INFLUENCE OF PRE-EXERCISE SOYBEAN-BASED BEVERAGE ON BIOCHEMICAL AND PHYSIOLOGICAL RESPONSES IN HEALTHY MEN

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CENTRE FOR SPORT AND EXERCISE SCIENCES
UNIVERSITY OF MALAYA
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INLUENCE OF PRE-EXERCISE SOYBEAN-BASED BEVERAGE ON BIOCHEMICAL AND PHYSIOLOGICAL RESPONSES IN HEALTHY MEN

ABSTRACT

Whey protein, when added with carbohydrate (CHO) beverage, has been shown to lower the postprandial blood glucose and insulin, which may help to attenuate postprandial reactive oxygen species (ROS) and inflammation. Whey added CHO beverage consumption also has shown to reduce oxidative stress, muscle inflammation and damage as well as improved exercise performance. Soybean contains isoflavones which may provide the possibility of having similar positive effects or better than whey protein when added with CHO beverage. The first study aimed to investigate the effects of soybean added CHO beverage consumption in comparison to whey added CHO beverage on postprandial glycemic, insulinemic and ROS in healthy men. Eight males [age 20 (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, separated by a one-week period. Venous blood samples were collected after an overnight fast (baseline) and at 30, 60, 90 and 120 min after consumption of the beverage. The area under the insulin curve was lower in SOY+CHO compared to WHEY+CHO. Similarly, SOY+CHO tended to have a lower postprandial ROS response than WHEY+CHO. However, no significant difference was observed between all beverages. The 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. The second study was to investigate the effects of pre-exercise SOY+CHO as compared with WHEY+CHO consumption on antioxidant activity, oxidative stress, muscle damage and inflammation, total cycling workload and fatigue perception post cycling at 85% VO_2peak and subsequent cycling in physically active males. In randomized

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counterbalanced order, seven physically active males [age 20 (0.9) years, peak oxygen consumption 49.3 (0.3) L/min] performed three cycling exercise at 85%VO_2peak after the consumption of 500 ml SOY+CHO, WHEY+CHO and CHO (Day 1) and repeated the same experimental protocol with the same trial on the next day (Day 2). On each trial, blood samples were collected after an overnight fast and at pre-exercise, post exercise and 1-hour post exercise after consumption of the beverage for the analysis of ferric reduced antioxidant power (FRAP), glutathione (GSH), oxidized glutathione (GSSG), glutathione ratio (GSH/GSSG), interleukin-6 (IL-6) and creatine kinase (CK). The rate of perceived exertion (RPE) and total workload (kilopond) were recorded at post exercise. There was no significant main effect between SOY+CHO and WHEY+CHO for FRAP, IL-6, GSH, GSSG, glutathione ratio, CK, total workload and RPE in Day 1 and subsequent Day 2. The differences between Day 1 and Day 2 at post cycling and 1 hour post exercise were not significantly different between SOY+CHO and WHEY+CHO for all the parameters. Compared to the CHO, no significant difference in all parameters was observed. These preliminary results suggest that soybean-based beverage may have a similar impact on antioxidant activity, oxidative stress, muscle inflammation, muscle damage, perceived fatigue and exercise performance, compared to whey protein when co-ingested with CHO.

Keywords: Soybean, antioxidant, muscle inflammation, muscle damage, exercise performance.

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PENGARUH PENGAMBILAN MINUMAN KANDUNGAN KACANG SOYA PRA-SENAMAN TERHADAP TINDAKBALAS BIOKIMIA DAN FISIOLOGIKAL SEMASA SENAMAN DI KALANGAN LELAKI YANG SIHAT

ABSTRAK

Pengambilan minuman karbohidrat (CHO) yang ditambah dengan protein whey berkesan dalam menurunkan glukosa dan insulin darah posprandial, yang boleh membantu mengurangkan spesies oksigen reaktif posprandial (ROS) dan keradangan. Pengambilan minuman CHO yang ditambah dengan protein whey juga menunjukkan pengurangan tekanan oksidatif, keradangan dan kerosakan otot serta peningkatan prestasi senaman. Kacang soya mengandungi isoflavon yang mungkin memberikan kesan positif yang sama atau lebih baik berbanding protein whey apabila ditambah dengan minuman CHO. Kajian pertama bertujuan untuk mengkaji kesan-kesan pengambilan minuman CHO ditambah dengan kacang soya berbanding minuman CHO ditambah dengan protein whey terhadap glisemik, insulinemik dan ROS posprandial pada lelaki yang sihat. Lapan lelaki [umur 20 (1.2) tahun, berat badan 59.2 (6.2) kg] mengambilan minuman 500 ml CHO ditambah dengan kacang soya (SOY+CHO), CHO ditambah dengan protein *whey* (WHO+CHO) setelah berpuasa semalaman, dalam rekaan rawak *counterbalanced*, dipisahkan oleh tempoh satu minggu. Sampel darah venus dikumpulkan selepas berpuasa semalaman (*baseline*) dan pada 30, 60, 90 dan 120 min selepas pengambilan minuman. Kawasan di bawah keluk glukosa dan insulin adalah lebih rendah bagi SOY+CHO berbanding WHEY+CHO. Tambahan, SOY+CHO cenderung mempunyai tindakbalas ROS posprandial yang lebih rendah berbanding WHEY+CHO. Walau bagaimanapun, tiada perbezaan yang signifikan diperhatikan di antara semua minuman dalam semua parameter. Minuman berasaskan kacang soya boleh menghasilkan tindakbalas ROS yang lebih rendah mencadangkan tekanan oksidatif yang lebih rendah kerana tindakbalas insulinemik yang lebih rendah, berbanding dengan protein whey apabila diambil bersama

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CHO. Kajian kedua mengkaji kesan pengambilan minuman SOY+CHO sebelum senaman berbanding dengan pengambilan WHEY+CHO terhadap aktiviti antioksidan, tekanan oksidatif, kerosakan dan keradangan otot, jumlah beban kayuhan berbasikal pada 85% VO_2peak dan persepsi keletihan selepas latihan dan latihan berikutnya. Tujuh lelaki [umur 20 (0.9) tahun, berat badan 56.4 (4.8) kg] melakukan tiga kali aktiviti berbasikal pada 85% VO_2peak: selepas pengambilan minuman 500 ml SOY+CHO, WHO+CHO dan CHO sahaja (Kontrol) dalam rekaan rawak counterbalanced dipisah dengan tempoh satu minggu. Sampel darah dikumpulkan selepas berpuasa semalaman (*baseline*) dan selepas pengambilan minuman pada titik pra-senaman (120min), selepas latihan dan 1 jam selepas latihan untuk analisis penggurangan kuasa antioxidan Ferric (FRAP), glutathione (GSH), glutathione teroksidasi (GSSG), nisbah glutathione (GSH/GSSG), interleukin-6 (IL-6), creatine kinase (CK). Kadar persepsi senaman (RPE) beban kerja (kilopond) direkodkan selepas senaman. Kesan utama antara SOY+CHO dan WHEY+CHO untuk FRAP, IL-6, GSH, GSSG, nisbah glutathione, CK, jumlah beban kerja dan RPE pada Hari 1 dan Hari 2 tidak menunjukkan perbezaan signifikan. Semua parameter selepas senaman dan 1 jam selepas senaman pada Hari 1 dan Hari 2 antara SOY+CHO dan WHEY+CHO tidak menunjukkan perbezaan signifikan. Berbanding dengan CHO, tiada perbezaan signifikan diperhatikan untuk semua parameter. Kajian awal ini menunjukkan bahawa kacang soya mempunyai kesan yang sama dalam aktiviti antioksidan, tekanan oksidatif, keradangan dan kerosakan otot, perspsi keletihan senaman dan prestasi senaman dibandingkan dengan protein whey bila diambil bersama dengan CHO.

Kata kunci: Kacang soya, antioksidan, keradangan otot, kerosakan otot, prestasi senaman.

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LIST OF SYMBOLS AND ABBREVIATIONS

ABTS	:	3-ethylbenz-thiazoline-6-sulfonic acid
AGE	:	Advanced glycation end products
ANOVA	:	Analysis of variance
AOPP	:	Advanced oxidation protein products
AP-1	:	Activator protein-1
AUC	:	Area under the curve
BCAA	:	Branched-chain amino acid
CAS	:	Cooked after soaking
CHO	:	Carbohydrate
CK	:	Creatine Kinase
CO_2	:	Carbon dioxide
CWS	:	Cooked without soaking
DOMS	:	Delayed onset muscle soreness
DPPH	:	2,2-diphenyl1-picrylhydrazyl
EAA	:	Essential amino acid
ECG	:	Electrocardiograph
EDTA	:	Ethylenediamine tetraacetic acid
FAO	:	Food and agriculture organization
FRAP	:	Ferric reducing antioxidant power
GAE	:	Gallic acid equivalent
GSH	:	Glutathione
GSSG	:	Glutathione Disulfide
GXT	:	Graded exercise test
HRmax	:	Maximum heart rate

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Hs-CRP	:	High-sensitivity C-reactive protein
HSF-1	:	Heat shock factor protein-1
IL-1β	:	Interleukin-1 beta
IL-4	:	Interleukin-4
IL-6	:	Interleukin-6
IL-8	:	Interleukin-8
IL-10	:	Interleukin-10
LDH	:	Lactate dehydrogenase
MapK	:	Map Kinase
MDA	:	Malondialdehyde
NADPH	:	Nicotinamide adenine dinucleotide phosphate hydrogen
NFκB	:	Nuclear factor kappa beta
NO	:	Nitrate Oxide
NOX	:	NADPH oxidase
O_2	:	Oxygen
OD	:	Optical density value
PCG-1α	:	peroxisome proliferator-activated receptor-γ coactivator-1 alpha
PDCAAS	:	Protein Digestibility-Corrected Amino Acid Score
RER	:	Respiratory exchange ratio
ROS	:	Reactive oxygen species
RPE	:	Rating of perceived exertion
RPM	:	Rotations per minute
RSI	:	Relative strength index
SIRT1	:	NAD-dependant protein deacetylase sirtuin 1
SOY	:	Soybean
SPSS	:	Statistical Package for Social Sciences

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TAC	:	Total antioxidant capacity
TBARS	:	Thiobarbituric acid reactive substances
TFs	:	Transcription factors
TNF-α	:	Tumor necrosis factor alpha
TPC	:	Total polyphenol content
Trp	:	Tryptophan
VO_2mx	:	Volume of maximum oxygen consumption
VO_2peak	:	Volume of peak oxygen consumption
WHEY	:	Whey protein
WHO	:	World health organization
5-HT	:	5-hydroxytryptamine

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CHAPTER 1: INTRODUCTION

High carbohydrate (CHO) foods promote fast release of glucose into the blood and regular consumption of these high-CHO foods may cause hyperglycemic effects that increased the generation of free radical (Brownlee & Hirsch, 2006) and oxidative stress leading to cardiovascular complications (Monnier et al., 2006). In addition, chronic exposure to hyperglycaemic condition has been shown to induce reactive oxygen species (ROS) which increases inflammation and deterioration of the pancreatic β cells leading to insulin resistance (Lin, Li, Zhang, Yang, & Su, 2017). In view of this, it has been suggested that high-CHO food when co-ingested with protein could act as an anti-inflammatory and cardioprotective diet. In a study of healthy individuals, the ingestion of whey protein with pure glucose drink was shown to lower the post-prandial blood glucose area under the curve by 56%, and increased insulin response by 60% (Nilsson, Holst, & Bjorck, 2007). Another study on the co-ingestion of casein protein with glucose and maltodextrin in patients with type-2 diabetes reported similar results whereby glucose response was lowered by 23% and increased of insulin response by more than 90%. (Manders et al., 2014). This may help to attenuate post prandial ROS and inflammation.

On the other hand, strenuous and intense exercise could also cause overproduction of reactive oxygen species (ROS), and the inability of endogenous antioxidants to remove ROS under this conditions could lead to oxidative stress which may cause damage and inflammation to the muscles (Tee, Bosch, & Lambert, 2007; White & Wells, 2013). It was suggested that these adverse effects could be prevented through the consumption of diet consisting carbohydrate, protein and antioxidant (Sousa, Teixeira, & Soares, 2014; Tara, Park, Mathison, Kimble, & Chew, 2013) especially at pre-exercise (Tipton et al., 2001; van Wijck et al., 2011). In addition, there were studies that showed protein could improve fatigue sensation (Alghannam, 2011; Newsholme & Blomstrand, 2006).

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However, there are non-promising results from other studies (Goh et al., 2012; Goldfarb, Cho, Cho, Romano-Ely, & Kent Todd, 2009; Tara et al., 2013).

Several studies have shown that CHO added protein beverage improved endurance performance when consumed during and after exercise (Ivy, Res, Sprague, & Widzer, 2003; Niles et al., 2001; Saunders, Moore, Kies, Luden, & Pratt, 2009) and subsequence exercise (Hall, Leveritt, Ahuja, & Shing, 2013) while some studies failed to prove such benefits (Alghannam et al., 2016; Romano-Ely, Todd, Saunders, & Laurent, 2006; van Essen & Gibala, 2006) as compared to carbohydrate only beverage, despite some showed changes in physiological markers. From these studies, whey hydrolase was mostly used (Alghannam et al., 2016; Saunders et al., 2009), followed by whey isolate (McCleave et al., 2011; Roberts, Desbrow, Grant, Anoopkumar-Dukie, & Leveritt, 2013) and casein with leucine (Hall et al., 2013). The consumption of CHO added with 20g-40g of whey protein hydrolase and isolate, at interval of 10-20 minutes during exercise showed an improvement in glucose uptake and delayed muscle glycogen depletion (Morifuji, Kanda, Koga, Kawanaka, & Higuchi, 2011; Roberts et al., 2013; Saunders, Kane, & Todd, 2004), reduced heart rate and fatigue perception and improve time-to-exhaustion performance among endurance trained athletes (Hall et al., 2013; Saunders et al., 2009). Meanwhile intake of CHO added protein beverage after exercise has shown to augment insulin level (Ivy et al., 2003), increased muscle protein synthesis (Morifuji et al., 2011), and reduce muscle inflammation and muscle damage and improve subsequence time-to-exhaustion performance among endurance trained athletes (Hall et al., 2013; Romano-Ely et al., 2006; Saunders, 2007). Studies on the intake of CHO added protein prior to exercise were scarce and limited to animal model only (Morifuji et al., 2011; Roberts et al., 2013). Furthermore, the consumption of whey protein may cause digestive problems to people with whey protein intolerance (Lam et al., 2008; Parker & Watson, 2017). Hence, the

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effect of pre-exercise carbohydrate added with plant-based protein such as soybean on antioxidant activity, oxidative stress, muscle damage and inflammation are required.

Soybean or soya bean, also called *Glycine max* is a domestic plant in Asia countries and is a member of the leguminosae family. Soybean contained all eight essential amino acids along with a complex array of phytochemicals that can provide significant long-term health benefits (Messina, 2016). One of the phytochemicals present in soybean is a polyphenolic compound known as isoflavone. Isoflavones contain strong antioxidant properties and had been shown to reduce oxidative stress and inflammatory markers (Yu, Bi, Yu, & Chen, 2016). Soybean supplementation has been shown to improve blood glucose and serum lipid levels in diabetic patients (Chang, Kim, Kim, & Lee, 2008; Tatsumi et al., 2013). However, it is unknown whether the ingestion of CHO with soybean could reduce the hyperglycaemic effects and thus promote the attenuation of postprandial ROS and inflammation.

Soybean has also been proven to enhance lipid peroxidation, altering the lipid metabolism and profile (Berg et al., 2012). This may be beneficial for endurance exercise which emphasize on fat oxidation efficiency and sparing of muscle and liver glycogen. However, there is still scarcity in evidence linking protein intake to enhance endurance capacity and only a few studies conducted on chronic response during endurance exercise using soybean-based supplement (Berg et al., 2012; Celec et al., 2013; Peng-Fei & Lan, 2010). Another similar study using mixture of carbohydrate, protein and antioxidant by Romano-fly et al (2006) showed a lower muscle damage and inflammatory markers despite no significant different in performance. Therefore, this may provide the insight of the possibility of soybean-based beverage effectiveness as compared to carbohydrate added whey protein beverage in reducing muscle damage and inflammation and improve exercise performance.

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1.1 Problem Statements

1. The studies of CHO-protein beverage on the postprandial glycemic and insulinemic responses have been focusing on the use of whey and milk as the source of protein. However, study of CHO-protein beverage comparing whey and soybean as protein is limited.
2. The consumption of pre-exercise whey added CHO beverage showed positive effects on muscle damage, oxidative stress and muscle inflammation biomarkers. However, little study done using soybean added CHO beverage on the biomarkers in comparison to whey protein added CHO beverage.
3. Pre-exercise whey added CHO beverage consumption has shown to improve the physiological responses during high intense exercise and subsequent exercise performance. To date, there is limited study conducted comparing soybean added CHO and whey added CHO beverages on physiological response on high intense exercise.

