Effects of Ribose Supplementation Prior to and During Intense Exercise on Anaerobic Capacity and Metabolic Markers

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This study examined whether ribose supplementation before and during intense anaerobic exercise impacts anaerobic capacity and metabolic markers. Twelve moderately trained male cyclists ($22.3 \pm 2.2 \text{ y}$; $181 \pm 6 \text{ cm}$, $74.8 \pm 9 \text{ kg}$) participated in the study. Subjects were familiarized and fasted for 8 h after standardizing nutritional intake. In a double blind and crossover manner subjects ingested either a 150 mL placebo or ribose (3 g ribose + 150 μ g folate). Subjects rested for 25 min and completed 5×30 s anaerobic capacity tests with 3 min passive rest. Six capillary blood samples were taken prior to and after sprints for adenine nucleotide breakdown determination. The experiment was repeated 1 wk later with alternative drink. Data were analyzed by repeated measures ANOVA. No significant interactions were observed for any performance or blood variables. D-ribose supplementation has no impact on anaerobic exercise capacity and metabolic markers after high-intensity cycling exercise.

Key Words: ATP resynthesis, sport nutrition, ergogenic aids

Single bouts or repeated bouts of high-intensity sprint exercise have been shown to cause drastic reductions in maximal power output, total work produced, and changes in creatine phosphate concentration, lactate, ammonia, total adenine nucleotide (TAN) pool, and inosine-5'-monophosphate (IMP) which are all indications of extreme fatigue (9, 11, 16, 18). Using muscle biopsies, Zhao (18) and Tullson (16) concluded that purine influx and efflux (TAN, IMP) is greatly increased after a short-term supramaximal 30 s cycling exercise bout. Consequently, the ability to effectively handle higher levels of stress is improved. These changes are observed

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by improving the recovery and quantities of high-energy molecules, greater power output, work performance, and/or metabolic responses (9).

D-ribose is a naturally occurring 5-carbon carbohydrate that for the last several years has been marketed with little physiological rationale as an ergogenic aid to athletes who engage in high-intensity activity. Ribose is a vital molecule for de novo synthesis and salvage of adenine nucleotides (ATP, ADP, and AMP) which are intimately involved with energy metabolism. Substantial de novo synthesis of adenine nucleotides is a lengthy process which could limit the actual ability of any de novo pathway or ribose supplementation to actually increase ATP resynthesis during acute, intermittent bouts of high-intensity exercise. Research has suggested, however, that enhancing the availability of ATP, increasing salvage pathway activity, and/or maintaining the TAN pool to a greater degree during a single bout or repeated bouts of high-intensity sprint-type activities increases exercise capacity (9, 16, 18). Further, research has shown ribose supplementation to increase the availability of purine nucleotides through either enhanced synthesis or increased turnover via salvage pathways during exercise (5, 15).

Previous research in clinical populations has provided some promise for ribose supplementation as an energy-providing supplement (8), enhancing de novo synthesis of purine nucleotides (19), reducing muscle cramping (17), and increasing exercise tolerance (13). For example, Pliml et al. showed ingestion of ribose in doses of 60 g/d for 3 d prior to a maximal treadmill test significantly increased time until termination in previously diagnosed men with severe coronary artery disease (13). In addition, Wagner et al. supplemented patients with AMP deaminase deficiency with ribose to determine any possible changes in energy provision and exercise performance. Every 10 min patients were given 3 g of either placebo or ribose prior to completing an incremental maximal exercise test. While exercise performance was not changed, plasma concentrations of lactate and inosine were increased (P < 0.05). The authors concluded that ribose administration might have served as an energy source or enhanced the de novo synthesis of purine nucleotides. In this regard, and despite inconclusive evidence, ribose has been marketed heavily to athletes for its purported ability to maintain power production during repeated bouts of intense exercise or to increase the peak power production seen during these types of activities. Studies in support of ribose supplementation before or after high-intensity exercise have demonstrated a greater work production during the exercise bout in addition to an increased recovery of ATP levels in the muscle several days after exercise (10). To date, no conclusive, performance-enhancing effect has been reported.

Consequently, several studies have sought to determine an ergogenic property to ribose supplementation during high-intensity exercise. Results from these studies have concluded that supplementation with ribose for 3 to 6 d in doses ranging from 8 to 50 g/d while participating in repeated bouts of high-intensity exercise (e.g., Wingate sprints or maximal knee extensions) did not increase performance over those subjects who were taking a placebo (4, 11, 12). While some findings did indicate improved maintenance of total work (11) or peak power (4) with ribose, the authors' in these studies concluded that ribose did not significantly improve performance.

