the year. This may be one of the reasons for high PUFA levels that showed only moderate seasonal variation. It is presently unclear whether each additional increase in phospholipid PUFA content has proportional, linear, or possibly nonlinear effects. It is possible that a 7% increase in unsaturation towards winter, as found in skeletal muscles of hares, may have profound effects on tissue function.

Within PUFAs, arachidonic acid (C20:4n-6) showed the largest elevation from summer to winter. Because the amount of arachidonic acid in dietary plants is negligible (Malainey et al. 1999; Hill and Florant 1999), and since its precursor linoleic acid (C18:2n-6) correspondingly decreased, we conclude that these changes indicate increased active formation of arachidonic acid in winter. Unless this increase merely reflects the need for higher unsaturation, it points to a specific need for arachidonic acid during the winter season. These requirements may be related to local functions of C20:4n-6 in muscle phospholipid membranes, or could arise from certain functions of its derivates, namely prostaglandins, which affect physiological characteristics that undergo seasonal adjustments, such as thermoregulation and immune responses (e.g., Rothwell 1992; Takahata et al. 1996;

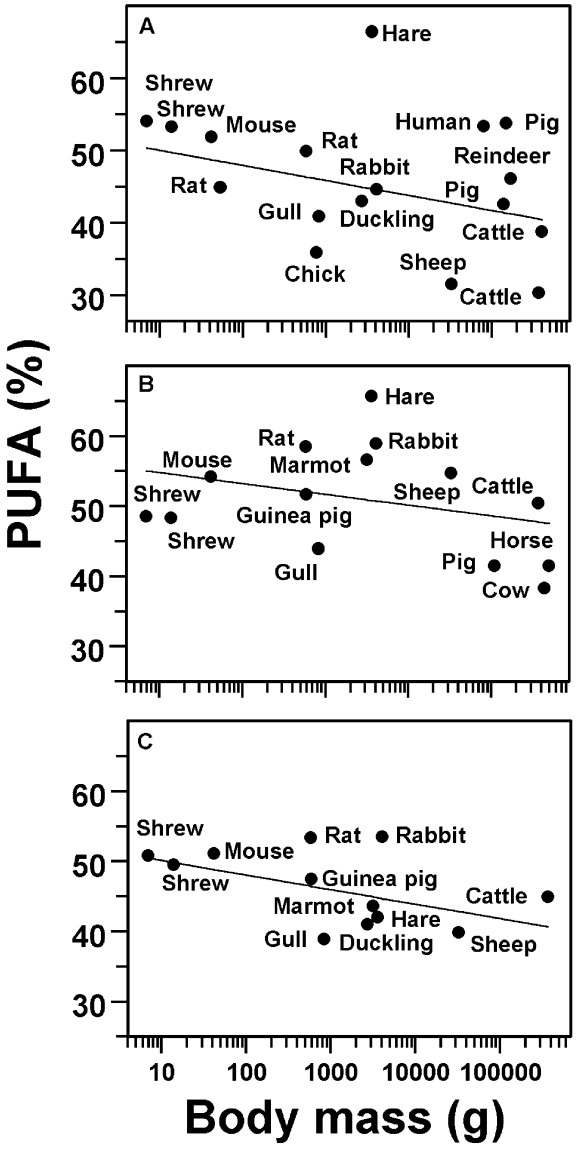

Fig. 3A–C Phospholipid PUFA content as a function of body mass in various birds and mammals. **A** Skeletal muscle (PUFA = 54.2–2.19logBody mass; $r^2 = 0.14$, $P = 0.15$). **B** Heart (PUFA = 56.2–1.52logBody mass; $r^2 = 0.09$, $P = 0.27$). **C** Liver (PUFA = 52.1–1.93logBody mass; $r^2 = 0.24$, $P = 0.10$). Note that the inclusion of hares in the regression did not cause major changes in levels of statistical significance. For references see Appendix

Pond and Mattacks 2002). Otherwise, changes in the proportions of certain FAs within the PUFA class, such as the relative increase of C18:3n-3 in winter, may, of course, mirror changes in the FA composition of dietary plants. A relative increase in green plant parts that contain large amounts of C18:3n-3 (Malainey et. al. 1999; F. Tataruch, unpublished results), and a proportional decrease of other parts, such as blossoms or seeds, may indicate changes in diet.

High levels of phospholipid PUFA contents in hares: origin and possible functions

A surprising result from this study was the extremely high amount of PUFAs in brown hare muscle phospholipids. A mean of 67% PUFAs is, to our knowledge, the highest PUFA proportion reported for any mammalian tissue. Most published data on PUFA content in muscle phospholipids have been obtained from livestock such as pigs, cattle, chicken and domestic rabbits (Andres et al. 2001; van Laack and Spencer 1999; Olomu and Baracos 1991; Lopez-Bote et al. 1997). In these species, PUFA proportions ranged from 35.9% to 53.8%.

There is evidence that FA composition in muscle phospholipids reflects nutrient composition (Geiser 1990, 1991; Pan et al. 1994; Cobos et al. 1995; Ayre and Hulbert 1996b). Correspondingly, we found that common food plants of brown hares, such as dandelion (*Taraxacum officinalis*) and white clover (*Trifolium repens*), contain 60–80% PUFAs (T.G. Valencak et al., unpublished observations). Phospholipids in our animals had high proportions of alpha-linolenic acid (C18:3n-3) and its derivate DPA (C22:5n-3). This is to be expected since C18:3n-3 is abundant in green food plants (Malainey et al. 1999).

