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Aging-Related Oxidative Stress Depends on Dietary Lipid Source in Rat Postmitotic Tissues

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We investigate mitochondrial-lipid peroxidation of mitotic (liver) and postmitotic (heart and skeletal muscle) tissues of rats fed lifelong on two different lipid sources: virgin olive oil (monounsaturated fatty acids) and sunflower oil ($n-6$ polyunsaturated fatty acids). Two groups of 80 rats each were fed over 24 months on a diet differing in the lipid source (virgin olive oil or sunflower oil). Twenty rats per group were killed at 6, 12, 18, and 24 months; liver, heart, and skeletal muscle mitochondria were isolated and the lipid profile, hydroperoxides, vitamin E, and ubiquinone as well as catalase activity measured. Lipid peroxidation was higher in postmitotic tissues, and sunflower oil led to a higher degree of polyunsaturation and peroxidation. The levels of α-tocopherol adapted to oxidative stress and preferentially accumulated during aging in heart and skeletal muscle. In conclusion, the type of dietary fat should be considered in studies on aging, since oxidative stress is directly modulated by this factor. This study confirms that postmitotic tissues are more prone to oxidative stress during aging and proposes a hypothesis to explain this phenomenon.

KEY WORDS: Aging; lipid peroxidation; dietary fat; olive oil; mitochondria; liver; heart; muscle; antioxidants.

INTRODUCTION

Aging, inevitable and universal in all organisms, is characterized by an endogenous and progressive decline in physiological function that leads to morbidity and mortality (Barja, 2002; Sohal *et al.*, 2002). The widely accepted free-radical theory of aging postulates that deleterious effects of reactive oxygen species (ROS), mainly those from mitochondria, are responsible for this functional deterioration associated with aging (Beckman and Ames, 1998; Harman, 1956; Salvioli *et al.*, 2001; Sohal *et al.*, 2002). However, although the relationships between ROS and aging have been extensively investigated, many studies have reported conflicting results (Barja, 2002; Beckman and Ames, 1998; Dogru-Abbasoglu *et al.*, 1997; Rikans and Hornbrook, 1997; Sohal *et al.*, 2002). Although increased oxidative stress and antioxidant protection frequently fail to occur, changes in these parameters appear to be species, strain, sex, and tissue specific (Barja, 2002; Rikans and Hornbrook, 1997). Moreover, it is evident that oxidative damage is focused mainly on mitochondria (Halliwell and Gutteridge, 1999; Salvioli *et al.*, 2001). Thus, large changes in mitochondrial oxidative stress could be "swamped out" by a lack of changes in other cell compartments if the whole cell or tissue is assayed (Halliwell and Gutteridge, 1999). In addition, another factor that contributes to contradictory results, and that has not been considered in most of the studies, is the fatty-acid profile of cell membranes, which is very important since the degree of fatty-acid unsaturation in the cell membrane has been correlated with the maximum longevity (Barja, 2002; Pamplona *et al.*, 1999, 2002). It is also well known that external modifications of membrane lipids (i.e., through the diet) effectively affects lipid-peroxidation levels (Mataix *et al.*, 1998; Ochoa-Herrera *et al.*, 2001; Quiles *et al.*, 1999).

The impact of the diet and dietary components on aging and age-associated degenerative diseases has been

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widely recognized in recent years (Ames *et al.*, 1993; Meydani, 2001). However, most of the studies on this subject have focused on the effect of caloric restriction or antioxidant nutrient supplementation (Ames *et al.*, 1993; Finkel and Holbrook, 2000; Meydani, 2001; Sohal *et al.*, 2002). Despite that dietary lipid modulates the membrane-lipid profile and peroxidation rate (Mataix *et al.*, 1998; Ochoa-Herrera *et al.*, 2001; Quiles *et al.*, 1999), its effect on aging has been only partially studied and focused primarily on short-term treatments based mainly on $n-3$ polyunsaturated fatty acids (Carrie *et al.*, 2000; Meydani, 2001).

The present study examines for the first time the dietary effect on lipid peroxidation in mitochondria of mitotic (liver) and postmitotic (heart and skeletal muscle) tissues of rats fed throughout their life on two different lipid sources frequently used in Europe: virgin olive oil (very rich in monounsaturated fatty acids) and sunflower oil (very rich in $n-6$ polyunsaturated fatty acids). Our major aim is to determine whether a diet of these specific lipid sources can modulate how organisms age, in terms of susceptibility to mitochondrial oxidative stress.

