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Effect of chronic exercise on DNA fragmentation and on lipid profiles in rat skeletal muscle

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The concentration and fatty acid composition of phospholipids in animal cells are important determinants of membrane function. Membrane function may influence apoptosis, a biological process that is crucial for the normal development and function of the body. Few and conflicting data exist regarding the effect of chronic exercise on apoptosis in skeletal muscle, and no data exist regarding the effect of chronic exercise on the fatty acid composition of individual muscle phospholipids. We therefore examined the effects of 8 weeks of voluntary wheel running on DNA fragmentation (an index of apoptosis) and on the concentration and fatty acid composition of individual muscle phospholipids and ceramide (a lipid involved in apoptotic signalling) in rat gastrocnemius muscle by comparing 11 trained and 14 untrained male Wistar rats. The trained animals had significantly ($P < 0.05$) higher cytochrome *c* oxidase activity (an index of aerobic adaptation) and lower phosphatidyl inositol concentration compared with their untrained counterparts. Groups did not differ in DNA fragmentation or any other lipid parameter. Our findings suggest that chronic wheel running did not affect apoptosis or the concentration and fatty acid composition of most phospholipids and ceramide in rat gastrocnemius muscle. Given the participation of several phospholipids and ceramide in apoptotic signalling, it appears that the lack of changes in the lipid parameters is in agreement with the lack of change in DNA fragmentation with exercise.

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Experimental Physiology

Programmed cell death, or apoptosis, is an important biological process for the normal development and function of the body. Apoptosis involves a series of biochemical events that lead to a variety of morphological changes, including chromosomal DNA fragmentation, a direct apoptotic index.

Cell membranes, in addition to their structural role, are dynamic components of the signal transduction system required to regulate cell survival and cell death (McMillin & Dowhan, 2002; Cui & Houweling, 2002). Several membrane phospholipids have been implicated in the apoptotic process. These include three glycerophospholipids (that is, phospholipids containing a glycerol backbone to which two acyl groups and a phosphate group are attached), namely, phosphatidyl choline (PC), phosphatidyl serine (PS) and cardiolipin (CL), as well as one sphingolipid (a phospholipid containing a sphingosine, rather than glycerol, backbone), namely, sphingomyelin (SM; Emoto *et al.* 1997; Farooqui *et al.* 2000; Cui & Houweling, 2002; McMillin & Dowhan, 2002; Gulbins, 2003). In addition, lysophospholipids (glycerophospholipids missing one acyl group), although minor membrane components, have revealed themselves to be important regulators of cell growth and survival (Ruiter *et al.* 1999).

Understandably, studies have focused on the association of phospholipid concentration with apoptosis. Nevertheless, since phospholipids may contain a variety of fatty acids in their structure, an additional parameter that merits attention is fatty acid composition. Indeed, studies have shown an association between the fatty acid composition of CL, a complex phospholipid of the inner mitochondrial membrane, and apoptosis. A decrease in the percentage of the unsaturated fatty acid, linoleate (18:2ω6), in CL promotes the release of cytochrome *c*, a peripheral protein of the inner mitochondrial membrane that activates caspase 9, one of a family of enzymes triggering apoptosis (Bernardi *et al.* 2002). Along the

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same line, cardiomyocytes grown in the presence of the saturated fatty acid, palmitate (16:0), exhibited decreased mitochondrial CL content, increased release of cytochrome *c* and enhanced apoptotic rate compared with cells grown in the presence of the unsaturated oleate (18:1ω9; Ostrander *et al.* 2001).

Physical exercise, either acute or chronic, has long been recognized as a potential apoptotic effector. Most of the research on the subject has focused on leukocytes and the heart. Regarding skeletal muscle, many studies have addressed the effect of acute exercise on apoptosis and all have found an increase (Sandri *et al.* 1995, 1997; Biral *et al.* 1998, 2000; Podhorska-Okolow *et al.* 1998; Arslan *et al.* 2002; Willoughby *et al.* 2003; Koçtürk *et al.* 2007). In contrast, only four studies have examined the effect of chronic exercise on apoptosis in skeletal muscle, with opposing data (Boffi *et al.* 2002; Lim *et al.* 2004; Siu *et al.* 2004; Song *et al.* 2006).

While there are data on the effect of chronic exercise on the concentration (Petridou *et al.* 2005) and fatty acid composition of total muscle phospholipids (Nikolaidis & Mougios, 2004), information on the effect of chronic exercise on the concentration and fatty acid composition of individual glycerophospholipids is limited and fragmentary. In particular, only one study has examined the effect of chronic exercise on the concentrations of the glycerophospholipids PC, PE, CL, phosphatidyl inositol (PI) and phosphatidyl serine (PS) in muscle (Górski *et al.* 1999). However, we could find no data on the effect of chronic exercise on the fatty acid profile of the individual glycerophospholipids.

Likewise, there are limited and conflicting data regarding the effect of chronic exercise on the concentration and fatty acid profile of the sphingolipids, SM and ceramide, in skeletal muscle. Dobrzyń *et al.* (2004) found a decrease and altered fatty acid profile in SM in soleus and red gastrocnemius muscles. Górski *et al.* (1999) found no changes in SM in the same muscles, as opposed to an increase in white gastrocnemius with exercise training. Finally, the only study on ceramide found that chronic exercise lowered its concentration and altered its fatty acid profile (Dobrzyń *et al.* 2004).

