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Effect of Exercise Duration on the Key Pathways of Ceramide Metabolism in Rat Skeletal Muscles

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ABSTRACT

Ceramide is the key compound on crossroads of sphingolipid metabolism. The content and composition of ceramides in skeletal muscles have been shown to be affected by prolonged exercise. The aim of this study was to examine the effect of exercise on the activity of key enzymes of ceramide metabolism in skeletal muscles. The experiments were carried out on male Wistar rats (200–250 g) divided into four groups: sedentary, exercised for 30 min, 90 min, and until exhaustion. The activity of serine palmitoyltransferase (SPT), neutral and acid sphingomyelinase (nSMase and aSMase), neutral and alkaline ceramidases (nCDase and alCDase) and the content of ceramide, sphingosine, sphinganine and sphingosine-1-phosphate were determined in three types of muscle. We have found that the activity and expression of SPT increase gradually in each muscle with duration of exercise. These changes were followed by elevation in the content of sphinganine. These data indicate that exercise increases de novo synthesis of ceramide. The aSMase activity gradually decreased with duration of exercise in each type of muscle. After exhaustive exercise the activity of both isoforms of ceramidase were reduced in each muscle. The ceramide level depends both on duration of exercise and muscle type. The ceramide level in the soleus and white gastrocnemius decreased after 30 min of running. After exhaustive exercise it was elevated in the soleus and red gastrocnemius. It is concluded that exercise strongly affects the activity of key enzymes involved in ceramide metabolism and in consequence the level of sphingolipid intermediates in skeletal muscles. J. Cell. Biochem. 105: 776–784, 2008.

KEY WORDS: SERINE PALMITOYLTRANSFERASE; SPHINGOMYELINASE; CERAMIDASE; SPHINGOLIPID INTERMEDIATES; SKELETAL MUSCLE; EXERCISE

Sphingolipids are ubiquitously distributed class of lipids that are present in all higher organisms. Ceramide (CER) is the key compound on crossroads of sphingolipid metabolism. The compound is very active biologically and mediate a number of biological processes, including apoptosis, proliferation, differentiation, growth arrest, inflammation and heat stress response [Ohanian and Ohanian, 2001; Kolesnick and Fuks, 2003; MacRae et al., 2006; Oh et al., 2006]. The variety of cellular effects of ceramide result from the fact that ceramide can alter the activity of kinases, phosphatases and transcription factors [Verheij et al., 1996; Wang et al., 1999; Schubert et al., 2000]. Other sphingolipid intermediates, such as sphingosine (Sph), sphingosine-1-phosphate (S1P), ceramide-1-phosphate are also important second messengers [Spiegel et al., 1994; Pyne and Pyne, 2000; Liang et al., 2003; Pettus et al., 2004; Phillips et al., 2007]. The content of ceramide is determined by a balance between the rate of its formation and degradation. Ceramide is produced in vivo by hydrolysis of sphingomyelin and by de novo biosynthesis (Fig. 1). Sphingomyelin is located in the plasma membrane and in lysosomes and endosomes. Its hydrolysis is catalyzed by the enzyme neutral Mg^{++}-dependent sphingomyelinase (nSMase) and acid sphingomyelinase (aSMase). Both enzymes may be activated by a number of stimuli, for example, inflammatory cytokines, tumor necrosis factor α (TNF-α), interleukin 1 (IL-1), dexamethasone, UV light [Jayadev et al., 1994; Tomiuk et al., 1998; Pfeilschifter and Huwiler, 2000]. De novo synthesis of ceramide occurs at the cytosolic surface of the endoplasmic reticulum [Merrill, 2002]. The process is initiated by condensation of serine with palmitoyl-CoA to generate 3-ketosphinganine. This, the rate-limiting step in de novo sphingolipid biosynthesis is catalyzed by the enzyme serine-palmitoyltransferase (SPT). 3-ketosphingosine is rapidly reduced to sphinganine (SFA) by action of the enzyme 3-ketosphinganine reductase. Next, SFA is acylated to form dihydroceramide by the action of dihydroceramide synthase. The last step of ceramide synthesis is conversion of dihydroceramide to ceramide by insertion of a 4,5-trans-double bond into dihydroceramide. This reaction is

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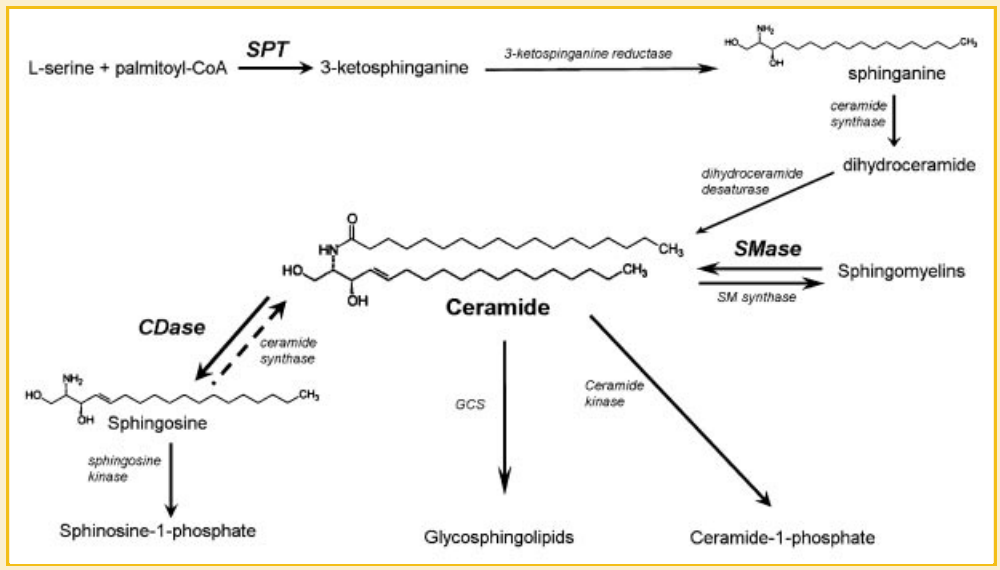

