Effects of soybean co-ingestion with carbohydrate on postprandial glycaemic, insulinaemic and reactive oxygen species in healthy men: A pilot study

Albert Tan Yi Wey a, Azlina Abdul Aziz b, Mohamad Shariff A Hamid c, Kuo Chia-Hua d, Sareena Hanim Hamzah ab,*

a Centre for Sport and Exercise Sciences, University of Malaya, 50603 Kuala Lumpur, Malaysia
b Department of Molecular Medicine, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia
c Sports Medicine Unit, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia
d Institute of Sports Science, University of Taipei, Taipei City 11153, Taiwan.

* Corresponding author: sareena@um.edu.my

ABSTRACT

Postprandial hyperglycaemia induced by excessive intake of high carbohydrate (CHO) foods and beverages promotes oxidative stress which can cause many health risks such as cardio-metabolic diseases and diabetes. Protein when co-ingested with CHO beverages, has been shown to lower the postprandial glycaemic and insulinaemic, which may help to attenuate postprandial reactive oxygen species (ROS) and oxidative stress. Soybean contains isoflavone which may provide potential benefits in regulating postprandial glucose and insulin levels as well as providing protection against ROS production. The aim of this study was to investigate the effects of soybean added CHO beverage on postprandial glycaemic, insulinaemic and reactive oxygen species responses in healthy men. Eight male [age 20.0 (1.2) years, body weight 59.2 (6.2) kg] consumed 500 ml of CHO added with soybean (SOY + CHO), CHO added with whey protein (WHEY + CHO) and CHO alone (Control) after an overnight fast, in a randomized counterbalanced order crossover design, separated by a one week period. Venous blood samples were collected after overnight fast (baseline) and at 30, 60, 90 and 120 min after consumption of the beverage. The mean area under the insulin curve was lower significantly between all beverages in all parameters. Soybean-based beverage may yield lower effect on postprandial ROS suggesting lower oxidative stress due to lower insulinaemic responses, compared to whey protein when co-ingested with CHO.

Keywords: Soybean; oxidative stress; postprandial; reactive oxygen species; glucose

INTRODUCTION

Trends in food consumption have undergone changes, largely attributed to factors such as income, urbanization, trade liberalization, food industry marketing and consumer attitudes and behaviour [1]. These coupled with the modern fast-paced lifestyle had attributed to the shift in nutrition transition from consuming natural food source to a higher calorie content food [1]. Most processed foods are highly-glycaemic index carbohydrate (CHO) that promote fast release of glucose into the blood [1]. Regular consumption of high-CHO foods may cause hyperglycaemic effects, which over time can lead to the obesity and diabetes [1]. Excessive use of easily digestible foods and beverages contributed to excessive blood glucose surges [2]. This surge of energetic substrate overwhels the metabolic capacities of mitochondria in the muscle with high acute glycerol concentration [2]. Glucose may flood the Krebs cycle, stimulating excess development of the reduced form of nicotinamide adenine dinucleotide (NADH) which may exceed the oxidative phosphorylation ability of the mitochondria and contributes to the transition of single electrons to oxygen, producing free radicals such as superoxide anion [3]. This postprandial oxidative stress can cause acute atherogenic changes, including increased oxidation of low-density lipoproteins, sympathetic tone, vasoconstriction and thrombogenicity [3].

Hyperglycaemia spikes artificially caused by infusions of intravenous glucose in lean nondiabetics have been shown to significantly increase free radical generation [4]. Hyperglycaemia has been associated with many health complications such as impairment of cardiovascular [5], renal [6], brain microvascular [7,8], nervous system, pancreas [9] and respiratory muscles [10] function. Moreover, hyperglycaemia has been shown to induce reactive oxygen species (ROS) which increases inflammation and apoptosis of the pancreatic β cells by targeting the NAD-dependant protein deacetylase sirtuin 1 also known as SIRT1 [8]. Chronic exposure to hyperglycaemic condition can cause deterioration of the pancreatic β cells [9], possibly leading to insulin resistance.

The addition of protein to CHO has proved to be an essential part of the cardioprotective and anti-inflammatory diet. Dairy protein such as whey protein has been claimed to provide the benefits of reducing
oxidative and inflammatory markers in both animal and human studies. In a study conducted on streptozotocin-induced diabetic rats, the biomarkers for oxidative stress such as malondialdehyde (MDA), nitrate oxide (NO) and ROS and pro-inflammatory cytokines such as interleukin-1β (IL-1β), tumour necrosis factor-α (TNF-α), IL-6 and IL-4 were reduced after 100 mg/kg whey protein was administered [11]. In healthy individuals, the ingestion of whey protein with pure glucose drink showed a 56% reduction in the post-prandial blood glucose area under the curve and a 60% increase in insulin response [12]. While in patients with type 2 diabetes, the co-ingestion of casein protein with glucose and maltodextrin showed similar findings in which glucose was decreased by 23% and insulin response increased by more than 90% [13]. This may help to attenuate postprandial ROS and inflammation. To date, there is scarcity of studying CHO-protein beverage using a non-dairy protein such as soybean.

