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RESEARCH ARTICLE | *Passive Properties of Muscle*

Effect of β-alanine supplementation during high-intensity interval training on repeated sprint ability performance and neuromuscular fatigue

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Milioni F, de Poli RA, Saunders B, Gualano B, da Rocha AL, Sanchez Ramos da Silva A, Muller PT, Zagatto AM. Effect of β-alanine supplementation during high-intensity interval training on repeated sprint ability performance and neuromuscular fatigue. *J Appl Physiol* 127: 1599–1610, 2019. First published October 17, 2019; doi: 10.1152/japplphysiol.00321.2019.—The study investigated the influence of β-alanine supplementation during a high-intensity interval training (HIIT) program on repeated sprint ability (RSA) performance. This study was randomized, double-blinded, and placebo controlled. Eighteen men performed an incremental running test until exhaustion (T_{INC}) at baseline and followed by 4-wk HIIT (10 × 1-min runs 90% maximal T_{INC} velocity [1-min recovery]). Then, participants were randomized into two groups and performed a 6-wk HIIT associated with supplementation of 6.4 g/day of β-alanine (Gβ) or dextrose (placebo group; GP). Pre- and post-6-wk HIIT + supplementation, participants performed the following tests: *1*) T_{INC}; *2*) supramaximal running test; and *3*) 2 × 6 × 35-m sprints (RSA). Before and immediately after RSA, neuromuscular function was assessed by vertical jumps, maximal isometric voluntary contractions of knee extension, and neuromuscular electrical stimulations. Muscle biopsies were performed to determine muscle carnosine content, muscle buffering capacity in vitro ($\beta m_{in\ vitro}$), and content of phosphofructokinase (PFK), monocarboxylate transporter 4 (MCT4), and hypoxia-inducible factor-1α (HIF-1α). Both groups showed a significant time effect for maximal oxygen uptake (Gβ: 6.2 ± 3.6% and GP: 6.5 ± 4.2%; $P > 0.01$); only Gβ showed a time effect for total (−3.0 ± 2.0%; $P = 0.001$) and best (−3.3 ± 3.0%; $P = 0.03$) RSA times. A group-by-time interaction was shown after HIIT + Supplementation for muscle carnosine (Gβ: 34.4 ± 2.3 mmol·kg^{-1}·dm^{-1} and GP: 20.7 ± 3.0 mmol·kg^{-1}·dm^{-1}; $P = 0.003$) and neuromuscular voluntary activation after RSA (Gβ: 87.2 ± 3.3% and GP: 78.9 ± 12.4%; $P = 0.02$). No time effect or group-by-time interaction was shown for supramaximal running test performance, βm, and content of PFK, MCT4, and HIF-1α. In summary, β-alanine supplementation during HIIT increased muscle carnosine and attenuated neuromuscular fatigue, which may contribute to an enhancement of RSA performance.

NEW & NOTEWORTHY β-Alanine supplementation during a high-intensity interval training program increased repeated sprint performance. The improvement of muscle carnosine content induced by β-alanine supplementation may have contributed to an attenuation of central fatigue during repeated sprint. Overall, β-alanine supplementation may be a useful dietary intervention to prevent fatigue.

anaerobic capacity; muscle buffering capacity; muscle carnosine; Western blot

INTRODUCTION

β-Alanine intake is common in recreational exercise practitioners to high-level athletes (46) and is an important precursor in muscle carnosine (β-alanyl-L-histidine) synthesis (40). Muscle carnosine is a dipeptide that performs several physiological roles including antioxidant scavenging, regulation of calcium sensitivity, and muscle buffering due its acid dissociation constant of 6.83 that makes it an efficient acceptor of hydrogen ions (for review, see Ref. 2). Supplementation of 4.0–6.4 g/day of β-alanine during 4–24 wk can increase muscle carnosine content (21, 22, 41) and muscle buffering capacity, although few studies have directly measured the latter (21, 22). Thus supplementation of β-alanine may be a promising strategy to minimize muscle acidosis (23, 40) induced by repeated all-out sprints.

The ability to repeatedly perform all-out sprints interspaced by incomplete recovery [termed repeated sprint ability (RSA)] is a fitness component of several sports modalities (6) and results in hydrogen accumulation and a drop in muscle pH (6, 32). This proton accumulation may inhibit anaerobic glycolysis since in vitro analysis has verified that phosphofructokinase (PFK; key regulatory glycolytic enzyme) activity is downregulated by a pH decrement (47, 48). In addition, muscle acidosis can impair the neuromuscular system (17) and has been suggested as an important trigger of the group III/IV muscle afferent

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feedback, which is responsible for restraining the central motor drive and preventing a critical threshold of peripheral fatigue to be reached (1, 25).

This impairment of anaerobic glycolytic activity (28, 47, 48) and subsequent neuromuscular fatigue induced (17) by muscle acidosis may impair RSA performance. Despite the potential to increase muscle buffering capacity, the effect of β-alanine supplementation on RSA performance and the likely mechanisms involved are unexplored. Sweeney et al. (43), Ducker et al. (13), and Milioni et al. (33) showed no ergogenic effect of β-alanine supplementation on RSA performed in running (nonmotorized treadmill, running track, and sprints with change of direction), while Brisola et al. (8) and Claus et al. (10) observed discrete improvement on RSA performed in swimming by professional and young water polo players.

Theoretically, the potential to delay acidosis due to increased muscle buffering from muscle carnosine would allow a greater effectiveness of anaerobic glycolysis, improving performance and consequently generating a higher blood lactate concentration. Recent evidence suggests that lactate is a powerful signaling molecule, able to alter the expression of PFK (28, 29) and upregulate hypoxia-inducible factor-1α (HIF-1α) (16, 35), which can increase anaerobic glycolysis (35) and lactate production and transport (16, 35), through the expression of the monocarboxylate transporter 4 (MCT4) (35, 45). Similarly, improved capacity to handle intramuscular hydrogen accumulation would protect against neuromuscular fatigue. However, there is no information regarding which mechanisms at the biomolecular level would be modulated by β-alanine supplementation, especially when administered concomitantly with a training program, and its potential contribution to performance.

High-intensity interval training (HIIT) is an efficient tool to improve anaerobic capacity (9, 44) as well as RSA performance (27). Although the mechanisms for this improved anaerobic capacity and RSA performance induced by HIIT are not completely clear, there may be a link between HIIT and the increment of muscle carnosine content (12) and buffer capacity (15). Also, studies investigating the practical relevance of β-alanine supplementation during controlled training routines (i.e., HIIT) and the possible benefits of the association of both strategies (HIIT + supplementation) reported likely beneficial effects (5, 19, 42).

We hypothesized that β-alanine supplementation during HIIT would increase the muscle carnosine content and consequently muscle buffering capacity, allowing greater molecular adaptation of PFK muscle content (i.e., key regulatory glycolytic enzyme) system during the RSA. Also, the increased capacity to handle hydrogen accumulation during a high-intensity effort (i.e., RSA) would protect against neuromuscular fatigue contributing to improve RSA performance. Thus the present study investigated whether β-alanine supplementation administered during HIIT could *1*) increase the muscle carnosine content and muscle buffering capacity; *2*) alter the content of PFK, MCT4, and HIF-1α; *3*) attenuate neuromuscular fatigue; and *4*) enhance RSA performance.

METHODS

Participants

The sample size was calculated using G*Power software (University of Düsseldorf, Düsseldorf, Germany), based on the assumption that 4 wk of 6.4 g/day of β-alanine supplementation can increase muscle carnosine content with an effect size of 1.96 (41). With a statistical power of 95% and alpha level of 0.05, the sample size calculated was $n = 9$ participants in each group. Thus 18 physically active males, were recruited to be part of the investigation [initial maximal oxygen uptake ($\dot{V}O_{2max}$): means ± SD: 43.7 ± 3.8 $mL \cdot kg^{-1} \cdot min^{-1}$; body weight: 74.3 ± 8.4 kg; height: 174.8 ± 6.4 cm; and age: 25 ± 5 yr]. Exclusion criteria included nonomnivore (i.e., vegetarian or vegan); regular user (in the last 6 mo) of creatine, β-alanine, and/or whey protein; and presence of any musculoskeletal disorder. The participants were asked to maintain their nutritional habits during participation in the study, as well as to abstain from strenuous activities and caffeine for 24 h and consume a light meal 2 h before each training/testing session. The study was approved by the Local Ethics Committee and was conducted according to the Declaration of Helsinki. All participants were informed about the procedures, benefits, and risks of the investigation before signing an informed consent form before beginning the study (Fig. 1).