Fig. 1. Overview of ceramide metabolism. SPT, serine palmitoyltransferase; SM synthase, sphingomyelin synthase; SMase, sphingomyelinase; CDase, ceramidase; GCS, glucosylceramide synthase.

catalyzed by the enzyme dihydroceramide desaturase [Merrill, 2002]. Ceramide is hydrolyzed by the enzyme ceramidase (CDase) to yield a free fatty acid and sphingosine. There are three isoforms of ceramidase: acid (aCDase), neutral (nCDase) and alkaline (alCDase). By the use of enzymatic assay and Northern blot analysis almost no activity and mRNA of aCDase was found in skeletal muscle [Li et al., 1998].

Ceramide has been shown to be present in skeletal muscle [Turinsky et al., 1990a; Dobrzyn and Gorski, 2002a; Adams et al., 2004]. Its content in the muscles depends on muscle type. The higher content of ceramide was observed in muscle composed mostly of oxidative fibers than in those composed mostly of glycolytic fibers [Dobrzyn and Gorski, 2002a]. Dobrzyn and Gorski [2002a] demonstrated that prolonged exercise of moderate intensity reduces the content of ceramide in each type of skeletal muscle and this was accompanied by a reduction in the activity of neutral, Mg^{++}-dependent sphingomyelinase in the soleus and red section of gastrocnemius. Different data was obtained in human skeletal muscle, where acute prolonged exercise elevated the content of ceramide [Helge et al., 2004]. It was also reported that prolonged exercise caused elevation in the content of sphingolipid intermediates: sphingosine and sphinganine in rat skeletal muscles [Dobrzyn and Gorski, 2002b].

The only available data on the activity of enzymes of ceramide metabolism in rat skeletal muscle refer to the activity of neutral and acid sphingomyelinase [Dobrzyn and Gorski, 2002a; Gorska et al., 2004]. There is no data on the expression and activity of SPT and the activity of ceramidases in rat skeletal muscle. Therefore aim of the present study was to examine the effect of physical exercise of various duration on the activity of key enzymes of ceramide metabolism in skeletal muscle. The content of selected sphingolipid intermediates was also studied.

MATERIALS AND METHODS

ANIMALS AND STUDY DESIGN

The investigation was approved by the Ethical Committee for Animal Experiments at the Medical University of Bialystok. The experiments were carried out on male Wistar rats (200–250 g) fed ad libitum on a commercial food pallet for rodents. Animals were housed in standard conditions ($21 \pm 2^{\circ}$C, 12 h light/12 h dark cycle) with free access to tap water and food pellets. The animals were randomly divided into four groups ($N = 6$ in each group): (1) sedentary (control), (2) exercised for 30 min, (3) exercised for 90 min, (4) exercised until exhaustion. The rats were made to run on a treadmill set at $+10^{\circ}$ and moving with the speed of 1,200 m/h. They were familiarized with running at the above conditions for 10 min daily during 1 week preceding the final experiment. Exhaustion was regarded as the point at which the rats refused any further running. The running time until exhaustion was 240 ± 30 min Immediately after the exercise, the rats were anesthetized with pentobarbital (80 mg/100 g), and the soleus, the red and white sections of the gastrocnemius were excised, cleaned of any visible adipose tissue, nerves, and fascias and frozen in liquid nitrogen. These muscles are composed predominantly of slow-twitch oxidative, fast-twitch oxidative-glycolytic, and fast-twitch glycolytic fibers, respectively [Sullivan and Armstrong, 1978; Dyck et al., 1997].

THE CONTENT OF SPHINGOSINE, SPHINGANINE AND SPHINGOSINE-1-PHOSPHATE

The content of Sph, SFA and S1P was measured as previously described by the method of Min et al. [2002]. Internal standards (C17-sphingosine and C17-S1P, Avanti Polar Lipids) were added to the samples before homogenization and ultrasonication. Sphingoid

bases were converted to their o-phthalaldehyde derivatives and analyzed on a HPLC system (ProStar, Varian, Inc.), equipped with a fluorescence detector and C18 reversed-phase column (Varian, Inc., OmniSpher 5, 4.6 mm × 150 mm). The isocratic eluent composition of acetonitrile (Merck): water (9:1 v/v) and a flow rate of 1 ml/min were used.

