Metabolic Responses to Sago and Soy Supplementations during Endurance Cycling Performance in the Heat

Daniel Tarmast¹, Asok Kumar Ghosh², Chee Keong Chen ³

¹ Department of Physical Education and Sport Sciences, Parand Branch, Islamic Azad University, Parand City, Tehran, Iran. E-mail: danieltarmast@piau.ac.ir
² Department of Sports Science, School of Rehabilitation Sciences and Physical Education, Ramakrishna Mission Vivekananda University, Belur Math, Howrah, West Bengal, India
³ Exercise and Sports Science Programme, School of Health Sciences, Universiti Sains Malaysia, Kota Bharu, Malaysia

ABSTRACT: This study examined the effects of Sago (Sa), Soy (So), combined iso-caloric Sago+Soy (SS) supplementations during cycling on metabolic responses as compared to placebo (P) in the heat (31°C, 70% relative humidity). Twelve well-trained male cyclists (Age: 19.0±5.6 yr, Height: 170.8±7.6 cm, Weight: 60.1±11.2 kg, and VO_{2max}: 56.5±6.5 mL.kg^{-1}.min^{-1}) participated in four experimental trials. The design of the trials was a randomized single-blind, placebo-controlled crossover trail comprising 90 min of steady-state cycling on an ergometer at 60% of VO_{2max} followed by a 20–km time trial performance (TT). The participants of the study were supplemented 5 times at 0, 20, 40, 60, and 80 minutes during the steady-state cycling. The supplements provided 7.5% Sago, 7.5% Soy, and 6.0% Sago + 1.5% Soy respectively. Plasma glucose concentrations (PG) reached a peak at 60 min after ingestion of Sa and SS as compared to baseline. At the end of the TT, PG reduced significantly to the baseline level. Plasma insulin concentrations (PI) increased in all trials, but reduced gradually to the baseline level. The concentration of plasma free fatty acids (FFA) increased gradually during the steady-state cycling and TT, and FFA was significantly higher in the P and So than the Sa and SS trials. At the end of the steady-state cycling, the plasma lactate concentration (LACT) reached its lowest concentrations and at the end of the TT was enhanced significantly in all trials. These results suggest that sago and soy supplements increase the PG and PI during endurance exercise in the heat. These data add to the growing body of knowledge concerning endurance athletes’ glycemic and insulinemic responses to carbohydrate consumptions during exercise in the heat.

KEY WORDS Sago, Soy, Supplementation, Glucose, Insulin, Free Fatty Acids, Lactate, endurance exercise, in the heat

INTRODUCTION

Carbohydrate (CHO) ingestion is frequently prescribed for endurance athletes to improve their performance during competitions [1, 2]. Study on the significance of CHO has been widely studied since the 1970s. The challenge for CHO consumption is to ingest the best sources and determine ideal diet plans by endurance athletes to improve performance. The healthy supplement is commonly less processed than imported diets, and the use of local daily food as supplements over expensive beverages is cost effective. It is now well established from a variety of studies, that locally-sourced and organic diets comprised further nutrients, including CHO [3, 4], and protein (PRO) [5].
Sago starch is one of the local sources of CHO in the tropical countries of Southeast Asia such as Malaysia, India, and Indonesia. The sago starch is generally used to make local dishes, biscuits, and consume sago together with potato, rice, corn, and in the manufacturing of pasta [3, 6, 7]. This starch is a simply digestible substance that is a fast absorbed nutrient in gastrointestinal tract into blood circulation. In addition, sago is a starch that holds a high glycemic index, about 88%, that ingestions elicit a high glycemic response to exercise [6]. Therefore, sago supplementations are able to be ingested before and/or during exercise by athletes as a CHO feeding. In Sports Science Unit Laboratory of Medical School, Universiti Sains Malaysia, sago starch has been extensively studied as CHO ingestion during physical activity [3, 5, 6, 8-12].

The addition of PRO to a CHO supplement has been reported to improve endurance performance [3, 13-16]. Soy is one of the commercially available local sources of PRO in a diet plan for athletic performance and this PRO is a combination of many essential amino acids such as valine, leucine, and isoleucine. As compared to other CHO and PRO supplementations, research into sago and soy ingestions are limited, and a few recent investigations have only considered them as a CHO and PRO supplementation for exercise. Dehydration and depletion of CHO sources in athletes are two main issues that may possibly impact on the endurance performance in the heat [17, 18]. Endurance athletes are able to improve their performance in the heat by adding CHO to the drinks during exercise [19, 20].

