## Effect of Acacia Honey Drink Consumed Pre and During Exercise on Glucose Metabolism, Total Antioxidant Status, and Running Performance in the Heat

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## ABSTRACT

INTRODUCTION: This study investigated the effects of Acacia honey drink on glucose metabolism, total antioxidant status, and running performance in the heat and compared it with a commercial sports drink. MATERIALS AND METHODS: In this randomised cross -over study, ten participants completed two endurance running trials in the heat (31°C, 70% relative humidity) with either the honey drink (H) or sports drink (SPD) consumption with a one-week interval between trials. The participants warmed up for 5 min at 50% of their respective maximum oxygen consumption (VO2max) and subsequently ran at 60% of VO2max for 1 hour, immediately followed by a 20-min time trial running performance. During the 20-min time trial, each participant's furthest running distance was recorded to determine the participants' running performance. Blood samples were analysed for glucose, insulin, cortisol, and total antioxidant status. RESULTS: There was no difference in running performance during the honey and sports drink trials. In addition, plasma glucose, insulin, and cortisol were also not statistically different at any time points between H and SPD trials. However, the total antioxidant status was significantly higher (p < 0.01) than the resting value in the H trial. **CONCLUSION**: Our findings indicate that H and SPD consumed before and during exercise elicited similar effects on running performance, glucose metabolism and total antioxidant status during prolonged running in the heat. Therefore, Acacia honey can be used as an alternative ergogenic aid for athletes who train and compete in a hot and humid environment.

Keywords

Blood glucose, Acacia honey, antioxidants, running performance in the heat.

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## INTRODUCTION

Carbohydrate is an important source of energy in moderate to high-intensity exercise.<sup>1</sup> Athletes are often advised to consume adequate carbohydrates before and during exercise and replenish the carbohydrate stores immediately after exercise.<sup>2-3</sup> Carbohydrate intake has been shown to delay muscular fatigue during prolonged exercise and enhanced power output.<sup>3-4</sup> In general, it is recommended that athletes consume sports beverages containing 6-8% carbohydrate to optimise gastric emptying and fluid absorption during exercise.<sup>5</sup>

Honey is a natural supersaturated sugar that is primarily composed of a complex mixture of carbohydrates.<sup>6</sup>

The primary carbohydrates present in honey are monosaccharides glucose ( $\sim$ 30–35%) and fructose ( $\sim$ 35–40%) depending on floral, geographical, entomological, seasonal, and environmental factors.<sup>7</sup> Previous studies have recommended that simultaneous consumption of carbohydrates from multiple sources such as glucose and fructose with a ratio of 2:1 (glucose: fructose) might increase the oxidation capacity by up to  $\sim$ 75% higher performance during prolonged exercise<sup>8</sup> compared to consuming an equivalent dose of pure glucose.<sup>2,9</sup> Therefore, given the natural variation in the composition of carbohydrates, honey might have a potential to be an energy source for athletes or people who exercise.

The honey used in the present study is Acacia honey. Acacia honey is a Malaysian local product produced by *Apis Mellifera*, cultured bees harvesting the extrafloral nectar of *Acacia Mangium* trees.<sup>10</sup> Acacia honey has a comparatively high concentration of fructose and a lower glycaemic index than other honey types.<sup>11-12</sup> Thus, Acacia honey does not crystallise easily due to the high concentration of fructose.<sup>12</sup> This allows it to stay in a liquid form much longer than other kinds of honey. Furthermore, the lower glycaemic index of Acacia honey might induce a lower insulinemic response and maintain blood glucose concentrations for a much longer duration during exercise, which is ideal for endurance sports as pre - and during exercise nourishment.<sup>13-15</sup>

In contrast, there are some conflicting findings on the disadvantage of consuming high glycaemic index carbohydrates, including most commercially available sports drinks, pre- and during prolonged strenuous exercise.<sup>16-17</sup> It has been suggested that consumption of high glycaemic index carbohydrates pre- and during exercise elicited a hypoglycaemic response or a sharp decline in the blood's glucose concentrations.<sup>13,18-19</sup>

