CLINICAL SCIENCE

PEPTIDE GLUTAMINE SUPPLEMENTATION FOR TOLERANCE OF INTERMITTENT EXERCISE IN SOCCER PLAYERS

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OBJECTIVE: To investigate whether supplementation of carbohydrate together with peptide glutamine would increase exercise tolerance in soccer players.

METHODS: Nine male soccer players (mean age: 18.4 ± 1.1 years; body mass: 69.2 ± 4.6 kg; height: 175.5 ± 7.3 cm; and maximum oxygen consumption of 57.7 ± 4.8 $ml \cdot kg^{-1} \cdot min^{-1}$) were evaluated. All of them underwent a cardiopulmonary exercise test and followed a protocol that simulated the movements of a soccer game in order to evaluate their tolerance to intermittent exercise. By means of a draw, either carbohydrate with peptide glutamine (CARBOGLUT: 50g of maltodextrin + 3.5g of peptide glutamine in 250 ml of water) or carbohydrate alone (CARBO: 50g of maltodextrin in 250 ml of water) was administered in order to investigate the enhancement of the soccer players' performances. The solution was given thirty minutes before beginning the test, which was performed twice with a one-week interval between tests.

RESULTS: A great improvement in the time and distance covered was observed when the athletes consumed the CARBOGLUT mixture. Total distance covered was 12750 ± 4037m when using CARBO, and 15571 ± 4184m when using CARBOGLUT ($p<0.01$); total duration of tolerance was 73 ± 23 min when using CARBO and 88 ± 24 min when using CARBOGLUT ($p<0.01$).

CONCLUSION: The CARBOGLUT mixture was more efficient in increasing the distance covered and the length of time for which intermittent exercise was tolerated. CARBOGLUT also reduced feelings of fatigue in the players compared with the use of the CARBO mixture alone.

KEYWORDS: Fatigue. Tolerance. Glutamine Supplementation. Carbohydrate Supplementation. Soccer players.

INTRODUCTION

Soccer is the most popular sport in Brazil. The International Federation of Football Association (FIFA) considers it to be the game that is most widely played worldwide by individuals with the widest diversity of socioeconomic status. According to data from the Brazilian Football Confederation (CBF), there are around 30 million soccer players in Brazil. Out of this total, 11,000 are registered players.[1]

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Soccer is a competitive acyclic activity of long duration practiced through intermittent movements with varying degrees of intensity that require participation by three energy-providing systems.[2] Today's players move at high speed and can cover an average distance of 10 to 14 km by the end of a game, mostly depending upon the player's position and the importance of the match, though the relative distribution of distance by speed has remained somewhat constant .[3-4]

This new profile presented by soccer demands the use of new strategies. One of these is a nutritional strategy that aims to compensate for the higher metabolic rates during training and the game itself. Currently, ergogenic nutritional supplements are used to increase energy production and, consequently, to compensate for the metabolic needs of athletes in different sports.[5-8]

Athletes in long-duration types of sports (e.g., runners, triathletes and cyclists) have been benefiting from the use of carbohydrate and glutamine together. Glutamine is an intermediate metabolite in the Krebs cycle and thus acts in gluconeogenesis by saving phosphocreatine (CP) deposits and glycogen in muscle fibers, particularly type I (aerobic) fibers, thereby increasing the tolerance to exercise.[9-11] This is the most likely hypothesis for its influence on performance.

Moreover, peptide glutamine is scientifically considered to be an essential nutrient for conditioning, and in sports nutrition, it plays an important role in preventing athlete fatigue and overtraining syndrome. The benefits of its use, in terms of the immune response to athlete nutrition and nitrogen balance, are well established.[12] For example, athletes consuming beverages containing glutamine following rowing competitions and marathons (5g glutamine after the activity and two hours later) experienced fewer upper respiratory tract infections (URTI).[13-15]

However, in contrast to other types of sports, there is a lack of research on the use of glutamine in relation to soccer performance. Therefore, the aim of the present study was to investigate whether supplementation of carbohydrate together with peptide glutamine would increase exercise tolerance in soccer players.

MATERIALS AND METHODS

Nine top-level male soccer players from professional soccer teams in the state of São Paulo (age: 18.4 ± 1.1 years; body mass: 69.2 ± 4.6 kg; height: 175.5 ± 7.3 cm; and maximum oxygen consumption 57.7 ± 4.8 $ml{\cdot}kg^{-1}{\cdot}min^{-1}$) participated in the study. All of these athletes were training for approximately 10 hours per week and were taking part in official competitions in Brazil.

