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Abstract
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Effect of Exogenous Creatine Supplementation on Muscle PCr Metabolism

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31P NMR was used to assess the influence of two weeks of creatine supplementation (21 g - d−1) on resting muscle PCr concentration, on the rate of PCr repletion (R_repl), and on the halftime of PCr repletion (t_1/2). Body mass (BM) and volume of body water compartments were also estimated by impedance spectroscopy. Fourteen healthy male subjects (20.8 ± 1.9 y) participated in this double-blind study. PCr was measured using a surface coil placed under the calf muscle, at rest and during two exercise bouts of duration of which was 1 min. They were interspersed by a recovery of 10 min. The exercises comprised of 30 plantar flexions-extensions against weights corresponding to 40% and 70% of maximal voluntary contraction (MVC), respectively. Creatine supplementation increased resting muscle PCr content by −20% (P = 0.002). R_repl was also increased by −15% (P < 0.001) and −10% (P = 0.026) among 40% and 70% MVC exercises, respectively. No change was observed in R_repl and t_1/2. BM and body water compartments were not influenced. These results indicate that during a standardized exercise more ATP is synthesized by the CK reaction when the pre-exercise level in PCr is higher, giving some support to the positive effects recorded on muscle performance.

Key words: Creatine monohydrate, phosphocreatine, skeletal muscle, magnetic resonance spectroscopy, body water.

Introduction

During strenuous exercise muscle phosphocreatine (PCr) acts as a temporary buffer of energy allowing a fast resynthesis of ATP [16] although other factors are attributed to PCr [29]. On this basis an increasing number of athletes are consuming dietary creatine expecting an improvement of their physical performance.

Several studies have shown that muscle free creatine and PCr increased after a few days of supplementation with creatine monohydrate (Cr) [9, 14, 17, 24, 27]. Recent reports (for review see [19]) have demonstrated that Cr supplementation improved performance in subjects performing high-intensity intermittent exercises [1] or repeated bouts of high-intensity isokinetic contractions [3, 10, 27]. No effect was reported either on long-term endurance performance [2] or in substrate utilization during and after incremental exercise [26], nor in power output and fatigue during continuous high-intensity bicycle exercise [5].

As the main effects on performance have been observed during high-intensity repeated exercises, the hypothesis of a faster rate of PCr resynthesis after creatine supplementation was drawn. Greenhaff et al. [9] indicated that the rate of PCr resynthesis was increased in some subjects during creatine supplementation whereas so-called “non-responder” subjects did not show this effect. However, another report did not confirm these results [28] in young healthy subjects suggesting that the effect observed in repeated exercise bouts cannot be attributed to a rise in the rate of PCr repletion.

The ergogenic effect of oral creatine supplementation has also been related to an increase in the rate of PCr utilization during a 30s maximal isokinetic cycling exercise [4] suggesting that creatine supplementation affects the rate of ATP synthesis during a maximal intensity exercise.

On the other hand an increase in body mass and more specifically in lean body mass was also observed [6]. This secondary consequence of creatine intake has been commonly attributed to water retention. Nevertheless this hypothesis has never been corroborated by experimental data. Recently, using bioimpedance spectroscopy, we showed that only 5% of the body mass rise observed after nine weeks of creatine intake are related to increase in body water content [7]. This finding suggests that the remaining 45% should be the consequence of a dry matter growth.

To clarify the discrepancies between the conclusions of previous studies performed in young healthy subjects [9, 28], the present study assessed the influence of two weeks creatine...
supplementation on the rate of initial PCR repletion ($R_{\text{prot}}$) and the half-time of PCR repletion ($t_{1/2}$) during the recovery period following repeated muscle contractions. The effect in the rate of PCR depletion ($R_{\text{depl}}$) during standardized exercises of various intensities was also considered to rest if a higher rate of ATP synthesis from PCR is also observed during sub-maximal exercises.

Body mass (BM) and volume of body water compartments were estimated to verify if the effect in water retention could be observed after a short period of creatine supplementation.

Materials and Methods

Subjects

Fourteen healthy male subjects (age 20.8 ± 1.9 years; height 174.2 ± 6.6 cm), physically active, volunteered to participate in this experiment which was approved by the Ethics Committees (Faculties of Medicine) of both the "Université Catholique de Louvain" and the "Université Libre de Bruxelles". Prior to the experiment the subjects were fully informed of the nature of the protocol, and their written approvals were obtained.

