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SHORT COMMUNICATION

Acute effect of betaine supplementation on muscular endurance during resistance exercise in trained men: a pilot and randomized study

Matheus Dantas, Victor Sabino de Queiros, Tatiane Andreza Lima da Silva, Alana Monteiro Bispo da Silva, Leonardo de Souza Fortes, Jason Cholewa, Anna Luiza Vasconcelos de Oliveira, Breno Guilherme de Araújo Tinôco Cabral

Objective: To analyse the acute effect of betaine supplementation on muscular endurance in weight training practitioners.
Design: An experimental, crossover, randomized and double-blind study.
Methods: The sample composed of 10 male subjects practicing resistance training (age: 23.71 ± 4.23 years old). Participants performed 2 sessions (i.e., Betaine x Placebo) with 3 sets of repetitions until failure with 70% of 1RM. The participants were provided 3 minutes of recovery between sets and 48 hours between sessions. The 24H food recall was evaluated before each exercise session.
Results: There were no significant differences in carbohydrates ($p = 0.732$), protein ($p = 0.684$), fat ($p = 0.271$), or in total energy consumption ($p = 0.865$). A time effect occurred for the training session ($F_{(2,18)} = 54.626$, $p < 0.0001$, $\eta^2 = 0.859$), with a linear reduction in the number of repetitions performed throughout the series for both conditions (1set > 2set > 3set). However, there was no interaction ($F_{(2,18)} = 0.625$, $p= 0.546$, $\eta^2 = 0.065$) or condition effect ($F_{(1,9)} = 0.045$, $p = 0.837$, $\eta^2 = 0.005$).
Conclusion: Acute betaine supplementation had no effect on muscular endurance performance in the bench press.
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Key words: total training volume ■ Betaine ■ supplementation ■ resistance training

INTRODUCTION

A dose-dependent relationship between the total training volume (TTV) and the magnitude of hypertrophic adaptations has been well established in the literature.[1] Therefore, strategies capable of increasing TTV may be beneficial for chronic hypertrophy.[2] Multi-ingredient pre-workout supplements (MIPS) have grown in popularity in the past decade and are designed to be consumed as an ergogenic aid prior to resistance training to enhance TTV. MIPS typically contain caffeine in addition to antioxidants and amino acids such as beta-alanine and betaine anhydrous.[3] While the effects of caffeine and beta-alanine are well documented in the literature,[4] the acute ergogenic potential of some of other popular ingredients in MIPS, such as betaine, requires further exploration[5].

Chronic (6 to 10 weeks) betaine supplementation with 2.5 g/day has been reported to enhance improvements in lean mass[6] and reductions in fat mass[7] that accompany resistance training. Sub-chronic (up to two weeks) betaine supplementation has been shown to improve upper and low body force output[8], power output[8], power output during cycle sprinting[9], strength[10], and muscular endurance[11]. However, within these aforementioned studies, improvements across variables and between studies is not consistent.[6,12] For example, Lee et al.[13] reported improvements in maximal force production after 14 days of betaine supplementation, while Hoffman et al.[8] found no improvements in 1 RM. It's hypothesized that betaine may be most ergogenic during resistance exercise paradigms that require muscular endurance (i.e.: greater than 10 repetitions per set) as betaine appears to stabilize contractile proteins and citric acid cycle enzymes against urea denaturation[5].

The betaine dosing protocol utilized in the aforementioned studies is also different from how betaine is consumed via MIPS. In most studies, betaine is consumed daily for 2 to 10 weeks, whereas consumers of MIPS consume betaine only on training days (i.e.: 3 to 5 days week). Previous studies have shown that supplementation of substances capable of metabolically signalling the alactic anaerobic system can acutely improve performance.[14,15] Only one study verified the effect of acute betaine supplementation in this regard, and showed a trend towards improvements in sprinting performance with a