Given the role of individual phospholipids and ceramide in apoptosis and the scarcity of data *vis-à-vis* the effect of chronic exercise on either apoptosis or these lipid molecules in skeletal muscle, the purpose of the present study was to examine the effect of 8 weeks of voluntary wheel running (a training model that eliminates the potential confounding influence of stress associated with forced exercise, hence, glucocorticoid-mediated apoptosis) on DNA fragmentation, as well as on the concentration and fatty acid composition of individual phospholipids and ceramide in rat gastrocnemius muscle.

Methods

Ethical approval

The study was approved by the University of Thessaloniki Ethics Committee. The animals were maintained according to the European Union guidelines for the care and use of laboratory animals.

Animals

Thirty-five male Wistar rats were purchased at the age of 7 weeks from Charles River Laboratories (Sulzfeld, Germany) and were housed under controlled environmental conditions (21°C, 12 h–12 h light–dark cycle). The rats had free access to water and standard rodent chow, in which the fat content was 3.5% and the predominant fatty acids were 18:2ω6, 16:0 and 18:1ω9, accounting for 40, 28 and 21% of total fatty acids, respectively (Nikolaidis *et al.* 2004).

Training

The animals were divided randomly into a group to be trained ($n = 20$) and an untrained group ($n = 15$). The members of the former group were housed individually in cages equipped with wheels, in which they exercised *ad libitum* for 8 weeks, while the members of the untrained group were housed individually in plain cages. The running activity of the former group was recorded continuously through the DasyLab 5.0 data collection system from Datalog (Mönchengladbach, Germany). Based on previous experience, we set 2 km day^{-1} as the minimal average running activity necessary for an animal to qualify as trained.

Specimen collection

Eleven rats that met the training criterion (having run, on average, 5.2 km day^{-1}) formed the trained group. These, along with 14 untrained animals (one died during the experimental period), were killed by dislocation of the neck at approximately the same time of day (14.00–16.00 h) in a counterbalanced order. Wheels and food had been removed from the cages 12 and 6 h earlier, respectively, to minimize the influence of the last exercise bout and the last feeding on the biochemical parameters of interest. The gastrocnemius lateralis muscle of the right hindlimb was surgically removed as fast as possible. This muscle contains 3% type I fibres, 6% type IIA fibres, 34% type IID/X fibres and 57% type IIB fibres (Delp & Duan, 1996). The muscle was stripped of visible fat, nerves and fasciae, and was immediately immersed in liquid nitrogen. Subsequently, all specimens were pulverized with a mortar

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and pestle in liquid nitrogen and stored at −80°C until analysis.

Cytochrome *c* oxidase assay

Cytochrome *c* oxidase (CCO) activity of gastrocnemius muscle was measured as an index of adaptation to training as previously described (Tsalouhidou *et al.* 2006). The intra-assay coefficient of variation was 3%.

DNA fragmentation

A cell death detection enzyme immunoassay (EIA) kit (number 11 774 425 001, Roche Molecular Biochemicals, Mannheim, Germany) was used to measure cytoplasmic histone-associated DNA fragments (mono- and oligonucleosomes) according to the manufacturer's instructions. Approximately 10 mg of muscle were homogenized on ice with 79 volumes of ice-cold lysis buffer contained in the kit. The homogenate was centrifuged at 100*g* for 1 min at 4°C to remove cell debris, and the supernatant was used in a sandwich EIA with an anti-histone mouse monoclonal antibody coated to the microtitre plate and an anti-DNA mouse monoclonal antibody coupled to peroxidase. The amount of peroxidase retained in the immunocomplex was determined photometrically by incubating with 2,2′-azino-di-(3-ethylbenzthiazoline-6-sulphonate) as substrate for 10 min at room temperature. The change in colour was measured at 405 nm by using an Anthos 2001 plate reader (Salzburg, Austria). The intra-assay coefficient of variation was 10%.

Lipid analysis

We determined the fatty acid composition of individual phospholipids and ceramide by gas chromatography after separation by thin-layer chromatography (TLC).

Lipids were extracted with a mixture of chloroform and methanol, 2:1 (v/v) according to Folch *et al.* (1957) from 125 mg of muscle in a test tube containing a fixed amount (36 μg) of diheptadecanoyl phosphatidyl choline (Sigma, St Louis, MO, USA) as internal standard for the determination of the phospholipid concentration and a fixed amount (3 μg) of heptadecanoyl ceramide (Sigma) as internal standard for the determination of the ceramide concentration. Phospholipids were separated by two-dimensional TLC. One-quarter of a lipid extract was spotted near a corner of a high-performance silica gel plate (Merck, Darmstadt, Germany) cut to dimensions of 10 cm × 10 cm. The plate was developed in a mixture of chloroform, methanol and acetic acid, 10:5:1 (v/v/v), dried, rotated by 90 deg, and redeveloped in a mixture of chloroform, acetone, methanol, acetic acid and water, 10:4:2:2:1 (v/v/v/v/v; Kester *et al.* 1984). Phospholipids were located by brief exposure to ultraviolet light after spraying with a solution of 0.2% (w/v) dichlorofluorescein in ethanol and were identified by comparison with the migration patterns of phospholipid standards from Sigma.

