

ORIGINAL SCIENTIFIC PAPER

Effect of β -Alanine Supplementation on Repeated Sprint Ability and Responses of Blood Lactate and Bicarbonate in Male Soccer Players

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Abstract

This study was designed to investigate the effect of β -alanine supplementation on sprint time during repeated sprint ability test and blood lactate and bicarbonate responses to the test. Eighteen male soccer players were randomly divided into two groups (β -alanine, $n=9$ (24.31 ± 2.14 yrs) or placebo, $n=9$ (23.98 ± 2.07)). We conducted a randomized, double-blind, parallel-group, placebo-controlled study in which participants ingested 4.8 g/day for four weeks of a β -alanine supplement or a placebo. Athletes completed seven repetitions of 30 m interspersed with 30 s recovery intervals. The test was performed before and after four weeks of supplementation. Blood samples were collected from each participant in both groups before and after the test, pre- and post-supplementation to measure lactate and bicarbonate levels. Data showed that the sixth and seventh repetitions were significantly faster after β -alanine supplementation than the placebo (sixth repetition: 3.74 ± 0.04 s vs 3.91 ± 0.09 s, seventh repetition: 3.91 ± 0.07 s vs 4.12 ± 0.14 s, $p=0.001$, $p=0.002$, respectively). Before supplementation, however, no differences existed between groups for any sprint time in all repetitions ($p>0.05$). Data revealed significantly higher lactate concentration in the β -alanine than the placebo after the finish of the test at both pre-supplementation ($p=0.022$), and post-supplementation ($p=0.017$). No differences noted between groups in bicarbonate at all measured points. In conclusion, β -alanine supplementation has a beneficial effect on repeated sprint performance in soccer players, probably due to effective vasodilatation mechanism.

Keywords: *carnosine, fatigue, hydrogen ion, fast-twitch fiber, glycolysis*

Introduction

Soccer players complete a competitive game with substantial high numbers of explosive powers, duels, accelerations, and repeated sprints (Varley & Aughey, 2013). These activities are performed with high-intensity effort that may be associated with the accumulation of muscle metabolites, such as adenosine diphosphate (ADP), inorganic phosphate (Smith et al., 2009), and hydrogen ion (H^+) (Hobson, Saunders, Ball, Harris, & Sale., 2012) contributing to muscle fatigue. Excessive amounts of H^+ impair the activity of calcium ions on the troponin-binding site (Lancha Junior, de Salles Painelli, Saunders, & Artioli, 2015) affecting excitation-contraction coupling. In

addition, the accumulation of intramyocellular H^+ represents the major cause of muscle fatigue during high-intensity exercise (de Salles Painelli et al., 2013) and has been shown to inhibit the resynthesis of phosphocreatine (PCr) (Hobson et al., 2012), glycolysis (Lancha Junior et al., 2015), and disrupt the buffering system (de Salles Painelli et al., 2013). Of importance, the muscle pH buffers are supported by the physicochemical buffering system (Smith et al., 2009) that includes free inorganic phosphate (Baguet, Koppo, Pottier, & Derave 2010), creatine phosphate (Baguet et al., 2010) bicarbonate (de Salles Painelli et al., 2013), and histidine residues, such as carnosine (Baguet et al., 2010; Hobson et al., 2012).



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Carnosine (β-alanyl-L-histidine) is a cytoplasmic dipeptide and one of the first lines of defence system against muscle acidosis (Hill et al., 2007) due to its imidazole that has an optimal pka value of 6.83 (Bate-Smith, 1938; Brisola, Artioli, Papoti, & Zagatto, 2016; Harris et al., 2006); high concentrations of it are found in human skeletal muscle (16 - 29.2 mmol/kg dry muscle) (Harris et al., 2006; Hobson et al., 2012; Smith et al., 2009). Additionally, carnosine functions are mostly associated with antioxidants, anti-ageing (Gallant, Semyonova, & Yuneva, 2000), protein glycosylation inhibition, and wound healing (Baguet et al., 2010). Since soccer players perform several changes of directions, with an average change of movements every 4 to 6 seconds (Devrnja & Matkovic, 2018), which may induce muscle acidosis, carnosine supplementation is likely to be beneficial.