Previously published studies on the effect of sago and sago+soy ingestions have shown that blood metabolic variables were improved during exercise. It has been shown that a CHO intake of sago results in high glucose and insulin responses to endurance exercise [3, 6, 21]. A study by Ghosh et al. (2010) indicated that the plasma insulin response was increased above that with placebo during sago and sago+soy supplementations in a thermoneutral environment. They concluded that a combination of sago and soy PRO was able to delay fatigue during 60 minutes of cycling. To determine the effects of sago supplements in a thermally stressful environment, Che Jusoh et al. (2016) conducted their study in the heat while participants ingested a sago gel (~30 °C; 78% relative humidity). During exercise, plasma glucose concentrations were maintained higher when the participants consumed sago gel as compared to control group. The authors of the study established that the sago ingestions may be beneficial to those endurance athletes who train in a hot and humid environment to improve performance.

Previously, our laboratory was the first, to our knowledge to report the use of combined sago+soy supplementation during exercise [3, 6, 10, 12, 22]. The current study follows the earlier studies to investigate the effects of iso-caloric sago and soy supplementations on exercise which is the first attempt in a hot and humid environment (~31 °C; 70 % relative humidity) [22]. This study will provide new evidence if a local and daily use diet, sago and soy iso-caloric supplementations can improve the metabolic variables in the heat. Therefore, this study was undertaken to examine the effects of sago, soy, and combined iso-caloric sago+soy as local supplementations during endurance cycling on metabolic variables in the heat, as compared to placebo.

**METHODS**

In this study 12 males recruited as participants were moderate, heat–adapted cyclists. Heat–adaptation in these participants was achieved through existing regular exercise in a tropical environment. They participated in local cycling competitions in Malaysia and were training regularly (>3 training sessions.week-1) covering almost 80 km prior to the study. The physical characteristics of them were (mean±SD), age: 19.0±5.6 y, height: 170.8±7.6 cm, weight: 60.1±11.2 kg, VO$_2$max: 56.5±6.5 mL.kg-1.min-1, maximal heart rate: 201±5.6 beats.min-1. All the participants were given a complete clarification of the study design and possible risks associated. Then, they read and signed an informed consent form. This study was funded by an e-ScienceFund from the Ministry of Science, Technology and Innovation Malaysia (MOSTI). The project number is USM/0000814 at Sports Science Unit, School of Medical Sciences, Health Campus, Universiti Sains Malaysia.

The design of the study was a randomized single–blind, placebo–controlled cross–over trial to examine the impact of iso–caloric sago and soy supplementations by pedaling. The protocol was similar to an actual race in which each participant ingested one of the supplementations per experimental trials with a one week washout period. There were 4 experimental trials where the participants were randomly assigned to drink either Sago, Soy, Sago+Soy, or Placebo before and during the experimental trials.

The sago flour and soy PRO isolate flour were obtained from Sim Company Sdn. Bhd. Penang [3]. All supplements of the present study were iso-caloric, and the energy value of them was estimated ~300 kcal and was randomized given to the participants 5 times at 0, 20, 40, 60, and 80 min during the 90 min steady state cycling. All of the supplements were prepared on the same day before each trial and kept at room temperature (25 °C). All the supplements and the placebo (Table 3.1) had a similar taste by mixing 5 mL of non–caloric chocolate flavor (Star Brand, Selangor, Malaysia) in 1000 mL of each drink. Only distilled water was used for placebo preparation.

All the participants visited the laboratory on 7 single cycling trials: 1) preliminary trials for submaximal and maximal tests, 2) familiarization trial, and 3 to 7) experimental trials. The purpose of the submaximal test was to determine the relationship between the workload of the pedaling and VO$_2$ and its results were used to calculate the workload during 90 minutes steady–state cycling at their respective VO$_2$max. The participants 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 [23, 24]. The maximal test was done to determine the VO$_2$max of each participant by using the electronically braked ergometer (Excalibur Sport, Lode, The Netherlands).

