

Short communication

Physical exercise affects the lipid profile of mitochondrial membranes in rats fed with virgin olive oil or sunflower oil

José L. Quiles^{1*}, Jesús R. Huertas¹, Mariano Mañas¹, Maurizio Battino² and José Mataix¹

¹*Institute of Nutrition and Food Technology (INYTA) and Department of Physiology, University of Granada, Spain*

²*Institute of Biochemistry, Faculty of Medicine, University of Ancona, 60131 Ancona, Italy*

(Received 9 February 1998 – Revised 13 July 1998 – Accepted 13 July 1998)

The effects of physical exercise on the lipid profile in mitochondrial membranes of liver and skeletal muscle were examined in rats fed with virgin olive oil or sunflower oil. Thirty male Wistar rats, 21 d old, were randomly assigned to four groups according to fat ingestion and physical activity over an 8-week period. For each type of oil, one group acted as a control group while rats from the other were trained to run for 40 min daily on a horizontal treadmill, at a speed of 35 m/min. The results show that diet affected the fatty acid profile of the mitochondrial membranes from skeletal muscle and liver. Physical exercise also modified the fatty acid profile of the mitochondrial membranes. Total monounsaturated fatty acids decreased ($P < 0.001$) in liver mitochondria of exercised animals. Total polyunsaturated fatty acids in mitochondrial membranes of liver increased ($P < 0.005$) after exercise but those in mitochondrial membranes of skeletal muscle decreased ($P < 0.05$). These changes due to the exercise may arise via several mechanisms, e.g. fluidity regulation; changes in the eicosanoid metabolism; differences in the availability or oxidation rate of the different fatty acids.

Liver: Skeletal muscle: Dietary fat: Exercise

Diet and exercise are both known determinants of good health. Many studies both in human subjects (Periago *et al.* 1990) and in animals (Innis & Clandinin, 1981; Suarez *et al.* 1996) have demonstrated that the fatty acid composition of plasma and different cellular membranes depends, partly, on the lipid composition of the diet. Physical activity, especially when combined with an appropriate diet, is also considered to be essential in maintaining good health. Many aspects of metabolism respond to exercise and several of these are also known to be modified by diet (Salting & Astrand, 1993). Few data are available, however, on the interaction between dietary lipids and physical training. In particular, there is a paucity of information on changes in mitochondrial membrane composition, an important factor in cellular aerobic metabolism.

The purpose of the present study was to test the effects of two different dietary fats plus physical training over an 8-week period on the lipid mitochondrial membrane patterns of rat liver and skeletal muscle.

Materials and methods

Experimental protocol

Thirty male Wistar rats, initially weighing 80–90 g, were allocated to four groups (three groups of eight and one group of six per cage) and maintained on a 12 h light–12 h dark cycle, with free access to food and drinking water. The study lasted 9 weeks (the first for animal selection followed by eight experimental weeks). During the selection week, all rats were fed on a non-purified diet and subjected to daily sessions on an exercise treadmill at a speed of 15 m/min, for 15 min.

The rats were then randomly assigned to the four groups and fed on semipurified and isoenergetic diets composed of (g/kg diet): casein 267, starch 135.3, sucrose 453, edible oil 80, mineral supplement 37, vitamin supplement 10, cellulose 1.8, choline 0.9, methionine 3. Half the rats received virgin olive oil and half sunflower oil as the dietary lipids (for the composition of the oils, see Mataix *et al.* 1998).

Abbreviations: MUFA, monounsaturated fatty acids; OOE, olive oil-fed, exercised animals; OOS, olive oil-fed, sedentary animals; PUFA, polyunsaturated fatty acids; SOE, sunflower oil-fed, exercised animals; SOS, sunflower oil-fed, sedentary animals.

* **Corresponding author:** Dr José Quiles, present address, Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK, fax +44 (0)1224 716687, email j1q@rri.sari.ac.uk

Each dietary group was further divided; sedentary (no exercise) and exercised (as described later). Thus, the groups were as follows: OOS, olive oil-fed, sedentary animals; OOE, olive oil-fed, exercised animals; SOS, sunflower oil-fed, sedentary animals and SOE, sunflower oil-fed, exercised animals.