While variation in nutrient composition may explain individual variation, seasonal variation, and the correlation in FA composition between tissues, it is clear that hare muscle phospholipid PUFA content is strongly enriched compared to WAT composition, and regulated within much narrower borders (Fig. 2). Note that the skeletal, as well as heart muscle phospholipid PUFA content, was extremely high, not only in comparison to WAT triacylglycerols, but also compared to phospholipid composition in other tissues such as liver (42.0%).

Together, the high muscle PUFA level and its low variability between individuals, compared to other tissues, suggest an important role for PUFAs in the function of muscle cells. Couture and Hulbert (1995) have demonstrated that membrane unsaturation is related to body mass, with smaller mammals having higher proportions of unsaturated phospholipids (Fig. 3). This suggests that increased proton leakage or other respiration-enhancing membrane effects induced by PUFAs contribute to the generation and adjustment of basal metabolic rate (Hulbert et al. 2002). Although this may well be an important function of PUFAs, the allometric increase of unsaturation with decreasing body mass (which in the data set analyzed here actually was statistically not significant; Fig. 3) does not sufficiently explain the extremely high PUFA content in both skeletal and heart muscles of *L. europaeus* (Fig. 3). We suggest that the high PUFA level of the hare skeletal and heart muscles is related to the high maximum running speed of brown hares, which is four times faster than that of rodents of the same body weight (Garland 1983).

There is increasing evidence for a relation between muscle function and PUFAs. For example, in rats, an essential FA-deficient diet caused an impairment of muscle performance, including lowered peak twitch tension and increased fatigue rates during high-frequency stimulation (Ayre and Hulbert 1996a). Similarly, endurance training in humans was accompanied by significant increases in n-3 PUFA contents of muscle phospholipids (Andersson et al. 2000; Helge et al. 2001). Also, very high amounts of one particular PUFA, docosahexaenoic acid (C22:6n-3), were found in phospholipids of high-frequency contraction muscles, such as hummingbird pectoral and rattlesnake shaker muscles (Infante et al. 2001). Taken together, these studies point to a possibly important role of PUFAs in sustaining and improving muscle function, by acting either directly on muscle cell performance (including signal transduction), or on energy supply, i.e., the metabolic rate of muscle cells. Thus, it should be interesting to investigate which fraction of the residual variation in phospholipid PUFA content between species can be explained by traits directly related to muscle function, such as maximum running speed.

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Appendix

The following table shows phospholipid PUFA content in skeletal muscle (% $PUFA_M$), heart (% $PUFA_H$), liver (% $PUFA_L$) and body masses of various mammals and birds. To allow for direct comparisons, only studies with identical sets of FAs are included (as shown in Table 2). Data from animals kept on PUFA-enriched or PUFA-deficient diets are also excluded (abbreviations: juv. = juvenile; dom. = domesticated).

Species	Body mass (kg)	Skeletal muscle (% $PUFA_M$)	Heart (% $PUFA_H$)	Liver (% $PUFA_L$)	Reference
Sorex araneus	0.007	54.1	48.5	50.8	Käkelä and Hyvärinen (1995)
Neomys fodiens	0.014	53.8	48.3	49.5	Käkelä and Hyvärinen (1995)
Mus musculus	0.042	51.9	54.2	51.1	Couture and Hulbert (1995)
Rattus norvegicus (juv.)	0.054	44.9	–	–	Ayre and Hulbert (1996b)
Rattus norvegicus	0.581	49.9	58.5	53.3	Couture and Hulbert (1995)
Cavia porcellus (dom.)	0.595	–	51.66	47.42	Abedin et al. (1999)
Gallus gallus (dom.)	0.773	35.9	–	–	Olomu and Baracos (1991)
Larus fuscus	0.837	–	43.9	38.9	Surai et al. (2000)
Cairina moscata (juv.)	2.721	43	–	41.0	Chainier et al. (2000)
Marmota marmota	3.194	–	56.6	43.6	F. Tataruch (unpublished data)
Oryctolagus cuniculus (dom.)	4.100	44.6	58.9	53.5	Couture and Hulbert (1995)
Lepus europaeus	4.191	66.8	65.7	42.0	Present study
Ovis aries (dom.)	32.9	31.5	54.7	39.8	Couture and Hulbert (1995)
Homo sapiens	80	53.4	–	–	Andersson et al. (2000)
Sus scrofa (dom.)	110	–	41.45	–	Pamplona et al. (1999)
Sus scrofa (dom.)	140	42.6	–	–	Andres et al. (2001)
Sus scrofa (dom.)	150	53.8	–	–	Muriel et al. (2002)
Rangifer tarandus	170	46.1	–	–	Wiklund et al. (2001)
Bos taurus (dom.)	369	30.3	50.4	44.9	Couture and Hulbert (1995)
Bos taurus (dom.)	405	38.78	–	–	Laborde et al. (2001)
Bos taurus (dom.)	440	–	38.28	–	Pamplona et al. (1999)
Equus caballus (dom.)	500	–	41.5	–	Pamplona et al. (1999)

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