Theoretically, this might be due to the surge of insulin secretion in conjunction with enhanced muscle glucose uptake and reduced liver glucose secretion. For example, better maintenance of blood glucose concentration was observed during the second half of simulated soccer match-play when an 8% solution of low glycaemic index isomaltose (GI: 32) was consumed during the pre-exercise warm-up and at half-time, if compared with an equivalent volume of high glycaemic index maltodextrin (GI: 90 - 100).<sup>13</sup> This finding suggested a potential role for a low glycaemic index carbohydrate such as Acacia honey as a carbohydrate source for athletes involved in intermittent team sports. This type of carbohydrate could release glucose slowly from the gut into the bloodstream throughout the exercise session.

It was reported that protein honey shakes sustained blood sugar for over 2 hours following exercise compared to a placebo trial.<sup>20</sup> Honey also elicits mild increases in blood sugar and insulin, demonstrating that honey could be an effective pre-workout energy source.<sup>21</sup> Additionally, consumption of honey has been shown to increase power and speed during a cycling performance test compared to dextrose, suggesting that honey may serve as a useful alternative form of carbohydrates for athletes.<sup>22</sup> Yosef & Shalaby (2010) demonstrated that honey intake resulted in a lower skin temperature and improved blood glucose among wrestlers.<sup>23</sup> In this regard, exercising in the heat causes profound thermoregulatory, cardiovascular, and metabolic changes that could impair performance and lead to heat injuries.<sup>24</sup> Therefore, a study on the consumption of honey drinks during exercise in a hot and humid environment is warranted.

Free radical production has been shown to increase with prolonged and strenuous exercises.<sup>25</sup> Free radicals have been postulated to be linked with muscular fatigue and reduced exercise performance.<sup>26</sup> Antioxidants may play an essential role for the sportspersons to enhance their performance by reducing the deleterious effect of free radicals.<sup>27</sup> Honey has strong antioxidant properties, which can help eradicate free radicals and alleviate oxidative stress.<sup>28</sup> The antioxidant activity of honey is attributed to its content of flavonoids and phenolic compounds.<sup>29</sup> A recent study demonstrated that supplementation of bee bread at a dosage of 20g daily for 8 weeks improved running performance and increased the total antioxidant status among recreational athletes.<sup>30</sup>

Two recent systematic reviews demonstrated that honey could provide multiple transportable carbohydrates recommended for endurance athletes to improve performance.<sup>15,31</sup> Moreover, honey exhibits natural antioxidant properties that could provide a balance between controlling the immunosuppressive response to exercise and maintaining the signalling pathways necessary for positive training adaptation. However, to the best of our knowledge, the potential beneficial effect of acute Acacia honey consumption at pre- and during endurance running has not been reported yet. Therefore, this study investigates the effects of Acacia honey drink consumed before and during exercise on glucose metabolism, total antioxidant status, and running performance in the heat.

## MATERIALS AND METHODS

## **Study Design**

This study employed the single-blind, randomised crossover design. The participants were provided with either a honey drink or a commercial sports drink as a supplement. As the randomisation and distribution procedure was conducted by a laboratory technologist, the researcher was not aware of the specific type of drinks consumed by each participant during each actual experimental trial. The participants then performed 2 separate experimental trials. The washout period between these supplements was one week.

## **Study Participants**

Ten male recreational athletes aged between 20-30 were recruited as participants. Each participant was briefed about the experimental design and protocol and the possible risk before giving their written informed consent. They were also given a personal details form to confirm their overall health status. The inclusion criteria for the participants were, 1) aged between 20-30, 2) have exercised at least twice per week for more than 30 min per session before the study, 3) healthy with no common chronic illnesses and not under any medication prescription, 4) non-smokers and did not consume any type of honey supplement prior to the study, 5) able to run at 60% VO<sub>2max</sub> for at least 60-min in a hot and humid environment. The experimental protocol was approved by Human Research Ethics Committee, Universiti Sains Malaysia (USMKK/PPP/JEPeM[228.3(0.3)]).