THE CONTENT OF CERAMIDE

Small volume of lipid extract obtained after chloroform extraction was transferred to a fresh tube. Ceramide contained in the organic phase was hydrolyzed in 1 M KOH in 90% at 90°C for 60 min. This digestion procedure does not convert complex sphingolipids, such as sphingomyelin, galactosylceramide or glucosylceramide, into free sphingoid bases [Bose et al., 1998]. Sphingosine liberated from CER was analyzed by means of HPLC as described above. The calibration curve was prepared using N-palmitoylsphingosine (Avanti Polar Lipids) as a standard. The chloroform extract used for the analysis of CER level also contains small amounts of free sphingoid bases. Therefore, the content of ceramide was corrected for the level of free sphingosine determined in the same sample. The efficiency of ceramide hydrolysis was estimated with the use of [N-palmitoyl-1-^{14}C]-sphingosine (Moravek Biochemicals). The values were corrected for the 88% efficiency of ceramide hydrolysis.

THE ACTIVITY OF SPHINGOMYELINASES

The activity of n- and aSMase was determined according to Liu and Hannun [2000]. The activity of both sphingomyelinases was measured with the use of radiolabeled substrate, [N-methyl-14C]-sphingomyelin (Perkin-Elmer Life Sciences). The product of reaction—^{14}C-choline phosphate—was extracted with $CHCl_3$/methanol (2:1, v/v), transferred to scintillation vials and counted using a Pacard TRI-CARB 1900 TR scintillation counter.

THE ACTIVITY OF CERAMIDASES

The activity of alCDase and nCDase was measured by the method of Nikolova-Karakashian and Merrill [2000]. The activity of the enzymes was determined with the use of radiolabeled [N-palmitoyl-1-^{14}C]-sphingosine (Moravek Biochemicals) as a substrate. Unreacted ceramide and liberated ^{14}C-palmitate were separated with the basic Doyle solution (isopropanol/heptane/1 N NaOH, 40:10:1, v/v/v). Radioactivity of the ^{14}C-palmitate was measured by scintillation counting.

THE ACTIVITY OF SERINE PALMITOYLTRANSFERASE

The activity of SPT was examined as described by Merrill [1983] with the use of radiolabeled substrate, [3H]-L-serine (Moravek Biochemicals). Briefly, rat skeletal muscle microsomal fraction was obtained by ultracentrifugation at 150,000*g* for 40 min. Microsomes were incubated for 10 min at 37°C in the reaction buffer (100 mM HEPES (pH 8.3), 5 mM DTT (dithiothreitol), 2.5 mM EDTA (pH 7.0), 50 μM pyridoxal phosphate, 200 μM palmitoyl-CoA and 2 mM L-serine, 44,000 dpm/nmol). The labeled lipid product 3-ketosphinganine was extracted with $CHCl_3$/methanol (1:2, v/v), and the radioactivity was measured by scintillation counting.

EXPRESSION OF SERINE PALMITOYLTRANSFERASE

RT-PCR. The content of serine palmitoyltransferase catalytic subunit (SPT2) mRNA was measured with the use of quantitative real-time PCR. Total RNA was isolated from skeletal samples using TriReagent (Sigma) according to the manufacturer's instructions. Following RNA purification, DNase treatment (Ambion) was performed according to the manufacturer's directions to ensure that there was no contaminating genomic DNA. Extracted RNA was solubilized in RNAse-free water and stored at −80°C until use. The RNA was reverse transcribed into cDNA using iScript cDNA Synthesis Kit (Bio-Rad) with oligo(dT)$_{18}$. Specific primers for the SPT2 subunits were designed using the Beacon Designer Software. The housekeeping gene β-actin was used as the reference gene for quantification. Quantitative real-time PCR was performed with SYBR Green Supermix Kit (Bio-Rad) using a Bio-Rad Chromo4 system. The following primers were used (SPT2) forward 5′ TGTAGAATAAGGCCACAAGGCA-3′ and reverse 5′ AGGACAGAGGACCTGGGTAACA-3′ (β-actin) forward 5′-GAAGATCCTGACCGAGCGTG-3′ and reverse 5′-CGTACTCCTGCTTGCTGATCC-3′. Amplification was carried out for SPT2: 40 cycles, each consisting of 10 s at 95°C, 10 s at 58°C, and 20 s at 72°C. The results were normalized to β-actin expression measured in each sample.

Western blotting. SPT2 amount was determined as described previously [Carton et al., 2003]. Equal amounts of protein (25 μg) were separated by 10% SDS–PAGE. Separated proteins were transferred on nitrocellulose membranes (BioRad). The membrane was probed with anti-SPT2-specific antibody (Cayman Chemicals). Bound antibody was detected after incubation with an alkaline phosphatase-conjugated secondary antibody (Sigma). Protein bands were scanned and quantified using a Gel Doc EQ system (Bio-Rad).

Protein content and plasma free fatty acids (FFA) concentration. Protein content was measured with the BCA protein assay kit (Sigma). Bovine serum albumin (fatty acid free, Sigma) was used as a standard. FFA concentration was determined using the Wako NEFA C kit (Wako Chemicals).

Statistical analysis. All data are presented as means ± SD. Data were analyzed by one-way analysis of variance—ANOVA, followed by Newman–Keuls post-hoc test. P values <0.05 were taken to indicate statistical significance.