Experimental Design

The participants first performed an incremental running test until exhaustion (T_{INC}) to determine $\dot{V}O_{2max}$ and maximal aerobic velocity. Thereafter, participants initiated a 4-wk HIIT program detailed below. The 4-wk training stimulus, without supplementation, aimed to induce an initial positive performance adaptation to avoid HIIT overwhelming the potential effects of β-alanine supplementation as suggested by Cochran et al. (11). After 4 wk of training, participants were matched for $\dot{V}O_{2max}$ and randomized into a β-alanine (Gβ: 6.4 g/day of β-alanine; $n = 9$) or placebo (GP: 6.4 g/day of dextrose; $n = 9$) group and continued the HIIT-based training program associated with the supplementation protocol (HIIT + supplementation) during a further 6 wk. Pre- and post-HIIT + supplementation, the participants performed the following tests with 48-h recovery between them: *1*) T_{INC}; *2*) supramaximal running test until exhaustion (T_{SUPRA}); and *3*) two bouts of 6 × 35-m all-out sprints (RSAs). Before (i.e., resting condition) and immediately after (i.e., fatigued condition) RSA, neuromuscular function was assessed by maximum vertical countermovement jumps, maximal isometric voluntary contractions (MVCs) of the knee extensors, and peripheral neuromuscular electrical stimulations. All protocols (except RSAs) were performed on a stationary treadmill (ATL; Inbramed, Porto Alegre, Brazil), with 5 min warm-up at 8 km/h and 5 min of passive recovery before the effort. Participants underwent muscle biopsies at Pre- and post-HIIT + supplementation. Forty-eight to seventy-two hours following the final physical test to determine muscle carnosine content, muscle buffering capacity in vitro ($\beta m_{in\ vitro}$) and the content of PFK, MCT4, and HIF-1α. (Fig. 2).

Incremental Running Test

The protocol started at 8 km/h with increments of 1.5 km/h every 2 min until volitional exhaustion. $\dot{V}O_{2max}$ was considered to be the highest average of the oxygen uptake obtained during the final 30 s of each stage, assuming the oxygen uptake plateau (i.e., range <2.1 $mL \cdot kg^{-1} \cdot min^{-1}$ in the final 2 stages) as primary criteria and secondarily a respiratory exchange ratio >1.10, maximal heart rate $>90\%$ of maximum predicted HR (220 – age), and peak blood lactate concentration ≥ 8.0 mmol/L (24). Maximal aerobic velocity was determined as the highest velocity achieved during T_{INC}; however, when the participant remained <30 s in the final stage, maximal aerobic velocity was adjusted according to Kuipers et al. (26).

High-Intensity Interval Training

The HIIT sessions consisted of 10 × 1-min runs at 90% of maximal aerobic velocity with 1-min passive recovery [adapted from Little et al. (30)]. Heart rate (Polar RS400, Kempele, Finland) was measured

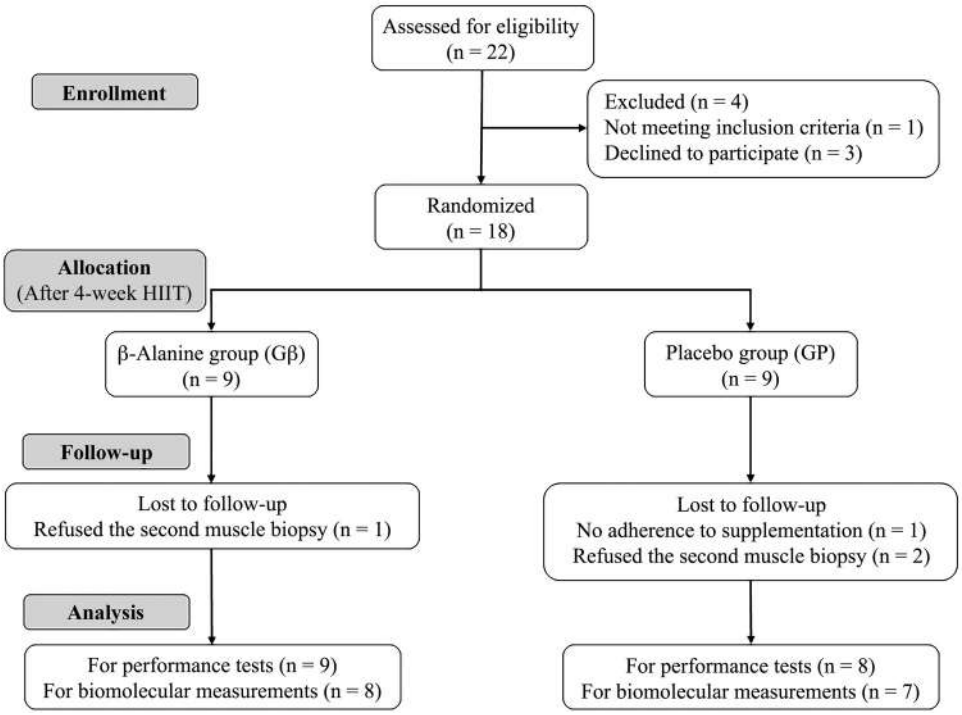

Fig. 1. Schematic recruitment of participants and progression of each stage of the study. HIIT, high-intensity interval training.

during each run and participants were required to reach 90% of maximal heart rate in the last five runs; if they did not, the intensity was increased by ~3% of maximal aerobic velocity in the subsequent HIIT session. Eleven training sessions were performed during the initial 4-wk of HIIT and 17 during the 6-wk HIIT + supplementation, with 36–72 h rest between sessions. All training sessions were supervised by a member of the research team, and participants presented excellent adherence to training program with 98% of all sessions completed.

Supplementation Protocol

Participants ingested 6.4 g/day of β-alanine (CarnoSyn β-alanine; NAI) or placebo (unflavored dextrose, Max Titanium; Supley, Matley, SP, Brazil) administered orally in 800 mg gastro-resistant capsules coated with hydroxypropyl-methylcellulose (Drcaps; Capsugel, Colmar, France). Participants were suggested to ingest supplements with meals (2 capsules per meal) to avoid paraesthesia (23, 41).

In the fifth week of supplementation, one participant (belonging to GP) was excluded from the study due to the impossibility of maintaining the supplementation protocol, and the remaining participants self-reported 100% of adherence to the supplementation protocol.

Supramaximal Running Test

The supramaximal running test was performed at 115% of previously determined individual maximal aerobic velocity, and participants were required to exercise until exhaustion. Oxygen uptake

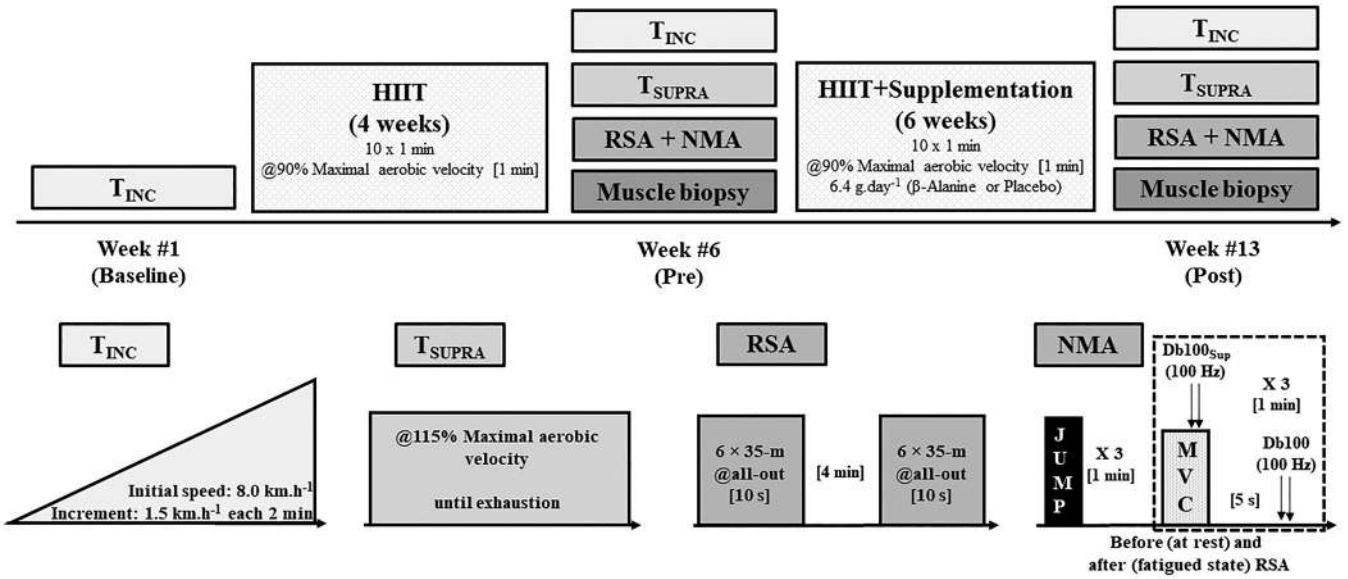

Fig. 2. Schematic chart of experimental design. T_{INC}, incremental running test; HIIT, high-intensity interval training; T_{SUPRA}: supramaximal running test; RSA, repeated sprints test; NMA, neuromuscular function assessment.

averaged over the final 30 s of the test and time-to-exhaustion were recorded as performance indicators. Blood lactate concentration was also measured.

Repeated Sprint Ability Test

The participants performed the running-based anaerobic sprint test (RAST) [intraclass correlation of RAST variables ~0.88 (49)] twice, with 4-min of passive recovery between sets. The RAST consists of 6 × 35-m all-out sprints with 10-s passive recovery between sprints. All sprints were recorded by two video cameras (GoPro Hero 3+ Black, San Mateo, CA) that were synchronized and positioned laterally to the track. Optical sensors which emitted light and a "beep" sound when the participants passed through were positioned at the finish lines. Sprint times were analyzed using Kinovea software (Kinovea 0.8.15 for Windows), and the variables measured from RSA were total time, best time, mean time, worst time, and fatigue index [100 × (total time/(best time × 12) – 100].

