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Phospholipid composition and longevity: lessons from Ames dwarf mice

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Abstract Membrane fatty acid (FA) composition is correlated with longevity in mammals. The "membrane pacemaker hypothesis of ageing" proposes that animals which cellular membranes contain high amounts of polyunsaturated FAs (PUFAs) have shorter life spans because their membranes are more susceptible to peroxidation and further oxidative damage. It remains to be shown, however, that long-lived phenotypes such as the Ames dwarf mouse have membranes containing fewer PUFAs and thus being less prone to peroxidation, as would be predicted from the membrane pacemaker hypothesis of ageing. Here, we show that across four different tissues, i.e., muscle, heart, liver and brain as well as in liver mitochondria, Ames dwarf mice possess membrane phospholipids containing between 30 and 60 % PUFAs (depending on the tissue), which is similar to PUFA contents of their normal-sized, short-lived siblings. However, we found that that Ames dwarf mice membrane phospholipids were significantly poorer in n-3 PUFAs. While lack of a difference in PUFA contents is contradicting the membrane pacemaker hypothesis, the lower n-3 PUFAs content in the long-lived mice provides some support for the membrane pacemaker hypothesis of ageing, as n-3 PUFAs comprise those FAs being blamed most for causing oxidative damage. By comparing tissue composition between 1-, 2- and 6-month-old mice in both phenotypes, we found that membranes differed both in quantity of PUFAs and in the prevalence of certain PUFAs. In sum, membrane composition in the Ames dwarf mouse supports the concept that tissue FA composition is related to longevity.

Keywords Ames dwarf mouse · Lipid peroxidation · Membrane phospholipids · Fatty acid composition · Membrane pacemaker hypothesis · Laboratory mice

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Introduction

Small mammals generally have cell membranes rich in polyunsaturated fatty acids (PUFAs), whereas larger

species contain tissues with less PUFAs but more monounsaturated and saturated FAs (reviewed in Hulbert et al. (2008)). Certain PUFAs are essential dietary compounds in the mammalian diet, make up cell membranes and affect a suite of cellular functions (Pond and Mattacks 1998). PUFAs, however, are susceptible to peroxidation and, therefore, may potentially reduce lifespan. PUFAs in the mitochondrial membrane are particularly vulnerable to oxidative damage and form highly reactive products that cause further oxidative damage (Esterbauer et al. 1991; Hulbert et al. 2007). Finally, PUFAs lead to the formation of hazardous DNA adducts and have a potential role for genome stability (Gruz and Shimizu 2010). The idea that membrane composition influences lifespan is encapsulated in the "membrane pacemaker hypothesis of ageing" (Hulbert et al. 2007; Hulbert 2008; Pamplona and Barja 2007; Pamplona and Barja 2011). Whilst this hypothesis emphasises impact of total PUFA content and membrane peroxidisability, we concluded previously, based on a comparison across a wide range of mammalian species, that it is the ratio between the n-3 and n-6 PUFA subclasses that best explains the association between FA composition of membranes and maximum lifespan (Valencak and Ruf 2007). Differentiating between the n-3 and n-6 PUFA subclass when relating membrane composition to certain physiological traits has also been proven successful in the context of seasonal changes in membrane composition (Valencak et al. 2003) for maximum running speed in mammals (Ruf et al. 2006) and occurrence and characteristics of torpor and hibernation (reviewed in Ruf and Arnold 2008). Due to genetic basis of the many traits involved in ageing, however, tests of hypotheses in the context are arguably better performed within a species (Speakman 2005). Fortunately, ageing research in the past years has generated long-lived genotypes such as the Ames dwarf mouse that represents an interesting model to test the concept. Ames dwarf mice are mutant mice that are homozygous for a spontaneous mutation and were shown to live almost 50 % longer than their normal siblings (Brown-Borg and Bartke 2012; Bartke 2012), as they carry a "longevity gene", *Prop1*^{df}. Ames dwarf mice reportedly show a reduced body size, lower plasma levels of insulin, lower levels of the insulin-like growth factor IGF-1, lower glucose and lower thyroid hormone (Brown-Borg and Bartke 2012; Bartke 2012). Interestingly, they were shown to have a consistently lower body core temperature throughout the circadian cycle than their heterozygous siblings (Hunter et al. 1999). Together, the impact of all these traits relevant for a long lifespan has been identified in Ames dwarf mice, but to our knowledge, membrane FA composition or the content of PUFAs in this long-living mouse model has not been explored yet. We, thus, aimed to compare tissue phospholipid FA composition of homozygous, long-lived Ames dwarf mice (*Prop1df*/*Prop1df*) with heterozygous, wild type (Prop1+/df) animals as controls. According to the "membrane pacemaker hypothesis of ageing", we predicted that homozygous Ames dwarf mice might show less membrane PUFA content than heterozygous siblings. Thereby, the long-lived phenotype might avoid excess lipid peroxidation in the membranes, which could favour the long life span. Amongst PUFAs, the n-3 subclass, and in particular docosahexaenoic acid (DHA), a PUFA with six double bonds, stands out for being highly susceptible to peroxidative damage (Turner et al. 2003). As a very dominant n-3 PUFA in membranes of small mammals, DHA is eight times more prone to peroxidation than linoleic acid (LA), which has only two double bonds and belongs to the n-6 PUFAs (Hulbert et al. 2007). We, thus, hypothesised that n-3 PUFA contents might be lower in the long-lived phenotype than in their normal-sized siblings, as would be expected from the general association found in mammals (Valencak and Ruf 2007). To control for potential growth effects when individuals mature, we sampled tissues at 1, 2 and 6 months of age. Similarly, to avoid generalisations arising from the study of single tissues, we analysed membrane phospholipids in four different tissues, namely heart, muscle, brain and liver, and finally in isolated liver mitochondria.