Experimental protocol

The first day of experimentation (day 0) the subjects (placebo- and creatine-group) came into the laboratory at 8.30 a.m. after a 12 hours fast without exercising. They were instructed to maintain a normal hydration and to avoid alcohol and caffeine 24 hours prior to the test. The volume of body water compartments was assessed by bio-impedance spectroscopy. Over the course of the day $^{31}$P NMR spectra were acquired in order to determine the muscle (PCR) at rest, during exercise, and during recovery.

The subjects were randomly divided into two groups. In this double-blind study each participant in the creatine-group was told to ingest 21 g creatine monohydrate daily, in the form of white powder which was diluted in hot/warm beverage. Creatine was distributed in 7 g doses in the early morning, at noon, and in the evening for the next 14 days. The placebo-group participants followed the same protocol but maltodextrine was ingested instead. At day 14, the protocol of day 0 was repeated for the two groups.

$^{31}$P NMR

$^{31}$P NMR spectra were acquired from the calf muscle of the left leg of every subject, at rest, during exercise, and during recovery, using a Bruker Biospec spectrometer (horizontal bore) working at 4.7 Tesla and 81 MHz for $^{31}$P. The experimental set-up was the same as the one presented by Goudemand et al. [8]. The leg was positioned in the center of the magnet and firmly immobilized over a 50 mm diameter surface coil by means of a specially designed set-up that allowed to study mainly the gastrocnemius muscle, with probable contribution of the neighbouring muscles. The left foot was placed on a pedal allowing a movement of flexion-extension of the ankle. The pedal was connected to a weight (or a dynamometer) by means of a pulley system. Prior to the NMR experiment the isometric force developed by a maximal voluntary contraction (MVC) of the calf muscle was measured by means of the mechanical dynamometer.

After fine tuning of the probe with the subject properly installed, the magnetic field was homogenised on the free induction decay (FID) of water protons. A first $^{31}$P NMR spectrum was recorded at rest. The signal was acquired on 1024 points (spectral width 6000 Hz) after a 130 µs excitation pulse, corresponding to an angle of 90° in the centre of the coil. This pulse was the optimum irradiation yielding the maximum intensity of the signal and allowing to record a signal mainly originating from deep muscle. To improve the signal/noise ratio, 40 free induction decays were accumulated with a repetition time of 8 s.

Afterwards the first exercise protocol was initiated. It consisted of 50 flexion-extensions of the ankle over 1 min (0.83 Hz) against a mass equal to 40% of MVC. The linear displacement of the weight was controlled. NMR spectra were recorded continuously over a period of 8 min (2 min at rest before the exercise, during the exercise itself and during the first 5 min of recovery). A spectrum was acquired every 15 s (6 FIDs accumulated with a 2.5 s repetition time).

After a recovery period of 10 min the same protocol was applied again but this time the exercise intensity was 70% of MVC.

The Fourier transform was calculated after exponential multiplication of the FID (line broadening = 5 Hz). The areas under the peaks were calculated by fitting in the time domain. They were properly corrected by saturation factors determined in a previous experiment [8]. The area under the [P-i-P]ATP peak at rest was used as reference and was set to 5.5 mmol·kg$^{-1}$ wet muscle [13].

During exercise and recovery muscle Pi and PCR concentrations were calculated from the rest values assuming that the sum of their concentration ([Pi] + [PCR]) was constant. $R_{\text{depl}}$ (mmol·kg$^{-1}$·min$^{-1}$) was calculated from the linear increase of PCR during the first minute of recovery. The half-time of PCR repletion ($t_{1/2}$), which is independent of the concentration, was calculated using a mono-exponential model [22]: $[\text{PCR}] = ([\text{PCR}]_{\text{est}}(1 - \exp(-0.5 \cdot t/t_{1/2}))) + [\text{PCR}]_{\text{est}}$ (where [PCR]$_{\text{est}}$ is the PCR concentration at rest or at the end of the recovery period, [PCR]$_{\text{est}}$ is the PCR concentration at the end of exercise, and $t_{1/2}$ is the half-time of PCR repletion, time zero being the end of exercise). Intracellular pH ($p_{\text{i}}$) was calculated from the difference between the frequencies of the Pi and PCR peaks (8 in ppm) using the following equation: $p_{\text{i}} = 6.75 + \log([8-3.27]/(5.69-8))$ [23]; $p_{\text{i}}$ was measured at rest and at the end of the exercise.

