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Dietary Fat Type and Regular Exercise Affect Mitochondrial Composition and Function Depending on Specific Tissue in the Rat

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Physical exercise and fatty acids have been studied in relation to mitochondrial composition and function in rat liver, heart, and skeletal muscle. Male rats were divided into two groups according to dietary fat type (virgin olive and sunflower oils). One-half of the animals from each group were subjected to a submaximal exercise for 8 weeks; the other half acted as sedentary controls. Coenzyme Q, cytochromes b, $c + c_1$, $a + a_3$ concentrations, and the activity of cytochrome c oxidase were determined. Regular exercise increased ($P < 0.05$) the concentration of the above-mentioned elements and the activity of the cytochrome c oxidase by roughly 50% in liver and skeletal muscle. In contrast, physical exercise decreased ($P < 0.05$) cytochrome c oxidase activity in the heart (in μmol/min/g, from 8.4 ± 0.1 to 4.9 ± 0.1 in virgin olive oil group and from 9.7 ± 0.1 to 6.7 ± 0.2 in sunflower oil animals). Dietary fat type raised the levels of coenzyme Q, cytochromes, and cytochrome c oxidase activity in skeletal muscle ($P < 0.05$) among the rats fed sunflower oil. In conclusion, dietary fat type, regular exercise, and the specific tissue modulate composition and function of rat mitochondria.

KEY WORDS: Virgin olive oil; sunflower oil; cytochrome *c* oxidase; cytochromes; coenzyme Q.

INTRODUCTION

To fulfill the energetic requirements of physical exercise, increased aerobic capacity and VO_{2max} of active muscle is needed (Robinson *et al.*, 1994). This is directly related with greater O_2 delivery (cardiac output), but also with the augmented ability of the muscle to utilize a greater fraction of the O_2 delivered (Saltin and Gollnick, 1983). Factors affecting the use of the O_2 delivered include an enriched capillary network, an increased myoglobin concentration of the myocyte, and changes at the mitochondrial level (Robinson *et al.*, 1994).

The role of mitochondria in relation to exercise has been studied in different tissues, such as skeletal muscle (Huertas *et al.*, 1992a; Robinson *et al.*, 1994), heart (Farrell *et al.*, 1991; Paulson *et al.*, 1987), and white adipose tissue (Stallknecht *et al.*, 1991), in fishes, rats and humans (Farrell *et al.*, 1991; Huertas *et al.*, 1992b; Kolok, 1992; Paulson *et al.*, 1987; Stallknecht *et al.*, 1991). Some of the effects found have been related to changes in the number, volume, and distribution of mitochondria (Papa, 1996; Stallknecht *et al.*, 1991), increased activity in certain enzymes of the tricarboxylic acid cycle, fatty acid transport, and in some of the respiratory-chain enzymes (Huertas *et al.*, 1992a). Moreover, it has been found that, at least in the rat, the growth in electron-transport capacity is a specific requisite to support the increase in the muscle VO_{2max} resulting from physical exertion (Robinson *et al.*, 1994).

Adaptations of electron transport system in relation to dietary fat type have been widely reported (Huertas *et al.*, 1991a; Innis and Clandinin, 1981; McMillin *et al.*, 1992; Yamaoka *et al.*, 1988, 1990). The importance of fatty acids resides in the finding that mitochondrial membranes adapt their lipid composition to dietary fat

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(Charnock *et al.*, 1992; Giron *et al.*, 1992; Periago *et al.*, 1988; Quiles *et al.*, 1999). In this sense, Yamaoka *et al.* (1988) have reported that fatty acid composition of heart mitochondria in rats drastically changed after sardine oil ingestion. These mitochondria showed diminished respiratory function due to a lower cytochrome *c* oxidase (CCO) activity. Similarly, we previously reported changes in liver mitochondrial CCO after treatment with adriamycin in rats fed a diet based on olive or corn oil (Huertas *et al.*, 1991b). Furthermore, it has been reported how other fatty acids alter mitochondrial function in different organisms and tissues (Barzanti *et al.*, 1994; McMillin *et al.*, 1992; Stillwell *et al.*, 1997). In addition, an interaction between fatty acids and genes has recently been credited with determining mitochondrial function (Kim and Berdanier, 1998).

