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Isocaloric Supplements of Whey Protein and Carbohydrate on Responses of Cardiorespiratory and Metabolic Systems and Blood Glucose Levels during Acute Progressive Exhaustive Exercises

Pei-Ni Chen[1], Amornpan Ajimaporn[1], Alisa Nana[1], Ai-Lun Yang[2], Mark Willems[3], Rungchai Chaunchaiyakul[1]

[1]College of Sports Science and Technology, Mahidol University, Nakhonpathom, Thailand, [2]Institute of Sports Sciences, University of Taipei, Taipei, Taiwan, [3]Institute of Sport, University of Chichester, Chichester, United Kingdom

ABSTRACT

Chen PN, Ajimaporn A, Nana A, Yang AL, Willems M, Chaunchaiyakul R. Isocaloric Supplements of Whey Protein and Carbohydrates on Responses of Cardiorespiratory and Metabolic Systems and Blood Glucose Levels during Acute Progressive Exhaustive Exercises. **JEPonline** 2023;26(3):1-14. The purpose of this study was to determine the effects of isocaloric supplements of whey protein and carbohydrate on physiologic functions and performance. Three separated randomized ingestions of whey protein (PRO), carbohydrate (CHO), and mixing of PRO-CHO (MIX) were provided to healthy males prior to performing progressive exercises to exhaustion. Blood glucose levels and physiological variables of the cardiorespiratory and metabolic functions were determined. Higher blood glucose was detected only in the ingestion of CHO. Most of gas exchange and cardiorespiratory variables measured during exercise were similar in all trails, except for the lower diastolic BP in PRO than in CHO and MIX throughout the test. MIX induced a higher VO_2 peak than did the other trials. In conclusion, immediate isocaloric ingestions of CHO, PRO, and MIX had exerted no impact on cardiorespiratory functions during high physical demands. Only the ingestion of CHO showed metabolic changes via blood glucose level; whereas, the ingestion of MIX induced a higher endurance performance on VO_2 peak.

Key Words: Blood Glucose, Cardiac Hemodynamic, Oxygen Uptake, Whey Protein

INTRODUCTION

Nutritional supplementations strategies are widely used among athletes to improve physical performance and gain competitive advantage (8). Glucose markedly influences on capacity of working muscles to sustain prolonged or mild-to-high intensity physical activity (21,23). However, numerous studies have reported that adding protein to carbohydrate intake results in an improvement in endurance exercise performance than carbohydrate (CHO) alone (9,17). Not only the chronic ingestion of protein (PRO) and carbohydrate on physiological and performance changes (7), but some studies also reported a longer time-to-exhaustion and time-trial from single doses of CHO-PRO versus CHO ingestion for both during and post-exercise (4,5,7).

To our knowledge, there are very few studies that have examined the effects of acute pre-exercise CHO-PRO ingestion on endurance performance. In 2011, Alghannam (1) reported the effect of pre-exercise ingestions of CHO and CHO-PRO, which resulted in a 7-minute longer running time in CHO-PRO than CHO alone. Hence, it is speculated that the additional protein supplement provides some extra benefit in physiological changes than when just CHO is consumed.

Theoretically, whey protein is widely used and regarded as a potent macronutrient to regenerate muscle function (5,12) and to improve sports performance (24,31). It contains various essential and non-essential amino acids that the body can rapidly absorb within 30 minutes along with positive effects on specific biological processes (20,25). For example, some investigators (5,22,23) have proposed that this protein will exert an additional effect on the level of insulin response and glycogen storage, which will enhance the subsequent ability to exercise. Moreover, long-term effects of whey protein on cardiovascular adaptation have been previously reported to improve vessel function while also increasing plasma volume and albumin in elderly patients (4,8). To our knowledge, very few studies have examined the effects of whey protein on cardiovascular hemodynamic responses in healthy sedentary subjects during exercise. A single dose of whey protein may possibly cause additional changes either in cardiac function centrally or peripherally on vascular resistance and thus affects O_2 uptake during exercise.

