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Addition of Protein in Carbohydrate Supplementation Does not Improve Performance of Amateur Runners in Exercise above the Anaerobic Threshold

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Abstract There is no consensus in the literature that supports the inclusion of protein in the carbohydrate supplement in endurance exercise. The purpose of this study was to compare the physical performance of amateur runners under three different supplementation protocols: placebo (PLA), carbohydrate (CHO) and carbohydrate plus protein (CHO + PTN). Twelve amateur runners performed three exercise protocols on separate occasions consisting of 60 initial minutes with intensity referring to the Anaerobic Threshold (AT) and then 10% above the AT until exhaustion. Supplements (150 mL) were ingested 15 minutes before starting the activity and every 20 minutes until the first hour of exercise. Biochemical analyzes (blood glucose and lactate) and rating of perceived exertion (RPE) were measured before, during and after exercise protocols. Total caloric intake (Kcal) and macronutrients (g) were evaluated in the 24 hours preceding each exercise protocol. The time of exhaustion was higher for the CHO group when compared to the PLA group (24.6±13.6 vs. 15.2±8.9 minutes, p = 0.001) and the CHO + PTN group (24.6±13.6 vs. 18.6±8.4 minutes, p = 0.01). In general, glycemia was higher for the CHO and CHO + PTN groups when compared to the PLA group at all times whereas lactate, RPE and dietary assement did not show great differences. Our results suggest that, unlike supplementation with CHO alone, the addition of PTN in CHO supplements does not result in improved performance for the studied population and exercise intensity.

Keywords: *dietary supplements, running, anaerobic threshold, performance*

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1. Introduction

The importance of carbohydrate (CHO) for skeletal muscle to remain active during exercise is something that has been studied for a long time [1]. It has been increasingly elucidated in the literature that the longer the duration of endurance exercise, greater is the ergogenic effect resulting from supplementation with CHOs [2]. For exercises with a duration between 60 and 120 minutes, the supply of 30 to 60 grams per hour (g/h) of CHO in solution with a concentration ranging from 6 to 8%, may result in an improvement of physical performance due to a greater availability of blood glucose [3] sparing glycogen stores during activity [4], and increased oxidation of glucose by skeletal muscle [5].

Moreover, the addition of 1g of protein (PTN) to every 3 to 4g of CHO (1: 3-4 ratio) seems to increase athlete exhaustion time by virtue of attenuating central fatigue and improve the insulin response [6], thereby sparing endogenous carbohydrates [7] which resulted in a lower oxidation of CHO during exercise [8]. However, this aspect is not fully elucidated in the literature yet. Study with trained cyclists verified that those who received CHO+PTN supplementation in an interval activity characterized by 45 to 75% of the Maximum Oxygen Consumption (VO2Max) with 3 hours of duration obtained a longer time to exhaustion (TTE) in a subsequent activity to 85% VO2Max when compared to groups that received only CHO or placebo (PLA) [9]. In line of this, other studies also found an improvement in performance after the addition of PTN in the CHO supplement [10,11,12]. On the other hand, other authors [13,14,15] did not observe improvement in the performance of individuals who received CHO+PTN supplementation. Perhaps the intensity, duration, and type of exercise may favor the benefit of adding PTN to the CHO supplement [16], since the studies cited that resulted in improved performance after PTN addition were performed with very long exercise duration (>3 hours) and milder intensity (80 to 85% VO2Max) when compared to those who did not find benefits (approximate duration of 2 hours and intensities closer to VO2Max).

As much as running is one of the most popular types of physical activity in the world [17], there is a lack in the literature about supplementation protocols in races, since most of the results are from studies performed with cycling [18]. Until then, only two studies [13,14] with methodological design time trial, were proposed to analyze the effect of the addition of PTN to the CHO supplement in running and found no benefits. However, as seen in cycling surveys, there is no study analyzing such a strategy of supplementation in TTE of runners, since treadmill TTE may be a reliable tool to evaluate the human endurance capacity in trained individuals [19].

Although Pfeiffer et al. [20] found that exogenous CHO oxidation rates are similar when comparing both sports modalities, Capostagno and Bosch [21] verified a lower fat oxidation for cycling ate the same intensity of exercises while De Oliveira et al. [22] pointed out greater gastric discomfort for runners. Therefore, the main objective of the present study was to compare the exhaustion time of amateur running above the Anaerobic Threshold (AT) under three different supplementation protocols: PLA, CHO and CHO+PTN.