1.2 Hypothesis

1. Soybean-CHO consumption may reduce postprandial glycemic, insulinemic and ROS response in healthy men in comparison to whey-CHO consumption.
2. Pre-exercise soybean-CHO beverage consumption may enhance antioxidant, reduce oxidative stress, muscle damage and inflammation post exercise and post subsequent exercise in comparison to whey-CHO beverage consumption.
3. Pre-exercise soybean-CHO intake may improve total cycling workload at 85% VO_2max and fatigue perception at post exercise and subsequent exercise in comparison to whey-CHO intake.

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1.3 Research Objectives

1. To investigate the effects of soybean added CHO beverage consumption in comparison to whey added CHO beverage on postprandial glycemic, insulinemic and ROS responses in healthy men.
2. To examine the effects of pre-exercise soybean added CHO beverage consumption as compared to whey added CHO beverage consumption on antioxidant activity, oxidative stress, muscle damage and inflammation post cycling at 85% VO_2max and subsequent cycling in physically active male.
3. To examine the effects of pre-exercise soybean added CHO ingestion as compared to whey added CHO beverage on the total cycling workload and fatigue perception post cycling at 85% VO_2max and subsequent cycling in physically active male.

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CHAPTER 2: LITERATURE REVIEW

2.1 Effects of postprandial glycemic on oxidative stress and inflammation

Trends in food consumption have undergone changes, largely attributed to factors such as income, urbanization, trade liberalization, food industry marketing and consumer attitudes and behavior (Kearney, 2010). 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. Most processed food are high-glycemic index carbohydrate (CHO) that promote fast release of glucose into the blood. Regular consumption of these high-CHO foods may cause hyperglycemic effects, which over time can lead to the obesity and diabetes phenomenon worldwide, including Asia. Excessive consumption of calorie-dense, easily digestible foods and drinks caused abnormal surges in blood glucose (Ceriello et al., 2005). This surge of energetic substrate overwhelms the metabolic capabilities of mitochondria in the muscle and adipose tissues that have high acute concentration of glycogen and triglyceride respectively. Glucose could flood the Krebs cycle, stimulating excess production of the reduced form of nicotinamide adenine dinucleotide (NAD), which may exceed the oxidative phosphorylation capacity of the mitochondria and drives the transfer of single electrons to oxygen, creating free radicals such as superoxide anion (Monnier et al., 2006). This post-prandial oxidative stress can acutely trigger atherogenic changes, including increases in low-density lipoprotein oxidation, sympathetic tone, vasoconstriction, and increase thrombogenicity (Monnier et al., 2006).

Hyperglycaemic spikes artificially induced through intravenous glucose infusions in lean nondiabetic individuals have been shown to markedly increase free radical generation (Brownlee & Hirsch, 2006). Hyperglycaemia has been associated with many health complications such as impairment of cardiovascular (Mapanga & Essop, 2016), renal (Busik, Mohr, & Grant, 2008), brain microvascular (Shao & Bayraktutan, 2014; Su

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et al., 2013), nervous system, pancreas (Lin et al., 2017) and respiratory muscles (Callahan & Supinski, 2014) 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-dependent protein deacetylase sirtuin 1 also known as SIRT1 (Su et al., 2013). Chronic exposure to hyperglycaemic condition can cause deterioration of the pancreatic β cells (Lin et al., 2017), possibly leading to insulin resistance.

2.2 Exercise-induced oxidative stress, muscle damage and inflammation

Exercise-induced muscle damage (EIMD) commonly attributed either to after experiencing a bout of unaccustomed physical activity or following physical activity of greater than normal duration or intensity (Tee et al., 2007). During high intensity or high duration exercise, the metabolic stress and reactive oxygen species (ROS) production increases at the mitochondria of skeletal muscle. This leads to lipid peroxidation, structural cell damage, and alters the redox status of the cell. Several transcription factors (TFs), such as nuclear factor kappa B (NFκB), Map Kinase (MapK), activator protein-1 (AP-1), heat shock factor protein-1 (HSF-1), and peroxisome proliferator-activated receptor-γ coactivator (PCG)-1α, are redox sensitive and their function may be altered by the change in redox status. Some of these TFs involved in muscle adaptation pathways and some produces and secretes cell signaling molecules such as interleukin-6 (IL-6) and interleukin-8 (IL-8). These cytokines, involved in the regulation of leukocytes may also contribute to ROS production at the muscle cell, contributing to structural damage and propagates the positive feedback pattern of the inflammatory response during exercise. Figure 2.1 showed the metabolic and mechanical pathways leading to the increase of metabolic rate and structural damages that attribute to muscle adaptation. In addition, high temperatures induced by exercise may also increase the production of ROS from NADPH oxidase (NOX), contributing to the structural damage, change in redox status,

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nuclear signaling and positive feedback signaling associated with the other forms of exercise stress (White & Wells, 2013).

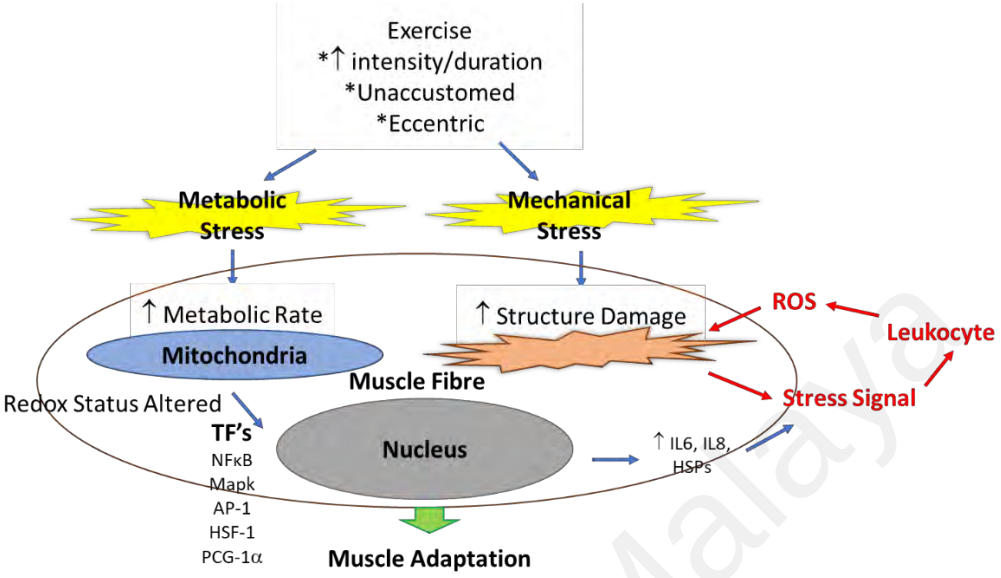

Figure 2.1: Exercise stress on the metabolic and mechanical pathways

2.3 Carbohydrate added protein beverage consumption and biochemical responses during endurance exercise

Exercise-induced muscle damage, oxidative stress and inflammation can be delayed with the right nutrition strategy, leading to faster recovery and improvement of performance post exercise. Diet combination that ensure the delivery of protein, carbohydrates, antioxidants and anti-inflammatory nutrients provide better adaptation both from metabolic and mechanical stress (Sousa et al., 2014). Milk-based supplementation containing carbohydrate and protein has been proven to be effective to attenuate the exercise-induced muscle damage by delaying protein degradation and promote protein synthesis (Cockburn, Stevenson, Hayes, Robson-Ansley, & Howatson, 2010). The study observed that the pre-exercise milk-based beverage consumption was able to limits the increase of muscle damage biomarker (creatine kinase, CK) despite no

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changes in delayed muscle onset soreness (DOMS), peak torque, reactive strength index (RSI) post exercise-induced muscle damage among group 8 healthy males.

Recent studies showed that adding protein into carbohydrate beverages can promote higher insulin response post exercise indicating glucose uptake by muscle for glycogen synthesis (Niles et al., 2001), and reduce muscle damage (Romano-Ely et al., 2006), improving muscle soreness rating (Alghannam, 2011; Hall et al., 2013; Saunders et al., 2009) and exercise performance (Alghannam, 2011; Hall et al., 2013; Ivy et al., 2003; McCleave et al., 2011; Niles et al., 2001; Roberts et al., 2013; Saunders et al., 2004).

2.3.1 Effects of carbohydrate added protein beverage on exercise induced oxidative stress and muscle inflammation

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), tumor necrosis factor-α (TNF-α), IL-6 and IL-4 were reduced, while glutathione levels increased, after the administration of 100 mg/kg whey protein (Ebaid, Salem, Sayed, & Metwalli, 2011). On the other hand, the lymphocyte glutathione levels were increased after 45 g/day of whey protein supplementation in a bar format in healthy individuals (Zavorsky, Kubow, Grey, Riverin, & Lands, 2007).

However, human exercise studies have shown inconsistent results in whey protein attenuating oxidative stress and inflammatory markers. It has been shown that the consumption of 40 g whey protein reduced lipid peroxidation in female endurance athletes who performed one-hour run per day, 5 days a week for 6 weeks (Tara et al., 2013). However, this study found no effect of whey protein on inflammatory markers after 6 weeks of training. Another study by Goldfarb et al (2009) also showed no effect

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on reducing oxidative stress nor inflammatory markers post cycling exercise at 80% VO_2peak after consuming a whey protein and vitamin c added isocaloric beverage among twelve trained men. This shows that whey protein may not be effective in reducing inflammation post endurance exercise.

2.3.2 Effects of carbohydrate added protein beverage on exercise induced muscle damage

Whey protein have been shown to reduce muscle damage and reduce the recovery time after an exercise induced-muscle damage routine. It has been reported that whey protein isolate supplementation reduced plasma CK, lactate dehydrogenase (LDH) and elicited better maintenance of muscle strength in the days following exercise-induced eccentric muscle damage (Cooke, Rybalka, Stathis, Cribb, & Hayes, 2010). This may be due to increase protein synthesis attributed to the essential amino acid (EAA) contained within the whey protein supplement. Meanwhile, in carbohydrate added protein studies, Romano-Ely et al. (2006) reported that the carbohydrate and protein supplementation was able to attenuate post exercise CK and LDH as compared to carbohydrate only supplementation despite no performance difference in a time-to-exhaustion exercise. In contrast, other studies found no changes in CK response (Saunders et al., 2009; White et al., 2008). Saunders et al. (2009) showed no significant difference in CK between 200ml carbohydrate added with protein beverage (6% carbohydrate and 1.8% whey protein hydrolase), and 200ml carbohydrate only (6% carbohydrate) beverage after 60km time trials in 13 male cyclists.

2.3.3 Carbohydrate added protein beverage and fatigue

Whey protein also well-known for its branched-chain amino acid (BCAA) on brain function and decrease fatigue perception based on the tryptophan (Trp)–5-hydroxytryptamine (5-HT)–central fatigue theory. During endurance exercise, there is an

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uptake of Trp by the brain, which may increase the synthesis and release of 5-HT in the brain that leads to fatigue. An oral intake of BCAAs will decrease the ratio of free Trp:BCAAs and hence decreases the transport of Trp into the presynaptic neuron in the brain which may reduce the uptake and also brain 5-HT synthesis and release, thereby delaying the level of fatigue by the brain (Newsholme & Blomstrand, 2006).

Lower rating of perceived exertion (RPE) score was reported in protein added carbohydrate group in comparison to the carbohydrate only and placebo groups after an exhaustive exercise (Alghannam, 2011; Hall et al., 2013; Saunders et al., 2009). In contrast, there was no significant difference in RPE during 20km time trial after a 20min cycling at 70% VO_2peak in 12 male cyclists consuming either the carbohydrate and protein or carbohydrate only beverages (Goh et al., 2012). The lower RPE in the former study may due to lower pre-exercise intensity which spared the muscle glycogen after consuming a 75g of carbohydrate beverage which is above the maximum carbohydrate oxidation rate at 60g/h.

Carbohydrate added with protein beverage has shown to prolong time-to-exhaustion exercise. Niles et al. (2001) showed 21% longer time-to-exhaustion after a glycogen lowering diet and exercise bout in the experimented group (112g CHO and 40.7g Protein) as compared to control group (152.7g of CHO) in 10 male runners. Similar finding on time-to-exhaustion was shown by the study conducted by Ivy et al. (2003) and Saunders et al. (2004). Ivy et al., (2003) reported that time to exhaustion cycling at 85%VO_2max was 111.8% longer in carbohydrate added protein group (7.75% CHO, 1.94% protein) as compared to 55.1% in carbohydrate group (7.75% CHO) among 10 trained male cyclists. Whilst, Saunders et al. (2004) observed a 40% longer time-to-exhaustion cycling at 85%VO_2peak after a 75% VO_2peak cycling 12-15 hours in carbohydrate added protein group (7.3% carbohydrate and 1.8% protein) in comparison with carbohydrate only (7.3%

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CHO) group. In contrast, van Essen (2006) reported no difference in terms of cycling performance between CHO group and placebo group as compared to the carbohydrate added protein group. However, most studies show that improved in time-to-exhaustion performance may be due to the extra calories from protein when carbohydrate content was matched (Ivy et al., 2003; Saunders, 2007; Saunders et al., 2004).

However, in a more recent studies no difference in time-to exhaustion was observed when isocaloric beverages (CHO + Pro vs CHO only) was consumed (Alghannam et al., 2016; Greer, Price, & Jones, 2014; Romano-Ely et al., 2006). Romano-Ely et al. (2006) showed no difference in time-to-exhaustion between CHO added protein group (7.5% CHO + 1.8% Pro) and CHO only group (9.5% CHO) on a subsequent exercise at 80% VO_2peak after a 70% ride for at least 60 minutes 24 hours prior in 14 male volunteers. This was supported by studies by Greer et al. (2014) and Alghannam et al. (2016) which showed no difference in time to exhaustion during interval running and 70%VO_2max, respectively, between CHO added protein and CHO only groups. This may be due to the intensity applied in these studies that was not high enough to promote glycogen depletion and muscle damage and hence no difference observed.

2.3.4 Timing of supplementation

In most studies, CHO added protein was ingested at 15 min interval during endurance exercise (Alghannam, 2011; Hall et al., 2013; Ivy et al., 2003; McCleave et al., 2011; Niles et al., 2001; Roberts et al., 2013; Romano-Ely et al., 2006; Saunders, 2004 & 2007; van Essen & Gibala, 2006) and some studies at immediately after exercise (Saunders, 2004 & 2007). However, only a few studies look at ingestion of carbohydrate added protein beverage before exercise (Morifuji et al., 2011; Roberts et al., 2013) but limited to animal model. One human study showed some physiological and physical performance

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changes when carbohydrate added protein was consumed before, immediate after and post-24hour exercise (Cockburn et al., 2010).

Pre-exercise ingestion of carbohydrate added protein beverage on the energy metabolism during endurance exercise is not well understood as compared to ingestion during and after exercise. Exhaustive exercise normally is accompanied by a redistribution of blood flow to skeletal muscle tissue, resulting in hypoperfusion of the gut (van Wijck et al., 2012) which induces intestinal damage and impairs dietary protein digestion and absorption kinetics during early post-exercise recovery (van Wijck et al., 2011). Therefore, dietary protein ingestion before and during exercise may provide a more effective feeding strategy to improve amino acid availability during early post-exercise recovery.

A study by Tipton et al. (2001) suggested that the ingestion of a mixture of 6 g of essential amino acids and 35 g of sucrose before exercise was more effective for the simulation of post exercise muscle protein synthesis than ingesting the same mixture immediately after exercise in well-trained athletes. The authors hypothesized that the greater stimulation of muscle protein synthesis may be attributed to the combination of increased amino acid levels at a time when blood flow is increased during exercise, thereby offering greater stimulation of muscle protein synthesis by increasing amino acid delivery to the muscle.

2.3.5 Type of protein used

Most protein used in these studies are mainly dairy protein such as whey protein hydrolysate (Alghannam et al., 2016; Morifuji et al., 2011; Saunders, 2004, 2007 & 2009), whey protein isolate (Betts & Williams, 2010; McCleave et al., 2011; Roberts et al., 2013), casein and leucine (Hall et al., 2013) while others are commercial carbohydrate beverage containing whey protein (Goldfarb et al., 2009; Romano-Ely et al., 2006).

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Despite the benefits, some individuals may have digesting whey protein problem due to the inability of the body to produce enough lactase to breakdown lactose in the whey protein, which is known as lactose intolerance. In addition, some individuals may just have dairy protein intolerance. Lactose intolerance can lead to symptoms such as stomach cramp, bloating, and diarrhea (Parker & Watson, 2017) while whey protein intolerance can lead to symptoms which includes hives, rashes, facial swelling, throat and tongue swelling and a runny or stuffy nose (Lam et al., 2008). The alternative to people with lactose or whey protein intolerance would be to consume non-dairy protein such as soybean/protein.