Accordingly, the purpose of the present crossover study was to estimate exercising metabolism, cardiorespiratory function, and aerobic capacity changes from an acute ingestion of carbohydrate, whey protein, or a combined supplementation in sedentary healthy adults. To avoid the variation in energy content, the present study used an isocaloric design.

METHODS

Participants

Healthy males, aged between 20 to 30 years (n = 13), voluntarily participated. The Inclusion criteria required that the participants: (a) had a recreationally active lifestyle (i.e., light exercise <3 days per week over the last 6 months); (b) had a body mass index (BMI) between 18.5 to 24.9 $kg \cdot m^{-2}$; (c) had no known history of cardiovascular, respiratory, and kidney diseases; (d) were non-allergic to dairy products; (e) were non-smokers; and (f) were non-vegetarian.

Procedures

All participants visited the laboratory for 3 randomized trials of the following isocaloric (100 kcal) supplements: (a) carbohydrate (CHO, 25 g); (b) whey protein (PRO, 25 g); and (c)

carbohydrate plus whey protein (MIX, CHO 12.5 g plus PRO, 12.5 g). Each visit was separated by 7 days. The cardiovascular variables that were measured included heart rate (HR), stroke volume (SV), end-diastolic volume (EDV), systemic vascular resistance (SVR), cardiac output (CO), end-systolic volume (ESV), total peripheral resistance (TPR), ejection fraction (EF) at rest, during the exercise test, and after the exercise test using a non-invasive impedance cardiograph instrument (PhysioFlow® PF07 Enduro™, France). A telemetry gas analyzer (Oxycon mobile®, Germany) was used to determine the respiratory and metabolic variables, which included oxygen consumption (VO_2), carbon dioxide production (VCO_2), respiratory exchange ratio (RER), respiratory rate (RR), minute ventilation (V_E), and tidal volume (V_T). The mobile gas analysis unit was routinely calibrated prior to the test using high-low (21% O_2 and 0% CO_2 and 16% O_2 and 4% CO_2). The turbine flowmeter was standardized with a 3-L calibration syringe. Blood samples via the fingertips were analyzed for glucose concentrations. Hydration status as urine specific gravity (USG) was estimated using a handheld visual analog refractometer (URC-NE: Atago; Tokyo, Japan) with a scale range of 1.000 to 1.050. The blood pressure (BP) and rate of perceived exertion (RPE using the modified Borg 10-point scale) were also obtained.

An incremental exercise protocol on a cycle ergometer (Monark 828E, USA) was conducted in a temperature-controlled laboratory (25 ± 1ºC). An initial workload of 50 watts (W) at the pedal rate of 60 rev·min^{-1} was used for a 5-minute warm-up period, then progressive workload was serially increased by 0.5 W·kg^{-1} BW for every 3-minute interval until exhaustion. During the test, the participants were verbally encouraged to continue the ride as long as possible. The data were then followed up for another 5-minute cool down period. Exhaustive criteria were determined from one of the followings: (a) signs and symptoms of exhaustion (i.e., cannot maintain pedal speed of 60 rev·min^{-1} for 2 consecutive workloads); (b) approaching target HR of 95% age-related maximal heart rate; (c) an RPE >8; or (d) volitional termination. Determinations of VO_2 peak were repeatedly performed on a four-time basis, this is, at rest after inclusion, and once every three acute supplements. Throughout the trial, changes in pulmonary and metabolism were recorded continuously using breath-by-breath gas analyzer via facemask. The data at the last 30 seconds of each stage were collected for further analysis. VO_2 peak was estimated as the highest 15 sec-averaged before exhaustion.

Three specific supplement products included carbohydrate (CHO), whey protein (PRO), and carbohydrate plus whey protein (MIX), which were packed in identical foil sachets (labeled with study codes) were randomly provided in the 250 ml liquid forms as prescribed in a previous study by Knuiman and colleagues (16).