Before undergoing the tests, the players were given explanations about the assessment procedures, the study objectives, and the possible benefits and risks. They all signed a statement of consent in accordance with the requirements of the Institution's Ethics Committee (National Health Board Resolution No. 196/96), under CAPPESQ protocol No. 938/05. The subjects were permitted to withdraw at any time for any reason. The following inclusion criteria were used: 1) age between 17 and 20 years; 2) officially registered soccer players; and 3) laboratory test results (hemogram, cholesterol and fractions, triglycerides, total protein and fractions, urea, creatinine, uric acid and minerals) within the normal standard ranges.

Exercise capacity was tested on a motorized treadmill (ATL – 10.200, Inbramed Instruments, Porto Alegre, Brazil) using an incremental protocol and a fixed inclination of 3%. Under this protocol, the players remained at rest for two minutes, and then warmed up for three minutes at speeds of 4.8, 6.0 and 7.2 $km{\cdot}h^{-1}$ per minute. The test began at 8.4 $km{\cdot}h^{-1}$ with increments of 1.2 $km{\cdot}h^{-1}$ every two minutes until the subjects reached volitional fatigue.[16] Their subjective perception of their own effort was determined for each test period using Borg's 15-point linear scale for rating perceived exertion.[17] Moreover, verbal encouragement was used during the tests.[18]

Pulmonary ventilation (V_E), oxygen consumption (VO_2), carbon dioxide production (VCO_2) and respiratory exchange ratio (RER) were continuously monitored by means of a breath-by-breath system (MedGraphics CPX/D, St. Paul, MN, USA). This metabolic device measures expired airflow by means of a pneumotach connected to the mouthpiece. A sample line was connected to the pneumotach, from which air was continuously pumped to O_2 and CO_2 gas analyzers. Prior to testing, the pneumotach was calibrated with ten samples from a 3-L calibration syringe (5530, Hans Rudolph, USA). The gas analyzers were also calibrated before and after each test in relation to room air and medically certified calibration gases (11.9% and 20.9% O_2 and 5.12% CO_2, respectively), balanced with nitrogen (N_2)[19-20]. Heart rate (HR) was continuously recorded during exercise by means of electrocardiography (6.4, HeartWare Instruments, Belo Horizonte, Brazil). Maximal oxygen uptake was assessed when the following criteria were satisfied: a) no increase in VO_{2max} greater than 2.0 $ml{\cdot}kg^{-1}{\cdot}min^{-1}$ at maximum effort; b) maximal respiratory exchange ratio (RER) ≥1.10; c) maximal HR within 10 beats/min of the age-predicted maximum (208 – [age x 0.7]); and d) more than 18 on Borg's scale.[21-25] All the subjects evaluated satisfied at least two criteria.

To test the level of tolerance to intermittent exercise, the soccer players followed a specific protocol on the motorized treadmill (Table 1) that simulated movements during a game (walking, running, jogging and sprinting) on the field. Each five-phase battery of tests lasted 25 minutes. To verify the players' feelings of fatigue and their volitional exhaustion, Borg's scale was used.[13] The athletes served as their own controls, taking the test twice with an interval of one week between the tests.

The athletes took either carbohydrate with glutamine peptide (named CARBOGLUT) at a concentration of 50g of maltodextrin + 3.5g of glutamine peptide in 250 ml of water, or a single carbohydrate (named CARBO) at a con-

Table 1 – Design of the intermittent tolerance exercise protocol used by the soccer players.

ACTIVITY	Phase 1 ($km{\cdot}h^{-1}$)	Phase 2 ($km{\cdot}h^{-1}$)	Phase 3 ($km{\cdot}h^{-1}$)	Phase 4 ($km{\cdot}h^{-1}$)	Phase 5 ($km{\cdot}h^{-1}$)
Walking	3.0 – 1’	4.0 – 1’	3.0 – 1’	4.0 – 1’	5.0 – 1’
Running	12.0 –1’	13.0 –1’	12.0 –1’	13.0 –1’	15.0 –1’
Jogging	8.0 – 2’	9.0 – 2’	8.0 – 2’	9.0 – 2’	10.0 – 2’
Sprinting	18.0 – 1’	19.0 – 1’	18.0 – 1’	19.0 – 1’	19.0 – 1’

Note: A complete battery is composed of five phases with a covered distance equivalent to 4418 m.

centration of 50g of maltodextrin in 250 ml of water. These supplements were administered thirty minutes before beginning the test. Carbohydrate was used as the control because it is a commonly used supplement in soccer and its effect on athletes’ performance is well known. Thus, the aim was to investigate whether the addition of glutamine to a carbohydrate beverage could enhance the performance even further. The soccer players were randomized in a double-blind manner, i.e., none of the staff responsible for or involved in the experiment or the evaluation of the procedures, or the athletes themselves, had any knowledge of which solution had been consumed. These solutions were prepared by an independent evaluator and all had the same taste (grape).