## **Beverages**

The beverages used in the present study comprised an Acacia honey drink and a commercial sports drink which contains 27kcal energy, 6.8g carbohydrate, 42mg sodium,

running 19mg potassium, 4mg calcium, 40mg chloride, and 17 mg phosphate for every 100mL.

Table 1: Results of Acacia honey analysis (Analysis Laboratory, Department of Molecular Medicine, University Malaya, 2010)

Acacia Honey	Per 100g
Energy (kcal)	302
Carbohydrate (g)	75.0
a) Fructose (g)	31.2
b) Glucose (g)	22.9
c) Sucrose (g)	9.9
d) Maltose (g)	3.3
Protein (g)	0.1
Fats (g)	0
Cholesterol (mg)	0
Sodium (mg)	13
Dietary Fiber (g)	0
*(Ratio of Fructose/ Glucose $(F/G) = 1.36$ )	
Ratio F/G for pure Malaysian honey is within the range of 0.90-1.35 (Blossom honey)	

To ensure that the honey drink contains the equivalent amount of carbohydrates to the sports drink, 100mL of the honey drink should contain 6.8mL of honey (6.8% concentration of CHO in the honey drink).

## i) Volume of honey drink one hour before the running trial.

500 mL of honey drink was consumed one hour before running trials.

## ii) Volume of honey drank during running trials

3 mL of honey drink per kg of body weight were consumed at 20-min intervals throughout 60-min of running.

#### **Procedures**

#### **Experimental Design**

The present study started with participants' recruitment, followed by preliminary tests, familiarisation trials, and actual trials. The time interval between preliminary tests, familiarisation trials, and actual trials was one week. Then, the 2 actual trial sessions were performed with each participant consuming either the honey drink or sports drink during exercise. Both trial sessions were carried out at a one-week interval.

## i. Preliminary Tests

The preliminary tests consisted of sub-maximal and maximal oxygen uptake (VO<sub>2max</sub>) tests. The participants performed two separate tests on a motorised treadmill, i.e. i) a 16-min incremental sub-maximal test for establishing the relationship between running speed and oxygen uptake (VO<sub>2</sub>), and ii) an uphill incremental maximal oxygen uptake (VO<sub>2max</sub>) test where the participants have to run until exhaustion to determine the participants' VO<sub>2max</sub>. Based on the results of these two tests, the running speed, which elicited 50% VO<sub>2max</sub> and 60% VO<sub>2max</sub> for each participant, was identified to determine the appropriate running intensity for the actual experimental trials.

The  $VO_{2max}$  test was terminated if the participant could not continue running despite verbal encouragement.  $VO_{2max}$  value of each participant during this test was accepted when 2 of 3 criteria were met: 1) maximum heartbeat is within 10 beats.min<sup>-1</sup> of their respective agepredicted maximum heart rate, 2) a plateau in oxygen uptake despite the increasing intensity, and 3) respiratory exchange ratio more than 1.15.<sup>32</sup>

### ii. Familiarisation Trial

Prior to the actual experimental trials, the participants were required to run in the heat at 60% VO<sub>2max</sub> for 60min to familiarise them with the endurance testing protocol in the heat (31°C, 70% relative humidity) and immediately followed by a 20-min time trial. The participants were required to cover as much distance as possible within 20-min. The heated environment was produced in an improvised climatic chamber. During the trial, halogen lamps (Philips-500W, France) were used to raise the ambient temperature to 31°C, the relative humidity was set at 70%, and a heated water bath (Memmet W350t, Germany) was placed in the chamber.

The room temperatures and relative humidity were constantly measured throughout the trial using a digital psychrometer (Extexch Instruments RH300, China). In addition, a standing fan was placed and directed to the participants during the trial to mimic airflow in an open environment.

#### iii. Actual Experimental Trials

Actual experimental trials were carried out after familiarisation trials. After arriving at the Sports Science Laboratory of Universiti Sains Malaysia, the participants' body height and weight were measured using a stadiometer (Seca 220, Germany) and a weighing scale (Tanita, TBF-410, Japan) respectively. At one hour before the warm-up, the participants were cannulated for repeated blood withdrawals during the trials. Then, the participants consumed 500 mL of either cool (~8°C) honey or sports drink. Participants rested for an hour before the commencement of the experimental trials, wherein they were required to run in the heat (31°C) with a relative humidity of 70%.