The spot corresponding to each phospholipid was excised and transferred to a screw-capped tube containing a fixed amount (8 μg) of pentadecanoic acid (Sigma) as external standard to be used in the determination of the concentration of the phospholipid in conjunction with the PC internal standard described in the previous paragraph. To produce fatty acid methyl esters, we added 1 ml of methanolic boron trifluoride (Fluka, Buchs, Switzerland) to the tube containing SM and heated it at 100°C for 75 min. To the tubes containing the other phospholipids we added 0.5 ml of methanolic sodium methoxide (Sigma) and heated them at 50°C for 10 min. Then we added 0.5 ml of methanolic boron trifluoride and heated for another 10 min at 50°C (Kramer *et al.* 1997).

Ceramide was isolated by one-dimensional TLC according to Dobrzyń & Górski (2002). One-quarter of each lipid extract was spotted onto adjacent lanes of a silica gel plate as for the phospholipidsand developed to one-third of the plate's height in a mixture of chloroform, methanol and 25% NH_3, 20:5:0.2 (v/v/v). The plate was dried and redeveloped in a mixture of heptane, isopropyl ether and acetic acid, 60:40:3 (v/v/v). Heptadecanoyl ceramide was run adjacent to the samples to aid in locating ceramide after spraying with dichlorofluorescein solution. The spot corresponding to ceramide in each sample lane was scraped off the plate and was transferred into a screw-capped tube. Fatty acid methyl esters were produced from ceramide as described above for SM.

The products of the transmethylation reactions of the individual phospholipids and ceramide were extracted with 1.5 ml of hexane and were separated in a Hewlett Packard 5890 Series II gas chromatograph (Waldbronn, Germany) equipped with a 30-m-long AT-WAX capillary column from Alltech (Deerfield, IL, USA) and a flame ionization detector. The column temperature was programmed from 160 to 250°C at 5°C min^{-1}. The carrier gas was helium at a flow rate of 1 ml min^{-1} (at 160°C). Methyl esters of individual fatty acids were identified with the aid of the HP 3365 ChemStation software from Hewlett Packard in the chromatograms by comparing their retention times to those of pure methyl esters from Sigma and were quantified by comparing the area under their peaks to that of methyl heptadecanoate (derived from the internal standard) in the case of PC and ceramide, and that of methyl pentadecanoate (derived from the external standard) in the chromatograms of the other phospholipids after normalizing for the area of methyl pentadecanoate in the chromatogram of PC.

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Statistics

Values are expressed as the means (SD). Differences between untrained and trained rats in the concentrations of CCO, cytoplasmic nucleosomes, fatty acids in each phospholipid and fatty acids in ceramide were examined by Student's unpaired *t* test. Differences in fatty acid percentages between untrained and trained rats, as well as among different lipids, were examined by two-way ANOVA (exercise × lipid) with repeated measures on lipid, followed by simple main effect analysis for pairwise comparisons. Correlation analysis was performed by Pearson's product–moment correlation. The level of statistical significance was set at $\alpha = 0.05$ for all analyses, which were performed with the SPSS software (version 13.0, SPSS Inc., Chicago, IL, USA).

Results

Cytochrome *c* oxidase

The gastrocnemius muscle of the trained rats had a 40% higher catalytic concentration of CCO compared with the untrained rats ($P = 0.003$; Fig. 1*A*).

DNA fragmentation

There was no significant difference between trained and untrained animals in cytoplasmic nucleosome concentration in the gastrocnemius muscle (Fig. 1*B*) and no significant correlation between cytoplasmic nucleosome concentration and either distance run or CCO concentration in the trained group (data not shown).

Lipids

Seven phospholipids, namely, PC, PE, CL, PI, PS, SM and lysophosphatidyl choline (LPC), were detected in gastrocnemius muscle by TLC. The methyl esters of 22 fatty acids, namely, laurate (12:0), myristate (14:0), myristoleate (14:1ω9), 16:0, palmitoleate (16:1ω7), stearate (18:0), 18:1ω9, *cis*-vaccenate (18:1ω7), 18:2ω6, γ-linolenate (18:3ω6), α-linolenate (18:3ω3), stearidonate (18:4ω3), arachidate (20:0), gondoate (20:1ω9), dihomo-γ-linolenate (20:3ω6), arachidonate (20:4ω6), timnodonate (20:5ω3), behenate (22:0), adrenate (22:4ω6), all-*cis*-4,7,10,13,16-docosapentaenoate (22:5ω6), all-*cis*-7,10,13,16,19-docosapentaenoate (22:5ω3) and all-*cis*-4,7,10,13,16,19-docosahexaenoate (22:6ω3), were detected in considerable amounts (above 0.1% of total) by gas chromatography of the phospholipids and ceramide. In addition, the dimethyl acetals produced by methylation of 4 aldehydes, namely, 16:0, 18:0, 18:1ω9 and 18:1ω7, were detected in PC and PE. These dimethyl acetals are derived from phosphatidal choline and phosphatidal ethanolamine (also known as plasmalogens), i.e. compounds carrying an alkenyl, rather than acyl, group at position *sn*1 of glycerol. Phosphatidal choline and phosphatidal ethanolamine comigrated with PC and PE, respectively, during two-dimensional TLC and are therefore included in these phospholipids in the subsequent data presentation.