The exercised animals (OOE and SOE) underwent training sessions on a horizontal treadmill throughout the 8 weeks: for the first 2 weeks the rats were exercised 5 d/week, once daily at a steadily increased rate, until running 40 min/d at a speed of 35 m/min. These conditions, equivalent to 65–70% of the maximum O₂ uptake were maintained during the remaining 6 weeks. Food intakes for each group were monitored daily. The protocols were approved by the Ethical Committee of the Interministerial Commission of Science and Technology.

Sample analysis

The rats were killed by decapitation and the mitochondria of the liver and skeletal muscle (*vastus lateralis*) were isolated as previously described (Mataix *et al.* 1998). The relative composition of mitochondrial fatty acids was determined by the method of Lepage & Roy (1986). A gas–liquid chromatograph Model HP-5890 Series II (Hewlett Packard, Palo Alto, CA, USA) equipped with a flame ionization detector was used to analyse fatty acids as methyl esters. Chromatography was performed using a 60 m long capillary column, 32 mm i.d. and 20 mm thickness impregnated with SpTM 2330 FS (Supelco Inc. Bellefonte, Palo Alto, CA, USA). The injector and the detector were both maintained at 275°C; N₂ 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.

Statistical analysis

The results are means with their the standard errors for six (OOS) or eight (OOE, SOS and SOE) animals. A two-way ANOVA was performed for effects of dietary fat and physical activity on each fatty acid. Significant effects were considered for $P < 0.001$, $P < 0.005$ and $P < 0.05$. Before any statistical analysis, all variables were checked for normal and homogeneous variance using the Levene test (Snedecor & Cochran, 1980). When a variable was found not to have a normal distribution it was log-transformed and reanalysed.

RESULTS AND DISCUSSION

Body weights were similar for all the groups at the beginning of the study (day 1), but the sedentary animals reached 371 (SE 8.2) g and the exercised rats 307 (SE 21.5) g at the end of the study (day 56; $P < 0.001$). Dietary intake did not vary significantly among groups. The lipid profiles of the mitochondrial membranes of liver and skeletal muscle are shown in Tables 1 and 2 respectively (ANOVA analyses are only shown for main effects of dietary fat and physical exercise; no effect was observed for the interaction between fat and exercise).

Saturated fatty acids

Dietary treatment had no effect on this fraction in the liver and only altered 18:0 values ($P < 0.05$) in skeletal muscle. This lack of modification by diet in the proportion of saturated fatty acids is in accordance with the findings of Suarez *et al.* (1996).

The training programme enhanced the proportions of 18:0 ($P < 0.001$) and total saturated fatty acids ($P < 0.005$) in

Table 1. Fatty acid profile (mol/100 mol) of liver mitochondrial membranes in rats subjected to physical exercise and fed on diets containing virgin olive oil or sunflower oil†

(Values are means for six (OOS) or eight (OOE, SOS, SOE) animals, with their pooled standard errors. Residual degrees of freedom were 26)

Fatty acid	Experimental groups				SE	Statistical significance of effect of: (two-way ANOVA)	
	OOS	OOE	SOS	SOE		Fat	Exercise
16:0‡	17.49	17.69	17.12	15.94	0.71	NS	NS
18:0	12.85	15.42	12.89	18.11	0.58	NS	***
ΣSaturated‡	32.35	36.39	32.05	36.91	0.82	NS	**
16:1n-7	4.32	2.45	2.71	0.88	0.30	***	***
18:1n-7	9.82	5.52	6.03	3.65	0.77	*	*
18:1n-9	26.06	23.66	9.82	6.46	1.71	***	*
ΣMUFA	41.19	32.35	21.92	11.41	2.39	***	***
18:2n-6	4.73	5.84	17.25	17.62	1.19	***	NS
20:4n-6	12.33	15.61	20.32	24.48	1.03	***	**
ΣPUFAn-6	20.68	24.73	43.91	49.37	2.40	***	**
20:5n-3	0.24	0.15	0.31	0.23	0.02	NS	*
22:6n-3	2.76	3.56	0.81	1.19	0.22	***	**
ΣPUFAn-3	3.22	3.91	1.27	1.76	0.22	***	**
ΣPUFA	22.83	31.26	45.54	51.71	2.44	***	**

