The Effect of Sprint Training on Skeletal Muscle Carnosine in Humans

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It has been suggested that histidine-containing dipeptide carnosine (β-alanyl-L-histidine), which is believed to act as a cytosolic buffering agent, is present predominantly in skeletal muscle. The purpose of this study was to investigate the effects of sprint training (30-s maximal cycle ergometer sprinting) on muscle carnosine concentration. Six untrained males trained 2 days per week for 8 weeks on an electronic-braked cycle ergometer. Muscle biopsy samples were taken from the vastus lateralis before and two days after the last training session and were analyzed for carnosine concentration by the use of an amino acid autoanalyzer. The carnosine concentration was significantly increased after sprint training (P < 0.05). The mean power during 30-s maximal cycle ergometer sprinting was significantly increased following training. When dividing the 30-s sprinting into 6 phases (0-5, 6-10, 11-15, 16-20, 21-25, 26-30 s), the magnitude of increase in mean power was significantly larger for the last 2 phases than the first phase (P < 0.05). These results suggest that the increases in skeletal muscle carnosine concentration following sprint training may be associated with the increase in sustainability of high power during 30-s maximal cycle ergometer sprinting.

Keywords: Short duration-high intensity exercise, Wingate test, Mean power, Muscle biopsy, Muscle buffering capacity

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1. Introduction

Carnosine (β-alanyl-L-histidine) is a dipeptide containing histidine and is found in vertebrate skeletal muscles. It has been reported that carnosine contributes to physico-chemical buffering to maintain acid-base balance during high-intensity exercise, which produces large quantities of H+ in association with lactic acid [Hultman and Sahlin (1980); Parkhouse and McKenzie (1984); Parkhouse et al. (1985)]. Physico-chemical buffering during the whole buffering process within the muscle cells depends on the uptake of H+ created by the buffer substance. The buffering capacity is determined by the buffer substance concentration and the hydrogen ion sequestering capability [Larsen and Burnell (1976)]. Since the pKa of carnosine is 6.83 [Bate–Smith (1938); Tanokura et al. (1976)], it is considered to serve as a powerful buffering agent. Therefore, it can be assumed that a high carnosine concentration will prevent intracellular pH decrease during high-intensity exercise which produces large quantities of lactic acid. Since the decrease of intracellular pH brings about muscle fatigue through several mechanisms such as decreased skeletal muscle tension and relaxation [Sahlin et al. (1981)], and the inhibition of phosphofructokinase activity [Trivedi and Danforth (1966)], it appears to be important in preventing the decrease of intracellular pH during exercise to achieve high performance.

In a recent study, Suzuki et al. (2002) recognized that there is a significant positive correlation between the skeletal carnosine concentration and the mean power output during a 30-second maximal cycle
ergometer sprint, especially the mean power output at the final two phases when a 30-s sprint is divided into six phases. Parkhouse et al. (1985) suggested that sprinters have a higher skeletal muscle buffering capacity ($\beta$) and carnosine concentration, and performed significantly better than either marathon runners or untrained subjects in anaerobic speed tests (high-intensity running performance). These results indicate that the skeletal muscle carnosine concentration has an effect on power output during the latter half of a short duration-high intensity exercise, which leads to the accumulation of large quantities of lactic acid. Therefore, it is suggested that carnosine concentration increase is probably involved in improving the short duration-high intensity exercise performance during sprint training.

So far, however, only Mannion et al. (1994) have examined carnosine concentration fluctuation during a 16-week knee extension training, and no study has yet been conducted to investigate carnosine concentration fluctuation during sprint training. Therefore, the purpose of the present study was to examine the effect of sprint training on skeletal muscle carnosine in humans.

2. Methods

2.1. Subjects

Subjects were six healthy male university students; age 22.4 ± 2.2 years; height 171.4 ± 4.3 cm; weight 64.1 ± 7.5 kg. The purpose, contents, and possible dangers of this study were explained to all the participants and each participant’s verbal and written consent was obtained prior to the experiment. This study was conducted with the approval of the Ethics Committee for the Institute of Health and Sport Science, University of Tsukuba.

2.2. Sprint Training

The subjects participated in an 8-week sprint training program. The program consisted of sixteen training sessions, and subjects trained two days per week. This involved single or double bouts of 30-s maximal sprinting on an electronic-braked cycle ergometer (TKK1254a Takei Scientific Instruments Co., Ltd.). During the first 2-week (four sessions), a single bout of sprinting was performed. In the last 6-week (twelve sessions), double bouts of sprinting were performed with 20 minute rest intervals. The training sessions were carried out with at least a two-day interval between training days and scheduled on the same days of the week as far as possible. The following warm-up exercises were performed:

1) A 2-min cycle sprint with 60 W resistance load,
2) A 2-min cycle sprint with 120 W resistance load,
3) Two sets of 5-s maximal cycle sprint with 300 W resistance load, taking a 30-s rest between the sets.