2. Materials and Methods

2.1. Participants

Twelve long-term male amateur runners aged 20-35 participated in the study. The characteristics of the subjects are listed in Table 1. All subjects practiced this sport modality for at least 4 years and had test time to complete 10 km between 30 and 40 minutes. Individuals who were injured throughout the study were excluded from the sample. All the subjects gave their written informed consent. The study was approved by Research Ethics Committee of the Clinical Hospital of Ribeirão Preto Medical School, University of São Paulo, SP, Brazil (CAAE: 15441113.0.0000.5440).

Table 1. Anthropometry, body composition and physiological variables of runners

Variable	Mean ± SD
Age (years)	27.7±4.8
Weight (Kg)	69.1±9.7
Height (m)	1.8±0.1
Body Mass Index (Kg.m^{-2})	22.1±1.7
Body Fat (%)	6.5±2.5
VO2Max (mL.Kg^{-1}.min^{-1})	56.8±3.0
Initial Speed (Km.h^{-1})	11.3±0.8
Final Speed (Km.h^{-1})	14.4±1.0

2.2. Experimental Design

After determination of the VO2Max and AT of everyone, all of them performed a physical exercise protocol following a double-blind cross over design under three different random supplementation interventions with a seven-day interval between each: 1. PLA: placebo consisting of artificial juice sweetened with aspartame, 2. CHO: solution with 8% carbohydrate and 3. CHO+PTN: solution with 6% of carbohydrate and 2% of protein.

The PLA was composed of non-caloric artificial juice (Clight™, Mondelez International, East Hanover, USA), while CHO was composed of maltodextrin (Maltodextrin™; Atlhetica Nutrition, Matão, Brazil) and CHO+PTN utilized hydrolyzed whey protein (Pepto Fuel Hidrowhey™; Performance Nutrition, Santo André, Brazil) in addition to the carbohydrate mentioned above in the 1:3 ratio.

The caloric value and the amount of macronutrient of each supplement used is showed in Table 2. All preparations had tangerine taste and were offered in dark bottle so that neither the athletes nor the researches knew what supplement in question would be. On test days, each individual ingested 150 mL of the supplement in four moments: 15 minutes before starting the exercise and 20, 40 and 60 minutes after the beginning of the activity.

Table 2. Energy and macronutrient content of the supplementation protocols

	PLA	CHO	CHO + PTN
Kilocalories (Kcal)	0	192	192
Carbohydrate (g)	0	48	36
Protein (g)	0	0	12
Fat (g)	0	0	0

The physical exercise was performed on a treadmill (Life Fitness™ model T91 club series; Life Fitness, Rosemont, USA) and constituted for 60 minutes at an initial speed rate of 4% inclination, corresponding intensity to AT (≈ 80% VO2Max). After this period, there was an increase in velocity for intensity corresponding to 10% (final speed) above the AT and the slope was maintained. The exhaustion occurred at the end of tests when the participants could no longer maintain the required speed, and TTE was timed for the different supplementation protocols. The runners arrived at the place of fasting and made a standard breakfast 90 minutes before the beginning of the tests, composed of maltodextrin solution with hydrolyzed whey protein, respecting the recommendations (1g CHO/Kg and 0,25g PTN/Kg) proposes by the guidelines [23], and started the exercise always at the same time, 8:00 AM.

It is worth mentioning that the tests took place in July, so that all athletes were already in the middle of the season, thus avoiding any possible improvement in the performance if they were at the beginning of the season.