In light of these previous studies, we studied the interactions between regular exercise (training) and dietary fat type in relation to mitochondrial composition and function in rats. In addition, we compared the responses of liver, heart, and skeletal muscle, for three reasons. First, these tissues serve different roles during exercise, i.e., the active skeletal muscle is directly related to locomotive functions, the heart controls cardiac output (related to O_2 deliver), and the liver increases many of its metabolic functions. Second, recent studies in our laboratory demonstrate that physical exercise may influence the way in which fatty acid composition of mitochondrial membranes in different organs adapt to dietary fat (Quiles *et al.*, 1999). Finally, little information is available concerning the events in tissues other than the skeletal muscle during physical exercise. The rationale of the use of the two different oils differing in their unsaturation degree (virgin olive oil, rich in monounsaturated fatty acids and sunflower oil, rich in polyunsaturated fatty acids) is based on evidences suggesting that changes in the fat of the diet alter mitochondrial membrane properties. Moreover, these oils are two of the most frequently used fat sources in Europe and additional studies are needed to clarify some of the beneficial properties attributed to the olive oil in the so-called Mediterranean Diet.

MATERIALS AND METHODS

Experimental Protocol

Male Wistar rats, initially weighing 80 to 90 g, grouped 10 per cage, were maintained on a 12 h light/12 h darkness cycle, with free access to food and water. The study lasted 9 weeks (the first for animal selection followed by 8 experimental weeks). During the selection week, all rats were fed a unpurified diet and subjected to daily treadmill exercise at a speed of 15 m/min, for 15 min. Only the rats able to run on the treadmill without requiring any external stimuli were chosen at the end of this selection period (about 35% of the initial animals).

Table I. Fatty Acid Composition of the Experimental Diets

Fatty acid composition	Virgin olive oil (%)	Sunflower oil (%)
16:0	11.32	7.19
16:1 (*n*-9)	0.11	0.02
16:1 (*n*-7)	0.84	0.19
18:0	4.34	4.51
18:1 (*n*-9)	74.12	32.08
18:2 (*n*-6)	7.64	54.26
18:3 (*n*-3)	0.61	0.10
Total saturated	16.02	12.93
Total unsaturated	83.98	87.07
Total monounsaturated	75.06	32.29
Total polyunsaturated	8.92	54.78

The selected rats were randomly assigned to four groups and fed semisynthetic and isoenergetic diets composed of (in g/kg of diet): 267 casein, 135.3 starch, 453 sucrose, 80 edible oil, 37 mineral supplement, 10 vitamin supplement, 1.8 cellulose, 0.9 choline, 3 methionine. One half of the rats received virgin olive oil (V) and half sunflower oil (S) as the dietary lipids (Table I). Each dietary group was further divided into sedentary (no exercise) and exercised (as described below). Thus, the groups were as follow: VS, virgin olive oil sedentary animals; VE, virgin olive oil exercised animals; SS, sunflower oil sedentary animals, and SE, sunflower oil exercised animals.

The exercised animals (VE and SE) underwent regular sessions of exercise on a horizontal treadmill throughout the 8 experimental weeks: the first 2 weeks, the rats were exercised 5 days/week, once per day at a steadily increasing rate until running 40 min/days at a 35 m/min speed. These conditions, equivalent to 65 to 70% of the VO_{2max} (Amstrong *et al.*, 1983) were maintained over the remaining 6 weeks. Average intake for each group was monitored daily. The Ethical Committee of the Spanish Interministerial Commission of Science and Technology approved the different protocols used in this experiment.

Sample Analysis

Before decapitation, the rats were weighed. Liver, heart, and skeletal muscle (*vastus lateralis*) were removed, carefully weighed, and their mitochondria isolated according to Fleischer *et al.* (1979). Blood was collected

into EDTA-coated tubes, plasma was separated by centrifugation at $1750 \times g$ for 10 min and plasma levels of triglycerides were measured by enzymic colorimetric methods using commercial kits (Boehringer Mannheim, Germany).

The concentration of mitochondrial cytochrome $c + c_1$, 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). Exactly 200 μl of sodium deoxycholate 10% (w/v) plus the sample (the equivalent volume to 2 mg of mitochondrial protein), together with KH_2PO_4 7 mM until a final volume of 1.7 ml, were gently mixed in a spectrophotometer cuvette. After 10 μl of 20 mM potassium fericyanide were added to the mix, to allow the total oxidation of the cytochromes, the oxidized spectra between 650 and 500 nm was recorded. Afterward, 20 μg of sodium dithionite were added to reduce the cytochromes completely, acquiring again the spectra between 650 and 500 nm. The differential spectra (reduced minus oxidized) were recorded to calculate the concentration of cytochromes, using the following extinction coefficients: $\varepsilon_{c+c1}(A_{550}-A_{540}) = 20 \quad \text{mM}^{-1} \quad \text{cm}^{-1}$; $\varepsilon_b(A_{561}-A_{575}) = 25\ \text{mM}^{-1}\ \text{cm}^{-1}$; $\varepsilon_{a+a3}(A_{605}-A_{630}) = 24\ \text{mM}^{-1}\ \text{cm}^{-1}$.

