Published in final edited form as:

Mech Ageing Dev. 2006 August ; 127(8): 653–657. doi:10.1016/j.mad.2006.03.002.

Extended longevity of wild-derived mice is associated with peroxidation-resistant membranes

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Abstract

Two lines of mice, Idaho (Id) and Majuro (Ma), both derived from wild-trapped progenitors, have previously been shown to have extended lifespans in captivity when compared to a genetically heterogenous laboratory line of mice (DC). We have examined whether membrane fatty composition varies with lifespan within the species *Mus musculus* in a similar manner to that previously demonstrated between mammal species. Muscle and liver phospholipids from these long-living mice lines have a reduced amount of the highly polyunsaturated omega-3 docosahexaenoic acid compared to the DC mice, and consequently their membranes are less likely to peroxidative damage. The relationship between maximum longevity and membrane peroxidation index is similar for these mice lines as previously observed for mammals in general. It is suggested that peroxidation-resistant membranes may be an important component of extended longevity.

Keywords

Lipid peroxidation; Membranes; Docosahexaenoic acid; Maximum lifespan

1. Introduction

While long-living mutant strains of mice have been produced in the laboratory (Bartke et al., 2001), significant lifespan-extension has also been observed in strains of wild-derived mice (Miller et al., 2002). Two lines of mice, Idaho (Id) and Majuro (Ma) both derived from wild-trapped progenitors, have been bred in captivity and compared with a genetically heterogenous mouse stock (DC) representative of the laboratory-adapted gene pool. Under identical laboratory conditions, Id mice have a maximum longevity (mean longevity of the longest-lived 10% of mice) that is 16% longer than DC mice, while the maximum lifespan of Ma mice exceeds that of DC mice by 9%. The longest-lived Id mouse has lived for 1501 days (4.11 years) of age (Harper and Miller, unpublished observations) and this degree of lifespan extension in mice achieved by nature is equal to (and possibly greater than) that yet achieved in any laboratory-derived mutant mouse strain when fully fed (i.e. not calorie-restricted).

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The “oxidative stress” theory (Beckman and Ames, 1998), the most accepted explanation of aging and maximum lifespan determination, proposes that reactive oxygen species (ROS), produced inevitably from aerobic metabolism, damage cellular nucleic acids, proteins and fats. Different fatty acids vary dramatically in their susceptibility to ROS attack; those that are highly polyunsaturated being much more vulnerable than less polyunsaturated fatty acids, with monounsaturates and saturates being essentially incapable of peroxidation (Holman, 1954). This fact, coupled with the finding in the last decade that fatty acid composition of cellular membranes varies systematically with species body size, both in mammals (Couture and Hulbert, 1995; Hulbert et al., 2002b) and birds (Hulbert et al., 2002a) has led to an increasing awareness of a potentially important link between membrane fatty acid composition and maximum species lifespan (MLSP) (e.g. Pamplona et al., 1996, 1998). This extension of the oxidative stress theory of aging and its implications has been recently described in some detail as the “membrane pacemaker” theory of aging (Hulbert, 2005). The “membrane pacemaker” theory highlights fatty acid composition of membrane phospholipids and its effects on lipid peroxidation in aging. Because many of the products of lipid peroxidation are themselves highly reactive ROS species, the susceptibility of membranes to lipid peroxidation may be a pivotal determinant of longevity. The current study was designed to investigate whether the correlation between membrane fatty acid composition and maximum lifespan so far described between species of mammal and birds, also exists within wild-derived mice exhibiting significant extended longevity compared to genetically heterogenous lab-derived mice.

2. Materials and methods

2.1. Animals

The Idaho and Majuro stocks used in this study were derived from wild-caught progenitors as described in Miller et al. (2000). The liver and skeletal muscle (quadriceps) tissues analysed here are from 3- to 4-month-old mice that had been used in the study previously reported by Harper et al. (2005). The details concerning animal care are described in that paper. The tissues had been kept frozen at -80°C from that study and were transferred to City College of New York where total lipids were extracted. The total lipids were then transported to University of Wollongong where phospholipid fatty acid composition was measured.

