Original Article

# Carbohydrates plus protein reduces oxidative stress after single bout of aerobic exercise

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**ABSTRACT**

The aim of this study was to investigate the effect of CPA in muscle damage and oxidative stress induced by aerobic exercise. Participate in the study ten healthy young (24 ± 4 years), eutrophic (23.2 ± 1 kg/m2),VO2max = 44.9 ± 10 ml/kg/min, four women performed three aerobic exercise sessions lasting 50 minutes randomly supplemented with water (WAT), isolated carbohydrate (CHO) or carbohydrate associated with proteins and antioxidants (CPA) every 10 minutes of exercise. Blood samples were taken before, immediately and 24 hours after each exercise session for analysis markers of muscle damage creatine kinase (CK) and oxidative stress malondialdehyde (MDA). Blood glucose was measured before, during and after the exercise. After testing the data for normality and homogeneity through the Shapiro-Wilk and Levine tests, one-way ANOVA or two-way analyses were made to compare the initial and the answers to the experimental procedure respectively, or their corresponding non-parametric. CHO and CPA resulted in maintaining or increasing glucose, respectively, during exercise, whereas WAT resulted in glycemia reduction. CHO or CPA did not affect CK post exercise concentration. MDA values were very similar immediately after exercise between CHO and CPA, however occurred significant reduction from post exercice to 24 hours after exercise in CPA procedure (4.8 ± 1.8 to 2.5 ± 0.8, p <0,05), while CHO (5.1 ± 0.8 to 4.6 ± 0.9) and WAT (4.9 ± 0.9 to 5.1 ± 0.6) did not promotes the same phenomenon. This study revealed that carbohydrates associated

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with proteins and antioxidants have ergogenic effect by increasing blood glucose during a single bout of aerobic exercise and accelerate the restoration of oxidative stress. **Key words:** CARBOHYDRATES, PROTEINS, ANTIOXIDANTS, AEROBIC EXERCISE, OXIDATIVE STRESS.

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## INTRODUCTION

Despite the efficacy of supplementation be something controversial in the literature, the isolated carbohydrate supplementation can be a consensus among researchers to delay fatigue in prolonged exercise (Tsintzas e Williams, 1998; Patterson e Gray, 2007) and accelerates muscle glycogen resynthesis after training (Betts e Williams, 2010; Jensen et al., 2011). Therefore, supplementation of carbohydrates is widely used both athletes and recreational practicing subjects of the various modalities of exercise. Whereas the most studies demonstrated delaying fatigue and post exercise recovery with cyclic exercise modalities protocols (running, cycling, swimming), these supplements are widely used in gyms for practitioners who use methods of cyclic aerobic exercises.

The concept of carbohydrates has been enhanced with the combination of other nutrients, and then developed by manufacturers of carbohydrate supplement enriched with proteins is already a reality in the sporting environment. This supplementation has been extensively researched and its benefits have shown an improvement in optimizing the recovery of muscle glycogen (Betts et al., 2005; Berardi et al., 2006), reduction in muscle damage markers (Skillen et al., 2008; Saunders et al., 2009), and protein balance (Koopman et al., 2009). Subsequently, it was enriched with antioxidants, promoting improvements in the response of muscle damage markers (Romano-Ely et al., 2006; Luden et al., 2007).

Despite of the presence of antioxidants and of the fact that this preparation is available in commercial products, has not yet clear whether this preparation influences the acute oxidative activity, normally induced by aerobic exercise sessions, although studies have already shown that supplementation with high doses of antioxidants isolates (without the presence of carbohydrates) reduce the oxidative stress induced by this exercise modality (Nakhostin-Roohi et al., 2008; Taghiyar et al., 2013). Among the most used as sports supplements antioxidants are vitamins C and E which are recognized as non-enzymatic or exogenous antioxidants agents by reacting with reactive oxygen species, thereby preventing the reaction of these species with the lipid layers of cells and the consequent deleterious oxidative effect (McGinley et al., 2009; Taghiyar et al., 2013).

Despite of the antioxidant action of the vitamins, available commercial carbohydrates products are enriched more with proteins than antioxidants. In fact, these commercial products frequently contain three parts of carbohydrate for one part of protein. When are enriched with antioxidants, these substances are in low concentrations.