The participants' blood samples were collected again immediately before the warm-up. The participants warmed up for 5 min at 50% of their respective VO<sub>2max</sub>. Blood samples were also collected during the final minute of the warm-up. Then, the running intensity was increased to 60% of their respective VO2max. Blood samples were collected at intervals of 20-min during the trials. Following each blood withdrawal, participants were required to consume 3 mL.kg body weight-1 of either the cool honey drink or the sports drink. The 20-min running time trial was carried out immediately after 60-min of running at 60% of VO2max.33. The longest distance covered in 20-min was used to indicate endurance running performance. During the actual experimental trials, the participants were required to drink the beverage at five different time points, resting, post-warm-up, 20, 40, and 60-min of running. As this study is a randomised cross-over study, the participants were required to return to the lab 7 days after the first trial for their second trial. The second trial was similar to the first trial, except for the drink consumed by the participants.34

#### Blood sample collection and analysis

During the experimental trials, 4 mL of blood was

withdrawn from the participants at 7 different time points, specifically at resting, immediately after warm-up, and every 20-min during the trials and at the end of the time trial. Blood parameters measured in the present study were blood glucose, cortisol, insulin, and total antioxidant status.

Two mL of the blood sample was transferred into the sodium fluoride (NaF) anticoagulant tubes to determine the plasma glucose, insulin, and cortisol concentration. The remaining 2 mL blood samples were transferred into plain tubes for serum separation. The blood was centrifuged using Ratina 46 RS refrigerated centrifuge (Hettich Zentrifugen GmbH, Germany) at a rotational speed of 4000 revolutions per min at 4°C for 10 minutes to separate the plasma from the blood. The plasma was then pipetted into a test tube in equal proportions and stored at -80°C (Thermo Forma -86 ULT Freezer, USA) for subsequent analysis of plasma glucose, cortisol, and insulin. Another 2 ml of blood was transferred into a plain tube and separated by centrifugation. The serum was stored at -80° C for the analysis of the serum total antioxidant status.

Plasma glucose concentration was determined using a chemistry analyser (Selectra E, The Netherlands) and a glucose reagent kit (Randox, United Kingdom). Plasma cortisol and insulin concentrations were determined using electrochemiluminescence immunoassay 'ECLIA' kits (Roche, USA). Total antioxidant power ELISA kit (Oxford Biomedical Research, USA) was used to determine the concentration of the total antioxidant status.

## **Statistical Analysis**

All statistical analyses were performed using Statistical Package for Social Sciences (SPSS version 22). Descriptive statistics were used to calculate the mean  $VO_{2max}$  and maximum heart rate. The differences in the time trial performance and serum TAS between the trials with honey and sports drinks were analysed using a paired t-test. The two-way repeated measure ANOVA with the Post hoc Bonferroni test was used to determine the differences in plasma glucose, insulin, and cortisol

concentrations between the honey and sports drink trials at each time point and the changes in these parameters over time. Two-way repeated measure ANOVA was also used to determine the differences in total antioxidant status at pre- and post-exercise between trials. The statistical significance was set at p < 0.05. Results are expressed as means  $\pm$  and standard deviations (SD).

## RESULTS

# Physical and physiological characteristics of the participants and endurance running performance

The participants' physical characteristics and physiological capacities (n=10) are presented in **Table 2**. There was no significant difference in the endurance running performance between the trials with a sports drink (SPD) and honey drink (H). The mean running distance covered during the SPD and H trials in 20-min was  $3.25\pm0.4$  and  $3.34\pm0.3$  km, respectively.

