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PHOSPHORYLATION POTENTIAL IN THE DOMINANT LEG IS LOWER, AND [ADP_{FREE}] IS HIGHER IN CALF MUSCLES AT REST IN ENDURANCE ATHLETES THAN IN SPRINTERS AND IN UNTRAINED SUBJECTS

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It has been reported that various types of mammalian muscle fibers differ regarding the content of several metabolites at rest. However, to our knowledge no data have been reported in the literature, concerning the muscle energetic status at rest in high class athletes when considering the dominant and non-dominant leg separately. We have hypothesised that due to higher mechanical loads on the dominant leg in athletes, the metabolic profile in the dominant leg at rest in the calf muscles, characterized by [PCr], [ADP_{free}], [AMP_{free}] and ΔG_{ATP}, will significantly differ among endurance athletes, sprinters and untrained individuals. In this study we determined the ΔG_{ATP} and adenine phosphates concentrations in the dominant and non-dominant legs in untrained subjects (n = 6), sprinters (n = 10) and endurance athletes (n = 7) at rest. The (mean ± SD) age of the subjects was 23.4 ± 4.3 years. Muscle metabolites were measured in the calf muscles at rest, by means of ^{31}P-MRS, using a 4.7 T superconducting magnet (Bruker). When taking into account mean values in the left and right leg, phosphocreatine concentration ([PCr]) and ΔG_{ATP} were significantly lower ($p<0.05$, Wilcoxon-Mann-Whitney test), and [ADP_{free}] was significantly higher ($p = 0.04$) in endurance athletes than in untrained subjects. When considering the differences between the left and right leg, [PCr] in the dominant leg was significantly lower in endurance athletes than in sprinters ($p = 0.01$) and untrained subjects ($p = 0.02$) (25.91 ± 2.87 mM; 30.02 ± 3.12 mM and 30.71 ± 2.88 mM, respectively). The [ADP_{free}] was significantly higher ($p = 0.02$) in endurance athletes than in sprinters and untrained subjects ($p = 0.02$) (42.19 ± 13.44 μM; 27.86 ± 10.19 μM; 25.35 ± 10.97 μM, respectively). The ΔG_{ATP} in the dominant leg was significantly lower ($p = 0.02$) in endurance athletes than in sprinters and untrained subjects ($p = 0.01$) (-60.53 ± 2.03 kJ·M^{-1}; -61.82 ± 1.05 kJ·M^{-1}, -62.29 ± 0.73 kJ·M^{-1}, respectively). No significant

differences were found when comparing [PCr], [ADP_{free}], [AMP_{free}], [Mg^{2+}_{free}], ΔG_{ATP} in the dominant leg and the mean values for both legs in sprinters and untrained subjects. Moreover, no significant differences were found when comparing the metabolites in non-dominant legs in all groups of subjects. We postulate that higher [ADP_{free}] and lower ΔG_{ATP} at rest is a feature of endurance-trained muscle. Moreover, when studying the metabolic profile of the locomotor muscles in athletes one has to consider the metabolic differences between the dominant and non-dominant leg.

Key words: *adenine phosphates, athletes, human muscles, NMR, phosphorylation potential*

INTRODUCTION

It is well known that human skeletal muscles are composed of various types of muscle fibers that possess different histological (1, 2) and functional characteristics (3 - 5). Moreover, it is well documented that muscle fiber composition of the calf muscles differs significantly when comparing sprinters, endurance athletes and untrained subjects (1, 6).

It has been reported that various types of mammalian muscle fibers also differ regarding the content of several metabolites at rest (7), and regarding the regulation of metabolism during exercise (8, 9). It is well established that ATP concentrations in various types of human muscle fibers at rest are almost identical (10 - 12), however phosphocreatine (PCr) concentration in type II muscle fibers is significantly higher than in type I muscle fibers (10, 12 - 14).

It has been shown that the expression of various types of myosin heavy chain isoforms, determining the functional characteristics of muscles, can be modified already in a relatively short period of time by various kinds of physical training (15 - 18). Moreover, it has been reported that training-induced transformation of fast-to-slow muscle fibers was accompanied by a significant decrease in [PCr] and [ATP]/[ADP_{free}] ratio in the transformed muscles, as compared to the control (19).

However, little is known regarding the metabolic status at rest expressed by phosphorylation potential (ΔG_{ATP}), concentrations of free adenosine diphosphate (ADP_{free}) and adenosine monophosphate (AMP_{free}) in the locomotor muscles in highly trained athletes of various specializations (*e.g.* sprint athletes *vs.* endurance athletes). Moreover, surprisingly, to our knowledge no data have been reported in the literature concerning the muscle energetic status at rest in high class athletes when taking into consideration the dominant and non-dominant leg separately. Since, as demonstrated previously, the mechanical load exerted on the muscles can modify muscle fibres composition (15 - 18) and muscle metabolic status at rest (19), in the present study we hypothesized that due to higher mechanical loads on the dominant leg in athletes, the metabolic profile in the dominant leg at rest in the calf muscles, characterized by [PCr], [ADP_{free}], [AMP_{free}] and ΔG_{ATP}, will significantly differ from that of endurance athletes, sprinters and untrained individuals.

METHODS

Subjects

Twenty three subjects: sprinters (9 men and 1 woman), endurance athletes (7 men) and untrained subjects (6 men) participated in this study. The mean (± S.D.) age of sprinters was 22.2 ± 2.7 years, of endurance athletes 24.7 ± 6.4 years and untrained subjects 23.8 ± 3.4 years. Body mass of sprinters was 77.3 ± 5.1 kg, of endurance athletes 66.4 ± 5.0 kg and untrained subjects 75.2 ± 7.3 kg. Body height of sprinters was 184.2 ± 7.3 cm, of endurance athletes 178.4 ± 6.8 cm and untrained subjects 183.6 ± 7.7 cm.