Body water content

The space of the body water compartments was assessed using a BODYSTAT DUALSCAN 2005 which was validated in a previous study [12]. This method is based on the fact that the water containing electrolytes conducts the electrical current well. At low frequency (5 kHz) the capacitive nature of the cell membrane does not allow the current to penetrate into the cell so the impedance is related only to the extracellular water (ECW). At high frequency (200 kHz) the current is able to pe-
nate cell membranes, and the impedance is a measure of the total body water (TBW). By deduction the intra-cellular water (ICW) can be calculated.

After accurate determination of the height (± 0.1 cm) and the BM (± 0.1 kg), disposable electrodes (areas 15 cm²) were placed on the right side of the body, behind the knuckles, on the wrist next to the ulna head, behind the toes and on the ankles at the level of and between the medial and lateral malleoli. Then the subject was placed in the supine position for at least 10 min. Afterwards a sinusoidal current of 500 μA (R.M.S.) was applied to the subjects at frequencies of 5 kHz and 200 kHz successively, and the respective impedance values were recorded (± 1 Ω). A software supplied by the manufacturer of the analyser was used to estimate the sizes of TBW, ECW, and ICW from the impedance values.

Statistics

The results are expressed as means ± standard deviation. The statistical significance of differences observed between the means was assessed by an ANOVA design for repeated measures after verifying the normality of the distribution and the lack of difference between variances. The limit of statistical significance was taken to be 0.05.

Results

\[ \text{PP NMR} \]

The results of the NMR measurements at rest are presented in Table 1. When the fourteen subjects are considered all together, the muscle PCR and Pi concentrations were at day one, 19.5 ± 1.9 mmol·kg⁻¹ and 19.5 ± 0.9 mmol·kg⁻¹, respectively, the pH being 7.04 ± 0.04 units. After two weeks of maltodextrine intake the placebo group did not show any difference in these values. However, after the same period of creatine supplementation (21 g·d⁻¹), PCR increased by 20% in the creatine group (P = 0.002) but the Pi and muscle pH remained unchanged. The magnitude of PCR increase was negatively related to its concentration at day 0 (R = -0.77; P = 0.044).

The PCR time course during exercise and recovery is illustrated in Fig. 1. The values of R

\[ \text{R}_{\text{peak}} \] and \[ \text{R}_{\text{rest}} \] are given in Table 2. When the subjects developed 40% of their MVC at a frequency of 0.83 Hz, \[ \text{R}_{\text{peak}} \] was about 10.5 mmol·kg⁻¹, and the PCR at the end of the exercise dropped to about half of the initial concentration. As indicated by the low value of the standard deviations, \[ \text{R}_{\text{peak}} \] was very similar in all subjects in day 0. Although PCR at rest was higher after creatine supplementation, the concentration measured at the end of the exercise was almost identical at day 0 and day 14 (Fig. 1, upper part). This means that 1% was increased (~15%) after creatine intake (P < 0.001).

but remained unchanged in the placebo subjects (Table 2, upper part).

As expected, when the intensity of dynamic muscle contractions was increased to 70% of MVC, \[ \text{R}_{\text{peak}} \] was accelerated to reach at day 0 13.9 ± 1.9 and 14.0 ± 3.5 mmol·kg⁻¹·min⁻¹ in placebo and creatine groups, respectively, these values being not statistically different. At the end of this heavy exercise PCR was decreased down to ~30% of its resting concentration. \[ \text{R}_{\text{peak}} \] was here also significantly affected (~10%) for the creatine group (P = 0.026) but it remained unchanged in the placebo group (Fig. 1 and Table 2, lower parts).

\[ \text{R}_{\text{peak}} \] was calculated from the linear increase of PCR observed during the first minute of recovery (Fig. 1). \[ \text{R}_{\text{peak}} \] was not affected by creatine loading at 40% of 70% MVC exercise.