Materials and methods

Animals and housing

Prior to the study, we established a colony of mice consisting of males and females heterozygous for the gene *Prop1* (Prop1+/Prop1df), purchased from Charles River Laboratories, Bad Sulzfeld, Germany. We crossed heterozygous individuals and selected the offspring that was homozygous for *Prop1df*. Homozygosis of *Prop1* phenotypically results in dwarfism and extended lifespan. All mice were pair-housed by gender and genotype at 22±2 °C on a 16 h:8 h L:D photoperiod in standard cages (Eurostandard Type II Long, Tecniplast, Italy). They were provided with a high-energy diet

"V118x" (Ssniff, Soest, Germany), described in Table 1, and water ad libitum. Major murine pathogens were monitored regularly using co-housed sentinel animals. Dwarf offspring (*Prop1df/Prop1df*) could be easily distinguished from normal siblings by body size; thus, we refrained from genotyping in our study. Further, there was no need for genotyping the heterozygous littermates as heterozygous Prop1+/df and homozygous wild type mice show no phenotypic difference but can both be used as controls for comparison with Ames dwarf (Prop1df/df) mice (Helms et al. 2010). Total hearts, brains and livers along with the hindleg musculus vastus were sampled from a total of 21 heterozygous and 18 Ames dwarf mice. Both genders were used in our study both in control animals and Ames dwarf mice. Liver mitochondria were isolated from another 16 6-month-old animals (six Ames dwarf, 10 controls) to make sure the sampled material (liver tissue) would allow sufficient analyses of FA composition. All mice came from both sexes, all originating from the F1 generation of the colony. Note that we kept mothers and offspring together for 4 weeks after birth to make sure that even the Ames dwarf offspring would be viable.

Tissue collection, preparation and analysis

All animals were killed by cervical dislocation and tissues were rapidly removed and stored in Eppendorf tubes at −18 °C until lipid extraction and analysis (<2 months). Tissue sampling, lipid extraction, analysis and computation of indices have been detailed in previous publications (Valencak et al. 2003; Valencak and Ruf 2007; Valencak and Ruf 2011). Briefly, lipids were extracted using chloroform and methanol (2:1 v/v), separated on silica gel thin layer chromatography plates (Kieselgel 60, F254, 0.5 mm, Merck) and then made visible under ultraviolet light with the phospholipid fraction isolated. Phospholipid extracts were transesterified by heating (100 °C) for 30 min, extracted into hexane and were analysed by gas liquid chromatography (GLC) (Perkin Elmer Autosystem XL with Autosampler and FID; Norwalk, CT, USA). FA methyl esters were identified by comparing retention times with those of FA methyl standards (Sigma-Aldrich, St. Louis, MO, USA). Liver mitochondria were isolated according to standard isolation methodologies. In brief, livers were quickly harvested and then liver tissues were chopped with scissors and minced with a scalpel blade on a cold tile prior to homogenisation and differential centrifugation. Isolated mitochondria samples were stored in Eppendorf cups at −18° until analysis (<2 weeks). In all tissues and in liver mitochondria examined, we have measured the composition of total phospholipids that obviously combines all subcellular membranes in one measurement. All experiments described here were approved by the ethics committee of the University of Veterinary Medicine, Vienna (No. 10/12/97/2009) and comply with the current laws in Austria, where the experiments were performed.

Table 1 Diet composition (weight%); percentages of fatty acids are given in relation to the percentage of fat, and not to the whole diet

Protein (%)	23.0
Fat (%)	6.0
Fibre (%)	3.3
Nitrogen free extracts (%)	49.2
Metabolisable energy content (MJ kg^{-1})	14.3
C: 14-0	0.19
C: 16-0	11.38
C: 16–1 n-7	0.58
C: 18-0	3.51
C: 17:0	0.01
C: 18–1 n-9	23.81
C: 18–2 n-6	53.39
C: 18–3 n-3	5.94
C: 20-0	0.43
C: 20-1	0.17
C: 22-1	0.03

Statistical analysis

Statistical analyses were conducted in R for Mac (2.13.1; R Development Core Team 2011). We compared individual FA contents and PUFA classes between mouse phenotypes using body weight, tissue type and age as covariates. Due to the fact that all four tissues were sampled from the same animals, we adjusted for repeated measurements by computing linear mixed effects models using individual intercepts as the random factor (library nlme; Pinheiro et al. 2012). F and p values for analyses of variances (ANOVAs) from these models were computed using marginal sums of squares. Interestingly, mouse phenotype still explained variance after the effect of body weight had been accounted for. In addition, we computed a principal component analysis, which indicated that the first principle component explained 82.7 % of the variance in the data set and was reflected by the ratio between the most abundant n-3 FA docosahexaenoic acid (DHA) (C 22:6 n-3)

and LA (C 18:2 n-6; as well as arachidonic acid (AA C 20:4 n-6). When the principle component analysis was run amongst all four tissues (including the brain) the first principle component explained 48.8 % of the variance only but the analysis basically provided the same result. Therefore, we mostly concentrated on comparing DHA and LA contents. Multiple comparisons of FA contents at specific time points were computed using Tukey-type test with the R package "Multcomp" (Hothorn et al. 2008).