MATERIAL AND METHODS

Experimental Protocol

A total of 160 male Wistar rats (*Rattus norvegicus*) initially weighing 80–90 g (supplied by the Laboratory Animal Service of the University of Granada) were maintained 10 per cage on a 12-h light/12-h darkness cycle, with free access to food and water. The rats were randomly assigned into two experimental groups and fed over 24 months on a semisynthetic and isoenergetic diet (in g/100 g of diet): 26.7 casein, 13.53 starch, 45.29 sucrose, 1.0 vitamin mixture, 3.68 mineral mixture, 1.84 cellulose, 0.09 choline, 0.3 methionine, and 8.0 fat. Experimental diets differed in the nature of the lipid source (Table I): virgin olive oil (olive group) and sunflower oil (sunflower group). The diets were prepared fresh every week and stored in darkness at 4°C to avoid autoxidation of the lipid source.

Twenty rats per group were killed at 6, 12, 18, and 24 months from the start of the experiment. Rats were always decapitated consistently at the same time of the day (between 8:00 and 9:00) to avoid any circadian fluctuations. The Ethical Committee of the Spanish Interministerial Commission of Science and Technology approved the different protocols used in this study, and the animals were handled according to the guidelines for care and use of laboratory animals of the Spanish Society for Laboratory Animal Sciences.

Table I. Fatty-Acid Composition of Experimental Diets

	Virgin olive oil	Sunflower oil
Palmitic acid (16:0) (%)	8.9	12.6
Palmitoleic acid (16:1) (%)	1.1	0.2
Stearic acid (18:0) (%)	2.0	1.9
Oleic acid (18:1*n*9) (%)	78.7	24.1
Linoleic acid (18:2*n*6) (%)	8.4	60.1
Linolenic acid (18:3*n*3) (%)	1.0	1.0
SFA	10.9	14.6
PUFA	9.4	61.1
MUFA	79.8	24.3

Note. SFA – Saturated fatty acids; PUFA – Polyunsaturated fatty acids; MUFA – Monounsaturated fatty acids.

Sample Analysis

Before decapitation, rats were weighed, after which the liver, heart, and skeletal muscle (vastus lateralis) were removed and weighed, and their mitochondria isolated according to Fleischer *et al.* (1979). Protein concentration of the samples was determined by the technique by Lowry *et al.* (1951), using bovine albumin as standard.

The fatty-acid profile of mitochondrial membranes was measured by gas–liquid chromatography as described by Lepage and Roy (1986). A gas–liquid Chromatograph model HP-5890 Series II (Hewlett Packard, Palo Alto, CA) equipped with a flame-ionization detector was used to analyze the fatty acids as methyl esters. Chromatography was performed using a 60-m-long capillary column; 32-mm id and 20-mm thickness impregnated with Sp 2330^{TM} FS (Supelco Inc., Bellefonte, Palo Alto, CA). The injector and the detector were maintained at 250 and 275°C, respectively; nitrogen was used as carrier gas, and the split ratio was 29:1. Temperature programming (for a total time of 40 min) was as follows: initial temperature, 160°C for 5 min, 6°C/min to 195°C, 4°C/min to 220°C, 2°C/min to 230°C, hold 12 min, 14°C/min to 160°C.

The hydroperoxide content was determined following the procedure of Jiang *et al.* (1992). *tert*-Butyl hydroperoxide was used to construct a standard curve.

Ubiquinone and α-tocopherol in mitochondrial membrane were assayed by high-performance liquid chromatography (HPLC), after extraction with ethanol:petroleum ether (60:40) using the method of Kröger (1978). The HPLC system consisted of an apparatus equipped with a Diode Array detector, model 168 (Beckman Instruments, Inc. Fullerton, CA) and the column was a reverse-phase C18 Spherisorb ODS 1 of 25 $\times$ 0.46 cm. Ubiquinone and α-tocopherol were identified by predetermining the retention times of individual standards.

Catalase activity was determined following the method described by Aebi (1984), based on monitoring at 240 nm the H_2O_2 decomposition, as a consequence of the catalytic activity of catalase. The activity was calculated from the first-order rate constant K (s^{-1}).

All chemical products and solvents, of the highest quality available, were acquired from Sigma (St. Louis, MO) and Merck (Darmstadt, Germany). The homologues of ubiquinone were kindly provided by Eisai Company (Tokyo, Japan). Virgin olive oil and sunflower oil were kindly provided by Coosur S.A. (Jaen, Spain).

Statistical Analysis

Results are presented as means ± standard error (SEM) ($n = 20$ rats). Before any statistical analysis, all variables were checked for normality and homogeneous variance using the Kolmogorov–Smirnoff and the Levene tests, respectively. When a variable was found not to follow normality, it was log-transformed and reanalyzed. One-way analysis of variance was used to test the time-dependent change. Statistically significant differences ($P < 0.05$) between groups were determined with the Student's t test and Bonferroni correction was used in case of multiple comparisons. A two-way ANOVA was used to analyze the effects of dietary fat and age period on each variable. Data were analyzed using the SPSS statistical package software (SPSS for Windows, 9.0.1, 1999, SPSS Inc., Chicago, IL).