The subjects were instructed to pedal as fast as possible from the start and were encouraged to maintain maximum pedaling speed throughout the 30-s period. Power output was calculated and expressed in watts per kilogram each second of the duration of the 30-s maximal cycle ergometer sprint. Two parameters were determined: mean power, which referred to the average power performed during the sprint, and peak power, which referred to the highest power during the sprint. Training resistance load was set at weight × 0.075 kp in the first bout, and weight × 0.05 kp in the second bout based on the weight measured in the first training session. It was observed that a 30-s cycle sprint could not always be accomplished when the load was 7.5% at the second bout in the preliminary experiment. Therefore, the resistance load of the second bout was determined to be less than the first bout. Since the repetition training method, which consisted of 30–90s strenuous exercise with a resting period between the exercise sets, was applied for rest periods of approximately 10–30 min, a 20 minute rest period used in this study.

2.3. Measured procedure

Mean power and peak power during the first bout of 30-s maximal cycle ergometer sprint were recorded continuously in sixteen training sessions. This 30-s maximal cycle sprint with resistance load of weight × 0.075 kp has been commonly applied and is known as the Wingate test, measuring anaerobic capacity [Bar-Or (1987)]. Additionally, the maximum oxygen uptake and carnosine concentration was measured before and after training.

2.3.1. Maximum oxygen uptake

Maximum oxygen uptake ($V_O_2 max$) was measured by an incremental cycle exercise test. The subjects performed a 4-min warm-up at 30 W and then resistance load was increased by 30 W every minute until exhaustion. The pedaling rate was set
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-at 60 rpm. Oxygen uptake (\(\dot{V}O_2\)) was measured every 30-s interval during the incremental exercise test using an automatic gas analyzer (Mijnhardt, OXYCON-GAMMA). \(\dot{V}O_2\) max was defined as the highest \(\dot{V}O_2\) measured during the test.

2.3.2. Muscle biopsy

The needle biopsy technique was applied to collect the muscle samples. The muscle samples (20-30 mg) were taken from the vastus lateralis muscle of the right femoral region, 15-20 cm proximal to the upper end kneecap and were 3-4 cm in depth. Biopsies were taken one week before (Before) and 2 days after (After) the sprint training. All the samples were stored at -80˚C until assayed.

2.3.3. Skeletal muscle carnosine concentration

Skeletal muscle carnosine concentration was measured according to the procedure described by Suzuki et al. (2002). The muscles were accurately weighed on a microbalance and then homogenized at 0 ℃ in 70 % ethanol. The homogenate was extracted at 100 ℃ and filtered through a 0.45 µm filter. The filtered sample was volatilized at 100 ℃, and was added diethylether. The supernatant fluid was discarded, and the sample liquid was desiccated in a centrifugal machine. The desiccated samples were added to 0.01 N sodium hydroxide, and the mixture was left at room temperature for 4 hours. The sample to which 0.02 N hydrochloric acid was added was then analyzed using an amino acid analyzer (JEOL, JLC-300).

2.4. Statistical analysis

All data are expressed as means ± SD. Differences between before and after training were examined using student’s paired t- test. Differences among the rate of change in mean power at six phases of the 30-s maximal cycle ergometer sprint were examined using repeated measurements of the analysis of variance (ANOVA) and Fisher’s PLSD post hoc comparison. Statistical significance level was set at \(P < 0.05\).

3. Results

Significant change was not observed in body mass (before: 64.1 ± 3.1 kg, after: 64.2 ± 4.4 kg) and in \(\dot{V}O_2\) max (before: 48.1 ± 4.5 ml·min⁻¹·kg⁻¹, after: 50.7 ± 4.3 ml·min⁻¹·kg⁻¹).

Table 1 shows skeletal muscle carnosine concentration before and after training when compared to before training (\(P < 0.05\)).
Table 2 shows the mean power, peak power of the 30-s maximal cycle sprint (the first bout; Wingate test) in the first (tr 1) and the last (tr 16) session. Both mean power and peak power were significantly higher in the last session than in the first session. The sixth phase showed a significantly higher mean power than the second phase (P < 0.05).

4. Discussion

The major finding of this study is that skeletal muscle carnosine concentration increased significantly by the 8-week sprint training. Further, this is the first study to report that sprint training elevates skeletal muscle carnosine concentration.

Carnosine is found in the brain, heart, skin, liver, kidney, and skeletal muscle of various animals [Chan et al. (1994); Crush (1970)]. Skeletal muscle in particular contains a high quantity of carnosine. However, the content varies greatly among different species and different regions of muscle [Davey (1960)]. Since it has been indicated that carnosine is found more in Type II fiber than Type I [Bump et al. (1990); Harris et al. (1998); Suzuki et al. (2002)] and that sprinters have higher carnosine concentration and show longer endurance time in anaerobic speed test than either marathon runners or untrained people [Parkhouse et al. (1984)], it can be inferred that sprint training increases carnosine concentration.

Mannion et al. (1992) measured carnosine concentration in vastus lateralis muscle of 50 ordinary healthy males and females, noting that carnosine concentration in males is significantly higher than in females. However, the concentration differs greatly across individuals. Such differences among individuals were also observed in the present study, with initial values of carnosine concentration ranging from 3.86 to 7.50 mmol·kg⁻¹ wet muscle. However, Mannion et al. (1992) reported that significant differences were not observed between the values of carnosine concentration at rest when measured twice at two-monthly intervals. This indicates that skeletal muscle carnosine concentration does not increase significantly in the daily lives of ordinary healthy people. Therefore, it can be inferred that the increase of carnosine concentration observed in the present study can be attributed to the sprint training exercise, although it should be noted that a control group was not used in the study.