To assess the velocity constant of PCR repopulation, an exponential function was fitted through the data acquired during the recovery period. The slope of the function corresponding to the half-time of PCR repopulation (t½), as compared to the initial rate of creatine repopulation, is independent of the concentration and has been calculated from the whole curve of the recovery data. The values of t½ are reported in Table 2. Despite a higher level off in PCR under creatine supplementation, there was no difference in t½ value between the creatine and placebo group.

Intra-cellular pH value was estimated from the chemical shift of the peaks of Pi and PCR (Table 2). As expected, pH decreased in function of the exercise intensity. Nevertheless the values observed at the end of the exercise (pH_{end}) were not different when comparing the two groups.

Body water content

As indicated in Table 3, although muscle phosphorylcreatine content was increased by about 20%, neither BM nor the volume of the body water compartments were significantly modified by 14 days of creatine supplementation (21 g·d⁻¹).

Discussion

The results of the present investigation confirm that the daily intake of 21 g of creatine monohydrate (3 g·d⁻¹) increases muscle PCR by 20% (P = 0.002). The plasma creatine concentration is usually around 20–30 μM in healthy omnivorous subject. Following the intake of a single dose of creatine (5 g), it rises rapidly to reach a value of about 700 μM one or two hours after the ingestion. Then it decreases to return to basal values within 8 hrs after intake (unpublished data). Creatine penetrates into the muscle cells by an active transporter Na⁺-dependent, the amino acid sequence of which is known both in

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Inorganic phosphate (Pi), phosphoryl-creatine (PCR), and muscle pH after two weeks of creatine supplementation (3 g·d⁻¹) or placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo group (n = 7)</td>
</tr>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>[Pi]</td>
<td>(mmol·kg⁻¹)</td>
</tr>
<tr>
<td>7.03 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>[PCR]</td>
<td>(mmol·kg⁻¹)</td>
</tr>
<tr>
<td>20.2 ± 1.14</td>
<td>20.3 ± 1.13</td>
</tr>
<tr>
<td>[PCR]</td>
<td>(mmol·kg⁻¹)</td>
</tr>
<tr>
<td>1.74 ± 1.17</td>
<td>2.03 ± 0.90</td>
</tr>
<tr>
<td>pH</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 1 Effect of two weeks creatine supplementation (3 x 7 g•d^-1) or placebo on PCR during dynamic exercises and recovery. (●) and (○) represent the pre- and the post-treatment conditions, respectively. The hatched area denotes exercise.

Table 2 Rates of PCR depletion (R_{depl}) and of initial repletion (R_{repl}) and muscle pH at the end of the exercise (pH_{max}) after two weeks of creatine supplementation (3 x 7 g•d^-1) or placebo. t_{1/2} is the half-time of repletion

<table>
<thead>
<tr>
<th></th>
<th>Placebo group (n=7)</th>
<th>40% MVC</th>
<th>Creatine group (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 14</td>
<td>P</td>
</tr>
<tr>
<td>R_{depl} (mmol•kg_{dry}^{-1}•min^{-1})</td>
<td>10.7±1.0</td>
<td>10.7±1.3</td>
<td>NS</td>
</tr>
<tr>
<td>R_{repl} (mmol•kg_{dry}^{-1}•min^{-1})</td>
<td>8.2±2.6</td>
<td>6.7±3.9</td>
<td>NS</td>
</tr>
<tr>
<td>t_{1/2} (s)</td>
<td>37.3±13.9</td>
<td>45.3±27.7</td>
<td>NS</td>
</tr>
<tr>
<td>pH_{max}</td>
<td>6.81±0.04</td>
<td>6.96±0.05</td>
<td>NS</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 14</th>
<th>70% MVC</th>
<th>P</th>
<th>Day 0</th>
<th>Day 14</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>R_{depl} (mmol•kg_{dry}^{-1}•min^{-1})</td>
<td>13.9±1.8</td>
<td>14.7±3.5</td>
<td>NS</td>
<td>14.0±3.5</td>
<td>16.2±1.8</td>
<td>0.026</td>
<td></td>
</tr>
<tr>
<td>R_{repl} (mmol•kg_{dry}^{-1}•min^{-1})</td>
<td>12.3±3.9</td>
<td>11.1±4.7</td>
<td>NS</td>
<td>10.7±2.0</td>
<td>11.9±2.2</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>t_{1/2} (s)</td>
<td>30.5±6.9</td>
<td>47.0±20.8</td>
<td>NS</td>
<td>34.6±8.0</td>
<td>40.0±13.0</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>pH_{max}</td>
<td>6.74±0.15</td>
<td>6.74±0.14</td>
<td>NS</td>
<td>6.85±0.17</td>
<td>6.81±0.09</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Effect of two weeks of creatine supplementation (3 × 7 g·d⁻¹) or placebo in body composition estimated by bio-impedance spectroscopy. ECW, ICW, and TBW are the intra-cellular, the extra-cellular, and the total body water, respectively. 25 kHz and 2 200 kHz are the impedance measured using current frequencies of 5 and 200 kHz, respectively.