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From the Graduate Program in Physical Education, Federal University of Rio Grande do Norte, Brazil (M.D., V.S.de Q., A.M.B.da S., B.G.de A.T.C.), Health Science graduate program, Federal University of Rio Grande do Norte, Brazil (T.A.L.da S.), Associate Graduate Program in Physical Education, Federal University of Paraíba, Brazil (L.de S.F.), Department of Exercise Physiology, College of Health Sciences, University of Lynchburg, Lynchburg, VA 24501 (J.C.), and Department of Nutrition, Health Science Center, Federal University of Rio Grande do Norte (A.L.V. de O.)
Communicated by Takashi Abe, Ph.D.
Correspondence to: Dr. Matheus Dantas, Physical activity and health (AFISA – room 5), Department of Physical Education, Federal University of Rio Grande do Norte, Brazil
E-mail: matheusp_dantas@ufrn.edu.br

reduction in the lactate concentration.[16] To our knowledge, there are no studies investigating whether acute betaine ingestion can induce performance improvement in resistance exercise. The purpose of this study was to investigate the acute effects of betaine supplementation on muscular endurance during resistance exercise in trained male participants. We hypothesize that total volume will be greater with betaine supplementation versus placebo.

MATERIALS and METHODS

Experimental design

The manuscript is written according to CONSORT guidelines for pilot studies.[17] This was an experimental, crossover, randomized and double-blind study carried out in the Biodynamics laboratory (Figure 1). Participants reported to the laboratory at 3 different times. On the first visit, all research steps were explained, and the 1 repetition maximum (RM) test was performed for the bench press. The participants returned to the laboratory after 48 hours and a third evaluator (PFAN) performed a randomized condition allocation draw and provided the substance (Betaine or Maltodextrin) for ingestion. After the draw to determine the substance that would be ingested, the participants consumed 5 capsules blindly (i.e., the pills of both conditions have the same weight, size and color). The participant was to perform as many repetitions as possible on the bench press with 70% of 1RM 1 hour after ingesting the first substance (Betaine or Maltodextrin). Consuming the supplement 1 hour prior to exercise was selected as plasma betaine has been shown to peak 60 minutes following a 3 g dose of betaine.[18] This betaine supplementation time was also used in a previous acute study.[16] The session consisted of 3 sets on the bench press performing repetitions to momentary muscular failure. The participants returned to the laboratory again 48 hours after the session for the same procedure, but with the substance that was not previously ingested (Betaine or Maltodextrin). The session was performed in an indoor gym with a non-controlled environment (temperature: ~26ºC, humidity: 77%). The study registered on the *Registro Brasileiro de Ensaios Clínicos* (ReBEC) has been published (register number: RBR-7k79kd).

Participants

The participants were recruited by digital media disclosure. To participate in the study, the participants should: (i) have experience in strength training for more than 6 months, (ii) never used any type of dietary supplement (e.g., Caffeine or creatine), and (iii) not use energy drinks or caffeine at least 48 hours before the session. Participants were recruited during the second semester of 2019. Ten healthy male university (Age: 23.71 ± 4.23 years, height: 1.76 ± 0.09 m, mass: 79.63 ± 5.79 kg, 1RM= 85.40 ± 13.13 kg) students participated in this study. Participants were asked to maintain their usual dietary routine throughout the study. They were also asked to avoid training in the 24 hours prior to the reporting to the laboratory. All participants signed an informed consent form. The research was approved by a local ethics and research committee (no. 3.616.247).

Procedures

The randomization was performed via coin flip by a third evaluator (PFAN). Participants ingested the pills with the supplementation and received water ad libitum throughout the training session. The participant then went to the laboratory gym after ingestion where he performed the resistance strength protocol after 1 hour. The physical evaluation was performed by the independent evaluators of the randomization process (MD and VSQ). Data were collected and passed to a third evaluator to fill out the spreadsheet. The procedure was then repeated in the second moment, except changing the nutrient (betaine or maltodextrin) (i.e., the participants ingested the other substance).

Supplementation was performed using 600ml absorbable capsules. Five capsules of the same weight and colour were used to guarantee blinding of the study. The betaine condition was composed of 2.5 g of betaine + 500 mg of Maltodextrin