The soybean or soya bean, also called Glycine max is a domestic plant in Asia countries and is a member of the leguminosae family, plants that form root nodules that house symbiotically nitrogen-fixing soil bacteria (Rhizobia). A raw soybean contains all the three macronutrients and essential micronutrients required for good nutrition. It contains 33.8% protein, 25.5% carbohydrate, 18.9% fat, 11.5% water or moisture and 5.5% dietary fibre as well as 4.8% in total for both vitamins and minerals (Nutrient Composition of Malaysian Food, NutriPro Database). Soybean provides all eight essential amino acids along with a complex array of phytochemicals that can provide significant long-term health benefits [14]. One of the phytochemicals present in soybean is a polyphenolic compound known as isoflavone. Isoflavones have high antioxidant properties and demonstrated a reduction in oxidative stress and inflammatory markers [15]. Soybean supplementation in diabetic patients has been shown to improve blood glucose and serum lipid levels [16,17]. However, it is unknown whether the ingestion of CHO with soybean could reduce the hyperglycaemic effects in physically active and healthy men and thus promote the attenuation of postprandial ROS and inflammation. The aim of this preliminary study was therefore to investigate the effects of soybean added CHO beverage consumption on postprandial glycaemic, insulinemic and ROS responses in healthy men.

**EXPERIMENTAL**

**Participants**

Eight healthy men aged 18 and 25 years old were recruited from higher learning institutions around Lembah Pantai, Kuala Lumpur. The participants were recreationally active individuals who exercise at least three times per week, not consuming any form of dietary supplements and do not have any metabolic disorders. This study was carried out with the approval of the University of Malaya Research Ethics Committee (UM.TNC2/RC/H&E/UMREC-115) and all participants gave written consent.

**Study design**

Each participant, in randomized counterbalanced order, participated in three dietary intervention trials lasting for 3 hours each. The participants consumed 500 ml of either CHO added with soybean (SOY+CHO), CHO added with whey protein (WHEY+CHO) or CHO alone (Control), after an overnight fast separated by a washout period of one week between trials as described by previous studies [12,18]. The participants were asked to refrain from any intense physical activity and from taking any medication and food rich in antioxidants 24 h prior to each intervention trial.

**Experimental protocol**

Participants arrived at the Sports Nutrition laboratory after a 12-hour overnight fast, at approximately 0830 hours. Their body weight, fat percentage (% fat) and fat free mass (FFM) were measured using Bioimpedance Analysis (InBody, USA) while height was measured using a portable stadiometer (SECA, Germany). Participants were asked to rest for 10 minutes in a seated position until the baseline blood sample was taken. Thereafter, the participants consumed the SOY+CHO, WHEY+CHO or CHO beverage, within 10 minutes. Blood samples were obtained at 30-, 60-, 90- and 120-minute after the beverages were consumed using the same blood sampling procedure. The participants remained within the Sports Nutrition laboratory during the duration of the test, performing only sedate behaviour like sitting, reading and studying.

**Experimental beverages**

The 500 ml SOY+CHO beverage contained 2% (10g) soybean, 4% (20g) rice, 4% cane sugar (20g), the 500 ml WHEY+CHO beverage contained 2% (10g) whey protein concentrate, 4% (20g) rice, 4% (20g) cane sugar while the CHO only (Control) beverage consists of 6% (30g) rice and 4% (20g) cane sugar. The formulated iso-caloric SOY+CHO, WHEY+CHO and CHO only beverages which provided 199 kcal, 192 kcal and 196 kcal respectively, were prepared by the researcher prior to the experimental trial.

**Blood collection and plasma preparation**

Blood samples were collected through a cannula (G-15, Venflon) that was inserted into the participant’s antecubital vein and transferred into 6 ml heparinised and EDTA tubes (BD vacutainer), and centrifuged at 3000 RPM for 15 min at 4° C. After centrifugation, plasma aliquots were transferred into labelled Eppendorf tubes and stored at -80°C prior to glucose, insulin and ROS analysis. Blood samples were taken at 30-, 60-, 90- and 120-minute after consuming the beverages. To ensure cannula patency, the cannula was flushed with heparinised saline after each blood sampling.