The sprint training in this study consisted of performing a 30-s maximal cycle ergometer sprint twice a week. It has been reported that lactic acid value in the muscles would reach more than 100
mmol·l⁻¹ immediately after the end of such exercise. Additionally, the muscle pH level would decrease to around 6.7 in 30-s maximal cycle sprint [Bogdanis et al. (1996); Rodas et al. (2000)]. This indicates that glycolysis is the primary energy suppliers during 30-s maximal cycle sprint, and this is supported that the subjects did not show significant increase in VO₂ max after training, even though initial values obtained from the subjects were relatively low (48.1 ± 4.5 ml·min⁻¹·kg⁻¹). It is also assumed that buffering action works positively during 30-s maximal cycle sprint where a large quantity of lactic acid is produced and pH level decreases to a great extent. Therefore, it is possible that carnosine is increased due to performing such exercise for long term.

The histidine-containing dipeptide carnosine has a pKa value of 6.83 [Bate–Smith (1938), Tanokura et al. (1976)], and serves as a buffering agent to neutralize the acidosis in associated with generation of lactic acid during high intensity exercise. Since lactic acid is dissociated almost completely to H⁺ and La⁻ in the physiological pH range, the accumulation of lactic acid causes a decrease in intracellular pH. It has been reported that decrease of intracellular pH inhibit the activity of PFK [Trivedi and Danforth (1966)], decrease the calcium ion (Ca²⁺) emission from the sarcoplasmic reticulum and decrease binding constant troponin [Fuchs et al. (1970)]. These results indicate that a decrease of intracellular pH cause the decrease of muscle contraction force. Therefore, it is inferred that the increase in skeletal muscle carnosine concentration, which augments buffering capacity, will minimize the decrease of intracellular pH during the latter half of short duration-high intensity exercise that accumulate large quantities of lactic acid. Consequently, high power can be elicited in the latter half of exercise.

This study assessed the training effect on performance by using the results of first bout of the 30-s maximal cycle sprint (Wingate test). Significantly higher values were observed for both mean power and peak power in the last session (tr 16) when compared to the first session (tr 1). When dividing 30-s maximal cycle sprint into 6 phases of 5-s each, the calculated mean power of each phase showed significantly higher values at every phase for tr 16 as compared to tr 1. The magnitude of increase in mean power was significantly larger for the last 2 phases than the first phase in which peak power is seen (P < 0.05, Figure 1). These results suggest that augmented mean power at tr 16 greatly accounts for the increase in power during the latter half of 30-s maximal cycle sprint, and that augmented physico-chemical buffering caused by the increase of skeletal muscle carnosine concentration can be involved in augmented power in the latter period.

Davey (1960) reported that carnosine accounts for approximately 40 % of the buffering capacity within muscle cells. However, in later research, Mannion et al. (1992) indicated that theoretical buffer value of carnosine in physico-chemical buffering accounts for 7 %. Hultman and Sahlin (1980) pointed out that muscular protein, bicarbonate ion, phosphate and carnosine are contributive substances in physico-chemical buffering in high-intensity exercise, accounting for about 50 %, 30 %, 13 % and 6 % of the buffering, respectively. This could suggest that the degree of carnosine accounting for the physico-chemical buffering is not so high.

It is difficult to apply these values directly to the result of the 30-s maximal cycle sprint used in the present study, because the exercise period of 5–10 minutes in the study of Hultman and Sahlin (1980) is comparatively long for high intensity exercise. Mannion et al. (1992) indicated that carnosine is essential to determine the skeletal muscle buffering capacity and that the skeletal muscle buffering capacity can be improved by increasing carnosine concentration because the carnosine concentration variations among individuals is greater than that of other buffer substances. Mannion et al. (1994) also indicated a significant positive correlation between the rates of change in muscle buffering capacity and the rate of change in carnosine concentration during the 16-week knee extension training. Moreover, Gore et al. (2001) suggested that an increase in carnosine could play a part in the improvement of skeletal muscle buffering capacity because muscular protein, a potent buffer substance in physico-chemical buffering, did not change significantly, although muscle buffer capacity improved significantly according to the "Living high-training low" effect. After considering these facts, carnosine seems to be an important factor affecting buffer capacity. It is assumed that carnosine increase can improve the muscle buffering capacity, resulting in the prevention of intracellular pH decrease during the latter half of short duration-high intensity exercise.
In conclusion, the present study observed significant increase in skeletal muscle carnosine concentration after 8-week of short duration-high intensity sprint cycle training. It is observed that mean power during 30-s maximal cycle ergometer sprinting was significantly increased following training and that the magnitude of increase in mean power was significantly larger for the latter half of exercise. These results suggest that the increases in skeletal muscle carnosine concentration may be associated with improving performance following high intensity sprint training.

References