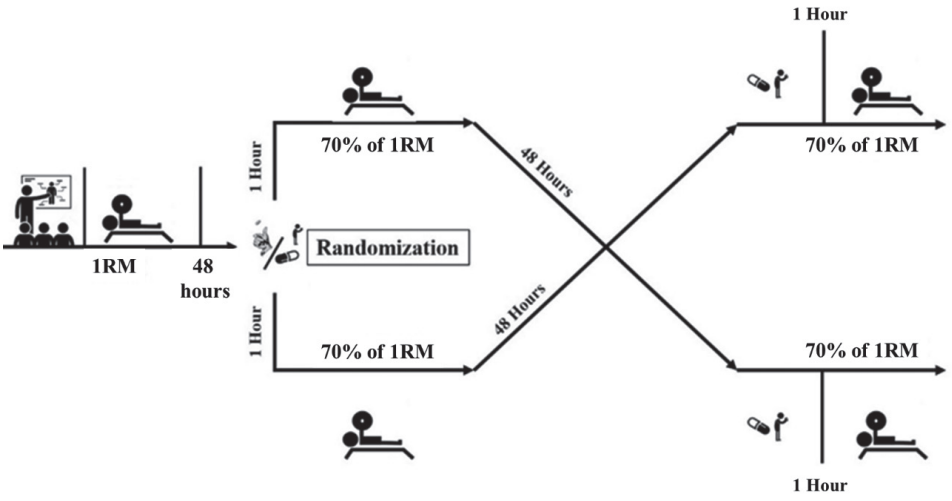

Figure 1 Experimental design.
Note. 1RM = one repetition maximum (i.e., Maximum load for performing just one repetition in a given exercise).

(i.e., 500 mg of betaine + 100 mg of Maltodextrin/capsule). The control condition used 2.5 g of cellulose + 500 mg of Maltodextrin during the same period (i.e., 500 mg of cellulose + 100 mg of carbohydrate/capsule). Participants were instructed to maintain a dietary pattern throughout the protocol. A food record of the last 24 hours prior to the test was maintained in order to ensure consistency. The food record was analysed to calculate the macronutrient intake and total caloric intake.

The 1RM load was determined for the bench press. First, participants warmed-up with 1 set of 5 repetitions with 80% of 1RM estimated for free barbell bench press exercise (i.e., Participants were asked the load they lift in the bench press exercise and the repetitions in the normal training routine), adopting rest intervals of 180 seconds before the next warm-up set. Next, the participants performed another set with 90% of 1RM. The participants then began the 1RM test after a 180-second rest period. Participants had three attempts to achieve the 1RM load with a three to five-minute interval between attempts. Verbal encouragement was given throughout the 1RM test.

Training session

Muscular endurance was assessed by TTV on the bench press exercise. The participants performed a 5 min aerobic warm-up in bicycle ergometer at 6 on CR10 Rating Perceived of Exertion scale. Subjects then performed as many repetitions as possible with 70% of 1RM (i.e., moderate load) and a cadence of 2:0:2:0 (i.e., concentric and eccentric actions for 2 seconds and without isometry between the change in muscle contraction) to momentary muscular failure with 3 minutes of rest between sets. The TTV was considered as the product between the number of sets, number of repetitions and load (TTV= Sets × Repetitions × Load$_{(kg)}$). Muscle endurance is the ability of the muscle to perform to maintain sub-maximal force output until exhaustion. The greater the muscular endurance, the greater the total training volume, which is related to muscular hypertrophy.[1,2] A previous study showed that the bench press could be sensitive to detect ergogenic effects of betaine supplementation[10]. Furthermore, bench press is an exercise commonly used in several training programs[6,13].

Statistical analysis

Data normality was verified by the Shapiro-Wilk test and z-score of asymmetry and kurtosis (-1.96 to 1.96). The 1st set of the placebo condition did not present normal data. The repetition data between the sets were transformed by LOG$_{10}$. Two-way analysis of variance (ANOVA) of repeated measures was used to verify the interaction effect ([2] condition × [3] sets) for TTV. Partial eta-squared was used for measurement effect size (ηp^2). Bonferroni post-hoc correction was used to check the punctual differences. The student's t-test for paired samples was used to compare dietary patterns between conditions and TTV. Effect size for pair comparison was performed by Cohen's d: ((M1 – M2)/ (SD_{pooled})) and the confidence intervals were calculated by equation 1. An effect size of greater than 0.2, 0.5m and 0.8 were interpreted as small, medium, and large, respectively.[19] We adopted $p < 0.05$ for all analyses. All analyses were performed in R studio.[20]

Equation 1. Cohen's d Confidence interval. In which, d= effect size, σ = variance.

$$95\%CI = d - 1.96 \times \sigma$$

Equation 2. Cohen's d Variance. In which, N1= Group 1 sample size, N2= Group 2 sample size, d= effect size.

$$\sigma(d) = \sqrt{\frac{N_1 + N_2}{N_1 x N_2} + \frac{d^2}{2\{N_1 + N_2\}}}$$

RESULTS

Dietary pattern

There was no significant difference in the subjects' dietary patterns in the last 24 hours preceding the tests for both carbohydrates (t = 0.354, $p = 0.73$, $d = 0.2$, 95%CI = -0.71 – 1.04), protein (t = 0.422, $p = 0.684$, $d = 0.2$, 95%CI = -0.68 – 1.07) and lipids (t = 1.183, $p = 0.27$, $d = -0.5$, 95%CI = -1.40 – 0.38). There was also no significant difference for the total energy consumption (t = 0.176, $p = 0.87$, $d = 0.1$, 95%CI = -0.97 – 0.79).