Cytochrome *c* oxidase activity was assayed at 25°C using cytochrome *c* (90 μM; in 10 mM Tris) reduced by sodium dithionite. After reduction, cytochrome *c* was purified in a Sephadex G-25 column (Battino *et al.*, 1986; Degli Esposti *et al.* and Lenaz, 1982), so that the ratio between the extinction at 500 and 565 nm was between 8 and 10. Cytochrome *c* was then mixed with 10 mM Tris, 50 mM KCl, 1 mM EDTA, and added with 0.3 mg/ml of antimicin A. Samples were poured into the cuvette and mixed, monitoring the absorbance decrease of cytochrome *c* upon oxidation at 417–409 nm every 10 s for 2 min; the extinction coefficient used for cytochrome *c* was 40.7 $\text{mM}^{-1}\ \text{cm}^{-1}$ (Battino *et al.*, 1986).

After extraction with methanol and light petroleum, according to the method of Lang and Packer (1987), mitochondrial coenzyme Q (CoQ) homologs (coenzyme Q_9 and coenzyme Q_{10}, that are present in the rat in an approximate ratio of 10:1) were determined by reversed-phase HPLC using a Spherisorb S5 ODS1 (Merck, Darmnstadt, Germany) column and ethanol:purified water 97:3 (v/v) as mobile phase. The HPLC system was a Beckman in-line Diode Array Detector; model 168 (Fullerton, CA) connected to a Water (Milford, MA) 717 plus autosampler. Determinations were done at 275 nm. Coenzyme Q homologs were identified by predetermining the retention times of individual standards and the sums of both homologs were expressed as total CoQ. In order to assay total coenzyme Q as oxidized CoQ, samples were treated with 1,4-benzoquinone (2 mg/ml); CoQ was then assayed. Thus, the results showed here are the total amount of coenzyme Q. 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.

The fatty acid profile of mitochondrial membranes was measured by gas liquid chromatography as described by Lepage and Roy (1986). Briefly, 50 μl of sample were precisely weighed in glass tubes and dissolved in 2 ml of methanol/benzene (4:1, v/v); 9 μM of BHT were added to the samples as antioxidant. After 200 μl of acetyl chloride were slowly added, the tubes were closed and subjected to methanolysis at 100°C for 1 h. After tubes were cooled in water, 5 ml of 0.43 MK_2CO_3 solution was slowly added to stop the reaction and neutralize the mixture. The tubes were then shaken and centrifuged. The benzene upper phase was removed and transferred to another glass tube to be dried under nitrogen and resuspended to 100 μl with hexane. 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™ FS (Supelco Inc. Bellefonte, Palo Alto, CA). The injector and the detector were each maintained at 275°C; nitrogen was used as carrier gas and the split ratio was 29:1. Temperature programming was as follows: initial temperature, 80°C, 15°C/min to 165°C, 3°C/min to 211°C, hold 10 min.

All chemical products and solvents, of the highest quality available, were acquired from Sigma (St. Louis, MO) and Merck (Darmstadt, Germany). The homolog of coenzyme Q (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 6 (VS) or 8 (VE, SS, and SE) animals. Except for the lipid profile (expressed in terms of percentage), results are referred per gram of wet tissue. A two-way ANOVA was used to analyze effects of dietary fat and physical activity on each variable. Effects were considered significant at $P < 0.05$. Prior to any statistical analysis, all variables were checked for normal and homogeneous variance using the Levene test.

RESULTS

Food Intake, Rat Weight, and Mitochondrial-Protein Content

Dietary intake did not vary significantly among groups and at the end of the experiments the weight of the liver, heart, and skeletal muscle were not affected by experimental treatments (data not shown). Body weight was similar for all the groups at the beginning of the study, but at the end of the experimental time, sedentary animals had a significantly higher weight than rats subjected to physical training (359.5 ± 7.2 and 318.1 ± 9.1, respectively, $P < 0.05$). According to the two-way ANOVA analysis, the weight-gain effect was a consequence of physical exercise.

Mitochondrial–protein content (Fig. 1) in the liver was higher for all the exercised groups, with no differences between dietary treatments, either for sedentary or for exercised groups. Regarding the heart, exercise increased mitochondrial protein in the olive oil group, but had no effect on the sunflower groups. The sedentary groups registered no differences between dietary treatments. For skeletal muscle, the rats fed sunflower oil had higher mitochondrial–protein content than did those fed virgin olive oil, both at rest and after exercise. Two-way ANOVA analysis confirmed that diet had no effect on liver and heart mitochondrial protein, while exercise affected liver and skeletal muscle values.