MUFA, monounsaturated fatty acids; OOE, olive oil-fed, exercised animals; OOS, olive oil-fed, sedentary animals; PUFA, polyunsaturated fatty acids; SOE, sunflower oil-fed, exercised animals; SOS, sunflower oil-fed, sedentary animals.

* $P < 0.05$, ** $P < 0.005$, *** $P < 0.001$.

† For details of diets and procedures, see pp. 21–22.

‡ Data were log-transformed to perform the statistical analysis.

Table 2. Fatty acid profile (mol/100 mol) of skeletal muscle mitochondrial membranes in rats subjected to physical exercise and fed on diets containing virgin olive oil or sunflower oil†

(Values are means for six (OOS) or eight (OOE, SOS, SOE) animals, with their pooled standard errors. Residual degrees of freedom were 26)

Fatty acid	Experimental groups				SE	Statistical significance of effect of: (two-way ANOVA)	
	OOS	OOE	SOS	SOE		Fat	Exercise
16:0‡	19.51	21.42	20.98	27.03	1.46	NS	NS
18:0‡	15.01	12.23	9.18	9.27	1.03	*	NS
ΣSaturated‡	59.71	43.39	39.4	48.55	2.23	NS	NS
18:1n-9	18.37	20.47	12.65	9.06	1.43	*	NS
24:1n-9	0.97	0.99	2.17	2.34	0.18	***	NS
ΣMUFA	30.15	37.79	32.02	24.89	1.74	*	NS
18:2n-6	8.61	8.11	15.91	13.46	0.90	***	NS
20:4n-6‡	8.08	6.62	9.52	9.88	0.64	*	**
ΣPUFA _{n-6}	20.24	15.21	26.48	24.49	1.24	**	*
22:5n-3	1.08	0.26	0.67	0.38	0.17	*	*
22:6n-3	3.88	2.25	1.43	1.71	0.21	***	**
ΣPUFA _{n-3}	4.96	2.51	2.11	2.07	0.29	***	**
ΣPUFA‡	28.09	18.82	31.95	26.56	2.11	***	*

MUFA, monounsaturated fatty acids; OOE, olive oil-fed, exercised animals; OOS, olive oil-fed, sedentary animals; PUFA, polyunsaturated fatty acids; SOE, sunflower oil-fed, exercised animals; SOS, sunflower oil-fed, sedentary animals.

* $P < 0.05$, ** $P < 0.005$, *** $P < 0.001$.

† For details of diets and procedures, see pp. 21–22.

‡ Data were log-transformed to perform the statistical analysis.

liver under both dietary treatments. Exercise had no effect in skeletal muscle.

Monounsaturated fatty acids (MUFA)

Animals fed on virgin olive oil had higher values of all MUFA in comparison with those fed on sunflower oil, both in liver and in skeletal muscle ($P < 0.05$ or better). Many studies have reported that different subcellular membranes reflect the consumption of a MUFA-rich diet (e.g. Giron *et al.* 1992; Seiquer *et al.* 1996).

In contrast, physical exercise decreased liver 16:1n-7 and total MUFA ($P < 0.001$) as well as 18:1n-7 and 18:1n-9 ($P < 0.05$) with both dietary treatments, while skeletal muscle was unaffected. It has been documented that some MUFA (e.g. 18:1n-9) are preferentially metabolized (Seiquer *et al.* 1996); thus, the decrease may arise from the higher energetic requirements caused by physical exercise. This might affect availability for structural functions in the mitochondrial membrane. That this mechanism appears to occur only in the liver may be related to the higher metabolic rate of this tissue.

n-6 Polyunsaturated fatty acids (n-6 PUFA)

The polyunsaturated sunflower oil diet led to a higher proportion of n-6 PUFA in membranes of liver ($P < 0.001$) and skeletal muscle (18:2n-6 ($P < 0.001$), 20:4n-6 ($P < 0.05$), total n-6 PUFA ($P < 0.005$)) compared with animals fed on olive oil, both for the control and exercise treatments. Again, this finding confirms the influence of diet, as has been widely reported (e.g. Suarez *et al.* 1996).