<table>
<thead>
<tr>
<th></th>
<th>Placebo group (n = 7)</th>
<th>Creatine group (n = 7)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 14</td>
<td></td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>61.3 ± 4.1</td>
<td>61.7 ± 4.5</td>
<td>NS</td>
</tr>
<tr>
<td>ECW (1)</td>
<td>16.4 ± 1.2</td>
<td>16.6 ± 1.4</td>
<td>NS</td>
</tr>
<tr>
<td>ICW (1)</td>
<td>18.8 ± 1.2</td>
<td>18.6 ± 1.0</td>
<td>NS</td>
</tr>
<tr>
<td>TBW (1)</td>
<td>35.2 ± 2.3</td>
<td>35.2 ± 2.3</td>
<td>NS</td>
</tr>
<tr>
<td>Z 5 kHz (Ω)</td>
<td>631 ± 65</td>
<td>604 ± 82</td>
<td>NS</td>
</tr>
<tr>
<td>Z 200 kHz (Ω)</td>
<td>480 ± 43</td>
<td>467 ± 48</td>
<td>NS</td>
</tr>
</tbody>
</table>

The present study also confirms that individuals with the lowest prefeeding levels reach the largest increases in PCR (R = 0.07; P = 0.044) suggesting the existence of an up-limited creatine level.

Intense repeated muscle contractions deplete PCR (Fig. 1). It was hypothesized that in this case a higher pre-exercise PCR could increase the amount of ATP synthesized by the CK reaction and therefore explain the positive effect of creatine supplementation observed during high-intensity exercises of a few seconds [21]. This hypothesis was corroborated by findings showing an increased ATP resynthesis during maximal exercise due to an increased PCR content in type II muscle fibres. The results of the present study complete these observations by showing that creatine supplementation increases Rₚₑₛₚ nearly only during repeated muscle contractions of high intensity but also during sub-maximal exercise (Table 2). In the pre-treatment conditions the PCR decreased by about the half of its resting level during the 40% MVC exercise, Rₚₑₛₚ being ~10 mmol·kg⁻¹·min⁻¹. As expected, Rₚₑₛₚ increased during the 70% MVC exercise to reach ~14 mmol·kg⁻¹·min⁻¹ leading to a PCR decrease down to ~6 mmol·kg⁻¹·min⁻¹ (~30% of the resting level) over 50 contractions performed over a period of one minute. Although PCR at rest was clearly higher after creatine ingestion, the PCR concentration measured at the end of the two exercises were not statistically different from the pre-treatment values. This means that PCR was utilized at a higher velocity by the contracting muscles after creatine supplementation as opposed to a recent study using isometric muscle contraction [28] instead of dynamic movements. No argument can be advanced to explain the discrepancy between these results.

When the rate of creatine utilization was calculated assuming a linear decrease of PCR over the duration of the exercise (1 min), Rₚₑₛₚ was augmented in both 40% MVC and 70% MVC conditions by 3.3 mmol·kg⁻¹·min⁻¹ (P < 0.001) and 2.2 mmol·kg⁻¹·min⁻¹ (P = 0.026), respectively (Table 2). This finding indicates that during standardized exercises of different intensities more ATP is synthesized by the CK reaction when the pre-exercise level in PCR is increased. Consequently the other metabolic pathways are probably less solicited. These results and the data of the literature suggest that creatine supplementation exerts an ergogenic effect via an increase in the rate of ATP synthesis during both maximal [4] and sub-maximal exercises.