Results

Whilst total PUFA content was similar in both phenotypes (Tables 2 and 3), other aspects of membrane composition in the long-lived Ames dwarf mice differed from that of heterozygous normal-sized siblings across all four tissues analysed. Figures 1, 2, 3 and 4 illustrate the proportion of phospholipid DHA (C 22:6 n-3) and LA (C 18:2 n-6) of freshly weaned (1 month old), young adult (2 months old) and adult (6 months old) Ames dwarf mice compared to the heterozygous controls from the same strain. These two FAs were the most abundant PUFA in all tissues (except for LA in the brain) (Table 3). In heart, skeletal muscle and liver, we found increasing differences between the phenotypes as age increased, with lower amounts of DHA and higher proportions of LA in Ames dwarf mice. The relationship between these two FAs largely corresponds to the first principle component (based on heart, muscle and liver phospholipids of 6-month-old animals) that explained 82.9 % of the total variance in the data set (Table 4). Please note that some of the loadings also point to a close relationship between AA and DHA (Table 4). Proportions of individual FAs in heart, skeletal muscle, liver and brain phospholipids are given in Tables 2 and 3. An ANOVA including all age classes and all four tissues along with body weight and mouse phenotype showed that the proportion of each single FA was dependent on tissue type (e.g., DHA: $F_{3,108}$=173.6; p<0.0001). We observed a significant interaction between age of individual mice and tissue type for all single FAs (e.g., DHA: $F_{3,108}$=7.78; p=0.0001), except for C 14:0, C 17:0 and C 18:0. The proportions of seven out of 13 FAs were affected by phenotype (C 18:0, C 18:1 n-9, C

Table 2 Fatty acid composition of phospholipids in skeletal muscle and heart of adult Ames dwarf mice (AD, 6 months old) and same-aged normal-sized littermates (C); values are given in weight%, means±SEM

	Muscle		Heart	
	AD	C	AD	C
N	6	7	6	7
C 14:0	0.16±0.005	0.12±0.006	0.08±0.006	0.036±0.002
C 15:0	0.10±0.005	0.11±0.003	0.05±0.003	0.04±0.001
C 16:0	21.3±0.3	24.5±0.1	13.45±0.13	13.03±0.14
C 17:0	0.39±0.01	0.38±0.01	0.35±0.02	0.36±0.01
C 18:0	16.1±0.2	14.37±0.13	20.6±0.25	21.5±0.13
C 16:1n-7	0.79±0.07	1.0±0.05	0.238±0.05	0.05±0.004
C 18:1n-9	7.8±0.2	5.5±0.2	7.32±0.7	5.46±0.3
C 18:2n-6	19.5±0.7	12.9±0.3	19.9±0.4	14.8±0.5
C 18:3n-3	0.5±0.03	0.48±0.01	0.08±0.03	0.097±0.03
C 20:4n-6	10.6±0.4	12.5±0.3	12.4±0.6	9.4±0.12
C 20:5n-3	0.2±0.01	0.13±0.005	0.12±0.01	0.04±0.003
C 22:5n-3	4.5±0.4	2.3±0.05	3.5±0.33	1.5±0.04
C 22:6n-3	18.02±0.8	25.7±0.5	21.9±1.2	33.7±0.77
∑SFA	38.03±0.2	39.5±0.04	34.6±0.2	34.9±0.2
∑MUFA	8.6±0.3	6.5±0.3	7.6±0.7	5.5±0.25
∑PUFA	53.4±0.3	54±0.3	57.9±0.9	59.5±0.3
∑N6	30.1±0.8	25.4±0.4	32.3±0.7	24.2±0.5
∑N3	23.3±0.5	28.6±0.5	25.6±1.3	35.3±0.7
UI	223.3±1.5	250.2±2.1	246.6±5.9	282.76±3.2
PI	235.6±3.1	284.4±3.6	266.6±9.5	331.4±5.4

Table 3 Fatty acid composition of phospholipids in liver, liver phospholipids and brain of adult Ames dwarf mice (AD, 6 months old) and same-aged normal-sized littermates (C); values are given in weight%, means±SEM. Please note that samples for liver and brain stem are from the same individuals, while for liver phospholipids, other individuals were sampled but at the same age of 6 months