β-alanine (BA), a non-essential amino acid, is the rate-limiting precursor of the carnosine synthesis in muscle cells (Glenn et al., 2015). It has been demonstrated that BA supplementation is used by athletes to delay fatigue and improve performance (Baguet et al., 2010) through increased non-bicarbonate myocyte buffering capacity (Baguet et al., 2010; Suzuki, Ito, Mukai, Takahashi, & Takamatsu, 2002) and increased vasodilatation (Ririe, Roberts, Shouse, & Zaloga, 2000). Previous studies have shown that intake of BA supplement with a dose of 3.2–6.4 g/day for 4–10 weeks (Hoffman, 2010; Kendrick et al., 2008) could increase intramyocellular carnosine content by 40–80% (Hill et al., 2007). Also, irrespective of different exercise modes, some studies have revealed that BA supplementation resulted in improved physical performance in high-intensity exercise (Claus et al., 2017), repeated maximal contractions (Derave et al., 2007), and during time-to-exhaustion (Glenn et al., 2015). In contrast, the others have failed to find a beneficial effect on strength exercise (Kendrick et al., 2008), repeated treadmill sprint test (Sweeney, Wight, Brice, & Doberstein, 2010), Wingate anaerobic testing (Al-horani & Alzoubi, 2017), and oxygen deficit (Baguet et al., 2010).

It is plausible that the increase in muscle carnosine content brought about by BA might be of benefit to soccer players. Although some studies have examined the effect of BA in repeated sprint modes (Brisola et al., 2016; Danaher, Gerber, Wellard, & Stathis, 2014; Sweeney et al., 2010), no studies have examined BA supplementation effect on treadmill repeated sprint ability (RSA) test in soccer players or on the field.

Consequently, the present study aimed to investigate the effect of BA supplementation on RSA test and blood lactate and bicarbonate responses to the test in soccer players. We speculate that BA supplementation may improve performance by the features of physicochemical buffers.

Methods

Subjects

Eighteen well-trained male soccer players from a premier Jordan division team participated in the study. All athletes trained once a day (approximately 110–120 min), six times per week. The study was done during the early period of the participants' off-season. Before signing the informed consent form, the athletes were informed about the potential risks and benefits involved in participation. Informed consent was obtained from all participants involved in the study. This study was approved in advance by the Local Scientific Research Committee (protocol no. 09/2019 M A)

Experimental design

A randomized, double-blind, parallel-group placebo-controlled design was used in this study. Participants were randomly divided into two groups: β-alanine (BA) group (n=9) and dextrose as the placebo (Pla) group (n=9). Participants' demographic data in both groups are presented in Table 1. Before beginning the study, each athlete visited the laboratory on two separate days. Day one consisted of fill-out and signing an informed consent form and completion of a background health information form to ensure that all participants met the inclusion criteria (normal vital signs). Day Two consisted of the measurements of characteristics of participants as well as information about possible risks and benefits related to participation. Before beginning the supplementation period, each athlete in both groups performed the RSA test. After that, athletes were instructed to ingest BA in the BA group and dextrose in the Pla group, both for four weeks. After one day of the completion of supplementation, the test was performed in the same order for all athletes in both groups. The test was performed at the same time of the day (9.30 am) in both groups. Randomization was equalized by skills, position, and experience to ensure the homogeneity between groups. The homogeneity of the demographic variables of participants between groups was equal (p>0.05) (Table 1).

Table 1. Subjects demographic data

variable	Pla	BA	p
	M±SD	M±SD	
Age (years)	23.98±2.07	24.31±2.14	0.741
Height (cm)	178.11±3.33	180.00±2.55	0.196
Mass (kg)	71.89±3.18	72.33±2.78	0.756
BMI (kg/m ²)	22.65±0.44	22.30±0.82	0.281
resting HR (bpm)	61.89±2.67	60.67±2.45	0.326
VO2max (ml/kg/min)	54.70±2.00	55.52±2.26	0.426
Training experience (years)	9.56±2.24	8.89±1.96	0.512

Legend: M-mean; SD-standard deviation; Pla-placebo, BA-β-alanine; p<0.05

No differences existed between groups for any demographic variable.