Statistical Analysis

The data analysis was performed using SPSS software (Statistical Package for the Social Sciences) for Windows, version 11.5. The means on Borg’s scale for the CARBOGLUT and CARBO groups were compared. These means were then subjected to Wilcoxon’s test[26] to determine their statistical significance. To investigate the effect of the supplementation on both the distance covered and the duration of tolerance, the means for the players in the CARBOGLUT and CARBO groups were compared. Statistical significance was determined using the paired Student’s t test and the data are presented as means ± standard deviation (SD). For both tests, a significance level of 5% was set.

RESULTS

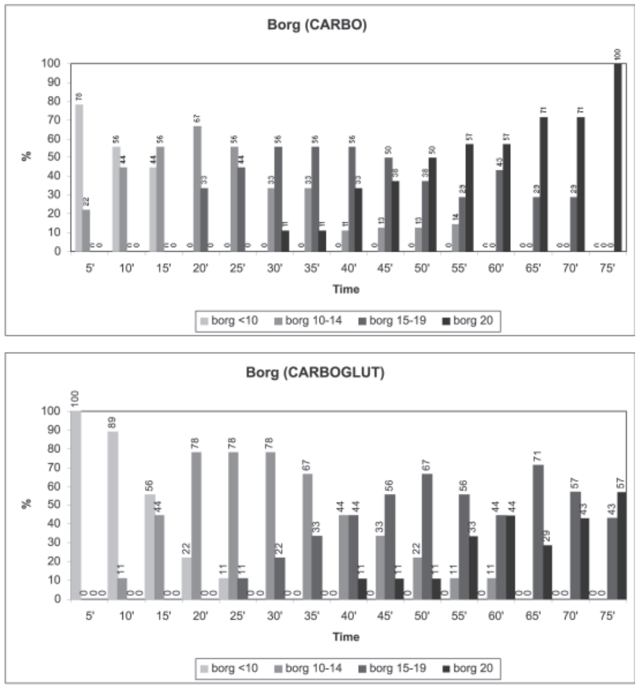
The means on Borg’s scale for the CARBOGLUT and CARBO groups were different. The CARBOGLUT group experienced less fatigue. This fatigue was significantly less when both the first and the second batteries were analyzed (p=0.047 and p=0.008, respectively), but the difference was not significant at the significance level of 0.05 when the third battery was considered (p=0.125) (Table 2). It should be noted that this low tendency towards feelings of fatigue in the CARBOGLUT group was observed during the entire test. Over the course of the test, the subjective perception of fatigue was measured at the end of each stage (after 5 minutes). Figures 1.A and 1.B present the distribution on Borg’s scale for the CARBOGLUT and CARBO groups at the end of each stage, and show a low tendency towards fatigue in the CARBOGLUT group. Figures 1.A and 1.B show that 65% of the athletes in the CARBOGLUT group had still not achieved a score of 15 on the Borg scale 30 minutes after the test (the mean time for the CARBOGLUT athletes to achieve a score of 15 on Borg’s scale - see Table 4); 55 minutes after the test (the mean time for the CARBO athletes to achieve a score of 20 on Borg’s scale – see Table 4), only 33% of the athletes in the CARBOGLUT group achieved a score of 20 on Borg’s scale, and 11% of them still presented a score on Borg’s scale that was lower than 10.

The mean distances covered by the CARBOGLUT and CARBO groups were different (Table 3). It should be noted that when the mean distances covered (maximum distance)

Table 2 – Perception of fatigue verified by the subjective Borg scale in three batteries of exercise in soccer players using carbohydrate (CARBO) and the mixture carbohydrate and peptide glutamine (CARBOGLUT).

	1st Battery		2nd Battery		3rd Battery	
	CARBO	CARBOGLUT	CARBO	CARBOGLUT	CARBO	CARBOGLUT
N	9	9	9	9	7**	9
Minimum	10	9	14	13	20	16
Maximum	18	15	20	20	20	20
Mean	14.00	12.33	18.67	16.56	20.00	19.11
*P (difference)	0.047		0.008		0.125	

* Wilcoxon’s test; ** Two GRII athletes did not tolerate up to third battery.