	Liver		Liver mito		Brain	
	AD	C	AD	C	AD	C
N	6	7	6	10	6	7
C 14:0	0.12±0.01	0.06±0.005	0.15±0.01	0.06±0.003	0.1±0.002	0.1±0.004
C 15:0	0.08±0.06	0.06±0.004	0.09±0.01	0.03±0.002	0.06±0.002	0.05±0.002
C 16:0	22.5±0.6	19.3±0.3	22.3±0.4	20.6±0.6	31.1±0.7	32.9±1.05
C 17:0	0.48±0.5	0.4±0.2	0.5±0.04	0.30±0.02	0.2±0.004	0.3±0.004
C 18:0	16.9±0.7	22.5±0.3	15.80±0.96	15.5±0.8	20.1±0.3	19.8±0.4
C16:1n-7	0.8±0.2	0.37±0.3	0.56±0.08	0.4±0.04	0.5±0.03	0.4±0.02
C 18:1n-9	9.9±1.4	6.7±0.6	8.22±0.6	5.5±0.4	19.3±0.4	19.7±0.3
C 18:2n-6	20.9±0.5	15.3±0.5	23.4±0.3	16.6±0.4	0.9±0.04	0.8±0.04
C 18:3n-3	0.3±0.03	0.1±0.007	0.3±0.02	0.09±0.008	0.03±0.002	0.04±0.001
C 20:4n-6	16.4±0.5	21.3±0.2	15.7±0.4	25.6±0.5	10.6±0.3	9.3±0.2
C 20:5n-3	0.7±0.06	0.4±0.04	0.90±0.09	0.65±0.04	0.02±0.001	0.002±0.001
C 22:5n-3	1.5±0.05	0.5±0.03	2.08±0.23	0.655±0.04	0.24±0.005	0.18±0.008
C 22:6n-3	9.3±0.4	13.1±0.2	9.97±0.6	13.94±0.7	16.8±0.6	16.5±0.5
∑ SFA	40.1±0.9	42.3±0.3	38.9±0.7	36.5±0.4	51.7±0.5	53.2±0.6
∑ MUFA	10.8±1.6	7.1±0.6	8.8±0.7	5.9±0.4	19.8±0.4	20.04±0.3
∑ PUFA	49.2±0.9	50.7±0.5	52.4±0.4	57.6±0.5	28.6±0.8	26.8±0.6
∑ N6	37.4±0.7	36.6±0.4	39.1±0.23	42.2±0.5	11.5±0.3	10.1±0.2
∑ N3	11.8±0.3	14.01±0.2	13.3±0.3	15.3±0.6	17.1±0.6	16.7±0.5
UI	186.1± 2.8	205.9 ±1.1	194.01±16.2	233.9±32	166.2± 4.2	158.8± 3.4
PI	175.2± 4.8	210.6 ±1.7	185.2±21.5	243.09±52.2	180 ± 6	171.7± 4.3

18: 2 n-6, C 18:3 n-3, C 20:5 n-3, C 22:5 n-3 and C 22:6 n-3), even after the influence of body weight was accounted for ($p<0.05$ each time). Note that the amount of C 18:2 n-6 in brain phospholipids was below 1 % in both phenotypes (Fig. 4, Table 2), so brain differed in tissue composition from all the others. DHA content significantly differed between tissues ($p<0.0001$) in both phenotypes, except for brain and muscle amongst the Ames dwarf mice, where the proportions were similar (Tables 2 and 3). Also, all tissues differed significantly in the amount of n-6 PUFAs ($p<0.0001$) with one exception. Amongst the heterozygous control animals, total n-6 PUFAs were not different between skeletal muscle and heart ($z=-1.8$; $p=0.44$, Tables 2 and 3) but reached significance in the long-lived phenotype ($z=3.1$; $p=0.02$; Tables 2 and 3). We did not include isolated liver mitochondria FA composition in the above models, as we used another batch of animals to harvest mitochondria. The results from the liver mitochondria revealed again the same pattern with more n-3 PUFAs, namely DHA in the control animals compared with the Ames dwarf mice (Table 3). Interestingly, the proportion of DHA in liver mitochondria phospholipids amounted to 9 % and, thus, was equal to its proportion in liver tissue phospholipids (Table 3).

Discussion

PUFAs are most susceptible to lipid peroxidation and thus, if peroxidation significantly affects ageing, exceptionally long-lived mammals and birds should have tissues with low PUFA content according to the membrane pacemaker hypothesis of ageing (reviewed in Hulbert (2010). Indeed, membranes containing smaller proportions of highly unsaturated PUFAs have been reported from the extremely long-lived naked mole rat (Hulbert et al. 2006a), from the short-beaked echidna (Hulbert et al.

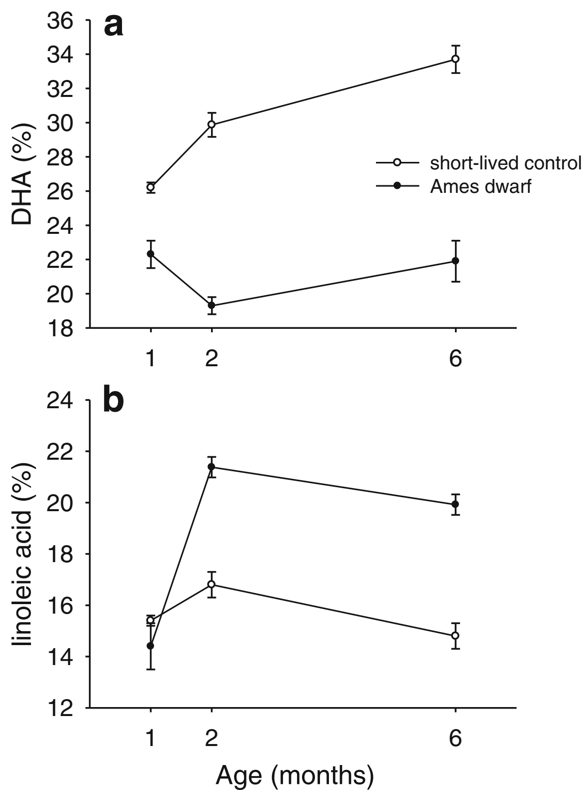

Fig. 1 Heart phospholipid docosahexaenoic acid (DHA) content (**a**) and linoleic acid content (**b**) in 1-, 2- and 6-month-old Ames dwarf mice and normal-sized littermates. Total $n_{\text{Ames dwarf mice}}$=18, total $n_{\text{normal littermates}}$=21; means±SEM