Physiological Variables

Throughout the T_{INC} and T_{SUPRA}, oxygen uptake was measured breath-by-breath using an ergospirometer (Quark PFT, Cosmed, Rome, Italy) calibrated before each test according to standard procedures. For oxygen uptake analysis, data were smoothed every five points and interpolated every 1 s to eliminate outlying data. Heart rate was measured using a transmitter belt coupled to the gas analyzer (Wireless HR 138 monitor; Cosmed). Blood samples (25 μL) were collected from the earlobe preexercise and 3, 5, and 7 min following exercise (T_{INC}, T_{SUPRA}, and RAST), stored in 1.5-mL plastic tubes containing 50 μL of sodium fluoride (1%), and immediately frozen at −20°C for later analyses using an electrochemical analyzer (YSI 2900; Yellow Spring Instruments, Yellow Spring, OH) (measurement error: ±2%).

Neuromuscular Function Assessments

Vertical jumps. Three countermovement jumps, with 1-min rest between jumps, were performed on a jump platform (Jump test; CEFISE, Nova Odessa, SP, Brazil), and the highest jump attempt of each participant was used. The intraclass correlation and coefficient of variation for jump height were 0.90 (0.80–0.95) and 5.3%.

MVC measurements and femoral nerve electrical stimulations. One minute after the final jump, participants performed three MVCs separated by 1-min rest. A custom-designed chair maintained a 90° flexion of participants' hips and knees; participants were firmly fixed to the chair with straps across the chest, hip, and thigh. The ankle of the dominant leg was attached to a load cell (SDK200; Miotec, Porto Alegre, RS, Brazil), and the force signal of MVC was acquired at 2000 Hz. Supramaximal, square-wave, electrical pulses were delivered on the femoral nerve by a constant current electrical stimulator (Bioestimulador; Insight, Ribeirão Preto, SP, Brazil; 400 V max). Electrodes (5 × 5 cm) with conductive gel were placed in the femoral triangle (cathode) and the gluteal fold (anode) and marked with ink for precise replacement after the RSA. The optimal intensity of stimulation was determined by consecutive and incremental doublet pulses (100 Hz; Db100) to the relaxed muscle until reaching the twitch force plateau (34). Supramaximal stimulation was ensured by increasing the stimulation intensity by 20%. The femoral nerve electrical stimulations were composed by a Db100 superimposed to MVC ($Db100_{sup}$) and potentiated Db100 on relaxed muscle 5 s after MVC termination (Fig. 2).

Force signal analysis was carried out according to Milioni et al. (34) using specific MatLab algorithms (The Math Works, Natick, MA). The voluntary activation (VA) was calculated as VA = [1 − ($Db100_{sup}$ × (force level at stimulation/MVC)/Db100)] × 100 (34). The intraclass correlations and coefficient of variations measured for MVC, Db100, and VA were 0.82 (0.59–0.92) and 4.8%; 0.70 (0.39–0.87) and 5.7%; and 0.62 (0.25–0.83) and 3.2%.

Muscle Biopsy and Biomolecular Measurements

Muscle biopsy. Participants underwent muscle biopsies at pre- and post-HIIT + supplementation 48–72 h following the last physical test. Following local anesthesia of the m. vastus lateralis using 2% lidocaine without vasoconstrictor, an incision of ~0.5–1.0 cm was made. Muscle samples were obtained using a modified Bergstrom needle with suction; approximately 60- to 100-mg fragments were immediately frozen in liquid nitrogen and stored at −80°C. Three participants refused to participate in the post-HIIT + supplementation biopsy (1 from Gβ and 2 from GP), resulting in a final sample size for biomolecular measurements of Gβ: $n = 8$; GP: $n = 6$.

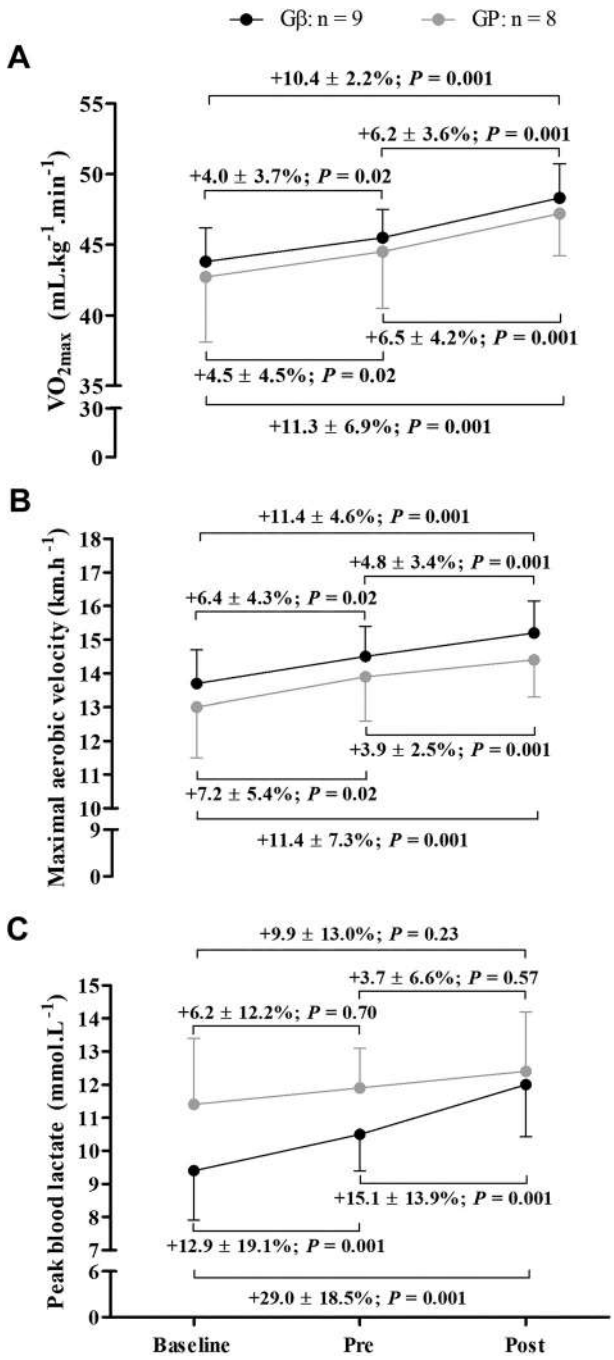

Fig. 3. Incremental running test results. *A*: maximal oxygen uptake. *B*: maximal aerobic velocity. *C*: peak blood lactate. β-Alanine (Gβ): $n = 9$; placebo group (GP): $n = 8$.

Chromatographic determination of muscle carnosine content. Muscle carnosine was determined by using HPLC (Hitachi, Hitachi, Tokyo, Japan), as thoroughly described by Saunders et al. (41). Ten random samples were quantified in duplicate to verify the coefficient of variation (coefficient of variation = 6.5%).

Western blot measurements. Approximately 20 mg of wet muscle were used as previously described by da Rocha et al. (37, 38). The antibodies used were PFK (no. 8164s; Cell Signaling Technology), MCT4 (AB3316P; Millipore), HIF-1α (no. 14179s; Cell Signaling Technology), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH; no. 2118s; Cell Signaling; dilution 1:1,000). The transfer efficiency onto nitrocellulose membranes was confirmed by brief staining of the blots with the Ponceau red stain. The protein/enzymatic content measured at each time point (pre- and post-HIIT + supplementation) were normalized by the respective standard protein content (i.e., GAPDH).

Muscle buffering capacity in vitro. Muscle buffering capacity was performed according to Bishop et al. (7). An aliquot of dry muscle (~2.0 mg) was homogenized in a solution of sodium fluoride (NaF; 10 mM; 33 μL $NaF \cdot g^{-1} \cdot dm^{-1}$). This homogenate was maintained at 37°C (Thermomixer, Eppendorf, Germany) and adjusted to a pH of 7.2 by the addition of NaOH (0.02 mM). The monitoring of pH was performed by a glass microelectrode (Microelectrodes; Mettler Toledo, Greifensee, Switzerland) connected to a pHmeter (FiveEasy Plus; Mettler Toledo). The homogenate was then titrated by a sequence of additions of 2 μL HCl (10 mM) until a pH of 6.2 was reached. All pH measurements were performed three times, and the mean value was used. From these data, the number of moles of hydrochloric acid required to change the pH from 7.2 to 6.5 was adjusted by the amount of dry muscle aliquoted in the sample for the determination of $\beta m_{in\ vitro}$.

Statistical Analysis

Performance data were reported as means ± SD while biomolecular data were reported as means ± SE. Homogeneity was confirmed by the Shapiro-Wilks test. A general linear model was applied for T_{INC} (time [baseline × pre × post] × group [Gβ × GP]); T_{SUPRA}, RSA, and biomolecular measurements (time [pre × post] × group [Gβ × GP]); and neuromuscular function assessment (condition [rest × fatigue] × time [pre × post] × group [Gβ × GP]) variables. Sidak's post hoc was used in case of significant F value. The percentage of change between pre- to post-HIIT + supplementation (Δ%) of T_{SUPRA}, RSA, muscle carnosine content, $\beta m_{in\ vitro}$, PFK, MCT4, and HIF-1α of each group was analyzed using independent samples t test. Pearson's correlations were used to determine the relationship between the improvement of RSA variables and absolute change of muscle carnosine content and $\beta m_{in\ vitro}$. Significance level was set at $P \leq 0.05$. The analyses were performed using the statistical package SPSS 17.0 for Windows.