2.2. Lipid extraction and fatty acid composition

Total lipid was extracted from frozen tissues using ultra-pure grade chloroform:methanol (2:1 v/v) containing butylated hydroxytoluene (0.01% w/v) as an antioxidant. Phospholipids were separated from neutral lipids by solid phase extraction on silicic acid columns (Waters Corp. Milford, MA, USA.). Phospholipids were transmethyated (Lepage and Roy, 1986) and fatty acid methyl esters were separated by gas-liquid chromatography on a Shimadzu GC-17A gas chromatograph (Shimadzu corp. Kyoto, Japan) with a fused silica capillary column. Individual fatty acids were identified by comparing each peak's retention time to those of external standards and then expressed as the mol% of total fatty acids. Peroxidation index (PI) is calculated as $\text{PI} = (0.025 \times \% \text{ monoenoics}) + (1 \times \% \text{ dienoics}) + (2 \times \% \text{ trienoics}) \pm (4 \times \% \text{ tetraenoics}) + (6 \times \% \text{ pentaenoics}) + (8 \times \% \text{ hexaenoics})$.

2.3. Statistical analyses

Significant effects were determined by ANOVA conducted using JMP v5.1 statistical package (SAS Institute Inc.). Where ANOVA revealed a significant effect, Tukey's *post hoc* test was used to identify significant differences.

3. Results

Significant differences in the fatty acid composition of phospholipids from both skeletal muscle and liver were evident among these mouse strains (Tables 1 and 2 and Fig. 1A and B). The largest difference is manifest in the amount of the highly polyunsaturated omega-3 docosahexaenoic acid (22:6 $n-3$) present in phospholipids and thus in the cellular membranes of these mouse strains lines. In both skeletal muscle and liver, the longer-living Id mice had a significantly smaller amount of 22:6 $n-3$ than the DC laboratory strain. This difference resulted in significantly lower $n-3$ polyunsaturated fatty acid (PUFA) in Id mice compared to DC mice both as a percent of total fatty acids and as a percent of total polyunsaturates. Consistent with their intermediate maximum life span Ma mice had $n-3$ PUFA values in both liver and muscle that were between those seen in the Id and DC mice.

Slight tissue-specific differences were also noted. No significant differences between strains were observed in the percent of $n-6$ polyunsaturates, monounsaturates or total unsaturated fatty acids in liver phospholipids. However, in skeletal muscle phospholipids Id mice had a significantly greater level of monounsaturates and $n-6$ polyunsaturates than DC mice although there was no significant difference between strains in the percentage of total unsaturates (Tables 1 and 2 and Fig. 1A and B). The increased abundance of $n-3$ polyunsaturates in Id mice compared to DC mice overwhelmed the opposite trend for $n-6$ PUFA, resulting in a significantly higher percentage of total PUFA in muscle membranes of Id mice. The difference in 22:6 $n-3$ content meant that in both liver and muscle, the percent long-chain (20 + 22 carbon) fatty acids, and the average chain length, were significantly less for Id mice compared to DC mice. The 20:4/18:2 ratio also revealed that muscle phospholipid $n-6$ PUFA was significantly skewed to the less polyunsaturated linoleic acid (18:2 $n-6$) in Id mice compared to DC mice, thereby further reducing its susceptibility to peroxidation, for arachidonic acid (20:4 $n-6$) is four-times more predisposed to peroxidation than 18:2 $n-6$ (Holman, 1954). There was no such trend in liver phospholipids.

The highly polyunsaturated omega-3 docosahexaenoic acid (22:6 $n-3$) is extremely susceptible to peroxidation, being eight-fold more prone to peroxidation than the omega-6 linoleic acid (18:2 $n-6$) and 320-fold more peroxidation-prone than the monounsaturated oleic acid (18:1 $n-9$). The peroxidation index (PI) of membrane phospholipids is a measure of the susceptibility of the membrane to lipid peroxidation. It is calculated by combining the peroxidation susceptibilities of individual fatty acids with the percent fatty acid composition of the pooled phospholipids (see Section 2). When this is done, in both liver and muscle, the long-living Id mice have membrane lipids that are significantly more resistant to peroxidation than the DC mice, with Ma mice having intermediate PI values (Tables 1 and 2 and Fig. 1C).

4. Discussion

The differences in 22:6 $n-3$ content among mice strains are almost solely responsible for the different PI values. Since mice were all fed the same diet, these differences are not diet-based. Indeed laboratory rodent diets generally have a negligible 22:6 $n-3$ content and so differences in 22:6 $n-3$ content of membranes likely result from differences in biochemical processes among the mice lines. It is not known if these differences are related to biosynthesis of 22:6 $n-3$ or its incorporation into membrane phospholipids (or both). The biosynthetic pathway of 22:6 $n-3$ is considerably more complex than that of other polyunsaturates and appears to involve peroxisomes (Sprecher, 2000).