<table>
<thead>
<tr>
<th>Table 3.1. The basic nutrient composition of the supplements.</th>
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<tbody>
<tr>
<td><strong>Nutrient</strong></td>
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<td>---------------</td>
</tr>
<tr>
<td>Calories</td>
</tr>
<tr>
<td>Carbohydrates</td>
</tr>
<tr>
<td>Protein</td>
</tr>
<tr>
<td>Fat</td>
</tr>
<tr>
<td>Fiber</td>
</tr>
<tr>
<td>Sodium</td>
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<tr>
<td>Potassium</td>
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</table>
The familiarization trial was similar to the experimental trials, but participants did not drink the supplements of the experimental trials. Instead, distilled water was given to them every 20 minutes during this trial [25]. The room temperature and relative humidity were measured during the familiarization experimental trials by using a Digital Psychrometer (Extech Instrument RH300, USA). The room temperature in the improvised climatic chamber was maintained at 31°C via halogen lamps (Philips - 500 W, France) and air conditioner (York®, Malaysia). The relative humidity was maintained at 70% via a heated water–bath (Memment, Germany) which was located in the chamber. In the chamber, a standing fan was placed to direct air to the participants to mimic the airflow in an open–air environment. The speed of the fan was maintained at level 2 all over the trials.

Before each experimental trial, all the participants were asked to avoid 24 hours from taking any other supplements or from any exercise. A food diary form was given to each participant to record their food consumption 3 days before each experimental trial to repeat the same diet plan over three days before the next experimental trials. All the participants reported to the laboratory after 10 to 12 hours of the overnight fast. On arrival to the laboratory for the experimental trials at 07:30 am, the participants were cannulated with an indwelling cannula which was inserted into a subcutaneous forearm vein. Almost, 0.8 mL of heparinized saline was introduced into the extension tube to keep the patency of blood well for each blood sampling. For every blood withdrawal, 8 mL of blood was drawn in a 10 mL sterile syringe. Subsequently, a standardized breakfast was given to the participants, which consisted of two slices of white bread (Gardenia®, Malaysia), and 250 mL distilled water (± 8°C).

Almost 20 minutes after the first blood collection, participants entered the heat chamber that was conducted in a thermally stressful situation. During all the experimental trials, the participants warmed up for 5 minutes at 50% of their respective VO2max and 90 minutes pedaling at 60% of their respective VO2max. After 5 min passive rest, the pedaling was followed by a 20-km cycling time trial on another bike (One Series Aluminium, Trek Road Bikes, USA). This bike was maintained by a trainer (CycleOps Power JetFluid Pro Trainer, USA) [23, 26] to form real–life accelerations, surroundings, and offering real–world condition for endurance pedaling in the heat chamber. In the meantime, participants were free to control the speed of the cycling by a digital cyclometer (CatEye Strada Wireless, Japan).

In the process of biochemical analysis in the laboratory, 2 mL of the blood was placed into sodium fluoride–Na Heparin tubes (Omnifix®, Germany) as the anticoagulant for lactate and glucose analysis. Then, about 6 mL was transferred to Gel and Clot Activator tubes (Terumo®, Philippines) for insulin and FFA analyses. For glucose, insulin, FFA, and lactate analyses the blood plasma was separated by centrifugation (Hettich-Rotina, Germany) for ten minutes at with 4000 rpm in the laboratory after each trial completion in the same day. Following this, the plasma samples were stored at ~80°C (Heto Ultra Freeze, Denmark) for future analyses. Randox glucose reagent (Glucose, Randox Laboratories®, UK) was used to measure the plasma glucose concentrations. To determine the plasma insulin concentrations an electrochemiluminescence immunoassay (Roche Insulin reagent, USA) with Modular Analytics E170 (Elecys module) immunoassay analyzers (Roche Diagnostics 1010/2010, Germany) was used. The concentrations of free fatty acids (FFA) were measured by using a non–esterfied fatty acids (NEFA) reagent (Wako, NEFA C Reagent, Japan). NEFA was obtained by determining the absorbance of the blue–purple color at 550 nm spectrophotometrically. The analysis of plasma lactate was prepared through commercial reagent kit (Cobas, USA) and indicated by using the auto-chemistry analyzer (Roche Hitachi 912 Analyzer, USA).

Data were examined for normality with a Shapiro–Wilks test. All the data were expressed in mean±standard deviation. Significance level was set at p<0.05. To determine the differences in the metabolic variables over time between four experimental trials continuously, analysis of variance (ANOVA) with repeated measurement was used. All these statistical analyses were done using IBM SPSS Statistics v.24 for windows operation system.