2.3 Cardiorespiratory Assessment

Before initiating exercise protocols, subjects reported to the laboratory to determine VO2Max and AT. The dynamic physical exercise test was performed on a treadmill (Master™; Inbramed, Porto Alegre, Brazil) with incremental power ramp type protocol. After verbal command from researchers, the volunteer started the effort with a velocity of 5 km.h-1 and 0% of slope. The subjects did not receive any information about the instant that the ramp power elevation started. The velocity and slope of the treadmill were increased every minute (1 km.h^{-1} and 0.5%, respectively) until the moment of physical exhaustion. The average duration of the effort was between 8 and 12 minutes. During the procedure, the exhaled air was analyzed to verify the values of oxygen uptake (VO2) and carbon dioxide production through an metabolic analyzer (Ultima CPX™; MedGraphics, Saint Paul, USA) using Breeze Suite of the same company. From this test, the velocity (Km.h^{-1}) and slope (%) were obtained in three different intensity levels: AT (initial speed) that was determined at the point where there was loss of linearity of the production of carbon dioxide production (VCO2) in relation to VO2, 10% above the AT (final speed) and VO2Max which was calculated as the mean of the VO2 values of the last 30 seconds of effort.

2.4 Anthropometric Evaluation

Individuals were weighed in a digital scale (Premium BK50-FA™, Balmak, Santa Bárbara do Oeste, Brazil). Height was assessed with a stadiometer (Standard™; Sanny, São Bernardo do Campo, Brazil) according to the method recommended in the literature [24]. For the body composition evaluation, the body density was previously determined according to the method of seven skinfolds proposed by Jackson and Pollock [25] using a skinfold caliper (Lange™; Beta Thecnology, Tullytown, USA) and later the percentage of body fat a was determinate according to the equation proposed by Siri [26].

2.5 Biochemical Analysis

For analysis of blood glucose (mmol.L^{-1}) and blood lactate (mmol.L^{-1}), 2 µl of blood was collected from the distal phalanx of the right index finger at four different times: T0 (before starting physical exercise), T2 (60 minutes after exercise), T3 (at the time of exhaustion) and T4 (5 minutes after exhaustion). A portable glycosometer (Accu-Chek Active™, Roche, Basel, Switzerland) was used to measure glucose, whereas a portable lactometer (Accutrend Plus™, Roche, Basel, Switzerland) was used for lactate. The two devices have already been validated in previous studies [27, 28].

2.6 Rating of Perceived Exertion

In order to evaluate the Rating of Perceived Exertion (RPE), the scale proposed by Borg [29] (values from 6 to 20) was used at three different moments: T1 (30 minutes after onset of exercise), T2 and T3.

2.7 Diet and Exercise

All subjects were instructed to maintain the diet and routine of workouts in addition to repeat the breakfast performed on the first exercise protocol for the remaining days. The dietary intake was evaluated by 24-hour recall performed on the day before the start of each physical test. A specific program (Dietpro 5.1™, Viçosa, Brazil) was used to calculate the amount of total caloric (Kcal) and macronutrients intake. In addition, runners were asked not to exercise in the days prior to the test days.

2.8 Statistical Analysis

Data are showed as mean±standard deviation. To evaluate the normality of the data distribution, the Shapiro-Wilk test was used. Analysis of variance (ANOVA) of repeated measures with Bonferroni post hoc was used to verify inter and intragroup differences. The significance level adopted was $p \leq 0.05$, and all analyzes were performed in the Statistical Package for Social Science (SPSS) version 20.0 (SPSS Inc, Chicago, USA).

3. Results

3.1. Time to Exhaustion

The time to exhaustion in the different supplementation protocols is showed in Figure 1. The TTE on CHO protocol was significantly higher when compared to PLA (24.6±13.6 vs. 15.2±8.9 minutes, p=0.001, denoted by *) and with CHO+PTN (24.6±13.6 vs. 18.6±8.4 minutes, p=0.01, denoted by †). However, no significant difference was observed when comparing CHO+PTN and PLA (18.6±8.4 vs. 15.2±8.9 minutes, p = 0.064).

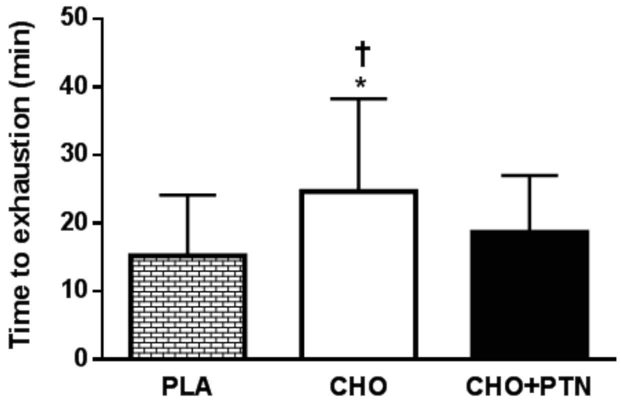

Figure 1. Time to exhaustion according to placebo (PLA), carbohydrate (CHO) and carbohydrate plus protein (CHO + PTN)

