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Ageing-Related Tissue-Specific Alterations in Mitochondrial Composition and Function Are Modulated by Dietary Fat Type in the Rat

José L. Quiles,[1,2] Estrella Martínez,[1] Susana Ibáñez,[1] Julio J. Ochoa,[1] Yolanda Martín,[1] Magdalena López-Frías,[1] Jesús R. Huertas,[1] and José Mataix[1]

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This study investigated the way in which feeding rats with two fat sources (olive or sunflower oils) affected electron-transport components and function of mitotic (liver) and postmitotic (heart and skeletal muscle) tissues during ageing. Rats adapted the mitochondrial-membrane-lipid profile to dietary fat throughout the study, suggesting that the benefits to eat either of the two fats might be maintained lifelong. Liver was more resistant to dietary changes and ageing than heart and skeletal muscle, which showed higher levels of coenzyme Q, cytochrome *b*, and cytochrome $a + a_3$ with ageing and lower cytochrome *c* oxidase and complex IV turnover. Dietary fat differentially modulated the response of tissues during ageing, with sunflower oil leading to the highest levels of coenzyme Q and cytochromes *b* and $a + a_3$. Since high levels of cytochrome *b* have been related to increased age, it could be hypothesized that olive oil could lead to less aged mitochondria.

KEY WORDS: Ageing; olive oil; sunflower oil; cytochrome *c* oxidase; cytochromes; coenzyme Q; liver, heart, and skeletal muscle.

INTRODUCTION

Ageing is usually defined as the progressive loss of function accompanied by decreasing fertility and increasing mortality with advancing age (Kirkwood and Austad, 2000). Ageing in humans is manifest not only by the stereotypical changes in phenotype but also by a large increase in the onset of many diseases (Guarente and Kenyon, 2000). Over 300 theories of ageing have been proposed to explain the events that lead to the death of an organism (Medvedev, 1990). Mitochondria may play a key role in causing ageing and may be one of the most adversely affected organelles during this process (Kwong and Sohal, 2000), and mitochondrial-respiratory-chain failure has been implicated as a factor during ageing (Miquel *et al.*, 1980; Wallace, 1992). This phenomenon seems to be more relevant in tissues lacking regenerative capacity, such as heart, skeletal muscle, or brain (Kwong and Sohal, 2000; Ojaimi *et al.*, 1999), and may be related to alterations in mitochondrial DNA, which has been proposed to be much more sensitive to alterations, such as mutations, deletions, rearrangements, etc., than is nuclear DNA (Miquel, 1991; Ojaimi *et al.*, 1999).

It is well known that the dietary fat source deeply influences several biochemical parameters in mitochondrial membranes (Mataix *et al.*, 1998; Quiles *et al.*, 1999). The importance of fatty acids resides in the fact that mitochondrial membrane adapts its lipid composition to dietary fat (Charnock *et al.*, 1992; Quiles *et al.*, 1999; Yamaoka *et al.*, 1988), and adaptations of electron-transport system in relation to the dietary fat type have been widely reported (Battino *et al.*, 2002; Huertas *et al.*, 1991a,b; Quiles *et al.*, 2001).

The present study was designed to assess the way in which the electron-transport components and function of mitotic (liver) and postmitotic (heart and skeletal muscle) tissues are affected by feeding rats throughout their life span with two different dietary fat sources.

[1] Department of Physiology, Institute of Nutrition and Food Technology, University of Granada, C/Ramón y Cajal 4, 18071 Granada, Spain.
[2] To whom correspondence should be addressed; e-mail: jlquiles@goliat.ugr.es.

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These fats (two of the most frequently used fat sources in Europe) were different in their lipid profile: olive oil, rich mainly in the monounsaturated oleic acid, and sunflower oil, rich primarily in the polyunsaturated linoleic acid. Results from this study might contribute to a better understanding of the ageing process with respect to the mitochondrion and might help clarify whether the intake of a specific fat source determines how organisms undergo ageing.

MATERIALS AND METHODS

Experimental Protocol

A total of 120 male Wistar rats (*Rattus norvegicus*) initially weighting 80–90 g (supplied by the laboratory Animal Service of the University of Granada) were divided into groups of 10 per cage and maintained on a 12-h-light/12-h-darkness cycle, with free access to food and water. The study lasted 24 months, the normal lifespan of a rat being around 30 months. During the first week, the rats were fed a nonpurified diet (Diet A.03 supplied by Panlab S.L., Barcelona, Spain). The rats were randomly assigned into two experimental groups and fed for 24 months on a semisynthetic and isoenergetic diet composed of (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, 0.30 methionine, and 8.0 fat. Experimental diets differed in fat source (Table I): olive oil and sunflower oil.