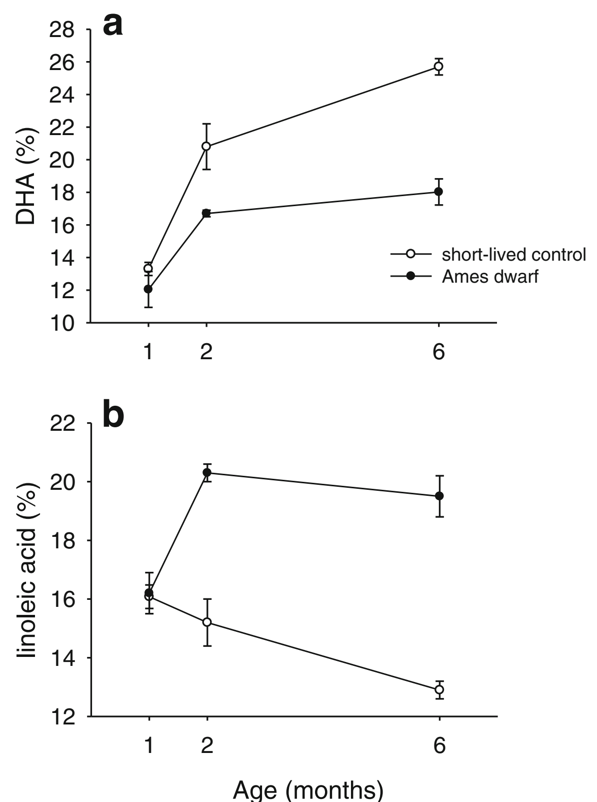

Fig. 2 Skeletal muscle phospholipid docosahexanenoic acid (DHA) content (**a**) and linoleic acid content (**b**) in 1-, 2- and 6-month-old Ames dwarf mice and normal-sized littermates. Total $n_{\text{Ames dwarf mice}}$=18, total $n_{\text{normal littermates}}$=21; means± SEM

2008), from long-lived galliform birds (Buttemer et al. 2008) and, most recently, from bivalves (Munro and Blier 2012). Similarly, short-lived worker bees have been shown to have highly polyunsaturated membranes, whereas the long-lived queen has few PUFAs in the membrane (Haddad et al. 2007). Amongst rodents, wild-derived mice also show a membrane unsaturation correlated with their maximum lifespan (Hulbert et al. 2006b). Yet, ageing research in recent years has revealed that multiple mechanisms can explain the outstanding lifespan of long-lived mouse mutants such as the Ames dwarf mouse (reviewed recently in Bartke 2012). Ames dwarf mice are very low in circulating insulin-like growth factor 1 (IGF-1) (Bartke and Brown-Borg 2004) and low in insulin levels whilst, at the same time, having high insulin sensitivity (Sharp and Bartke 2005). Similarly, they have been shown to have reduced mammalian Target of Rapamycin (mTOR) signalling (Sharp and Bartke 2005), whilst anti-oxidative defence systems are up-regulated (Brown-Borg and Bartke 2012) as is the resistance to various forms of oxidative, toxic and metabolic stresses (reviewed in Bartke 2012; Brown-Borg and Bartke 2012).

Does the tissue lipid profile of Ames dwarf mice also contribute to their extended lifespan amongst mice? According to the prediction of the membrane pacemaker hypothesis of ageing, Ames dwarf mice should have significantly lower PUFA contents in their membranes than wild type, non-mutant control animals. In our study, which to our knowledge is the first to address this question, in a mutant mouse model, we found that overall PUFA content was not significantly different between the two phenotypes in muscle, heart, liver and brain phospholipids (Tables 2 and 3). The only exception was liver mitochondrial composition, which revealed that PUFA content was significantly lower in the Ames dwarf mice than in the control animals (Table 3). Thus, liver mitochondrial phospholipids from Ames dwarf mice contain 5 % less PUFAs than those from controls (but notably

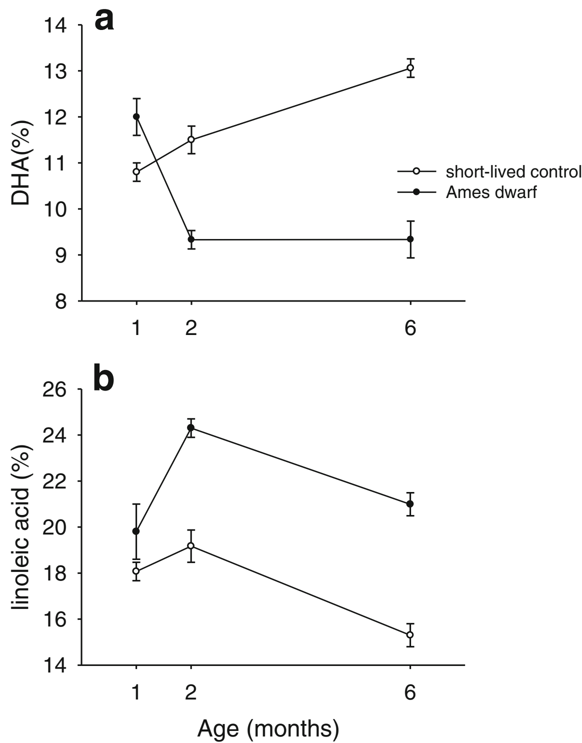

Fig. 3 Liver phospholipid docosahexanenoic acid (DHA) content (**a**) and linoleic acid content (**b**) in 1-, 2- and 6-month-old Ames dwarf mice and normal-sized littermates. Total $n_{\mathrm{Ames\ dwarf\ mice}}$=18, total $n_{\mathrm{normal\ littermates}}$=21; means±SEM