RESULTS

PLASMA FFA CONCENTRATION

The plasma FFA concentration increased gradually throughout the exercise. At 30 min of running the concentration of FFA increased from 260 ± 17 nmol ml^{-1} (control group) to 457 ± 65 nmol ml^{-1} ($P < 0.05$). Next at 90th min of exercise the FFA concentration further elevated by 144% ($P < 0.05$) as compared to control group and it reached 636 ± 100 nmol ml^{-1}. The highest concentration of plasma FFA was observed at the point of exhaustion (731 ± 105 nmol ml^{-1}), and it was 2.8-fold higher compared to the control group.

THE CONTENT OF SKELETAL MUSCLE SPHINGOLIPIDS

Sphinganine. Both in the soleus and in the red section of gastrocnemius the level of SFA increased gradually with the duration of the exercise and at the point of exhaustion it was over twofold higher than in the control animals (Fig. 2A). In the white section of gastrocnemius there were no changes in the content of SFA until 90th min of running and at the point of exhaustion it increased by 76% ($P < 0.05$) as compared to the sedentary rats.

Ceramide. The content of ceramide in the soleus decreased after 30 min of exercise (Fig. 2B). Than at 90th min of running it returned to the baseline level and at the point of exhaustion the content of CER increased by 27% ($P < 0.05$) in relation to the sedentary animals.

The level of ceramide in the red section of gastrocnemius was stable until 90th min of exercise, but at the point of exhaustion its content increased by 27% ($P < 0.05$) as compared to the control animals.

In white section of gastrocnemius the ceramide content decreased gradually until 90th min of exercise where the CER level was 36% ($P < 0.05$) lower than in the control group, but at the point of exhaustion the content of ceramide returned to the control value.

Sphingosine. The level of Sph in the soleus increased after 30 min of running and remained at this level until exhaustion (Fig. 2C).

In the red section of gastrocnemius at 30th min of exercise, the content of Sph increased by 73% ($P < 0.05$) as compared to the control. Next at 90th min of running the sphingosine level decreased and remained at this level until exhaustion being still higher than at rest.

In the white section of gastrocnemius the content of Sph increased by 22% ($P < 0.05$) after 30 min of exercise and was stable at this level until exhaustion.

Sphingosine-1-phosphate. In the soleus there were no changes in the content of S1P until 90th min of running and at the point of exhaustion it was twofold higher than in the sedentary animals (Fig. 2D).

In the red section of gastrocnemius the level of S1P slightly increased after 30 min of exercise. Then it returned to the control level after 90 min of exercise. However, at the point of exhaustion the content of S1P increased again and was 81% ($P < 0.05$) higher as compared to the sedentary rats.

In the white section of gastrocnemius the level of S1P decreased at 90th min of running by 72% ($P < 0.05$), but it returned to the control value at the point of exhaustion.