Figures 1A and 1B - Percentage of perceived effort distributions on the Borg scale during test utilizing supplementation with CARBO and CARBOGLUT.

in the tolerance test performed by athletes supplemented with CARBO (12750 ± 4037m) were compared with the same athletes supplemented with CARBOGLUT (15571 ± 4187m), the CARBOGLUT group covered a significantly greater distance (2822 ± 2771m; p=0.016). This was also noticeable when the distances covered up to a rating of 15 on Borg's scale, in which the distance covered by the CARBOGLUT group increased by 1973 ± 1471m (p=0.004). In addition, when the mean distances covered up to 20 on Borg's scale were measured, the CARBOGLUT group covered 3155 ± 1607m (p<0.001) more than the CARBO group did.

Table 4 presents a comparison between the results from the mean time elapsed for the players in the CARBOGLUT and CARBO groups. This shows that, on average, the players in the CARBOGLUT group persisted for significantly longer (15.6 ± 15.6 min; p=0.017) than the CARBO group players did. This also occurred when times to scores of 15 and 20 on Borg's scale were compared, in which the players in the CARBOGLUT group presented increases of 11.1 ± 8.2 min (p=0.004) and 17.2 ± 10.0 min (p=0.001), respectively.

Table 3 – Comparison of the distance covered in relation to the perception of fatigue verified by the subjective Borg scale at three moments of exercise in soccer players supplemented with carbohydrate alone (CARBO) and the mixture of carbohydrate and peptide glutamine (CARBOGLUT).

		Borg = 15 (tired)		Borg = 20 (maximum effort)		End of the test (maximum tolerance)	
		CARBO	CARBOGLUT	CARBO	CARBOGLUT	CARBO	CARBOGLUT
	N	9	9	9	9	9	9
	Mean	5516	7489	9261	12416	12750	15571
	SD	2341	2197	2862	3663	4037	4187
	IC 95%	(3716; 7316)	(5800; 9177)	(7061;11462)	(9601;15232)	(9647;15852)	(12353;18790)
Difference	N	9		9		9	
	Mean	1973		3155		2822	
	SD	1471		1607		2771	
	IC 95%	(842 ; 3104)		(1920 ; 4390)		(692 ; 4952)	
	P	0.004		< 0.001		0.016	

Table 4 – Comparison of the lapse of time to the perception of fatigue verified by the subjective Borg scale at three moments of exercise in relation to the soccer players with supplementation of carbohydrate (CARBO) and the mixture of carbohydrate and peptide glutamine (CARBOGLUT).

		Borg = 15 (tired)		Borg = 20 (maximum effort)		End of the test (maximum tolerance)	
		CARBO	CARBOGLUT	CARBO	CARBOGLUT	CARBO	CARBOGLUT
	N	9	9	9	9	9	9
	Mean	31.7	42.8	52.8	70.0	72.8	88.3
	SD	13.2	12.5	16.0	21.1	22.9	23.7
	CI 95%	(21.5; 41.8)	(33.2; 52.4)	(40.5; 65.1)	(53.8; 86.2)	(55.2; 90.4)	(70.1; 106.6)
Difference	N	9		9		9	
	Mean	11.1		17.2		15.6	
	SD	8.2		10.0		15.6	
	CI 95%	(4.8 ; 17.4)		(9.5 ; 24.9)		(3.6 ; 27.6)	
	P	0.004		0.001		0.017	

DISCUSSION

This study shows for the first time the effect of combined supplementation of carbohydrate and glutamine on soccer players' performances with regard to intermittent physical activity. As such, feelings of fatigue were measured using an intermittent effort protocol that simulated soccer players' activities. The measurements obtained were compared between the CARBOGLUT and CARBO groups using Borg's scale. The results obtained showed the substantial effect of the CARBOGLUT mixture on the soccer players' physical performance when compared with the use of CARBO alone.

The results presented in Table 2 show that the CARBOGLUT group presented a low tendency towards feelings of fatigue. This fatigue was significantly lower when both the first and second batteries were analyzed, but it was not significant at the level of 0.05 when the third battery was considered. However, the test on the third battery was probably compromised because of the small size of the sample, considering that not all of the players in the CARBO group were able to participate. Thus, a more conclusive test should be conducted with a larger number of athletes.