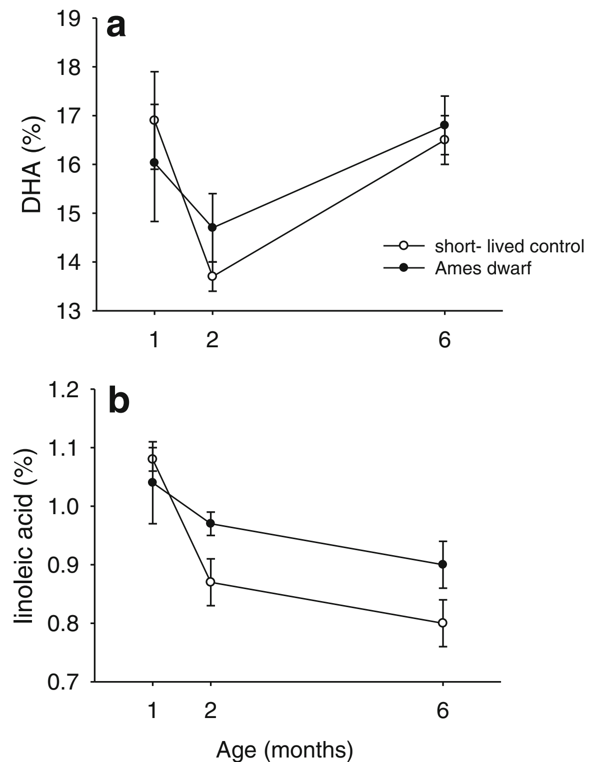

Fig. 4 Brain phospholipid docosahexanenoic acid (DHA) content (**a**) and linoleic acid content (**b**) in 1-, 2- and 6-month-old Ames dwarf mice and normal-sized littermates. Total $n_{\mathrm{Ames\ dwarf\ mice}}$=18, total $n_{\mathrm{normal\ littermates}}$=21; means±SEM

still contain 52 % PUFAs). This difference in mitochondrial phospholipids clearly supports the membrane pacemaker hypothesis of ageing in isolated mitochondria, but total PUFA contents in other tissues gave no evidence for differences between phenotypes. Still, our data from heart, skeletal muscle and liver also seem to support a relation between membrane composition and ageing, since Ames dwarf mice had lower contents of DHA and, hence, lower degrees of unsaturation in these tissues. As illustrated in Fig. 5 (upper panel), the relation between membrane composition and maximum lifespan amongst strains of laboratory mice is very weak, however, and even factors that did differ between phenotypes in our study, e.g., n-3 PUFA content, have only little predictive power (Fig. 5 lower panel). In particular, the Ames dwarf mouse is much longer lived than predicted from its membrane composition alone. It should be noted that the large scatter shown in Fig. 5 is not due to large variation within strains. Generally, tissue phospholipid composition is a regulated trait in mammals and muscle phospholipid PUFA content varies from 34.54 % in cattle to 70 % in the ibex (Valencak and Ruf 2007). Within a species, strain differences are much smaller (Valencak and Ruf 2007, c.f. SEMs in Tables 2 and 3). Whilst our study from 2007 revealed that phospholipid DHA contents did not correlate with maximum lifespan in mammals, our recent data from the Ames dwarf mouse

Table 4 Component loadings from a principle component analysis of phospholipid fatty acid proportions in Ames dwarf and heterozygous control mice skeletal muscle, heart and liver

	Component loadings		
	PC 1	PC 2	PC 3
C 18:2 n-6	−0.187	−0.687	−0.689
C 18:3 n-3			0.104
C 20:4 n-6	−0.363	0.663	−0.480
C 20:5 n-3			
C 22:5 n-3		−0.267	0.406
C 22:6 n-3	0.912	0.131	−0.344
Variance explained by components (%)	82.9	14.04	2.12
Total variance explained (%)	82.9	97.02	99.1