RESULTS

HIIT Efficacy

The HIIT led to significant improvement from baseline to post-HIIT + supplementation (i.e., 10 wk) of $\dot{V}O_{2max}$ (Gβ: +10.4 ± 2.2%; GP: +11.3 ± 6.9%) and maximal aerobic velocity (Gβ: +11.4 ± 4.6%; GP: +11.4 ± 7.3%). However, only Gβ presented significantly increased blood lactate concentration after T_{INC} (Gβ: +29.0 ± 18.5%; GP: +9.9 ± 13.0%; Fig. 3).

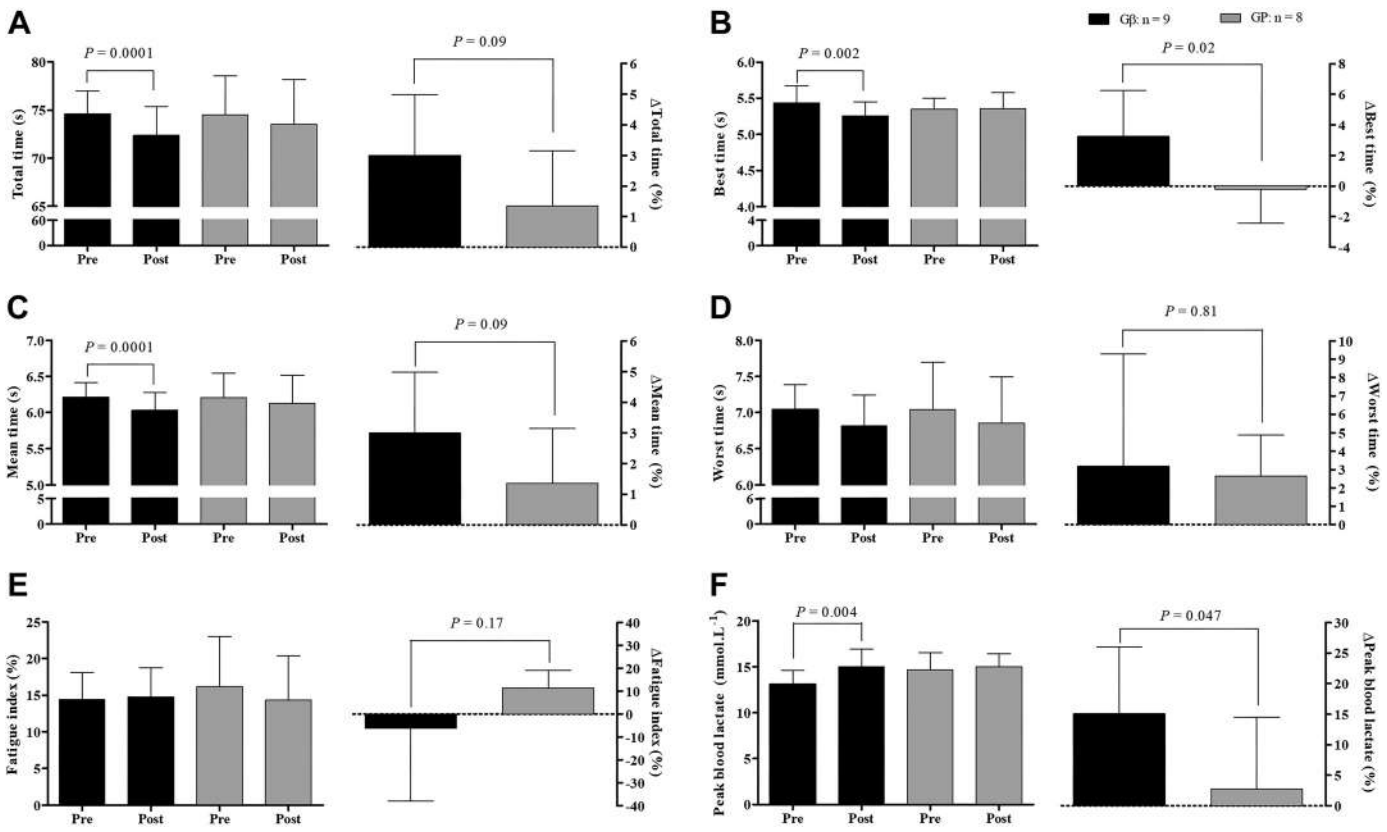

Fig. 4. Repeated sprints performance. *A*: total time. *B*: best time. *C*: mean time. *D*: worst time. *E*: fatigue index. *F*: peak blood lactate. β-Alanine (Gβ): $n = 9$; placebo group (GP): $n = 8$.

Repeated Sprints Performance

Significant decrement of total time ($P = 0.0001$), best time ($P = 0.002$), and mean time ($P = 0.0001$) were observed only for Gβ. Also, only Gβ increased the RSA blood lactate concentration after HIIT + supplementation ($P = 0.004$). The Gβ worst time showed a significant time effect ($P = 0.02$), although post hoc analysis comparing pre- and post-HIIT + supplementation did not reach statistical significance ($P = 0.06$). Fatigue index showed no time effect ($P = 0.34$), and no time × group interaction was observed for any RSA variable (all $P > 0.09$). The percentage of change of best time ($P = 0.02$) and peak blood lactate ($P = 0.047$) was significantly higher for Gβ compared with GP (Fig. 4).

Muscle Carnosine Content and $\beta m_{in\ vitro}$

There was a significant time effect ($P = 0.001$) for muscle carnosine content with significant increases only for Gβ at post-HIIT + supplementation ($P = 0.0001$). There was a significant time × group interaction ($P = 0.001$) with higher values of muscle carnosine content for Gβ compared with GP at post-HIIT + supplementation ($P = 0.003$). The percentage of change between pre- and post-HIIT + supplementation of muscle carnosine content was greater for Gβ compared with GP ($P = 0.01$).

There was no time × group interaction ($P = 0.87$) for $\beta m_{in\ vitro}$, although there was a significant effect of time ($P = 0.02$) with greater overall values at post-HIIT + supplementation. However, the percentage of change of $\beta m_{in\ vitro}$ between groups was not statistically different (Fig. 5).

Correlations between Absolute Change of Muscle Carnosine Content and $\beta m_{in\ vitro}$ with the Improvement of RSA Variables

Significant associations were shown between muscle carnosine content and the improvement of RSA total time ($r = 0.62$; $P = 0.02$) and mean time ($r = 0.62$; $P = 0.02$) (Supplemental Fig. S1; Supplemental Material is available at https://doi.org/10.6084/m9.figshare.9914432). The $\beta m_{in\ vitro}$ was not significantly associated with any RSA variable ($-0.17 < r < 0.11$; $P > 0.23$).

Running Performance at 115% of Maximal Aerobic Velocity

Time to exhaustion ($P = 0.33$) and peak blood lactate concentration ($P = 0.20$) showed no significant time effect after HIIT + supplementation. Oxygen uptake at exhaustion ($P = 0.001$) was improved only for GP. There was no significant group × time interaction for any T_{SUPRA} variable measured ($P > 0.08$). The percentage of change of all T_{SUPRA} variables did not present significant differences (Fig. 6).

Western Blot Measurements

The content of PFK, MCT4, and HIF-1α did not show any significant time ($P > 0.17$) or interaction ($P > 0.09$) effects. However, the percentage of change of HIF-1α was higher for Gβ compared with GP ($P = 0.03$), while MCT4 also showed a trend toward greater increases with Gβ ($P = 0.08$) (Fig. 7).

Neuromuscular Function

MVC (Gβ: 604 ± 96 N; GP: 630 ± 75 N; $P = 0.54$), Db100 (Gβ: 268 ± 37 N; GP: 294 ± 18 N; $P = 0.09$), VA (Gβ:

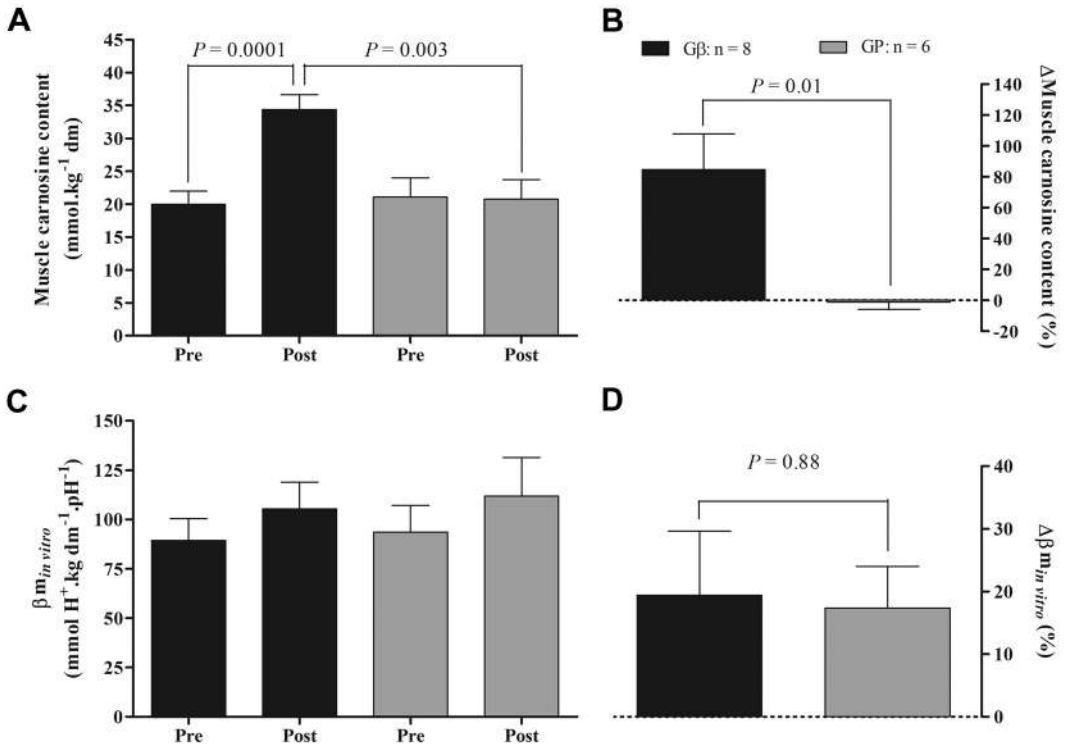

Fig. 5. *A*: muscle carnosine content. *B*: percentage of change of muscle carnosine content between pre- and post-high-intensity interval training (HIIT) + supplementation for β-alanine (Gβ) and placebo group (GP). *C*: muscle buffering capacity in vitro ($\beta m_{in\ vitro}$). *D*: percentage of change of $\beta m_{in\ vitro}$ between pre and post-HIIT + supplementation for Gβ and GP. Gβ: $n = 8$; GP: $n = 6$.

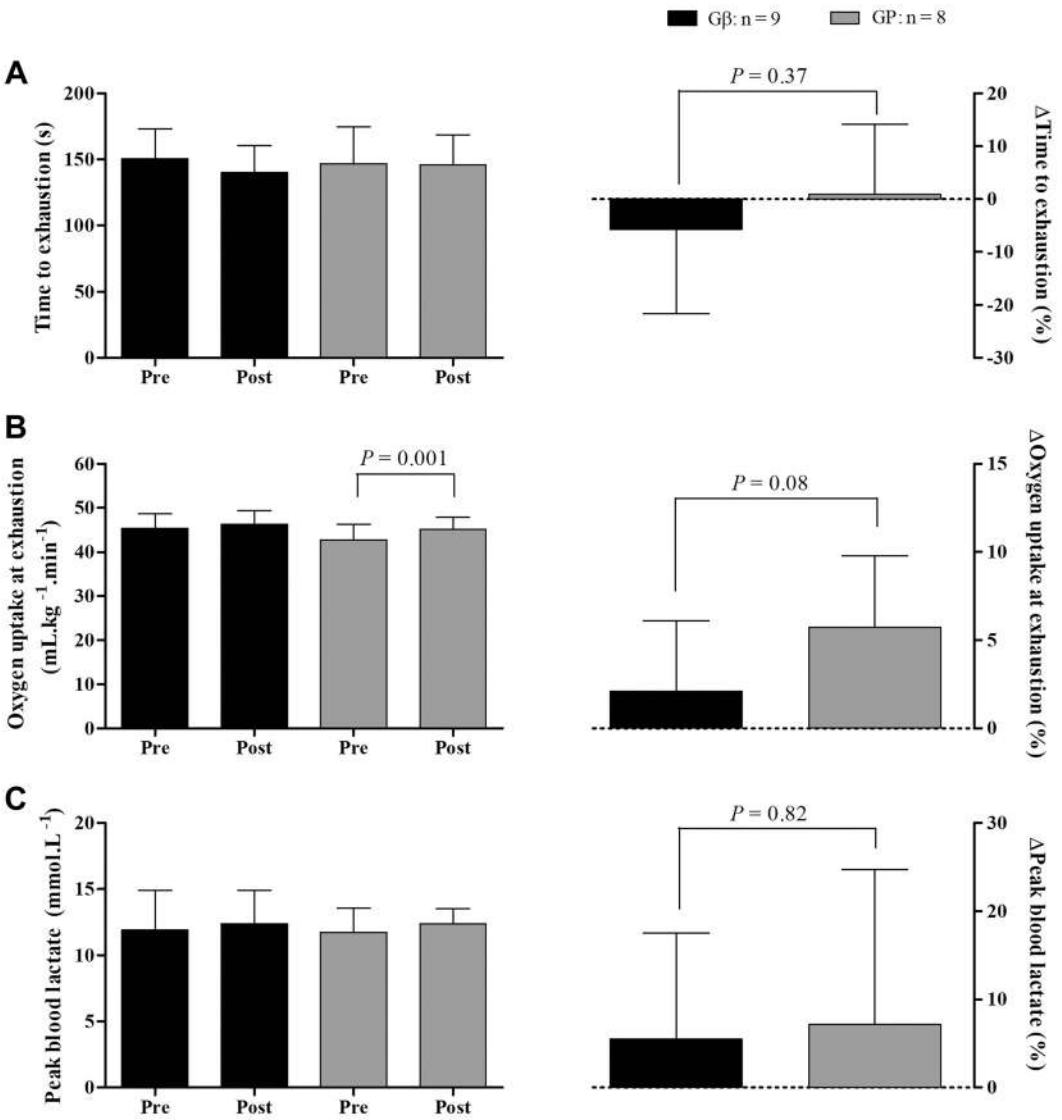

Fig. 6. Supramaximal running test performance. *A*: time to exhaustion at 115% of maximal aerobic velocity. *B*: oxygen uptake at exhaustion. *C*: peak blood lactate. β-Alanine (Gβ): $n = 9$; placebo group (GP): $n = 8$.

88 ± 9%; GP: 88 ± 8%; $P = 0.97$), and jump height (Gβ: 38 ± 5 cm; GP: 37 ± 5 m; $P = 0.64$) were not different between Gβ and GP at pre-HIIT + supplementation.

MVC and jump height showed a significant condition effect ($P = 0.0001$) with significantly lower values in the fatigued state compared with rest for both groups ($P < 0.02$) at both times (pre- and post-HIIT + supplementation). There was no significant time ($P = 0.66$) or interaction ($P = 0.57$) effect for either variable.

The Db100 showed a significant condition effect ($P = 0.0001$), with lower values in the fatigued state compared with rest for GP at both times (pre: $P = 0.01$; post: $P = 0.01$) and for Gβ only at pre-HIIT + supplementation ($P = 0.02$). A significant time effect was observed only for GP ($P = 0.01$), with lower values in the fatigued state of post-HIIT + supplementation compared with fatigued state at pre-HIIT + supplementation ($P = 0.02$). No significant interaction ($P = 0.28$) was shown for Db100.

There was a significant condition effect ($P = 0.003$) for VA, with lower values in the fatigued state compared with rest for GP at both times (pre: $P = 0.02$; post: $P = 0.01$) and for Gβ only at pre-HIIT + supplementation ($P = 0.02$). There was a significant interaction ($P = 0.04$) with higher values for Gβ in the fatigued state of post-HIIT + supplementation compared with GP ($P = 0.02$). A significant time effect was shown ($P = 0.003$), and Gβ presented significant higher value at the fatigued state at post-HIIT + supplementation compared with the fatigued state at pre-HIIT + supplementation ($P = 0.02$), while GP presented significant lower values in the fatigued state at post-HIIT + supplementation compared with fatigued state at pre-HIIT + supplementation ($P = 0.03$; Table 1).

DISCUSSION

The aim of the present study was to investigate the influence of β-alanine supplementation during a HIIT program on RSA performance. Following the 6-wk HIIT + supplementation