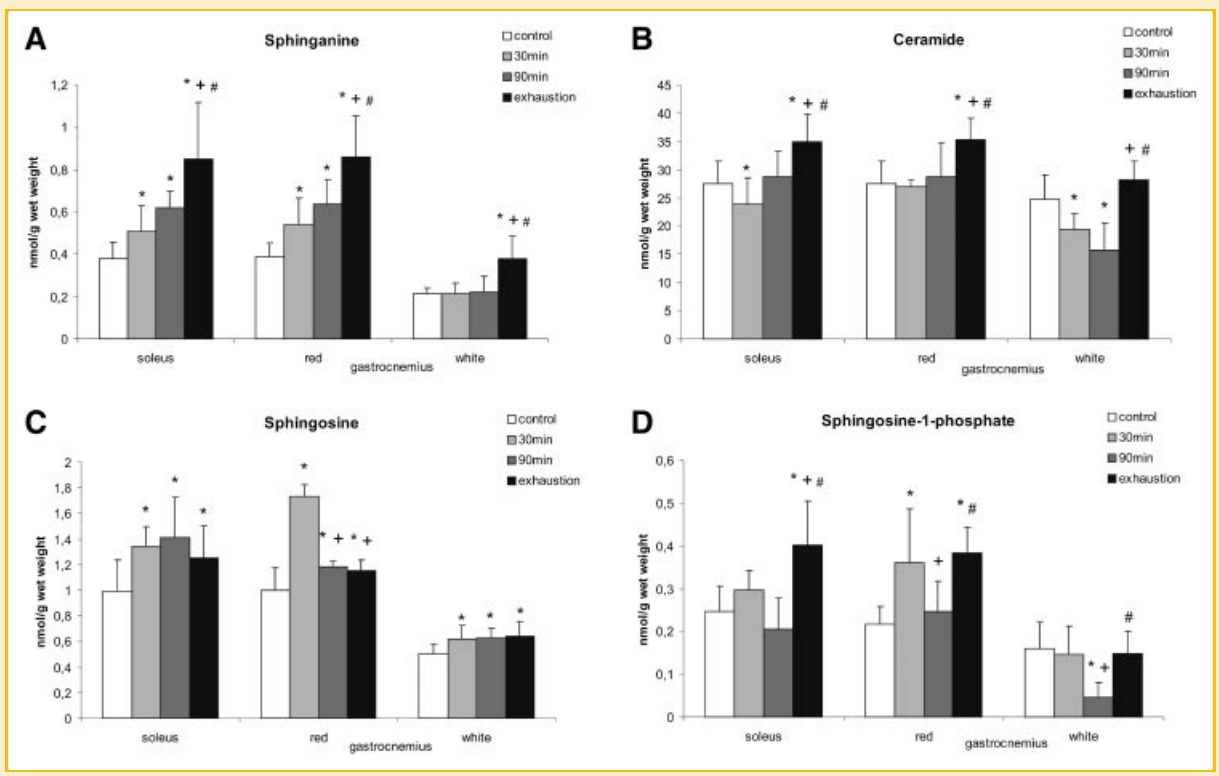

Fig. 2. Effect of exercise of various duration on the content of sphinganine (A), ceramide (B), sphingosine (C) and sphingosine-1-phosphate (D) in three types of rat skeletal muscles. Values are means ± SD (n = 6 in each group). The rats were either sedentary (control) or exercised for 30 min, 90 min or until exhaustion on the electrically driven treadmill as described in Materials and Methods Section. $^{*}P < 0.05$ versus the control group, $^{+}P < 0.05$ versus the group exercised for 30 min, $^{\#}P < 0.05$ versus the group exercised for 90 min.

THE ENZYMES OF CERAMIDE METABOLISM

Serine palmitoyltransferase activity. The activity of SPT in the soleus increased gradually with the duration of the exercise (Fig. 3A). After 30 min of running the activity of SPT increased by 41% ($P < 0.05$), at 90th min of exercise by 82% ($P < 0.05$) and at the point of exhaustion it was twofold higher than in the control group.

In red section of gastrocnemius, the activity of SPT increased after 30 min of exercise by 42% ($P < 0.05$). At 90th min of running the enzyme activity was 61% ($P < 0.05$) higher as compared to the sedentary animals and it increased further at the point of exhaustion exceeding the control value by 78% ($P < 0.05$).

In the white section of gastrocnemius the activity of SPT increased gradually during exercise, but it was significantly higher than the control value only at exhaustion.

Expression of SPT. The content of SPT mRNA in the soleus increased with the duration of exercise. After 30 min of running the mRNA content was higher by 26% ($P < 0.05$) than in the control group. At 90th min and at the point of exhaustion the mRNA content was two- and threefold higher, respectively than in the sedentary animals (Fig. 3B). The content of SPT2 protein increased gradually by 16%, 39%, and 64% ($P < 0.05$) after 30 min, 90 min, and exhaustion, respectively, as compared to the control value (Fig. 3C).

The level of SPT2 mRNA in the red section of gastrocnemius also increased. After 30 min of exercise the mRNA content was 52% higher than the control value. After 90 min, and after exhaustive exercise the mRNA content was respectively two- and threefold higher than in the control animals (Fig. 3B). The content of SPT2 protein increased along with the duration of exercise by 20%, 34%, and 53% respectively at 30th, 90th min and at the point of exhaustion (Fig. 3C).

The content of mRNA in the white section of gastrocnemius increased gradually with the duration of the exercise and at 30th min, 90th min and at the point of exhaustion it was 20%, 41%, and 87% ($P < 0.05$) higher than the respective control value (Fig. 3B). The level of SPT2 protein was also elevated, but only after exhaustive exercise the value was statistically higher than the control value (Fig. 3C).

SPHINGOMYELINASES

Neutral sphingomyelinase activity. In the soleus the activity of nSMase decreased by 19% ($P < 0.05$) after 30 min of exercise and remained at this level until exhaustion (Fig. 4A).

The first 90 min of exercise did not change the activity of nSMase in the red section of gastrocnemius. At the point of exhaustion the activity of the enzyme decreased in this muscle by 24% ($P < 0.05$) as compared to the control value.

In the white section of gastrocnemius, 30 min of running reduced the activity of nSMase by 23% ($P < 0.05$) but at the point of exhaustion the activity of this enzyme returned to control value.

Acid sphingomyelinase activity. In the soleus the activity of aSMase decreased at 90th min of exercise by 41% ($P < 0.05$) and at the point of exhaustion by 61% ($P < 0.05$) compared to the control rats (Fig. 4B).

In the red section of gastrocnemius the activity of aSMase decreased significantly only at exhaustion.

After 90 min of exercise the activity of aSMase in the white section of gastrocnemius was lower by 28% ($P < 0.05$) and at the point of exhaustion it was 54% ($P < 0.05$) lower compared to the control rats.

CERAMIDASES

Neutral ceramidase activity. The activity of nCDase in the soleus increased by 65% ($P < 0.05$) after 30 min of exercise, then at 90th min it returned to the control value and at the point of exhaustion the activity of the enzyme decreased, being 24% ($P < 0.05$) lower as compared to the control rats (Fig. 5A).

In both section of gastrocnemius the activity nCDase decreased only at the point of exhaustion ($P < 0.05$).

Alkaline ceramidase activity. The activity of alkaline ceramidase (alCDase) in the soleus decreased by 21% ($P < 0.05$) after 90 min of exercise and remained at this level until exhaustion (Fig. 5B).

In the red section of gastrocnemius the activity of alCDase increased by 21% ($P < 0.05$) after 30 min of exercise but at 90th min of running it returned to the control value and at the point of exhaustion it was 43% lower as compared to the sedentary animals.

The activity of alCDase in the white section of gastrocnemius decreased by 15% ($P < 0.05$) after 90 min of exercise and this trend continued to the point of exhaustion where the enzyme activity dropped below the control value by 24% ($P < 0.05$).