2.4 Soybean

The soybean or soya bean, also known as *Glycine max* is a domestic plant in Asia countries and is a member of the leguminosae family, plants that form root noodles that house nitrogen-fixing soil bacteria (*Rhizobia*) in a symbiotic relationship. Fermented soybean products such as miso, soy source, and tempeh, as well as soy-milk, natto and tofu are frequently consumed by Asians which is known for its health benefits (Barnes, 2010).

Soybean has been proven to have various medicinal properties that can prevent and cure human diseases (Verma, Sharma, Argawal, Arggawal, & Singh, 2014). The consumption of soy foods may contribute to a lower incidence of coronary artery disease (Rostagno et al., 2010), hypertension (He et al., 2011), type 2 diabetes mellitus (Sathyapalan et al., 2017), certain cancers such as breast and prostate and prevent osteoporosis (Bawa, 2010), obesity, allergy and also help to relief women of menopausal symptoms (Ahsan & Mallick, 2017). Many of the health benefits of soybean are derived from its secondary plant metabolites such as flavones, phyto-sterols, lecithins, saponins and so forth along with its comprehensive branch-chain amino acids (BCAAs), making it

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one of the most valuable beans for its bioactive components and proteins (Verma et al., 2014).

2.4.1 Nutritional value of soybean

A raw soybean contains all the three macronutrients and important minerals required for good nutrition as shown in Table 2.1. It contains 33.8% protein, 25.5% carbohydrate, 18.9% fat, 11.5% water or moisture and dietary fiber 5.5% as well as 4.8% in total for both vitamins and minerals. It is an excellent source of minerals especially calcium, iron, magnesium, phosphorus, potassium, sodium, zinc and copper. It is also a good source of vitamins like ascorbic acid (vitamin C), Retinol (Vitamin A), alpha-tocopherol (Vitamin E), thiamin, riboflavin, niacin, and folate. Soy also contains all the essential amino acid that cannot be synthesized *de novo* by human body like tryptophan, threonine, isoleucine, leucine, lysine, methionine, phenylalanine, valine and histidine.

Table 2.1. Macronutrients and micronutrients content of raw soybean per 100g value

Protein	(g)	33.792		Iron	(mg)	5.996
Carbohydrate	(g)	25.503		Thiamine	(mg)	0.871
Fat (total)	(g)	18.907		Riboflavin	(mg)	0.339
Dietary Fibre (total)	(g)	5.503		Niacin	(mg)	0.998
Moisture	(g)	11.499		Folate	(ug)	53.623
Sodium	(mg)	45.855		Pyridoxine (Vit B6)	(mg)	0.233
Potassium	(mg)	398.589		Phosphorus	(mg)	440.917
Vitamin A	(RE)	17.989		Magnesium	(mg)	85.362
Vitamin C	(mg)	7.513		Zinc	(mg)	1.139
Vitamin E	(mg)	1.933		Copper	(mg)	0.406
Calcium	(mg)	197.884				

Source: Nutrient Composition of Malaysian Food, NutriPro.

The raw and cooking process of soybean into consumable product has shown in Table 2.2 to have different antioxidant properties concentration on polyphenol, 2,2-diphenyl1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS) and FRAP. The raw organic soybean has higher gallic acid equivalent (GAE) compared

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to inorganic soybean. The total polyphenol content (mg GAE/100 g) was also higher in both cooked without soaking (CWS) and cooked after soaking (CAS) in organic compared to inorganic soybean. The DPPH and ABTS radical scavenging capacity (μmol TE/100 g) of raw and cooked both CWS and CAS for organic soybean were higher compered to raw and cooked inorganic beans with exception to CWS of DPPH. The Ferric reducing antioxidant power (FRAP) (μmol TE/100 g) of raw and cooked organic is higher than that of inorganic beans. Organic soybean has higher antioxidant property compared to inorganic and cooking without soaking with preserved the antioxidant property than soaking before cooked (Hanis Mastura, Hasnah, & Dang, 2017).

Table 2.2. Total polyphenol content, DPPH, ABTS and FRAP in raw, cooked organic and inorganic soybean

Soybean	RAW	CWS	CAS
	TPC (GAE/100g)		
Organic	209.31±6.41	168.57±8.33	147.25±4.24
Inorganic	156.54±5.78	135.24±4.81	91.73±8.48
	DPPH (μmol TE/100g)		
Organic	331.84±2.38	191.22±5.99	201.50±8.36
Inorganic	299.15±3.66	215.02±4.95	201.52±4.95
	ABTS (μmol TE/100g)		
Organic	2721.68±33.68	2352.19±20.97	2049.08±16.66
Inorganic	2124.47±22.04	2066.05±22.04	1907.67±30.04
	FRAP (μmol TE/100g)		
Organic	724.14±7.14	490.83±5.45	432.42±4.12
Inorganic	503.92±8.25	400.36±5.45	340.83±12.54

Values are expressed as mean ± standard deviation of three replication.
TPC: Total polyphenol content; GAE: Gallic acid equivalent; DPPH: 2,2-diphenyl1-picrylhydrazyl; ABTS: 3-ethylbenz-thiazoline-6-sulfonic acid; CWS: cooked without soaking; CAS: cooked after soaking. (Table reproduced using data extracted from Hanis Mastura et al., 2017)

Many especially male athletes perceived soybean-based products as more inferior to whey protein due to the perception of it has lower quality protein in comparison to whey

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protein. However, the Protein Digestibility-Corrected Amino Acid Score (PDCAAS) recommended by FAO/WHO 1989, on soybean shows that soybean protein achieved the highest possible PDCAAS score of 1.00 which is equivalent to whey protein and casein protein, making it more favorable protein source as it takes into account on human amino acid requirements and digestibility of the protein (Hughes, Ryan, Mukherjea, & Schasteen, 2011). Other than the nine essential amino acid, importantly BCAAs like leucine, isoleucine and valine that will be oxidized by the body and provide energy during exercise, soybean also contain high conditional amino acid such as arginine and glutamine that promote muscle synthesis and recovery post exercise recovery (Hughes et al., 2011).

2.4.2 Soy isoflavones

Soybean contains isoflavones which are a subclass of phytochemicals group called flavonoids and soybean has abundant source of isoflavones of up to 3 mg/g dry weight in nature (Dixit, Antony, Sharma, & Tiwari, 2011). There are three types of isoflavones in soybean mainly genistein, daidzein and glycitein, (Figure 2.2) that normally occur in four chemical forms (Figure 2.3). They exist in soybeans either as glucosides or in free form (aglucones) as shown in Figure 2.3. The glucosides of daidzein, glycitein, and genistein are called daidzin, glycitin, and genistin, respectively. Six derivatives of the glucosides also exist in soybeans: 6''-O-acetyl-daidzin, -glycitin, -genistin; and 6''-O-malonyl-daidzin, -glycitin, -genistin (Kudou, Shimoyamada, Imura, Uchida, & Okubo, 1991; Wang, Ma, Pagadala, Sherrard, & Krishnan, 1998). In intact, minimally processed soybean 6"-O-malonylgenistin is the major isoflavone followed by genistin, 6"-O-malonyldaidzin, and daidzin respectively. These four components contribute about 83% to 93% of the isoflavones while the remaining eight isoflavones represent about 7% to 17%. All of these isoflavone compounds have been considered as non-nutrients, because they neither yield any energy nor function as vitamins. However, they play significant

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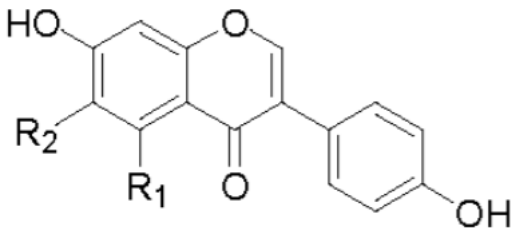
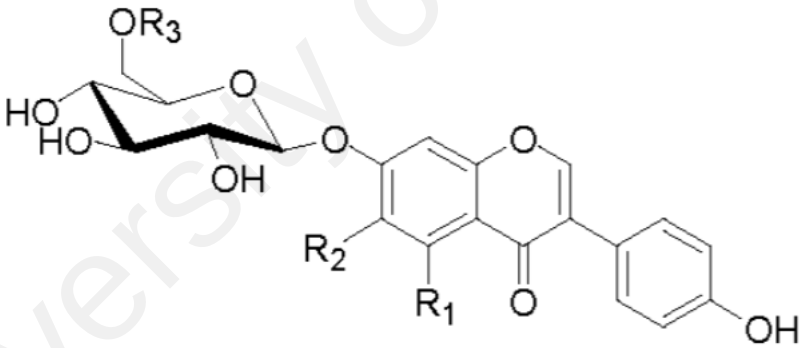
roles in the prevention of heart diseases and cancers, so they may become the vitamins of the twenty-first century (Messina, 2016).



$R_1 = R_2 = H$, Daidzin
$R_1 = OH$, $R_2 = H$, Genistein
$R_1 = H$, $R_2 = OCH_3$, Glycitein

Figure 2.2: Structural difference between genistein, daidzein and glycitein (adopted from Dixit et al., 2011)



$R_1 = R_2 = R_3 = H$: Daidzin
$R_1 = OH$, $R_2 = R_3 = H$: Genistin
$R_1 = H$, $R_2 = OCH_3$, $R_3 = H$: Glycitin
$R_1 = H$, $R_2 = H$, $R_3 = COCH_3$: 6"-O-Acetyldaidzin
$R_1 = OH$, $R_2 = H$, $R_3 = COCH_3$:, 6"-O-Acetylgenistin
$R_1 = H$, $R_2 = OCH_3$, $R_3 = COCH_3$: 6"-O-Acetylglycitin
$R_1 = H$, $R_2 = H$, $R_3 = COCH_2COOH$: 6"-O-Malonyldaidzin
$R_1 = OH$, $R_2 = H$, $R_3 = COCH_2COOH$: 6"-O-Malonylgenistin
$R_1 = H$, $R_2 = OCH_3$, $R_3 = COCH_2COOH$: 6"-O-Malonyldiadzin

Figure 2.3: Soybean isoflavones in its glucosides form (adopted from Dixit et al., 2011)

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2.4.3 Effects of soybean intake on oxidative stress, muscle damage, muscle inflammation and exercise performance

Despite the benefits of soybean, there are few studies conducted on soybean-based diet on exercise performance in relation to oxidative stress (Celec et al., 2013; Peng-Fei & Lan, 2010), energy metabolism (Berg et al., 2012) and exercise performance (Peng-Fei & Lan, 2010). This may provide an evidence to athletes that soybean-based diet or beverage can yield similar if not better response than whey physiologically and performance wise.

Celec et al. (2013) observed an increased in total antioxidant capacity by soybean intake for both 55 young women and 33 young men aged 18-25 years old who were given 2 g/kg bodyweight of soybean daily for one week. They measured plasma oxidative stress markers such as thiobarbituric acid reactive substances (TBARS), advanced oxidation protein products (AOPP) and total antioxidant capacity (TAC); and carbonyl stress markers such advanced glycation end products (AGE-specific fluorescence) and plasma fructosamine at the beginning and at the end of one-week soybean intake and after another week of a wash-out period. The authors found that there were decreased levels of AOPP in women, but not in men. On the contrary, lipoperoxidation was increased only in men while no effects on carbonyl stress markers were found for both genders. The authors concluded that soybean intake has gender-specific effects on oxidative stress in young healthy men and women potentially due to divergent action and metabolism of phytoestrogens between the genders. However, the relationship on reducing oxidative stress to enhance exercise performance was not clear.

In a study by Berg et al. (2012), 15 healthy sports students consumed soy-based supplement (53.3 g protein, 30.5 g carbohydrates, 2.0 g fat, 354 kcal per 100 g solubilized in 200 ml water) and performed endurance training at aerobic threshold for the duration

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of 6 weeks. This study observed an increase in running exercise and lower lactate values in the supplemental group. However, no significant changes in the exercise-induced stress and inflammatory reaction throughout the 6 weeks intervention. The lower response in exercise-induced stress and inflammatory markers Creatine Kinase (CK), Lactate dehydrogenase (LDH), myoglobin, high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), and interleukin-10 (IL-10) in this study may attribute to adaptation to the training intervention.

The above-mentioned findings may provide the insight of the possibility of soybean-based beverages as a better alternative to whey protein supplementation in reducing oxidative stress, muscle damage and inflammation, delayed liver glycogen depletion and improve endurance exercise performance in men. Soy derived protein has proven to enhance protein synthesis and promote lipid peroxidation, altering the lipid metabolism and profile (Berg et al., 2012) and improve performance (Peng-Fei & Lan, 2010). While the exercise performance study by Peng-Fei & Lan (2010) on mice sounds promising, the acute pre-exercise soybean-based beverage consumption in lowering exercise-induced oxidative stress, muscle damage and inflammation while improving exercise performance in men in comparison to whey-based beverage still requires more investigation.

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CHAPTER 3: METHODS

Two studies were conducted 1) To investigate the effects of soybean-CHO beverage consumption on postprandial glycaemic, insulinemic and ROS responses in healthy men and 2) To examine pre-exercise carbohydrate added with soybean or whey protein on physiological responses, muscle damage, inflammation and oxidative stress post cycling at 85% VO_2max and subsequent cycling in physically active men.

3.1 Study 1: The Effects of Soybean Co-ingestion with Carbohydrate on Post-prandial Glycemic, Insulinemic and Reactive Oxygen Species in Healthy Men

3.1.1 Participants

Eight healthy men aged between 18 to 25 years old were recruited from higher learning institutions around Lembah Pantai, Kuala Lumpur. The participants are recreationally active, exercising at least three times per week and not consuming any supplements This study was conducted with the approval of the University of Malaya Research Ethics Committee (UM.TNC2/RC/H&E/UMREC-115) (Appendix A) and the participants provided written consent (Appendix B).

3.1.2 Study Design

Each participant, participated in a dietary intervention trial lasting for 3 hours, in a randomized counterbalanced order. The participants consumed 500 ml of either CHO added with soybean (SOY+CHO), CHO added with whey protein (WHEY+CHO) or CHO alone as the control group, after an overnight fast, each type of beverage separated by a one-week period. The participants were asked to refrain from participating in any vigorous physical activity and from taking any medications, supplements, and food rich in antioxidants 24h prior to the intervention trial.

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3.1.3 Experimental Protocol

Participants arrived at the Sports Nutrition laboratory after a 12-hour overnight fast, at approximately 08:30 hours. They were weighed using Bioimpedance Analysis (InBody, USA) and a cannula (G-15, Venflon) was inserted in the anticubital vein. Participants then rested in a seated position for 10 minutes before the baseline blood sample was drawn. Then, the participants consumed the SOY+CHO, WHEY+CHO or CHO beverage within 10 minutes. Blood samples were collected at 30, 60, 90 and 120 min after the consumption of the beverages using the same blood sampling procedure (Figure 3.1). The participants remained within the Sports Nutrition laboratory during the duration of the test, performing only sedate behavior like sitting, reading and studying.

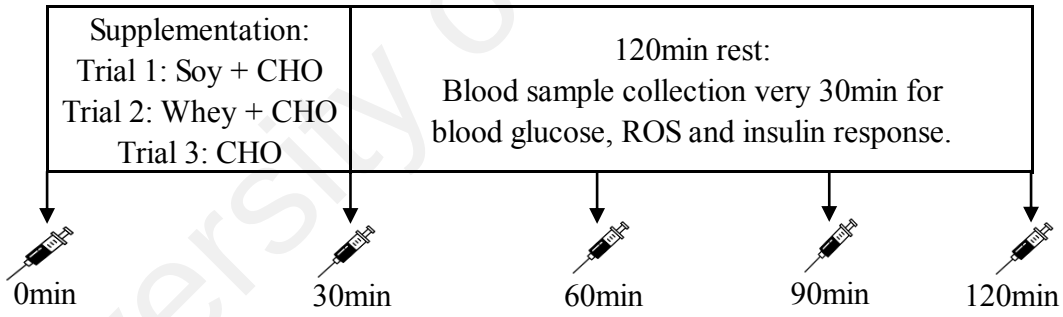

Figure 3.1: The Schematic of the experimental protocol.

3.1.4 Experimental Beverage

The beverage were prepared fresh at the Sport Nutrition Laboratory prior to each experimental trial. The 500ml SOY+CHO beverage contained 2% (10g) soybean, 4% (20g) rice, 4% cane sugar (20g), the 500ml 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 beverages

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were prepared fresh at the Sports Nutrition Laboratory prior to each experimental trial. The total calories provided by each beverage are shown in Table 3.1.

Table 3.1: Calorie content of the beverages consumed by the participants.