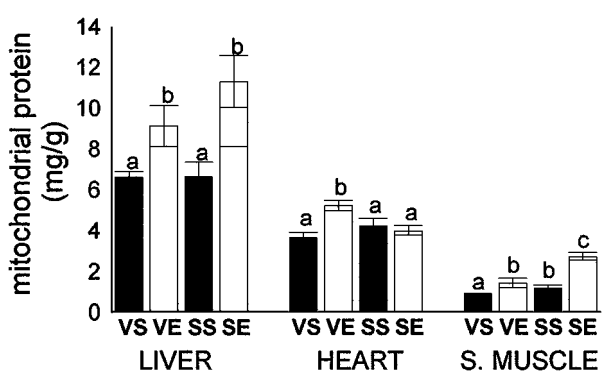

Fig. 1. Effects of physical exercise and dietary fat on mitochondrial protein content in liver, heart, and skeletal muscle mitochondrial membranes of rats. Group abbreviations: VS, virgin olive oil-fed and sedentary rats; VE, virgin olive oil-fed and exercised rats; SS, sunflower oil-fed and sedentary rats; SE, sunflower oil-fed and exercised rats. Values are means ± S.E. For each tissue, values labeled with different letters are significantly different ($P < 0.05$). A two-way ANOVA analysis was performed for fat (F), exercise (E), and the interaction between fat and exercise (F × E) effects. Significant effects ($P < 0.05$) are noted by S and nonsignificant ones by NS. For liver: F, NS; E, S; F × E, NS. For heart: F, NS; E, NS; F × E, S. For skeletal muscle: F, S; E, S; F × E, NS.

Mitochondrial–Lipid Profiles

Figure 2 shows the proportions of the major lipid groups from the mitochondrial membranes. For the saturated fraction (Fig. 2A), neither dietary fat nor physical exercise altered the relative proportions of these fatty acids for any of the groups studied. For both dietary treatments, the levels of saturated fatty acids were similar. In terms of monounsaturated fatty acids (MUFA; Fig. 2B), rats fed on virgin olive oil had higher levels than those fed on sunflower oil. On the other hand, regular exercise decreased the MUFA levels in the liver. For the polyunsaturated fatty acids (*n*-6) (PUFAn-6) (Fig. 2C), sunflower oil-fed rats had higher levels than did those fed virgin olive oil, and the levels of PUFAn-6 increased in the liver of all the exercised animals compared with sedentary controls.

Coenzyme Q and Cytochromes Composition

Coenzyme Q (Fig. 3) in the liver was affected by physical exercise but not by dietary fat. All exercised animals registered higher values than did sedentary animals. Diet and the interaction between diet and regular exercise affected CoQ levels in the heart, this effect being similar in sedentary and exercised animals. In skeletal muscle, physical exercise increased CoQ levels with both diets, and the sunflower-oil group had higher values of CoQ than did the virgin-olive oil groups for each state of activity. Diet, exercise, and diet plus exercise influenced muscle CoQ.

With respect to cytochrome *b* (Fig. 4A), in liver, exercised groups had higher concentrations than did the sedentary groups. No differences appeared between dietary treatments and only exercise was attributable to the changes found. In the heart, no changes were found between sedentary and exercised rats; dietary treatment led to differences between exercised animals, with higher amounts of cytochrome *b* for the sunflower group. In skeletal muscle, diet, exercise, and the interaction between both factors changed the cytochrome *b* concentration. Exercise and sunflower oil resulted in higher levels than did olive oil and the sedentary condition.

Figure 4B shows the concentrations of cytochromes $c + c_1$. In liver, regular exercise led to increased values. The rats fed virgin olive oil had higher amounts than did the rats fed sunflower oil, both for sedentary and exercised groups. Both factors (dietary fat type and regular exercise) affected the cytochrome $c + c_1$ content, according to the two-way ANOVA. In heart, no differences were found between the four experimental groups. In skeletal muscle, physical exercise had no effect. Sunflower oil led to higher amounts of cytochromes $c + c_1$ both in sedentary and exercised animals.

