



Effects of Sago Consumption Associated with Endurance Exercise on Oxidative Stress in Recovery from 20–km Cycling Time Trial Performance in the Heat

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Abstract

Exercise in hot and humid conditions cause remarkable physiologic challenges for endurance athletes. The aim of the current study was to investigate the effects of carbohydrate consumption during endurance cycling performance on F₂-isoprostanes as an oxidative stress biomarker. Sago flour is a commercial starch source that is frequently used in tropical countries, like Malaysia, as carbohydrate ingestion. The consumptions of sago have been shown to improve endurance performance. Twelve well-trained male cyclists (age: 19.0±5.6 years, body weight: 60.1±11.2 kg, height: 170.8±7.6 cm, and VO₂max: 56.5±6.5 mL.kg⁻¹.min⁻¹) pedaled at 60% of VO₂max for 1.5 hours followed by a 20–km cycling time trial in the heat (31°C; 70% relative humidity). From the start of the cycling and at 20–minute intervals during 1.5 hours cycling participants ingested 200 mL of 7.5% of the sago supplement. There was a significant reduction of plasma F₂-isoprostanes concentrations after endurance cycling as compared to the baseline levels. This study has shown that sago supplementation was able to lower the oxidative stress in the recovery after endurance cycling performance in the heat.

Keywords

Oxidative Stress, Sago, 20–km Cycling Time Trial, F₂-Isoprostane, Heat

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Introduction

It is well known that carbohydrate (CHO) supplementation during endurance exercise leads to performance improvements in a hot and humid environment (Rothenberg & Panagos, 2008). Supplementation plays a significant role in response to exercise performance since it can prolong endurance capacity (Learsi et al., 2019; Tarmast, Ghosh, & Chen, 2017) and improve recovery from exercise (Alghannam, Gonzalez, & Betts, 2018; Moore, 2015). Evidence suggests that exercise causes excessive production of reactive oxygen species (ROS) resulting in oxidative stress (OS), which has been implicated in the endurance performance and also subsequent exercise (Souza-Silva et al., 2016; Sureda et al., 2015). In endurance exercise, the production of ROS causes muscular fatigue, and it has been shown that OS contributes to muscular fatigue (McKenna et al., 2006).

In recent years, there has been an increasing interest in using herbal supplements during exercise (Sellami et al., 2018). These healthy meals are commonly less processed naturally than imported diets which some athletes are tending to ingest herbs instead of industrial supplements. One of the herbal origins of CHO is sago starch in the tropical region of Southeast Asia. In Malaysia and India, sago is used to prepare foods locally that are cost-effective (Ahmad, Singh, & Ghosh, 2009; Faizal, Ooi, Ghosh, Ang, & Rosli, 2012; Ghosh, Rahaman, & Singh, 2010; Tarmast et al., 2017). The current study follows the former works to study the effects of different sago meals during exercise which is the first attempt on OS in a hot and humid environment (~31°C; 70% relative humidity).

Studies have shown that endurance exercise in a hot and humid condition is associated with increased blood OS compared to training in a cooler condition (Hillman et al., 2011; Sureda et al., 2015). The combination of exercise in the heat and fluid loss causes acute stress physiologically, which consists of the equilibrium between oxidants and antioxidants cellular agents (Paik et al., 2009). Therefore, in order to guarantee a higher level of endurance capacity, it is recommended to counteract the effects caused by OS with different strategies. There have been some efforts to lower exercise-induced OS via different kinds of supplements, which have indicated mixed outcomes (McAnulty et al., 2007). CHO intake during endurance exercise might moderate the OS as some evidence has investigated the effects of consuming CHO drinks on OS and antioxidant status (Klapcinska et al., 2002; McAnulty et al., 2005). The efficacy of sago supplementation on the exercise-induced OS remains unclear, some studies demonstrating no effect with different kinds of CHO ingestions although sago has not been used.

The current study was the first attempt that aimed to determine the effects of sago supplementation on the OS status in the heat. One of the sensitive biomarkers of OS is F₂-Isoprostanes concentration in the blood (Il'yasova et al., 2018). Previously, our earlier study has suggested that the sago supplementation can improve the metabolic variables in the heat. Hence, the current study was carried out to investigate the effects of sago supplementation as a local CHO supplementation on plasma concentration of F₂-Isoprostanes following endurance cycling performance in the heat.