Table 2: Physical characteristics and physiological capacities of the participants  $(n\!=\!10)$ 

Parameters	Mean ± SD
Age (years)	$22.3 \pm 1.3$
Body Weight (kg)	$63.2 \pm 5.7$
Standing Height (cm)	$168.7 \pm 2.6$
Maximum heart rate (HRmax)	$197.7 \pm 1.3$
VO <sub>2max</sub> (ml.kg <sup>-1</sup> .min <sup>-1</sup> )	$51.4 \pm 4.2$
Body fat percentage (%) Body mass index (BMI)	$18.7 \pm 3.9$ 22.2 ± 1.9

Data are expressed as mean (SD). VO<sub>2max</sub> = maximum oxygen uptake.

## Endurance running performance

There was no significant difference (p=0.120) in endurance running performance between the trials with a sports drink (SPD) and the honey drink (H). The mean distance running covered during the SPD and H trials in 20-min was  $3.25\pm0.4$  and  $3.34\pm0.3$  km, respectively.

## Plasma glucose

There was no significant interaction in plasma glucose concentration (p=0.845) between SPD and H trials (**Fig. 1A**). However, during the 1-hour resting phase, plasma

glucose concentration increased non-significantly in the SPD trial but not in the H trial. Plasma glucose decreased at the onset of exercise before increasing after 40-mins until the end of both trials, despite the lack of statistical significance. At the end of the trials, the plasma glucose concentration of SPD and H trials was  $6.0\pm0.4$  and  $6.0\pm0.5$  mmol.L<sup>-1</sup>, respectively.

## Plasma insulin

During the trials, it was observed that there was no significant interaction between trials on plasma insulin concentration (p=0.742). (Fig. 1B). However, a significant main effect of time on plasma insulin concentration during SPD trials (p<0.001) was observed. The plasma insulin increased significantly during the 1-hour resting phase of the SPD trial, but not in the H trial. At the end of the time trial, the plasma insulin level was significantly

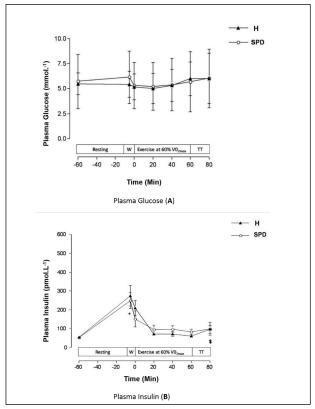


Figure 1: Plasma glucose concentrations (mmol. L<sup>-1</sup>) (A) and plasma insulin concentrations (pmol. L<sup>-1</sup>) (B) in sports drink (SPD) and honey (H) trials (Mean  $\pm$  SD)

W= Warm up, TT= Time Trial.

 $^{+}$  plasma insulin significantly different from respective resting value at -60 min in SPD trial (p < 0.05).

 $^{\rm $$}$  plasma insulin significantly different from respective resting value at -5 min in SPD trial (p < 0.01).

#### Plasma cortisol

There was no significant interaction in the plasma cortisol concentration at each time interval between trials (p=0.589) (Fig. 2). However, it was revealed that there was a significant main effect of time on plasma cortisol concentration during the H trial (p<0.001). During the H trial, the plasma cortisol concentration was significantly lower at 40-min compared to its resting value. There was a decreasing trend in plasma cortisol from resting to the beginning of warm-up in both the trials despite the lack of statistical significance. In both trials, plasma cortisol decreased from 0 min to 40-min. Then, it increased from 40-min until the end of the time trial, despite the absence of statistical significance. At the end of the trial, the plasma cortisol concentration for SPD and H trials was 505.0  $\pm$ 27.3 and 530.2 $\pm$ 32.6 nmol.L-1 respectively.

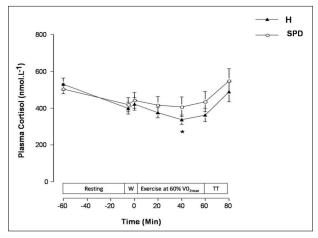


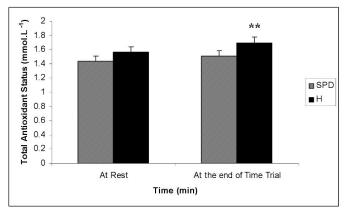
Figure 2: Plasma cortisol concentration (nmol.L-1) in sports drink (SPD) and honey (H) trials (Mean  $\pm$  SD).