The caloric assessments from diet were estimated and analyzed using an in-house dietary software program (INMUCAL, Institute of Nutrition, Mahidol University, Thailand) using a 4-day food recording (i.e., three weekdays and one weekend day). Validity and reliability of INMU-CAL were specified from previous investigations (32). The participants were instructed to send digital food photos, with ruler scale, on daily basis. Participants were excluded for anyone who failed to complete the food records for less than 3 days.

Specific questionnaire for family and personal health and physical activity history were derived from the International Physical Activity Questionnaire (IPAQ; Craig CL et al., 2003; IPAQ, 2005). Following the physical examination, an informed consent form was completed. Then, each subject received instructions for the next visit. This experiment was approved by the Ethic

Committee of the Mahidol University Central Institutional Review Board (MU-CIRB 2019/ 243.1809).

Statistical Analysis

Sample size was calculated by G Power v3.1 with data from a previous study (Ferguson-Stegall et al., 2011) with a power analysis (F-test, ANOVA, repeated measure), an effect size of 0.89, $P < 0.05$, and a desired power value of 0.8 using 3 treatments. This was a double-blinded study design. All the data were expressed as mean ± standard deviation (SD) and analyzed using the SPSS program (v21, IBM, Chicago, IL, USA) with a one-way ANOVA to assess body composition and anthropometric data. The data of 2 x 3 two-way repeated measures ANOVA (with time and intervention as independent factors) were used to assess exercise performance, body composition, exercise performance, as well as cardiac and metabolism variables during the 3 randomized interventions.

RESULTS

Subjects' Participation and General Characteristics.

A total of 13 volunteers were randomly assigned to the interventions, of whom 12 completed the study. One subject failed to join the last intervention due to time constraints. Table 1 summarizes the 3-trial baseline characteristics of subjects. All the resting physiologic variables are in normal ranges of subjects as young adults. There were no significant differences for all variables among the 3 baselines over time ($P > 0.05$). USG during post-ingestions showed a slight increase but was not significantly different between trails. After ingestion, blood glucose concentrations in CHO showed a significant increase from resting level ($P < 0.05$) with a non-significant increase in MIX and a non-significant decrease in the PRO trail. Immediately after exhaustion, blood glucose levels in all ingestions were significantly decreased from the resting levels in all three trails ($P < 0.05$). Blood glucose levels were significantly further reduced in the PRO at 30 minutes post-exhaustion.

Table 1. Baselines Characteristics and Resting Physiological Variables of Subjects during Three Visits prior to Randomized Interventions of Isocaloric Supplements.

	Visit 1 (n = 12)	Visit 2 (n = 12)	Visit 3 (n = 11)	P-value
Anthropometric Data				
Age (yrs)	22.6 ± 3.1	-	-	
Height (cm)	171.9 ± 4.6	-	-	
Weight (kg)	67.8 ± 2.4	68.5 ± 2.3	67.7 ± 2.6	NS
BMI	23 ± 2.1	23.2 ± 2.1	22.8 ± 2	
Blood Glucose ($mg \cdot dL^{-1}$)	105.4 ± 2.2	102.3 ± 3.5	105.2 ± 3.1	
USG	1.015 ± 0.002	1.017 ± 0.002	1.018 ± 0.002	
Resting Cardiac Functions				
SV (ml)	76 ± 2.8	80.2 ± 3.4	78.7 ± 4.9	
HR ($bpm \cdot min^{-1}$)	83.3 ± 4.4	81.3 ± 3.9	83.4 ± 3.7	
CO ($L \cdot min^{-1}$)	6.3 ± 0.2	6.4 ± 0.3	6.5 ± 0.2	NS
CI ($L \cdot min^{-1} \cdot m^{2}$)	3.4 ± 0.2	3.5 ± 0.1	3.7 ± 0.2	
SVR ($dyn \cdot s \cdot cm^{-5}$)	1130.2 ± 34.6	1114.3 ± 58.2	1109 ± 56.3	