Materials and Methods

Subjects and Design



The sample size of the current study was calculated by using PS Power and Sample Size Calculation v.2.1.30. The power of the study was set at 80% with 95% confident interval while standard deviation (σ) observed was 1, and the difference in population means (δ) was set at 2, and calculated sample size was 15 subjects including the drop-out rate at 20 %. Twelve moderate, heat-adapted cyclists were recruited for this randomized single-blinded study. Heat-adaptation in these subjects was obtained through existing regular training in a tropical condition. They involved in Malaysian cycling events and were training regularly (>3 drill sessions.week-1) covering almost >80 km without any recent breaks in drill before the study. Table 1 displays the main characteristics of the subjects in the study. All the subjects were given a complete explanation of the study design and the possible risks associated. All subjects were nonsmokers and normotensive and had no overt history of metabolic, cardiovascular, hepatic, thyroid, or other types of diseases in the cycling trials. Then, they read and signed an informed consent form. This study was supported by an *e-Science* Fund from the Ministry of Science, Technology and Innovation Malaysia (MOSTI). The project number was USM/0000814 at Sports Science Unit, School of Medical Sciences, Health Campus, Universiti Sains Malaysia.

Table 1. The main characteristics of the subjects (n=12).

Variables	Mean \pm SD
Age (years)	19.0 \pm 5.6
Height (cm)	170.8 \pm 7.6
Body weight (kg)	60.1 \pm 11.2
Body fat (%)	16.6 \pm 4.4
Body mass index (kg.m ⁻²)	20.5 \pm 3.0
Maximal oxygen consumption (mL.kg ⁻¹ .min ⁻¹)	56.5 \pm 6.5
Maximum heart rate (beats.min ⁻¹)	201 \pm 5.6

Sago Supplementation

The sago flour was obtained from Sim Company Sdn. Bhd. Penang (Tarmast et al., 2017). The sago supplement of the present study was an isocaloric drink with 75 grams of sago flours per liter of distilled water. The amount of each supplement was estimated ~ 300 kcal, and was randomized given to be ingested 5 times at 0, 20, 40, 60, and 80 min during the 90min steady-state cycling. The supplement was prepared on the same day before each trial by a similar taste by mixing 5 mL of non-caloric chocolate flavor (Star Brand, Selangor, Malaysia) in 1000mL of each drink, and stored at room temperature (25 °C).

Experiment Procedure



The protocol was similar to a real competition in which each subject consumed a sago supplementation as it is illustrated in Appendix A. All the subjects visited the laboratory on 4 separate cycling trials: 1) preliminary testing for submaximal and maximal assessments, 2) familiarization trial, and 3) experimental trials. The purpose of the submaximal test was to establish the relationship between the workload of the pedaling and VO_2 and its results were applied to determine the workload during 90 minutes steady-state cycling at their respective VO_{2max} . The subjects of the study pedaled four stages at the different workloads of 50, 80, 110, and 140 w at 60 rpm during 16 min of cycling (Tarmast et al., 2017). The maximal test was done to determine the VO_{2max} of each subject by cycling on an electronically braked ergometer (Excalibur Sport, Lode, The Netherlands).

The room temperature and relative humidity environment ($\sim 31^\circ\text{C}$; 70% relative humidity) were determined throughout the familiarization and experimental trials by using a Digital Psychrometer (Extech Instrument RH300, USA). The chamber temperature in the improvised climatic place was kept at 31°C via halogen lamps (Philips - 500 W, France) and air conditioner (York®, Malaysia). The relative humidity was kept at 70% via a heated water-bath (Memmert, Germany) which was situated in the chamber. In the chamber, a standing fan was located with speed at level 2 to direct the motions of air to the subjects to mimic the airflow in an open-air environment. During the familiarization trial, which was similar to the experimental trials, subjects did not drink the sago supplements of the experimental trials. As a replacement for the sago supplementations, distilled water was given to them every 20 minutes during this trial (Robinson et al., 1995). The subjects who were not able to cope with this test were excluded from the study. Before each experimental trial, all the subjects were asked to avoid 24 hours from taking any other supplements or from any exercise. A food diary form was given to each subject to note their food intake 3 days before each experimental trial. All the subjects were asked to report to the laboratory after 10 to 12 hours of the overnight fast. They were then guided to follow the same diet before the subsequent trial to minimize the differences in resting muscle glycogen concentrations.

On arrival to the laboratory at 07:30am for the experimental trials, a standardized breakfast was given to the subjects, which consisted of 2 pieces of white bread (Gardenia®, Malaysia), and 250mL distilled water ($\pm 8^\circ\text{C}$). Almost 20 minutes after the first blood collection, subjects entered the heat chamber that was conducted in a thermally stressful situation. During all the experimental trials, the subjects warmed up for 5 minutes at 50% of their respective VO_{2max} , and 90 minutes pedaling at 60% of their respective VO_{2max} . After 5 min passive rest, the cycling was followed by a 20-km cycling time trial on another cycle (One Series Aluminium, Trek Road Bikes, USA). This cycle was maintained in a vertical position by a trainer (CycleOps Power JetFluid Pro Trainer, USA) to form real-life accelerations, surroundings, and offering a real-world condition for endurance pedaling in the heat chamber (Tarmast, Ghosh, & Chen, 2012). In the meantime, subjects were permitted to manage the speed of cycling via a digital cyclometer (Cateye Strada Wireless, Japan).