Table 1 presents the concentrations of the seven muscle phospholipids in untrained and trained rats. The only significant difference between groups was a 31% lower PI concentration in the trained rats ($P = 0.009$). There was no significant correlation between any phospholipid concentration and either distance run or CCO concentration (data not shown).

There was no significant difference between untrained and trained rats in the muscle ceramide concentration [0.33 (0.16) *versus* 0.27 (0.06) μmol g^{-1}, respectively].

The percentage distribution of fatty acids in the individual phospholipids, total phospholipids and ceramide of the gastrocnemius muscle of untrained and trained rats are presented in Table 2. No significant differences were found between groups. In contrast, there were significant differences among the seven phospholipids and ceramide in most fatty acids.

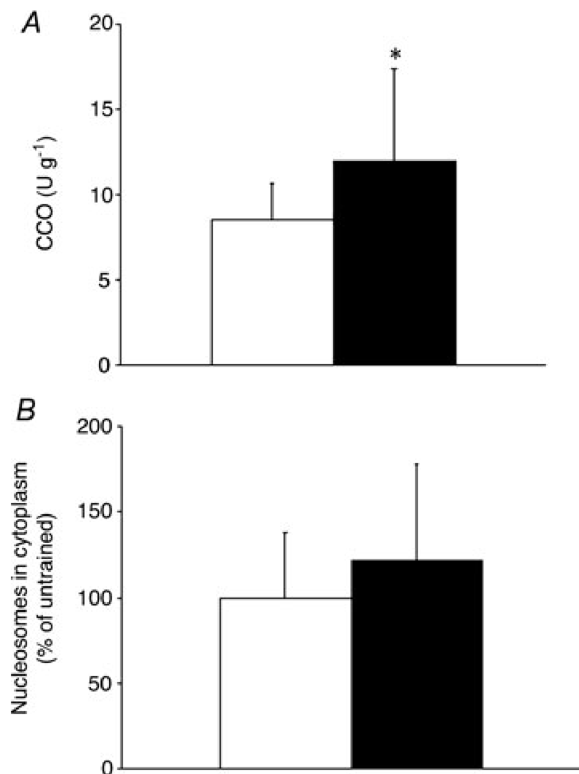

Figure 1. Catalytic concentration of cytochrome *c* oxidase (*A*) and nucleosomes in the cytoplasm (*B*) in rat gastrocnemius muscle of untrained (open bars) and trained rats (filled bars)
Error bars represent S.D. *Significantly different from untrained ($P = 0.003$).

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Table 1. Concentrations of individual muscle phospholipids in untrained and trained rats

	Acyl and alkenyl groups (μmol g^{-1})		Phospholipid (μmol g^{-1})	
Phospholipid	Untrained	Trained	Untrained	Trained
PC	12.31 (1.45)	12.85 (2.15)	6.16 (0.72)	6.42 (1.07)
PE	4.51 (1.60)	4.53 (1.85)	2.26 (0.80)	2.26 (0.93)
CL	1.43 (0.52)	1.62 (0.67)	0.36 (0.13)	0.41 (0.17)
PI	1.19 (0.36)	0.82 (0.25)*	0.59 (0.18)	0.41 (0.13)*
PS	0.61 (0.53)	0.73 (0.51)	0.30 (0.26)	0.37 (0.25)
SM	0.40 (0.24)	0.54 (0.35)	0.40 (0.24)	0.54 (0.35)
LPC	0.40 (0.28)	0.47 (0.35)	0.40 (0.28)	0.47 (0.35)
Total	20.86 (2.94)	21.56 (2.95)	10.47 (1.39)	10.89 (1.52)

Data are expressed as means (S.D.). Data in the final two columns were calculated by dividing the corresponding data of the preceding columns by the number of acyl/alkenyl groups in the structure of each phospholipid, that is, 2 for phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), phosphatidyl inositol (PI) and phosphatidyl serine (PS), 4 for cardiolipin (CL) and 1 for sphingomyelin (SM) and lysophosphatidyl choline (LPC). * Significantly different from untrained ($P = 0.009$).

Table 3 presents the aldehyde profiles of PC, PE and total phospholipids in untrained and trained rats. There were no significant differences between groups.

Discussion

In the present study, we have attempted to contribute to the elucidation of whether and how chronic exercise affects the concentrations and fatty acid profiles of skeletal muscle lipids, some of which play roles in DNA fragmentation, an integral part of apoptosis. The training stimulus was sufficient to elicit adaptive responses, as judged from the higher catalytic concentration of CCO in the muscles of the trained rats. The novelty of the present work lies in the examination of the effect of chronic exercise on the concentration of all individual muscle phospholipids and the thorough characterization of their fatty acid profiles, which is missing from the literature.

Several of the phospholipids have been implicated, in one way or another, in apoptotic signalling, although none of the evidence derives from skeletal muscle. A perturbation of PC homeostasis has been shown to induce cell death in ovary cells (Cui *et al.* 1996). Migration of PE from the inner to the outer leaflet of the plasma membrane of T cells can trigger processes leading to DNA fragmentation (Emoto *et al.* 1997). Cardiolipin is involved in the mitochondria-mediated pathway of apoptosis in several tissues, including heart muscle, through its binding of cytochrome *c*, an activator of caspase 9 (McMillin & Dowhan, 2002). Finally, SM and ceramide participate in a signal transduction pathway in which the former is hydrolysed by a sphingomyelinase to the latter and phosphocholine. Physiological apoptotic stimuli, such as hormones and cells of the immune system, promote, through binding to plasma membrane death receptors, the movement of sphingomyelinase from the cytosol to the membrane and the production of ceramide from SM. Ceramide then causes a change in membrane structure that results in caspase 8 activation (Gulbins, 2003).