When the mean distances in the soccer players' tolerance tests were compared, the CARBOGLUT group covered a significantly greater distance than the CARBO group did. The players with CARBOGLUT supplementation covered, on average, 3155m more than did those who received CARBO alone. Recently, in a study of soccer players at a high competitive level in Europe, Di Salvo et al.[4] found that they covered a mean distance of 11 km by the end of a game. In the present study, the athletes receiving CARBO alone covered a mean distance of around 12.7 km, while those who received the CARBOGLUT mixture covered 15.5 km. This is 41% more than the distance covered in high-level matches that was determined by Di Salvo et al.[4] The same was also observed in relation to the duration of exercise tolerance following the ingestion of CARBOGLUT. Table 4 presents the results from comparing the mean distances covered by the players in the CARBOGLUT and CARBO groups, showing that, on average, the players in the CARBOGLUT group could endure significantly longer periods of exercise. On average, the maximum tolerance of the players in the CARBOGLUT group was 21% higher than the tolerance of players in the CARBO group.

There are few studies that associate the effect of glutamine with the performance of athletes because the mechanisms involved are still unclear. However, one interesting study[27] carried out among triathletes found that glutamine supplementation was efficiently absorbed and was not used for enterocyte proliferation, thus making it easier for the athletes receiving the supplementation to maintain their glycemic levels and ensuring greater exercise tolerance in these athletes. Additionally, in these athletes, a tendency towards a lower subjective perception of submaximal effort was observed among those who received glutamine and maltodextrin supplementation in comparison with those who received maltodextrin supplementation alone. Another remarkable finding was that there was higher maximum oxygen consumption (VO_{2max}) at the second ventilatory threshold (VT_2) (maximal respiratory compensation point) among the athletes who received supplementation. This indicated greater efficiency in this parameter for extracting O_2 for equal maximal aerobic power. It can therefore be said that the athletes who received glutamine and maltodextrin supplementation presented a higher aerobic capacity for submaximal effort.

Studies developed among cyclists and runners have also found greater tolerance to effort when these athletes were supplemented with peptide glutamine and carbohydrate. This is because glutamine is an intermediate metabolite of the Krebs cycle, and is thus thought to act in gluconeogenesis, thereby increasing the efficiency of this metabolic process.[9-10] Hence, a higher efficiency of gluconeogenesis for energy production by muscle glycogen is of fundamental importance for higher performance in sports.

A study in which muscle biopsies were taken to investigate the effects of CARBO ingestion with regard to phosphocreatine (PC) degradation in muscle fiber types I (aerobic) and II (anaerobic) during submaximal effort found that ingestion of CARBO significantly ($p<0.05$) accentuated the decline of PC concentration by 46% and 36% in type I and type II fibers, respectively.[11] These authors also observed a 56% reduction in the use of muscle glycogen in type I fibers, but not in type II fibers. They concluded that CARBO supplementation during exhaustive sprints accentuated the decline of ATP oxidative resynthesis of type I fibers, as indicated by the greater PC and muscle glycogen storage. In contrast, this response was not observed in muscle glycogen in relation to type I fibers, which may reflect different forms of recruitment.[28]

This response has important metabolic significance because in our study the combined supplementation of CARBOGLUT probably boosted the capability of the soccer players' muscles to use muscle glycogen more slowly. Through this effect, glycogen content would be saved for the more intense times of demand seen in soccer matches. Tsintzas et al.[11] showed that there was a slower CP reduction in type I muscle fibers (resistant to fatigue) and type II (nonresistant to fatigue), and also a slower reduction in glycogen in type I muscle fibers.

Soccer is a long-duration sport practiced with a variety of intensities of aerobic and anaerobic stimuli throughout the match, using both types of muscle fibers. For this reason, greater muscle glycogen storage would surely bring benefits in terms of better performance. The results from this study offer exciting prospects in the field of peptide glutamine utilization as an ergogenic aid for improving the performance of athletes who take part in continuous and intermittent activities of long duration.

In conclusion, the CARBOGLUT mixture was more efficient in increasing athletes' distance and duration of tolerance to intermittent exercise, and in lowering feelings of fatigue among the players when compared with the use of CARBO alone. Thus, supplementation with both carbohydrate and peptide glutamine improved the physical performance of these soccer players.

It is important to critically evaluate the results and the whole study. The present study has certain limitations that need to be taken into account when considering the study itself and its contributions to the field. Peptide glutamine has often been used for immunological support for immunosuppressed patients. Few scientific studies have used peptide glutamine as an ergogenic aid for improving athletic performance. In our study, we used this supplement in an acute manner with excellent results, but in a small sample. However, we do not know whether chronic use of this supplement would lead to the same results in our study population. Thus, we suggest that more research with other study designs should be carried out in order to compare whether other models of peptide glutamine use might be more efficient in increasing the levels of tolerance among soccer players. This field of research remains open, and some of the limitations of this current study may be seen as fruitful avenues for future research on this topic.

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