More research is needed to investigate the potential ergogenic value of ribose supplementation particularly at the dosages recommended by various supplement manufacturers. Currently, studies need to be conducted to investigate any possible role of ribose administration prior to maximal exercise and/or during recovery from a maximal exercise bout. Subsequently, the purpose of this study was to determine if acute supplementation of ribose in dosages that are commonly marketed as an ergogenic aid (2 doses of 3 g ribose + 150 μ g folate) prior to a maximal exercise bout or during the recovery period prior to a subsequent exercise bout has any beneficial impact on the performance of repeated sprint exercise with limited recovery.

Methods

Subjects

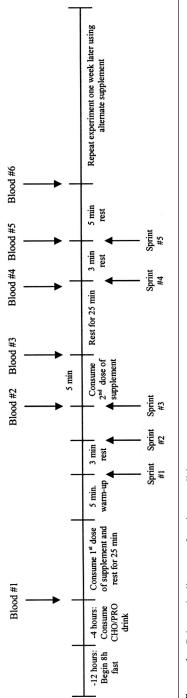
Twelve apparently healthy moderately trained male cyclists between the ages of 18 and 40 were recruited for this investigation. All subjects signed informed consent documents and the study was approved by the Baylor University Institutional Review Board prior to any data collection. To qualify for participation, all subjects were: 1) experienced cyclists who had been previously involved in a cycling program for at least 3 h/wk for 3 months and were part of a competitive club cycling program at large National Collegiate Athletic Association Division I institution; 2) not taking any nutritional supplements purported to have ergogenic effects (e.g., creatine monohydrate, ribose, caffeine, sodium phosphates, bicarbonates, etc.); and 3) not taking or have never taken any anabolic steroids.

During initial familiarization sessions, subjects were informed of testing procedures, completed all necessary paperwork, and were familiarized to all exercise testing on two different occasions prior to beginning the experimental protocol. Descriptive characteristics of the subjects were as follows: age, 22.3 ± 2.2 y; body weight, 74.9 ± 9.6 kg; height, 180.9 ± 6.1 cm; and body fat, $18.2 \pm 5.4\%$. Prior to data collection, all subjects had been training for competitive events which were typically shorter distance (20 to 30 miles), higher-paced events which led them to maintain an average of 1.8 ± 0.5 h/d, 3.1 ± 1.3 d/wk for 76.8 ± 51.6 miles/wk.

Experimental Design

Throughout two familiarization sessions, subjects completed informed consent documents, medical and training history questionnaires, and personal information sheets, and were then familiarized with the testing protocol. Subjects were instructed to record their training for 5 d and food and fluid intake for 24 h prior to their initial testing session (T1). All training and dietary intake was then subsequently replicated prior to the second testing session (T2). Subjects were required to refrain from exercise for 48 h and fast for 8 h prior to their testing sessions. All subjects consumed a standardized carbohydrate/protein (240 kcals, 40 g protein, 16 g carbohydrate, 3 g fat) meal replacement drink (RTD40 Met-Rx, Boca Raton, FL) 4 h prior to their testing session to standardize nutritional intake for 12 h prior to testing.

Figure 1 illustrates the testing design. Subjects provided their first blood sample from a clean, pre-warmed finger prior to consuming the first dose of the supplement. Pre-exercise blood samples were taken within 15 min of reporting to the lab and followed by 25 min of quiet rest. Subjects warmed up for 5 min on a





bicycle ergometer at a standardized work rate (2 kg @ 60 rpm; 120 W) and rested for 5 min. Subjects then performed 3×30 s Wingate anaerobic capacity tests each separated by 3 min of standardized passive recovery. Immediately after the third sprint, a second blood sample was taken and a second identical dose of the supplement was ingested. The second dose of the supplement was consumed within 5 min of completion of the third sprint and all subjects consumed the drink within 30 s in view of the investigators. Five minutes after ingestion of the supplement, a third blood sample was taken and subjects then sat at quiet rest for a total of 25 min. A fourth blood sample was then obtained. Subjects began to free spin at 80 rpm with no resistance for 30 s prior to the each Wingate test. Subjects then completed the fourth and fifth Wingate tests with 3 min passive rest between both trials; a fifth blood sample was taken immediately upon completion of the fifth sprint. A sixth and final blood sample was taken 5 min after completion of the last sprint.