Exercise enhanced the liver content of 20:4n-6, total n-6 PUFA and total PUFA ($P < 0.005$), for both dietary treatments, but had no effect on 18:2n-6 values. In skeletal muscle, exercise also enhanced 20:4n-6 values ($P < 0.005$) after the 8-week training programme but decreased the

relative quantities of total n-6 PUFA and total PUFA ($P < 0.05$).

n-3 Polyunsaturated fatty acids

Dietary treatment affected liver membrane 22:6n-3 and total n-3 PUFA values ($P < 0.001$), with higher values for the animals fed on monounsaturated olive oil. In skeletal muscle, all the animals fed on olive oil (both control and trained) had higher proportions of 22:5n-3 ($P < 0.05$), 22:6n-3 ($P < 0.001$) and total n-3 PUFA ($P < 0.001$) relative to total fatty acid content. The competition between n-6 and n-3 series fatty acids for elongase and desaturase enzymes has been widely documented (e.g. Periago *et al.* 1990), with n-6 enrichment leading to a subsequent n-3 inhibition, whereas when the n-9 series is prevailing, the n-3 series is not affected. This situation is confirmed by the current study.

As far as n-3 PUFA are concerned, physical exercise enhanced 22:6n-3 and total n-3 PUFA ($P < 0.005$) and decreased 20:5n-3 ($P < 0.05$) in both dietary groups in liver mitochondrial membranes. In skeletal muscle, exercise resulted in a decrease of 22:5n-3 ($P < 0.05$), 22:6n-3 ($P < 0.005$) and total n-3 PUFA ($P < 0.005$) with both diets, except for 22:6n-3 in animals fed on sunflower oil.

In relation to physical exercise and PUFA, Vapaatalo *et al.* (1984) described increases in 20:4n-6 in the plasma of young subjects after short-term heavy exercise. However, Masumura *et al.* (1992), from a study comparing the effects of season and exercise on the concentration of plasma PUFA in young rats, reported the opposite effect. The results of the present study provide support for both of these apparently contradictory reports in that the response may vary depending on the fatty acid and tissue studied. However, why the response to exercise should differ according to the tissue and the fatty acid is unclear at present.

n-6 and n-3 PUFA are not only involved in membrane composition but are also precursors of the eicosanoids.

Whether the diet or the physical exercise resulted in different rates of eicosanoid production is unknown, but it is possible that the effects reported here may result from changes at this level (Ayre & Hulbert, 1997).

Data previously reported (Mataix *et al.* 1998) show that physical exercise led to an increased production of free radicals in these same rats. Therefore, it is possible that there was mobilization by phospholipase A₂ of the oxidized fatty acids in the mitochondrial membrane.

Alternatively, even though opposite changes in MUFA and PUFA composition of liver mitochondrial membranes occurred after physical exercise, membrane fluidity remained the same (results not shown), which suggests that a counterbalance effect was achieved as has been postulated by other authors (Periago *et al.* 1990; Huertas *et al.* 1992).

Finally, the weight gain of the exercised rats was less than that of the sedentary animals. It could be that some of the changes found in the membrane fatty acid profiles of rats after exercise resulted from this lower weight gain. However, exercised animals ate the same amount of diet as the sedentary groups. Therefore, this lower weight gain resulted from the increase in energy expenditure and not from an exercise-induced anorexia or a food restriction effect; similar observations have been reported by other authors (Lewis *et al.* 1993). The increase in fatty acid oxidation for energetic purposes will result in a lower availability of fatty acids for structural functions, as has been discussed earlier.