3.2. Blood Analyses and Rating of Perceived Exertion

The glycemia (mmol.L^{-1}) of the CHO group at the time T0, T2, T3 and T4 (6.4±0.9, 5.5±0.4, 6.1±1.1 and 6.9±0.7) was significantly higher (p=0.02, denoted by *) when compared to the PLA group (5.6±0.7, 4.6±0.6, 5.6±1.0 and 5.9±0.7), except for T3 (p=0.13) and for the CHO+PTN (6.7±0.8, 5.6±1.0, 6.5±1.2 and 7.1±1.4) group when compared to the PLA group at all times (p=0.01, denoted by †). There was no statistical difference at any time when comparing the CHO and CHO+PTN groups (Figure 2).

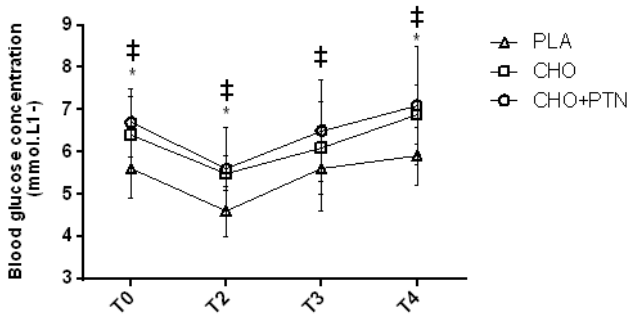

Figure 2. Blood glucose concentration at different moments of exercise

Blood lactate (mmol.L^{-1}) did not present a significant difference at any point of the study when comparing the three supplementation protocols (p=0.79). On the other hand, the RPE was significantly lower for the CHO group when compared to the PLA and CHO+PTN groups (p=0.005) only at time T2 (Table 3).

Table 3. Variation lactate and rating of perceived exertion during exercise protocols

		PLA	CHO	CHO + PTN
Lactate (mmol.L^{-1})	T0	2.3±0.6	2.5±0.7	2.8±0.9
	T2	2.9±2.3	2.3±0.9	2.6±1.5
	T3	6.9±3.2	7.4±3.0	6.9±2.5
	T4	5.1±1.6	4.9±2.4	4.8±2.1
Perceived Exertion	T1	12±1	12±2	12±2
	T2	13±1	12±1[a,b]	13±1
	T3	19±1	19±1	19±1

[a] CHO significantly different compared to PLA; [b] CHO significantly different compared to CHO+PTN.

3.3. Dietary Evaluation

Total caloric and macronutrients intake did not present statistical difference for the different supplementation protocols (p=0.57) (Table 4).

Table 4. Caloric and macronutrient intake in the 24 hours prior to exercise protocols in the different groups

	PLA	CHO	CHO + PTN
Energy (Kcal)	2923.5±512.4	2874.3±441.8	2784.8±683.4
Carbohydrate (g)	439.3±78.2	401.9±102.3	409.4±150.5
Protein (g)	105.42±39.4	122.0±48.7	105.6±41.5
Fat (g)	81.4±24.6	79.4±22.2	81.2±12.9

4. Discussion

The literature lacks studies involving protocol of supplementation with running [18]. Our findings corroborate in part with other researches with runners [13,14] and cyclists [15,30] who also found no advantages in adding PTN to the CHO supplement. Colleta et al. [13] observed similar results in the performance of recreational athletes who, after running 18 km under controlled intensity, covered 1.92 km in the shortest possible time, receiving supplements containing CHO or CHO + PTN before and during exercise. Likewise, Van Essen and Gibala [15] observed no difference in time that cyclists took to travel 80 km when receiving CHO (6%) or CHO+PTN (6 and 2%, respectively) solutions during the exercise. In a more recent study [30], the authors also did not found improvements in the performance of cyclists (40 km and 10 km time trial) when adding PTN in supplement with multiple CHO sources (dextrose, fructose and maltodextrin).