In a double blind, randomized, and crossover manner, subjects were administered either 3 g of D-ribose with 150 μ g of the vitamin folate (folic acid) or 3 g of a maltodextrin placebo. Folate was included for its role in normal ribose metabolism but it does not possess any known ergogenic properties. All supplements were prepared in powdered form and packaged in ready-to-mix containers for double blind administration by Royal Numico Research B.V. (Wageningen, The Netherlands) to ensure similar taste, color, consistency, and texture. Supplements were mixed immediately prior to ingestion with 150 mL of cold water and consumed in front of researchers to ensure proper administration. Subsequent testing sessions were completed 1 wk later at the same time of day in an identical fashion as described above.

Procedures

Total body mass was measured on a calibrated digital scale with a precision of \pm 0.02 kg (Sterling Scale Co., Southfield, MI). Skinfold body composition measures were taken prior to each testing session for descriptive purposes using standard skinfold techniques (Lange calipers) following American College of Sports Medicine guidelines (2). Standard finger-stick phlebotomy techniques from a clean, pre-warmed finger were used to collect each 200 to 400 μ L whole blood sample into lithium heparin-treated Microtainer tubes (Becton Dickinson, Franklin Lakes, NJ). The tubes were centrifuged for 15 min using a bench-top centrifuge (VanGuard V6500, Hamilton Bell Co., Montvale, NJ). Plasma was assayed for ammonia, lactate, and glucose using an Analox MicroStat GM7 analyzer (Analox Instruments, Ltd., London, UK). Inter-assay variances for ammonia, lactate, and glucose were $\pm 10 \ \mu$ mol/L, ± 0.05 -0.07 mmol/L, and 1.4% at 10 mmol/L, respectively.

Wingate anaerobic capacity tests were performed on a computerized Lode Excalibur Sport (Lode BV, Groningen, The Netherlands) cycle ergometer equipped with toe clips at a standardized torque factor 0.7. The torque factor setting was set to the manufacturer's guidelines relative to the population being tested. Seat position, seat height, handlebar height, and handlebar position were determined during familiarization sessions and repeated for both testing sessions. Subjects were instructed to begin sprinting 5 s prior to beginning of data collection to ensure optimal force and power production at the beginning of the test and to remain

sprinting for the entire duration of the test. All visual feedback was removed during testing and subjects were instructed to remain in the saddle for the entire duration of the test while researchers provided verbal encouragement. The ergometer was connected via an RS-232 parallel interface to a Dell Optiplex GX 260 computer (Dell Computer Corp., Austin, TX) using Wingate for Windows software version 1 (Lode BV, Groningen, The Netherlands). Crank frequency was measured using magnetic encoders (4/revolution). The Excalibur Sport has a range of 0 to -2000 W with typical variation of measurement less than 2% with the sampling frequency of data at 5 times/s. Test to test variability in performing repeated Wingate tests in our lab has yielded correlation coefficients of $r = 0.981 \pm 15\%$ for mean power.

Statistical Analysis

Descriptive variables and all other data were analyzed using a 2×5 (group × sprints) repeated measures ANOVA for all performance variables and a 2×6 (group × blood sample) repeated measures ANOVA using SPSS for Windows version 11.5 (SPSS Inc., Chicago, IL) with an added correction factor to control test effect bias. Alpha level was set at 0.05. Effect sizes were calculated for any significant trends using the mean differences. Data was considered significantly different when the probability of a Type I error was 0.05 or less. Data are presented as means \pm standard deviation.

Results

Side Effects

No subjects reported adverse events or responses to the supplementation and training protocol. No reports of medical problems or symptoms were indicated in post-study questionnaires administered in a blinded manner.

Sprint Performance

Peak power (P = 0.006) and total work (P = 0.005) both significantly decreased across sprint trials with no significant differences between groups. No significant interactions were found (P > 0.05) for average power, peak power, time to peak power, rate of fatigue, and total work between the two groups. No significant group x time interactions were found for any of the five sprint tests throughout the study.

Metabolic Markers

The Wingate sprint tests significantly increased lactate (P < 0.001) across time for both groups. No significant group × time interactions (P > 0.05) were observed among groups in these metabolic parameters. Due to an inability to retrieve an adequate amount of sample and/or to analyze some samples within a few hours after exercise, ammonia analysis was only performed on 9 subjects (ribose = 4, placebo = 5). Complete analysis was conducted on all other variables. Figure 2 indicates the changes in peak power, mean power, rate of fatigue, glucose, lactate, and ammonia levels throughout all five sprint tests for both the ribose and placebo groups.