At 6 and 24 months after the start of the experiment, 20 rats per group were decapitated, at the same time of day in all cases (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 experiment, 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 the Experimental Diets

Fatty-acid composition	Virgin olive oil(%)	Sunflower oil(%)
16:0	8.92	12.6
16:1(*n*-7)	1.06	0.2
18:0	1.97	1.9
18:1 (*n*-9)	78.73	24.1
18:2(*n*-6)	8.36	60.1
18:3 (*n*-3)	0.96	1
Total saturated	10.89	14.6
Total unsaturated	89.11	85.4
Total monounsaturated	79.79	24.3
Total polyunsaturated	9.32	61.1

Sample Analysis

Before decapitation, the rats were weighed. Liver, heart, and skeletal muscle (*vastus lateralis*) were removed, weighed, and their mitochondria isolated according to Fleischer *et al.* (1979). The concentration of mitochondrial protein in liver, heart, and skeletal muscle was assayed according to Lowry *et al.* (1951) using bovine serum albumin as a standard. For the analysis of the mitochondrial-lipid profile by gas–liquid chromatography, the fatty-acid methyl esters of mitochondrial membranes were formed according to Lepage and Roy (1986). Gas chromatography was performed as previously described in detail (Quiles *et al.*, 2001). The concentration of mitochondrial cytochrome *b* and $a + a_3$ was evaluated by differential spectra in a λ16-Perkin Elmer double-beam spectrophotometer, according to Vanneste (1966) and Nicholls (1976). Cytochrome *c* oxidase (CCO) activity was assayed at 25°C using cytochrome *c* reduced by sodium dithionite (Degli Esposti *et al.*, 1982), monitoring the absorbance decrease of cytochrome *c* upon oxidation at 417–409 nm every 10 s for 2 min using an extinction coefficient for cytochrome *c* of 40.7 $mM^{-1} \cdot cm^{-1}$ (Battino *et al.*, 1986). Mitochondrial coenzyme Q (CoQ) isomers (Coenzyme Q_9 and Coenzyme Q_{10}, which are present in the rat in an approximate ratio of 10:1) were extracted and determined by reversed-phase HPLC, as previously described (Quiles *et al.*, 2001).

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

Statistical Analysis

The results represent the mean and the standard error of 20 animals. Except for the lipid profile (expressed in terms of percentage), results are expressed per gram of wet tissue. Significant interaction terms were evaluated by a Student's *t* test. Results were considered significant at $P < 0.05$. Data were analysed using the SPSS/PC statistical software package (SPSS for Windows, 9.0.1, 1999, SPSS Inc., Chicago, IL).

RESULTS

The present study investigates mitotic (liver) and postmitotic (heart and skeletal muscle) rat tissues for any possible effect on electron-transport components and function in relation to the dietary fat source. These dietary fats, olive oil and sunflower oil, differ markedly in their lipid profile [olive oil is rich in monounsaturated fatty acids (MUFA) and sunflower oil in polyunsaturated fatty acids (PUFA)]. Results from the present research could help us to determine whether the dietary intervention may help to modulate the ageing process with respect to the mitochondria.

Food Intake, Rat Weight, and Mitochondrial-Lipid Profile

Dietary intake did not vary significantly among the groups during the experiment (data not shown). Body weight was similar for both diets for the different time intervals (olive oil vs. sunflower oil, for 6 months: 535.5 ± 12.6 vs. 543.1 ± 12.2, and for 24 months: 597.3 ± 18.7 vs. 544.2 ± 19.7). To test whether the rats were properly adapted to dietary fat, we analysed the lipid profile of mitochondrial membranes, Figure 1 shows the proportion of MUFA in mitochondrial membranes of liver (Fig. 1(A)), heart (Fig. 1(B)), and skeletal muscle (Fig. 1(C)). For the three tissues studied, and for both age periods, the animals fed on olive oil had the highest MUFA level. The opposite was found for PUFA (data not showed). Age only affected MUFA proportion in heart, with a decrease in the proportion of these fatty acids for both dietary groups at 24 months.

Concentrations of Coenzyme Q and Cytochrome *b*

Figure 2 shows the levels of coenzyme Q (CoQ). In liver (Fig. 2(A)), CoQ levels for the group fed olive oil compared with sunflower-oil group were higher at 6-month age and lower at 24 months. Age affected only the sunflower-oil group, with an increase at 24 months. In heart (Fig. 2(B)), for both age periods, olive led to lower levels of CoQ than did sunflower oil. An increase with time was found for the olive-oil group. In skeletal muscle (Fig. 2(C)), olive-oil group showed lower levels of CoQ than did the sunflower-oil group in both age groups. With respect to age, there was an enhancement at 24 months for both dietary groups.