Little is known of the mechanisms that regulate membrane fatty acid composition within a species and nothing is known of the mechanisms responsible for differences in membrane composition between species. Enzymes involved in membrane remodelling (e.g.

acyltransferases) are likely important. For example, despite there being hundreds of different molecular species of phospholipid in the membranes of rat hepatocytes, only four molecular species of phosphatidylcholine are synthesised *de novo* and membrane remodelling is responsible for producing the remaining hundreds of molecular species (Schmid et al., 1995). The importance of membrane remodelling is also illustrated by the fact that, although oxidative stress in rat hepatocytes results in the destruction of membrane phospholipid polyunsaturates, membrane fatty acid composition is unaltered because of the rapid replacement of peroxidised polyunsaturates with new polyunsaturated acyl chains from the hepatocyte triacylglycerol pool (GironCalle et al., 1997).

The PI of both skeletal muscle phospholipids and liver mitochondrial phospholipids is inversely related to the maximum lifespan of mammalian and bird species (Hulbert, 2005). Using data for six mammal species ranging from mice to sheep (Hulbert et al., 2002b; Porter et al., 1996), we have calculated that, on average, the PI for liver phospholipids is ~90% of the PI value for liver mitochondrial phospholipids from the same species. When PI and maximum longevity data for the three mice lines are superimposed on these relationships for mammals (Fig. 2), the relationships between PI and lifespan of the mice approximates the relationships observed for mammals in general. The slopes of these log-log plots can be used to calculate the relative change in PI associated with a doubling of lifespan. When this is done for skeletal muscle, a doubling of maximum lifespan is associated with a 68% decrease in PI between mice strains compared to a 20% decrease among mammals in general. Within the mice, a 38% decrease in PI of liver phospholipids is associated with a doubling of lifespan compared to a 25% decrease in the PI of liver mitochondrial phospholipids for mammals in general. If average lifespan values (Miller et al., 2002) are used instead of maximum longevity, then a doubling of average lifespan of mice is associated with a 56% decrease in the PI of muscle phospholipids and a 24% decrease in PI of liver phospholipids.

It is of interest that a senescence-prone substrain of senescence-accelerated mice have brain mitochondrial membranes with a significantly elevated peroxidation index compared to those from a senescence-resistant substrain of these mice (Choi et al., 1996).

Extended longevity of Id and Ma mice is also associated with delayed maturation and both of the wild derived lines are smaller than the DC mice (Miller et al., 2002). The smaller body size of Id and Ma mice is associated with significantly lower serum IGF-1 levels in both mice lines and a lower T4 concentrations in Ma mice but not Id mice. Id mice have reduced glycated hemoglobin levels compared to DC mice but have similar tolerance to glucose loads and fasting serum concentrations of glucose and insulin (Harper et al., 2005). However Ma mice are hyperglycaemic, glucose intolerant and have elevated glycated hemoglobin levels compared to both DC and Id mice, suggesting neither reduced blood glucose nor enhanced glucose tolerance are responsible for the extended longevity of these mice strains. The tissues analysed in this present study were harvested from 3 to 4 months animals that were part of the study described above. The lack of difference in insulin and T4 levels between these mice strains at that age suggest that the membrane fatty acid differences we report here are not due to differential expression of these hormones even though both thyroid hormones and insulin are known to influence membrane fatty acid composition (Hulbert, 2000; Sanz et al., 2005). It is presently not known whether IGF-1 influences membrane fatty acid composition.

Here we report an association between membrane fatty acid composition and longevity of three different lines of mice. We cannot ascertain from these results whether this association is one of "cause and effect". Experimental manipulation of membrane fatty acid composition in these mice lines (possibly by diet) that also altered lifespan appropriately would be necessary to confirm a cause and effect relationship between these two parameters.