Given this perspective, this study was conducted to verify if a commercial compound of carbohydrate enriched with proteins and antioxidants in low concentration may minimize muscle damage and oxidative stress induced by an aerobic exercise session.

## MATERIALS AND METHODS

### *Participants*

The study was conducted with ten healthy young, four women, mean age of 24 ± 4 years, goers gyms. They could do any type of exercise, but should necessarily perform aerobic exercise regularly for at least three months and perform at least 50 minutes in each session. Were excluded of the study the volunteers who not involved in all experimental procedures or presented gastrointestinal discomfort when tested supplement.

***Procedures***
The project previously approved by ethics committee on human research of the Hospital Lauro Wanderley (University Federal of Paraíba), by Protocol 690/10. All of subjects who agreed to participate the study signed the free consent term according to Resolution 196/96 of the National Health Council.

***Design of the study***
All volunteers performed three sessions of aerobic exercise in cycle ergometer in different days and separate by at least 48 hours between sessions. They consumed carbohydrate alone (CHO), carbohydrate enriched with proteins and antioxidants (CPA) and water (WAT) every 10 minutes exercise being one session for each nutritional experiment. Blood samples were taken before, immediately after exercises and within 24 hours after each exercise session for analysis of muscle damage markers creatine kinase (CK) and malondialdehyde oxidative stress (MDA). Glycemia was measured before, during exercise and the end of each session.

***Preparation of the Subjects***
Were instructed to suspend the exercise 72 hours before the start of the protocols and do not perform exercise between experimental procedures Additionally, it was requested abstinence from alcohol consumption, nutritional supplements and food sources of antioxidant vitamins in this period. Dietary intake was assessed using the 24-hour food recordatory to verify the habitual intake of foods rich in antioxidants (Gibson, 1990). They were instructed to make the last meal of the day before the experimental procedures between 07:00 PM and 10:00 PM.

***Supplementation***
The experimental procedures were performed in the first half of the morning. The subjects performed a standardized breakfasting (sandwich with cheese and fruit juice 200ml) containing 55,9 g of carbohydrates, 14,3 g proteins, 9,4 g fat and 365,5 calories 30 minutes before each workout.

To experimental nutritional procedures, CHO supplementation was made through the maltodextrin compound (Atletica®, São Paulo - Brazil). CPA supplementation was made through of the commercial product Endurox (Pacific Health®, São Paulo - Brazil). The two products were isocaloric, but CHO consisted solely of carbohydrates, while CPA consisted of four pairs of carbohydrates to one-part protein. Carbohydrate product only contained 281 kcal and 73 g of carbohydrate. Meanwhile, the carbohydrate enriched with proteins contained 280 Kcal, 53 g of carbohydrate, 13 g of proteins, 1 g of total fats, 0.5 g of saturated fats and 10 mg of cholesterol. Additionally, this product contained the minerals sodium (180 mg), potassium (100 mg), calcium (122 mg) and magnesium (240 mg). The enrichment to antioxidants was made by addiction of vitamin C (45 mg) and vitamin E (10 mg).

The supplements were diluted in water to a concentration of 6 to 8%. To ensure that the powder of the two compounds were well diluted, this process was done using a blender, initially placing one part of water, the powder above this water and a further portion of water and powered blender. Then the diluted product was transported to containers, ensuring that parts of the product had not been fixed in the blender jar. After this process, the supplements were chilled between 4° C and 8° C until the time of use. The dilution was always done on the same day of each procedure. The volunteers were supplemented with 200 ml of solution every 10 minutes of exercise. In the control session the volunteers ingested the same volumes of water at the same moments of the exercise.