Calories (Kcal)	CHO	SOY+CHO	WHEY+CHO
Carbohydrate	185.9	161.6	154.4
Protein	7.9	20.7	39.6
Fat	2.0	16.7	7.5
Total	195.8	199.0	191.5

3.1.5 Blood Collection and Plasma Preparation

Blood Samples were collected into 6 ml heparinized and Ethylenediamine tetraacetic acid (EDTA) tubes (BD vacutainer), and centrifuged at 3000 RPM for 15 min at 4°C. After centrifugation, aliquots of plasma were transferred into labelled Eppendorf tubes and stored at -40°C before analysis of glucose, insulin and ROS.

3.1.6 Analysis of Blood Glucose, Insulin and ROS

3.1.6.1 Glucose and Insulin

Post-prandial 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 were performed according to the manufacturer's instructions.

3.1.6.2 Reactive Oxygen Species (ROS)

Plasma (5 µl) was added with 100 µl of 100 µM 2,7-dichlorofluorescein diacetate in a black 96-well plate. The mixture was shaken on a shaker for 1 minute followed by incubation for 30 minutes at 37°C. Fluorescence reading was taken with the excitation

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(EX) and emission (EM) wavelengths set at 485 nm and 530 nm, respectively using a Multiplex ELISA System (Bioplex 200, Bio-Rad, USA). All results were expressed as relative fluorescence unit.

3.1.7 Statistical Analysis

Data were expressed as mean ± standard error mean (SEM) unless otherwise stated. The distribution of data normality was assessed using the Shapiro-Wilk test. All statistical analysis was performed 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 trial, WHEY+CHO trial and CHO trial were compared using one-way repeated measures analyses of variance (ANOVA). Two-way ANOVA (Between-Within Trials) was performed on all groups to determine group and time differences. Significant main effects and interactions were further analyzed using Tukey's post hoc test. Differences were considered significant if $p < 0.05$.

3.2 Study 2: The Effects of Soybean Co-ingestion with Carbohydrate on Biochemical and Physiological Responses Post Exercise and Subsequent Exercise in Physically Active Men: A Preliminary Study.

3.2.1 Participants

Participants were recruited via an advertisement in the University's emails, posters and social media. Seventeen participants interested to participant, however only seven participants decided to take part in this study, while the remaining ten had to withdraw due to various reasons. The committed participants were given the study information sheet (Appendix C) and the procedures of the study including possible risks and discomforts involved was explained via e-mail, telephone or in person. They also completed a physical activity readiness questionnaire (PAR-Q) form (Appendix D) prior

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to the study to ensure that the participants were healthy and have no medical conditions. This study was conducted with the approval of the University of Malaya Research Ethics Committee (UM.TNC2/RC/H&E/UMREC-115) and the participants provided consent (Appendix E).

To be eligible for inclusion in all studies, participants had to be aged between 18-30 years old, body mass index (BMI) of between 18.5 to 23 $kg.m^{-1}$, maximum oxygen consumption (VO_2max) between 40-50 $mL.kg^{-1}.min^{-1}$, recreationally active and exercising at least three times per week. All participants should be non-vegetarian, non-smokers, not on weight reducing diet and not consuming medication or drugs. Participants who were diagnosed with neurological, metabolic, and/or cardiovascular diseases, and presenting high risk for performing maximal intensity exercises were excluded. They will also be excluded if they are unable to perform cycling exercises whether it's due to injury or other reasons.

3.2.2 Study Design

Each participant, in single blind and randomized counterbalanced order, performed three cycling exercise to exhaustion at 85%VO_2 max after the consumption of 500 ml of either CHO mixed with soybean (SOY+CHO), CHO mixed with whey protein (WHEY+CHO) or CHO alone (Control) (Day 1) . The participants then repeated the same experimental protocol with the same trial on the next day (Day 2). The exercise trials were separated by a one-week washout period. They were informed to refrain from taking any soy based and protein related supplement before the first trial until the completion of all trials. For two days before the trials, the participants were asked to limit themselves to activities of daily living and slow walking or cycling for personal transport. All cycling trials were performed under similar experimental and environmental conditions. Medical doctor was present during all the trials should participants collapse or fall ills during the

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trials. Before the main cycling trial, the participants undertook the submaximal and maximum aerobic capacity (VO_2max) test. Then the cycling load correspond to 85%VO_2max were predicted and familiarization trial was conducted to allow the participants to become familiar with the experiment protocol. Figure 3.2 presents the schematic of the study design.

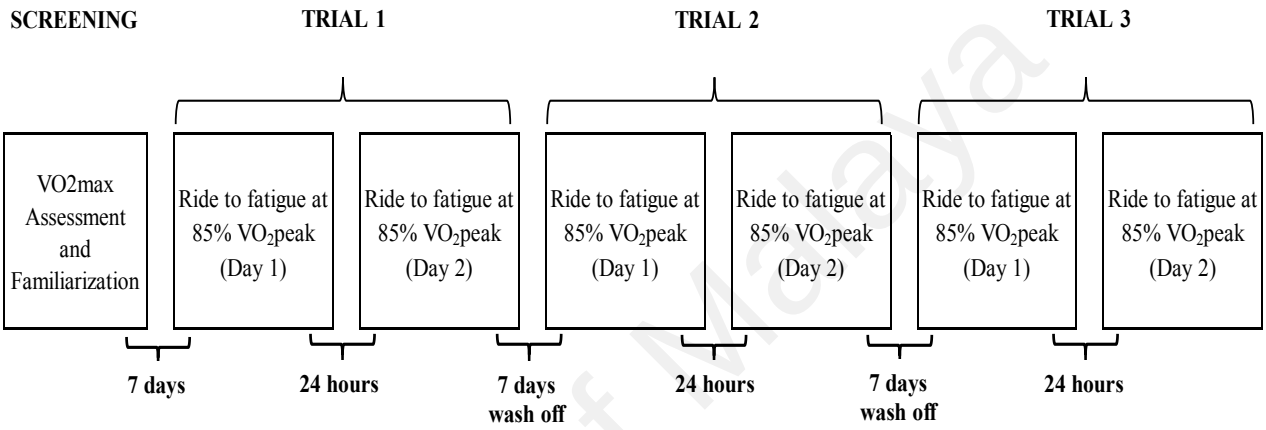

Figure 3.2: Schematic representation of the study design

3.2.3 Determinant of VO_2max and Exercise Intensity

The submaximal and maximum oxygen consumption (VO_2max) tests were conducted using a graded exercise test (GXT) protocol on Monark 839 E ergometer (Vansbro, Sweden) to determine the participant's VO_2 max and the cycling load which correspond to 85% VO_2max. For submaximal test, the participant performed a continuous cycling exercise starting at 50 watts with 25 watts increment every 4 minutes at 50-60rpm. During the last minute of every 4 min stage, heart rate was monitor using short-range telemetry (Polar FT4M, Polar Electro, Finland) and oxygen consumption (VO_2) was recorded using metabolic analyzer (Quark Cpet, Cosmed, Italy). Submaximal test was terminated when the participant's heart rate reached 85% of maximum heart rate (HRmax).

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Participant was given 30 minutes rest before the start of VO_2max test where they pedaled at 75 watts for 1 minute with 25 watts increment every one minute until volatile exhaustion. The VO_2max were determined when the participant met at least two out of three criteria described by the American College of Sports Medicine (2001):

1) Respiratory exchange ratio (RER) above 1.1,

2) VO_2 reached plateau, and

3) 95% predicted HRmax.

The maximum value of oxygen consumption recorded as VO_2peak if no plateau is observed. The VO_2peak value is then used to calculate the intensity in power (watt) at 85% of individual's VO_2peak.

3.2.4 Experimental beverages

The experimental beverages were prepared as described in 3.1.4.

3.2.5 Soybean Antioxidant analysis

Raw soybean was sent to UNIPEQ laboratory, Malaysia National University (UKM) for the analysis of total polyphenol content (TPC) as gallic acid equivalent (GAE) (mg/100g) and 2,2-diphenyl1-picrylhydrazyl (DPPH) as % inhibition (Wu et al., 2006) and FRAP in 1ml using method by (lkay Koca & Gençcelep, 2011).

3.2.6 Main Exercise Trials

Participants arrived at the Sports Nutrition laboratory after a 12-hour overnight fast, at approximately 08:30 hours (Day 1). They were weighed using Bioelectrical Impedance Analysis (InBody, USA) and a cannula (G-15, Venflon) was inserted in an antecubital vein by a medical doctor. Participants then rested in a seated position for 10 minutes before the baseline blood sample was drawn. Then the participants consumed either SOY+CHO, WHEY+CHO or CHO within 10 minutes. The participants were asked to

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stay within the testing area, executing only sedate behavior like sitting, reading and studying for 120 minutes before another blood sample was drawn (pre-exercise).

After a brief warm-up for 5 min, the participants cycle on an ergometer (Monark 839 E) at a workload equivalent to 85% VO_2peak until volitional exhaustion. Heart rate was monitored using Polar FT4M (Polar Electro, Finland). Exhaustion was defined as the point at which participants were no longer able to maintain the cycling load. At this point, rating of perceived exertion (RPE) were recorded using Borg scale (Borg, 1982), and the workload were recorded. The work done (kilopond per minute) and the total duration spent was used to calculate the total work done (kilopond). Blood samples were taken at exhaustion (post-exercise) and at 1-hour post exercise. The same protocol was repeated after 24 hours period to determine the recovery effect of the experimental beverages (Day 2). Figure 3.3 presents the schematic of the main exercise trial on Day 1 and the same protocol was repeated after 24 hours (Day 2).

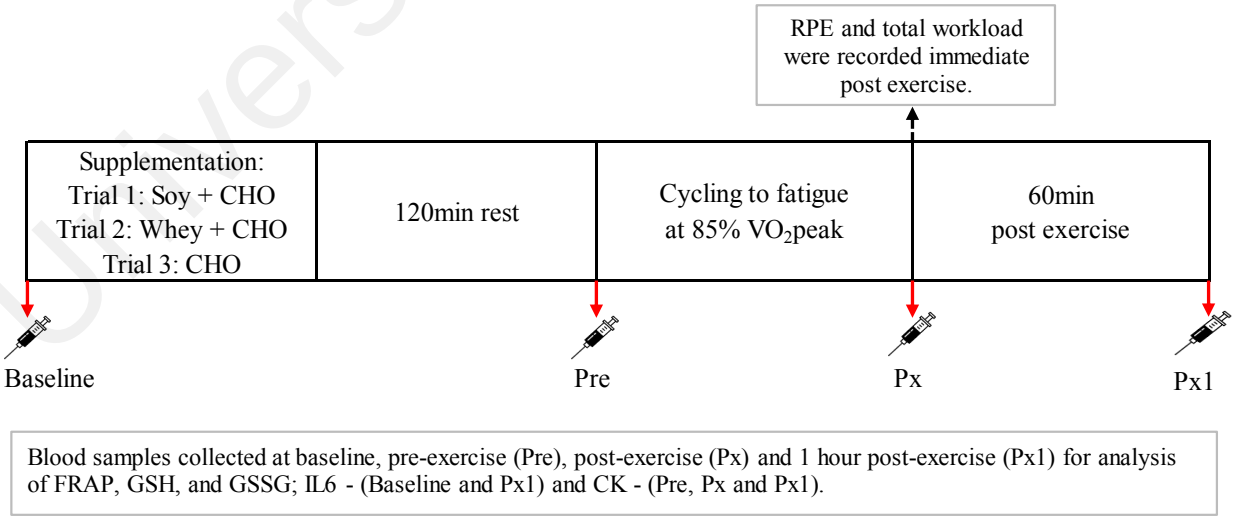

Figure 3.3: Schematic representative of the experimental protocol for Day 1 and Day 2

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3.2.7 Plasma Preparation and Analysis

Blood samples were collected into pre-cooled appropriate tubes (EDTA, Heparin or plain) and centrifuged at 3000rpm for 15 minutes at 4°C. After centrifugation, aliquots of plasma were transferred using a disposable plastic Pastured pipette into labeled 1.5-ml Eppendorf tubes. The aliquoted plasma was then stored at -40°C for later analysis of oxidative stress biomarkers (FRAP, and reduced to oxidized glutathione (GSH/GSSG) ratio), inflammatory biomarker (Interleukin-6 (IL-6)) and muscle damage marker (Creatine Kinase (CK)).

3.2.7.1 Ferric Reducing Anti-Oxidant Power (FRAP)

The plasma for FRAP was analyzed using OxiSelectTM Ferric Reducing Antioxidant Power Assay Kit (Cell Biolabs, INC, USA). For the preparation of assay buffer, 12ml of deionized water was added into a 3ml of assay buffer, mixed thoroughly until homogeneous. This buffer was used for preparing kit reagents and within the assay. As for reaction reagent, the colorimetric probe was diluted 1:10 and the iron chloride solution diluted 1:10 in the added assay buffer and vortexed thoroughly.

Each standard, sample and control were assayed in duplicate or triplicate. 100 µL of each standard, plasma samples or control was added to a 96-well plate. Then 100 µL of the reaction reagent was added to all wells and incubated for 10 minutes at room temperature before the absorbance of each well was read on Tecan microplate reader (Infinite 200 PRO, Switzerland) using 540-600 nm as the primary wavelength.

The average absorbance values were determined for each sample, control, and standard. The net optical density value (OD) was calculated by subtracting the zero-standard value from samples and standards. This was the background correction. The standard curve was then generated on a graph. The net OD of each sample was compared to the standard curve to determine the quantity of antioxidant potential, as µM Fe2+ iron

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equivalents (FRAP value), present in the sample. Only values within the range of the standard curve were used.

3.2.7.2 Glutathione (GSH) and Oxidized Glutathione (GSSG)

Glutathione and oxidized Glutathione response is measured using Glutathione Fluorometric Assay Kit (BioVision, USA). The reducing agent mix and GSH Quencher were done by adding 0.85ml water (H2O) to the OPA probe and mixed thoroughly before dissolved both in 1.05ml of distilled water (dH2O) separately for the reagent reconstitution on O-phthalaldehyde (OPA) Probe.

For standard curve, 10ul of the 20ul standard GSH stock was added to 990µl of Assay Buffer to generate a 0.2ug/ul working standard solution. A 0,2,4,6,8,10 ul were added to a 96-well plate to generate 0, 0.4, 0.8, 1.2, 1.6, 2.0 µg/well GSH. The volume is then increased to 90ul with Assay Buffer.

As of preparation of samples for Assays, 20ul of ice cold 6N KOH was added to 40µl of PCA preserved samples to precipitate PCA and neutralize the samples. The ice was kept for 5min before spun for 2 min at 13,000 G at 4°C. 10ul of the neutralized samples were then transferred to a 96-well plate.

For detection of GSH, the volume was increased to 90µl with Assay Buffer; whilst for detection of GSSG, the sample well volume was increased to 70µl with Assay Buffer and a background control without sample was created. A 10µl of GSH Quencher was added, mixed well and incubated at room temperature for 10min to quench the GSH. Then a 10µl of reducing agent mix was added to destroy the excess GSH Quencher and convert GSSG to GSH.

For Assay, 10µl of OPA probe was added into the standard and sample wells, mixed well, incubated at room temperature for 40min. The samples and standard were read on a

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fluorescence plate reader equipped with Ex/Em = 340/420 nm. The plate reader setting was adjusted so that the background reading without glutathione is at about 50-150 FRU.

For calculations, the background reading was subtracted from the sample readings. RFU vs GSH standard curve was plotted and the sample readings are applied to the standard curve to get glutathione amount in each sample.

Glutathione Concentration = Ga/Sv ug/ml.

Ga = glutathione amount from standard curve (in ug).

Sv = original sample volume added to the sample wells (in ml).

3.2.7.3 Interleukin-6 (IL-6)

Plasma interleukin-6 was measured using eBioscience Human IL-6 High Sensitivity Elisa (Vienna, Austria). The required number of microwell strips was determined and washed twice with wash buffer.

For the standard dilutions on the microwell plate, a 100µl sample diluent, in duplicate, was added to all standard wells. 100µl prepared standard was then pipetted into the first wells and standard dilutions was created when 100µl was transfer from well to well. 100µl from the last wells were discarded. 100µl sample diluent in duplicate was added into the blank wells, 50µl sample diluent to sample wells, and 50µl sample in duplicate to designated sample wells.

Biotin conjugate was prepared and 50µl was added into each well. The microwell strips were then covered and incubated for 2 hours at room temperature (18°C to 25°C). Streptavidin-HRP was prepared and 100ul diluted Streptavidin-HRP was added to all wells after the microwell strips were emptied and wash 6 times with wash buffer. The

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microwell strips were covered again for second time and incubated at room temperature for 1 hour.

The microwell strips were then washed 6 times again with wash buffer before added with 100µl Amplification solution I to all wells, covered and incubated for 15min at room temperature. The wash process was repeated after 15min and 100ul added with Amplification solution II to all wells before covered and incubated for 30min at room temperature. The microwell strips were washed 6 times with wash buffer again and added with 100µl TMB substrate solution to all wells, incubated for 10-20 minutes at room temperature before a 100µl of stop solution was added to all wells for the final process. The microwell was then been emptied and measured for color intensity at 450nm.