RESULTS

Rat Weight

Body weight (Fig. 1) was similar for both diets at the different time periods. In both groups, the body weight increased for 12 and 18 months compared with 6 months. Finally, at 24 months old, rats decreased in body weight. Two-way ANOVA analysis (Table III) confirmed that only age had a significant effect on body weight.

Mitochondrial Fatty-Acid Profile

Table II shows the fatty-acid profile in mitochondrial membranes of liver, heart, and skeletal muscle. The proportion of saturated fatty acid (SFA) in liver did not change with age in either group, although there was a higher proportion of these fatty acids in the sunflower group at 12, 18, and 24 months. Only dietary fat affected liver SFA (Table III). In heart (Table II), the sunflower group increased SFA with age. Differences between diets were found at 12 and 18 months, with animals fed on sunflower oil showing the highest levels. An effect from the lipid source as well as an interaction between lipid source and age were found (Table III). In skeletal muscle, differences between groups were detected only at 6 months, with the olive group registering the highest SFA proportion. The effect on age proved significant in the rats fed virgin olive oil having lower SFA levels at 12, 18, and 24 months (Table II). Age also affected skeletal muscle SFA (Table III).

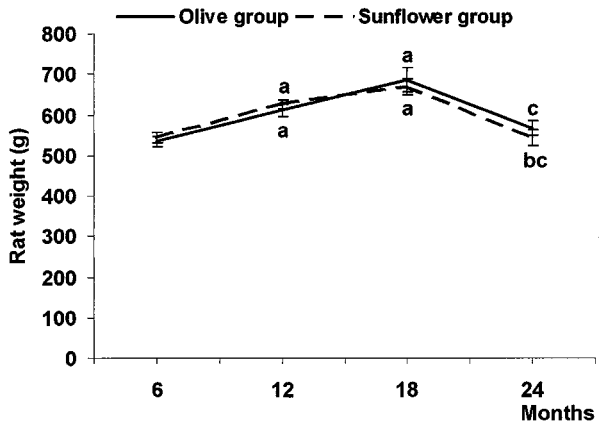

Fig. 1. Effect of dietary lipid source and aging on rat weight. Results are mean ± SEM of 20 animals. Statistical significance: ∗ differences between dietary lipid source for a particular time period. Differences related to aging for each dietary lipid source: a – 12, 18, or 24 months versus 6 months; b – 18 or 24 months versus 12 months; c – 18 months versus 24 months.

Animals fed on olive oil had a higher levels of monounsaturated fatty acids (MUFA) in all tissues, except for 6 months in skeletal muscle (Table II). Aging affected the proportion of MUFA differentially, depending on the tissue. In liver, age did not affect olive group, while in the sunflower group the rats 18 and 24 months old showed significantly lower values than in rats 12 months old. In heart, MUFA decreased in both dietary groups at 18 and 24 months with respect to 6 months. In skeletal muscle, age led to an increase of MUFA in both groups. The lipid source significantly affected the three tissues studied, and age was significant only for heart and muscle (Table III).

The proportion of polyunsaturated fatty acids (PUFA, Table II), for all the tissues studied, was higher in the sunflower group (except for heart at 18 months). No age effect was detected in liver. In heart, the olive group increased in PUFA at 18 and 24 months with respect to 6 and 12 months. The sunflower group registered a significant decrease at 12 months with respect to 6 and 24 months. In skeletal muscle, both groups showed a similar response, with the highest value at 18 months.

Table II. Fatty-Acid Profile (%) in Mitochondrial Membranes of Liver, Heart, and Skeletal Muscle of Rats Fed Diets Enriched in Virgin Olive Oil or Sunflower Oil for 6, 12, 18, or 24 Months