Our data regarding the total concentrations of the individual phospholipids of rat gastrocnemius muscle are very similar to those of Górski *et al.* (1999) on the same species and muscle. Remarkably, our data (and those of Górski *et al.* 1999) are very similar even to those of Masoro *et al.* (1966) on monkey gastrocnemius muscle and Therriault *et al.* (1973) on dog biceps femoris. With the exception of a decrease in PI concentration, chronic exercise had no effect on the concentrations and fatty acid profiles of the individual muscle phospholipids (as well as ceramide) in the present study. Górski *et al.* (1999) found that exercise training did not affect the content of any individual phospholipid in rat soleus, while it increased PI and SM in white gastrocnemius, PE and CL in red gastrocnemius and PI in diaphragm. Dobrzyń *et al.* (2004), in contrast, found decreased SM and ceramide in rat soleus and gastrocnemius after exercise training. There is no obvious explanation for these differences across studies and muscles regarding the effect of exercise on phospholipid concentrations and no apparent physiological significance. We could find no data regarding the effect of chronic exercise on the fatty acid profiles of PC, PE, CL, PI and PS to permit a comparison with our data. A comparison could be made only with the study by Dobrzyń *et al.* (2004), who studied SM and ceramide in rat soleus and gastrocnemius and reported altered fatty acid profiles of both sphingolipids with treadmill exercise training, in contrast to no changes with wheel running in the present study. These discrepancies may be due to the different exercise types employed in each study.

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Table 2. Molar percentage distribution of fatty acids in muscle phospholipids and ceramide of untrained and trained rats