Muscular endurance

Figure 2 shows the effect of betaine supplementation on number of repetitions per set (A and B) and total volume

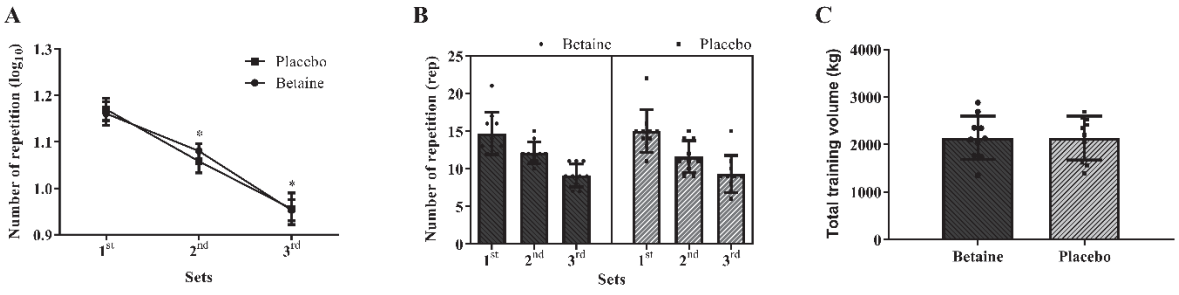

Figure 2 Effect of betaine supplementation on number of repetitions per set (A and B) and total training volume (C).
Note. (A) Data per set transformed by log$_{10}$, (B) Clinical data obtained from the subjects, (C) total training volume of session. The analysis was performed on log transformed data. *Significant difference to the previous series ($p < 0.05$).

training (C). There was a significant time effect for the muscular endurance ($F_{(2, 18)} = 54.63$, $p < 0.0001$, $\eta p^2 = 0.86$), but there was no significant condition ($F_{(1, 9)} = 0.05$, $p = 0.84$, $\eta p^2 = 0.01$) or interaction ($F_{(2, 18)} = 0.63$, $p = 0.55$, $\eta p^2 = 0.07$) effect. There was a significant reduction in the number of log-transformed repetitions from the first to third set for both experimental conditions ($p \leq 0.05$). Total training volume was not significantly different between betaine or placebo supplementation (Figure 2, C: $t = 0.075$; $p = 0.942$; $d = 0.01$).

DISCUSSION

The aim of our study was to determine the effect of acute betaine supplementation on bench press muscular endurance performance. We hypothesized that supplementation would have a positive ergogenic effect on the TTV. We reject our hypothesis as there were no differences in TTV between betaine and placebo conditions.

When betaine is ingested by healthy subjects it is freely filtered in the kidney, reabsorbed into the circulation, and stored in the tissues.[21] Betaine is rapidly absorbed and reaches maximal plasma concentrations in approximately 40-80 minutes and returns to baseline values following approximately 23 hours.[18] These data are dose dependent and a large dose of betaine supplementation peaks later (1g = ~40min, 3g = ~60min, 6g = ~80min).[18] If betaine is to function acutely, it is likely to provide protection against metabolic and external hypertonic stress via its osmotic effects.[22] To the best of our knowledge, the only study to evaluate the ergogenic effect of acute betaine supplementation was conducted by Armstrong et al.[16]. Subjects consumed 5 g of betaine, ran for 75 minutes at 65% of VO2max, and then sprinted to exhaustion at 84% of VO2max in a hot environment. Plasma lactate was reduced and plasma volume increased in the betaine condition, but there were no significant improvements in performance. Our results are in agreement with Armstrong et al.[16] and suggest that betaine is not ergogenic when consumed acutely.