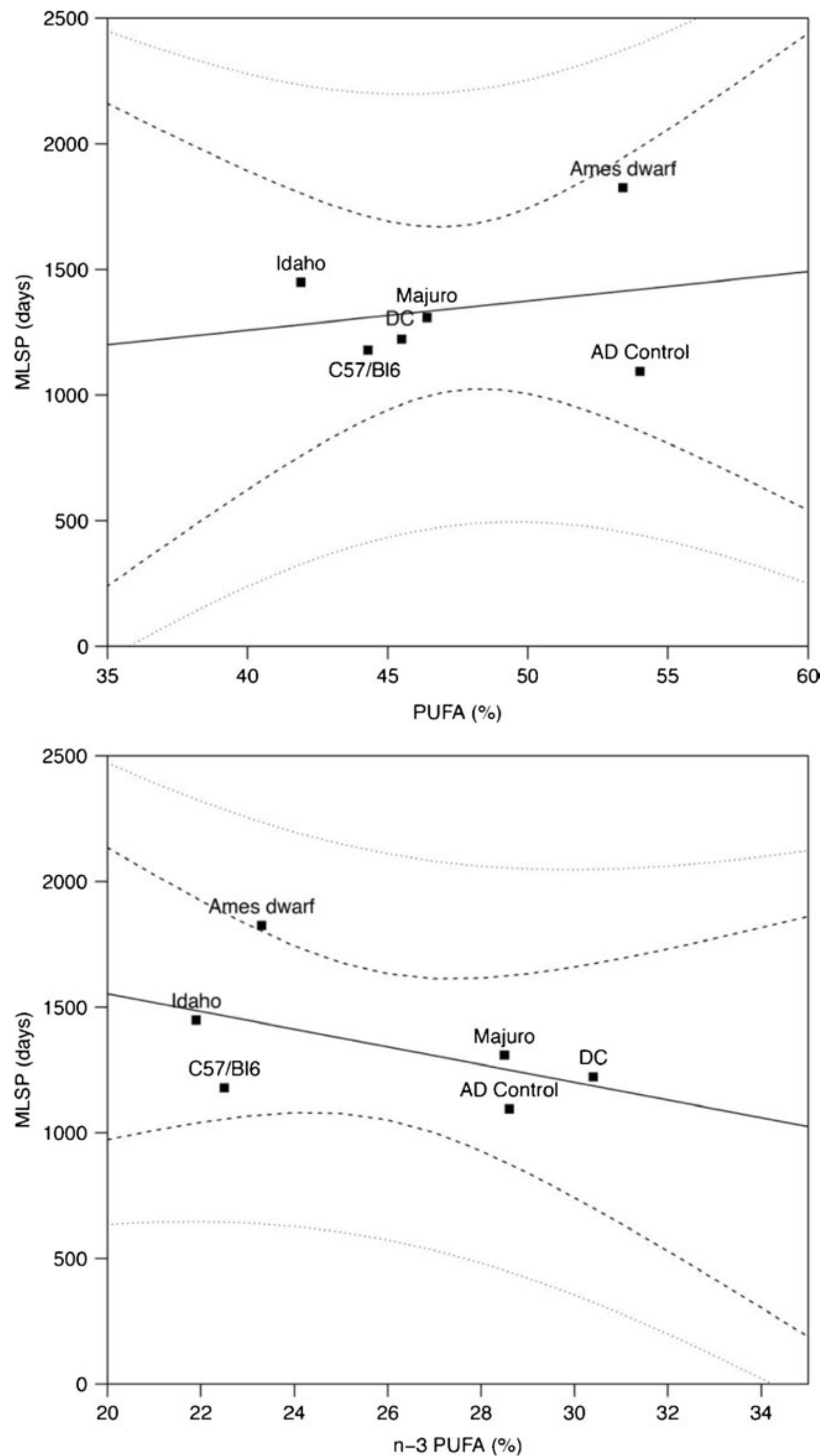

Fig. 5 Relationship and prediction intervals between maximum lifespan (MLSP) and muscle phospholipid PUFA content (*top panel*) and n-3 PUFA content (*bottom panel*) of five different strains of laboratory mice. Data obtained in this study are from Ames dwarf mouse tissues and controls, and all other data points from Hulbert et al. (2006a, b). AD control refers to heterozygous control Ames dwarf mice

contradict our earlier findings (Valencak and Ruf 2007) with the exact reason for this being unclear to us. It is possible that, again, some interspecific observations are not confirmed intraspecifically just as with the relationship between energy expenditure and lifespan (Speakman 2005).

Generally, Ames dwarf mice, indeed, have been reported to have a lower reactive oxygen species (ROS) production along with increased resistance to oxidative stress (Murakami 2006; reviewed in Brown-Borg and Bartke (2012)) although this has not been assessed in context with lipid peroxidation. Maybe Ames dwarf mice aged 2 months or older always, thus, were found to have lower levels of DHA than heterozygous controls, which should make them less susceptible to peroxidation, as DHA is thought to be eight times more prone to peroxidation than LA, for instance (Hulbert et al. 2007). This is often expressed as a peroxidation index, i.e., the relative susceptibility of the acyl chains, which was indirectly determined via oxygen consumption (Holman 1954; reviewed in Hulbert et al. 2007). The peroxidation index largely reflects the DHA content of a given membrane. A potential problem with this simple index is that research in humans and animal models has demonstrated that probably the most damageing and reactive product of lipid peroxidation is the aldehyde 4-hydroxy-2-nonenal (HNE) (Esterbauer et al. 1991; Lakatta and Sollott 2002; Juhaszova et al. 2005). In contrast to other ROS species, HNE is relatively long-lived and acts not only in the immediate proximity of membranes but can diffuse from the site of its origin and damages even distant targets (Esterbauer et al. 1991; Lakatta and Sollott 2002). Importantly, HNE originates not from n-3 PUFAs, such as DHA, but is formed by superoxide reaction with n-6 PUFAs. Further, it has been shown that PUFAs are involved in the production of DNA adducts and, thus, the susceptibility for endogenous tissue DNA damage (Chung et al. 2000; reviewed in Gruz and Shimizu 2010). Finally, there is increasing evidence challenging the hypothesis that ageing is related to ROS production (reviewed in Speakman and Selman (2011)). Therefore, to infer a causal relationship between low membrane n-3 PUFAs levels and increased longevity, direct experimental research is required. Specifically, we suggest identifying the potential detrimental effects of certain single FAs such as DHA on lifespan.