On the other hand, our research found improvement in the TTE of runners who received CHO only during the exercise protocol. Similarly, Gui et al. [14] found that eleven female recreational endurance improved performance in 21 Km time-trial running when comparing intake 36 grams of CHO per hour versus PLA. Such improvement was not sustained at a time when participants received CHO+PTN.

As we did not analyze substrate oxidation during exercise, here is a limitation of our study, we speculate that this may have happened because the CHO group received a carbohydrate amount (36g/hour) which is already seen as ergogenic for activities lasting between 1 and 2 hours [2,23], while the CHO+PTN group received lower than that recommended for the same nutrient (27g/hour). In addition, we cannot disregard a possible gastric discomfort caused by protein intake during the activity that could impair physical performance [31]. Among the twelve participants in our study, three reported gastrointestinal symptoms during CHO+PTN intervention that, according De Oliveira et al. [22], these disadvantages may affect sports performance.

It is interesting to note that the differences among the results may be due to the different methodologies addressed. Some authors proposed an initial exercise protocol with a much longer time (≥ 120 minutes) and an intensity for the athletes to reach exhaustion at the end of exercise much lower (80 to 85% VO2Max) [9,11,12] when compared to the present study (60 minutes and 90% VO2Max, respectively). Another study [10] that also showed a positive effect on protein addition used this intervention in a chronic way (for 8 days) with ad libitum intake of the supplement making it difficult to compare with our research. Possible explanations for this fact are that prolonged exercises require a greater contribution of PTN as an energy source [32] whereas activities with intensities above AT use predominantly CHO to produce ATP through the oxidation of glucose by glycolytic pathway [33].

When we analyzed the blood glucose response against different supplementation protocols, it was expected that the acute concentration of this nutrient in the blood would increase after ingestion of any solution containing carbohydrate. The maintenance and/or increase of glycemia from CHO supplementation is essential to prevent episodes of hypoglycemia and to preserve glycogen during physical exercise [2]. However, at time T3, there was no significant difference of glycemia between the PLA and CHO groups. This can be explained by the fact that the CHO group had a higher TTE and it may cause a decrease in glycemia close to the PLA group. Somewhat consolidated in the literature for a long time is that the strenuous exercise favors a greater oxidation of glucose by the skeletal muscles in activity [34].

Regardless of the supplement used, blood lactate concentration increased with the advancement of exercise duration, as occurred in other studies [35,36]. However, we did not find statistical differences among supplements used. Considering that the final part of the exercise was above the AT, this result was expected since this intensity is characterized by predominance of anaerobic metabolism for energy production [37]. Moreover, all lactate concentrations at time T3 are greater than 4 mmol.L^{-1}, a value that is used as AT by Onset of Blood Lactate Accumulation (OBLA) [38]. Indeed, the decrease in lactate concentration 5 minutes after the end of the activity is mediated by the monocarboxylate transporters (MCTs), since these proteins take this molecule to the liver to be excreted and/or transformed into energy by gluconeogenesis [39].

All subjects reported an increased on RPE with advancing of physical exercise duration and/or intensity. These findings corroborate with other studies [11, 40] and can be explained by the high correlation that RPE has with other indicators of exercise intensity, such as lactate [41]. At time T2, the CHO group reported less fatigue when compared to the other two groups. This may have occurred because CHO supplementation contributed to a possible lower glycogen depletion at this stage of exercise [42] or even because this nutrient activates brain areas related to motor control and reward that would make them feel less fatigued [43].

There was no difference on daily caloric intake among the types of intervention. It is known that carbohydrate intake in large quantities (8-12 g/Kg) in the days prior to physical exercise may increase glycogen storage due to carbohydrate loading process that could result in improved performance [44,45]. However, none of the groups (PLA, CHO or CHO+PTN) had a carbohydrate intake equal to or greater than this recommendation (6.2±1.7, 6.0±1.9 and 5.8±2.4 g/Kg, respectively).

5. Conclusions

In summary, the present study found that, although isocaloric supplements (CHO and CHO+PTN) favor increased blood glucose circulation before, during and after exercise when compared to placebo, only the supplementation of CHO increased the time of exhaustion of amateur runners with intensity above the anaerobic threshold. Therefore, adding PTN to the CHO supplement appears to result in no ergogenic advantage for the population and exercise intensity studied.

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