National and top international level athletes, including Olympic champion and a finalist of the world championship in athletics participated in this study. The subjects reported to the laboratory in the morning after a night's rest and usual breakfast. The day before only mild training was allowed. ^{31}P-MRS spectra were acquired from both legs in a random order. The dominant leg was identified as the leg the subject would use to kick a ball and step up onto a step. Additionally the athletes (sprinters and endurance athletes) were asked which leg they preferred to execute the take-off while performing a long and/or high jump. The leg that was used to perform the tasks was deemed dominant.

The subjects were aware of the aims of the study and gave informed written consent. Local Ethic Committee approved this study.

^{31}P NMR spectroscopy

^{31}P MR signal was acquired on a MRI research system equipped with a 4.7 T/310 mm horizontal bore superconducting magnet (Bruker) and a digital MARAN DRX console (Resonance Instruments). A dedicated, home built ^{31}P/^{1}H (81.05 MHz/200 MHz) double tuned MR probehead with a two turn 50 mm ID surface coil for muscle study was used. The probehead was placed in the magnet in such a position that the surface coil was located 10 cm distal of the *fossa poplitea* under the calf to acquire MR signal from the *gastrocnemius* and *soleus* muscles.

MR signal was acquired with a pulse-acquisition sequence using a single square RF pulse with repetition time TR = 5 s. 16 k data points of ^{31}P MR signal (FID) were recorded with the dwell time of 10 μs. Power and duration of RF pulse were calibrated using phantoms to obtain the ^{1}H and ^{31}P signals from the region of interest 15 mm under the surface of the calf. The main magnetic field over the volume of interest was corrected manually (so called "shimming") to achieve homogeneity of about 0.1 - 0.2 ppm using proton signal from muscle water. Signal acquisition for a single volunteer was performed twice, separately for both legs. MR signal from the resting calf muscles was accumulated for 10 min to obtain the best signal to noise ratio.

MR data sets were analyzed in the time-domain using the MRUI 97.1 software package. The signals were filtered to obtain the 130 ppm spectrum width. Homonuclear reference deconvolution was performed using fitted PCr signal. Then the signals were fitted in the time domain using the AMARES algorithm and prior knowledge of ATP signal components. α and γ ATP were fitted as doublets with amplitude ratio 1:1 and β ATP as a triplet with amplitude ratios 0.5:1:0.5 all with line splitting of 17 Hz and equal line width and phase. The amplitudes were T_1 corrected and relative concentrations [P_i]/[ATP], [PCr]/[ATP] and chemical shifts of every spectral component were assessed.

Calculations of the derivative parameters

Absolute concentrations of PCr and P_i were obtained by assuming that the concentration of ATP in calf muscle at rest is 8.2 mM (20 - 23) and of [TCr] - 42 mM (21, 22).

Intracellular pH was calculated applying the Henderson-Hasselbalch equation with pK = 6.75, δ_1 = 3.27, δ_2 = 5.69 (20), which utilizes dependence of pH on P_i chemical shift δ_{Pi} relative to PCr chemical shift δ_0.

$$pH = 6.75 + \log\left(\frac{\delta - 3.27}{5.69 - \delta}\right) \qquad (1)$$

where: δ is an experimental value $\delta = \delta_{Pi} - \delta_0$ expressed in ppm.

pMg and hence concentration of free cytosolic magnesium ($[Mg^{2+}]$) was calculated using the equation (*2*) and calibration constants provided by Iotti *et al.* (24). It involves the dependence of free magnesium concentration on chemical shift of βATP.

$$pMg = y_0 + \frac{c_1}{c_2 - \exp\left(\frac{\delta_\beta - \delta_0}{d}\right)} + a_1\left(\delta_\beta - \delta_0\right) + a_2\left(\delta_\beta - \delta_0\right)^2 + b\left([PCr] - 27\right) \qquad (2)$$

where:
$d = D_1 + D_2\,(pH - 7)^2$
$a_1 = A_1 + A_2\,(pH - 7)$

with calibration constants:

y_0 = 10.43, A_1 = - 0.20 ppm^{-1}, A_2 = - 0.29 ppm^{-1}, D_1 = 0.105 ppm^{-1}, D_2 = 0.22 ppm^{-1}, a_2 = 0.128 ppm^{-2}, b = - 0.005 mM^{-1}, c_1 = - 25.66, c_2 = 3.728

and with experimental values pH, [PCr] and δ_β, δ_0 (chemical shifts of βATP and PCr respectively).

$[ADP_{free}]$ was calculated using the formulas (*3* - *7*) and constants according to Kemp *et al*. (23). $[K^+]$ was assumed as a constant value of 0.11 M.

$$\left[ADP_{free}\right] = \left[ATP\right] \cdot \left(\frac{\left[TCr\right]}{\left[PCr\right]} - 1\right) \cdot \frac{1}{K_{CK}^{app}} \cdot \frac{1}{\left[H^+\right]} \qquad (3)$$

$$K_{CK}^{app} = K_{CK}^{true} \cdot \frac{f_{ADP} \cdot f_{PCr}}{f_{ATP}} \qquad (4)$$

$$\frac{1}{f_{ATP}} = 1 + \frac{\left[Mg^{2+}\right]}{K_{Mg\,ATP}} + \frac{\left[K^+\right]}{K_{K\,ATP}} + \frac{\left[H^+\right]}{K_{H\,ATP}} \cdot \left\{1 + \frac{\left[Mg^{2+}\right]}{K_{Mg\,HATP}} + \frac{\left[H^+\right]}{K_{H_2\,ATP}}\right\} \qquad (5)$$

$$\frac{1}{f_{ADP}} = 1 + \frac{\left[Mg^{2+}\right]}{K_{Mg\,ADP}} + \frac{\left[K^+\right]}{K_{K\,ADP}} + \frac{\left[H^+\right]}{K_{H\,ADP}} \cdot \left\{1 + \frac{\left[Mg^{2+}\right]}{K_{Mg\,HADP}} + \frac{\left[H^+\right]}{K_{H_2\,ADP}}\right\} \qquad (6)$$

$$\frac{1}{f_{PCr}} = 1 + \frac{\left[Mg^{2+}\right]}{K_{Mg\,PCr}} + \frac{\left[K^+\right]}{K_{K\,HPCr}} + \frac{\left[H^+\right]}{K_{H\,PCr}} \cdot \left\{1 + \frac{\left[H^+\right]}{K_{H_2PCr}} + \frac{\left[K^+\right]}{K_{KH_2PCr}}\right\} \qquad (7)$$