DISCUSSION

There are very few data in the literature concerning the effect of exercise on the activity of the key enzymes of ceramide metabolism in skeletal muscle. Our study is the first to describe the activity and expression of SPT in rat skeletal muscle both at the rest and during exercise. At rest, the activity of the enzyme in the soleus and the red gastrocnemius was similar and was higher than in the white gastrocnemius. The activity of SPT increased gradually during exercise in the soleus and the red section of gastrocnemius. In the white section of gastrocnemius the activity of SPT increased only after exhaustive exercise. It has been also observed that the content of sphinganine (the key intermediate of ceramide synthesis de novo) corresponded to SPT activity in each type of muscle. The accumulation of SFA in skeletal muscles of rats subjected to exhaustive exercise was observed also in the former study [Dobrzyn and Gorski, 2002b]. It was suggested that the de novo ceramide synthesis is rather a slow process [Mathias et al., 1998; Schmitz-Peiffer et al., 1999]. This study shows that the SPT activity, in the soleus and in the red section of gastrocnemius, increases already after 30 min of running. We also showed that not only SPT activity but also the content of mRNA SPT2 and protein SPT2 increased with duration of running. It suggests that exercise-induced activation of SPT was a result of the increased expression of SPT2 (catalytic subunit). Moreover in the study by Gorski et al. [2002] on incorporation of the blood-borne labeled fatty acids into skeletal muscle ceramide, the label was found in the ceramide pool already in 10 min after its administration into the tail vein. It indicates that

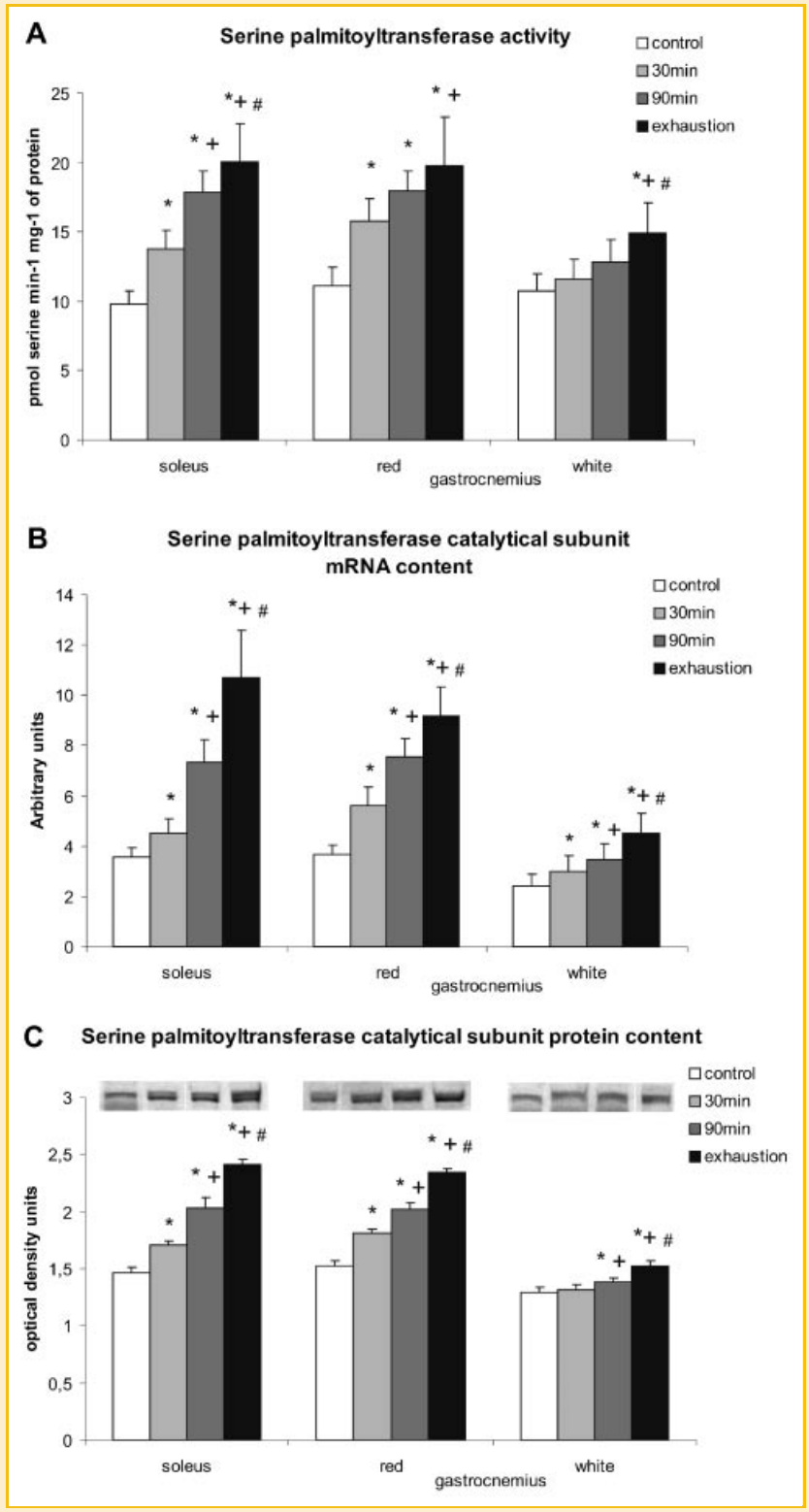

Fig. 3. Effect of exercise of various duration on the activity (A) and expression (B,C) of serine palmitoyltransferase in three types of rat skeletal muscles. Values are means ± SD (n = 6 in each group). The rats were either sedentary (control) or exercised for 30 min, 90 min or until exhaustion on the electrically driven treadmill as described in Materials and Methods Section. $^{*}P < 0.05$ versus the control group, $^{+}P < 0.05$ versus the group exercised for 30 min, $^{\#}P < 0.05$ versus the group exercised for 90 min.