EDV (ml)	109.2 ± 4.4	120.7 ± 7.2	113.6 ± 4.8	
EF (%)	67.2 ± 2.8	67.5 ± 2.3	67.8 ± 2.2	
SBP (mmHg)	118.3 ± 2.3	118.8 ± 2.4	117.6 ± 1.8	
DBP (mmHg)	79 ± 3.2	79.1 ± 3.5	76.5 ± 2.01	
Resting Respiratory and Metabolism Functions				
$\mathbf{V_E}$ ($L \cdot min^{-1}$)	12 ± 2.1	12.3 ± 1.6	12.4 ± 2.4	
$\mathbf{VO_2}$ ($ml \cdot min^{-1}$)	371.1 ± 42.3	374.1 ± 69.9	378.9 ± 75.6	
$\mathbf{VCO_2}$ ($ml \cdot min^{-1}$)	315.8 ± 68.5	327.6 ± 59	322.8 ± 69.2	
RER	0.86 ± 0.22	0.88 ± 0.08	0.85 ± 0.06	NS
BF (breaths·min^{-1})	17.4 ± 1.1	18.7 ± 1.0	18.7 ± 0.7	
EE	2480 ± 131	2454.5 ± 170	2581.1 ± 131	
$\mathbf{V_{Tin}}$ (L)	0.7 ± 0.05	0.6 ± 0.04	0.6 ± 0.05	

All values are means ± SD. **Abbreviations: BMI** = Body Mass Index; **USG** = Urine Specific Gravity; **SV** = Stroke Volume; **HR** = Heart Rate; **CO** = Cardiac Output; **CI** = Cardiac Index; **SVR** = Systemic Vascular Resistance; **EDV** = End-Systolic Volume; **EF** = Ejection Fraction; **SBP** = Systolic Blood Pressure; **DBP** = Diastolic Blood Pressure; $\mathbf{V_E}$ = Minute Ventilation; **RER** = Respiratory Exchange Ratio; **BF** = Breath Frequency; **EE** = Energy Expenditure; **VTin** = Inspiratory Tidal Volume; **TTE** = Time To Exhaustion; **RPE** = Rating of Perceived Exertion.

Dietary Daily Macronutrient Intake

The means of the 3 days daily intake from a week prior to each test (Table 2) showed no significant differences of caloric intakes and proportions of % carbohydrate, protein, and fat of each intervention (visit 1, 2, and 3). In addition, none of subjects reported any additional supplements during the duration of the study.

Table 2. Average Three-Day Dietary Evaluations on Macronutrients Prior to the Tests.

	Visit 1 (n = 12)	**Visit 2** (n = 12)	**Visit 3** (n = 11)	**P-value**
Energy (kcal)	1618.6 ± 85.4	1628.4 ± 71	1667.9 ± 52.8	
Carbohydrate (g)	213 ± 17.4	212.1 ± 17.9	218.3 ± 11.4	
% Energy from CHO	52.6 ± 2.2	52.0 ± 3.1	52.3 ± 3.22	
Protein (g)	64.2 ± 6.9	62.2 ± 5.6	67.1 ± 7.5	NS
% Energy from PRO	15.9 ± 1.6	15.3 ± 0.9	16.1 ± 1	
Fat (g)	56.6 ± 3.5	59.3 ± 4.2	58 ± 7.8	
% Energy from fat	31.5 ± 2.0	32.8 ± 0.9	31.3 ± 0.8	

All values are means ± SD.

Cardiorespiratory Responses

Hemodynamic respiratory and metabolic changes showed normal values at rest (prior to), with no significant differences after ingestion during the warm-up, stages 1-2-3 until exhaustion and recovery (Table 3). In all trails, the cardiac variables consecutively increased with the progressive increase in exercise intensity, with the exceptions of SVR. Peaks of all the above variables, with no significant differences among all the supplements, appeared during the exhaustive phases in all the trials. It is recognized that the HR responses were approximately 1.5 to 2.0 times of the resting values; whereas, the SV values were about 1 to 1.5 times.