### *Exercise protocol*

After warming up lasting five minutes the volunteers exercised in a cycle ergometer for 50 minutes at 65% of maximum heart rate, obtained through the protocol proposed by Karvonen et al. (1957). Whereas for this aerobic exercise prescription protocol is required heart rate at rest, this variable was obtained in the first time after the arrival of the volunteers at the site of experiments, without any physical activity has been performed previously on this day. For this, they were seated in a quiet and peaceful environment, with temperature between 22° C and 26° C for 10 minutes and instrumented with a heart rate monitor. After these 10 minutes, the heart rate was monitored for over five minutes, registering the lowest value obtained in this period. Heart rate was monitored through heart rate monitor (Training Fitness, Geratherm - model 552). During exercise, the volunteers were informed about heart rate every five. If the heart rate was not within the prescribed zone for training, the volunteers were instructed to increase the frequency of pedaling or the researchers increased the tension of the pedal.

### *Analysis*

Before, immediately after and 24 hours post exercises procedures, ten milliliters of blood were collected from antecubital vein by suitably experienced nurse. For purposes CK measures of blood volume was added in dry tubes (no anticoagulant). To measure MDA another portion of the blood was added in tubes containing anticoagulant EDTA. Blood samples without anticoagulant were centrifuged 20 minutes after collection. Blood sample containing anticoagulant was centrifuged immediately after collection. Both samples were centrifuged at 3000 rpm for 15 minutes, then refrigerated at -20 ° C until analysis.

To CK analysis was used a specific commercial kit (Labtest, Lagoa Santa-MG, Brazil). A volume of 20 ml of sample was added to 1 ml of work reagent, following the manufacturer's recommendations. The reading was performed in a spectrophotometer (Biospectro, SP-220 model / Brazil) at a wavelength of 340nm.

MDA was analyzed to access oxidative stress through lipid peroxidation by thiobarbituric acid reaction (TBARS) with hydroperoxide decomposition products, according to the method described by Ohkawa et al. (1979). For this purpose, 250 µl sample was incubated in a water bath at 37 ° C for 60 minutes. Then, the sample was precipitated with perchloric acid 35% and centrifuged at 14,000 rpm for 20 minutes at 4°C. The supernatant was transferred to microtubes and added 400µl of 0.6% thiobarbituric acid and incubated at 100 C° for 60 minutes. After cooling down, the material was read in a spectrophotometer at a wavelength of 532nm.

For glucose analysis, were collected drops of arterial blood of the digital lobe at rest situation (before heating) during exercise (after 25 minutes) and at the end of the exercise. Glycaemia was assessed by enzymatic method with reagent strips, using a portable glucometer (Roche Advantage, São Paulo, Brazil).

### *Statistical analysis*

Data are expressed as mean and standard deviation. After testing the data for normality and homogeneity through the Shapiro-Wilk and Levine tests, one-way ANOVA or two-way analyses were made to compare the initial and the answers to the experimental procedure respectively, or their corresponding non-parametric. Analyses were performed using Instat 3.0 software (GraphPad, San Diego, CA, USA), a significance of $p < 0.05$.

## RESULTS

The characteristics of the participants are shown in Table I. They were young adults, eutrophic and with good aerobic capacity for non-athletes adult standards, according to the American College Sports American College of Sports Medicine (2003). Pre-experimental values were similar to glucose and CK at three sessions of aerobic exercise, although it has been encountered a tendency for higher baseline value in CHO procedure in relation to CPA ($p < 0.051$). For the MDA, subjects began the experiments with significantly higher values in the CPA procedure over the other.

Table 1. Anthropometric and physiological characteristics and baseline conditions to each procedure.