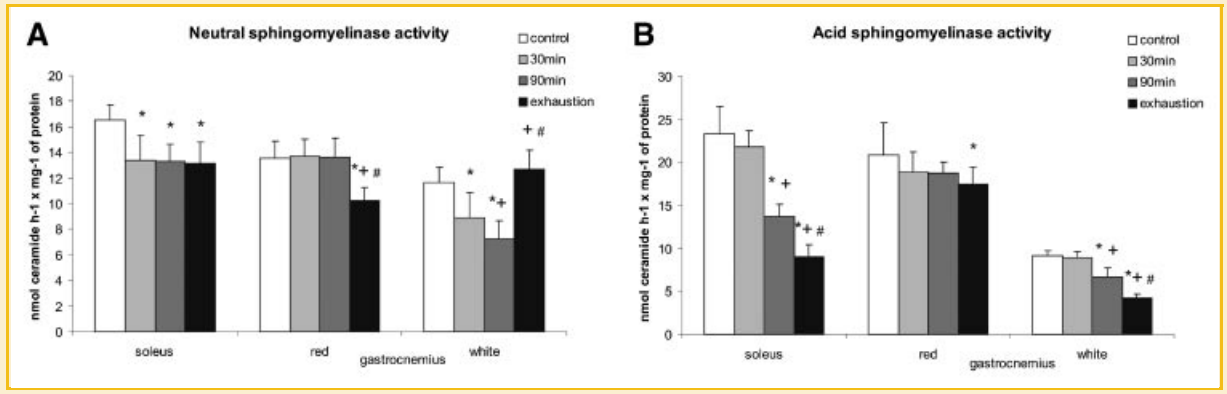

Fig. 4. Effect of exercise of various duration on the activity of neutral (A) and acid (B) sphingomyelinase in rat skeletal muscle. Values are means ± SD (n = 6 in each group). The rats were either sedentary (control) or exercised for 30 min, 90 min or until exhaustion on the electrically driven treadmill as described in Materials and Methods Section. $^{*}P < 0.05$ versus the control group, $^{+}P < 0.05$ versus the group exercised for 30 min, $^{\#}P < 0.05$ versus the group exercised for 90 min.

activation of de novo synthesis of ceramide is rather a fast process. As mentioned in introduction, the main substrate in de novo ceramide synthesis, beside serine, is palmitoyl-CoA. It is widely accepted that increased availability of extracellular fatty acids leads to accumulation of ceramide in muscle tissue due to augmentation of its de novo synthesis [Summers, 2006]. In vitro studies on rat astrocytes and pancreatic islets have shown that incubation with palmitate increases the activity and expression of SPT [Shimabukuro et al., 1998; Blazquez et al., 2001]. Therefore, activation of SPT, observed in our study, could be a result of increased plasma FFA availability which occurred during the exercise. The elevation in the activity of SPT and the SFA content during exercise strongly indicate that physical exercise augments de novo ceramide synthesis in the muscles.

The changes in nSMase activity during exercise observed in our study are compatible with those observed by Dobrzyn and Gorski [2002a]. In both works, prolonged exercise caused a reduction in the enzyme activity in the soleus and the red section of gastrocnemius, but not in the white gastrocnemius. It should be added that also in human skeletal muscle the reduction of nSMase activity after exercise was observed [Helge et al., 2004]. There are no data available on the effect of exercise on activity of aSMase in skeletal muscles. At rest, the activity of aSMase was reported to be highest in the soleus and lowest in the white section of the gastrocnemius [Gorska et al., 2004] and it was confirmed presently. Now, we showed that the activity of aSMase gradually decreased in each type of muscle during exercise. The reduction in the activity of both neutral and acidic isoform of SMase would lead to reduced hydrolysis of sphingomyelin and thus decreased production of ceramide on this way. In the present work, for the first time, the data on the activity of ceramidases in rat skeletal muscle at rest and during exercise are provided. We demonstrated, that the activity of alCDase decreased with the duration of exercise in each type of muscle. Acidification of skeletal muscle during exercise is the most likely reason for the inhibition of the enzyme activity. The reduction in the activity of CDase would contribute to reduction in hydrolysis of CER.