Concerning cytochrome *b* (Fig. 3) in liver (Fig. 3(A)), the olive-oil group showed higher values at 6 months and

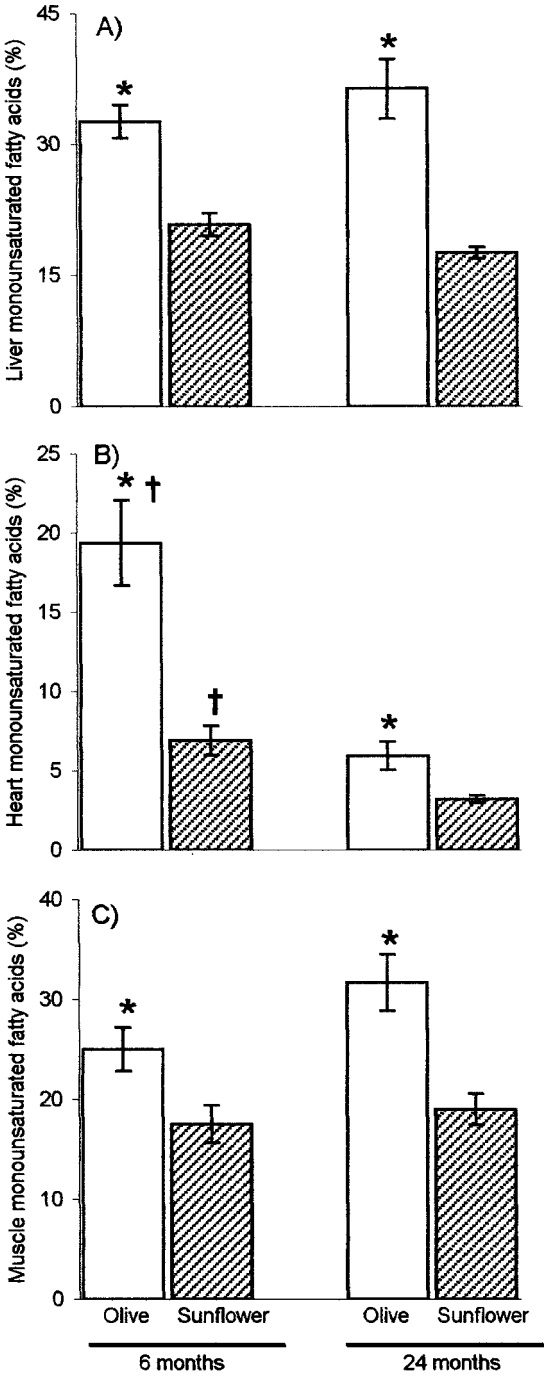

Fig. 1. Effects of dietary fat type and ageing on the proportion of monounsaturated fatty acids (MUFA) in liver (A), heart (B), and skeletal muscle (C) of rats. Results are mean ±SEM of 20 animals. Statistical significances: *olive-oil group vs. sunflower-oil group for 6 or 24 months; †6 months vs. 24 months for olive-oil group or sunflower-oil group. Note that y-axis has a different scale, depending on the tissue.