$[AMP_{free}]$ was calculated by using the formulas (*8* - *10*) and constants according to Kemp *et al.* (23).

$$\left[AMP_{free}\right] = \frac{K_{AK}^{app} \cdot \left[ADP_{free}\right]^2}{\left[ATP\right]} \quad (8)$$

$$K_{AK}^{app} = K_{AK}^{true} \cdot \frac{\left[f_{ADP}\right]^2 \cdot f_{AMP}}{f_{ATP}} \quad (9)$$

$$\frac{1}{f_{AMP}} = 1 + \frac{\left[Mg^{2+}\right]}{K_{Mg\,AMP}} + \frac{\left[K^{+}\right]}{K_{K\,AMP}} + \frac{\left[H^{+}\right]}{K_{H\,AMP}} \quad (10)$$

ΔG_{ATP} was calculated by using the formulas (*11 - 13*) and constants according to Kemp *et al.* (23).

$$\Delta G_{ATP} = \Delta G_{ATP}^{0} + RT \ln \frac{\left[ADP_{free}\right] \cdot \left[P_i\right]}{\left[ATP\right]} \quad (11)$$

$$-\Delta G_{ATP}^{0} = RT \left[\ln\left(K_{ATP}^{true}\right) + \ln\left(\frac{f_{ATP}}{f_{ADP}\ f_{Pi}} \cdot \frac{1}{\left[H^{+}\right]} \right) \right] \quad (12)$$

$$\frac{1}{f_{Pi}} = 1 + \frac{\left[Mg^{2+}\right]}{K_{Mg\,Pi}} + \frac{\left[K^{+}\right]}{K_{K\,Pi}} + \frac{\left[H^{+}\right]}{K_{H\,Pi}} \cdot \left\{ 1 + \frac{\left[H^{+}\right]}{K_{H_2\,Pi}} + \frac{\left[K^{+}\right]}{K_{KH\,Pi}} \right\} \quad (13)$$

where:
R is a gas constant = 8.3145 $J \cdot K^{-1}\ M^{-1}$
T is an absolute temperature in Kelvin = 310 K

Statistics

Data are presented as mean (± S.D.). Significance in differences was tested using the Wilcoxon-Mann-Whitney test (exact, 2-sided p-value) and Wilcoxon Signed Rank test (exact, 2-sided p-value).

RESULTS

(a). The mean (± S.D.) values of muscle pH, [PCr], [P_i], [ADP_{free}], [AMP_{free}], [$Mg^{2+}{}_{free}$], ΔG_{ATP} determined in the calf muscle at rest, expressed as a mean value for both legs in sprinters, endurance trained athletes and untrained subjects

Muscle pH expressed as a mean value for both legs at rest amounting to 7.052 ± 0.016 in sprinters, 7.038 ± 0.018 in endurance athletes and 7.043 ± 0.029 in untrained subjects was not significantly different in all groups of subjects. PCr concentration in endurance athletes amounting to 26.74 ± 2.79 mM was significantly ($p = 0.04$) lower than in untrained subjects (30.37 ± 3.25 mM). No significant difference was found between the [PCr] in sprinters amounting to 29.85 ± 3.06 mM and in endurance athletes (*Fig. 1*). P_i concentration amounting

to 3.60 ± 0.74 mM in sprinters, 4.50 ± 2.14 mM in endurance athletes and 3.36 ± 0.65 mM in untrained subjects was not significantly different among all groups of subjects. ADP_{free} concentration in endurance athletes amounting to 39.26 ± 12.34 μM was significantly higher (p = 0.04) than in untrained subjects (25.43 ± 12.31 μM). No significant difference was found between [ADP_{free}] in sprinters (28.86 ± 9.33 μM) and in endurance athletes (*Fig. 2*). AMP_{free} concentration in endurance athletes amounting to 0.148 ± 0.092 μM was significantly higher (p = 0.04) than in untrained subjects (0.067 ± 0.071 μM) (p = 0.04) (*Fig. 3*).The AMP_{free} in sprinters amounting to 0.080 ± 0.039 μM was not significantly different when compared to endurance athletes and untrained subjects. Muscle [Mg^{2+}_{free}]

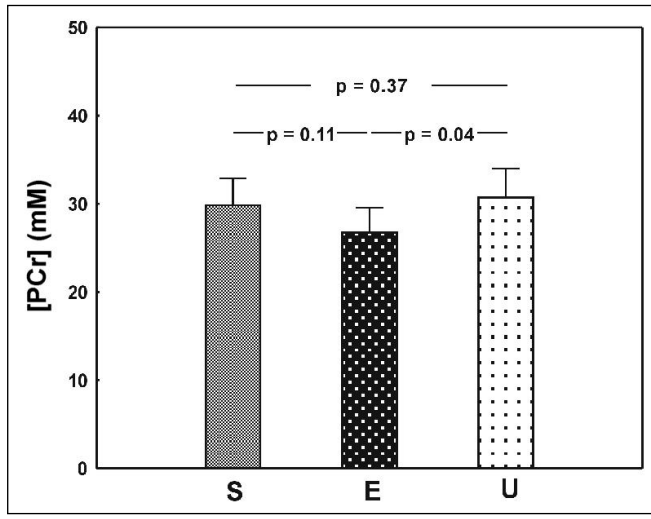

Fig. 1. Mean (± S.D.) concentration of PCr in calf muscles, expressed as mean values for both legs, in sprinters (S), endurance athletes (E) and untrained subjects (U). The Wilcoxon-Mann-Whitney test was used to test for significance of differences.