**Analysis of plasma glucose, insulin and ROS**

Postprandial plasma insulin was measured using commercially available enzyme-linked immunosorbent assays (ELISA) kit (Insulin ELISA Kit, LDN, Germany) and plasma glucose was determined using ADVIA 2400 Analyzer (Siemens, Germany). Analysis was carried out according to instructions from the manufacturer.

For ROS analysis, plasma (5 µl) was mixed with 100 µl of 100 µM 2,7-dichlorofluorescein diacetate in a black 96-well plate. The mixtures were shaken on a shaker for 1 minute and were incubated for 30 minutes in a water bath (37°C). The reading of fluorescence was taken with the wavelengths of excitation and emission set at 485 and 530 nm, respectively using Multiplex ELISA System (Bioplex 200, Bio-Rad, USA). All results were expressed as relative fluorescence unit.

**Statistical analysis**

Data were expressed as mean ± standard error mean (SEM) except where otherwise indicated. The Shapiro-Wilk test was used to assess the distribution of data normality. All statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS) Version 22.0 (SPSS, Inc., Chicago, IL). The area under the curve (AUC) for glucose, insulin and ROS were calculated using the trapezoidal formula and the differences between SOY+CHO, WHEY+CHO and CHO trials were compared using one-way repeated measures analyses of variance (ANOVA). Two-way mixed ANOVA (Mixed Between-Within Subjects) was performed on all trials to determine group and time differences. Significant main effects and interactions were further analysed using Tukey’s post hoc test. Differences were considered significant if $p < 0.05$. The sample size was estimated using G-Power version 3.1.9.2 based on results from a study by Chang et al (2008). The G-Power indicated that a minimum sample of 7 produced 95% confidence level with effect size of $f = 1.10$, $\alpha = 0.05$ and $\beta = 0.80$.
RESULTS AND DISCUSSION

Participants

All eight participants completed the experiment. The physical characteristics of the participants are presented in Table 1.

Table 1 Physical characteristics of the participants.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>20.3 (1.2)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>171.3 (5.0)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>59.5 (6.2)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>20.2 (1.4)</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>13.9 (2.2)</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>28.8 (3.4)</td>
</tr>
</tbody>
</table>

Values are mean (SD) (n=8). BMI = body mass index; FFM = fat free mass.

Area under the curve (AUC)

The mean AUC₀₋₁₂₀ for glucose, insulin and ROS over 120 minutes after the consumption of SOY+CHO, WHEY+CHO and CHO beverages are presented in Table 2. The AUC₀₋₁₂₀ for postprandial glucose was higher in SOY+CHO trial compared to WHEY+CHO and CHO trials. The difference in the AUC₀₋₁₂₀ between trials however was not statistically significant (p = 0.253). SOY+CHO showed a lower AUC₀₋₁₂₀ for insulin compared to WHEY+CHO and CHO, however there were no significant differences among all trials (p = 0.956). The AUC₀₋₁₂₀ for ROS was lower in SOY+CHO than WHEY+CHO and CHO. No significant difference was observed among all beverages (p = 0.677).

Table 2 Mean Area under the curve (AUC) for postprandial glucose, insulin and reactive oxygen species (ROS) after consumption of beverage consisting of carbohydrate added with soybean (SOY+CHO), carbohydrate added with whey protein (WHEY+CHO) and carbohydrate only (CHO).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control</th>
<th>SOY</th>
<th>WHEY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>589.1 ± 18.8</td>
<td>608.9 ± 27.9</td>
<td>548.1 ± 28.9</td>
</tr>
<tr>
<td>Insulin</td>
<td>3982.4 ± 742.0</td>
<td>3493.7 ± 381.5</td>
<td>3693.0 ± 863.5</td>
</tr>
<tr>
<td>ROS</td>
<td>7986.9 ± 824.4</td>
<td>7499.4 ± 478.6</td>
<td>8319.4 ± 611.9</td>
</tr>
</tbody>
</table>

Values are mean ± standard error of the mean (SEM) (n=8).

Postprandial plasma glucose, insulin and ROS concentrations after consumption of the beverages

The glucose, insulin and ROS concentrations at baseline and at 30-, 60-, 90- and 120-minute following SOY+CHO, WHEY+CHO and CHO consumption are presented in Fig. 1-3, respectively. There were no significant difference between all trials for glucose (F₆,₈₄=0.604, p = 0.772), insulin (F₆,₈₄=0.326, p = 0.954) and ROS (F₆,₈₄=0.326, p = 0.954).