3.2.7.4 Creatine Kinase (CK)

Plasma CK was measured using Vitros Chemisty Products CK Slides (Ortho-clinical diagnostic, INC, New York, USA). The slide was inserted into a slide channel of the DTSC II module, Johnson & Johnson clinic diagnostic, USA. Plasma CK was extracted using an electronic pipette and inserted into a funnel of the DTSC II where the plasma CK will be injected upon signal from the analyzer. Result will be printed after 30sec.

3.2.8 Statistical Analysis

Data were expressed as mean ± standard error mean (SEM) unless otherwise stated. The distribution of data normality was assessed using the Shapiro-Wilk test. Two-way mixed analyses of variance (ANOVA) (Mixed Between-Within Subjects) was performed on FRAP, IL-6, GSH, GSSG and GSH to GSSG ratio to determine trials and time differences between SOY+CHO, WHEY+CHO and CHO trials. Significant main effects and interactions were further analyzed using Tukey's post hoc test. Differences were considered significant if $p < 0.05$. One-way repeated measure ANOVA was conducted to determine differences between SOY+CHO, WHEY+CHO and CHO trials on post

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exercise and 1-hour post exercise for Day 1 and Day 2. All statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) Version 22.0 (SPSS, Inc., Chicago, IL).

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CHAPTER 4: RESULTS

4.1 The Effects of Soybean Co-ingestion with Carbohydrate on Post-prandial Glycemic, Insulinemic and Reactive Oxygen Species Responses in Healthy Men

4.1.1 Participants

All the participants (n=8) completed the experiment. The physical characteristics of the participants are presented in Table 4.1.

Table 4.1. Antropometric characteristics of the participants

Variables	Mean (SD)
Age	20.3 (1.2)
Height (cm)	171.3 (5.0)
Weight (kg)	59.5 (6.2)
BMI (kg/m^2)	20.2 (1.4)
%Fat (%)	13.9 (2.2)
FFM (kg)	28.8 (3.4)

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

4.1.2 Soybean Antioxidant analysis

The Soybean content analysis displayed a 5.01mg GAE, 56.83% DPPH inhibition and 5.81 µM of FRAP as in Appendix G.

4.1.3 Area under the Curve (AUC)

The mean AUC for glucose, insulin and ROS over 120 minutes after the consumption of SOY+CHO, WHEY+CHO and CHO beverages are presented in Table 4.2. The AUC for postprandial glucose was higher in SOY+CHO compared to WHEY+CHO and CHO. No significant difference was observed among all beverages (p=0.253). SOY+CHO

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showed a lower AUC for insulin compared to WHEY+CHO and CHO, however there were no significant differences among all groups (p=0.956). The AUC for ROS was lower in SOY+CHO than WHEY+CHO and CHO. No significant difference was observed among all beverages (p=0.677).

Table 4.2. Mean Area under the curve (AUC) for postprandial glucose, insulin and reactive oxygen species (ROS)

	CHO	**SOY+CHO**	**WHEY+CHO**
Glucose (mmol/L x min)	589.1 ± 18.8	608.9 ± 27.9	548.1 ± 28.9
Insulin (uIU/mL x min)	3982.4 ± 742.0	3493.7 ± 381.5	3693.0 ± 863.5
ROS (RFU x min)	7986.9 ± 824.4	7499.4 ± 478.6	8319.4 ± 611.9

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

4.1.4 Post-prandial Plasma Glucose, Insulin and ROS after Consumption of the Beverages

Glucose, Insulin and ROS concentrations at baseline and at 30, 60, 90, 120 minutes following SOY+CHO, WHEY+CHO and CHO consumption are presented in Figure 4.1-4.3, respectively. There was no significant main effect between all trials for glucose ($F_{8,84}$=0.604, p=0.772), insulin ($F_{8,84}$=0.326, p= 0.954) and ROS ($F_{8,84}$=0.326, p=0.954).

Postprandial glucose responses within trials are significantly different for the various time points ($F_{1.7,\ 36.1}$=29.489, p=0.0001) (Figure 4.1). 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 34.5% and 31.9% decreased from 30 to 90 minutes respectively after which the concentration remained the same until 120 min. Glucose concentrations in the WHEY+CHO trial decreased beyond the baseline level by 2.6%, 5.4% and 6.6% at the 60, 90 and 120 time points respectively. Glucose concentration in

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the WHEY+CHO trial was significantly lower in the 90 and 120 min time point compared to the 30-min time point.

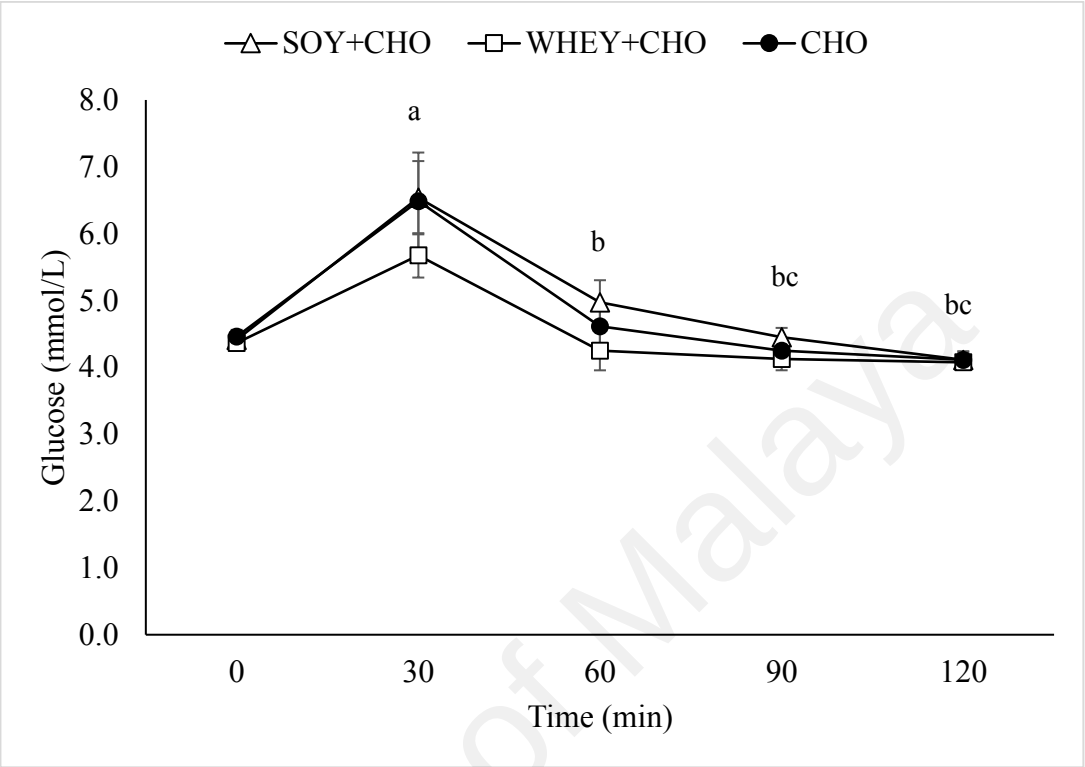

Figure 4.1. Plasma glucose concentration before (baseline) and 30 min, 60min, 90min and 120min after consumption of carbohydrate added soybean (SOY+CHO), carbohydrate added with whey protein (WHEY+CHO) and carbohydrate only (CHO) beverages. Values are mean ± SEM (*n=8*)

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

Postprandial insulin responses showed significant differences within trials for the various time point ($F_{1.8, 37.6}$=23.829, p=0.001) (Figure 4.2). Plasma insulin concentrations in SOY+CHO, WHEY+CHO and CHO trial significantly increased by 330.3%, 481.1% and 505.4% from baseline to 30 minutes. 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

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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.

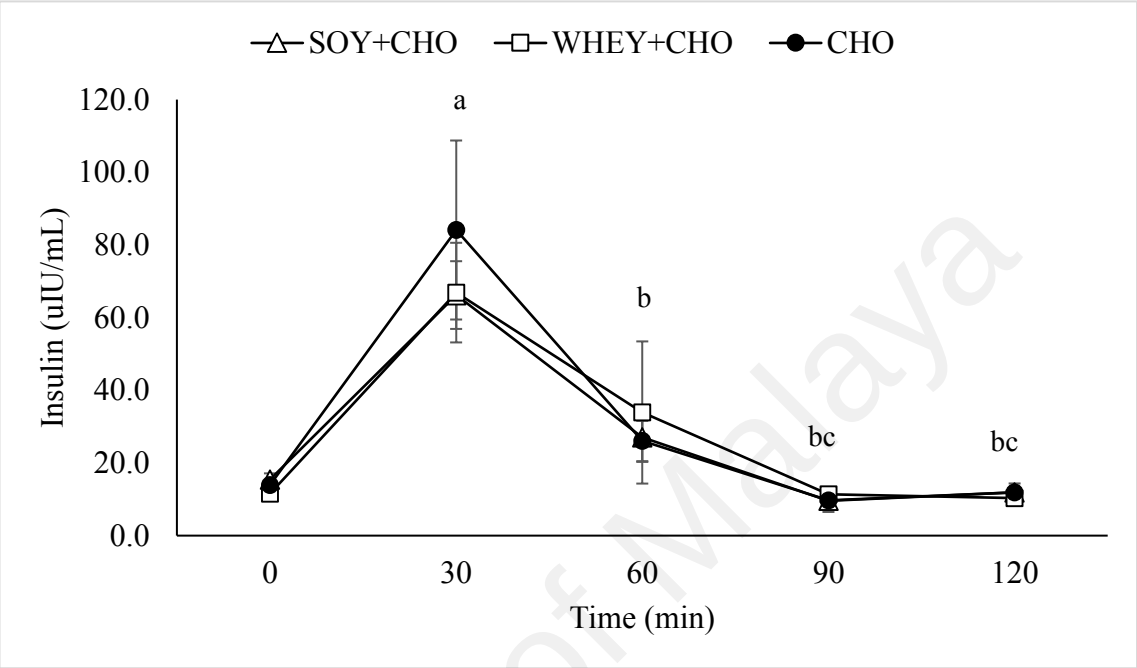

Figure 4.2. Plasma insulin concentration before (baseline) and 30 min, 60 min, 90 min and 120 min after consumption of carbohydrate added with soybean (SOY+CHO), carbohydrate added with whey protein (WHEY+CHO) and carbohydrate only (CHO) beverages. Values are mean ± SEM (*n=8*)

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

Figure 4.3 shows the plasma ROS values at the various points following consumption of the three beverages. The relative fluorescence unit (RFU) value for ROS was lower in SOY+CHO compared to WHEY+CHO and CHO at 0, 30, 60 and 120 min. However, there were no significant differences within trials for all time points ($F_{4,84}=1.336$, $p = 0.263$). Although RFU values for SOY+CHO and CHO beverages were lower at 30 and 60 min compared to baseline, this observation was not statistically significant.

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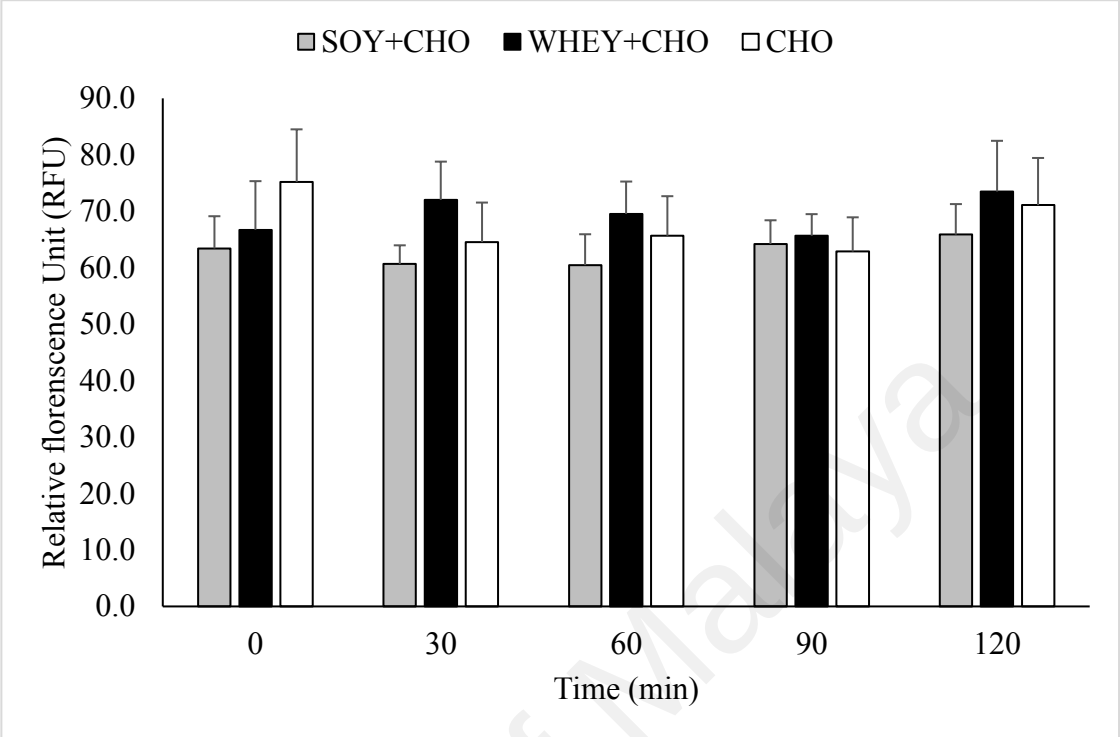

Figure 4.3. Plasma reactive oxygen species (ROS) concentration at before (baseline) and at 30 min, 60min, 90min and 120min after carbohydrate added soybean (SOY+CHO), carbohydrate added whey protein (WHEY+CHO) and carbohydrate only (CHO) consumption. Values are mean ± SEM (*n=8*)

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4.2 The Effects of Soybean Co-ingestion with Carbohydrate on Biochemical and Physiological Responses Post Exercise and Subsequent Exercise in Physically Active Men: A Preliminary Study

4.2.1 Participants

All the participants (n=7) completed the experiment. The anthropometric and physiological characteristics of the participants are presented in Table 4.3.

Table 4.3. Antropometric and physiological characteristics of the participants

Variables	Mean (SD)
Age	20 (0.9)
Height (cm)	167.7 (4.4)
Weight (kg)	56.4 (4.8)
BMI (kg/m^2)	20.1 (1.6)
%Fat (%)	11.4 (2.6)
FFM (kg)	28 (2.2)
VO_2peak (L/min)	2.79(0.3)
VO_2peak (mL/kg/min)	49.3 (6.2)

Values are mean (Standard deviation) (*n=7*). BMI = body mass index, FFM = fat free mass, VO_2peak = peak oxygen consumption.

4.2.2 Biochemical Response

4.2.2.1 Ferric Reducing Antioxidant Power (FRAP)

Plasma Ferric reducing antioxidant power (FRAP) concentrations at baseline (0 min), pre exercise (120 min), post exercise (Px) and 1-hour post exercise (Px1) following SOY+CHO, WHEY+CHO and CHO consumption on Day 1 and Day 2 are presented in Table 4.4. There was no significant main effect between all trials for FRAP in Day 1 ($F_{6,36}$=1.47, p=0.215) and subsequent Day 2 ($F_{6,36}$=1.35, p=0.263).

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Table 4.4. Ferric Reducing Antioxidant Power (FRAP) responses at baseline (0 min), pre exercise (120min), post exercise (Px) and 1-hour post exercise (Px1) for Day 1 and Day 2 after consumption of carbohydrate added soybean (SOY+CHO), carbohydrate added whey protein (WHEY+CHO) and carbohydrate only (CHO) beverages.

FRAP (μM)	Day 1			
Trial	0min	120min	Px	Px1
SOY+CHO	679.2 ± 40.1	647.5 ± 41.0	624.4 ± 41.9	777.1 ± 27.8*
WHEY+CHO	643.2 ± 40.4	649.9 ± 31.7	648.9 ± 23.8	715.2 ± 40.4
CHO	692.3 ± 21.2	656.1 ± 23.3	670.6 ± 16.8	729.9 ± 28.3
	Day 2			
Trial	0min	120min	Px	Px1
SOY+CHO	660.3 ± 48.4	700.1 ± 31.2	677.0 ± 34.5	733.4 ± 51.0
WHEY+CHO	701.8 ± 27.0	706.6 ± 18.8	673.7 ± 29.8	728.0 ± 33.6*
CHO	698.6 ± 37.3	656.2 ± 21.7	649.8 ± 16.1	719.4 ± 27.9*

Values are mean ± standard error of the mean (SEM) ($n=7$).
*significantly different ($p<0.05$) from Px.