**STATISTICAL RESULTS**

As shown in Figure 1, plasma glucose concentrations (A) significantly increased during 30 min of cycling as compared to the baseline in the sago, and sago+soy trials, but the placebo and soy trials were significantly decreased (p<0.001). At 60–min, the plasma glucose concentration hit a peak in the sago and sago+soy trials, and then remained as the highest levels at 90–min of cycling when compared to placebo and soy trials. Plasma glucose levels in sago and sago+soy trials did not differ significantly at any point of time. Strong evidence of sago and sago+soy supplementation was found when plasma glucose concentrations were improved during cycling. At the end of time trial, plasma glucose concentrations returned to baseline in all of four trials.

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**Table 2.** Combination of sago and soy-protein supplementation during endurance cycling exercise and subsequent high-intensity endurance capacity. *International Journal of Sport Nutrition & Exercise Metabolism, 20(3), Table 2 on page 217.*

<table>
<thead>
<tr>
<th>Type of the supplement</th>
<th>Amount (mL)</th>
<th>CHO (g)</th>
<th>PRO (g)</th>
<th>Concentration (%)</th>
<th>Total energy (kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sago</td>
<td>200</td>
<td>15</td>
<td>0</td>
<td>7.5</td>
<td>0</td>
</tr>
<tr>
<td>Soy</td>
<td>200</td>
<td>0</td>
<td>15</td>
<td>0</td>
<td>7.5</td>
</tr>
<tr>
<td>Sago + Soy</td>
<td>200</td>
<td>12</td>
<td>3</td>
<td>6.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Placebo</td>
<td>200</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Content per serving consumed during each experimental trial**

<table>
<thead>
<tr>
<th>Type of the supplement</th>
<th>Total consumed during each experimental trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sago</td>
<td>1000</td>
</tr>
<tr>
<td>Soy</td>
<td>1000</td>
</tr>
<tr>
<td>Sago + Soy</td>
<td>1000</td>
</tr>
<tr>
<td>Placebo</td>
<td>1000</td>
</tr>
</tbody>
</table>

**Nondex et al., (2010). Combination of sago and soy-protein supplementation during endurance cycling exercise and subsequent high-intensity endurance capacity. *International Journal of Sport Nutrition & Exercise Metabolism, 20(3), Table 2 on page 217.*"
In all trials plasma insulin concentrations following the warm-up increased ($p < 0.001$), and reached peak values at 30-min in sago and sago+soy trials as compared to the placebo and soy trials ($p < 0.001$). At 60-min as plasma insulin concentrations was reduced in all trials, and in placebo and soy trials the insulin levels were the lowest values, but in the sago and sago+soy trials were significantly higher than placebo and soy trials ($p < 0.001$).

Plasma FFA concentration was significantly decreased in all trials after the warm-up ($p < 0.001$), but at 30-min it was increased, however it was significantly lower than the baseline level ($p = 0.036$). At 60-min, plasma FFA concentration was significantly higher in the placebo trial compared to the sago+soy trial ($p = 0.044$). The concentration of plasma FFA elevated gradually at the end of the 90-min, but it was not significant when compared to the baseline level. At the end of the 20-km time trial, plasma FFA concentration was significantly lower in the sago and sago+soy trials than the placebo and soy trials ($p < 0.001$).

During the 90-min cycling, there was no significant difference between the four trials in the plasma lactate concentration, and at the end of the 90-min, it declined to its lowest value during the cycling ($p = 0.05$). The plasma lactate concentration at the end of the 20-km time trial reached the highest values in all trials ($p < 0.001$), but only sago trial was significantly higher than soy trial ($p = 0.022$).

**DISCUSSION**

Prior studies that have noted the performance improvement by examining sago and soy ingestion during exercise were conducted in a thermoneutral environment. As mentioned in the introduction, our laboratory at Universiti Sains Malaysia was first to investigate the use of combined iso-caloric sago, soy, and sago+soy supplementations during exercise in the heat. In this study, sago and soy supplements were found to cause metabolic responses during endurance exercise. The most interesting findings are glycemic and insulinemic responses when the participants of the current study consumed iso-caloric sago and sago+soy supplements during exercise in the heat. In this study, sago and soy supplements were found to cause metabolic responses during endurance exercise. The most interesting findings are glycemic and insulinemic responses when the participants of the current study consumed iso-caloric sago and sago+soy supplements during exercise in the heat. These results are in agreement with Ahmad et al. (2009) and Ghosh et al. (2010) findings which showed the increment of plasma glucose concentrations when participants were fed by sago and sago+soy supplements. Therefore, this study confirms that iso-caloric sago and sago+soy supplementations are quickly absorbed and metabolized to glucose in the blood flow showing that they were effective to maintain the plasma glucose during endurance performance in the heat.