W= Warm-up, TT= Time Trial.

\* significantly different from respective resting values at -60 min in H trial (p < 0.05).

## Serum Total Antioxidant Status

Two-way ANOVA with repeated measures revealed that there was no significant interaction on serum total antioxidant status between both trials (p=0.450). However, during the H trial, there was a significantly higher level of serum TAS at the end of time trial compared to its resting value (p<0.01) (**Fig. 3**). The serum TAS at the end of the SPD and H trials was  $1.57\pm0.08$  and  $1.69\pm0.09$  mmol.L<sup>-1</sup>, respectively.



**Figure 3**: Total antioxidant status (mmoL<sup>-1</sup>) for honey (H) and sports drink (SPD) trials at rest and at the end of the time trial (Mean  $\pm$  SD).

\*\*significantly different from respective resting values in the H trial (p<0.01 respectively).

## DISCUSSION

A notable finding in the present study is that the honey drink elicited a similar effect as a sports drink on running performance since there was no difference between distances recorded during the 20-min time trial. Furthermore, given that honey contains multiple sources of carbohydrates (i.e., primarily fructose and glucose), this natural substance may theoretically exert positive effects as a "food-first" approach for carbohydrate supplementation. Thus, consuming the Acacia honey drink has similar endurance running performance as commercial sports drinks in the present study. It is believed that the same concentration of carbohydrates in these two drinks may have caused similar effects on the running performance.

Several studies examined the influence of acute honey supplementation on exercise or sports skill performance during team sports<sup>35</sup>, running<sup>14</sup>, and cycling<sup>36</sup>. However, findings were inconsistent, the these whereby performance benefits could be observed when honey is compared to placebo or plain water.14,36 A previous study examined people consuming Acacia honey solution during a 2 hr recovery period to optimise restoration. It was found that rehydration with a honey drink after 150% of body mass loss following a 60-min run in the heat improved the subsequent 20-min running time trial performance compared to a trial with plain water.14 Another study reported no significant differences between honey or dextrose treatment for the time taken to

complete a 64km cycling time trial. However, consuming 15 g of either honey or dextrose in a gel form, every 16 km maintained the average 16 km time throughout the exercise and increased the average power output during the final 16 km compared with the preceding 16 km segments.<sup>36</sup> In contrast, when compared to placebo, the finishing time was quicker in both carbohydrate treatments, and the cycling performance in the last 16km significantly declined when placebo was consumed.<sup>36</sup>

Since the present study revealed no significant difference in distance covered during 20-min time trial performance with honey supplementation compared to sports drinks, the finding suggests that honey could be a useful form of carbohydrate for endurance athletes. Therefore. consuming honey pre and during exercise might serve as an effective source of carbohydrates to enhance performance. Nevertheless, further study with different types of honey is recommended to investigate the effects of honey supplementation on exercise performance because the actual composition of honey varies, depending on many factors such as the pollen source, climate, environmental conditions, and the processing it undergoes.

The absence of a significant difference between plasma glucose concentration during exercise in the heat between SPD and H trials indicated that the honey and sports drink might have a similar effect on maintaining blood glucose concentration when exercising in the heat. This observation is consistent with findings from other studies that reported no significant differences in the blood glucose level following supplementation with carbohydrate-electrolyte drink regardless of the number of carbohydrates and types of carbohydrates consumed.<sup>37-38</sup>

However, as shown in Fig. 1A, there was an increasing trend in plasma glucose one hour before exercise after sports drink consumption compared to honey drink. It could be speculated that the high glycaemic index of the commercial sports drink might induce the increasing trend in plasma glucose when consumed one hour before the running trial.<sup>39-40</sup> In line with the previous findings, the high glycaemic index foods ensued in higher blood glucose concentrations than the low glycaemic index

foods if consumed one hour before cycling<sup>41</sup>, running<sup>42</sup>, and simulated soccer match.<sup>43</sup>