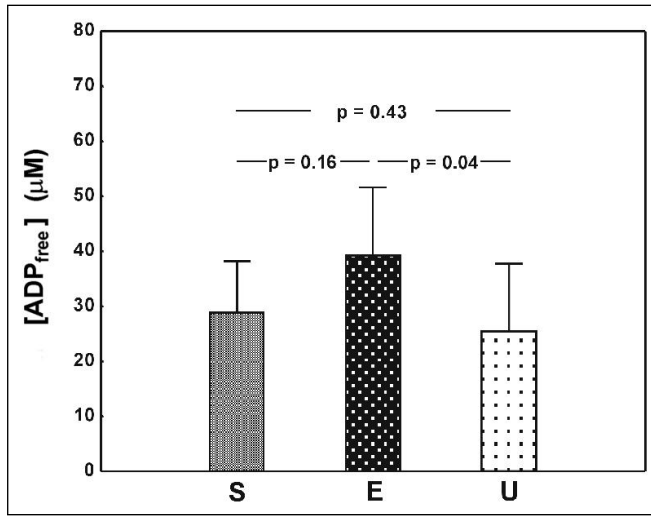

Fig. 2. Mean (± S.D.) concentration of free ADP in calf muscles, expressed as mean values for both legs, in sprinters (S), endurance athletes (E) and untrained subjects (U). The Wilcoxon-Mann-Whitney test was used to test for significance of differences.

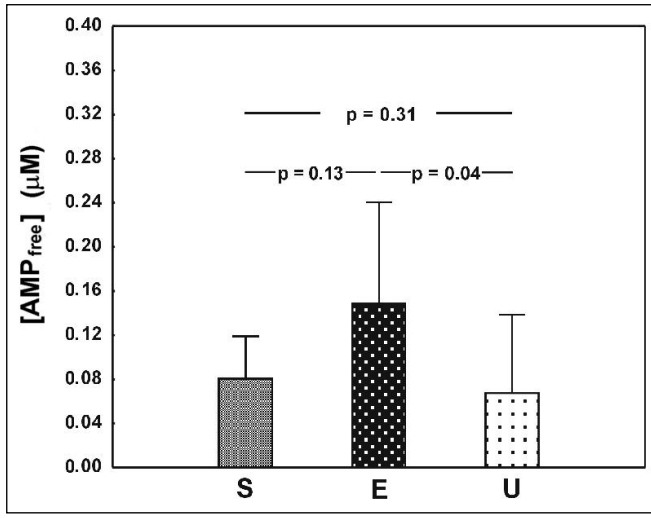

Fig. 3. Mean (± S.D.) concentration of free AMP in calf muscles, expressed as mean values for both legs, in sprinters (S), endurance athletes (E) and untrained subjects (U). The Wilcoxon-Mann-Whitney test was used to test for significance of differences.

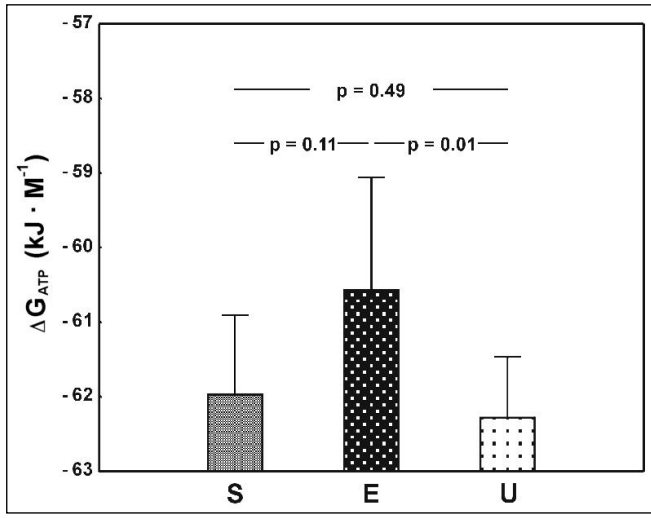

Fig. 4. Mean (± S.D.) values of ΔG_{ATP} in calf muscles, expressed as mean values for both legs, in sprinters (S), endurance athletes (E) and untrained subjects (U). The Wilcoxon-Mann-Whitney test was used to test for significance of differences.

amounting to 0.74 ± 0.47 mM in sprinters, 0.61 ± 0.09 mM in endurance athletes and 0.64 ± 0.09 mM in untrained subjects, was not significantly different in all groups of subjects. ΔG_{ATP} in endurance athletes amounting to -60.57 ± 0.09 mM was significantly lower ($p = 0.01$) than in untrained subjects (-62.29 ± 0.82 $kJ \cdot M^{-1}$). The ΔG_{ATP} in sprinters amounting to -61.98 ± 1.07 $kJ \cdot M^{-1}$ was not significantly different than in endurance athletes (*Fig. 4*). *Figs 1 - 4* show the mean (± S.D.) values of [PCr], $[ADP_{free}]$, $[AMP_{free}]$, and ΔG_{ATP}, respectively, determined in the calf muscle at rest, expressed as a mean value for both legs in sprinters, endurance trained athletes and untrained subjects.

(b). The mean (± S.D.) values of muscle pH, [PCr], [P_i], [ADP_{free}], [AMP_{free}], [Mg^{2+}_{free}] and ΔG_{ATP} determined in the calf muscle at rest, expressed as a mean value determined in the dominant leg in sprinters, endurance trained athletes and in untrained subjects