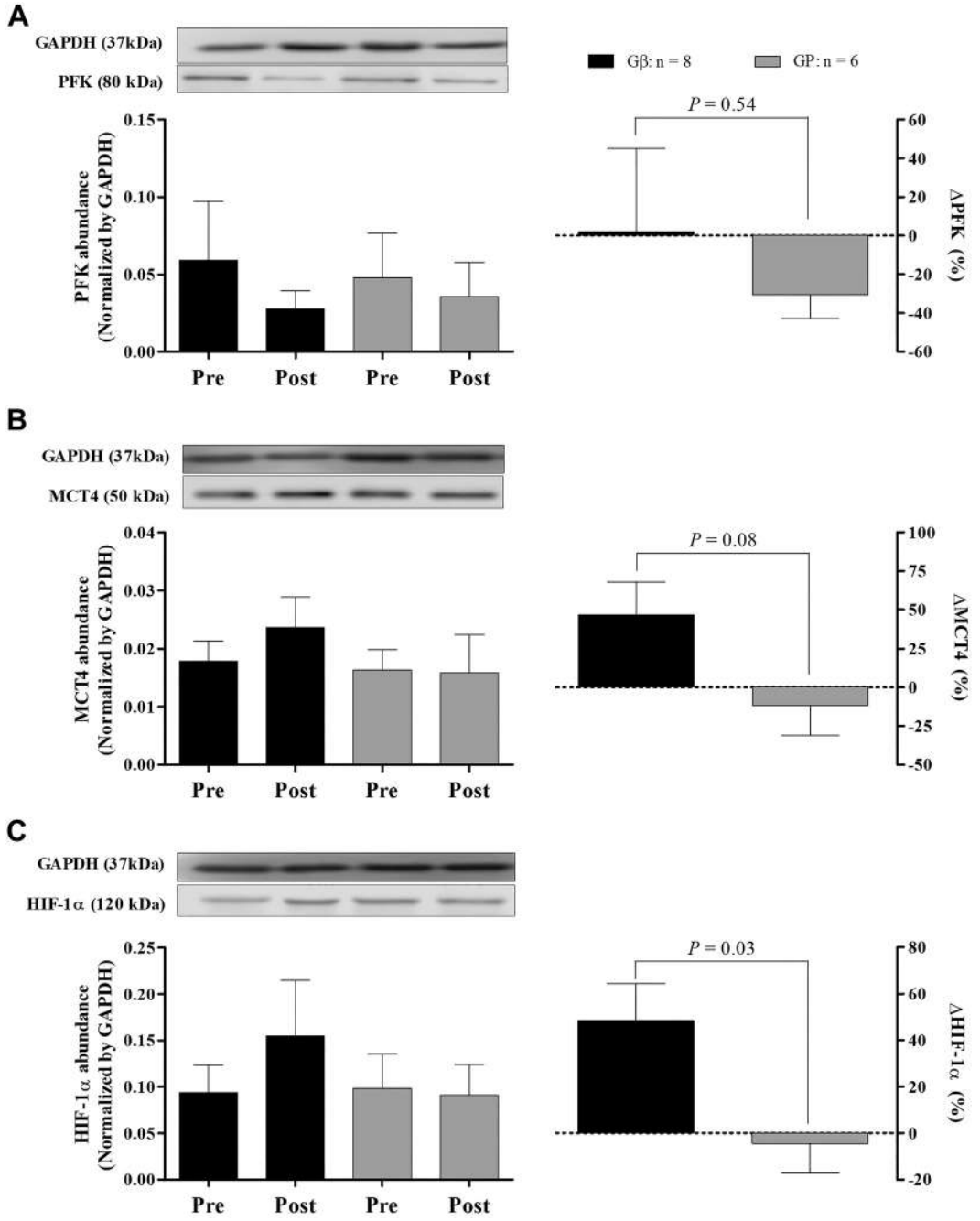

Fig. 7. *A* and *B*: content normalized by GAPDH of phosphofructokinase (PFK; *A*) and monocarboxylate transporter 4 (MCT4; *B*). *C*: hypoxia-inducible factor-1α (HIF-1α). β-Alanine (Gβ): $n = 8$; placebo group (GP): $n = 6$.

protocol, the Gβ group increased muscle carnosine content without a concomitant significant increase in $\beta m_{in\ vitro}$. Gβ, but not GP, improved some measures of RSA performance (i.e., total time, best time, and mean time) and increased blood lactate concentration after the RSA test. No significant alterations in supramaximal test performance or PFK content were shown; however, percent increases in HIF-1α content for Gβ were significant compared with GP, while MCT4 presented a trend ($P = 0.08$) for the same outcome. Finally, we observed a potential neuromuscular protection of β-alanine supplementation, as evidenced by lower central neuromuscular deficit after the RSA. Collectively, this study demonstrated the potential of β-alanine supplementation as an ergogenic aid to individuals engaged in HIIT.

In the current study, the supplementation protocol was administered concomitantly to a HIIT program aiming to reproduce the common practice of β-alanine users. The RSA total time, best time, and mean time were reduced for Gβ, and the percentage of change of Gβ best time was higher, compared with GP, suggesting a positive influence of β-alanine supplementation on these markers of RSA performance. Muscle carnosine content increased by ~85%, with no change in GP (~1%), corroborating previous research showing an increase in muscle carnosine content with β-alanine supplementation (21,

Table 1. *Outcomes from neuromuscular function assessment normalized by their respective rest values at pre-HIIT + supplementation*

	Gβ							GP						
		Pre-HIIT + Supplementation			Post-HIIT + Supplementation				Pre-HIIT + Supplementation			Post-HIIT+Supplementation		
	Raw	Rest	Fatigued	Δ%	Rest	Fatigued	Δ%	Raw	Rest	Fatigued	Δ%	Rest	Fatigued	Δ%
MVC	603.9 ± 95.7 N	100	93.0 ± 8.5*	−7.0 ± 8.5	102.4 ± 10.32	95.8 ± 9.7*	−6.4 ± 3.4	630.0 ± 75.0 N	100	88.2 ± 9.2*	−11.8 ± 9.2	97.6 ± 10.8	88.9 ± 12.8*	−8.9 ± 8.3
Db100	268.1 ± 36.6 N	100	95.1 ± 3.9*	−4.9 ± 3.9	93.4 ± 12.2	89.3 ± 12.9	−4.0 ± 10.2	293.8 ± 18.3 N	100	94.0 ± 6.9*	−6.0 ± 6.9	93.6 ± 8.4	83.2 ± 12.5*#	−11.4 ± 8.0
VA	88.2 ± 8.5%	100	94.2 ± 7.7*	−5.8 ± 7.7	100.6 ± 4.2	99.1 ± 7.1#	−1.2 ± 8.9	88.0 ± 8.4%	100	94.3 ± 4.4*	−5.7 ± 4.4	98.4 ± 4.1	89.3 ± 8.1*#$	−9.2 ± 6.9
Jump height	38.2 ± 5.4 cm	100	87.4 ± 5.9*	−12.6 ± 5.9	104.6 ± 3.7	88.6 ± 7.0*	−15.0 ± 9.3	36.9 ± 5.0 cm	100	86.2 ± 8.1*	−13.8 ± 8.1	100.1 ± 5.7	83.2 ± 7.2*	−16.7 ± 8.4

MVC, maximal voluntary isometric contraction of knee extension; Db100, amplitude of doublet 100 Hz; VA, voluntary activation; HIIT, a high-intensity interval training; Gβ, β-alanine; GP, placebo group. *Significant difference of respective rest values ($P < 0.03$); #Significant difference of fatigued condition at pre-HIIT + supplementation ($P < 0.03$); $Significant difference of Gβ in fatigued condition at post-HIIT + supplementation.

22, 41) but not with HIIT (3). On the other hand, a recent study did show that HIIT could increase muscle carnosine content in a vegetarian population (39). While it remains disputable whether exercise training per se can increase muscle carnosine content in omnivorous individuals, the noticeable β-alanine-induced increased muscle carnosine content may have contributed to the further improvement of RSA performance shown, as there was a significant correlation between muscle carnosine content and improvement of RSA. Since there were no similar improvements in RSA in the placebo group, this suggests that the increases in muscle carnosine content may have been responsible for these performance gains and highlights the importance of muscle carnosine content for high-intensity exercise performance.

Despite an increase in muscle carnosine content, $\beta m_{in\ vitro}$ was not changed in either group. Harris et al. (21) and Hill et al. (22) estimated from the Henderson-Hasselback equation that the contribution of muscle carnosine content to muscle buffering capacity is approximately +9%, which increases to ~14% after 4 wk of β-alanine supplementation with an increment of ~64% in muscle carnosine content (21). Only Gross et al. (19) used the $\beta m_{in\ vitro}$ technique to measure muscle buffering capacity after β-alanine supplementation and similarly reported no changes. This result is somewhat surprising in light of carnosine's role as a muscle buffer. Nonetheless, McGinley and Bishop (31) and De Salles Painelli et al. (39) recently questioned the validity of the $\beta m_{in\ vitro}$ technique due to high intersample and intrasubject variability. Indeed, in the current study there was a similar +15% increase in buffering capacity in the placebo group; it is unclear as to whether this is due to the training stimulus or methodological issues. The sample homogenization process may lead to an overestimation of buffering capacity due to the inclusion of a pool of buffer substances from different intracellular compartments, as well as extracellular proteins (31). Thus, in light of the limitations inherent to this method, these data should be interpreted with caution.

The improved RSA performance could not be explained by alterations in supramaximal running performance (T_{SUPRA} outcomes) or any alterations in PFK content. This outcome may be due to the HIIT protocol adopted in the present investigation, since recent evidence suggests that this protocol only leads to discrete improvements in anaerobic capacity (36). The current protocol was adopted due to its applicability to the real-world. Most HIIT programs are extremely demanding, making them intolerable and unappealing to most individuals (18). It is important to highlight that an intense open-ended HIIT model combined with β-alanine supplementation loading before the HIIT block led to greater improvements in RSA performance, although this was in trained individuals (4). Bellinger and Minahan (4) recruited trained cyclists and administered 28 days of β-alanine supplementation loading (6.4 g/day) followed by 5 wk of sprint interval training twice a week (SIT; 4 × 1-km maximal cycling sprints with 4 min of active recovery) alongside a maintenance dose of 1.2 g/day of β-alanine. The authors observed increased training intensity throughout SIT as well as additional benefits during exhaustive extreme-intensity cycling compared with placebo group (sprint interval training alone). Since our training protocol clamped training intensity, it cannot be ruled out that further or different

adaptations may have occurred if the participants could increase their training intensity.