Determination of F_2 -Isoprostanes Concentration

Before having breakfast, the subjects were cannulated with an indwelling cannula which was injected into a subcutaneous forearm vein. Almost, 0.8 mL of heparinized saline was introduced into the extension tube to keep the patency of blood fine for each blood sampling. Blood samples were collected 30 min pre-exercise, immediately post-exercise, and 24 hours post-exercise. For each blood



withdrawal, 5 mL of blood was drawn in a 10mL sterile syringe and was transferred to the yellow color tubes (5 mL, Gel and Clot Activator, 13×100 mm, ST750CG) for F₂-Isoprostanes determination. The blood samples were centrifuged (Hettich-Rotina 46 RS, Germany) in sodium heparin tubes, and plasma was aliquoted into cryotubes, and stored at -80°C (Heto Ultra Freeze 3410, Denmark) until analysis for F₂-isoprostanes.

An enzyme immunoassay (EIA) was applied to determine the presence of the F₂-Isoprostanes (8-iso Prostaglandin F₂α) in plasma by considering enzyme-linked immunosorbent assay (ELISA) method. To decide the F₂-Isoprostanes concentration, the reagents of the EIA (Cayman Chemical's ACE™ EIA Kits, F₂-Isoprostanes, Catalog No. 516351, USA), were provide after defrosting the plasma, the determination of concentration was done by a photometric microplate reader (Molecular Devices; Versamax Tunable Micro Plate Reader, USA). 50μL of plasma samples was added into sample wells which were pre-coated with mouse anti-rabbit antibody. Standards were prepared at 8 different concentrations as stated in the assay protocol and 50μL of each standard were added to standards wells. Subsequently, 50μL of F₂-Isoprostane AChE Tracer was added into each well, except for Total Activity and Blank wells.

50μL of F₂-Isoprostane EIA Antiserum was added into each well, except for the Total Activity, the Non-Specific Binding and Blank wells. Covering the plate with plastic film, and incubated for 18 hours at 4°C. Following 18 hours of incubation, the wells were emptied and rinsed for five times with Wash Buffer. 200μL of Ellman's Reagent was added to each well whereas 5μL of tracer was added to the Total Activity well. Covering the plate by the plastic film before it was allowed to develop on microtitre plate mixing apparatus (IKA Vibrax® VXR basic, U.S.A.) in dark at room temperature for 2 hours. The plate was measured at a wavelength of 405–420nm.

Statistical Analysis

IBM SPSS Statistics v.24 for windows operation system was used for all statistical analyses. The statistical significance was accepted at p<0.05. All the data were expressed as means±standard deviations (SD). Data were tested for normality with a Shapiro-Wilks test. In order to investigate whether the sago supplementation effects on the OS biomarker after exercise, a dependent t-test was used to compare the means between time points at 30 min pre-exercise, immediately post-exercise, and 24 hours post-exercise.

Results of the Study

The descriptive statistics and a brief of statistical analyses are shown in Table 2 and Table 3 respectively. Also, the plasma F₂-Isoprostanes concentrations during the experimental trials are shown in Figure 1.

Table 2. The results of the descriptive statistics (n=12).

The plasma F ₂ -Isoprostanes concentrations	Mean	SD
At 30 min pre-exercise	481.83	34.1
At immediately post-exercise	424.08	49.0
At 24 hours post-exercise	402.35	55.2



There was a reduction in the concentrations of plasma F₂-Isoprostanes after 20-km cycling time trial. The immediately post-exercise and the 24 hours post-exercise levels were significantly decreased as compared to the 30 min pre-exercise level (p<0.001). Additionally, the difference between the immediately post-exercise and the 24 hours post-exercise levels was significant (p<0.001).

Table 3. The results of the dependent t-test analyses (n=12).