Muscle pH in the dominant leg at rest amounting to 7.050 ± 0.019 in sprinters, 7.045 ± 0.016 in endurance athletes and 7.043 ± 0.037 in untrained subjects was not significantly different in all groups of subjects. PCr concentration in the dominant leg in endurance athletes amounting to 25.91 ± 2.87 mM was significantly lower (p = 0.01) than in sprinters (30.02 ± 3.12 mM) and than in untrained subjects (30.71 ± 2.88 mM) (p = 0.02) (*Fig. 5*). P_i concentration in the dominant leg amounting to 3.70 ± 0.73 mM in sprinters, 4.03 ± 1.49 mM in endurance athletes and 3.34 ± 0.70 in untrained subjects was not significantly different in all groups of subjects. ADP_{free} concentration in the dominant leg in endurance athletes amounting to 42.19 ± 13.44 μM was significantly higher (p = 0.02) than in sprinters (27.86 ± 10.19 μM) and than in untrained subjects (25.35 ± 10.97 μM) (p = 0.02) (*Fig. 6*). AMP_{free} concentration in the dominant leg in endurance athletes amounting to 0.165 ± 0.108 μM was significantly (p = 0.03) higher than in sprinters (0.074 ± 0.043 μM) and than in untrained subjects (0.064 ± 0.057 μM) (p = 0.04) (*Fig. 7*). Muscle [Mg^{2+}_{free}] in the dominant leg amounting to 0.90 ± 0.93 mM in sprinters, 0.63 ± 0.14 mM in endurance athletes and 0.65 ± 0.24 mM in untrained subjects, was not significantly different in all groups of subjects. ΔG_{ATP} in the dominant leg in endurance athletes amounting to -60.53 ± 2.03 $kJ \cdot M^{-1}$ was significantly lower (p = 0.01) than in untrained subjects (-62.29 ± 0.73 $kJ \cdot M^{-1}$) and it was significantly lower (p = 0.02) than in sprinters (-61.82 ± 1.05 $kJ \cdot M^{-1}$) (*Fig. 8*). *Figs 5 - 8* show the mean (± S.D.) values of [PCr], [ADP_{free}], [AMP_{free}], and ΔG_{ATP}, respectively, determined in the calf

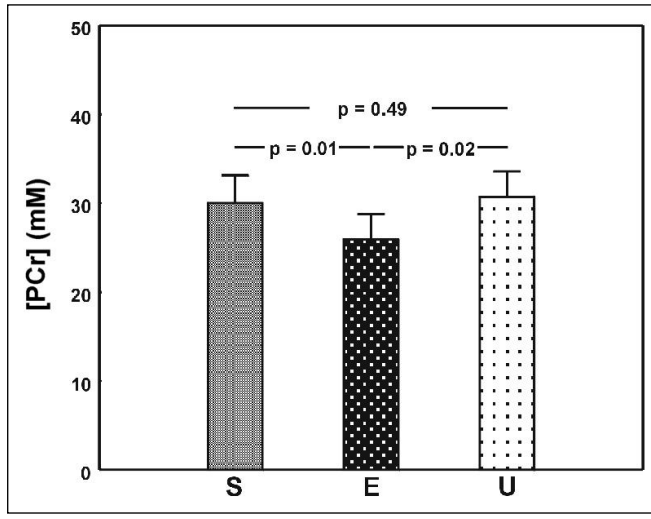

Fig. 5. Mean (± S.D.) concentration of PCr in calf muscles in the dominant leg in sprinters (S), endurance athletes (E) and untrained subjects (U). The Wilcoxon-Mann-Whitney test was used to test for significance of differences.

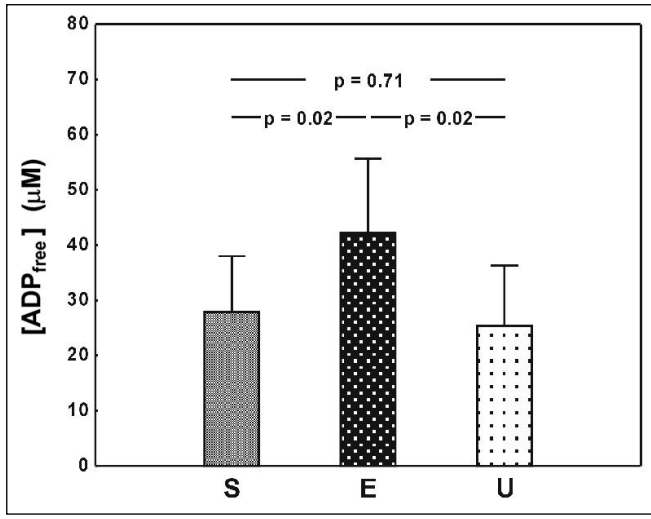

Fig. 6. Mean (± S.D.) concentration of free ADP in calf muscles in the dominant leg in sprinters (S), endurance athletes (E) and untrained subjects (U). The Wilcoxon-Mann-Whitney test was used to test for significance of differences.

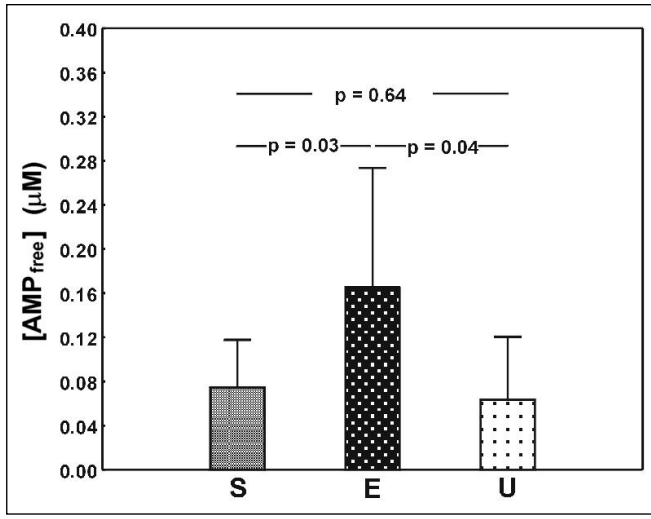

Fig. 7. Mean (± S.D.) concentration of free AMP in calf muscles in the dominant leg in sprinters (S), endurance athletes (E) and untrained subjects (U). The Wilcoxon-Mann-Whitney test was used to test for significance of differences.

muscle at rest, expressed as a mean value determined in the dominant leg in sprinters, endurance trained athletes and untrained subjects.