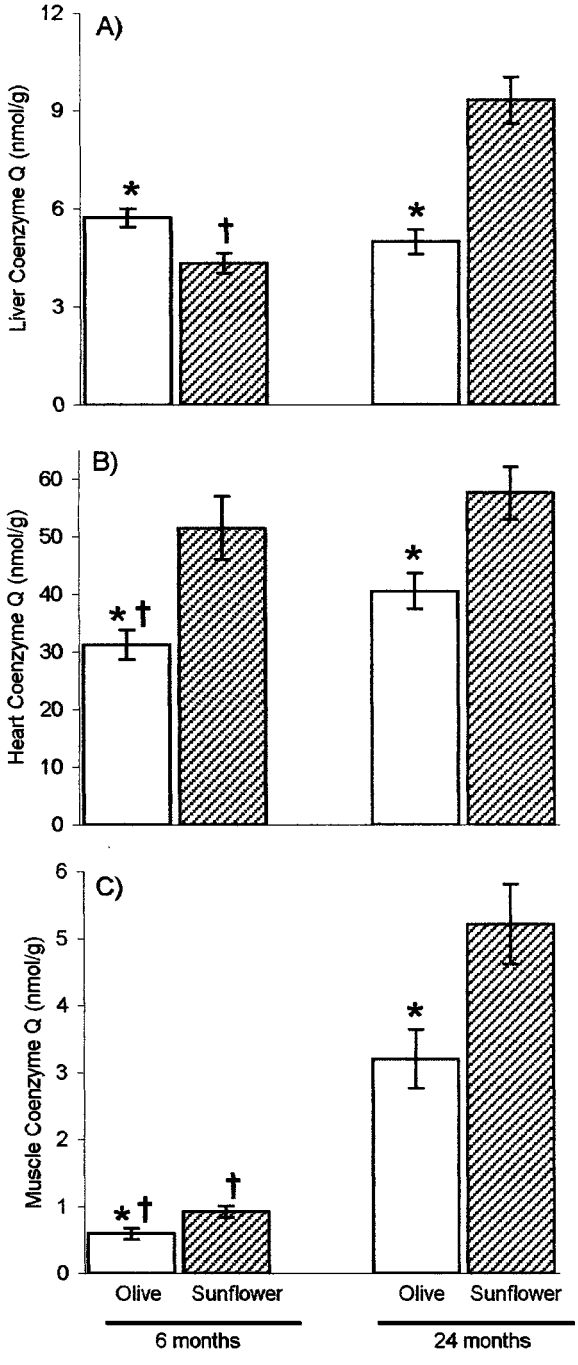

Fig. 2. Effects of dietary fat and ageing on coenzyme Q levels in liver (A), heart (B), and skeletal muscle (C) of rats. Results are mean ±SEM of 20 animals. Statistical significances: *olive-oil group vs. sunflower-oil group for 6 or 24 months; †6 months vs. 24 months for olive-oil group or sunflower-oil group. Note that y-axis has a different scale, depending on the tissue.

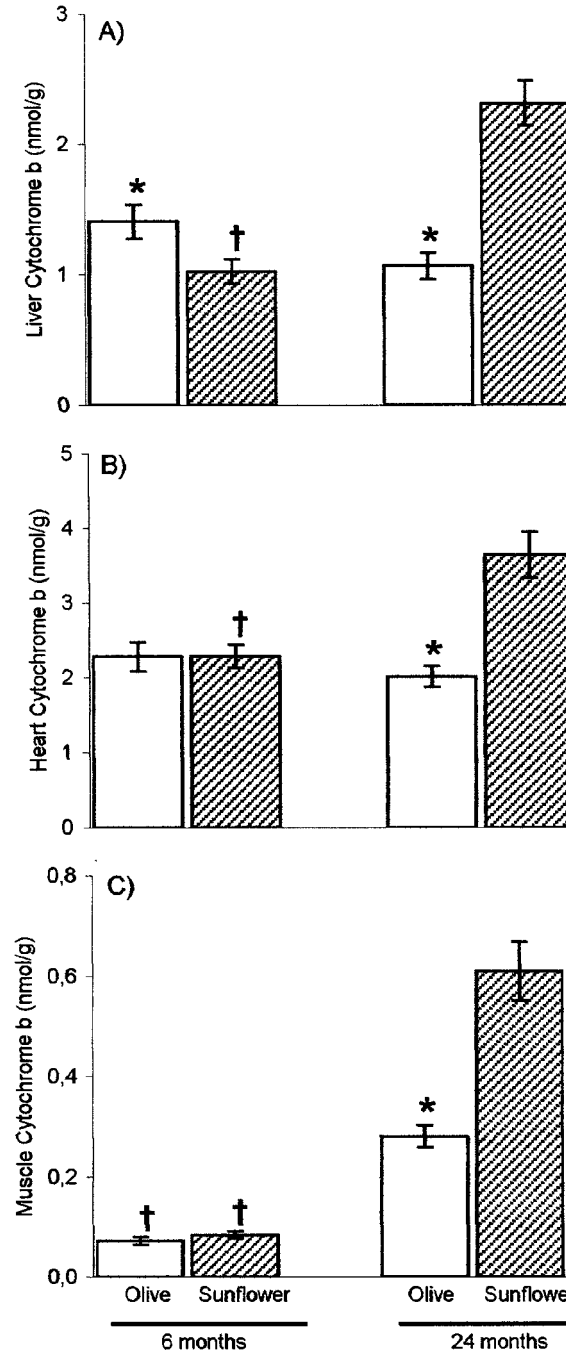

Fig. 3. Effects of dietary fat and ageing on cytochrome *b* levels in liver (A) heart (B), and skeletal muscle (C) of rats. Results are mean ±SEM of 20 animals. Statistical significances: *olive-oil group vs. sunflower-oil group for 6 or 24 months; †6 months vs. 24 months for olive-oil group or sunflower-oil group. Note that y-axis has different scale, depending on the tissue.

lower levels at 24 months, compared with sunflower-oil group. Age affected only the sunflower-oil group, with a sharp increase at 24 months. For heart (Fig. 3(B)), no differences were found between dietary treatments at 6 months of age, and higher levels were found in the sunflower-oil group at 24 months. For skeletal muscle (Fig. 3(C)), both groups were similar at 6 months and the olive-oil group differed from the sunflower group at 24 months (with lower values). Aging led to higher cytochrome *b* values in both groups at 24 months.

Complex IV Concentration, Activity, and Turnover

Cytochrome $a + a_3$ concentration is shown in Fig. 4. For liver (Fig. 4(A)), dietary treatment showed an effect only at 24 months, with higher levels found in the sunflower-oil group. Age affected only the sunflower-oil group, with higher values at 24 months. In heart (Fig. 4(B)), no differences between diets were found for the two age categories. Age affected both dietary groups, lowering cytochrome $a + a_3$ levels at 24 months. In skeletal muscle (Fig. 4(C)), there were no differences between dietary treatments. Ageing was reflected in higher values at 24 months in both groups.