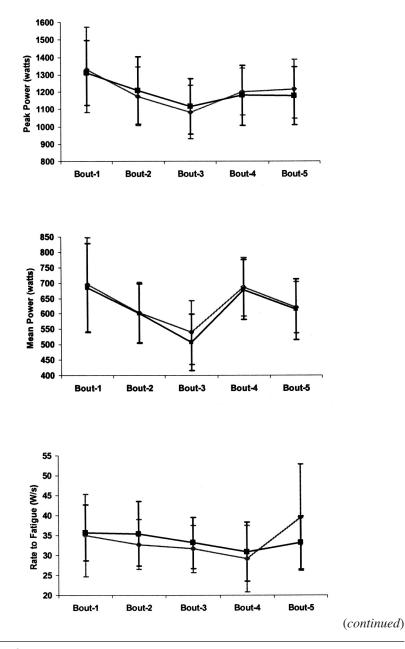
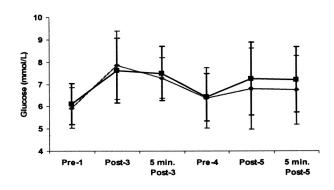
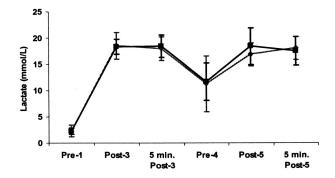


Figure 2—Peak power, average power, rate to fatigue, glucose, lactate and ammonia values observed for the ribose (solid square) and placebo (grey diamond) groups prior to and following supplementation. Peak power, average power and total work are shown top to bottom in the left panel; plasma glucose, lactate and ammonia are shown top to bottom in the right panel. Data are means \pm standard deviation.





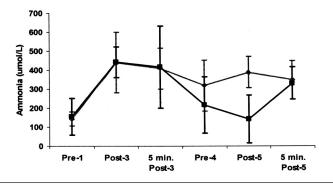


Figure 2—(continued)

Discussion

This study was developed to determine if the pattern and timing of ribose supplementation at acute dosage levels (2 doses of 3 g ribose + 150 μ g folate) of what is commonly marketed as an ergogenic aid affected the outcome of repeated sprint performance with limited recovery. The timing of administration and dosages were chosen to mimic both the dosage amounts marketed to athletes as well as provide a similar layout to what is commonly experienced by many competitive athletes (e.g., heats in swimming, sprinting, etc.). The pharmokinetics of ribose suggest that 88 to 100% of an oral dose (up to 200 mg \times kg⁻¹ \times h⁻¹) is absorbed from the small intestine and distributed to various tissues including skeletal muscle (1). The dosage used in the present study (3 g) was only 20% of this expected upper limit of ribose bioavailability, suggesting the doses of ribose were indeed able to be absorbed to some degree prior to the exercise bouts. It was expected that the Wingate anaerobic capacity tests would be a good, reliable measure of anaerobic capacity (6, 9). It is possible that slight reductions in peak power performance might have occurred due to a possible loss of optimal neuromuscular coordination and resulting force production as a result of extremely high cadence rates (typically 180 to 200 rpm during the 5 s "superspin" period prior to the beginning of the test). The authors, however, believe that this effect is negligible due to the sprint cycling experience of the participants and the standardization of the testing procedures. Furthermore, the tests and protocol were anticipated to stimulate a degradation of the TAN pool in addition to changes in metabolic activity based on previous research (6, 9, 11).

Following the theoretical rationale and marketing campaigns of nutritional supplement companies for ribose supplementation, we hypothesized that if acute ribose ingestion improves the availability of ATP (increase de novo synthesis or increased salvage of these nucleotides) during the sprints and/or during recovery, then: 1) an increase in peak power would have been determined after the first or fourth sprints; 2) a greater maintenance of power (average power) or total work output would be observed after either group of sprints; or 3) an improved maintenance of the metabolic markers (i.e., glucose, lactate, and ammonia) measured would have been observed between groups throughout the protocol. While many different considerations could have been made that might have elucidated varying conclusions, the rest periods and end points (i.e., peak ammonia, glucose, and lactate levels) chosen in this study were hypothesized to provide the most realistic picture of what would be experienced by athletes using ribose supplementation throughout their workouts (4, 6).

Results from the present study indicate that the exercise protocol employed was successful at producing a significant metabolic challenge. A 23% decrease in total work output, a 16.5% decrease in peak power, a six-fold increase in blood lactate, and a 180% increase in ammonia levels from the first to the third sprint was found, which was similar to the findings of Hargreaves et al. when they had subjects complete 3×30 s sprints on a cycle ergometer with 4 min rest between each sprint. In this study, a 34% decrease in total work was found in addition to a thirteen-fold increase in serum lactate (9). While the supplementation protocols were different, the changes in the present study for total work, lactate, and ammonia were similar to changes reported by Kreider et al. (11) in which they used only 2×30 s anaerobic capacity tests. Furthermore, the magnitude of change in the