In the current study, only GP showed an increased oxygen uptake at exhaustion during the T_{SUPRA} with no other significant alterations. Conversely, Bellinger and Minihan (4) showed only the group supplemented with β-alanine improved anaerobic capacity ad increased blood lactate concentration and supramaximal cycling performance at 120% of $\dot{V}O_{2max}$. This suggests that improvements in anaerobic capacity may be dependent on training intensity (4). On the other hand, similar to Bellinger and Minihan (4), we also showed an increased blood lactate concentration in Gβ after RSA (and the incremental test) at post-HIIT + supplementation. Lactate has been suggested to be a powerful signaling molecule (16) and has the potential to reciprocally control mechanisms with HIF-1α (35). HIF-1α is a key transcription factor that, in addition to mediate adaptation of anaerobic glycolysis, can upregulate the content of MCT4 (35, 45). This is somewhat in line with the results of the present investigation since we showed significant increases in blood lactate concentration, a higher percentage of change of HIF-1α and a trend toward the same result for MCT4 only for Gβ.

The improvements in neuromuscular outcomes further support the hypothesis that increased muscle carnosine content delayed muscle fatigue in this study. The RSA induced a significant drop in all neuromuscular variables at pre-HIIT + supplementation, corroborating previous literature which demonstrated that both peripheral fatigue and central fatigue contribute significantly to the decline of RSA performance (25). However, after the intervention, only GP continued to present significant peripheral and central neuromuscular deficits (drop of Db100 and VA) after RSA, and Gβ presented higher values of VA after RSA than GP. The potential contribution of a pH reduction during repeated sprints for neuromuscular fatigue remains controversial (32), although there is evidence to suggest it occurs (17). It is possible to speculate that the increased muscle carnosine content in Gβ may have prevented the negative effects of hydrogen accumulation on contractile function at the cross bridge level (17), or the disruption of the so-called "critical fatigue threshold," theoretically responsible for constraining central motor drive via feedback from group III/IV neural afferents (1), contributing to the maintenance of Db100 and VA.

Muscle carnosine may also protect against neuromuscular fatigue due to changes in calcium sensitivity. Dutka and Lamb (14) showed an interaction between carnosine and facilitation of the muscle excitation-contraction process in isolated muscle fibers by increasing the sensitivity of the muscle fiber to calcium ion, although Hannah et al. (20) did not confirm these results in vivo. Further work should determine the mechanistic role of muscle carnosine in calcium sensitivity.

An important limitation of the present study is the time-delay between the cessation of the exercise and the MVC and peripheral electrical stimulation (~3.5 min), which may have led to an underestimation of the actual state of neuromuscular fatigue induced by RSA, since substantial recovery may occur within minutes (34). This might be the reason for the absence of a significant group × time interaction, especially of peripheral fatigue variables. The strengths of this study include the use of β-alanine supplementation during a HIIT routine mimicking a "real-world" condition where this supplement is commonly applied. Also, the comprehensive assessments employed in this study allowed determining the role of β-alanine on physiological, performance and mechanistic outcomes. The limitations of the study involve the relatively short-term follow-up period, the low sample size, and the subjects' characteristics (i.e., recreationally trained individuals not engaged in sports competitions). Further studies should address some of these limitations.

Conclusions

β-Alanine supplementation throughout a HIIT program improved some measures of repeated sprint performance. The mechanisms underlying these improvements are linked to an increased muscle carnosine content although we showed no significant alteration in muscle buffering capacity. The attenuation of neuromuscular fatigue, especially central fatigue, may also have contributed to the improvement of repeated sprints performance. Overall, this study provides evidence that β-alanine supplementation may be a useful dietary intervention to prevent fatigue in individuals undergoing HIIT.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

F.M. and A.M.Z. conceived and designed research; F.M., R.A.B.d.P., A.L.d.R., A.S.R.d.S., and P.d.T.G.M. performed experiments; F.M., R.A.B.d.P., B.S., B.G., A.L.d.R., A.S.R.d.S., and A.M.Z. analyzed data; F.M., R.A.B.d.P., B.G., A.S.R.d.S., and A.M.Z. interpreted results of experiments; F.M. prepared figures; F.M. and B.S. drafted manuscript; F.M., B.S., and A.M.Z. edited and revised manuscript; F.M., R.A.B.d.P., B.S., B.G., A.L.d.R., A.S.R.d.S., P.d.T.G.M., and A.M.Z. approved final version of manuscript.

REFERENCES

1. **Amann M, Blain GM, Proctor LT, Sebranek JJ, Pegelow DF, Dempsey JA.** Implications of group III and IV muscle afferents for high-intensity endurance exercise performance in humans. *J Physiol* 589: 5299–5309, 2011. doi:10.1113/jphysiol.2011.213769..
2. **Artioli GG, Gualano B, Smith A, Stout J, Lancha AH Jr.** Role of β-alanine supplementation on muscle carnosine and exercise performance. *Med Sci Sports Exerc* 42: 1162–1173, 2010. doi:10.1249/MSS.0b013e3181c74e38.
3. **Baguet A, Everaert I, De Naeyer H, Reyngoudt H, Stegen S, Beeckman S, Achten E, Vanhee L, Volkaert A, Petrovic M, Taes Y, Derave W.** Effects of sprint training combined with vegetarian or mixed diet on muscle carnosine content and buffering capacity. *Eur J Appl Physiol* 111: 2571–2580, 2011. doi:10.1007/s00421-011-1877-4.
4. **Bellinger PM, Minahan CL.** Additive benefits of β-alanine supplementation and sprint-interval training. *Med Sci Sports Exerc* 48: 2417–2425, 2016. doi:10.1249/MSS.0000000000001050.
5. **Bellinger PM, Minahan CL.** The effect of β-alanine supplementation on cycling time trials of different length. *Eur J Sport Sci* 16: 829–836, 2016. doi:10.1080/17461391.2015.1120782.