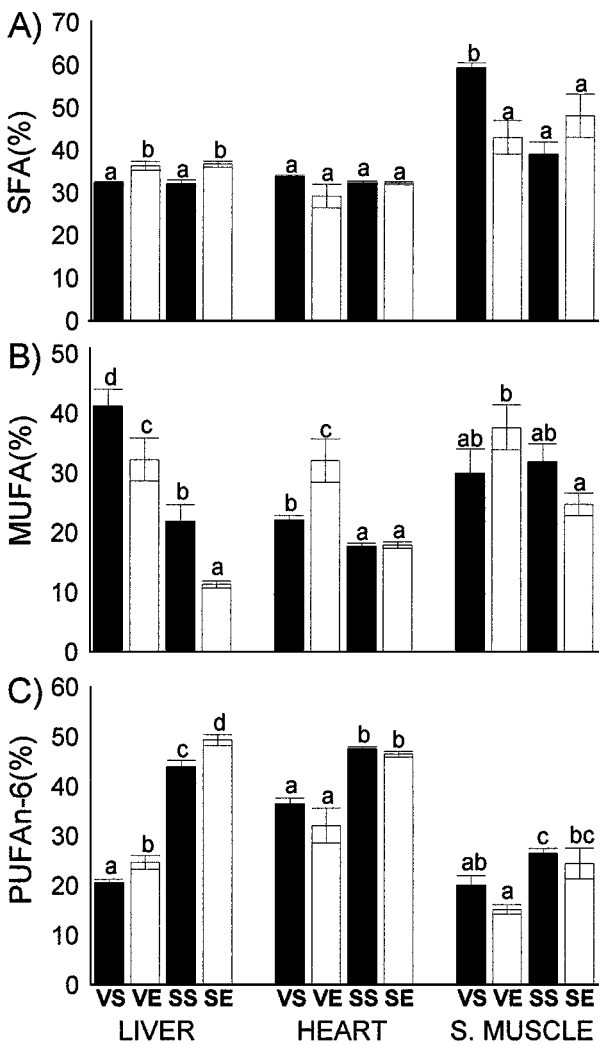

Fig. 2. Effects of physical exercise and dietary fat on (A) saturated fatty acid (SFA), (B) monounsaturated fatty acid (MUFA), and (C) polyunsaturated *n*-6 fatty acid (PUFAn-6) profiles in mitochondrial membranes of liver, heart, and skeletal muscle of rats. Group abbreviations: VS, virgin olive oil-fed and sedentary rats; VE, virgin olive oil-fed and exercised rats; SS, sunflower oil-fed and sedentary rats; SE, sunflower oil-fed and exercised rats. Values are means ± S.E. For each lipid fraction and tissue, values labeled with different letters are significantly different ($P < 0.05$). A two-way ANOVA analysis was performed for fat (F), exercise (E), and the interaction between fat and exercise (F × E) effects. Significant effects ($P < 0.05$) are noted by S and nonsignificant ones by NS. For SFA, in liver: F, NS; E, NS; F × E, NS. In heart: F, NS; E, NS; F × E, NS. In skeletal muscle: F, S; E, S; F × E, NS. For MUFA, in liver: F, S; E, S; F × E, NS. In heart: F, S; E, S; F × E, NS. In skeletal muscle: F, S; E, S; F × E, NS. For PUFAn-6, in liver: F, S; E, S; F × E, NS. In heart: F, S; E, NS; F × E, NS. In skeletal muscle: F, S; E, NS; F × E, S.

Cytochromes $a + a_3$ concentrations are shown in Fig. 4C. In liver, exercised animals showed higher amounts than did sedentary groups, for both experimental diets. No differences appeared between dietary treatments. Physical

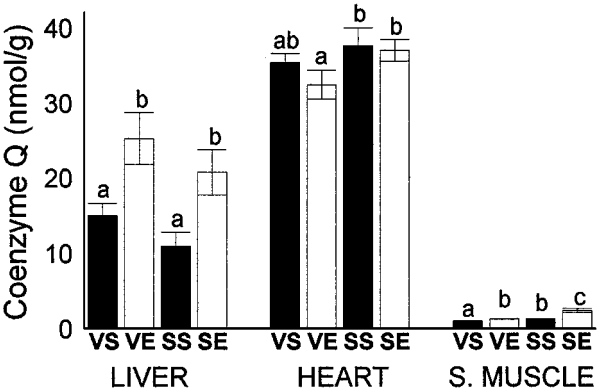

Fig. 3. Effects of physical exercise and dietary fat on coenzyme Q (sum of coenzyme Q_9 plus coenzyme Q_{10}) concentration in liver, heart, and skeletal muscle mitochondrial membranes of rats. Group abbreviations: VS, virgin olive oil-fed and sedentary rats; VE, virgin olive oil-fed and exercised rats; SS, sunflower oil-fed and sedentary rats; SE, sunflower oil-fed and exercised rats. Values are means ± S.E. For each tissue, values labeled with different letters are significantly different ($P < 0.05$). A two-way ANOVA analysis was performed for fat (F), exercise (E), and the interaction between fat and exercise (F × E) effects. Significant effects ($P < 0.05$) are noted by S and nonsignificant ones by NS. For liver: F, NS; E, S; F × E, NS. For heart: F, S; E, NS; F × E, S. For skeletal muscle: F, S; E, S; F × E, S.

exercise, but not dietary fat type, affected liver levels of cytochromes $a + a_3$. In heart, no differences were found between the four experimental groups. In muscle, diet, exercise, and their interactions affected cytochromes $a + a_3$ levels. Exercised animals had higher amounts than did sedentary groups, but, at the same time, for a similar activity, sunflower oil led to higher values than did virgin olive oil.