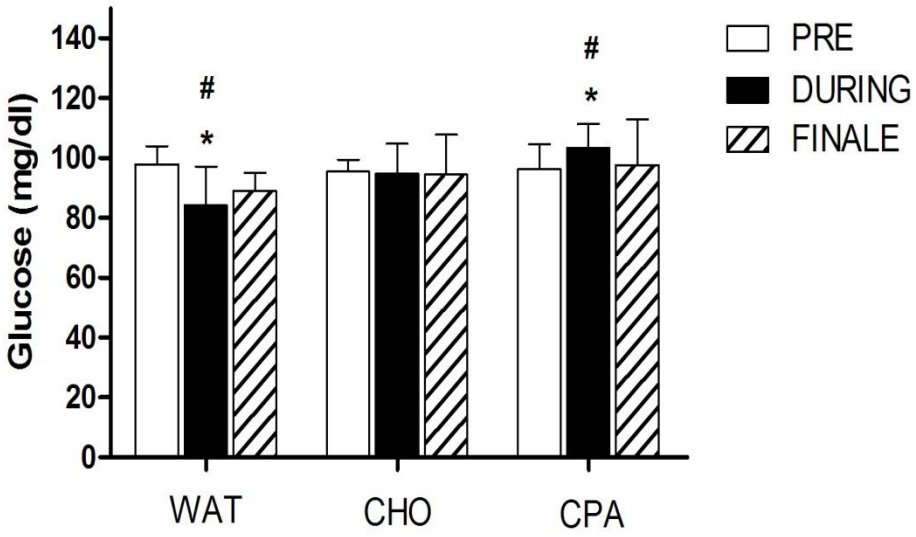
| Variables | | | |
|---|---|---|---|
| Age (years) | | | 24±4 |
| Height (cm) | | | 177,6±17 |
| BMI (kg/m²) | | | 23,2±1,2 |
| Peak VO2 (ml/kg/min) | | | 44,9±10 |
| | WAT | CHO | CPA |
| Glucose (mg/dl) | 97,9±6 | 95,4±4 | 96,3±8 |
| CK(U/mL) | 290,7±183 | 454,14±201 | 256,5±165 |
| MDA (µM) | 3,3 ± 0,8 | 3,4 ±0,8 | 5,0 ± 1,6* |

*Data are mean and standard deviation. BMI = body mass index; WAT = water; CHO = carbohydrate; CPA = carbohydrate enriched with protein and antioxidants. * Indicates difference compared to other procedures (one-way ANOVA).*

In the procedure with water ingestion occurred a significant reduction in blood glucose compared to baseline during exercise, with the values having returned to initial conditions at end of the workout (Figure 1). Meantime, blood glucose remained unchanged during CHO exercise and increased significantly during the exercise with CPA ingestion, being statistically higher than the CHO procedure, and returning to baseline values at end of the workout.

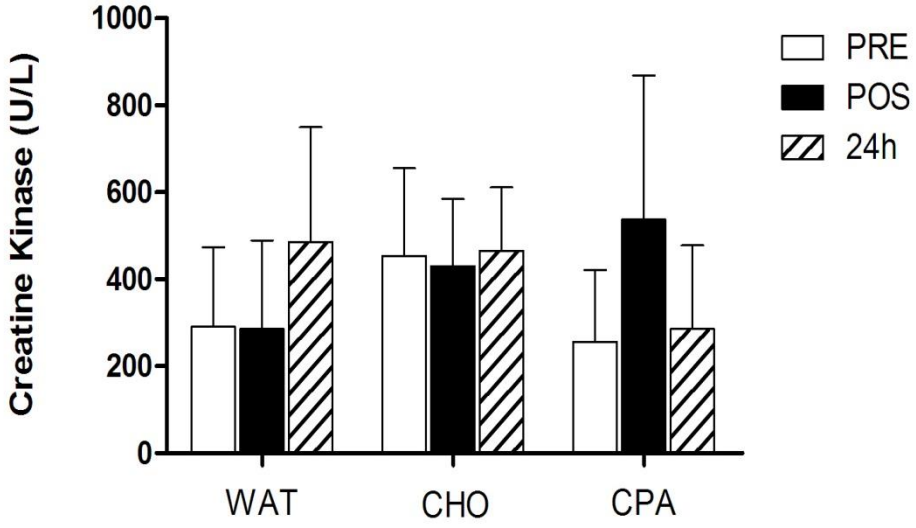
The procedures with CHO or CPA did not affect muscle damage after or 24 hours post exercise, as can be visualized in Figure 2. Meanwhile, in WAT or CHO procedures, the exercise session resulted in a significant increase of 48.5% and 50% respectively in the concentration of lipid peroxidation marker MDA considering the measure performed immediately after exercise (Figure 3). Conversely, this increase was not observed in CPA procedure, although it should be considered that in this procedure the volunteers started the experiment with high levels compared to WAT and CHO.