6. **Bishop D, Edge J, Goodman C.** Muscle buffer capacity and aerobic fitness are associated with repeated-sprint ability in women. *Eur J Appl Physiol* 92: 540–547, 2004. doi:10.1007/s00421-004-1150-1.
7. **Bishop, D, Edge, J, Thomas, C, Mercier, J.** Effects of high-intensity training on muscle lactate transporters and postexercise recovery of muscle lactate and hydrogen ions in women. *Am J Physiol Regul Integr Comp Physiol* 295: R1991–R1998, 2008. doi:10.1152/ajpregu.00863.2007.
8. **Brisola GM, Artioli GG, Papoti M, Zagatto AM.** Effects of four weeks of β-alanine supplementation on repeated sprint ability in water polo players. *PLoS One* 11: e0167968, 2016. doi:10.1371/journal.pone.0167968.
9. **Buchheit M, Laursen PB.** High-intensity interval training, solutions to the programming puzzle: Part I: cardiopulmonary emphasis. *Sports Med* 43: 313–338, 2013. doi:10.1007/s40279-013-0029-x.
10. **Claus GM, Redkva PE, Brisola GM, Malta ES, de Araujo Bonetti de Poli R, Miyagi WE, Zagatto AM.** Beta-alanine supplementation improves throwing velocities in repeated sprint ability and 200-m swimming performance in young water polo players. *Pediatr Exerc Sci* 29: 203–212, 2017. doi:10.1123/pes.2016-0176.
11. **Cochran AJ, Percival ME, Thompson S, Gillen JB, MacInnis MJ, Potter MA, Tarnopolsky MA, Gibala MJ.** β-Alanine supplementation does not augment the skeletal muscle adaptive response to 6 weeks of sprint interval training. *Int J Sport Nutr Exerc Metab* 25: 541–549, 2015. doi:10.1123/ijsnem.2015-0046.
12. **Derave W, Everaert I, Beeckman S, Baguet A.** Muscle carnosine metabolism and β-alanine supplementation in relation to exercise and training. *Sports Med* 40: 247–263, 2010. doi:10.2165/11530310-000000000-00000.
13. **Ducker KJ, Dawson B, Wallman KE.** Effect of beta alanine and sodium bicarbonate supplementation on repeated-sprint performance. *J Strength Cond Res* 27: 3450–3460, 2013. doi:10.1519/JSC.0b013e31828fd310.
14. **Dutka TL, Lamb GD.** Effect of carnosine on excitation-contraction coupling in mechanically-skinned rat skeletal muscle. *J Muscle Res Cell Motil* 25: 203–213, 2004. doi:10.1023/B:JURE.0000038265.37022.c5.
15. **Edge J, Bishop D, Goodman C.** The effects of training intensity on muscle buffer capacity in females. *Eur J Appl Physiol* 96: 97–105, 2006. doi:10.1007/s00421-005-0068-6.
16. **Ferguson BS, Rogatzki MJ, Goodwin ML, Kane DA, Rightmire Z, Gladden LB.** Lactate metabolism: historical context, prior misinterpretations, and current understanding. *Eur J Appl Physiol* 118: 691–728, 2018. doi:10.1007/s00421-017-3795-6.
17. **Fitts RH.** The role of acidosis in fatigue: pro perspective. *Med Sci Sports Exerc* 48: 2335–2338, 2016. doi:10.1249/MSS.0000000000001043.
18. **Gibala MJ, Little JP, Macdonald MJ, Hawley JA.** Physiological adaptations to low-volume, high-intensity interval training in health and disease. *J Physiol* 590: 1077–1084, 2012. doi:10.1113/jphysiol.2011.224725.
19. **Gross M, Boesch C, Bolliger CS, Norman B, Gustafsson T, Hoppeler H, Vogt M.** Effects of beta-alanine supplementation and interval training on physiological determinants of severe exercise performance. *Eur J Appl Physiol* 114: 221–234, 2014. doi:10.1007/s00421-013-2767-8.
20. **Hannah R, Stannard RL, Minshull C, Artioli GG, Harris RC, Sale C.** β-Alanine supplementation enhances human skeletal muscle relaxation speed but not force production capacity. *J Appl Physiol (1985)* 118: 604–612, 2015. doi:10.1152/japplphysiol.00991.2014.
21. **Harris RC, Tallon MJ, Dunnett M, Boobis L, Coakley J, Kim HJ, Fallowfield JL, Hill CA, Sale C, Wise JA.** The absorption of orally supplied β-alanine and its effect on muscle carnosine synthesis in human vastus lateralis. *Amino Acids* 30: 279–289, 2006. doi:10.1007/s00726-006-0299-9.
22. **Hill CA, Harris RC, Kim HJ, Harris BD, Sale C, Boobis LH, Kim CK, Wise JA.** Influence of β-alanine supplementation on skeletal muscle carnosine concentrations and high intensity cycling capacity. *Amino Acids* 32: 225–233, 2007. doi:10.1007/s00726-006-0364-4.
23. **Hobson RM, Saunders B, Ball G, Harris RC, Sale C.** Effects of β-alanine supplementation on exercise performance: a meta-analysis. *Amino Acids* 43: 25–37, 2012. doi:10.1007/s00726-011-1200-z.
24. **Howley ET, Bassett DR Jr, Welch HG.** Criteria for maximal oxygen uptake: review and commentary. *Med Sci Sports Exerc* 27: 1292–1301, 1995. doi:10.1249/00005768-199509000-00009.
25. **Hureau TJ, Ducrocq GP, Blain GM.** Peripheral and central fatigue development during all-out repeated cycling sprints. *Med Sci Sports Exerc* 48: 391–401, 2016. doi:10.1249/MSS.0000000000000800.
26. **Kuipers H, Verstappen FT, Keizer HA, Geurten P, van Kranenburg G.** Variability of aerobic performance in the laboratory and its physiologic correlates. *Int J Sports Med* 6: 197–201, 1985. doi:10.1055/s-2008-1025839.
27. **Lee CL, Hsu WC, Cheng CF.** Physiological adaptations to sprint interval training with matched exercise volume. *Med Sci Sports Exerc* 49: 86–95, 2017. doi:10.1249/MSS.0000000000001083.
28. **Leite TC, Coelho RG, Da Silva D, Coelho WS, Marinho-Carvalho MM, Sola-Penna M.** Lactate downregulates the glycolytic enzymes hexokinase and phosphofructokinase in diverse tissues from mice. *FEBS Lett* 585: 92–98, 2011. doi:10.1016/j.febslet.2010.11.009.
29. **Leite TC, Da Silva D, Guimarães Coelho R, Zancan P, Sola-Penna M.** Lactate favours the dissociation of skeletal muscle 6-phosphofructo-1-kinase tetramers down-regulating the enzyme and muscle glycolysis. *Biochem J* 408: 123–130, 2007. doi:10.1042/BJ20070687.
30. **Little JP, Safdar A, Wilkin GP, Tarnopolsky MA, Gibala MJ.** A practical model of low-volume high-intensity interval training induces mitochondrial biogenesis in human skeletal muscle: potential mechanisms. *J Physiol* 588: 1011–1022, 2010. doi:10.1113/jphysiol.2009.181743.
31. **McGinley C, Bishop DJ.** Distinct protein and mRNA kinetics of skeletal muscle proton transporters following exercise can influence interpretation of adaptations to training. *Exp Physiol* 101: 1565–1580, 2016. doi:10.1113/EP085921.
32. **Mendez-Villanueva A, Edge J, Suriano R, Hamer P, Bishop D.** The recovery of repeated-sprint exercise is associated with PCr resynthesis, while muscle pH and EMG amplitude remain depressed. *PLoS One* 7: e51977, 2012. doi:10.1371/journal.pone.0051977.
33. **Milioni F, Redkva PE, Barbieri FA, Zagatto AM.** Six weeks of β-alanine supplementation did not enhance repeated-sprint ability or technical performances in young elite basketball players. *Nutr Health* 23: 111–118, 2017. doi:10.1177/0260106017700436.
34. **Milioni F, Vieira LH, Barbieri RA, Zagatto AM, Nordsborg NB, Barbieri FA, Dos-Santos JW, Santiago PR, Papoti M.** Futsal match-related fatigue affects running performance and neuromuscular parameters but not finishing kick speed or accuracy. *Front Physiol* 7: 518, 2016. doi:10.3389/fphys.2016.00518.
35. **Nalbandian M, Takeda M.** Lactate as a signaling molecule that regulates exercise-induced adaptations. *Biology (Basel)* 5: 38, 2016. doi:10.3390/biology5040038.
36. **Panissa VL, Fukuda DH, Caldeira RS, Gerosa-Neto J, Lira FS, Zagatto AM, Franchini E.** Is oxygen uptake measurement enough to estimate energy expenditure during high-intensity intermittent exercise? Quantification of anaerobic contribution by different methods. *Front Physiol* 9: 868, 2018. doi:10.3389/fphys.2018.00868.
37. **da Rocha AL, Pereira BC, Pauli JR, de Souza CT, Teixeira GR, Lira FS, Cintra DE, Ropelle ER, Júnior CR, da Silva AS.** Downhill running excessive training inhibits hypertrophy in mice skeletal muscles with different fiber type composition. *J Cell Physiol* 231: 1045–1056, 2016. doi:10.1002/jcp.25197.
38. **da Rocha AL, Pereira BC, Pauli JR, Cintra DE, de Souza CT, Ropelle ER, da Silva AS.** Downhill running-based overtraining protocol improves hepatic insulin signaling pathway without concomitant decrease of inflammatory proteins. *PLoS One* 10: e0140020, 2015. doi:10.1371/journal.pone.0140020.
39. **De Salles Painelli, V, Nemezio, KM, Jéssica, A, Franchi, M, Andrade, I, Riani, LA, Saunders B, Sale C, Harris RC, Gualano B, Artioli GG.** HIIT augments muscle carnosine in the absence of dietary beta-alanine intake. *Med Sci Sports Exercise* 2018 Jun 21. [Epub ahead of print]. doi:10.1249/MSS.0000000000001697.
40. **Saunders B, Elliott-Sale K, Artioli GG, Swinton PA, Dolan E, Roschel H, Sale C, Gualano B.** β-Alanine supplementation to improve exercise capacity and performance: a systematic review and meta-analysis. *Br J Sports Med* 51: 658–669, 2017. doi:10.1136/bjsports-2016-096396.
41. **Saunders B, DE Salles Painelli V, DE Oliveira LF, DA Eira Silva V, DA Silva RP, Riani L, Franchi M, Gonçalves LS, Harris RC, Roschel H, Artioli GG, Sale C, Gualano B.** Twenty-four weeks of β-alanine supplementation on carnosine content, related genes, and exercise. *Med Sci Sports Exerc* 49: 896–906, 2017. doi:10.1249/MSS.0000000000001173.
42. **Smith AE, Walter AA, Graef JL, Kendall KL, Moon JR, Lockwood CM, Fukuda DH, Beck TW, Cramer JT, Stout JR.** Effects of β-alanine supplementation and high-intensity interval training on endurance performance and body composition in men; a double-blind trial. *J Int Soc Sports Nutr* 6: 5, 2009. doi:10.1186/1550-2783-6-5.
43. **Sweeney KM, Wright GA, Glenn Brice A, Doberstein ST.** The effect of beta-alanine supplementation on power performance during repeated sprint activity. *J Strength Cond Res* 24: 79–87, 2010. doi:10.1519/JSC.0b013e3181c63bd5.

44. **Tabata I, Nishimura K, Kouzaki M, Hirai Y, Ogita F, Miyachi M, Yamamoto K.** Effects of moderate-intensity endurance and high-intensity intermittent training on anaerobic capacity and VO2max. *Med Sci Sports Exerc* 28: 1327–1330, 1996. doi:10.1097/00005768-199610000-00018.
45. **Thomas C, Bishop DJ, Lambert K, Mercier J, Brooks GA.** Effects of acute and chronic exercise on sarcolemmal MCT1 and MCT4 contents in human skeletal muscles: current status. *Am J Physiol Regul Integr Comp Physiol* 302: R1–R14, 2012. doi:10.1152/ajpregu.00250.2011.
46. **Trexler ET, Smith-Ryan AE, Stout JR, Hoffman JR, Wilborn CD, Sale C, Kreider RB, Jäger R, Earnest CP, Bannock L, Campbell B, Kalman D, Ziegenfuss TN, Antonio J.** International society of sports nutrition position stand: beta-alanine. *J Int Soc Sports Nutr* 12: 30, 2015. doi:10.1186/s12970-015-0090-y.
47. **Trivedi B, Danforth WH.** Effect of pH on the kinetics of frog muscle phosphofructokinase. *J Biol Chem* 241: 4110–4112, 1966.
48. **Ui M.** A role of phosphofructokinase in pH-dependent regulation of glycolysis. *Biochim Biophys Acta* 124: 310–322, 1966. doi:10.1016/0304-4165(66)90194-2.
49. **Zagatto AM, Beck WR, Gobatto CA.** Validity of the running anaerobic sprint test for assessing anaerobic power and predicting short-distance performances. *J Strength Cond Res* 23: 1820–1827, 2009. doi:10.1519/JSC.0b013e3181b3df32.