Intraspecific differences in membrane composition correlate with observed differences in maximum lifespan in wild-derived mice and provide strong support for the “membrane pacemaker theory” of aging. This theory suggests that the current measurement of mitochondrial ROS production solely as $O_2^{\bullet-}$ and H_2O_2 production might be analogous to measuring a “pilot light” on a stove as an indicator of heat production and may be less informative than studies that also measure membrane lipid peroxidation. The latter, together with $O_2^{\bullet-}$ and H_2O_2 production better represents the “full furnace” of ROS production and highlights the potential importance of peroxidation-resistant membranes in extended longevity. Unfortunately, it is currently not known if the in vivo or in vitro lipid peroxidation levels differ in these lines of mice, however we would expect this to be the case from the different fatty acid composition of their membranes we report here.

Acknowledgments

This research was supported by a Fulbright Senior Scholar award to AJH and grants from the Australian Research Council (DP0450178 & DP0557448 to AJH) and the US National Institute of Health and US National Institute of Aging (AG023122 to RAM, and NIH-AG022891-01 and NIGMS S06-GM08168 to RB).

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Fig. 1.

Fatty acid composition of phospholipids from skeletal muscle (A) and liver (B) from three strains of mice (Idaho, Majuro and DC) that differ in longevity. Values are shown for the following fatty acids (from bottom of each column): 16:0, 18:0, 18:1 $n-9$, 18:1 $n-7$, 18:2 $n-6$, 20:4 $n-6$, 22:5 $n-3$, 22:6 $n-3$. The columns do not add up to 100% because only data for the eight major fatty acids are shown. Individual fatty acids are identified by number of C atoms: number of double bonds followed by position of most terminal double bond. They are grouped in pairs as either saturated fatty acids (SFA), monounsaturates (MUFA), omega-6 polyunsaturates ($n-6$ PUFA) and omega-3 polyunsaturates ($n-3$ PUFA). Those fatty acids that significantly differ between mice strains have a different letter in the column ($P < 0.05$). (C) Shows the calculated Peroxidation index (mean PI $\pm$ S.E.M.) for the three mice strains. Lines indicate those values that are significantly different ($P < 0.05$) from one another.

Fig. 2.

The relationship between the maximum lifespan (MLSP) and peroxidation index (PI) of phospholipids from skeletal muscle (A) and liver (B) for three mice lines (Id: Idaho, Ma: Majuro and DC: laboratory mice line) that differ in longevity. The skeletal muscle data are compared to that for mammal species in general and the liver data for mice are compared to the relationship for liver mitochondrial phospholipids for mammals in general (both taken from Hulbert, 2005).

Table 1

Fatty acid composition of liver phospholipids from three strains of mice that differ in longevity

	Idaho (<i>N</i> = 8)	Majuro (<i>N</i> = 8)	DC (<i>N</i> = 8)	Significance of difference
16:0	25.2 ± 0.6	23.6 ± 0.3	24.9 ± 0.6	NS
16:1 <i>n</i> - 7	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.0	NS
18:0	16.9 ± 0.3	17.3 ± 0.5	16.2 ± 0.3	NS
18:1 <i>n</i> - 9	8.3 ± 0.3	7.6 ± 0.3	7.7 ± 0.2	NS
18:1 <i>n</i> - 7	1.6 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	NS
18:2 <i>n</i> - 6	19.0 ± 0.8	19.4 ± 0.6	17.6 ± 0.6	NS
20:3 <i>n</i> - 6	0.5 ± 0.1 a	0.9 ± 0.1 b	0.9 ± 0.1 b	0.010
20:4 <i>n</i> - 6	13.4 ± 0.2	13.6 ± 0.4	13.6 ± 0.2	NS
20:5 <i>n</i> - 3	0.4 ± 0.1 a	1.1 ± 0.2 b	0.5 ± 0.1 a	0.001
22:5 <i>n</i> - 3	0.6 ± 0.1 ab	0.6 ± 0.1 a	0.4 ± 0.0 b	0.027
22:6 <i>n</i> - 3	11.4 ± 0.4 a	11.3 ± 0.6 a	13.6 ± 0.4 b	0.002
% UFA	56.9 ± 0.7	58.1 ± 0.6	57.8 ± 0.8	NS
% MUFA	10.9 ± 0.4	10.3 ± 0.5	10.2 ± 0.2	NS
% PUFA	46.1 ± 0.9	47.8 ± 0.8	47.6 ± 0.8	NS
% <i>n</i> - 6 PUFA	33.5 ± 0.9	34.6 ± 1.0	32.9 ± 0.8	NS
% <i>n</i> - 3 PUFA	12.6 ± 0.5 a	13.2 ± 0.5 ab	14.7 ± 0.4 b	0.007
<i>n</i> - 3 PUFA (% total PUFA)	27.4 ± 1.1 a	27.7 ± 1.2 ab	31.0 ± 0.8 b	0.031
20:4/18:2	0.71 ± 0.03	0.71 ± 0.03	0.78 ± 0.03	NS
% C20-22	26.8 ± 0.5 a	28.6 ± 0.6 ab	29.9 ± 0.5 b	0.004
Average chain length	18.23 ± 0.02 a	18.31 ± 0.03 ab	18.35 ± 0.02 b	0.005
Peroxidation index	172.6 ± 3.5 a	178.6 ± 3.7 a	190.6 ± 3.3 b	0.003
Maximum longevity (days)	1323	1245	1144	
Maximum lifespan (days)	1450	1309	1223	