Control of pre-experimental status

The authors did not interfere with the athletes' training

sessions throughout the study. Athletes were asked to maintain their routine training sessions and were instructed to refrain from strenuous exercise 48 hours prior to each test. None of the athletes had ever consumed BA supplements. They were not permitted to ingest any nutritional and/or ergogenic sup-

plementation throughout the study. They were also requested to maintain their normal diet throughout the supplementation period and were instructed to fast three hours prior to the test for not to affect final analyses. Athletes wore the same attire for each test and wore the T-shirt and shoes in which they normally train.

Supplementation protocol

During the supplementation period, athletes in the BA group received 4.8 g/day of BA (CarnoSyn®, Capsule, Beta-alanine, BioSteel, Canada) taken three times per day throughout the supplementation. Athletes in the Pla group received 8 g dextrose in the same order. The selected dose was utilized to avoid the potential incidence of paraesthesia (Kendrick et al., 2008). The supplementation period was determined to ensure the increased content of muscle carnosine (Smith et al., 2009). The dose of BA supplement was taken after meal or snack interspersed minimally by three hours to circumvent paraesthesia. Similar opaque capsules were used by participants in both groups to preserve the design and participants' inability to distinguish between the two types. None of the athletes were affected by paraesthesia.

Repeated sprint ability test

Familiarization for the RSA test was done once in the study due to the fact the athletes are already adapted to short sprinting. The test was performed in the track (arena). Athletes performed a sprint test before and after four weeks of the supplementation period. It consisted of seven maximal sprint repetitions of 30 m (7×30 m) interspersed by a 30 s recovery. It was performed after a standardized warm-up of 10 min that had already been used by athletes before every training session. The distance of 30 m was measured electronically (30M Laser Digital Tape, Germany). A digital clock (Infrared Control System, Clock Counter, 63501IR, USA) was utilized to record the time of each repetition during the test. All participants were performed in the standing position beginning.

During the 30 s recovery intervals between repetitions, athletes were asked to rest passively. Verbal encouragement was given to each athlete to achieve the maximal speed of every repetition.

Blood sample analysis

The blood samples were collected from each participant in the BA and Pla groups to measure lactate and bicarbonate before and after five minutes of the RSA test. Venepuncture was used to obtain blood samples (5 ml). Lactate concentration was analysed using (Integral 400, Switzerland). A gas analyser (Cobas b 221 - Roche Diagnostics, Switzerland) was utilized to analyse bicarbonate. The period of five minutes was specified to ensure sufficient lactate clearance and bicarbonate buffering and, therefore, supposed the explanation for ingested BA effect. The reference ranges of variables were as follows: 0.63–2.44 mmol/L for lactate, 22.0–29.0 mmol/L for serum bicarbonate.

Statistical analysis

The Shapiro-Wilk test was applied to check for normal distribution. The variables were normally distributed ($p > 0.05$). A paired sample t-test was used to analyse the differences in blood lactate and bicarbonate within a group (between pre- and post-RSA test, and between before and after supplementation). Paired sample t-test was also used to analyse the differences between repetitions within a group. An independent t-test was utilized to analyse the differences in blood lactate and bicarbonate between groups. Two-way ANOVA with repeated measures on (pre vs post) was used to determine if any significant main effects were present between groups (BA and Pla). Statistical analyses were carried out by SPSS version 23.0. All data are reported as mean±SD. The level of statistical significance was set at $p < 0.05$.

Results

Table 2 illustrates the sprint time of each repetition during the RSA test before and after supplementation within a group.

Table 2. Sprint time of the 7 repetitions during repeated sprint ability test within a group before and after supplementation

RSA test	Pla group		p	BA group		p
	Pre-suppl	Post-suppl		Pre-suppl	Post-suppl	
	M±SD	M±SD		M±SD	M±SD	
Rep. 1	3.51±0.01	3.52±0.01	0.088	3.52±0.02	3.51±0.02	0.154
Rep. 2	3.54±0.02	3.53±0.01	0.316	3.55±0.03	3.53±0.02	0.070
Rep. 3	3.57±0.02	3.56±0.01	0.212	3.58±0.02	3.55±0.02	0.052
Rep. 4	3.62±0.03	3.61±0.02	0.056	3.63±0.03	3.59±0.02	0.010*
Rep. 5	3.66±0.04	3.65±0.02	0.738	3.67±0.03	3.63±0.03	0.002*
Rep. 6	3.89±0.10	3.91±0.09	0.009*	3.90±0.09	3.74±0.04	0.001*
Rep. 7	4.11±0.14	4.12±0.14	0.028*	4.10±0.13	3.90±0.06	0.001*