In conclusion, the main effect attributable to diet was an adaptation of the mitochondrial membranes, with animals fed on monounsaturated fat showing higher levels of these in their composition, and with a similar phenomenon for *n*-6 PUFA for rats fed on sunflower oil. Physical exercise affected the fatty acid profiles of both liver and skeletal muscle mitochondrial membranes. This effect was greater in liver. The changes due to the exercise are difficult to explain, but could depend on several mechanisms, e.g. fluidity regulation, eicosanoid metabolism, or differences in the fatty acid availability or in the oxidation rate of the different fatty acids. These possible mechanisms are under further investigation.

Acknowledgements

This work was supported by CICYT project ALI91-1113-C03-01. We also wish to acknowledge the University of Granada and COOSUR S.A. for a PhD grant to J. L. Quiles.

References

- Ayre KJ & Hulbert AJ (1997) Dietary fatty acid profile affects endurance in rats. *Lipids* **32**, 1265–1270.
- Giron MD, Mataix J & Suarez MD (1992) Long-term effects of dietary monounsaturated and polyunsaturated fatty acids on the lipid composition of erythrocyte membranes in dogs. *Comparative Biochemistry and Physiology* **102**, 197–201.
- Huertas JR, Battino M, Barzanti V, Maranesi M, Parenti-Castelli G, Littarru GP, Turchetto E, Mataix FJ & Lenaz G (1992) Mitochondrial and microsomal cholesterol mobilization after oxidative stress induced by adriamycin in rats fed with dietary olive and corn oil. *Life Science* **50**, 2111–2118.
- Innis SM & Clandinin MT (1981) Dynamic modulation of mitochondrial inner-membrane lipids in rat heart by dietary fat. *Biochemical Journal* **193**, 155–167.
- Lepage G & Roy CC (1986) Direct transesterification of all classes of lipids in a one-step reaction. *Journal of Lipid Research* **27**, 114–120.
- Lewis DE, Shellard L, Koeslag DG, Boer DE, McCarthy HM, McKibbin PE, Russell JC & Williams G (1993) Intense exercise and food restriction cause similar hypothalamic neuropeptide Y increases in rats. *American Journal of Physiology* **264**, E279–E284.
- Masumura S, Furui H, Hashimoto M & Watanabe Y (1992) The effects of season and exercise on the levels of plasma polyunsaturated fatty acids and lipoprotein cholesterol in young rats. *Biochimica et Biophysica Acta* **1125**, 292–296.
- Mataix J, Quiles JL, Huertas JR, Battino M & Mañas M (1998) Tissue specific interactions of exercise, dietary fatty acids, and vitamin E in lipid peroxidation. *Free Radical Biology and Medicine* **24**, 511–521.
- Periago JL, Suarez MD & Pita ML (1990) Effect of dietary olive oil, corn oil and medium-chain triglycerides on the lipid composition of rat red blood cell membranes. *Journal of Nutrition* **120**, 986–994.
- Salting B & Astrand PO (1993) Free fatty acids and exercise. *American Journal of Clinical Nutrition* **57**, 752S–758S.
- Seiquer I, Mañas M, Martínez-Victoria E, Ballesta MC & Mataix FJ (1996) Long-term influence of dietary fat (sunflower oil, olive oil, lard and fish oil) in the serum fatty acid composition and in the different lipidic fractions, in miniature swine. *International Journal for Vitamin and Nutrition Research* **66**, 171–179.
- Snedecor GW & Cochran WG (1980) *Statistical Methods*, 7th ed. Ames, IA: Iowa State University Press.
- Suárez A, Ramírez MCD, Faus MJ & Gil A (1996) Dietary long-chain polyunsaturated fatty acids influence tissue fatty acid composition in rats at weaning. *Journal of Nutrition* **126**, 887–897.
- Vapaatalo H, Laustiola K, Seppala E, Rauramaa R, Kaste M, Hillbom M & Kangasaho M (1984) Exercise, ethanol and arachidonic acid metabolism in healthy men. *Biomedica Biochimica Acta* **43**, S413–S420.