On Day 1, plasma FRAP activity in SOY+CHO trial decreased by 4.7% at pre-exercise compared to baseline and continue to decrease by 3.6% post exercise before an increment of 24.5% 1-hour post exercise. WHEY+CHO showed an increase by 1% at pre-exercise from baseline before decreased slightly by 0.2% at post exercise. The activity then increased by 10.2% 1-hour post exercise. Meanwhile in CHO trial, FRAP activity decreased by 5.2% at pre-exercise from baseline before an increment of 2.2% post exercise and further increment of 8.8% 1-hour post exercise. No significant changes were observed in plasma FRAP activities in all three trials from baseline to 1-hour post exercise ($p>0.05$), except between post exercise and 1-hour post exercise in SOY+CHO trial.

For Day 2, plasma FRAP activity in SOY+CHO trial showed a similar trend as the WHEY+CHO trial where FRAP increased from baseline to pre-exercise by 6.0% and 0.7%, then decreased by 3.3% and 4.7% at post exercise, respectively. FRAP activity subsequently increased by 8.8% (SOY+CHO) and 8.1% (WHEY+CHO) 1-hour post exercise. FRAP activity in CHO trial showed a maximum reduction of 7.1% at post exercise compared with baseline. The activity subsequently increased by 10.7% from post

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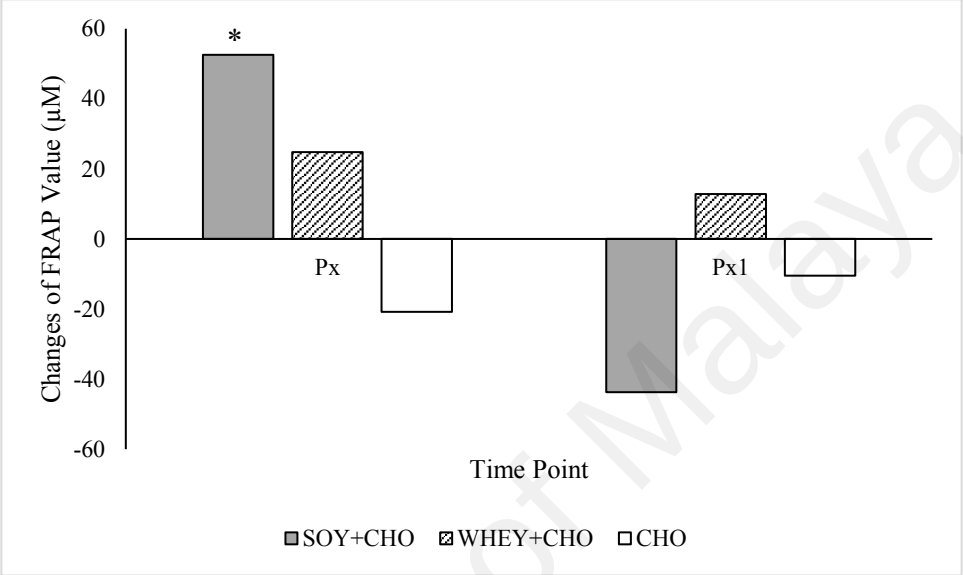
exercise to 1-hour post exercise. No significant difference was found within SOY+CHO trial from baseline to 1-hour post exercise. However, significant changes (p<0.05) were seen between post exercise and 1-hour post exercise in WHEY+CHO and CHO trials.



* Significantly different (p<0.05) between SOY+CHO and CHO.

Figure 4.4. The FRAP differences between Day 1 and Day 2 for post exercise (Px) and 1-hour post exercise (Px1) between the SOY+CHO, WHEY+CHO and CHO trial. Values are mean ± standard error of the mean (SEM) (n=7).

The antioxidant activity differences at post exercise and 1-hour post exercise between Day 1 and Day 2 were not significantly different between SOY+CHO and WHEY+CHO trials (Figure 4.4). At post exercise, antioxidant responses increased in both trials from Day 1 to Day 2 and the increment was higher in SOY+CHO as compared to WHEY+CHO trial. At 1-hour post exercise, antioxidant responses decreased in SOY+CHO trial while WHEY+CHO trial increased from Day 1 to Day 2 and these changes observed no significant difference (p>0.05) between two trials. When compared to CHO trial, the antioxidant responses was significantly different (p<0.05) with SOY+CHO trial post exercise only.

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4.2.2.2 The glutathione (GSH, GSSG, GSH to GSSG ratio)

Plasma glutathione (GSH), oxidized glutathione (GSSG) and glutathione to oxidized glutathione ratio (GSH/GSSG) at baseline (0 min), pre-exercise (120 min), post exercise (Px) and 1-hour post exercise (Px1) after consumption of SOY+CHO, WHEY+CHO and CHO beverages on Day 1 and Day 2 are shown in Table 4.5, 4.6 and 4.7, respectively.

There was no significant main effect between all trials for GSH in Day 1 ($F_{6,36}$=1.31, p=0.279) and subsequent Day 2 ($F_{6,36}$=0.72, p=0.635) (Table 4.5).

Table 4.5. Glutathione (GSH) responses at baseline (0 min), pre exercise (120 min), post exercise (Px) and 1-hour post exercise (Px1) for Day 1 and Day 2 after consumption of carbohydrate added soybean (SOY+CHO), carbohydrate added whey protein (WHEY+CHO) and carbohydrate only (CHO) beverages.

GSH (µmol/L)	Day 1			
Trial	0 min	120 min	Px	Px1
SOY+CHO	73.9 ± 10.5	73.5 ± 12.2	87.2 ± 7.8	62.0 ± 11.1
WHEY+CHO	90.4 ± 20.8	78.6 ± 16.0	110.7 ± 19.0^{+}	98.8 ± 20.1
CHO	79.9 ± 14.2	104.5 ± 16.1	101.3 ± 14.7	83.9 ± 14.0
	Day 2			
Trial	0 min	120 min	Px	Px1
SOY+CHO	59.9 ± 9.6	76.3 ± 11.1	80.8 ± 9.6	73.3 ± 11.2
WHEY+CHO	74.6 ± 14.5	101.5 ± 18.4*	98.0 ± 25.5	95.3 ± 19.7
CHO	75.6 ± 16.8	95.2 ± 12.8	110.6 ± 15.3	117.9 ± 24.0

Values are mean ± standard error of the mean (SEM) (n=7).
+significantly different (p<0.05) between Px and 120 min.
*significantly different (p<0.05) between 120 min and 0 min.

On Day 1, plasma GSH concentration in SOY+CHO and WHEY+CHO trials decreased from baseline to pre-exercise by 0.6% and 13.1%, respectively. GSH then increased (SOY + CHO, 18.7%; WHEY + CHO, 40.9%) at post exercise before decreased (SOY + CHO, 28.9%; WHEY + CHO, 10.7%) at 1-hour post exercise. The CHO trial however, showed an increment of GSH concentration by 13.1% from baseline to pre-exercise before decreased by 3.1% and 17.2% at post exercise and at 1-hour post exercise, respectively. There were no significant difference observed in GSH activities in all three

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groups between pre-exercise and post exercise (p>0.05). All groups showed an increase from post-exercise to 1-hour post exercise and significant difference was observed in WHEY+CHO trial (p<0.05) only.

For Day 2, plasma GSH concentration in SOY+CHO trial increased by 27.3% from baseline to pre-exercise and 5.9% increased at post exercise. The concentration then decreased by 9.2% at 1-hour post exercise. Plasma GSH concentration in WHEY+CHO trial shown the highest increment from baseline to pre-exercise by 36.0% before decreased by 3.4% and 2.8% at post-exercise and at 1-hour post exercise, respectively. Plasma GSH concentration in CHO trial increased by 25.9% at pre-exercise, 16.2% post exercise and 6.6% 1-hour post exercise. No significant changes was observed in GSH concentration for all three trials (p>0.05), except at between baseline and pre-exercise in WHEY+CHO trial (p<0.05).

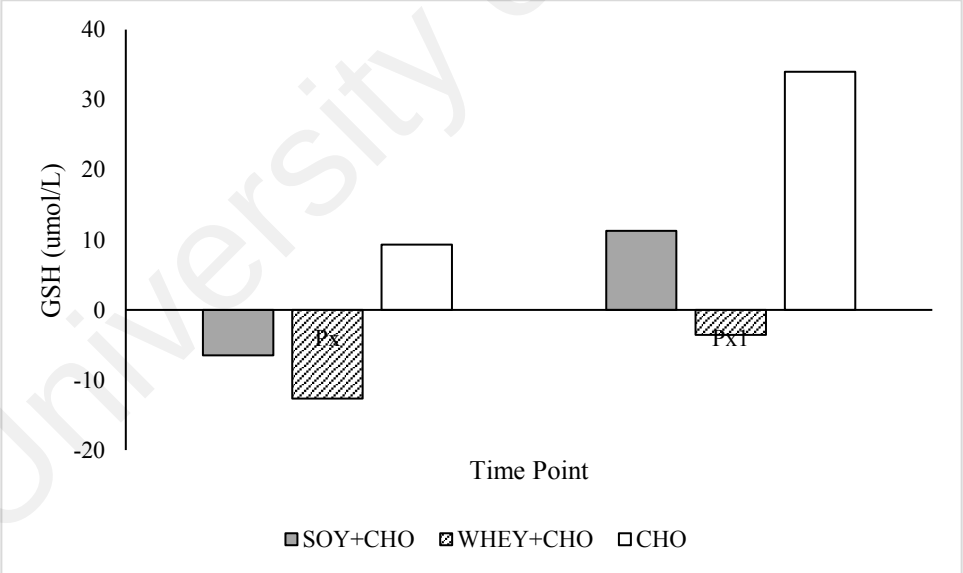

Figure 4.5. The GSH differences between Day 1 and Day 2 for post exercise (Px) and 1-hour post exercise (Px1) between the SOY+CHO, WHEY+CHO and CHO trial. Values are mean ± standard error of the mean (SEM) (n=7).

The antioxidant concentration of glutathione (GSH) differences at post exercise and 1-hour post exercise between Day 1 and Day 2 were not significantly different (p>0.05) between SOY+CHO and WHEY+CHO trials (Figure 4.5). At post exercise, GSH

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responses decreased in both trials from Day 1 to Day 2 and the decrement was less in SOY+CHO than in WHEY+CHO trial. At 1-hour post exercise, GSH responses in SOY+CHO increased while the WHEY+CHO trial decreased from Day 1 to Day 2 and the changes observed no significant difference (p>0.05) between the two trials. When compared to CHO trial, GSH was not significantly difference (p>0.05) with SOY+CHO and WHEY + CHO at both post exercise and 1-hour post exercise.

There was no significant main effect observed between all trials for GSSG in Day 1 ($F_{6,36}$=0.60, p=0.725) and subsequent Day 2 ($F_{6,36}$=1.32, p= 0.274) (Table 4.6).

Table 4.6. Oxidized glutathione (GSSG) responses at baseline (0 min), pre exercise (120 min), post exercise (Px) and 1-hour post exercise (Px1) for Day 1 and Day 2 after consumption of SOY+CHO, WHEY+CHO and CHO only beverages.

GSSG (µmol/L)	Day 1			
Trial	0 min	120 min	Px	Px1
SOY+CHO	874.9 ± 82.4	831.8 ± 77.8	894.0 ± 57.9	738.7 ± 60.4
WHEY+CHO	816.2 ± 59.1	773.1 ± 70.8	833.6 ± 55.0	738.0 ± 58.0*
CHO	832.0 ± 61.5	856.6 ± 67.8	871.7 ± 73.5	824.5 ± 77.5
	Day 2			
Trial	0 min	120 min	Px	Px1
SOY+CHO	713.1 ± 50.0	758.0 ± 24.3	746.3 ± 66.3	735.5 ± 53.2
WHEY+CHO	783.7 ± 97.7	844.9 ± 55.5	836.8 ± 45.6	761.2 ± 37.9
CHO	925.8 ± 80.8	767.6 ± 116.7	837.7 ± 111.5	857.9 ± 49.4

Values are mean ± standard error of the mean (SEM) (n=7).
*significantly different (p<0.05) between Px1 and Px.

On Day 1, plasma of oxidized glutathione (GSSG) concentration in SOY+CHO trial showed an increment of 4.9% and 7.5%, from baseline to pre-exercise and at post exercise respectively, followed by a decrement of 17.4% at 1-hour post exercise. Meanwhile, plasma GSSG in WHEY+CHO trial decreased by 5.3% at pre-exercise from baseline, then increased by 7.8% at post exercise before decreased by 11.5% at 1-hour post exercise. In CHO trial, plasma GSSG concentration increased by 3.0% at pre-exercise and 1.8% post exercise before 5.4% decreased at post 1-hour exercise. There were no significant changes of GSSG concentration in all three groups between pre-exercise and

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post exercise (p>0.05). Plasma GSSG concentration reduced from post-exercise to 1-hour post exercise in all trials and significant difference was observed only in WHEY+CHO trial (p<0.05).

For Day 2, plasma GSSG concentration in SOY+CHO trial increased from baseline to pre exercise by 6.3% before further 1.5% decreased at post exercise and 1.4% decreased 1-hour post exercise. In WHEY+CHO trial, the GSSG concentration showed an increment of 7.8% at pre-exercise compared to baseline followed by a decrement of 1.0% and 9.0% at post-exercise and 1-hour post exercise, respectively. Meanwhile in CHO trial, GSSG decreased by 17.1% at pre-exercise from baseline before 9.1% increased at post exercise and further 2.4% increased 1-hour post exercise. All three trials did not show any significant changes of GSSG concentration at all time points (p>0.05).

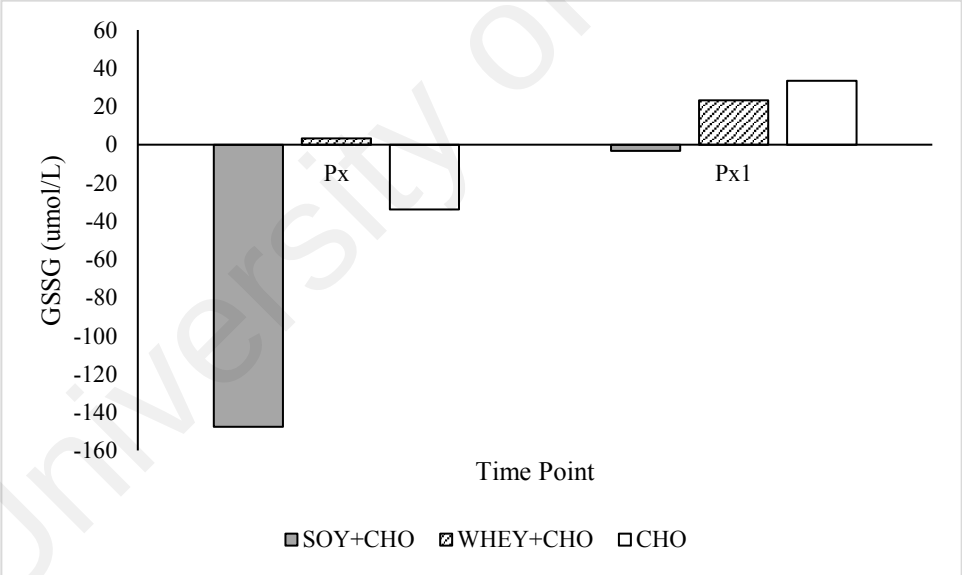

Figure 4.6. The GSSG differences between Day 1 and Day 2 for post exercise (Px) and 1-hour post exercise (Px1) between the SOY+CHO, WHEY+CHO and CHO trial. Values are mean ± standard error measurement (SEM) (n=7).

The oxidized glutathione concentration (GSSG) differences at post exercise and 1-hour post exercise between Day 1 and Day 2 were not significantly different (p>0.05) between SOY+CHO and WHEY+CHO trials (Figure 4.6). At both post exercise and 1-hour post exercise, GSH responses decreased in SOY+CHO trial but increased in

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WHEY+CHO from Day 1 to Day 2 and the changes observed no significant difference (p>0.05) between the two trials. Compared to CHO trial, GSSG was not significantly different (p>0.05) with SOY + CHO and WHEY+CHO at both post exercise and 1-hour post exercise.

As for the glutathione ratio (GSH/GSSG) which indicates oxidative stress, there was no significant main effect between all trials in Day 1 ($F_{6,36}$=1.52, p=0.199) and subsequent Day 2 ($F_{1.252,7.511}$= 0.76, p=0.442) (Table 4.7).

Table 4.7. The glutathione ratio (GSH/GSSG) at baseline (0 min), pre exercise (120 min), post exercise (Px) and 1-hour post exercise (Px1) for Day 1 and Day 2 after consumption of SOY+CHO, WHEY+CHO and CHO only beverages.