Legend: * - $p < 0.05$; RSA - repeated sprint ability; Pre - before, Post - after, suppl - supplementation, Rep - repetition

Two-way ANOVA showed that the sprint time of sixth and seventh repetitions were significantly ($p < 0.05$) faster before than after dextrose supplementation. Data also revealed that BA supplementation had a significantly beneficial ($p < 0.05$) effect in the sprint times of the fourth to seventh repetitions compared to pre-supplementation. In addition, the sixth and seventh repetitions were significantly faster ($p < 0.05$) after BA supplementation than the Pla (Table 3). Before supplementation, however, no differences existed between groups for any

sprint time in all repetitions.

The paired sample t-test showed that lactate concentration was significantly higher after the finish of the RSA test compared to pre-test before and after supplementation in both the BA and Pla groups ($p < 0.05$). These were measured pre- and post-sprint test before and after supplementation (BA: 2.30±0.12 vs 9.01±0.14 mmol/L, $t=190.45$, $p=0.001$; 2.40±0.07 vs 9.15±0.22 mmol/L, $t=105.89$, $p=0.001$; Pla: 2.37±0.30 vs 9.23±0.21 mmol/L, $t=51.41$, $p=0.001$; 2.38±0.05 vs 8.89±0.17

Table 3. Sprint time of the 7 repetitions during repeated sprint test before and after supplementation between groups

RSA test	Pre-suppl		p	Post-suppl		p
	Pla (M±SD)	BA (M±SD)		Pla (M±SD)	BA (M±SD)	
Rep. 1	3.51±0.01	3.52±0.02	0.395	3.52±0.01	3.51±0.02	0.468
Rep. 2	3.54±0.02	3.55±0.03	0.464	3.53±0.01	3.53±0.02	0.920
Rep. 3	3.57±0.02	3.58±0.02	0.593	3.56±0.01	3.55±0.02	0.325
Rep. 4	3.62±0.03	3.63±0.03	0.748	3.61±0.02	3.59±0.02	0.067
Rep. 5	3.66±0.04	3.67±0.03	0.562	3.65±0.02	3.63±0.03	0.186
Rep. 6	3.89±0.10	3.90±0.09	0.818	3.91±0.09	3.74±0.04	0.001*
Rep. 7	4.11±0.14	4.10±0.13	0.882	4.12±0.14	3.90±0.06	0.002*

mmol/L, $t=80.97$, $p=0.001$, for pre- and post-RSA before supplementation, pre- and post-RSA after supplementation, respectively). The values of bicarbonate were significantly ($p<0.05$) decreased within a group after the finish of the test compared to pre-test before and after supplementation (BA: 26.09 ± 0.42 vs 19.91 ± 0.24 mmol/L, $t=72.810$, $p=0.001$; 26.31 ± 0.20 vs 20.17 ± 0.23 mmol/L, $t=194.705$, $p=0.001$;

Pla: 26.14 ± 0.29 vs 19.93 ± 0.24 mmol/L, $t=73.566$, $p=0.001$; 26.23 ± 0.23 vs 19.96 ± 0.27 mmol/L, $t=71.568$, $p=0.001$, for pre- and post-RSA before supplementation, pre- and post-RSA after supplementation, respectively). The independent t test demonstrated significantly higher lactate concentration in BA than Pla after the sprint test, before ($p=0.022$), and after supplementation ($p=0.017$) (Figure 1).