Several studies reported a benefit of creatine supplementation in repeated bouts of high intensity interspersed with short periods of recovery [1, 3, 27]. The hypothesis of a higher Rₚₑₛₚ after exercise was drawn by Greenhaff et al. [9]. They observed a significantly higher PCR two minutes after the end of a standardized exercise in five subjects, the so-called “responders”. Although no difference was observed in the initial period of PCR repletion, these authors concluded that creatine intake enhanced PCR repletion after strenuous exercise. Recently Vandenberghe et al. [28] contradicted this observation by measuring ³¹P-NMR in young healthy subjects. Due to the biopsy methodology Greenhaff et al. [9] reported only few datapoints during the recovery assuming a linear relationship. In the present study PCR was recorded every 15 s. A monoeponential curve was fitted through the datapoints, and the half-time of PCR repletion (t₁/2) was calculated. t₁/2 is directly proportional to the velocity constant of PCR repletion. Clearly t₁/2 did not change after supplementation (Table 2). As PCR at rest is increased by 20% (Table 1), it is obvious that PCR recovers also to a higher level after exercise to reach a concentration similar to that at rest (Fig. 1). As t₁/2 did not change, the effect previously reported [9] is not due to an increase in the velocity constant of the reaction catalysed by creatine kinase but only to an increase in muscle PCR content at rest.

On the other hand the initial rate of PCR repletion (Rₚₑₛₚ) can be calculated during the first minute of recovery assuming the linearity of the PCR-time relationship (Fig. 1). Rₚₑₛₚ is similar between the pre- and the post-treatment conditions (Fig. 1 and Table 2) after the 40% and 70% MVC exercises, PCR being resynthesized faster when the intensity of exercise increases and therefore when its depletion is deeper.

When the results of the subjects showing a large increase of resting PCR (> 10%) were considered separately (n = 5), Rₚₑₛₚ re-
mained very similar during the recovery of the 40% and 70% MVC exercises between the pre- and the post-treatment conditions. It seems that R\text{ref} could only be improved in subjects showing a great increase of muscle PCR following creatine supplementation. Indeed a positive effect has been reported in R\text{ref} in middle-aged subjects whose resting PCR were increased by 30% after creatine supplementation whereas this effect was not found in young subjects [24].

The consequence of creatine supplementation is a gain in BM and more specifically in lean mass [6]. Up to now the origin of this effect has not been clearly identified. It was hypothesized that the rise in creatine concentration could increase the osmolarity of the intracellular milieu despite the large number of mechanisms allowing the cell to self-regulate its own osmolarity. This mechanism would imply water retention and cellular swelling. Ingwall [18] has proposed an "anaobic effect" of creatine that would stimulate the muscle protein synthesis. If the water retention hypothesis is correct, the increase in BM should be observed comitantly with the rise in muscle creatine content whereas protein synthesis should be a longer-term phenomenon. Recently we observed a weight-gain of about 2 kg in subjects having consumed a creatine enriched diet over a period of nine weeks [7]. No effect was observed in TBW and in ICW when they were expressed in percentage of body mass. The present study did not show any significant change in BM over a period of two weeks despite an increase in PCR (–20%) measured in the calf muscle of the subjects. Moreover no effect was found in the volume of body water compartments. These negative results cannot be explained by a lack of sensitivity of bioimpedance spectroscopy technique since we observed an increase in the absolute value of ICW and TBW after six and nine weeks of creatine supplementation [7].

The delay between the rise in muscle PCR and the gain in BM and in ICW argues against a retention of water responsible for the increase in weight regularly observed after medium-term creatine supplementation. Although the uptake of creatine modifies the osmolarity of the intra-cellular milieu, the cell is still able to normalize its osmolarity, i.e. by ions exchange [15]. Although bio-impedance spectroscopy is not able to detect any increase in net protein synthesis, the results previously reported in the literature [7,20] and the present data suggest that BM increases following a medium-term creatine supplementation, which is not due to intra-cellular water retention but probably to dry matter growth accompanied by a normal volume of intra-cellular water.

The results of the present study confirm that creatine supplementation increases muscle PCR content by –20%. However, no effect was found on R\text{ref} and t\text{1/2}. The data also suggest that during standardized exercises of different intensities more ATP is synthesized by the CK reaction when the pre-exercise level in PCR is higher, giving support to the positive effects recorded on muscle performance.

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