	PC		PE		CL		PI	
Fatty acid	Untrained	Trained	Untrained	Trained	Untrained	Trained	Untrained	Trained
12:0	0.04 (0.03)[bcdeg]	0.04 (0.03)[deg]	0.15 (0.13)[aceg]	0.10 (0.08)[deg]	0.31 (0.22)[abe]	0.21 (0.10)[eg]	0.33 (0.23)[ae]	0.34 (0.34)[abe]
14:0	0.32 (0.06)[cdefg]	0.32 (0.09)[defg]	0.38 (0.45)[efg]	0.20 (0.10)[defg]	0.72 (0.35)[aefg]	0.55 (0.17)[efg]	0.70 (0.34)[aefg]	0.76 (0.47)[abg]
14:1ω9	0.06 (0.06)[cdefg]	0.07 (0.06)[defg]	0.12 (0.09)[efg]	0.13 (0.11)[efg]	0.25 (0.22)[aeg]	0.17 (0.12)[eg]	0.24 (0.14)[aeg]	0.31 (0.23)[ag]
16:0	41.68 (3.83)[bcdefgh]	40.63 (5.11)[bcdefh]	9.14 (2.98)[afgh]	8.06 (2.19)[aefgh]	7.27 (4.24)[aefgh]	5.14 (2.81)[adefgh]	9.31 (4.95)[afgh]	10.11 (7.03)[acfgh]
16:1ω7	0.75 (0.21)[bcde]	0.58 (0.14)[d]	0.38 (0.27)[ac]	0.47 (0.32)	1.18 (0.45)[abdefg]	0.91 (0.19)[df]	0.23 (0.16)[ach]	0.16 (0.24)[aceg]
18:0	10.02 (2.46)[bdefgh]	12.16 (2.46)[bdefgh]	35.31(8.29)[acdef]	38.04 (6.68)[acdef]	11.75 (8.34)[bdefgh]	8.32 (5.76)[bdefgh]	51.46 (9.22)[abch]	51.79 (7.87)[abcgh]
18:1ω9	4.36 (0.52)[cd]	4.21 (0.49)[bd]	4.37 (1.08)[cd]	3.64 (1.46)[cd]	1.96 (0.78)[abeg]	1.56 (0.18)[abeg]	1.84 (0.54)[abeg]	2.01 (0.60)[ab]
18:1ω7	3.86 (1.17)[bdefg]	3.95 (0.45)[bdefg]	2.22 (0.48)[acdef]	1.85 (0.34)[acdef]	4.49 (0.63)[bdefg]	4.56 (0.50)[bdefg]	1.19 (0.77)[abc]	1.07 (0.39)[abc]
18:2ω6	19.41 (2.56)[bcdefgh]	18.11 (2.22)[bcdefgh]	8.21 (1.45)[acdefh]	8.25 (1.61)[acdefh]	66.77 (12.64)[abdefgh]	74.07 (8.61)[abdefgh]	2.69 (1.01)[abcg]	3.40 (1.26)[abc]
18:3ω6	0.10 (0.03)[efgh]	0.13 (0.04)[eh]	0.20 (0.13)[efh]	0.27 (0.20)[h]	0.18 (0.22)[efh]	0.09 (0.03)[eh]	0.20 (0.13)[efh]	0.27 (0.18)[h]
18:3ω3	0.19 (0.08)[bc]	0.18 (0.07)[c]	0.08 (0.03)[bc]	0.09 (0.03)[c]	0.44 (0.18)[abd]	0.43 (0.12)[abd]	0.10 (0.16)[c]	0.11 (0.25)[c]
18:4ω3	0.03 (0.04)[h]	0.02 (0.03)[fh]	0.16 (0.27)	0.08 (0.11)[f]	0.21 (0.22)	0.25 (0.30)	0.35 (0.55)	0.20 (0.22)
20:0	0.06 (0.04)[bcefgh]	0.06 (0.04)[fh]	0.12 (0.07)[aefh]	0.09 (0.07)[fh]	0.17 (0.14)[aefh]	0.12 (0.08)[h]	0.18 (0.15)[efh]	0.14 (0.16)[h]
20:1ω9	0.09 (0.04)[g]	0.12 (0.06)[d]	0.11 (0.07)[dg]	0.11 (0.06)[d]	0.13 (0.20)	0.05 (0.03)	0.02 (0.03)[b]	0.02 (0.06)[ab]
20:3ω6	0.62 (0.23)[bdf]	0.49 (0.10)[bd]	0.24 (0.10)[acd]	0.28 (0.14)[acd]	0.59 (0.18)[bf]	0.56 (0.11)[bdf]	1.62 (0.87)[abcef]	1.48 (0.36)[abcef]
20:4ω6	12.88 (4.14)[bcdefg]	13.05 (4.41)[bcdefg]	9.13 (3.32)[acdefg]	9.24 (3.04)[acdefg]	0.90 (0.62)[abde]	0.78 (0.40)[abdg]	22.87 (10.53)[abcefg]	21.54 (7.16)[abcefg]
20:5ω3	0.18 (0.09)[h]	0.18 (0.07)[h]	0.21 (0.07)[h]	0.30 (0.24)[h]	0.12 (0.33)[h]	0.02 (0.05)[h]	0.21 (0.47)[h]	n.d.
22:0	0.13 (0.20)[efh]	0.13 (0.16)[fh]	0.24 (0.32)[fh]	0.40 (0.48)[h]	0.48 (0.66)[fh]	0.44 (0.60)[h]	1.15 (1.34)[h]	0.91 (1.27)[h]
22:4ω6	0.34 (0.11)[bdefh]	0.32 (0.13)[bcdefh]	1.31 (0.33)[acfh]	1.18 (0.48)[acfgh]	0.27 (0.23)[bdeh]	0.13 (0.09)[abde]	0.89 (0.54)[acefh]	0.97 (0.61)[acefh]
22:5ω6	0.27 (0.19)[beh]	0.20 (0.16)[bh]	1.33 (0.71)[acdgh]	1.29 (0.61)[acdgh]	0.15 (0.17)[bdeh]	0.10 (0.09)[b]	0.44 (0.43)[bceh]	0.33 (0.23)[b]
22:5ω3	1.23 (0.57)[bcdef]	1.28 (0.65)[ace]	3.84 (1.34)[acdg]	3.15 (1.48)[acdg]	0.49 (0.27)[abdef]	0.47 (0.21)[abdefh]	2.08 (1.18)[abcg]	1.88 (0.97)[bcg]
22:6ω3	2.82 (1.56)[bce]	3.29 (1.95)[bce]	14.39 (6.00)[acdefgh]	14.19 (6.19)[acdefgh]	1.18 (0.66)[abde]	1.08 (0.57)[abde]	1.91 (1.04)[bce]	2.22 (0.88)[bce]
Total	99.43 (0.44)	99.52 (0.28)	91.45 (2.96)	91.43 (2.59)	100.00	100.00	100.00	100.00