(c). The mean (± S.D.) values of muscle pH, [PCr], [P_i], [ADP_{free}], [AMP_{free}], [$Mg^{2+}{}_{free}$] and ΔG_{ATP} determined in the calf muscle at rest, expressed as a mean value determined in the non-dominant leg in sprinters, endurance trained athletes and in untrained subjects are presented

Muscle pH determined at rest in the non-dominant leg amounting to: 7.053 ± 0.021, 7.031 ± 0.033 and 7.043 ± 0.027 in sprinters, endurance athletes and in

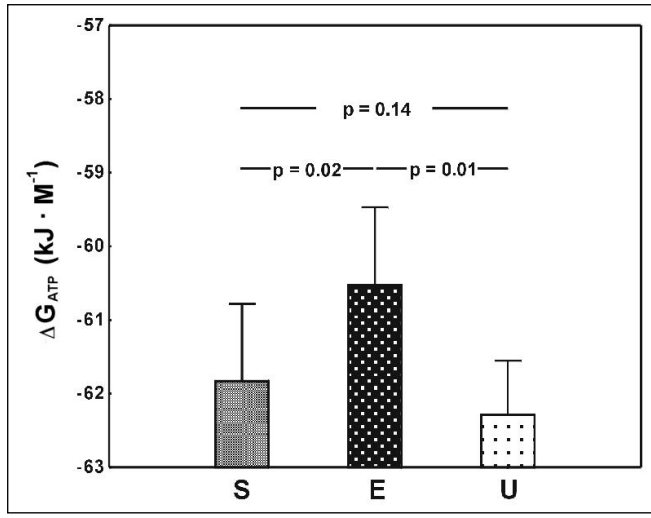

Fig. 8. Mean (± S.D.) values of ΔG_{ATP} in calf muscles in the dominant leg in sprinters (S), endurance athletes (E) and untrained subjects (U). The Wilcoxon-Mann-Whitney test was used to test for significance of differences.

untrained subjects respectively, was not significantly different in all groups of subjects. PCr concentration in the non-dominant leg amounting to 29.68 ± 4.03 mM in sprinters, 27.57 ± 3.89 mM in endurance athletes and 30.75 ± 3.85 mM in untrained subjects, was not significantly different in all groups of subjects. P_i concentration in the non-dominant leg amounting to 3.49 ± 0.95 mM in sprinters, 4.97 ± 2.99 mM in endurance athletes and 3.99 ± 0.60 mM in untrained subjects, was not significantly different in all groups of subjects. ADP_{free} concentration in the non-dominant leg amounting to 29.86 ± 10.89 μM in sprinters, 36.33 ± 17.33 μM in endurance athletes and 25.51 ± 14.67 μM in untrained subjects, was not significantly different in all groups of subjects. AMP_{free} concentration in the non-dominant leg amounting to 0.086 ± 0.042 μM in sprinters, 0.131 ± 0.132 μM in endurance athletes and 0.071 ± 0.091 μM in untrained subjects, was not significantly different in all groups of subjects. Mg^{2+}_{free} concentration in the non-dominant leg amounting to 0.58 ± 0.12 mM in sprinters, 0.59 ± 0.15 mM in endurance athletes and 0.63 ± 0.09 mM in untrained subjects, was not significantly different in all groups of subjects. ΔG_{ATP} in the non-dominant leg amounting to -62.12 ± 1.62 $kJ \cdot M^{-1}$ in sprinters, -60.62 ± 2.03 $kJ \cdot M^{-1}$ in endurance athletes and -62.28 ± 0.98 $kJ \cdot M^{-1}$ in untrained subjects, was not significantly different in all groups of subjects.

(d). The individual values of muscle pH, [PCr], [P_i], [ADP_{free}], [AMP_{free}], [Mg^{2+}_{free}] and ΔG_{ATP} determined in the calf muscle at rest in the best sprinter (finalist of the World Championships in Athletics – 200 m personal best (19:98 s)) and endurance athlete (Olympic gold medalist and world record holder in race walking) and in untrained subjects (n = 6) are presented. Data are expressed as a mean value for both legs (Table 1) and individual data for the dominant (Table 2) and non-dominant leg (Table 3)

Table 1. Muscle pH, [PCr], [P_i], [ADP_{free}], [AMP_{free}], [Mg^{2+}_{free}] and ΔG_{ATP} in the calf muscle at rest in the best sprinter, best endurance athlete and in untrained subjects (n = 6). Data are presented as a mean ± S.D. values for both legs.

Variable / Subject	pH	[PCr] (mM)	[P_i] (mM)	[ADP_{free}] (μM)	[AMP_{free}] (μM)	[Mg^{2+}_{free}] (mM)	ΔG_{ATP} (kJ · M^{-1})
Sprinter	7.047 ± 0.039	28.21 ± 0.00	2.92 ± 0.46	33.65 ± 2.22	0.099 ± 0.017	0.54 ± 0.02	- 61.80 ± 0.46
Endurance athlete	7.042 ± 0.028	23.63 ± 2.53	3.43 ± 0.51	53.74 ± 16.78	0.264 ± 0.165	0.55 ± 0.01	- 60.20 ± 0.28
Untrained subjects	7.043 ± 0.029	30.37 ± 3.25	3.36 ± 0.65	25.43 ± 12.31	0.067 ± 0.071	0.64 ± 0.09	- 62.29 ± 0.82

Table 2. Muscle pH, [PCr], [P_i], [ADP_{free}], [AMP_{free}], [Mg^{2+}_{free}] and ΔG_{ATP} in the calf muscle of the dominant leg at rest in the best sprinter, best endurance athlete and untrained subjects. The data for the sprinter and for the endurance athlete are presented as an individual result obtained in the dominant leg. The data for the untrained subjects (n = 6) are given as the mean ± S.D.

Variable / Subject	pH	[PCr] (mM)	[P_i] (mM)	[ADP_{free}] (μM)	[AMP_{free}] (μM)	[Mg^{2+}_{free}] (mM)	ΔG_{ATP} (kJ · M^{-1})
Sprinter	7.020	28.21	2.60	32.08	0.087	0.53	- 62.13
Endurance athlete	7.062	21.84	3.07	65.61	0.381	0.54	- 60.00
Untrained subjects	7.043 ± 0.037	30.71 ± 2.88	3.34 ± 0.70	25.35 ± 10.97	0.064 ± 0.057	0.65 ± 0.24	- 62.29 ± 0.73

Table 3. Muscle pH, [PCr], [P_i], [ADP_{free}], [AMP_{free}], [Mg^{2+}_{free}] and ΔG_{ATP} in the calf muscle of the non-dominant leg at rest in the best sprinter, best endurance athlete and untrained subjects. The data for the sprinter and for the endurance athlete are presented as an individual result obtained in the non-dominant leg. The data for the untrained subjects (n = 6) are given as the mean ± S.D.