CCO activity is shown in Fig. 5. In liver (Fig. 5(A)), differences between diets were found at 24 months (higher activity for sunflower oil). No age effect was found for the olive-oil group, but CCO activity increased at 24 months in the sunflower-oil group. In heart (Fig. 5(B)), no differences between diets were detected. Ageing led to a decrease in both groups. For skeletal muscles (Fig. 5(C)), diet showed an effect only at 6 months, with higher activity for the sunflower-oil group. Ageing reduced CCO activity in sunflower-oil group.

Complex IV turnover is shown in Fig. 6. For liver (Fig. 6(A)), no differences were found concerning diet or age. In heart (Fig. 6(C)), a higher turnover was found for both age periods in the sunflower-oil group. Ageing led to a sharp decline in turnover in both dietary groups. In skeletal muscle (Fig. 6(C)), diet triggered difference only at 24 months, with higher levels for the sunflower-oil group. Age diminished turnover in both dietary groups, showing lower values at 24 months.

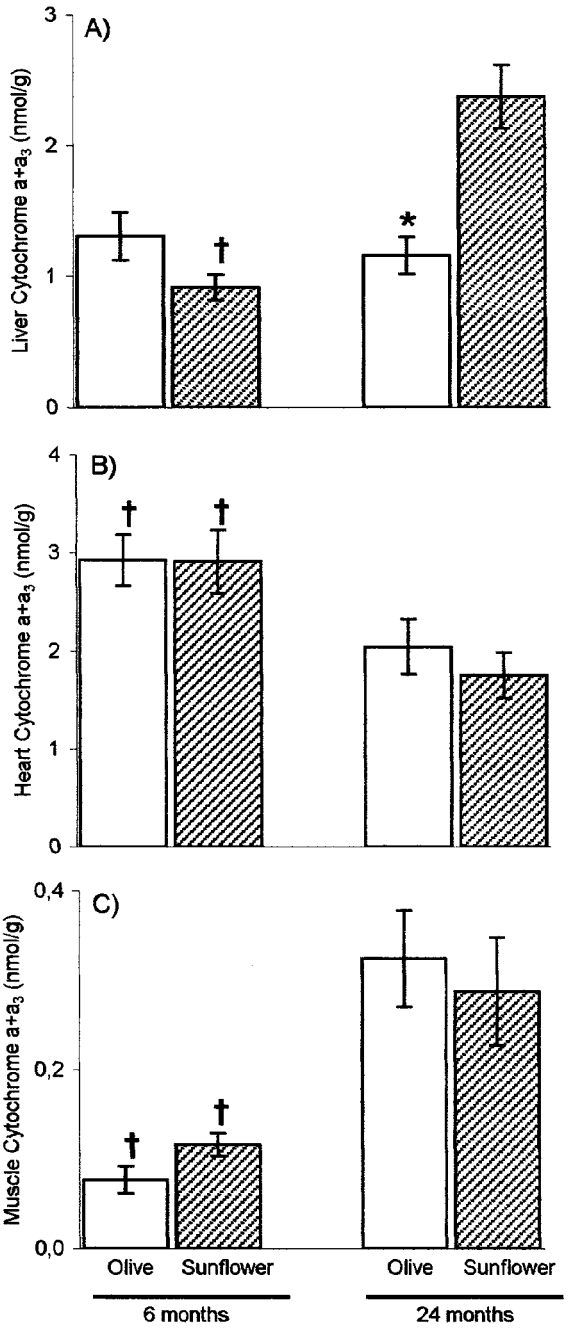

Fig. 4. Effects of dietary fat and ageing on cytochrome $a + a_3$ levels in liver (A) heart (B), and skeletal muscle (C) of rats. Results are mean ±SEM of 20 animals. Statistical significances: *olive-oil group vs. sunflower-oil group for 6 or 24 months; †6 months vs. 24 months for olive-oil group or sunflower-oil group. Note that y-axis has a different scale, depending on the tissue.

DISCUSSION

The present experiment was designed to study mitochondrial composition and function in various tissues