Despite differences to MDA response between CPA to CHO and WAT procedures, immediately post exercise values were very similar to three experimental conditions. However, only CPA procedure resulted in significant decrease in MDA value of 48% 24 hours after the exercise session while CHO (9,8%) and WAT (4%) reduction was discrete and non-significant. In addition, 24 post exercise MDA in the CPA procedure was significantly lower compared to CHO and WAT.




*WAT=water; CHO=carbohydrate; CPA= carbohydrates enriched protein and antioxidants. #= difference intra-group between pre, during and after exercise. *= difference compared to other procedures at the same time (ANOVA two-way).*

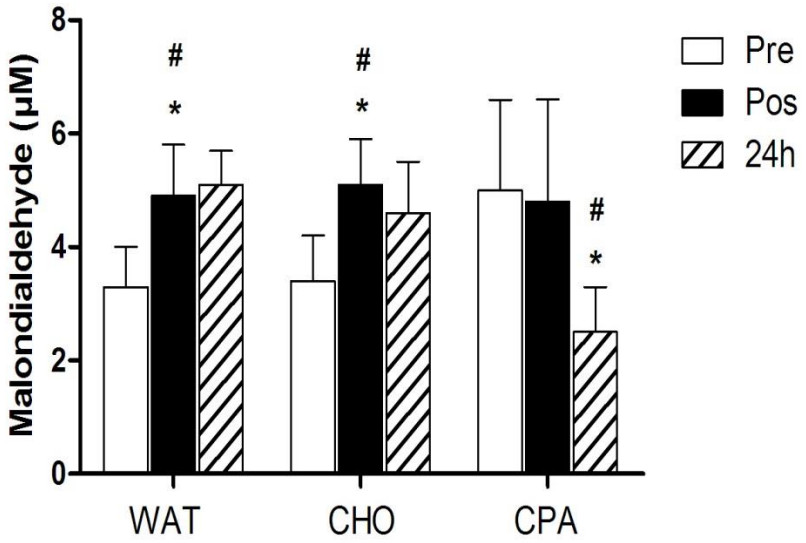
Figure 1. Blood Glucose in pre, during and at the end of the aerobic exercise sessions with the consumption of water, carbohydrate and carbohydrate plus proteins and antioxidants.




*WAT = water; CHO = carbohydrate; CPA = protein enriched carbohydrates and antioxidants. Whereas two-way ANOVA.*

Figure 2. CK behavior us moment before, after and 24 hours after the end of the aerobic exercise protocol with the consumption of water supplementation, CHO and CPA.




*WAT = water; CHO = carbohydrate; CPA = carbohydrate enriched with protein and antioxidants. # Indicates difference between pre, post and 24 hours after intra-group exercise. * Indicates difference compared to other procedures for the same time of the measurement. Whereas two-way ANOVA.*

Figure 3. MDA behavior pre, post and 24 hours at the end of the aerobic exercise protocol with the consumption of water supplementation, CHO and CPA.

## DISCUSSION

Data from this study have shown that supplementation of CHO or CPA was able prevent the fall blood glucose levels during aerobic exercise. Only carbohydrates enriched proteins and antioxidants prevented the increase in oxidative stress induced by exercise session and yet restored the activity of this enzyme to levels similar to baseline within 24 hours after exercise. This phenomenon could be observed even considering that the procedures were not able to provide protection against muscle damage assessed indirectly by serum concentration of a typical enzyme muscle cell.

The glycemic response of our study corroborates with previous studies in which carbohydrate supplementation singly (Campbell et al., 2008; Foskett et al., 2008) or protein enriched (Alghannam et al., 2011) resulted in elevated blood glucose levels during exercise. This difference in glycemic responses after supplementation with CHO or CPA compared to WAT confirms the success of the study supplementation protocol. The benefits of this greater glycemic activity have been demonstrated by retarding fatigue with isolated (Patterson et al., 2007; Foskett et al., 2008) or enriched carbohydrates (Valentine et al., 2008; Alghannam et al., 2011), and faster post exercise glycogen resynthesis (Jentjens et al., 2003; Berardi et al., 2006) which are potential ergogenic effect to aerobic exercise practitioners considering that one of the major problems of this exercise modalities is the slow recovery of muscle glycogen, which takes 24-48 hours (Burke et al., 2011).