Betaine concentrations in human tissues have not been well studied, and to our knowledge, there are no reports related to betaine concentrations in skeletal muscle in humans. The results from rodent studies demonstrate that muscle tissue accumulation of betaine is directly related to plasma betaine concentrations.[23] In young mice (21 to 56 days of age), betaine accumulation in skeletal muscle seems to be most rapid during the first 10 days of supplementation, but will continue to slowly rise for up to 6 weeks or longer.[24] Based upon this data and the ergogenic results reported in sub-chronic supplementation studies,[7] it would appear that at least 10 days of supplementation is necessary to reach muscle tissue concentrations whereby betaine can exert an ergogenic effect.

Betaine ingestion increases the availability of the universal methyl donor S-adenosylmethionine (SAM). Given that creatine synthesis accounts for about 40% of total SAM consumption,[25] betaine supplementation has been suggested to exert an ergogenic influence by increasing phosphocreatine availability[10]. While this mechanism has a good theoretical foundation, the results of Del Favero et al.[26] refute this theory as 10 days of betaine supplementation did not increase the phosphocreatine content in the skeletal muscle of untrained men. However, the subjects in Del Favero et al.[26] were untrained and not engaged in an exercise program that relied on phosphocreatine catabolism. As explained by Cholewa et al.,[7] it is therefore unlikely the demand for SAM to synthesize additional creatine was elevated. Nevertheless, even if betaine does act via increasing creatine availability, the effects of betaine on power and strength are ambiguous compared to creatine[26] and athletes would likely achieve greater ergogenic effects supplementing creatine monohydrate directly.

Some methodological aspects adopted in our study need to be highlighted as limitations: (i) no sample size calculated; (ii) Plasma betaine was not measured; (iii) there only one measure for neuromuscular performance. Sample size was collected by convenience and the data were analysed step-by-step in our study. The initial plan was to assess 10 subjects and analyse the behaviour of the data. As we had no evidence of effect after 10 subjects, we stopped data collection and returned to the project in order to understand the causes of the findings. The previous study that evaluated the plasma betaine concentration after acute supplementation was a primarily female sample (70%),[18] however, females tend to have lower betaine concentrations compared to males due to greater betaine-homocysteine methyltransferase activity[21]. While we were unable to measure plasma betaine, there is evidence that the betaine supplementation protocol used in this study elevated plasma betaine concentration, it is likely that tissue saturation, and not acutely available plasma betaine, are necessary to support an ergogenic benefit. Therefore, our data failed to demonstrate that a single dose of betaine supplementation

Table 1 Description of daily food consumption within 24 hours before testing (mean ± SD)

	Betaine (n = 10)	Placebo (n = 10)
1st set (Rep)	14.70 ± 2.83	15.00 ± 2.83
2nd set (Rep)	12.10 ± 1.45	11.60 ± 2.12
3rd set (Rep)	9.10 ± 1.52	9.30 ± 2.45
Carbohydrate	438.22 ± 286.33	400.89 ± 135.27
Protein	201.44 ± 93.20	186.44 ± 57.37
Lipids	136.22 ± 57.70	170.44 ± 74.48
Total energy	3770.22 ± 1508.00	3890.67 ± 1191.41

Note: SD, standard deviation. Rep, number of repetitions.

improves the session TTV (i.e., resistance exercise performance).

CONCLUSION

Acute betaine supplementation did not provide a significant ergogenic effect on muscular endurance performance or TTV in the bench press in trained male participants. TTV is an important marker of muscle stress related to muscle hypertrophy.[1,2] However, chronic supplementation seems to increase TTV,[9,10] so we cannot refute the idea that supplementation increases TTV. Studies investigating the time-course of tissue saturation in humans may be needed to determine the minimal duration of betaine supplementation to observe ergogenic effects.