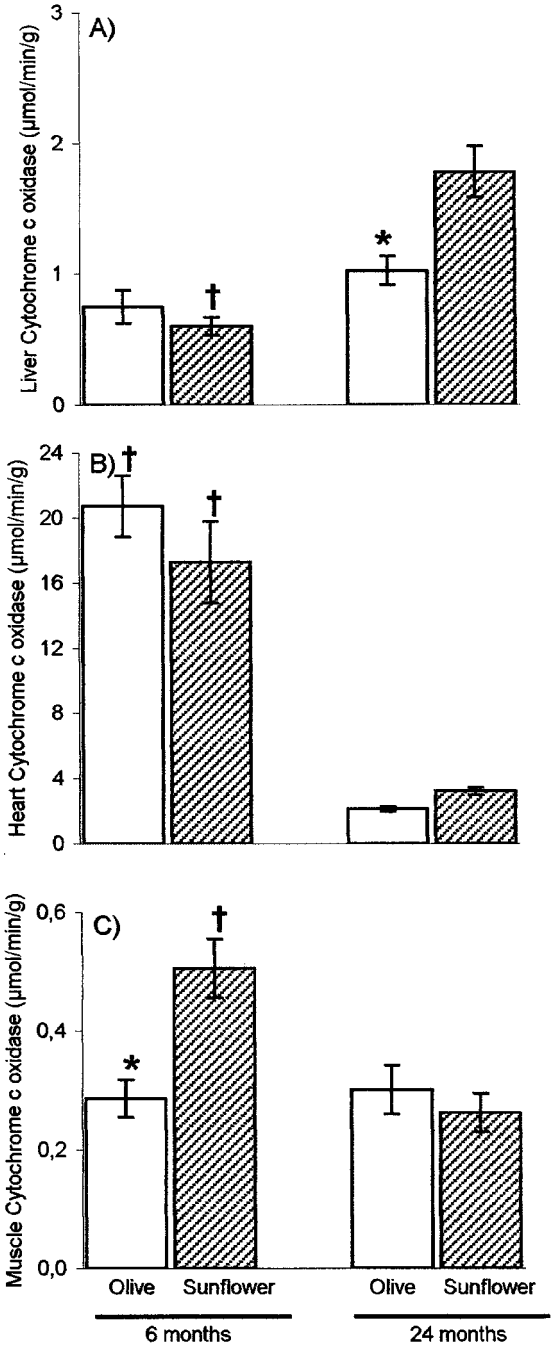

Fig. 5. Effects of dietary fat and ageing on cytochrome *c* oxidase activity in liver (A) heart (B), and skeletal muscle (C) of rats. Results are mean ±SEM of 20 animals. Statistical significances: *olive-oil group vs. sunflower-oil group for 6 or 24 months; †6 months vs. 24 months for olive-oil group or sunflower-oil group. Note that y-axis has a different scale, depending on the tissue.

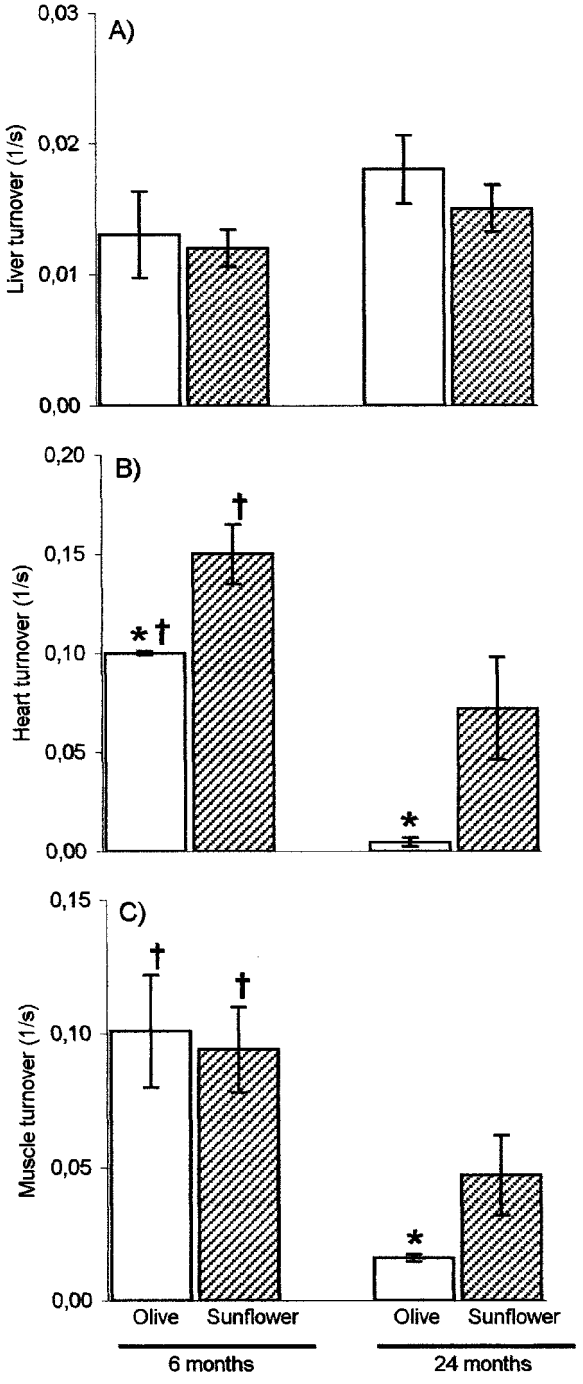

Fig. 6. Effects of dietary fat and ageing on complex IV turnover in liver (A) heart (B), and skeletal muscle (C) of rats. Results are mean ±SEM of 20 animals. Statistical significances: *olive-oil group vs. sunflower-oil group for 6 or 24 months; †6 months vs. 24 months for olive-oil group or sunflower-oil group. Note that y-axis has a different scale, depending on the tissue.