GSH:GSSG Ratio	Day 1			
Trial	0min	120min	Px	Px1
SOY+CHO	0.085 ± 0.012	0.088 ± 0.012	0.099 ± 0.008	0.084 ± 0.014*
WHEY+CHO	0.109 ± 0.023	0.102 ± 0.020	0.134 ± 0.023	0.139 ± 0.031
CHO	0.097 ± 0.017	0.125 ± 0.022	0.120 ± 0.019	0.103 ± 0.018
	Day 2			
Trial	0min	120min	Px	Px1
SOY+CHO	0.082 ± 0.009	0.100 ± 0.013^{+}	0.100 ± 0.015	0.100 ± 0.013
WHEY+CHO	0.095 ± 0.017	0.121 ± 0.021	0.113 ± 0.024	0.124 ± 0.024
CHO	0.080 ± 0.014	0.073 ± 0.041	0.100 ± 0.029	0.136 ± 0.027

Values are mean ± standard error of the mean (SEM) (n=7).
*significantly different (p<0.05) between Px1 and Px.
+significantly different (p<0.05) between 120 min and 0 min.

On Day 1, the glutathione ratio (GSH/GSSG) in SOY+CHO trial increased from baseline to pre exercise by 4.1% followed by an increment of 11.7% post exercise before decreased by 14.7% at 1-hour post exercise. GSH/GSSG in WHEY+CHO trial decreased by 6.4% at pre-exercise before increase by 30.6% and 3.6% at post exercise and at 1-hour post exercise, respectively. As for CHO trial however, GSH/GSSG showed an increment of 28.3% at pre-exercise from baseline followed by a decrement of 4.3% and 14.2% at post exercise and 1-hour post exercise, respectively. There were no significant changes in oxidative stress in all three trials despite of the different responses at pre-exercise, post

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exercise and 1-hour post exercise (p>0.05), except between post exercise and 1-hour post exercise in SOY+CHO trial (p<0.05).

On Day 2, GSH/GSSG for SOY+CHO trial increased by 21.3% from baseline to pre exercise and by 0.3% at post exercise before decreased by 1.0% at 1-hour post exercise. In WHEY+CHO trial, GSH/GSSG also showed an increment of 27.0% at pre-exercise, a decrement of 6.4% at post-exercise and then 9.6% increment at 1-hour post exercise. In the CHO trial however, decreased by 9.6% at pre-exercise from baseline before increased by 37.2% and 37.0% at post exercise and at 1-hour post exercise, respectively. All three trials did not show any significant changes of GSH to GSSG ratio value at all time points (p>0.05), except between baseline and pre exercise in SOY+CHO trial (p<0.05).

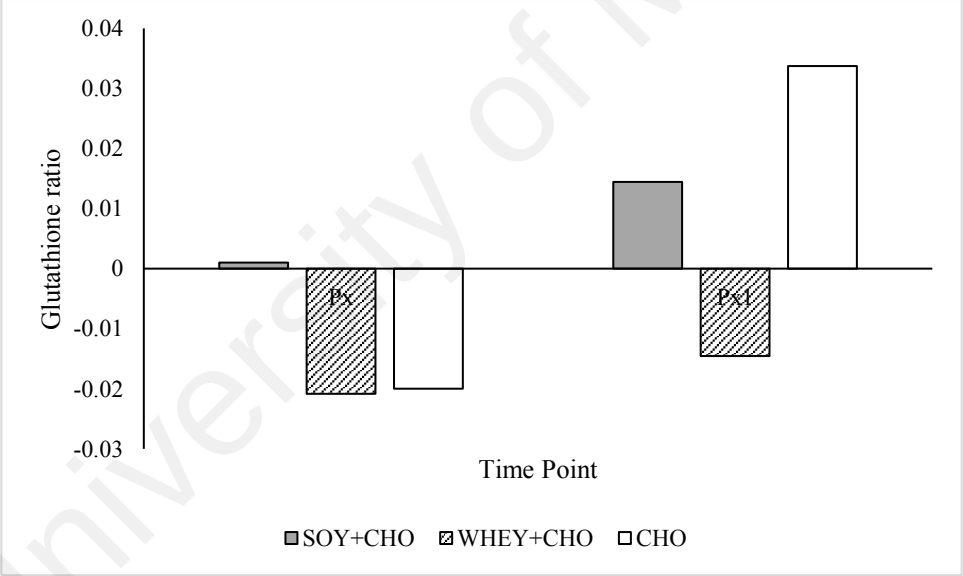

Figure 4.7. The glutathione ratio differences between Day 1 and Day 2 for post exercise (Px) and 1-hour post exercise (Px1) between the SOY+CHO, WHEY+CHO and CHO trial. Values are mean ± standard error of the mean (SEM) ($n=7$).

The glutathione ratio (GSH/GSSG) differences at post exercise and 1-hour post exercise between Day 1 and Day 2 were not significantly different (p>0.05) between SOY+CHO and WHEY+CHO trials (Figure 4.7). At post exercise and 1-hour post exercise, GSH/GSSG increased in SOY+CHO trials and decreased in WHEY+CHO from Day 1 to Day 2 and changes observed no significant difference (p>0.05) between the two

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trials. When compared to CHO trial, GSH/GSSG was not significantly different (p>0.05) with SOY+CHO and WHEY + CHO trials at both post exercise and 1-hour post exercise.

4.2.2.3 The interleukin-6 (IL-6)

Plasma interleukin-6 (IL-6) responses at 120min and at 1-hour post exercise on Day 1 and Day 2 after consumption of SOY+CHO, WHEY+CHO and CHO beverages are shown in Table 4.8. There was significant main effect between WHEY+CHO and CHO trials for IL-6 in Day 1 ($F_{2,12}$=8.297, p=0.005) and no significant for all trials in Day 2 ($F_{2,12}$=2.612, p=0.114).

Table 4.8. Interleukin-6 (IL-6) values at baseline (120 min) and 1-hour post exercise (Px1) for Day 1 and Day 2 after consumption of SOY+CHO, WHEY+CHO and CHO only beverages.

IL-6 (pg/mL)		Day 1	Day 2
Trial	120min	Px1	Px1
SOY+CHO	0.211 ± 0.06	0.365 ± 0.09	0.517 ± 0.18
WHEY+CHO	0.262 ± 0.09	0.684 ± 0.13*	0.524 ± 0.13
CHO	0.244 ± 0.04	0.518 ± 0.05*	0.429 ± 0.14

Values are mean ± standard error of the mean (SEM) (n=7).
*significantly different (p<0.05) between 120min and Px1

On Day 1, plasma IL-6 activity in SOY+CHO, WHEY+CHO and CHO trials increased by 73.1%, 161.4% and 112.4% respectively at 1-hour post exercise compared to baseline. Significant changes were observed from baseline to 1-hour post exercise (p<0.05) for WHEY+CHO and CHO trial.

For Day 2, plasma IL-6 activity in SOY+CHO, WHEY+CHO and CHO trials increased by 145.3%, 100.2% and 76.0% respectively at 1-hour post exercise from baseline. No significant changes were observed in IL-6 activities in all three trials from baseline to 1-hour exercise post (p>0.05).

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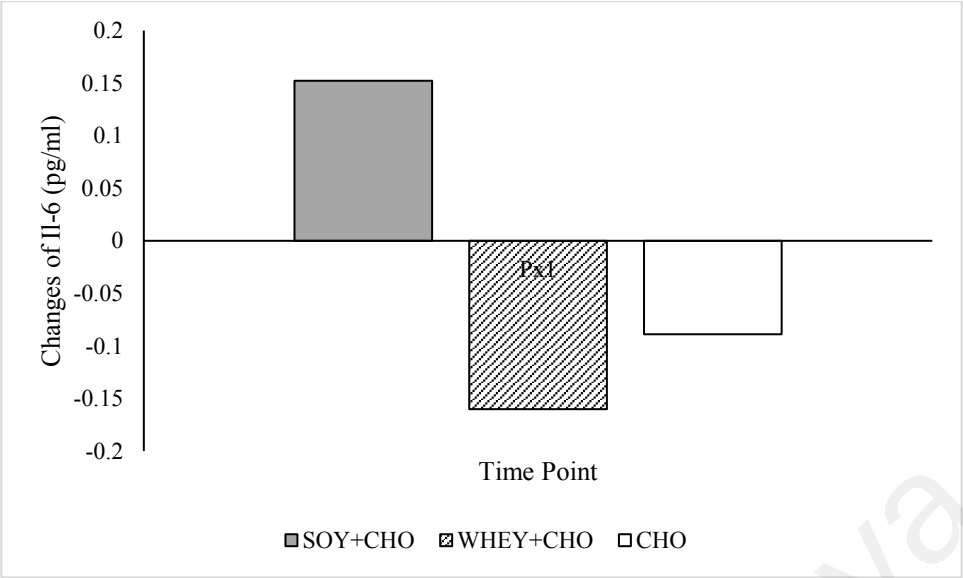

Figure 4.8. The IL-6 differences between Day 1 and Day 2 at 1-hour post exercise (Px1) between the SOY+CHO, WHEY+CHO and CHO trial. Values are mean ± standard error of the mean (SEM) (*n=7*).

There was no significant difference (P>0.05) in muscle inflammation responses at 1-hour post exercise between SOY+CHO and WHEY+CHO trials (Figure 4.8). Results showed that after 1-hour post exercise, muscle inflammation in SOY+CHO trial increased by 41.6% whilst in WHEY+CHO and CHO trials decreased by 23.4% and 17.2%, respectively. However, no significant difference was found between all trials (P>0.05). Muscle inflammation for post exercise was not measured due to funding limitation and 1-hour post exercise samples were analyzed for higher inflammation response.

4.2.2.4 Creatine kinase (CK)

The plasma CK concentration, an indicative of muscle damage, at baseline (0 min), pre exercise (120 min), post exercise (Px) and 1-hour post exercise (Px1) following SOY+CHO, WHEY+CHO and CHO consumption are presented in Table 4.9. There was no significant main effect observed between all trials for CK in Day 1 ($F_{1.988,11.926}$=0.67, P=0.527) and Day 2 ($F_{1.121,6.727}$=0.45, *p*=0.549).

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Table 4.9. The creatine kinase (CK) responses at pre-exercise (120 min), post exercise (Px) and 1-hour post exercise (Px1) for Day 1 and Day 2 after consumption of SOY+CHO, WHEY+CHO and CHO only beverages.

CK (U/L)		Day 1	
Trial	120min	Px	Px1
SOY+CHO	158.9 ± 15.6	186.1 ± 18.9^{+}	159.1 ± 14.5*
WHEY+CHO	184.4 ± 30.1	229.4 ± 42.8^{+}	196.3 ± 37.3*
CHO	149.6 ± 16.6	178.6 ± 25.2^{+}	150.0 ± 16.5*
		Day 2	
Trial	120min	Px	Px1
SOY+CHO	160.1 ± 12.2	186.1 ± 16.8^{+}	160.1 ± 12.4*
WHEY+CHO	165.7 ± 13.0	197.7 ± 19.2^{+}	167.7 ± 12.6*
CHO	194.6 ± 65.7	240.6 ± 91.8	192.1 ± 61.8

Values are mean ± standard error of the mean (SEM) (*n=7*).
+significantly different ($p<0.05$) between Px and 120 min.
*significantly different ($p<0.05$) between Px1 and Px.

On Day 1, the plasma creatine kinase (CK) concentration in SOY+CHO, WHEY+CHO and CHO trials significantly increased ($p<0.05$) from pre-exercise (120min) to post-exercise by 17.2%, 24.4% and 19.4%, respectively. The CK concentration then significantly decreased by 14.5% (SOY+CHO), 14.4% (WHEY+CHO) and 16.0% (CHO) at 1-hour post exercise.

On the subsequent Day 2, plasma CK concentration significantly increased ($p<0.05$) from pre-exercise (120min) to post-exercise in SOY+CHO (16.2%) and WHEY+CHO (19.3%) trials and significantly decreased ($p<0.05$) at 1-hour post exercise from post exercise (SOY + CHO, 14.0%; WHEY + CHO, 15.2%). There was no significant changes at all time point in CHO trial.

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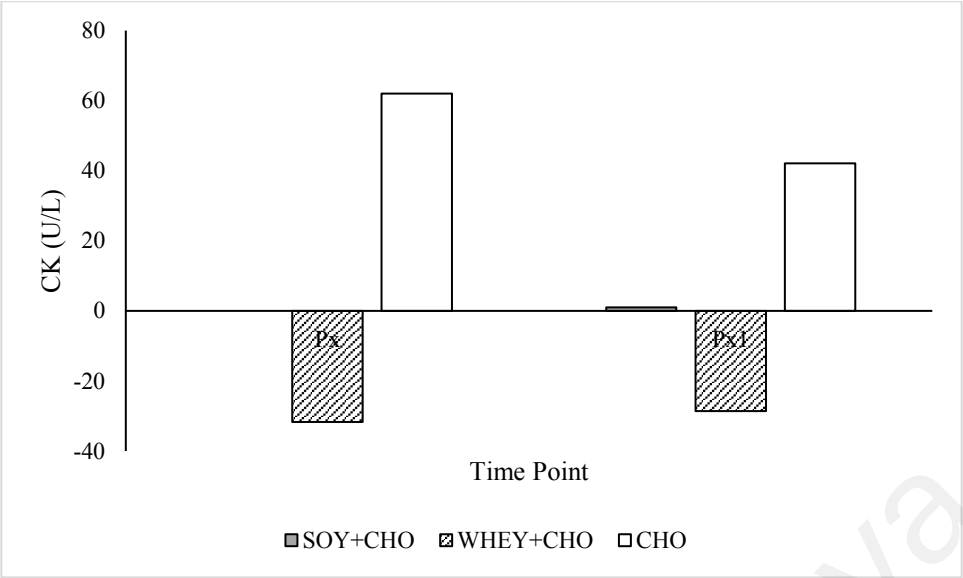

Figure 4.9. The CK response differences between Day 1 and Day 2 for post exercise (Px) and 1-hour post exercise (Px1) between the SOY+CHO, WHEY+CHO and CHO trial. Values are mean ± standard error of the mean (SEM) (*n=7*).

The muscle damage markers concentration of creatine kinase (CK) differences at post exercise and 1-hour post exercise between Day 1 and Day 2 were not significantly different (p>0.05) between SOY+CHO and WHEY+CHO trials (Figure 4.9). At both post exercise and 1-hour post exercise, CK responses were similar in SOY+CHO and decreased in WHEY+CHO trials from Day 1 to Day 2 and changes observed no significant difference (p>0.05) between the two trials. Compared to CHO trial, the increased in CK was no significantly different (p>0.05) with SOY+CHO and WHEY + CHO at both post exercise and 1-hour post exercise.

4.2.3 Physiological Responses

4.2.3.1 The rating of perceived exertion (RPE)

The post-exercise rating of perceived exertion (RPE) for Day 1 and Day 2 after consumption of SOY+CHO, WHEY+CHO and CHO beverages are shown in Table 4.10. There was no significant main effect between all trials for RPE in Day 1 ($F_{2,18}$= 5.12, p>0.05) and Day 2 ($F_{2,18}$=1.12, p>0.05).

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Table 4.10. The RPE responses at post exercise for Day 1 and Day 2 after consumption of SOY+CHO, WHEY+CHO and CHO only beverages.

RPE (6-20)	Day 1	Day 2
SOY+CHO	16.6 ± 0.3	16.7 ± 0.3
WHEY+CHO	16.9 ± 0.3	17.3 ± 0.4
CHO	17.0 ± 0.3	17.3 ± 0.2

Values are mean ± standard error of the mean (SEM) (*n=7*).

The rating of perceived exertion (RPE) for SOY+CHO, WHEY+CHO and CHO trials was higher by 0.86%, 2.54% and 1.68%, respectively at post exercise on Day 2 compared to Day 1. All increment of RPE did not show any significant difference ($p<0.05$). Despite containing branch-chained amino acid for the WHEY+CHO, it did not seem to be more effective in reducing the fatigue perception than SOY+CHO and CHO during an all-out effort exercise.