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REFERENCES

1. Schoenfeld BJ, Ogborn D, Krieger JW. Dose-response relationship between weekly resistance training volume and increases in muscle mass: A systematic review and meta-analysis. *J Sports Sci* 2017;35:1073-1082.
2. Schoenfeld BJ, Grgic J, Krieger J. How many times per week should a muscle be trained to maximize muscle hypertrophy? A systematic review and meta-analysis of studies examining the effects of resistance training frequency. *J Sports Sci* 2019;37:1286-1295.
3. Jagim AR, Harty PS, Camic CL. Common ingredient profiles of multi-ingredient pre-workout supplements. *Nutrients* 2019;11:254.
4. Eudy AE, Gordon LL, Hockaday BC et al. Efficacy and safety of ingredients found in preworkout supplements. *Am J Health Syst Pharm* 2013;70:577-588.
5. Cholewa JM, Guimaraes-Ferreira L, Zanchi NE. Effects of betaine on performance and body composition: a review of recent findings and potential mechanisms. *Amino Acids* 2014;46:1785-1793.
6. Cholewa JM, Wyszczelska-Rokiel M, Glowacki R et al. Effects of betaine on body composition, performance, and homocysteine thiolactone. *J Int Soc Sports Nutr* 2013;10:39.
7. Cholewa JM, Hudson A, Cicholski T et al. The effects of chronic betaine supplementation on body composition and performance in collegiate females: a double-blind, randomized, placebo-controlled trial. *J Int Soc Sports Nut*r 2018;15:37.
8. Hoffman JR, Ratamess NA, Kang J et al. Effect of 15 days of betaine ingestion on concentric and eccentric force outputs during isokinetic exercise. *J Strength Cond Res* 2011;25:2235-2241.
9. Czapla R, Swensen T, Craig SA. Effect of betaine on cycling sprint power. *J Int Soc Sports Nutr* 2010;7(Suppl 1):P23.
10. Hoffman JR, Ratamess NA, Kang J et al. Effect of betaine supplementation on power performance and fatigue. *J Int Soc Sports Nutr* 2009;6:7.
11. Trepanowski JF, Farney TM, McCarthy CG et al. The effects of chronic betaine supplementation on exercise performance, skeletal muscle oxygen saturation, and associated biochemical parameters in resistance trained men. *J Strength Cond Res* 2011;25:3461-3471.
12. Ismaeel A. Effects of betaine supplementation on muscle strength and power: a systematic review. *J Strength Cond Res* 2017;31:2338-2346.
13. Lee EC, Maresh CM, Kraemer WJ et al. Ergogenic effects of betaine supplementation on strength and power performance. *J Int Soc Sports Nutr* 2010;7:27.
14. Izquierdo M, Ibañez J, González-Badillo JJ et al. Effects of creatine supplementation on muscle power, endurance, and sprint performance. *Med Sci Sports Exerc* 2002;34:332-343.
15. Cox G, Mujika I, Tumilty D et al. Acute creatine supplementation and performance during a field test simulating match play in elite female soccer players. *Int J Sport Nutr Exerc Metab* 2002;12:33-46.
16. Armstrong LE, Casa DJ, Roti MW et al. Influence of betaine consumption on strenuous running and sprinting in a hot environment. *J Strength Cond Res* 2008;22:851-860.
17. Eldridge SM, Chan CL, Campbell MJ et al. CONSORT 2010 statement: extension to randomised pilot and feasibility trials. *Pilot Feasibility Stud* 2016;2:64.
18. Schwab U, Törrönen A, Meririnne E et al. Orally administered betaine has an acute and dose-dependent effect on serum betaine and plasma homocysteine concentrations in healthy humans [published correction appears in *J Nutr* 2007;137:1124]. *J Nutr* 2006;136:34-38.
19. Diener MJ. Cohen's d. in The Corsini Encyclopedia of Psychology. Eds Weiner IB, Craighead WE, John Wiley & Sons, 2010
20. Rstudio team URL. RStudio: integrated development for R. RStudio, Inc., Boston, MA URL http://www. rstudio.com. 2015; 42:14.
21. Lever M, Sizeland PC, Frampton CM et al. Short and long- term variation of plasma glycine betaine concentrations in humans. *Clin Biochem* 2004;37:184-190.
22. Willingham BD, Ragland TJ, Ormsbee MJ. Betaine Supplementation May Improve Heat Tolerance: Potential Mechanisms in Humans. *Nutrients* 2020;12:2939.
23. Slow S, Lever M, Chambers ST et al. Plasma dependent and independent accumulation of betaine in male and female rat tissues. *Physiol Res*. 2009;58:403-410.
24. Clow KA, Treberg JR, Brosnan ME, et al. Elevated tissue betaine contents in developing rats are due to dietary betaine, not to synthesis. *J Nutr* 2008;138:1641-1646.
25. Brosnan JT, da Silva RP, Brosnan ME. The metabolic burden of creatine synthesis. *Amino Acids* 2011;40:1325-1331.
26. Del Favero S, Roschel H, Artioli G, et al. Creatine but not betaine supplementation increases muscle phosphorylcreatine content and strength performance. *Amino Acids* 2012;42:2299-2305.