	Time points	Mean	SD	t-value	df	Significance level
Pair 1	Between 30 min pre-exercise and immediately post-exercise	57.8	7.77	7.4	11	<0.001
Pair 2	Between 30 min pre-exercise and 24 hours post-exercise	79.4	11.18	7.1	11	<0.001
Pair 3	Between immediately post-exercise and 24 hours post-exercise and	21.7	3.94	5.4	11	<0.001

Discussion

The current study was the first to our knowledge to determine the effects of sago supplementation during endurance cycling performance on OS in the heat. The current study found that the OS biomarker was lowered after exercise as compared to the 30 min pre-exercise levels when cyclists consumed sago ingestion during endurance performance in the heat. One of the end products of non-enzymatic free radical oxidation is plasma F₂-Isoprostanes (Mastaloudis, Leonard, & Traber, 2001) and shows the level of oxidative stress-induced lipid peroxidation (Nikolaidis, Kyparos, & Vrabz

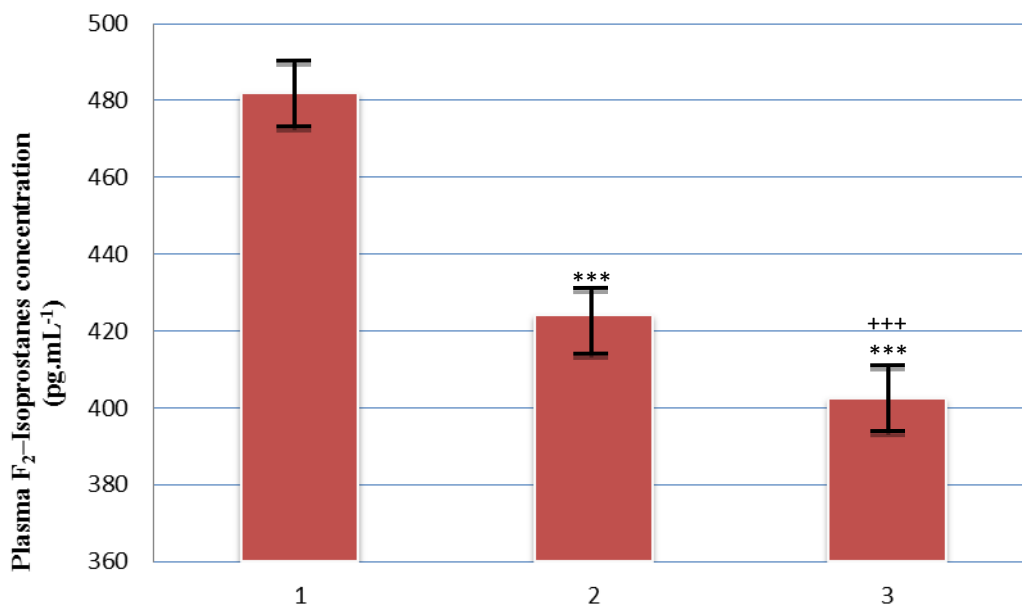


Figure 1. The plasma F₂-Isoprostanes concentration of the participants during the experimental trials when they consumed the sago supplement in the heat. *** Significantly different from 30 min pre-exercise level (p<0.001). +++ Significantly different from immediately post-exercise level (p<0.001).



These results seem to be consistent with other research which established that CHO intakes lowered the OS in exercise (Klappinska et al., 2002; McAnulty et al., 2005; McAnulty et al., 2003). Some of the supplements have some antioxidant properties in the diet that is a potential countermeasure against the blood OS during endurance exercise. CHO ingestion during exercise may diminish OS by lessening the stress-hormone response (Nieman et al., 2005). It is recognized that plasma concentrations of cortisol and epinephrine elevated in endurance exercise, and CHO feeding has been shown to decrease the rise of these hormones. A possible explanation for this might be that catecholamines are capable of auto-oxidation to form ROS (Mastorakos & Pavlatou, 2005). The higher concentrations of catecholamines have also been shown to mobilize neutrophils from body pools and augment the liberation of superoxide via those neutrophils (Nunes-Silva et al., 2014). Another possible explanation for this is that elevated cortisol concentration might significantly reduce cellular glutathione that is one of the main substrates in antioxidant defense (Walther, 2004).

In the current study, the sago supplementation might indirectly reduce the OS via improving maintenance of plasma glucose concentrations. This glycemic response was also reported previously by our work (Tarmast et al., 2017). These results support previous studies indicating that the reason for OS reduction was due to the blood glucose maintenance during endurance exercise once athletes ingested CHO (McAnulty et al., 2007; Nieman et al., 2005). In addition, the 24 hours post-exercise concentration of plasma F₂-Isoprostanes decreased when compared to the 20 min pre-exercise levels, suggesting that residual memory of these oxidant stimuli may provide a prolonged defense system against OS after exercise. Thus, OS decreased for several hours following the time trial cessation. Also, this observation was also reported in another study after a marathon race when the antioxidant status increased during the recovery period (Rokitzki et al., 1994).

Conclusions

The present study showed that sago supplementation during endurance exercise in the heat reduced OS in the recovery period. However, future studies are warranted to assess the nutritional and antioxidant status of the athletes along with other OS markers when performing endurance exercise in the heat.

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Appendix A