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Table 2. Continued

	PS		SM		LPC		Total PL		Ceramide	
Fatty acid	Untrained	Trained	Untrained	Trained	Untrained	Trained	Untrained	Trained	Untrained	Trained
12:0	0.56 (0.20)^{abcd}	0.62 (0.33)^{abcd}	0.79 (0.74)	0.1 (0.1)	0.87 (0.64)^{ab}	0.80 (0.48)^{abc}	0.1 (0.1)	0.1 (0.1)	n.d.	n.d.
14:0	1.10 (0.39)^{abcdf}	1.02 (0.31)^{abcg}	1.95 (0.77)^{abcdeh}	0.4 (0.2)	1.76 (0.87)^{abcdh}	1.86 (0.42)^{abcde}	0.4 (0.2)	0.4 (0.2)	0.25 (0.55)^{fg}	0.62 (1.55)
14:1ω9	0.47 (0.28)^{abcdh}	0.47 (0.24)^{abc}	0.46 (0.35)^{abh}	0.1 (0.1)	0.80 (0.59)^{abcdh}	0.78 (0.51)^{abcdh}	0.1 (0.1)	0.1 (0.1)	0.08 (0.23)^{efg}	0.13 (0.32)^{g}
16:0	12.00 (5.86)^{acfgh}	12.08 (4.22)^{abcfgh}	26.85 (8.20)^{abcde}	28.5 (3.8)	31.09 (7.47)^{abcde}	33.69 (5.87)^{bcde}	29.2 (4.0)	28.5 (3.8)	32.05 (8.21)^{abcde}	31.12 (7.32)^{abcde}
16:1ω7	0.37 (0.17)^{ac}	0.50 (0.23)^{d}	0.48 (0.53)^{c}	0.6 (0.1)	0.73 (0.45)^{c}	0.87 (0.34)^{df}	0.6 (0.1)	0.6 (0.1)	0.98 (0.81)^{d}	0.69 (0.31)
18:0	50.09 (6.59)^{abch}	53.04 (7.23)^{abcgh}	50.15 (10.86)^{abch}	21.8 (5.4)	42.19 (14.06)^{ach}	36.34 (10.10)^{acde}	20.5 (5.8)	21.8 (5.4)	30.62 (4.97)^{acdefg}	33.29(3.45)^{acdef}
18:1ω9	5.59 (2.20)^{cdfg}	3.83 (1.02)^{c}	2.48 (2.18)^{e}	3.7 (0.6)	3.08 (1.31)^{cde}	3.05 (0.80)c	4.0 (0.5)	3.7 (0.6)	4.04 (2.81)	3.63 (3.69)
18:1ω7	0.86(0.30)^{abc}	0.89 (0.44)^{abc}	0.73 (0.91)^{abc}	3.2 (0.4)	1.24 (0.83)^{a}	1.49 (0.91)^{ac}	3.2 (0.8)	3.2 (0.4)	4.21 (7.27)	3.68 (2.54)
18:2ω6	2.66 (0.77)^{abc}	3.66 (2.21)^{abc}	1.81 (1.28)^{abc}	18.5 (2.3)	6.24 (4.56)^{acd}	7.76 (4.79)^{ac}	18.2 (2.0)	18.5 (2.3)	4.57 (4.89)^{abc}	2.51 (1.18)^{abc}
18:3ω6	0.42(0.21)^{abcdh}	0.46 (0.19)^{ac}	0.67 (0.51)^{abcd}	0.2 (0.1)	0.53 (0.35)^{a}	0.41 (0.35)	0.2 (0.0)	0.2 (0.1)	1.39 (0.82)^{abcde}	1.16 (0.68)^{abcdf}
18:3ω3	0.21(0.37)	0.13 (0.23)	0.47 (0.66)	0.2 (0.1)	0.29 (0.55)	0.38 (0.78)	0.2 (0.1)	0.2 (0.1)	n.d.	n.d.
18:4ω3	0.61 (1.10)	0.71 (0.86)	0.27 (0.38)	0.1 (0.1)	0.79 (1.23)	1.19 (1.35)	0.1 (0.1)	0.1 (0.1)	0.41(0.36)^{a}	0.51 (0.25)^{a}
20:0	0.84 (0.71)^{abcd}	0.56 (0.40)	1.68 (0.97)^{abcdg}	0.1 (0.1)	0.57 (0.60)^{af}	0.57 (0.31)	0.1 (0.1)	0.1 (0.1)	1.06 (0.54)^{abcd}	1.15 (0.30)^{abcd}
20:1ω9	0.22 (0.37)	0.08 (0.18)	0.04 (0.15)	0.1 (0.0)	0.01 (0.04)^{ab}	n.d.	0.1 (0.0)	0.1 (0.0)	n.d.	n.d.
20:3ω6	0.69 (0.31)^{bdf}	0.50 (0.27)^{d}	0.23 (0.32)^{acde}	0.5 (0.1)	0.49 (0.86)	1.02 (1.91)	0.6 (0.2)	0.5 (0.1)	n.d.	n.d.
20:4ω6	3.55 (1.98)^{abcdf}	3.50 (2.14)^{abcd}	0.59 (0.62)^{abde}	10.9 (3.8)	2.45 (2.99)^{abd}	4.26 (3.13)^{abcd}	11.1 (3.8)	10.9 (3.8)	n.d.	n.d.
20:5ω3	1.70 (2.12)^{h}	2.37 (2.97)	1.32 (1.93)^{h}	0.3 (0.2)	0.11 (0.28)^{h}	0.30 (0.78)^{h}	0.3 (0.2)	0.3 (0.2)	11.48 (9.93)^{abcdefg}	12.16 (6.44)^{abcfg}
22:0	0.94 (1.16)^{ah}	0.48 (0.27)^{h}	2.30 (1.82)^{abch}	0.4 (0.3)	2.34 (4.53)	1.86 (3.05)	0.3 (0.4)	0.4 (0.3)	5.52 (2.51)^{abcdef}	5.34 (2.01)^{abcdef}
22:4ω6	1.77 (1.09)^{acdfgh}	1.96 (0.98)^{acdfh}	0.08 (0.14)^{abde}	0.5 (0.2)	0.87 (1.09)	0.24 (0.59)^{be}	0.6 (0.2)	0.5 (0.2)	n.d.	n.d.
22:5ω6	1.55 (1.03)^{acdgh}	0.89 (0.76)	0.67 (1.57)	0.4 (0.2)	0.58 (0.65)^{be}	0.32 (0.48)^{b}	0.6 (0.3)	0.4 (0.2)	n.d.	n.d.
22:5ω3	3.06 (2.08)^{acg}	2.88 (1.75)^{acg}	3.63 (2.20)^{acg}	1.7 (0.7)	1.05 (1.11)^{bdef}	0.91 (0.67)^{ade}	1.9 (0.8)	1.7 (0.7)	1.82 (2.08)^{c}	3.17 (2.35)
22:6ω3	10.72 (6.56)^{abcdfgh}	9.38 (5.35)^{abcdfgh}	2.35 (2.13)^{be}	5.5 (2.9)	1.92 (1.40)^{be}	1.90 (1.39)^{be}	5.4 (2.8)	5.5 (2.9)	1.52 (2.59)^{be}	0.84 (1.45)^{be}
Total	100.00	100.00	100.00	100.00	100.00	100.00	97.9 (8.1)	97.9 (8.1)	100.00	100.00