One experiment in this context, which has been carried out already, did not support such a causal relationship: whilst feeding C57BL/6 mice n-3- or n-6 PUFA-enriched diets significantly altered their membrane compositions, it had no effect whatsoever on lifespan, compared to controls (Valencak and Ruf 2011). Therefore, we suggest there may be an alternative explanation why Ames dwarf mice differ significantly in their membrane n-3 PUFA content from their heterozygous siblings. Recently, Ames dwarf mice have been found to have fully functional mitochondria but lower mitochondrial activity (Choksi et al. 2011). This decreased mitochondrial metabolism in the homozygous Ames dwarf mice might be linked to lower membrane n-3 PUFA contents, since certain PUFAs such as DHA correlate with the metabolic activity of tissues in mammals (Turner et al. 2003). DHA and other n-3 PUFA are known to up-regulate oxidative capacity (Weber 2009) and have been shown to be important activators for mitochondrial uncoupling proteins (Jezek et al. 1998). These functions of n-3 PUFAs could explain why their levels are decreased in Ames dwarf mice, if their reduction serves to decrease metabolism and enzyme activities rather than ROS production. Also, the lower body temperature of 34 °C found in Ames dwarf mice (Hunter et al. 1999) indicates that metabolism is lowered, possibly due to a different membrane FA composition. Finally, relating to the potentially altered lipid peroxidation in the Ames dwarf mice, they might have increased DNA stability (Gruz and Shimizu 2010).

In the present study, the single tissue deviating from the general pattern was the brain that contained almost no n-6 PUFAs but equal amounts of DHA in the Ames dwarf mice as in the wild type controls (Fig. 4). If Ames dwarf mice have lower DHA levels than heterozygotes in other tissues, why is it then not lower in the brain? DHA is a major structural component in sperm, retinal membranes and brain phospholipids, and regulates enzymes, receptors and transport proteins (Stillwell and Wassall 2003). Additionally, DHA is a precursor for important eicosanoids (Stillwell and Wassall 2003) and eicosanoids fulfil several functions in the brain (reviewed in Tassoni et al. (2008)). Therefore, it is likely that a high DHA content is essential for brain functionality and, thus, is observed in all mammals independent of their lifespan. Similarly, our new data confirm that brain tissue is very rich in oleic acid (C 18:1 n-9) due to it being a major constituent of myelin lipid (Rioux and Innis 1992). Hulbert et al. (2006b) also reported relatively high DHA contents of 11 % from the very long-lived naked mole rat and concluded that brain tissue requires high levels of n-3 PUFAs to ensure intracellular signalling processes (Hulbert et al. 2006b).

We found that membrane PUFA composition in all tissues studied, even in the brain, significantly changed with the age of animals (Figs. 1, 2, 3 and 4). Age-related changes in PUFA levels, especially of the n-3/n-6 PUFA

ratio, are also well known from humans (Lakatta and Sollott 2002). Also, older European hares have altered FA composition in comparison to young individuals (Valencak unpublished; Valencak et al. 2003) and the same effect was found in humans (Baur et al. 2000). However, it seems that in comparative studies (e.g., Hulbert et al. 2006b; Valencak and Ruf 2007; Munro and Blier 2012), the influence of age on membrane FA composition has been largely overlooked in the past. Our current data from the Ames dwarf mice presented here point to the need for including individual age in general models relating membrane FAs to certain traits. We assume that, as membrane composition is tightly regulated in mammals with little variance between individuals (Valencak and Ruf 2007), the differences between mice at 1, 2 or 6 months of age observed here are caused by differential up- and down-regulation of acyltransferases, elongases and desaturases involved in membrane remodelling (Sprecher 2000). More specifically, the FA-specific glycerol-3 phosphate acyltransferase 1 (GPAT1) represents a likely candidate enzyme causing the membrane compositional differences between 1-, 2- or 6-month-old mice (Coleman and Mashek 2011). Yet, this is speculative, as Coleman and Mashek (2011) refer to triacylglycerol metabolism and our tissues under test were membrane phospholipids. Thus, future studies to identify the role of all enzymes involved are needed and with specific attention to differences between membrane phospholipids and triacylglycerols.

Conclusions

We conclude that tissues as well as mitochondrial membranes from long-lived Ames dwarf mice have low proportions of n-3 PUFAs and, thus, may have lower oxidative stress due to their tissues being more resistant to lipid peroxidation (except for brain), specifically, tissue n-3 PUFAs related to lifespan. This observation does not, however, necessarily indicate a causal relationship between in vivo ROS production and membrane composition. Rather, given the lower body temperature (Hunter et al. 1999) and increased resistance to oxidative stress (Murakami 2006) in Ames dwarf mice, we suggest that its altered membrane composition is caused by altered activity of certain acyltransferases to down-regulate n-3 PUFA content and, hence, mitochondrial activity in the Ames dwarf mice in order to match their slow pace of life.

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