present study is 1 to 4 times greater than previously reported by other investigators. Op't Eijnde and colleagues (12) performed two bouts of isokinetic knee extensions $(15 \text{ sets} \times 12 \text{ contractions each with } 15 \text{ s rest})$ which promoted a significant 20 to 25% decrease in TAN. In comparison to these two previous studies, the present study used subjects who had been participating in a cycling program that included either sprint work or intense interval work compared to recreational subjects. The specific adaptations made in response to their training status are thought to explain the somewhat decreased magnitude of change in peak power and lactate response. The present study's findings are limited due to the absence of any direct assessment of muscle TAN levels via muscle biopsy. The present study, however, when compared with other published reports (11, 12), provides evidence (e.g., changes in indirect markers such as lactate, ammonia, and power output) that the study design used likely produced a decrease in TAN in addition to challenging the metabolic systems. For example, Zhao and colleagues had seven male subjects complete only one 30 s maximal sprint and reported a \sim 33% decrease in TAN using muscle biopsies (18) in addition to the findings by Hargreaves in which he noted an $\sim 11\%$ decrease in TAN after three maximal 30 s sprints (9)

In contrast to the present findings in which no significant increases or improvement in performance were noted, recent studies have suggested that ribose could be effective at maintaining or attenuating the amount of work completed in addition to promoting a greater maintenance of high-energy compounds (e.g., ATP, ADP, etc.) used during high-intensity exercise (3, 7, 10, 11, 14). Antonio and colleagues (3) concluded that ribose supplementation (10 g/d in 5 g doses prior to and following workouts) resulted in a greater number of repetitions performed during 10 sets to failure in the bench press. Kreider and colleagues (11) reported that subjects who were supplemented with ribose (50 g/d \times 5 d) were better able to sustain total work output after 2×30 s Wingate cycle ergometer sprints compared to a matched double-blind placebo, which resulted in a more drastic decrease in total work output. Lastly, Hellsten and colleagues (10) trained subjects for 7 d and then supplemented subjects for 3 d in a double-blind manner (600 mg \times kg⁻¹ \times d⁻¹) prior to completing an identical exercise bout used in the training period. Muscle biopsy samples were taken 5, 24, and 72 h after this exercise bout and found a significantly increased level of muscle ATP at 72 h post-exercise. Furthermore, ribose supplementation was found in two related studies (20 g/d for 3 d prior to training, during a 5 d training period, and for 3 d following training) to have no impact on performance but did help to attenuate the decrease in the TAN pool following acute, intense exercise as well as after a 65 h recovery period (7, 14). While limited evidence is provided for ribose to increase performance, these findings do support a possible benefit for ribose supplementation to sustain work production or promotion of long-term recovery by enhancing ATP availability. In summary, these previously published studies help to provide the collective evidence indicating why manufacturers of ribose have marketed these supplements to athletes (3, 7, 10, 11, 14).

While some studies have suggested ergogenic properties for ribose administration, the results from the present study do not support any ergogenic role for acute ribose supplementation compared with a placebo on markers of performance and metabolic activity before or during repeated high-intensity intermittent exercise. This provides additional support to previous research that has suggested no ergogenic benefit of ribose supplementation (8 to 50 g/d for 3 to 6 d) while completing various forms of high-intensity exercise (e.g., 6 to 15×10 to 30 s sprints with 60 to 180 s recovery as well as finding no difference in plasma metabolites (lactate, ammonia, uric acid, glucose, or creatine kinase) and muscle adenine nucleotides (ATP, ADP, AMP, IMP, or TAN) (4, 7, 11, 12). Lastly, and in accordance with the findings showing no performance increase after ribose supplementation, de novo synthesis of ATP is a slow process with limited evidence of its ability to increase resting muscle ATP levels during an acute bout of high-intensity, intermittent exercise (7, 10). Under these circumstances, it is possible that any acute administration of ribose in a single day or exercise bout would not have enough time to have a physiological impact; the popularity of ribose supplementation, however, warranted the investigation.

In summary, the results of this study indicate that acute ribose supplementation (2 doses of 3 g each) during five repeated, high-intensity, short-term (30 s) exercise bouts provides no ergogenic benefit. Previous findings have alluded that possible benefits from ribose supplementation might not be elucidated until several days after supplementation (10) suggesting additional studies should evaluate the impact on the TAN pool and performance of ingesting varying doses of ribose before, during, and after exercise. Investigations should also focus on the pharmokinetic pattern of ribose to the muscle during acute dosing studies and more research is needed to evaluate the effects of ribose supplementation on recovery from intense exercise and training adaptations. Nevertheless, results from this study indicate that acute ribose supplementation does not improve performance or recovery in trained cyclists.

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