At the onset of exercise (Fig. 1A), a rapid fall in plasma glucose was recorded, which might be due to higher muscle glucose uptake with the onset of exercise. However, it started to increase after 20-min and was maintained throughout the exercise. After 1 hour of exercise, the blood glucose concentration was higher in the honey trial than in the sports drink trial, even though it was not statistically significant. This could be because the low glycaemic index honey drink consumed by the participants only showed its potential to increase plasma glucose at a later stage of exercise. This might also be due to the utilisation of stored endogenous glucose in the liver, contributed by the earlier consumption of honey during the later stage of the exercise.

glucose and insulin concentrations Plasma are interrelated. The results of plasma insulin concentrations implied that both H and SPD had similar effects on insulin regulation in the participants. However, consuming a sports drink one hour before exercise caused a significant increase in insulin from the baseline level, while there was no significant increase after consuming the honey drink. This finding demonstrated that the insulin response in high glycaemic index drinks is higher than in low glycaemic index drinks. In this regard, the higher carbohydrate oxidation might trigger a higher quantity of insulin released one hour before exercise during the SPD trial. This metabolic finding is in agreement with previous works.43-44 With the increase in insulin (Fig. 1B), the glucose concentration in the blood was maintained (Fig. 1A) after consuming sports drinks. Even when blood glucose levels are normalised, insulin levels can remain high because the liver might not be able to remove the circulating insulin fast enough.45 These phenomena can be observed in the H trial, where the plasma glucose concentration was normalised (Fig. 1A) during warm-up, but the insulin concentration (Fig. 1B) remained high in the H trial.

Subsequently, the study observed a decreasing level of plasma insulin during the 1 hour run in both trials. The decline of plasma insulin during an exercise session is consistent with findings from other studies.<sup>44</sup> It is postulated that this decrease in plasma insulin concentrations could be necessary for preserving blood glucose for storage or utilisation.<sup>43-44</sup> Even though the insulin levels were not significantly different between H and SPD trials at any time point during the 1-hour run, it was shown that consuming low glycaemic index foods such as Acacia honey caused a lower insulinemic response compared to higher glycaemic food such as sports drink (Fig. 1B).

The results of plasma cortisol concentration during exercise between SPD and H trials imply that the honey drink and sports drink have exerted a similar effect on plasma cortisol concentration during exercise. The consumption of honey and sports drinks stimulated a similar stress response to exercise during the trials. The plasma cortisol levels were slightly maintained throughout the 60-min of exercise. This phenomenon is consistent with the previous observation that following the consumption of carbohydrates during prolonged cycling, running or resistance exercise reduced the cortisol level.46-47 The present study finding implies that the consumption of carbohydrates during exercise could reduce stress hormone levels. Moreover, the increase of cortisol at the end of both trials could be attributed to the stress and the high-intensity exercise48 where the participants were exerted to run the furthest distance possible during the time trial.

This study indicates that there was no difference between the serum TAS levels in both trials. This finding is similar to Keong et al. (2006), who reported that TAS was not significantly different between the group with tocotrienolrich palm vitamin E-supplement and the group with a placebo supplement.<sup>48</sup> The resting serum TAS recorded immediately before exercise did not demonstrate any significant difference between trials, indicating that the participants have similar TAS in their bodies before the experimental trials. At the end of the time trial, it was observed that there was also no significant difference between trials. However, TAS in the H trial at the end of the trial was significantly higher (p<0.01) than the resting value. This could be due to the antioxidant properties of Acacia honey. It has been shown that activities of antioxidant enzymes increase as a response to prolonged 2. exercise, consequently increasing the TAS. TAS is a combination of various antioxidant defenses, including enzymatic and nonenzymatic systems. As shown in this 3. present study (Fig. 3), TAS levels increased after an exhaustive exercise, and this antioxidant response to exercise is well documented.<sup>49-50</sup> This present study 4. concludes that the antioxidant properties of the Acacia honey might have contributed to the increased TAS level.

## CONCLUSION

The present study suggests that Acacia honey drink could be used as an alternative ergogenic aid before and during exercises. This is because the honey drink elicited similar positive effects on glucose metabolism, total antioxidant status, and running performance as sports drinks, which are deemed as an effective aid for enhancing endurance sports performance of athletes in a hot and humid environment.

## **CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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