It has been reported that different factors can influence on the ceramide level in skeletal muscles. It was elevated in the muscle of obese, insulin-resistant Zucker rats [Turinsky et al., 1990b]. It was

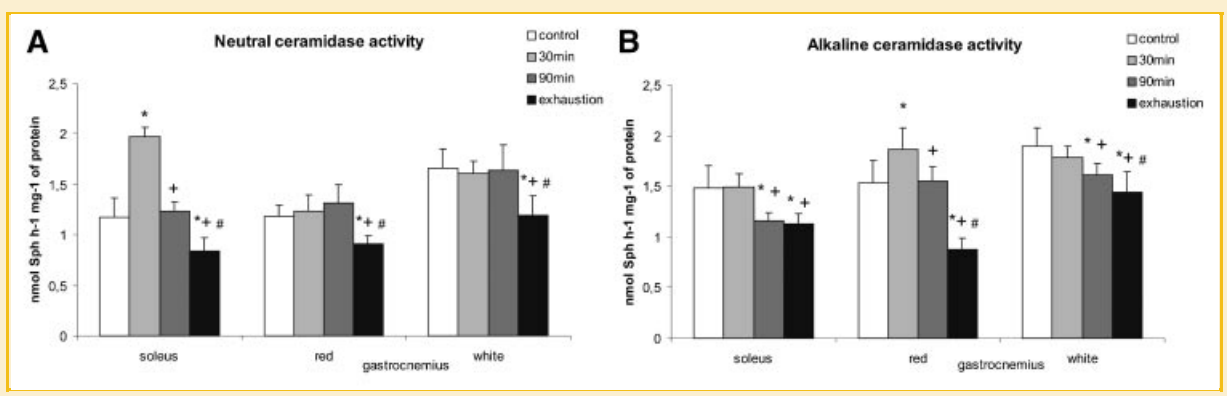

Fig. 5. Effect of exercise of various duration on the activity of neutral (A) and alkaline (B) ceramidase in rat skeletal muscle. Values are means ± SD (n = 6 in each group). The rats were either sedentary (control) or exercised for 30 min, 90 min or until exhaustion on the electrically driven treadmill as described in Materials and Methods Section. $^{*}P < 0.05$ versus the control group, $^{+}P < 0.05$ versus the group exercised for 30 min, $^{\#}P < 0.05$ versus the group exercised for 90 min.

also demonstrated in humans that the content of ceramide in skeletal muscle was inversely related to insulin sensitivity [Straczkowski et al., 2004]. There are few published data on the effect of physical activity on the ceramide content. The present study shows that the content of ceramide in the muscle depends both on duration of exercise and muscle type. It was reduced in the soleus and white gastrocnemius after 30 min exercise and elevated in the soleus and red section of the gastrocnemius at exhaustion. These data differ from those obtained by Dobrzyn and Gorski [2002a] where prolonged exercise resulted in a reduction in the content of ceramide in the muscles. An explanation for this discrepancy may be different analytical method chosen for the measuring of ceramide content. Dobrzyn and Gorski [2002a] determined the content of ceramide-fatty acid residues using gas–liquid chromatography. The ceramide fraction was isolated by means of thin layer chromatography (TLC). The procedure of TLC separation includes ceramides with different sphingolipid bases (including also dihydroceramide, phytoceramide and other) [Schulze et al., 2000; van Echten-Deckert, 2000; Signorelli and Hannun, 2002]. In the present work only the level of ceramide containing sphingosine backbone was measured. This fact could also explain the reason of higher ceramide content reported by Dobrzyn and Gorski [2002a] as compared to the present results. It is possible that prolonged exercise did not affect the total content of ceramide in skeletal muscle, but only specific pool of this compound containing sphingosine.

Experimental studies revealed that ceramide impairs insulin stimulated glucose uptake in the muscles by maintaining protein kinase B (PKB)/Akt in an inactive dephosphorylated state [Schmitz-Peiffer et al., 1999; Stratford et al., 2004]. A consequence of PKB/Akt inhibition is a reduction of glucose transporter 4 (GLUT4) translocation to the plasma membrane and a reduction in insulin-stimulated glucose uptake [Summers et al., 1998]. Numerous reports showed that short-term exercise improves the insulin action on glucose uptake [Wahren et al., 1978; Richter et al., 1988; Bloem and Chang, 2008]. The reduction in the content of ceramide seen after 30 min of exercise in the present study could contribute to this phenomenon. During long-term exercise the glucose uptake decreases [Stein et al., 1989]. Undoubtedly, it is a consequence of reduced glucose availability. Elevated plasma free fatty acid concentration is claimed to be responsible for the reduction in the glucose uptake during this type of exercise [Hargreaves et al., 1991]. The results obtained by Helge et al. [2004] and the present data strongly suggest that increased content of ceramide could add to the effect of fatty acids.

It was presently shown that prolonged exercise increases the content of S1P in the soleus and the red gastrocnemius. No data in the literature are available to be compared with these results. Danieli-Betto et al. [2005] demonstrated that sphingosine-1-phosphate exerts a protective effect against development of fatigue in skeletal muscle. Therefore, our data indicate that the accumulation of S1P could be a factor delayed the muscles fatigue during long-term exercise.

In summary, we have shown a gradual elevation in the SPT activity and SPT2 expression in different skeletal muscle types during prolonged exercise. It was accompanied by elevation in the content of sphinganine. These data strongly indicate that the exercise increases de novo synthesis of ceramide. Moreover, we showed gradual reduction in the activity of aSMase during exercise in each type of studied muscle. Besides this, exhaustive exercise caused a reduction in the activity of the neutral and alkaline ceramidase. It is concluded that exercise strongly affects the activity of the key enzymes involved in ceramide metabolism and in consequence the level of sphingolipid intermediates in skeletal muscles.

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