In our study, there was only one blood glucose measurement during exercise (assuming that the other measures were taken before and at the end of the session). Obviously, this data would be limited to establish

consistent information about glycemic effect of carbohydrate supplementation. However, this measure did more to confirm the success of the procedure than to set the ergogenic effect of supplementation in glycemic point of view, precisely because this ergogenic effect is already unequivocally demonstrated in previous studies.

Whereas these ergogenic effects are already well established, the most important issue of this study was to assess other possible ergogenic effects to carbohydrates enriched with proteins and antioxidants, which were muscle protection during exercise and the antioxidant effect. The lack of protection of the CPA or CHO supplementation on muscle damage found in our study corroborates with previous data of the Baty et al. (2007) and Cermak et al. (2009) who found that supplementation CHO + PRO, before and after the session exercise did not alter muscle damage induced by exercise. Additional information brought by our study is that the addition of antioxidants does not confer protection against muscle damage induced by exercise. However, it should be noted that small concentration of antioxidants in commercial product we tested does not completely discard any effect of these nutrients.

Another consideration that must be made is of methodological characteristic. The fact that the subjects were physically active and adapted to exercise may explain the fact that the exercise protocol did not promote increase in serum CK, an indirect indicator of muscle damage induced by exercise, as proposed Cermak et al. (2009) and Serrano et al. (2010). Evangelista et al. (2011) suggests that even after intense resistance exercise training no increase in serum CK activity in individuals previously trained occur. Thus, not having been increased activity of this enzyme, it can be understood that supplementation with carbohydrates isolated or plus proteins and antioxidants do not influences muscle damage in previously active subjects.

Although supplementation does not appear to result in muscle damage protection, data from this study showed that carbohydrates enriched protein and antioxidants have the ability to restore the oxidative stress induced by an exercise session. However, it should be noted that the compound used in this study was a commercial product containing vitamins C and E, which are known to be important antioxidants agents (Nakhostin-Roohi et al., 2008), however, the concentration of these vitamins in this product is much lower than dose that has been used in the studies that demonstrate the effectiveness of the antioxidant vitamins (over 200mg and 300 mg of vitamin C and E respectively). Therefore, data from this study allow us to consider the possibility that enriched carbohydrate protein solutions can generate a new ergogenic effect for these products even with only a slight addition of antioxidant vitamins, which is the main finding of the present study.

The low concentration of antioxidant may suggest that the effect of reducing oxidative stress may have been generated more by protein than for vitamins C and E, although this latter two substances are those which have the capacity to fight lipid peroxidation. Therefore, our data raise the need for replication of our methodological procedure with added control situations with carbohydrate enriched with antioxidants alone or only with proteins.

This study's main limitation is the fact that the sample size was small. Another aspect that can be improved is to conduct experiments that may also indicate activity of the enzymatic antioxidant system. The variable MDA evaluates the deleterious effects of oxidative stress through peroxidation that reactive oxygen species causes in cell membranes. However, it is reasonable to investigate whether antioxidant vitamins act only preventing peroxidation or also stimulate the enzymatic antioxidant defense system. This can be assessed in further studies by measuring the activity of superoxide dismutase, catalase and glutathione peroxidase (or by measuring the total enzyme activity through a technique known as total antioxidant capacity).

Taken together and considering the limitations of this study, our data provide a new benefit of carbohydrate supplementation enriched with proteins and antioxidants in low concentration. While the previous literature had already demonstrated greater retarding fatigue carbohydrates (Valentine et al., 2008; Alghannam et al., 2011), greater insulin (Betts et al., 2008; Roberts et al., 2013) and better glycogen resynthesis (Berardi et al., 2006), our data add a restoration of oxidative stress after aerobic exercise session as another ergogenic effect of the carbohydrate plus proteins with small amounts of antioxidant vitamins.

## CONCLUSION

In addition to avoiding the reduction of blood glucose during an aerobic exercise, this study showed that a compound of carbohydrate enriched with protein and small amounts of antioxidant vitamins accelerates the restoration of oxidative stress induced by exercise in young practitioners of recreational exercise.

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