		6 months		12 months		18 months		24 months	
		Olive group	Sunflower group	Olive group	Sunflower group	Olive group	Sunflower group	Olive group	Sunflower group
Saturated fatty acids (SFA)	Liver	42.9 ± 1.7	45.4 ± 1.4	40.9 ± 1.1*	45.1 ± 1.5	44.4 ± 0.9*	48.8 ± 1.8	42.3 ± 1.3*	48.2 ± 1.1
	Heart	67.0 ± 3.0	62.9 ± 1.7	65.9 ± 2.6*	73.0 ± 0.9[a]	63.1 ± 1.8*	72.9 ± 1.6[a]	69.5 ± 0.7	69.2 ± 1.5[a]
	Muscle	76.0 ± 2.9*	64.8 ± 2.1	54.4 ± 4.3[a]	58.8 ± 2.5	56.1 ± 3.4[a]	54.6 ± 2.2[a]	60.5 ± 3.6[a]	62.7 ± 1.4
Monounsaturated fatty acids (MUFA)	Liver	32.6 ± 1.9*	20.7 ± 1.3	33.1 ± 1.3*	21.1 ± 1.0	32.9 ± 1.3*	17.0 ± 0.4[b]	36.3 ± 3.4*	17.6 ± 0.7[b]
	Heart	19.3 ± 2.7*	6.9 ± 0.9	19.1 ± 1.2*	4.8 ± 0.3[a]	9.2 ± 1.4*[ab]	3.5 ± 0.3[a]	7.5 ± 0.6*[ab]	3.2 ± 0.2[a]
	Muscle	18.1 ± 2.2	17.5 ± 1.9	36.8 ± 3.8*[a]	21.6 ± 1.7	30.8 ± 2.8*[a]	23.1 ± 2.3	31.7 ± 2.8*[a]	19.0 ± 1.6
Polyunsaturated fatty acids (PUFA)	Liver	24.6 ± 0.9*	33.8 ± 1.0	25.8 ± 1.0*	33.7 ± 1.6	22.8 ± 0.9*	34.2 ± 1.7	21.4 ± 2.2*	34.2 ± 0.8
	Heart	18.2 ± 1.4*	30.2 ± 1.1	14.3 ± 2.7*	22.3 ± 0.7[a]	27.6 ± 1.1[ab]	24.6 ± 1.3[a]	23.8 ± 1.0*[ab]	27.6 ± 1.4[b]
	Muscle	6.4 ± 0.7*	18.8 ± 1.0	9.9 ± 0.8*[a]	21.0 ± 1.5	14.0 ± 0.8*[ab]	24.3 ± 1.5[a]	8.5 ± 0.8*[c]	19.8 ± 0.4[c]

Note. Results are mean ± SEM of 20 animals. Statistical significances ($P < 0.05$): *sunflower oil versus olive oil for the same period of time. [a]: 12, 18, or 24 months versus 6 months for the same oil type. [b]: 18 or 24 months versus 12 months, for the same oil type. [c]: 24 months versus 18 months, for the same oil type.

Table III. Two-Way ANOVA Analysis for the Different Parameters Studied ($P < 0.05$)

		Lipid source	Age	Lipid source × Age
Weight		NS	S	NS
SFA	Liver	S	NS	NS
	Heart	S	NS	S
	Muscle	NS	S	S
MUFA	Liver	S	NS	NS
	Heart	S	S	S
	Muscle	S	S	S
PUFA	Liver	S	NS	NS
	Heart	S	S	S
	Muscle	S	S	NS
Hydroperoxydes (nmol/mg protein)	Liver	NS	S	S
	Heart	S	S	S
	Muscle	S	S	S
α-Tocopherol (nmol/mg protein)	Liver	NS	S	NS
	Heart	S	S	S
	Muscle	NS	S	NS
Ubiquinone (nmol/mg protein)	Liver	NS	S	NS
	Heart	NS	S	S
	Muscle	S	S	S
Catalase (K(s^{-1})/ mg protein)	Liver	S	S	S
	Heart	S	S	NS
	Muscle	NS	S	NS

Note. S – significant effects; NS – nonsignificant effects.

Hydroperoxide Levels

In liver (Fig. 2(A)), differences between diets were found only at 12 months, with the sunflower group reaching the highest levels. Both groups showed a significant decrease at 24 months compared with 6 and 18 months. Postmitotic tissues (i.e., heart and skeletal muscle, Fig. 2(B) and (C), respectively), increased hydroperoxide levels with age. Animals fed on sunflower oil reached higher values for all time periods except for 6 months. The two-way ANOVA (Table III) revealed an effect from age in the three tissues studied. The lipid source had a direct effect only on heart and muscle hydroperoxides.

Concentration of α-Tocopherol and Ubiquinone

Liver α-tocopherol (Fig. 3(A)) decreased at 24 months in both dietary groups. The sunflower group showed lower levels of α-tocopherol at 24 months than did the olive group. In heart (Fig. 3(B)) and skeletal muscle (Fig. 3(C)), both groups increased α-tocopherol with aging. Animals fed on olive oil reached higher values at 6, 12, and 18 months for heart and at 6 and 12 months for skeletal muscle.

Liver ubiquinone (Fig. 4(A)) showed a similar pattern in both groups, reaching the lowest values at 24 months. In heart (Fig. 4(B)), both groups registered the highest levels at 24 months. In skeletal muscle (Fig. 4(C)), both groups showed a higher ubiquinone concentration at 24 months.