Insulin hormone inhibits glucose production by the liver and prevents lipolysis. When blood glucose levels increase above the normal condition, the pancreas delivers the insulin hormone to elicit glucose transport into its target cells. As a result, insulinemic responses are associated with plasma glucose concentrations. However, it is interesting to note that by the two iso-caloric sago and sago+soy supplements, plasma insulin concentration was increased during the first 30 min of steady state cycling, such changes were in response to the plasma glucose concentrations, and also caused by the standardized breakfast. Insulin hormone is affected by CHO ingestion, and is also one of the main factors in the adjustment of the balance between FFA/CHO oxidation in exercise [27]. The metabolic effects of CHO supplementation in sago and sago+soy trials indicated that glucose and insulin plasma levels extended to higher values. The rise of these concentrations prevents lipolysis of adipose tissue [28]. However, in both trials of sago and sago+soy...
plasma glucose concentrations were maintained to the end of the cycling in the heat, plasma glucose levels raised and FFA concentrations declined during the first hour cycling. In addition, hyperglycemia and hyperinsulinemia caused a greater extent of muscle glucose uptake compared to placebo and soy trials. The sago and sago+soy iso–caloric supplementations of extended insulin sensitivity for the duration of 45 to 60 minutes of cycling, and then decreased thereafter. A possible explanation for this decrease might be once the FFA concentration increased [29]. Besides, it has been shown that metabolism of FFA and triglycerides impair insulin signaling [30]. Moreover, it has been assumed that FFA can inhibit insulin to recruit the microvessel in the muscles, preventing both insulin and glucose from reaching muscle fibers [31]. This result may be explained by the fact that the standardized breakfast before the pedaling shifts metabolism toward CHO and likewise, diminishes the plasma FFA concentration [32]. An additional reason is that during the first couple minutes of exercise, plasma FFA concentration drops as a result of the delay in the enhanced uptake of fatty acids directly after the onset of activity and the trigger of fat hydrolysis [33]. It has been known that the supplement ingestions, especially CHO feeding instead of fat diet decide whether the ideal energy source is CHO or FFA [34]. The reason is that CHO supplement raises insulin production, which hinders the release of plasma FFA. Consequently, the plasma concentration of FFA falls off at the beginning of exercise [35].

There was a significant reduction in the plasma lactate concentration after the warm–up in all trials, and then it reduced gradually and reached the lowest level at the end of 90 minutes of pedaling. During exercise, lactate in liver and muscle is oxidized, and the liver removes half of the formed lactate by active muscles [35]. Lactate is produced of glycogen and glucose in the glycolysis process through the pyruvate conversion by lactate dehydrogenase [36]. The lactate secretion is not only as an energy source and a precursor for glycogen release by the liver, but also as an energy substrate for aerobic oxidation in active muscles [37]. The lactate reduction occurred due to the fact that active skeletal muscles were further generating lactate, different parts of the organism like liver and heart received lactate from blood flow, either to become transformed into glucose or oxidized to function as an energy substrate [35]. During prolonged exercise, in order to supply additional calorie by aerobic energy systems, accumulated lactate was removed gradually from the blood circulation. Additionally, training in hot and humid environment adjusts the blood circulation to deliver oxygen and nutrients, and it would have an influence on lactate removal as well [38, 39]. The intakes of sago and iso–caloric sago+soy supplementations elevated the plasma concentrations of lactate at the end of the 20–km time trial, showing that exercise intensity was enhanced progressively. Additionally, glycolysis resulted in the synthesis of a greater amount of pyruvate than that at which it could be removed. Accordingly, lactate accumulation was induced within active muscles at the end of the 20–km cycling time trial [40].

In conclusion, the present study confirms previous findings and contributes additional evidence that suggests locally–sourced and organic diets of the current study, the sago and soy iso–caloric supplementations can improve the metabolic variables in the heat. The sago and sago+soy ingestions during exercise in a hot and humid condition indicates the higher glycemic and insulinemic responses compared to placebo and soy treatments. Hence, their intake probably support improve endurance performance.

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