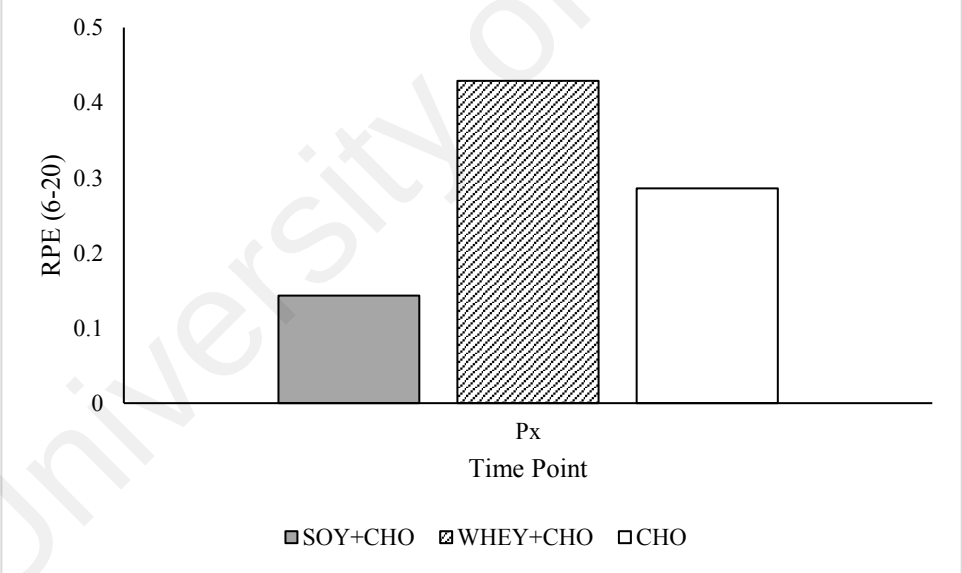

Figure 4.10. The RPE response differences between Day 1 and Day 2 at post exercise (Px) between the SOY+CHO, WHEY+CHO and CHO trial. Values are mean ± standard error measurement (SEM) (n=7).

The perceived exertion of fatigue (RPE) differences at post exercise between Day 1 and Day 2 were not significantly different ($p>0.05$) between SOY+CHO and WHEY+CHO trials (Figure 4.10). At post exercise, RPE increased from Day 1 to Day 2 in both SOY+CHO and WHEY+CHO trials and changes observed no significant

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difference (p>0.05) between the two trials. Compared to CHO trial, the increased in RPE was no significantly different (p>0.05) with SOY+CHO and WHEY + CHO trial at post exercise.

4.2.3.2 The total workload

There was no significant main effect between SOY+CHO, WHEY + CHO and CHO trials for total workload in Day 1 ($F_{2,18}$=0.011, p>0.05) and Day 2 ($F_{2,18}$=0.16, p>0.05) (Table 4.11).

Table 4.11. The total workload at post exercise for Day 1 and Day 2 after consumption of SOY+CHO, WHEY+CHO and CHO only beverages.

Workload (Kilopond)	Day 1	Day 2
SOY+CHO	11611.5 ± 1261.7	13496.2 ± 2016.8
WHEY+CHO	11733.2 ± 1395.4	14166.4 ± 1770.4
CHO	11945.3 ± 2047.8	12628.7 ± 20136.0

Values are mean ± standard error of the mean (SEM) (n=7).

The total workload (kpm) for SOY+CHO, WHEY+CHO and CHO trials increased from Day 1 to Day 2 by 16.2%, 20.7% and 5.7%, respectively. No significant difference were found in total workload for all three trials (p<0.05). Despite a similar RPE response on Day 1 and Day 2, the workload showed some increment on total workload on Day 2 as compared to Day 1 but not significant. The increment may be attributed to the learning effect.

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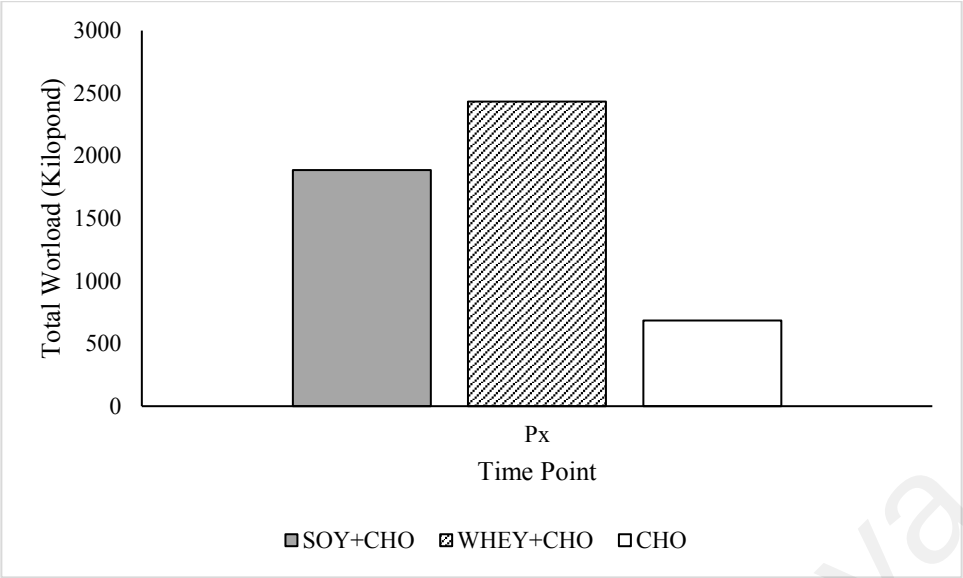

Figure 4.11. The total workload differences between Day 1 and Day 2 at post exercise (Px) between the SOY+CHO, WHEY+CHO and CHO trial. Values are mean ± standard error measurement (SEM) (n=7).

The total workload (kilopond) differences at post exercise between Day 1 and Day 2 were not significantly different (p>0.05) between SOY+CHO and WHEY+CHO trials (Figure 4.10). At post exercise, total workload was higher in both SOY+CHO and WHEY+CHO trials from Day 1 to Day 2 and changes observed no significant difference (p>0.05) between the two trials. When compared to CHO trial, the lower total workload was no significantly different (p>0.05) with SOY+CHO at post exercise.

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CHAPTER 5: DISCUSSION

The main findings of the first study was that the area under the insulin curve was lower in SOY+CHO compared to WHEY+CHO. Similarly, SOY+CHO tended to have a lower postprandial ROS response than WHEY+CHO. However, no significant difference was observed between the two beverages and when compared with CHO. 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.

Although there is no significant difference, CHO alone could induce higher glucose response compared to the protein-added CHO beverages. Similar response can be seen for the insulin and ROS. The rise in blood glucose levels following a carbohydrate-rich meal is known as postprandial hyperglycaemia. Hyperglycaemia can induce oxidative stress (Busik et al., 2008; Lin et al., 2017) and is typically accompanied with postprandial inflammation and impaired endothelial function (Busik et al., 2008; Ceriello, 2002). Postprandial hyperglycaemia is one of the risk factors for cardio-metabolic disease which is associated with oxidative imbalance.

Under normal physiological condition, the antioxidant defense systems are able to balance ROS production. However, excess ROS due to hyperglycaemia can alter redox balance, causing the activation of pro-inflammatory pathways such as the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB). This can impair cellular signaling (e.g. insulin signaling) and cause irreversible modifications of cellular and non-cellular components. Hyperglycaemia can promote non-enzymatic glycation of proteins such as low-density lipoprotein (LDL) (Nivoit, Wiernsperger, Moulin, Lagarde, & Renaudin, 2003; Sima et al., 2010), and can increase the susceptibility of LDL to

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oxidation, which is one of the risk factors for the progression and complications of atherosclerosis (Spagnoli, Bonanno, Sangiorgi, & Mauriello, 2007).

There are strategies that have been designed to modify postprandial glycaemia to favor lower blood glucose and ROS response and one of these strategies is to add protein to CHO diet or beverages. Studies involving addition of protein to CHO diet or beverages have reported lower glucose response and in contrast, higher insulin response (Akhavan, Luhovyy, Brown, Cho, & Anderson, 2010; Akhavan et al., 2014; Allerton et al., 2016). Soybean is rich in phytochemicals including isoflavones, saponins, triterpenoids and oligopeptides. These compounds have been reported to contain high antioxidant activities and are able to protect the body against oxidative stress (Callahan & Supinski, 2014; Fritz, Seppanen, Kurzer, & Saari Csallany, 2003). Genistein and daidzein which are the two main soy isoflavones, contain high antioxidant activities (Rahman Mazumder & Hongsprabhas, 2016). It was reported that consumption of soy beverage was able to increase FRAP activity 60 min post-consumption, although the increase was very small (approximately 3%) (Serrano, Martin-Gari, Cassanye, Granado-Serrano, & Portero-Otin, 2017). This same 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.

Differences observed in this study could be attributed to the dose of protein available in the beverages consumed. Kashima et al (2016) has shown that different doses of soy protein could yield different responses in AUC for plasma insulin, whereby higher concentrations of soy protein yielded lower AUC of plasma glucose and higher AUC of plasma insulin. Despite the similar weight of soybean and whey protein, the soybean,

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although not significant, contained 4.5% and 55% higher in total CHO and fat content as compared to whey protein. The amount of protein in the soybean-based beverage however was 47.7% less compared to the whey-based CHO beverage. This may explain why soybean-based beverage showed similar response as CHO alone beverage in terms of total AUC of plasma glucose while similar response was observed with whey protein-based beverage in terms of total AUC of plasma insulin.

The present study indicated the potential benefits of soybean-based CHO beverage in regulating post prandial glucose and insulin levels as well as providing protection against ROS and oxidative stress. Future studies looking into higher doses of protein and with higher numbers of participants may yield more significant results.

The results of the preliminary study on the effects of soybean co-ingestion with CHO beverage on biochemical and physiological responses in physically active men showed that soybean-based beverage may have a similar impact on antioxidant activity, oxidative stress, muscle inflammation, muscle damage, perceived fatigue and total workload at 85%VO_2peak post cycling and subsequent cycling, compared to whey protein when co-ingested with CHO. This study also showed an encouraging indication that the consumption of CHO beverage containing soybean may be effective in increasing antioxidant activity, reducing muscle inflammation and delayed muscle damage during high intense exercise compared to CHO beverage added with whey protein.

The FRAP values which indicate the antioxidant activity of the experimental beverages in the blood at 1-hour post exercise for Day 1 and Day 2 showed no significant difference between SOY + CHO and WHEY + CHO trials, and when compared to CHO trial. The small increase in SOY+CHO antioxidant activity post exercise in Day 1 may indicate some soy isoflavones response on the antioxidant activity in the blood. The content of soy isoflavones in this study may be low in yielding higher FRAP reading

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compared to WHEY+CHO and CHO trials. The efficacy of isoflavones is dependent on their relative enrichment in soy products, which can vary as much as 50-fold due to processing methods (Wang et al., 1998).

High intense aerobic exercise leads to excess production of ROS that caused oxidative stress. Glutathione is an antioxidant that act as a redox system that prevent oxidative damage induced by exercise onto tissues and cells in the body (Seifi-Skishahr, Damirchi, Farjaminezhad, & Babaei, 2016). In general, the relative amounts of reduced glutathione (GSH) and oxidized glutathione (GSSG) form determine the cellular redox state and more reduced redox represents healthy status (Jones & Liang, 2009), while more oxidized form predisposes individuals to aging and diseases (Chung et al., 2009). The additional antioxidants from the soybean did not seem to provide any additional protective benefits as compared to the WHEY+CHO trial and CHO trial (Control) in regulating the reduced glutathione. However, it did reduce the oxidized glutathione post exercise as compared to baseline for both Day 1 and Day 2. Similar response can be seen in the WHEY+CHO trial but not in the CHO trial. The small differences may due to the exercise protocol itself which may not able to yield a significant oxidative stress. The physical background of the participants may affect the oxidative stress and redox state in response to strenuous exercise. Trained participants may have better adaptation towards oxidative stress and redox state as compared to the untrained.

Interleukin-6 (IL6) measures inflammation in the muscles. The higher IL-6 responses in both SOY+CHO and WHEY+CHO trials compared to CHO trial (Control) at 1-hour post exercise for Day 1 and Day 2 though not significant may, along with insulin, act as a GLUT4 translocation modulator that potentiated glucose uptake when protein is added into CHO beverage especially whey protein. Neidert (2017) study supported this when they examined the effect of whey protein isolate and CHO supplementation on glucose

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uptake and IL-6 responses. This study observed higher increased in IL-6 and significant glucose clearance after exercise in the CHO added protein group as compared to CHO and control group. This may explain the faster clearance glucose in the WHEY+CHO trial in this study as compared to SOY+CHO and CHO trials. The higher IL-6 response may not seem to have negative impact on subsequent exercise trial as shown in this study.

Creatine Kinase (CK), indicative of muscle damage, was lower in both SOY+CHO and WHEY+CHO trials compared to the CHO trials at post exercise for Day 1 and Day 2. Similar result can be seen in study by Romano-Ely et al (2006) that found lower CK response in iso-caloric CHO beverage containing protein in comparison with carbohydrate alone beverage at subsequent exercise until exhaustion at 80%VO_2peak. The pre-exercise consumption of protein added beverage was able to provide free amino acid that can promote protein synthesis during high intense exercise and reduce muscle damage. However, White (2008) investigation on pre and post CHO added protein supplementation showed no significant difference in post-acute eccentric exercise. The added protein in the CHO beverage did not give additional benefit in delaying muscle damage. The CK response in this study was low and not significant between trials. Immediate and 24-hour post exercise CK may not provide the true difference in CK response. White (2008) study showed that there will be significant difference in CK response 48-hours post-eccentric exercise compared to immediate post exercise. Though there are differences observed in CK response between pre, post CHO-protein consumption and placebo group after 48 hours, the difference was not significant. The CHO content in White (2008) study was above 60gram and protein above 20gram which was higher than our beverages used in this study. This may yield different result in CK response as CHO-protein may not provide acute effect on delaying muscle damage.

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All the trials in this study did not show any significant difference in the rate of perceived exertion when performing the 85%VO_2peak cycling exercise. The added whey protein and soybean dosage of 10g in this study was insufficient to decrease the transportation of tryptophan to the brain and reduce the brain 5-HT synthesis and released. Studies from Saunders et al. (2009), Alghannam et al. (2011) and Hall et al. (2013) showed lower RPE score where the total protein intake was more than 10g throughout the exercising period. However, Goh (2012) found no significant difference in RPE despite having 25g added protein in the beverage consumed which did not justify that higher protein content will affect the perceived of muscle soreness rating. Hence no strong evidence suggesting that added protein alone will affect brain fatigue perception during exercise. Perhaps a greater difference can be seen in a steady state exercise design as compared to an all-out effort exercise and RPE may not be a good variable to measure under this condition.

Despite the similar result in RPE, the workload shown otherwise. The total workload in kilopond during 85%VO_2peak cycling exercise for both the SOY+CHO and WHEY+CHO trials were higher than the CHO trial and all three trials achieved higher workload in Day 2 compared to Day 1. The result was not significant indicating that this may be due to learning effects from the participants or simply the added protein dosage was not high enough to yield the extra benefits as claimed by other CHO added protein studies. Other possibility would be the CHO contain in the beverage is sufficient to provide the needed energy and the cessation of exercise was due to neuromuscular fatigue.

This preliminary study indicates the potential biochemical response benefits of soybean-based CHO beverage by increasing antioxidant activity, reduces muscle damage and inflammation, as well as improving physiological responses i.e. fatigue perception

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and exercise workload. The small and insignificant difference in all parameters measured between SOY+CHO and WHEY+CHO trials, and as compared to CHO trial may be due to the small dosage of soybean and whey protein content in the beverages consumed by participants. Study by Kashima et al (2016) showed that a higher dosage of soy can elicit higher response of insulin production when they compare a 10g, 20g and 40g of soy protein isolate. Other possible reason is the exercise protocol was not able to deplete the muscle glycogen or oxidized the consumed CHO in order to observe the response from the soybean and whey protein to take effect. Another limitation to this study was the small number of participants recruited due to participants' unavailability to commit a longer study design. Future studies looking into higher doses of protein and with higher numbers of participants may yield more significant results.

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CHAPTER 6: 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. Further investigation with a larger sample size is warranted to confirm this.

Soybean-based beverage may have a similar impact on antioxidant activity, oxidative stress, muscle inflammation, muscle damage, perceived fatigue and total workload at 85%VO_2peak post cycling and subsequent cycling, compared to whey protein when co-ingested with CHO. The consumption of CHO beverage containing soybean may be effective in increasing antioxidant activity, reducing muscle inflammation and delayed muscle damage during high intense exercise compared to CHO beverage added with whey protein. However, the effects of CHO added protein beverages on fatigue perception and intense exercise performance was not different between soybean and whey protein. These preliminary results suggest that soybean-based beverage can be used as an alternative to whey protein when consumed with CHO beverage for a more cost-effective supplementation and diary sensitive individuals.

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LIST OF PUBLICATIONS AND PAPERS PRESENTED

Publication

Albert TYW, Abdul Aziz A, Mohamad Shariff AH and Sareena HH. (2017). Effect of soybean co-ingestion with carbohydrate on postprandial glycaemc-induced reactive oxygen species in healthy men. Malaysian Journal of Nutrition, 23 (Supplement);S114 (SCOPUS-Indexed) - Abstract

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Effect of soybean co-ingestion with carbohydrate on postprandial glycaemic-induced reactive oxygen species in healthy men. The 1st Southeast Asia Health Nutrition Conference, May 14-17, 2017, Hotel Istana, Kuala Lumpur, Malaysia.

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