Data are expressed as means (S.D.) of acyl groups expressed as a percentage of the sum of acyl and alkenyl groups. Since PC and PE contain considerable amounts of alkenyl groups (presented in Table 3), the sum of fatty acids does not add up to 100%. Abbreviations: PC, phosphatidyl choline; PE, phosphatidyl ethanolamine; CL, cardiolipin; PI, phosphatidyl inositol; PS, phosphatidyl serine; SM, sphingomyelin; LPC, lysophosphatidyl choline; PL, phospholipids; and n.d., not detected. ^{abcdefgh} Significantly different from PC, PE, CL, PI, PS, SM, LPC and ceramide, respectively ($P < 0.05$).

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Table 3. Molar percentage distribution of aldehydes in muscle PC, PE and total PL of untrained and trained rats

	PC		PE		Total PL	
Aldehyde	Untrained	Trained	Untrained	Trained	Untrained	Trained
16:0	0.56 (0.44)	0.48 (0.28)	5.58 (3.49)[a]	5.38 (2.89)[a]	1.6 (1.1)	1.5 (0.9)
18:0	n.d.	n.d.	1.62 (1.09)	1.72 (1.40)	0.3 (0.2)	0.3 (0.3)
18:1ω9	n.d.	n.d.	0.69 (0.47)	1.09 (1.30)	0.1 (0.2)	0.2 (0.3)
18:1ω7	n.d.	n.d.	0.46 (0.27)	0.37 (0.22)	0.1 (0.1)	0.1 (0.0)
Total	0.56 (0.44)	0.48 (0.28)	8.55 (2.96)[a]	8.57 (2.59)[a]	2.1 (0.8)	2.1 (0.7)

Data are expressed as means (S.D.) of alkenyl groups expressed as a percentage of the sum of acyl and alkenyl groups. Abbreviations: PC, phosphatidyl choline; PE, phosphatidyl ethanolamine; PL, phospholipids; n.d., not detected. [a] Significantly different from PC ($P < 0.001$).

The lack of an effect of chronic exercise on the concentrations and fatty acid profiles of most muscle phospholipids suggests a rigorous homeostasis of structural lipids, in contrast to depot lipids (triacylglycerols), which are markedly affected by exercise training (e.g. Petridou *et al.* 2005). In contrast, it was remarkable to observe a plethora of differences in the percentages of most fatty acids among phospholipids. In fact, three-quarters of these differences have been found in a previous study from this laboratory (Tsalouhidou *et al.* 2006), thus reiterating the importance of the distinct fatty acid profile of each phospholipid in membrane function.

We estimated DNA fragmentation quantitatively by using a technique (EIA of mono- and oligonucleosomes) that is more sensitive than the DNA laddering usually employed (Salgame *et al.* 1997). The absence of difference between untrained and trained rats in the concentration of nucleosomes in the cytoplasm of muscle fibres is in agreement with the results of Siu *et al.* (2004), who found no change in apoptosis (estimated through both EIA and DNA laddering) in rat muscle after 8 weeks of treadmill exercise training. Song *et al.* (2006) found no change in DNA fragmentation (estimated through EIA) in muscle of young rats and reduced DNA fragmentation in muscle of old rats after 12 weeks of treadmill exercise training. In contrast, Boffi *et al.* (2002) found increased apoptosis in horse muscle with treadmill exercise training, while Lim *et al.* (2004) found increased apoptosis in rat muscle after 8 weeks of voluntary wheel running. Comparison with the study of Lim *et al.* (2004), which employed the same species and mode of exercise as ours, is hampered by the fact that Lim *et al.* (2004) did not report the daily running activity of the rats. Thus, the different outcome (increase *versus* no change in apoptosis) may be due to a difference in the amount of exercise stimulus. Alternatively, this and the other discrepancies in the literature cited above may be related to the age of the experimental animals: very young rats (4 weeks old) responded to exercise training by increasing apoptosis (Lim *et al.* 2004), young rats (7–12 weeks old) were unresponsive to exercise training in terms of apoptosis (Siu *et al.* 2004; Song *et al.* 2006 and present study), while old rats (24 months old) responded to exercise training by decreasing apoptosis (Song *et al.* 2006).

When considering the data of the present study as a whole, one may note that the absence of a difference in DNA fragmentation between trained and untrained rats was in accordance with the absence of differences in the concentrations and fatty acid profiles of lipids that have been associated with apoptosis, that is, PC, PE, CL, SM and ceramide. We therefore conclude that voluntary wheel running for 8 weeks did not influence DNA fragmentation and the concentrations or fatty acid profiles of lipids involved in apoptotic signalling in the gastrocnemius muscle of young rats. Given the two aforementioned reports of increased apoptosis with chronic exercise in muscle of very young rats (Lim *et al.* 2004) and decreased apoptosis in muscle of old rats (Song *et al.* 2006), it would be interesting to attempt to confirm the apparent biphasic age-dependent response of apoptosis to exercise in conjunction with an analysis of lipid profiles.

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