Fatty acids identified by number of C atoms: number of double bonds and position of terminal double bond. Only values for the major fatty acids (>0.5%) are shown. Values are shown as mean ± S.E.M. Values with different letters are significantly different. NS: no significant differences. Values for lifespan of mice are from Miller et al. (2002). Maximum longevity is the average lifespan of the longest-living 10% of individual in the mice line.

Table 2

Fatty acid composition of skeletal muscle phospholipids from three strains of mice that differ in longevity

	Idaho (<i>N</i> = 7)	Majuro (<i>N</i> = 7)	DC (<i>N</i> = 6)	Significance of difference
16:0	26.8 ± 0.5	26.9 ± 0.7	28.4 ± 0.2	NS (0.052)
16:1 <i>n</i> - 7	1.6 ± 0.1 ab	1.3 ± 0.1 a	1.8 ± 0.2 b	0.014
18:0	15.4 ± 0.9	14.7 ± 0.4	13.7 ± 0.7	NS
18:1 <i>n</i> - 9	8.7 ± 0.3 a	6.5 ± 0.4 b	5.8 ± 0.3 b	<0.0001
18:1 <i>n</i> - 7	3.5 ± 0.1 a	3.0 ± 0.2 b	3.3 ± 0.1 ab	0.021
18:2 <i>n</i> - 6	10.5 ± 0.4 a	8.7 ± 0.7 b	6.6 ± 0.6 c	0.0002
20:3 <i>n</i> - 6	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	NS
20:4 <i>n</i> - 6	7.6 ± 0.4	7.5 ± 0.4	6.9 ± 0.6	NS
20:5 <i>n</i> - 3	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	NS
22:5 <i>n</i> - 3	2.7 ± 0.1	2.6 ± 0.2	2.3 ± 0.1	NS
22:6 <i>n</i> - 3	18.4 ± 1.6 a	25.0 ± 0.9 b	27.4 ± 1.6 b	0.0003
% UFA	56.1 ± 1.2	57.4 ± 1.2	56.7 ± 0.5	NS
% MUFA	14.3 ± 0.2 a	11.0 ± 0.6 b	11.2 ± 0.5 b	<0.0001
% PUFA	41.9 ± 1.1 a	46.4 ± 1.5b	45.5 ± 0.4 ab	0.018
% <i>n</i> - 6 PUFA	20.0 ± 0.8 a	17.8 ± 1.1 ab	15.1 ± 1.2 b	0.009
% <i>n</i> - 3 PUFA	21.9 ± 1.6 a	28.5 ± 1.0 b	30.4 ± 1.5 b	0.0005
<i>n</i> - 3 PUFA (% total PUFA)	51.9 ± 2.7 a	61.7 ± 1.6 b	66.8 ± 2.9 b	0.0009
20:4/18:2	0.72 ± 0.03 a	0.87 ± 0.04 a	1.05 ± 0.07 b	0.0005
% C20-22	31.4 ± 1.4 a	36.7 ± 1.2 b	37.6 ± 1.8 b	0.0002
Average chain length	18.45 ± 0.06 a	18.72 ± 0.05 a	18.75 ± 0.04 b	0.0006
Peroxidation index	213.6 ± 11.3 a	263.3 ± 7.5 b	274.8 ± 9.2 b	0.0003

Fatty acids identified by number of C atoms: number of double bonds and position of terminal double bond. Only values for the major fatty acids (>0.5%) are shown. Values are shown as mean ± S.E.M. Values with different letters are significantly different. NS: no significant differences.