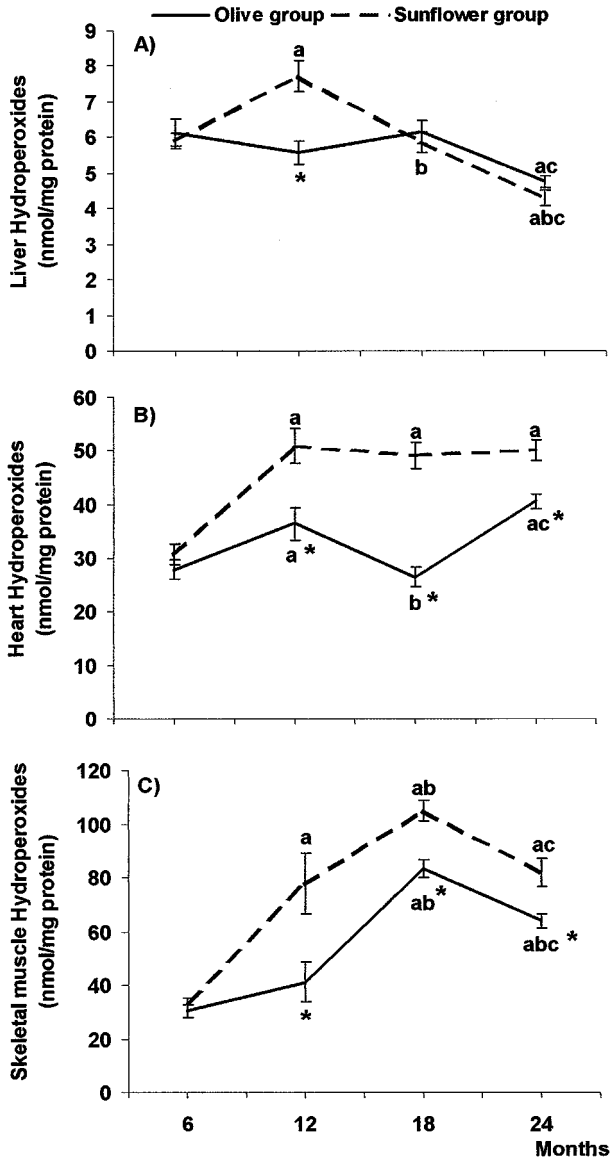

Fig. 2. Effect of dietary lipid source and aging on hydroperoxide levels in liver (A), heart (B), and skeletal muscle (C). Results are mean ± SEM of 20 animals. Statistical significance: ∗ differences between dietary lipid source for a particular time period. Differences related to aging for each dietary lipid source: a – 12, 18, or 24 months versus 6 months; b – 18 or 24 months versus 12 months, c – 18 months versus 24 months.

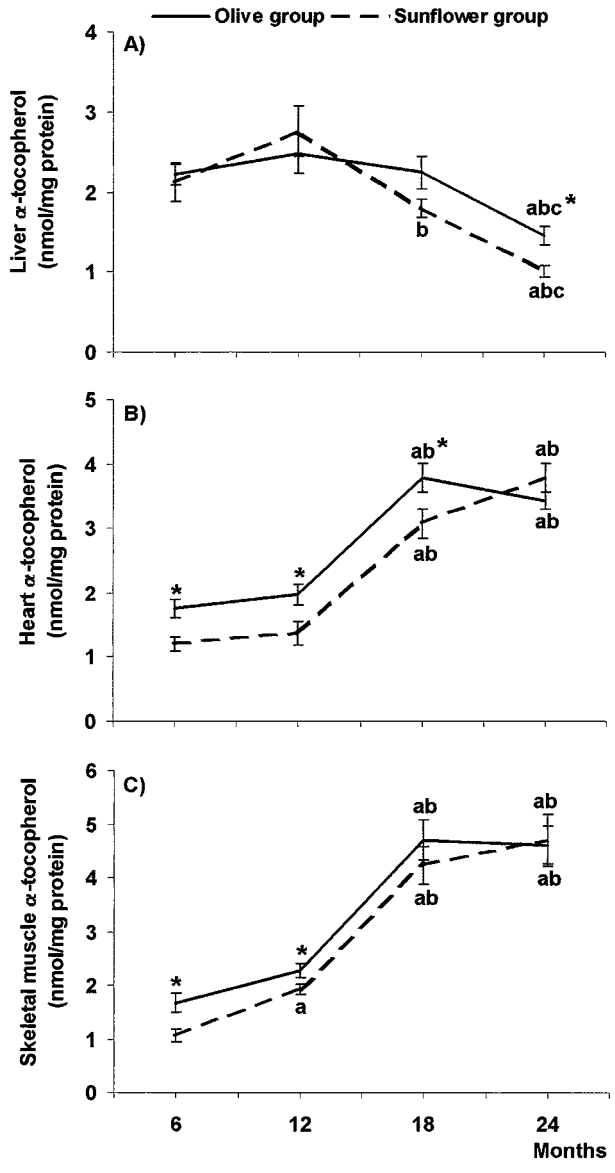

Fig. 3. Effect of dietary lipid source and aging on α-tocopherol levels in liver (A), heart (B), and skeletal muscle (C). Results are mean $\pm$ SEM of 20 animals. Statistical significance: $*$ differences between dietary lipid source for a particular time period. Differences related to aging for each dietary lipid source: a – 12, 18, or 24 months versus 6 months; b – 18 or 24 months versus 12 months; c – 18 months versus 24 months.