The postprandial glucose responses within trials were significantly different (F₂,₂₄=29.488, p = 0.0001) (Fig. 1). Glucose concentrations in SOY+CHO and CHO trials significantly increased by 45.4% and 48.2% from baseline to 30 minutes followed by a significant decrease by 34.5% and 31.9% from 30 to 90 minutes, respectively after which the concentration remained the same until 120 minutes. Glucose concentrations in the WHEY+CHO trial decreased beyond the baseline level at the 60-, 90- and 120-minute time points. Glucose concentration in the WHEY+CHO trial was significantly lower in the 90- and 120-minute time point compared to the 30 min time point.

Fig. 1 Plasma glucose concentration before (baseline) and 30 min, 60 min, 90 min and 120 min after consumption of carbohydrate added soybean (SOY+CHO), carbohydrate added with whey protein (WHEY+CHO) and carbohydrate only (CHO) beverages. Values are mean ± SEM (n=8)

a significantly different (p<0.05) from baseline in SOY+CHO and CHO trials
b significantly different (p<0.05) from 30 min in SOY+CHO and CHO trials
c significantly different (p<0.05) from 30 min in WHEY+CHO trial

Postprandial insulin responses showed significant differences within trials (F₂,₂₄=23.829, p < 0.000) (Fig. 2). Plasma insulin concentrations in SOY+CHO, WHEY+CHO and CHO trials were significantly increased by 330.3%, 481.1% and 505.4%, respectively from baseline to 30 min. Thereafter, insulin concentrations significantly decreased from the 30 minute time point to the 60-, 90- and 120-minute time points in the SOY+CHO (59.2%, 85.5% and 82%) and CHO (69%, 88.5% and 85.9%) trials only. The WHEY+CHO trial showed a significant reduction in plasma insulin at the 90- and 120-minute time points (82.9% and 84.6%) compared to the 30-minute time point.

Fig. 2 Plasma insulin concentration before (baseline) and 30 min, 60 min, 90 min and 120 min after consumption of carbohydrate added with soybean (SOY+CHO), carbohydrate added with whey protein (WHEY+CHO) and carbohydrate only (CHO) beverages. Values are mean ± SEM (n=8)

a significantly different (p<0.05) from baseline in SOY+CHO, WHEY+CHO and CHO trials
b significantly different (p<0.05) from 30 min in SOY+CHO and CHO trials
c significantly different (P<0.05) from 30 min in WHEY+CHO trial
There are strategies that have been designed to modify postprandial glycemia to favour lower blood glucose and ROS response balanced of the risk glycation of proteins such as low resistance alteration in the signal uptake in activation of glucose transporter 4 (GLUT4) and subsequent glucose kinase (PI3K) oxidative damage to cellular components [20]. Insulin regulates blood systems are able to balance ROS production, hence preventing postprandial inflammation and endothelial impairment can cause oxidative stress [6,9]. Hyperglycaemia has been reported that consumption of soy beverage was able to increase ferric reducing antioxidant power (FRAP) activity 60 min post-consumption, although the increase was very small (approximately 3%) [34]. This preliminary study also suggested that high levels of ROS, as a result of high glycolysis in the pancreatic β cells, may reduce insulin secretion. However, this was not observed in our study. In this present study however, although not significant, there is a trend of lowered insulin response and increased protection against ROS for CHO with added soybean beverage.

This preliminary study indicated the potential benefits of soybean-based CHO beverage in regulating postprandial glucose and insulin levels as well as providing protection against ROS production and oxidative stress.

CONCLUSION

Soybean-based beverage may yield lower effect on postprandial ROS suggesting lower oxidative stress due to lower insulinemic responses compared to whey protein when co-ingested with CHO. These preliminary results suggest that soybean-based beverage can be potentially used as an alternative to whey protein when consumed with CHO beverage for a more cost-effective supplementation and diary sensitive individuals. Future studies looking into higher doses of protein and with higher numbers of participants may yield more significant results.

ACKNOWLEDGEMENT

This study was funded by grants from University of Malaya (project no: BK003-2016) and University of Taipei, Taiwan.

REFERENCES

Postprandial Hypertriglyceridemia and Hyperglycemia on Endothelial
Ceriello S. Yu Med W Manders weakness is mediated by oxidative stress.
and 1 pancreatic B cells by targeting SIRT1.
thioredoxin F 701 Busik Am on cardiovascular function: spotlight on glucose metabolic pathways.
Mapanga - 2013.
Effects of soybean product intake on fasting and postload
Diabetes - 312 312.
Acute hyperglycaemia enhances oxidative stress and aggravates myocardial ischaemia/reperfusion injury: role of thio-redoxin-interacting protein. Journal of Cellular and Molecular Medicine, 17(1), 181-191