Cytochrome *c* Oxidase (CCO) Activity

For CCO activity (Fig. 5) in liver, physical exercise increased the activity of this enzyme with both dietary treatments. No differences were found between diets and physical exercise was the only factor responsible for changes in CCO activity in liver. In heart, there was decreased in activity after physical exercise with both dietary treatments. No differences were found to be due to diet. For skeletal muscle, diet, exercise, and diet plus exercise interaction affected CCO activity. The highest values were found in exercised animals fed sunflower oil, whereas the lowest concentration belonged to sedentary animals fed virgin olive oil.

DISCUSSION

The present experiment was designed to ascertain the interactions between regular exercise and dietary fat type

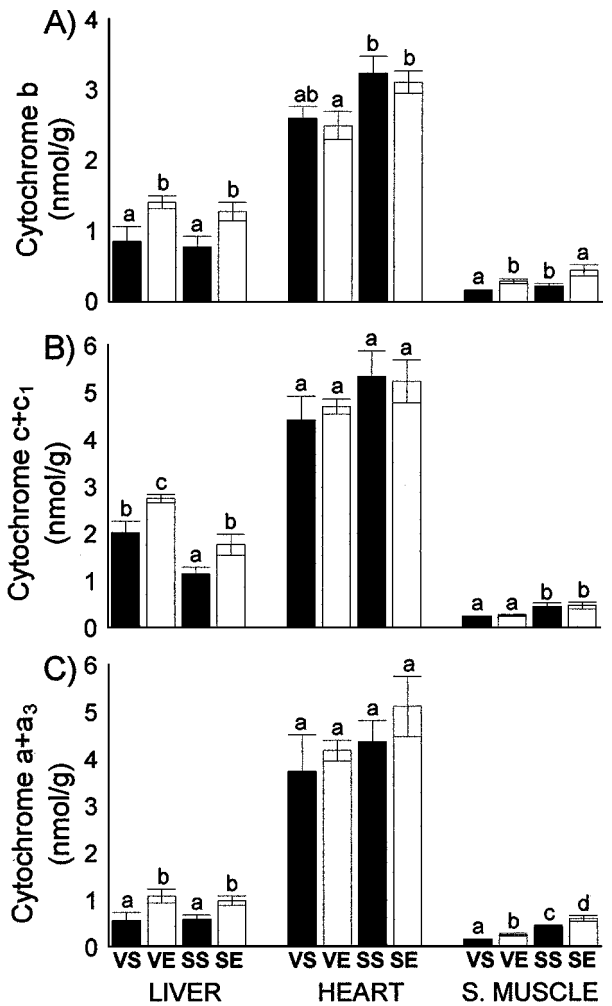

Fig. 4. Effects of physical exercise and dietary fat on (A) cytochrome b, (B) cytochrome $c + c_1$, and (C) cytochrome $a + a_3$ concentrations in mitochondrial membranes of liver, heart, and skeletal muscle of rats. Group abbreviations: VS, virgin olive oil-fed and sedentary rats; VE, virgin olive oil-fed and exercised rats; SS, sunflower oil-fed and sedentary rats; SE, sunflower oil-fed and exercised rats. Values are means ± S.E. For each cytochrome and tissue, values labeled with different letters are significantly different ($P < 0.05$). **A** two-way ANOVA analysis was performed for fat (F), exercise (E), and the interaction between fat and exercise (F × E) effects. Significant effects ($P < 0.05$) are noted by S and nonsignificant ones by NS. For cytochrome *b*, in liver: F, NS; E, S; F × E, NS. In heart: F, S; E, NS; F × E, S. In skeletal muscle: F, S; E, S; F × E, S. For cytochromes $c + c_1$, in liver: F, S; E, S; F × E, S. In heart: F, NS; E, NS; F × E, NS. In skeletal muscle: F, S; E, NS; F × E, NS. For cytochromes $a + a_3$, in liver: F, NS; E, S; F × E, NS. In heart: F, NS; E, NS; F × E, S. In skeletal muscle: F, S; E, S; F × E, S.