Variable / Subject	pH	[PCr] (mM)	[P_i] (mM)	[ADP_{free}] (μM)	[AMP_{free}] (μM)	[Mg^{2+}_{free}] (mM)	ΔG_{ATP} (kJ · M^{-1})
Sprinter	7.074	28.21	3.25	35.22	0.111	0.56	- 61.48
Endurance athlete	7.023	25.42	3.79	41.88	0.147	0.55	- 60.40
Untrained subjects	7.043 ± 0.027	30.75 ± 3.85	3.39 ± 0.60	25.51 ± 14.67	0.071 ± 0.091	0.63 ± 0.09	- 62.28 ± 0.98

DISCUSSION

The present study shows that [PCr] and ΔG_{ATP} in the calf muscles in endurance athletes were significantly lower, whereas [ADP_{free}] and [AMP_{free}] were significantly higher than in untrained subjects (*Figs. 1 - 4*). The main and original finding of this study was that [PCr] and ΔG_{ATP} in the dominant leg in endurance athletes was significantly lower than in sprinters and in untrained subjects (*Figs.*

5, 8). The [ADP_{free}] in the dominant leg in endurance athletes was significantly higher than in sprinters and in untrained subjects (*Fig. 6*). The differences in the metabolic status of the dominant legs were even more pronounced when comparing the values of [ADP_{free}], [AMP_{free}], [PCr] and ΔG_{ATP} in the muscles of the best sprinter (personal best in 200 m sprint: 19:98 s) and the best endurance trained athlete participating in this study (4 times Olympic gold medallist in race walking) (*Table 2*). The [ADP_{free}] in the dominant leg in the best endurance trained athlete was by ~100% higher (2 times greater) than in the dominant leg in the best sprinter and the ΔG_{ATP} was markedly lower in the endurance athlete than in the sprinter (-60.00 *vs*. -62.13 $kJ \cdot M^{-1}$).

The calculation of [ADP_{free}] and ΔG_{ATP} in the present study was based on the assumption that the concentration of [ATP] in the muscles is 8.2 mM (20 - 23, 25), and the total creatine concentration is 42 mM (21, 22) – *i.e.* values considered as typical concentrations of these metabolites in a mixed human muscle fibers population. The first assumption can be justified by the fact that ATP concentrations in type I and type II muscle fibers in humans are almost equal (10, 12, 26). Far less is known, however, regarding the total creatine concentration in type I and type II muscle fibers in humans. According to the data published by Karatzaferi *et al.* (27), the total creatine concentration in type II muscle fibers is by about 10 percent higher than in type I muscle fibers selected from the vastus lateralis muscle in humans. Assuming that the proportion of type I to type II muscle fibers in the calf muscles in sprint athletes was 20 and 80 percent respectively, and in endurance athletes 80 and 20 percent respectively (1), then the total creatine pool in the calf muscle in sprinters would be by about 6 percent higher than in endurance athletes. This hypothetical difference in creatine concentration would account only for about 30 percent of the difference in [ADP_{free}] found when comparing the dominant legs in endurance athletes and the dominant legs in sprinters. However, in the present study the differences in [ADP_{free}] in the dominant leg in sprinters and endurance athletes were much greater (*Table 2* and *Fig. 6)*.

The reason for the higher [ADP_{free}] and lower ΔG_{ATP} in endurance trained muscles compared with untrained muscles at rest is not obvious, but such a metabolic profile seems to be a feature of oxidative well-trained muscle. It has been reported that in heart muscle which is the most fatigue resistant muscle in the human body and is characterised by the highest metabolic stability during work (28), at low work intensity [ADP_{free}] is significantly higher than in the resting skeletal muscle (discussion in 29). Moreover, we postulated recently (9, 29, 30), using a computer model of oxidative phosphorylation (31) that the better homeostasis of [ADP_{free}] during rest-to-work (or low-to-high-work) transition in heart than in skeletal muscle, in oxidative muscle than in glycolytic muscle and in trained muscle than in untrained muscle (9), is due to differences in the intensity of parallel activation by some cytosolic factor of all oxidative phosphorylation complexes during muscle contraction (32). Assuming that the

maximal concentrations of ADP_{free} in endurance trained muscle are lower than in untrained muscle and that the maximal oxygen uptake in endurance trained muscle is higher than in untrained muscle, it seems that in oxidative skeletal muscle a higher increase in $\dot{V}_{O_2}$ during rest-to-work transition is accompanied by a smaller increase in [ADP_{free}] (33, 34, 35). This conclusion may seem paradoxical when it is assumed that the increase in [ADP_{free}] is the main mechanism responsible for the adjustment of the rate of [ATP] consumption to the current rate of [ATP] usage during muscle contraction, as proposed originally by Chance and Williams (36). However, as it was discussed above and elsewhere (9, 30), we postulate that in oxidative (endurance trained) skeletal muscle *in vivo* the main mechanism of the regulation of oxidative phosphorylation is the parallel activation of oxidative phosphorylation complexes by some cytosolic factor during muscle work, while the increase in [ADP_{free}] constitutes only a minor, fine-tuning mechanism (37). Therefore, oxidative skeletal muscle does not have to rely on changes in [ADP_{free}] so much as glycolytic skeletal muscle (9, 34, 37).