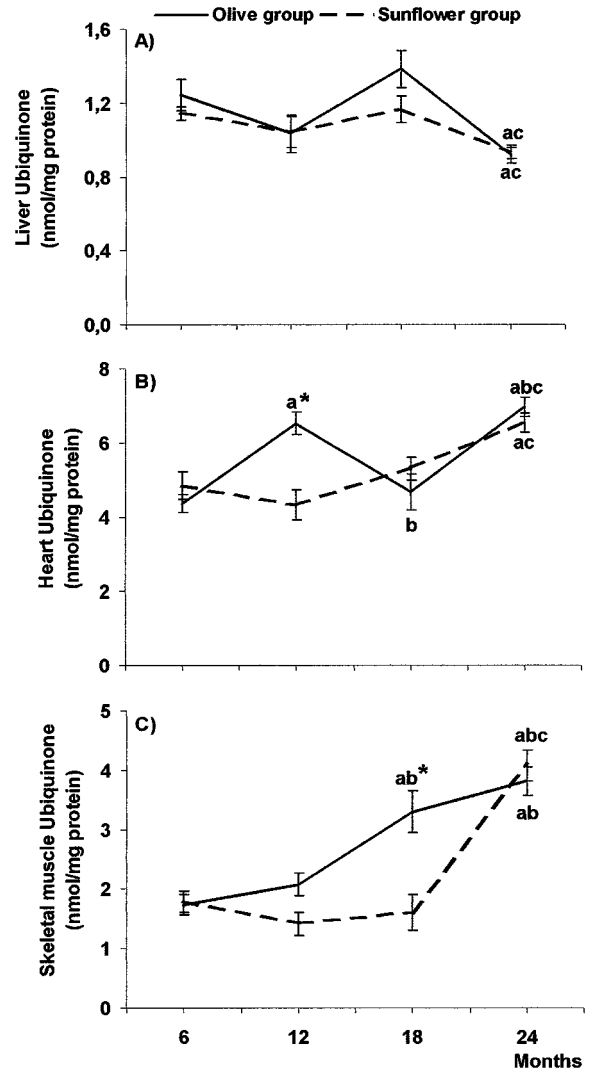

Fig. 4. Effect of dietary lipid source and aging on ubiquinone levels in liver (A), heart (B), and skeletal muscle (C). Results are mean $\pm$ SEM of 20 animals. Statistical significance: $*$ differences between dietary lipid source for a particular time period. Differences related to aging for each dietary lipid source: a – 12, 18, or 24 months versus 6 months, b – 18 or 24 months versus 12 months; c – 18 months versus 24 months.

The olive oil group showed higher ubiquinone values at 12 and 18 months of life. Age had a significant effect for the two antioxidants studied, while the lipid source only affected heart α-tocopherol and skeletal-muscle ubiquinone.

Catalase Activity

Cytosolic catalase activity in liver (Fig. 5(A)) showed the highest values for the olive group at 12, 18, and 24 months. Aging increased activity only for the olive group (at 18 and 24 months). In heart (Fig. 5(B)), catalase activity increased with age in both dietary groups after 12 months. The olive group showed the highest values at 12, 18, and 24 months. Skeletal muscle catalase (Fig. 5(C)) decreased at 24 months with both dietary treatments. Diet led to differences only at 6 months, with the highest values for the sunflower group. Two-way ANOVA (Table III) analysis showed effect for the lipid source in liver and heart. Age affected the three studied tissues.