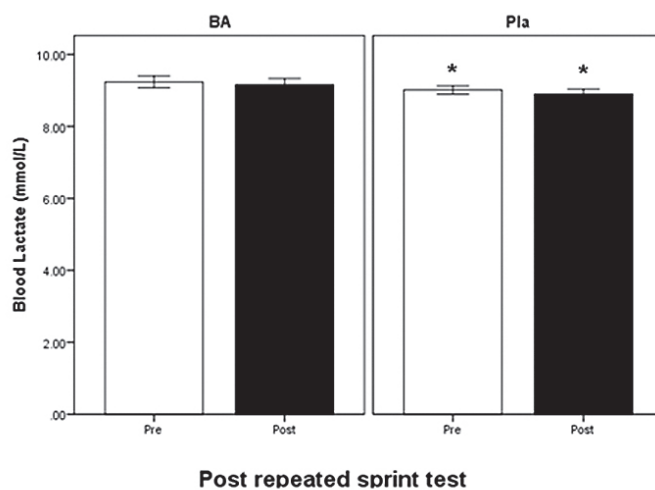


FIGURE 1. Lactate response to repeated sprint test between trials pre- and post-supplementation ($p<0.05$)

Figure 2 illustrates the insignificant ($p>0.05$) differences between groups in bicarbonate following sprint test, before and after supplementation.

No differences existed between groups ($p=0.894$, $p=0.104$ for pre and post supplementation, respectively). Significance level was set at $p<0.05$

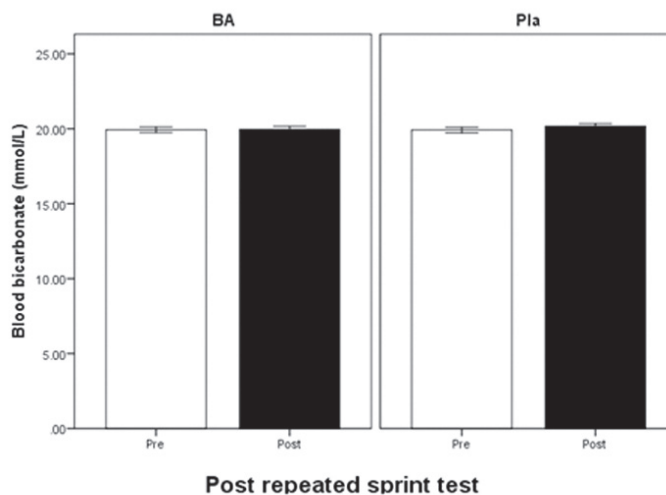


FIGURE 2. Bicarbonate response to repeated sprint test between trials pre- and post-supplementation.

Discussion

Our main finding is that the sprint times in the sixth and seventh repetitions during the RSA test were performed faster after BA supplementation than by the Pla group. This result could be explained by the beneficial effects of BA for improving performance by eliciting calcium release from the sarcoplasmic reticulum (Dutka & Lamb, 2004), by increasing calcium sensitivity of the contractile apparatus (de Salles Painelli et al., 2013), and by vasodilatation (Ririe et al., 2000). These mechanisms can occur as a result of increased muscle carnosine levels, making the muscle contractile force operate effectively (Hobson et al., 2012) and the subsequently delayed onset of fatigue (Hill et al., 2007). Significantly, a typical content of muscle carnosine is 17–25 mmol/kg dry muscle (Baguet et al., 2010). This amount has been proposed to elevate muscle carnosine content by about 60–80% (Lancha Junior et al., 2015).

Although we were unable to measure muscle carnosine content, the supplementation period and dosage utilized in our study (4.8 g/day for 4 weeks) are approximately equal in previous studies that have shown increase muscle carnosine levels. In line with this, Baguet et al. (2010) found, using proton magnetic resonance spectroscopy (1H-MRS), increased muscle carnosine content by 45.3% in the soleus and 28.2% in gastrocnemius after BA supplementation (5 g/day for 7 weeks) and showed the high correlation between speed and carnosine content as 4.3 s faster than the placebo after the completion of a 2000 m ergometer test in rowers. Derave et al. (2007) also observed significantly increased carnosine content in the soleus (+47%) and gastrocnemius (+37%) following BA supplementation (4.8 g/day for 4 weeks) and diminished fatigue during five bouts of 30 maximal voluntary leg extension in sprinters. Danaher and colleagues (2014) found elevated carnosine concentration in the gastrocnemius (+62%) and soleus (+88%) after supplementation period (4.8–6.4 g/day for 6 weeks) compared to placebo (7.42 mM, 6.33 mM, respectively).