On the other hand, the higher resting [ADP_{free}] in oxidative fibers (endurance trained muscle, dominant leg) than in glycolytic fibers (sprint training, non-dominant leg) or in untrained subjects may play a role in limiting free radical production. Higher resting [ADP_{free}] means lower ΔG_{ATP} and lower Δp (protonomotive force related to the proton gradient through the inner mitochondrial membrane). This results in a much smaller free radical production by the respiratory chain (especially complex I) (38, 39) that depends very steeply on Δp. Therefore, the high resting [ADP_{free}] encountered in oxidative fibers may constitute a mechanism that protects these fibers from damage caused by oxygen reactive species (free radicals).

It is noteworthy that, in the present study, the significant differences in the metabolic profile at rest were found only when comparing the dominant leg of sprinters and endurance athletes, but not between the non-dominant leg, presumably subjected to lower mechanical loads during locomotors and sports activities. No significant differences were found when comparing the pH, [ADP_{free}], [AMP_{free}], [$Mg^{2+}{}_{free}$], ΔG_{ATP} in the non-dominant legs in all study groups. The differences found in the [PCr], [ADP_{free}] and ΔG_{ATP} when comparing the dominant leg of sprint and endurance athletes are most likely caused by cellular adaptations induced by a specific training regime of various specializations. The dominant leg in sprinters (mainly the left one) is exposed to the most powerful short-term tasks as the take-off while starting the sprint or the take-off while jumping, whereas the dominant leg in endurance athletes (mainly the right one) is preferentially used while performing more difficult tasks (*e.g.* jumping) but in long lasting endurance training sessions of lower intensities (such as running or walking). Moreover, the dominant leg in sprinters and in endurance athletes may be more "mechanically loaded" during normal locomotors movements such as running or walking compared with the non-dominant one. Furthermore, endurance training involves mostly muscle work where oxidative

phosphorylation is the main source of ATP, whereas during sprint training most of ATP comes from creatine kinase reaction and glycolysis.

It was reported that muscle training/conditioning is accompanied by transformation from fast to slow muscle fibers (18). Moreover, the magnitude of this transformation depends upon training volume and its duration. Interestingly, in view of the available data any kind of muscle activities (strength or endurance mode of training) induces transformation of the fastest type IIX myosin heavy chain isoforms into slower isoforms (18, 40 - 42). The opposite effects *i.e.* transformation of MyHC slow isoforms into the fastest type IIX were observed when decreasing the mechanical load on the muscles (18, 40, 43).

Assuming that the dominant leg is the leg that performs more work during each training session compared with the non-dominant leg, it seams likely that the degree of transformation from fast to slow MyHC isoforms is greatest in the dominant leg in endurance athletes subjected to long lasting training sessions. However, to our knowledge no data have been published regarding the MyHC composition in the dominant and non-dominant legs in humans.

Interestingly, it was recently reported that the $[ATP]/[ADP_{free}]$ ratio at rest is by about 20% higher in fast-twitch than in slow-twitch rabbit muscles (19). Moreover, the training-induced conversion of fast-to-slow muscle fibers was accompanied by a significant reduction in [PCr] and $[ATP]/[ADP_{free}]$ ratio in the converted muscles, when compared with the control. According to these authors the drastically and persistently decreased $[ATP]/[ADP_{free}]$ ratio may be a trigger initiating the fast-to-slow transformation of myofibril protein isoform expression (19).

In view of the results obtained by Conjard *et al.* (19), the high $[ADP_{free}]$ and the low ΔG_{ATP} demonstrated in the present study in the dominant legs in endurance athletes may be an indication of a greater degree of transformation of the fastest to slowest MyHC isoforms in the dominant legs in endurance athletes as compared with sprinters or untrained subjects. It should be mentioned here that in our study $[ADP_{free}]$ in the dominant leg in the best endurance trained athlete was by ~100% higher (2 times greater) than in the dominant leg in the best sprinter (*Table 2*). Moreover, no difference was observed in $[ADP_{free}]$ in the dominant leg between sprinters and untrained subjects in whom the training load is rather low, when compared with endurance athletes (*Fig. 6*).

In the present study [PCr] at rest, expressed as a mean value for both legs in sprinters was by about 12 percent higher ($p = 0.11$) than in endurance athletes (29.85 ± 3.06 *vs.* 26.74 ± 2.79 mM) (*Fig. 1*). When comparing [PCr] concentration in the dominant leg in sprinters and in endurance athletes, we found a significant difference ($p = 0.0097$) (30.02 ± 3.12 *vs*. 25.91 ± 2.87, respectively, *Fig. 5*). This difference amounting to ~15% is similar to that reported in the literature when comparing [PCr] in type I and type II muscle fibers at rest (10, 12, 26). When comparing [PCr] in the dominant leg of the best sprinter (28.21 mM) and the best endurance athlete (21.84 mM) involved in this study, the difference was about 30 percent. Surprisingly, the differences in [PCr]

and [ADP_{free}] almost disappeared when comparing the non-dominant leg in both groups of athletes (*Table 3*).

The results for pH, [P_i] and [Mg^{2+}_{free}] at rest obtained in the present study were within the range published in the literature (23, 24, 44, 45). No differences were found regarding muscle pH, [P_i] and [Mg^{2+}_{free}] when comparing muscles of the dominant and the non-dominant leg in various groups of subjects. It is also of interest that [Mg^{2+}_{free}] expressed as a mean value for both legs in all subjects was 0.68 ± 0.31 mM and in most cases it was in the range between 0.44 and 0.81 mM, except for one subject in whom [Mg^{2+}_{free}] was 2.04 mM.

In conclusion: We postulate that elevated [ADP_{free}] and decreased ΔG_{ATP} in endurance-trained muscles at rest is a feature of oxidative well-trained muscles. The lower [PCr] and ΔG_{ATP} as well as higher [ADP_{free}] in the dominant leg in endurance athletes compared with sprinters may result from many years of endurance training involving continuous training sessions, leading to transformation of glycolytic fibers into oxidative ones. Moreover, when studying the metabolic profile and training-induced muscle adaptation in locomotor muscles in athletes one has to consider the metabolic differences between the dominant and non-dominant leg. The differences in the metabolic status at rest between the dominant and non-dominant leg seem to be interesting in view of the origin of metabolic and functional disorders in locomotor muscles that come with aging.

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