of rat depending on ageing and the intake of different dietary-fat sources. The first fact to demonstrate is that feeding rats with sunflower or olive oils leads to mitochondrial membranes of liver, heart, and skeletal muscle with a different fatty-acid profile, as shown in Fig. 1. Fatty-acid-profile adaptation to olive oil or sunflower oil has been previously reported (Battino *et al.*, 2002; Quiles *et al.*, 2001) but no studies are available that monitor the adaptation to these diets during ageing in different tissues in the rat, using a large sample size (20 animals per group). In the present study, such adaptation followed throughout all the time periods studied, and aged rats still had different mitochondrial membrane lipid profiles. This finding implies that the benefits (if any) after eating either of the two fat types might be maintained through the whole life.

Ageing modulated the adaptation to dietary fat in a different way, depending on the tissue. Liver and skeletal muscle were quite resistant to change its mitochondrial membranes while heart decreased its MUFA. In parallel, PUFA and saturated fatty acids (SFA) were differentially modulated in heart and muscle (data not shown). It is quite difficult to ascertain whether these relative variations in the fatty-acid fractions have any specific role in mitochondrial function through changes in fluidity, preparing mitochondria for the best work condition of membrane proteins, improving membrane transport, etc. (Feller *et al.*, 2002; Lutz *et al.*, 1999), or whether this is just a consequence of ageing that could change the capacity to incorporate specific fatty acids with age or could alter membrane fatty-acid metabolism (Kumar *et al.*, 1999; Toth and Tchernof, 2000). Whatever the reason for the changes described, the first finding of the study concerns the different response to ageing of the three tissues studied. Liver was almost unaffected by ageing, but postmitotic heart and skeletal muscle underwent the greatest changes.

Several studies have investigated the effect of ageing on the electron-transport system in various tissues (Cooper *et al.*, 1992; Gabbita *et al.*, 1997; Miquel *et al.*, 1995; Sastre *et al.*, 1997); however, few have included more than one tissue. It is widely believed that ageing changes are relatively more prevalent and severe in the postmitotic cells. The results of the present study indicate variations in the pattern of age-associated changes in the content of electron-chain components and the function of one of these components according to tissue. Overall, postmitotic tissues, and skeletal muscle in particular, seem to be the most drastically affected by age, as previously reported (Kwong and Sohal, 2000). On the other hand, heart was affected by age, depending on the parameter, with CCO being the most sensitive. Liver was the most resistant to age. Variations in tissue response to age have been attributed to more than a single factor (Kwong and Sohal, 2000). Thus, it has been noted that oxidative phosphorylation ratios differ in rat liver, heart, and brain mitochondria (Cairns *et al.*, 1998). Moreover, there are different tissue-specific isozyme forms for complex I, II, and IV (Capaldi *et al.*, 1988).

With regard to the particular age-related effects on the different components of the mitochondrial-electron chain studied here, it should be mentioned that in heart and skeletal muscle CoQ, cytochrome, *b*, cytochrome $a + a_3$ (in skeletal muscle but not in heart) increased overall while CCO and complex IV turnover underwent a general decrease. One of the objectives of this study was to evaluate the role of dietary fat in relation to possible changes in mitochondrial composition and function during ageing. The results show that dietary fat differentially modulated the response of tissues during the ageing process. Thus, although liver slightly reduced the content of CoQ and cytochrome *b* in the olive-oil group, the opposite occurred in the sunflower-oil group, which also showed higher levels of cytochrome $a + a_3$, CCO and a greater complex IV turnover. These findings confirm the role of dietary fat as a modulator of the structure and function of electron-chain components. Similarly, higher levels of CoQ and cytochrome *b* in heart and skeletal muscle of the sunflower-oil group were found at 24 months. Some authors have suggested that higher levels of cytochrome *b* might be good indicators of ageing (Kipling, 2001). In the present study, the highest levels of cytochrome *b* were found at 24 months in rats fed on sunflower oil (for rats fed olive oil, only skeletal muscle showed a greater cytochrome *b* concentration, but to a lower extent than for the sunflower-oil group). Thus, from this viewpoint, we could hypothesize that olive oil leads to less aged and less damaged mitochondrial membrances. In this sense, it is noteworthy that the lipid-peroxidation degree found in these membranes (data not shown) directly correlated with this hypothesis, finding that the olive-oil diet led to lower peroxides levels in aged rats.

In conclusion, dietary fat and ageing differentially affect the composition and function of rat mitochondria, depending on the tissue type. The most important effects of age were found in the postmitotic tissues, heart and skeletal muscle, with an overall increase in CoQ and cytochrome *b* together with a decrease in the activity and turnover of complex IV. Dietary fat can determine the mitochondrial fatty-acid composition throughout the life of the rat, representing a potential way to modulate effects of ageing in mitochondria through diet.