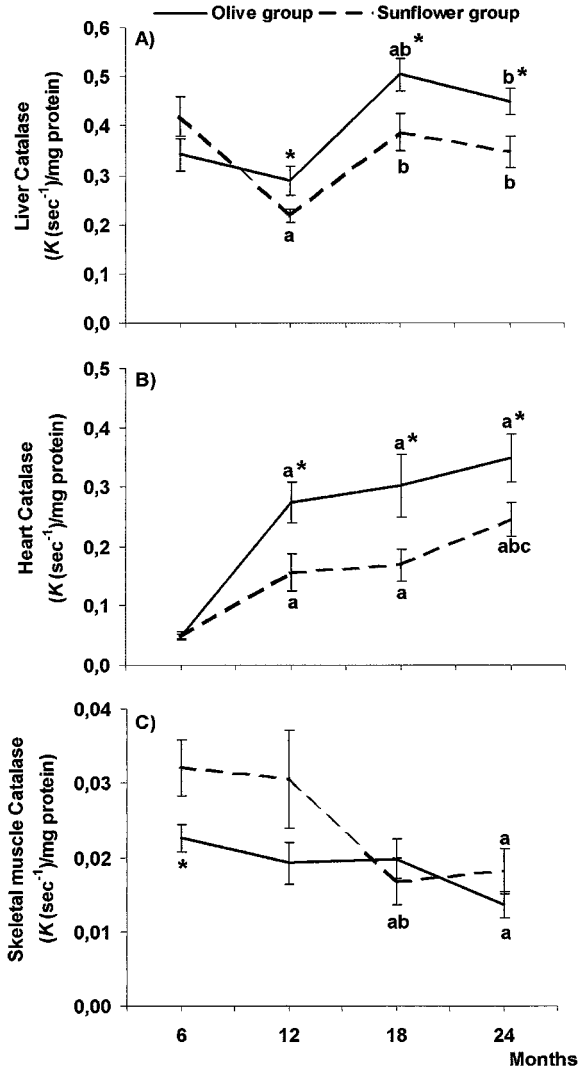

Fig. 5. Effect of dietary lipid source and aging on cytosolic catalase activity in liver (A), heart (B), and skeletal muscle (C). Results are mean ± SEM of 20 animals. Statistical significance: ∗ differences between dietary lipid source for a particular time period. Differences related to aging for each dietary lipid source: a – 12, 18, or 24 months versus 6 months; b – 18 or 24 months versus 12 months; c – 18 months versus 24 months.

DISCUSSION

Virgin olive oil, the main lipid source in the Mediterranean diet, offers greater protection of mitochondrial and microsomal membranes against damage caused by both exogenous and endogenous oxidative stress than do other lipid sources (Mataix *et al.*, 1998; Ochoa-Herrera *et al.*, 2001; Quiles *et al.*, 1999). However, the effect of this oil on aging is practically unknown. The present study was designed to study whether the intake through the life of two different dietary lipid sources (virgin olive oil and sunflower oil) can alter how an organism ages, from the standpoint of the oxidative stress.

The way in which the membrane fatty-acid profile adapts to dietary lipid source has been previously reported (Mataix *et al.*, 1998; Ochoa-Herrera *et al.*, 2001; Quiles *et al.*, 1999, 2001), but no studies are available to monitor the adaptation to the diets during aging in different rat tissues using a large sample size (20 animals per group) as in the present work. Our results show that such adaptation of mitochondrial membranes to dietary fat effectively occurred throughout all the studied time periods. Animals fed on olive oil showed the highest MUFA proportion in their mitochondrial membranes whereas those fed on sunflower oil registered the highest percentages of $n-6$ PUFA. This result is noteworthy because any benefit or damage derived from the intake of the two lipid sources is maintained throughout life, providing the opportunity to modulate aging through diet.

A generally accepted feature of aging is that tissue fatty acids become more saturated, this being associated with a decrease in the degree of polyunsaturation (Imre *et al.*, 2000; Ulmann *et al.*, 1991). We found that changes associated with aging in the mitochondrial-membrane lipid profile were tissue and lipid-source dependent. Consequently, it is not possible to consider a net increase or decrease in the level of unsaturation associated with aging and the three tissues and the two dietary treatments should be studied separately. It could be speculated that, depending on the dietary lipid source, each tissue changes its mitochondrial-membrane lipid profile and therefore its fluidity in different ways, to establish the best conditions for membrane proteins, for membrane transport, etc. (Feller *et al.*, 2002; Lutz *et al.*, 1999; Pamplona *et al.*, 1999). These modifications in the mitochondrial-membrane lipid profile can change its susceptibility to lipid peroxidation (Mataix *et al.*, 1998).

Lipid peroxidation during aging has been reported to increase, decrease, or remain unchanged, depending on the experiment (Barja, 2002; Beckman and Ames, 1998; Dogru-Abbasoglu *et al.*, 1997; Rikans and Hornbrook, 1997; Salvioli *et al.*, 2001; Sohal *et al.*, 2002). In the present study, lipid-peroxidation values, as the level of mitochondrial hydroperoxides, differed depending on the tissue, the dietary treatment, and the period. Liver, which has regenerative capacity, showed the lowest level of hydroperoxides among the three tissues studied. This tissue did not show an increase in lipid peroxidation with aging or even a decrease at 24 months with either treatments was found. Similar responses in liver have been reported by other authors (Dogru-Abbasoglu *et al.*, 1997; Feller *et al.*, 2002). By contrast, in heart and skeletal

muscle, hydroperoxides increased with aging in both dietary groups, although animals fed on olive oil showed lower hydroperoxides than did those fed on sunflower oil at 12, 18, and 24 months. The lipid peroxidation results reflect with a high precision differences found in mitochondrial-lipid profile. Thus, olive-oil treatment, which led to the less polyunsaturated membranes, resulted in lower increases in lipid peroxidation of postmitotic tissues, the most relevant tissues in aging (Barja, 2002).