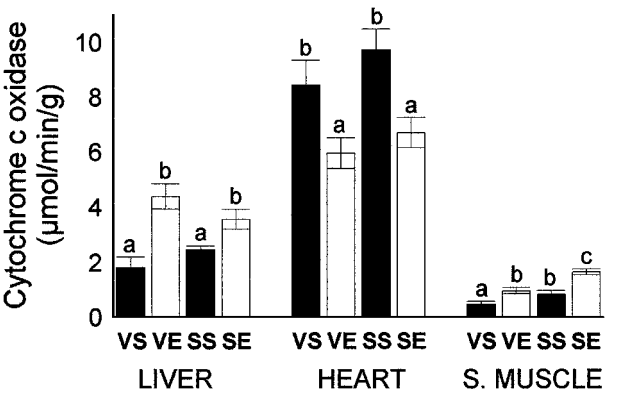

Fig. 5. Effects of physical exercise and dietary fat on cytochrome *c* oxidase activity in liver, heart, and skeletal muscle mitochondrial membranes of rats. Group abbreviations: VS, virgin olive oil-fed and sedentary rats; VE, virgin olive oil-fed and exercised rats; SS, sunflower oil-fed and sedentary rats; SE, sunflower oil-fed and exercised rats. Values are means ± S.E. For each tissue, values labeled with different letters are significantly different ($P < 0.05$). A two-way ANOVA analysis was performed for fat (F), exercise (E), and the interaction between fat and exercise (F × E) effects. Significant effects ($P < 0.05$) are noted by S and nonsignificant ones by NS. For liver: F, NS; E, S; F × E, NS. For heart: F, NS; E, S; F × E, NS. For skeletal muscle: F, S; E, S; F × E, S.

in relation to mitochondrial composition and function in different tissues of rats.

Physical exercise is known to change the number of muscle mitochondria and the levels of some of the elements involved in the production of energy, thus implying changes in the structure of the mitochondrial membrane (Gollnick and Saltin, 1982; Holloszy and Coyle, 1984). The molecular mechanism responsible for these events is related to increased energetic demands determined by the intensity and duration of the exercise (Robinson *et al.*, 1994). Moreover, mtDNA is likely to be involved because it codes for several polypeptides of the complexes I, III, and IV (Huertas *et al.*, 1992b). In addition, dietary fat induces changes in the mitochondrial membranes (Barzanti *et al.*, 1994; Huertas *et al.*, 1991a) In the present study, we found that regular exercise selectively increased mitochondrial components and functionality only in some tissues and that such changes were also selectively altered according to the dietary fat type and tissue.

Regarding the adaptation to the diet, the rats adapted the lipid profile of their mitochondrial membranes to the fat type. The saturated fraction was similar for all the groups, which agrees with the low rate of mobilization and adaptation to dietary changes of this fraction reported elsewhere (Charnock *et al.*, 1992). Rats fed virgin olive oil had a higher proportion of MUFA than those found for this fraction among animals fed sunflower oil. On the contrary, the highest PUEAn-6 concentration was found in rats fed sunflower oil.

The adaptation to the exercise program appears to be satisfactory. The results on body weight show that all exercised animals had a lower weight gain. Moreover, sedentary animals showed significantly higher

plasma triglyceride levels ($190.49 \pm 67.75\,\mu$ mol/ml for VS group and $348.56 \pm 51.55\,\mu$ mol/ml for SS group) than in exercised rats ($27.78 \pm 17.39\,\mu$ mol/ml for VE and 36.22 ± 14.01 mol/ml for SE). This also suggests good adaptation to exercise. Similarly, increases in the mitochondrial–protein content (Fig. 1) of the exercised animals agree with these results.

With respect to the changes found in the electron transport chain, the results suggest a dependency on the diet, the physical activity, and the studied tissue. For liver, there were no differences for the two diets with respect to the concentrations of CoQ, the cytochromes b, $c + c_1$and $a + a_3$ and for the activity of the enzyme CCO. However, all these elements and enzymic activity increased approximately twofold in the exercise groups. The response of the heart mitochondria to dietary treatment and exercise was practically unappreciable for CoQ and for the different cytochromes. This lack of responsiveness to the exercise is not surprising, since other studies employing treadmill running as a mode of exercise have found no effect on cardiac performance (Paulson *et al.*, 1987). Nevertheless, the activity of the heart CCO surprisingly decreased in all the exercised animals, despite a similar cytochrome $a + a_3$ content. For muscle, dietary fat affected all cytochromes, CoQ, and CCO. In all the cases, rats fed sunflower oil showed higher levels than those fed olive oil. Physical exercise raised the levels of these elements, except in the case of cytochromes $c + c_1$, which did not change.