In regard with repeated sprint performance, Brisola et al. (2016) demonstrated no significant group-time interaction for RSA test interspersed by 30-min swimming test after 28 days of BA supplementation (4.8–6.4 g/day) in water polo players. Danaher et al. (2014) showed that the performance during RSA test (5 × 6 s) separated by 24 s was not different between beta-alanine and placebo groups, although intramuscular carnosine levels were increased after supplementation. In a study conducted on physically active college men, BA supplementation (4–6 g per/day for 4 weeks) had no beneficial effect on repeated sprint protocol that consisted of 2 sets × 5 five-second sprints separated by 45 s between sprints and 2 min between sets (Sweeney et al., 2010). In the same study, they suggested that the recovery period was inadequate to resynthesize PCr. In contrast, Claus et al. (2017) showed that BA supplementation (6.4 g/day for 6 weeks) improved ball velocity shooting during RSA test in water polo players. Regarding endurance capacity, Glenn et al. (2015) showed that BA supplementation (3.2 g/day for 28 days) improved time to exhaustion compared to placebo in female cyclists. Surprisingly, one study revealed no existed difference in rating of perceived exertion between a BA and placebo, although the time to exhaustion during supra-maximal cycling test (120% VO₂ peak) was significantly increased following BA supple-

mentation (6.4 g/day for 4 weeks) in cyclists (Bellinger & Minahan, 2016).

In the present study, lactate concentration at the end of the RSA test pre- and post-supplementation was significantly higher in the BA than the Pla group. This could be explained by the fastest sprint time achieved by athletes in the BA group, specifically in the last two repetitions. Importantly, during repeated sprint bouts, fast-twitch fibers' reliance on both PCr and glycolysis (Sweeney et al., 2010), resulting in elevated lactate concentration. In the study of Derave et al. (2007), lactate concentration was not different between BA (16.3±0.8 mmol/L) and placebo (15.9±0.7 mmol/L) that was measured after 180 s from completion of a 400-m run. Sweeney et al. (2010) found no difference in lactate response to repeated sprint protocol before and after supplementation between a BA and placebo (12 mmol/L, 13 mmol/L, respectively). In the present study, after supplementation, lactate concentrations reached 9.15 mmol/L in the BA group compared to 8.89 mmol/L in the Pla group after the completion of the RSA test. We suggest that the measured lactate levels after 5 min might not allow for lactate clearance. Tobias et al. (2013) reported increased lactate levels (~13 mmol/L) after BA supplementation compared to placebo (~12 mmol/L) after 5-min recovery of four 30 s upper-body Wingate tests, separated by 3 min. The supplementation period in that study was 6.4 g/day for four weeks. Glenn et al. (2015) concluded that adequate recovery to determine a typical lactate concentration would be more than five minutes after the completion of the exercise. Sale et al. (2011) showed that lactate concentration remained elevated after five minutes of exercise in BA compared to the placebo. According to the lactate values in previous studies, we suggest that our athletes did not reach the point of "lactate-induced fatigue".

BA supplementation was likely to decrease bicarbonate after finishing the RSA test compared to Pla, though that was not statistically significant (p=0.104). The explanation of this result might be attributed to the effective buffering system in which the carbonic acid catalyses bicarbonate to H⁺. Basically, when lactic acid accumulates inside muscle fibers due to glycolysis and disassociates to lactate overcome the buffering capacity (Hobson et al., 2012), excessive production of H⁺ will occur (de Salles Painelli et al., 2013), the muscle pH declines, and subsequent, bicarbonate decreases. Intramuscular H⁺ can increase to 10-fold during intensive exercise, in which the muscle pH drops from 7.1 to 6.3 (Tobias et al., 2013). In addition, muscle acidosis is increased during activation of fast-twitch fibers rather than slow-twitch fibers (Zhen-He, Botinelli, Pellegrino, & Reggani, 2000). Therefore, in our design, the repeated sprinting bouts might be depending on fast-twitch fibers that may result in decreased bicarbonate (Derave et al., 2007).

These are the first data investigating the beneficial effects of β -alanine on repeated sprint performance in soccer players. Our main data indicate that β -alanine supplementation (4.8 g/day, 3 times per day for 4 weeks) can improve sprinting performance during RSA test (7 × 30 m) in soccer players. After the supplementation period, however, lactate response to the test was higher in BA than in the placebo group, though the bicarbonate concentration was not different.

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Conflict of interests

The authors declare that there is no conflict of interest.

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