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REFERENCES

Battino, M., Quiles, J. L., Huertas, J. R., Ramírez-Tortosa, M. C., Cassinello, M., Mañas, M., López-Frías, M., and Mataix, J. (2002). *J. Bioenerg. Biomembr.*

Battino, M., Rugolo, M., Romeo, G., and Lenaz, G. (1986). *FEBS Lett.* **199**, 155–158.

Cairns, C. B., Walther, J., Harken, A. H., and Banerjee, A. (1998). *Am. J. Physiol.* **274**, R1376–R1383.

Capaldi, R. A., Halphen, D. G., Zhang, Y.-Z., and Yanamura, W. (1988). *J. Bioenerg. Biomembr.* **20**, 291–311.

Charnock, J. S., McLennan, P. L., and Abeywardena, M. Y. (1992). *Mol. Cell. Biochem.* **116**, 19–25.

Cooper, J. M., Mann, V. M., and Schapira, A. H. V. (1992). *J. Neurol. Sci.* **113**, 91–98.

Degli Esposti, M., and Lenaz, G. (1982). *Biochim. Biophys. Acta* **682**, 189–200.

Feller, S. E., Gawrisch, K., and MacKerell, A. D. (2002). *J. Am. Chem. Soc.* **124**, 318–326

Fleishcher, S., McIntyre, I. O., and Vidal, J. C. (1979). *Methods Enzymol.* **55**, 32–39.

Gabbita, S. P., Butterfield, D. A., Hensley, K., Shaw, W., and Carney, J. M. (1997). *Free Radic Biol. Med.* **23**, 191–201.

Guarente, L., and Kenyon, C. (2000). *Nature* **408**, 255–262.

Huertas, J. R., Battino, M., Lenaz, G., and Mataix J. (1991a). *FEBS Lett.* **287**, 89–92.

Huertas, J. R., Battino, M., Mataix, F. J., and Lenaz, G. (1991b). *Biochem. Biophys. Res. Commun.* **181**, 375–382.

Kipling, D. (2001). *Maturitas* **38**, 25–38.

Kirwood, T. B. L., and Austad, S. N. (2000). *Nature* **408**, 233–238.

Kumar, V. B., Vyas, K., Buddhiraju, M., Alshaher, M., Flood, J. F., and Morley, J. E. (1999). *Life Sci.* **65**, 1657–1662.

Kwong, L. K., and Sohal, R. S. (2000). *Arch. Biochem. Biophys.* **373**, 16–22.

Lepage, G., and Roy, C. C. (1986). *J. Lipid Res.* **27**, 114–120.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. G. (1951). *J. Biol. Chem.* **193**, 265–275.

Lutz, M., Alvarado, J., Barraza, P., Bonilla, S., and Luna, L. (1999). *Lipids* **34**, S119.

Mataix, J., Quiles, J. L., Huertas, J. R., Battino, M., and Mañas, M. (1998). *Free Rad. Biol. Med.* **24**, 511–521.

Medvedev, J. A. (1990). *Biol. Rev.* **65**, 375–398.

Miquel, J. (1991). *Arch. Gerontol. Geriatr.* **12**, 99–117.

Miquel, J., Ecónomos, A. C., Fleming, J., and Johnson, J. E. (1980). *Exp. Gerontol.* **15**, 575–591.

Miquel, J., Ferrándiz, M. L., De Juan, E., Sevilla, I., and Martínes, M. (1995). *Eur. J. Pharmacol.* **292**, 333–335.

Nicholls, P. (1976). *Biochim. Biophys. Acta* **430**, 30–45.

Ojaimi, J., Masters, C. L., Opeskin, K., McKelvie, P., and Byrne, E. (1999). *Mech. Ageing Dev.* **111**, 39–47.

Quiles, J. L., Huertas, J. R., Mañas, M., Battino, M., and Mataix, J. (1999). *Br. J. Nutr.* **81**, 21–24.

Quiles, J. L., Huertas, J. R., Mañas, M., Ochoa, J. J., Battino, M., and Mataix, J. (2001). *J. Bionembr.* 33, 127–143.

Sastre, J., Pallardó, F. V., Plá, R., Pellon, A., Juan, G., O'Connor, J. E., Estrella, J. M., Miquel, J., and Viña, J. (1997). *Hepatology* **24**, 1199–1205.

Toth, M. J., and Tchernof, A. (2000). *Eur. J. Clin. Nutr.* **54**, S121–S125.

Vanneste, W. H. (1966). *Biochim. Biophys. Acta* **113**, 175–178.

Wallace, D. W. (1992). *Science* **256**, 628–632.

Yamaoka, S., Ride, R., and Kito, M. (1988). *J. Nutr.* **118**, 290–296.