Changes have been previously reported in muscle mitochondrial oxidative elements after physical exercise and the modulation by dietary fat types (see above) and we found here that both parameters interact according to the tissue. However, the following question may arise: why do different tissues have such different responses to dietary fat type and regular exercise?

Yamaoka *et al.* (1988, 1990) and Huertas *et al.* (1991b) previously reported that polyunsaturated fat increased the activity of CCO, finding the maximal activity when at least a 75% of the cardiolipin in the mitochondria was 18:2 (*n*-6)/18:2 (*n*-6), as theoretically can happen in the present study only for sunflower oil fed animals (Periago *et al.*, 1988; Yamaoka *et al.*, 1990). Here, only in the muscle was there an apparently direct effect derived from the differences in the type of fat. The animals fed sunflower oil reached the highest CCO activity, supporting the hypothesis of Yamaoka *et al.* (1988, 1990) and Huertas *et al.* (1991b) concerning the importance of 18:2 *n*-6 based cardiolipin for CCO activity. The lack of effect of dietary fat on heart mitochondrial CCO activity is normal, given the poor level of adaptation of this organ to the dietary treatment. Despite the high level of adaptation of liver mitochondria to dietary fat and the subsequent high level of PUFA*n*-6 in sedentary and exercised animals fed on sunflower oil, it was not possible to find an increased CCO activity in these animals compared with those fed virgin olive oil; thus no explanation for the response of the liver is possible with the present data.

Regarding the different effects of dietary fat type depending on the tissue, it should be borne in mind that correlations between changes in fatty acid composition and variations in enzyme activity are difficult. This is because tissues can accommodate cell membrane composition to the changing status and according to the many possible interactions between the lipid moieties and associated proteins. Thus, although varying dietary-lipid composition can alter membrane physicochemical properties through changes in *n*-6:*n*-3 PUFA, unsaturated indexes, and other compositional features (Stubbs and Smith, 1984), animals could slightly preserve such properties regardless of the lipid changes (Royce and Holmes, 1984).

Only a few of the many exercise-induced adaptive changes in biological parameters have been fully mechanistically explained and most of these changes can readily be considered useful for physical activity. In skeletal muscle, the increase in oxidative elements after exercise is assumed to be a local effect directly caused by the contractile activity (Henriksson *et al.*, 1982; Stallknecht *et al.*, 1991). However, other tissues that increased working load during exercise (i.e., heart) did not show such adaptation. On the other hand, the liver, which is not directly involved in exercise, increased its mitochondrial oxidative elements. This fact is noteworthy because liver is subjected to a substantial decrease in the oxygen supply during exercise (Astrand and Rodahl, 1986); the increase in its mitochondrial elements is directly related neither to the oxygen increase nor to the contractile activity, as it has been demonstrated for muscle (Henriksson *et al.*, 1982; Asson-Batres and Hare, 1991). The increase in the mitochondrial elements may be related to a greater nutrient mobilization and to a generally accelerated metabolic function of the liver as a consequence of exercise. In this sense, in the white adipose tissue of exercised rats, the activity of mitochondrial enzymes accelerates, probably to satisfy greater energy demands for the lipid mobilization, simultaneously boosting the potential for a fast replenishment of triglycerides stores between exercise bouts (Stallknecht *et al.*, 1991).

Decreases in CCO in heart mitochondria after exercise have not been previously reported. Evidence has emerged describing an opposite response between heart and other organs for CCO. For example, Kolok (1992), studying the changes in mitochondrial composition and functionality in largemouth bass between summer and winter, found that while CCO of liver and skeletal muscle diminished during the summer, the activity of this enzyme

rose over the same period in heart mitochondria; no explanation was provided for such behavior. In addition, we found no changes in the other parameters of the electron transport chain in heart, in accord with other studies showing no effect on cardiac performance by the use of treadmill running as a mode of exercise (Nutter *et al.*, 1981; Paulson *et al.*, 1987). We also found that the heart is poorly adapted to changes in physical activity and dietary fat (Mataix *et al.*, 1998; Quiles *et al.*, 1999).

From an integrative standpoint, it is worth noting the differences between the various tissues in response to physical exercise and dietary treatment, with regard to the content of CoQ, cytochromes, and, in particular, the CCO activity. In this sense, the evidence for CCO isoforms has prompted work on tissue specificity and developmental regulation of the enzyme (Kuhn-Nentwig and Kandebach, 1985; Capaldi, 1990) and could offer some clues for the changes found in the present work.

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