Enzymatic and nonenzymatic components of the antioxidant system followed a similar tissue-dependent response in both dietary groups, although the lipid dietary source differentially modulated the concentration and/or activity of these components. The α-tocopherol concentration decreased in both dietary groups in liver, but increased in heart and skeletal muscle. According to these results, the organism appears to accumulate this potent antioxidant at the sites at which it is needed in order to counterbalance oxidative damage (Van der Loo *et al.*, 2002). A similar response was found for ubiquinone concentration, which could be due both to a higher energy demand in postmitotic tissues or to the antioxidant role of this molecule (Linnane *et al.*, 2002; Mataix *et al.*, 1998; Quiles *et al.*, 1999).

Increased catalase activity is associated with a greater resistance to oxidative damage (Beckman and Ames, 1998; Brown-Borg and Rakoczy, 2000) and, in this sense, catalase seems to be important in overall antioxidant enzymatic defence systems with respect to lifespan (Beckman and Ames, 1998; Brown-Borg and Rakoczy, 2000). However, the results of catalase activity with respect to aging are quite varied, depending on the tissue and the age at which the analysis is performed (Beckman and Ames, 1998). Our results confirm that another factor must be considered when catalase activity during aging is considered, i.e., the dietary lipid source. Liver-catalase activity increased with virgin olive oil but remained unchanged with sunflower oil. In heart, both groups showed increased catalase activity with age, but this increase was higher in the olive-oil group. Finally, skeletal muscle decreased catalase activity in both dietary groups. With respect to heart, the increased activity found in olive group is important because it has been suggested that catalase can function as a major pathway for detoxification of H_2O_2 in cardiac tissue (Molina and García, 1997).

In general, oxidative stress, in terms of lipid peroxidation, was higher in such postmitotic tissues as heart and skeletal muscle, and dietary lipids with a different degree of polyunsaturation differentially modulated this lipid peroxidation. Sunflower oil intake led to more polyunsaturated mitochondrial membranes and, consequently, increases in lipid peroxidation with aging was higher in the heart and skeletal muscle of animals fed on this polyunsaturated oil. In liver, which has a normal cell turnover, this effect is lower probably due to dilution effects during cell division. In parallel, the α-tocopherol levels adapted to the oxidative stress in the sense that this antioxidant preferentially accumulated during aging in heart and skeletal muscle.

In addition, we have previously reported (Quiles *et al.*, 2002) that aging depresses cytochrome oxidase activity, the final electron acceptor at the mitochondrial electron transport chain (METC), in heart and skeletal muscle but not in liver. In parallel, the levels of other METC components such as cytochrome *b* increased, a situation that could lead to the uncoupling of the METC and subsequently augment free-radical production. Cytochrome *b* enhancement is considered a marker of aging (Kipling, 2001) and occurs at the main site of free-radical production in the METC (Lenaz, 1998). With the PUFA-rich diet, cytochrome oxidase decreased and cytochrome *b* increased more than with the MUFA-rich diet (Quiles *et al.*, in press). Data from the above-mentioned study together with those from the present work offer a possible mechanism to explain why dietary lipids can modulate the way in which an organism ages, depending on the tissue type. Such a mechanism would involve the mitochondrial-membrane lipid profile and bioenergetics, together with the particular regenerative capacity of the specific tissue. Briefly, aging, considered as an endogenous and progressive process (Barja, 2002), leads to oxidative alteration of mitochondria and mitochondrial components such as mitochondrial-DNA throughout life (Barja, 2002; Beckman and Ames, 1998; Finkel and Holbrook, 2000; Sohal *et al.*, 2002). These alterations deteriorate mitochondrial structure and function, and, depending on the tissue capacity to repair damage or to remove the cell, tissue function is affected in a different way (Quiles *et al.*, in press). Regenerative tissues such as liver appears to be able to buffer the damage, as suggested by the lack of loss in mitochondrial function (expressed in terms of cytochrome oxidase activity). However, a loss of function results in postmitotic tissues such as skeletal muscle or heart, with no capacity to replace cells or probably with a less effective repair system (differences in mitochondrial-DNA repair system between liver and heart have been described; Souza-Pinto *et al.*, 1999). This is reflected by decreased cytochrome oxidase activity, leading to a possible uncoupling of the METC and subsequently to an increase in free-radical production. Thus, the role of dietary lipids may reside in the building of an environment more or less susceptible to free-radical generation and propagation, especially when the METC fails.

In conclusion, dietary fat should be considered in studies on aging, since the intake of oils with different degrees of polyunsaturation directly modulates the final response of tissues to oxidative stress. This might help to explain why populations living in Mediterranean countries enjoy a longer life expectancy and lower rates of coronary heart diseases than in Northern Europeans (Trichopoulou and Vasilopoulou, 2000). Moreover, the present study confirms that postmitotic tissues are more prone to oxidative stress during againg, proposing a mechanism to explain this phenomenon.

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