Table 3. Changes in Hemodynamic, Respiratory, and Metabolic Variables in CHO, PRO, and MIX Supplements at Rest and During Progressive Exercise Until Exhaustion and Cool-Down.

Variables	Trials	Rest	Warm-Up	Stage 1	Stage 2	Stage 3	Exhaustion	Cool-Down
Respiratory System								
V_E ($L \cdot min^{-1}$)	PRO	12.4 ± 0.6	31.2 ± 1.3	38.6 ±1.2	49.3 ± 2.4	62.8 ± 2.2	89.9 ± 4.3	33.8 ± 1.5
	CHO	12.4 ± 0.6	28.2 ± 1.5	39.3 ±1.3	49.1 ± 1.7	65 ± 0.5	88.8 ± 4.4	33 ± 2.4
	MIX	12.1 ± 0.5	28.8 ± 1.2	36.5 ±1.4	47.8 ± 1.9	67 ± 2.5	94.6 ± 5.1	35.7 ± 2.9
VO_2 ($ml \cdot min^{-1}$)	PRO	376 ± 23	1027.4±40.5	1258.9±48.7	1546.8±51.5	1861.9±54.2	2236.3±124.7	751.3 ± 49.8
	CHO	389.4 ± 23.3	940.2±59.8	1283.3±53.8	1585.2±58.6	1907.6±46.2	2164.6±121.4	691.6 ± 59.6
	MIX	387.6 ± 13.4	958.7±41.1	1199.4±59.2	1469.4±54.8	1887.3±91.8	2255.7±117.2	750.4 ± 78.2
VCO_2 ($ml \cdot min^{-1}$)	PRO	313.8 ± 19.8	1022.4±49.6	1317.2±54.3	1700.2±69.2	2207.6±80.2	2833.3±185.4	854 ± 50.5
	CHO	356.3 ± 17.2	932.8±62.3	1354.4±45.8	1740.3±53.9	2244.3±62.3	2699.2±153.5	805.7 ± 65
	MIX	298.6 ± 8.7	953.8±41.9	1259.8±63.1	1645.4±62.8	2224±86.5	2871.5±147.4	884.7 ± 87.8
RER	PRO	0.8 ± 0.01	1 ± 0.02	1±0.01	1.1±0.02	1.2±0.02	1.3±0.03	1.2 ± 0.03
	CHO	0.9 ± 0.02	1 ± 0.02	1.1±0.02	1.1±0.03	1.2±0.01	1.3±0.04	1.2 ± 0.04
	MIX	0.9 ± 0.01	1 ± 0.01	1.1±0.02	1.1±0.02	1.2±0.02	1.3±0.03	1.2 ± 0.03
BF (breaths·min^{-1})	PRO	18.7 ± 1.1	25.4 ± 1.5	27.9±1	31.6±1.6	37±2	46.3±2.5	30.8 ± 1.6
	CHO	17.1 ± 1.2	25.6 ± 1	27.3±1.4	31.8±2.2	36.4±1.5	45.6±2.5	28.5 ± 2.2
	MIX	20.5 ± 0.8	24.7 ± 1.7	28.5±1.8	32±2.4	37.2±2.1	50.4±3.7	27.9 ± 2.7
EE	PRO	2602.5 ± 161.9	7429±303.9	9212.3±361.1	11456.6±392.1	14052.3±429.3	17174±990.4	5592.5±358.9
	CHO	2746.4 ± 158.6	6789.4±436.3	9393.8±367.5	11726.5±405.2	14358.9±360.1	16527.6±917.9	5173.8±437.5
	MIX	2397.9 ± 82.1	6929 ± 298.1	8779.4±432.8	10910.6±402.8	14157.8±628.3	17073.6±834	5615.4±580
V_{Tin} (L)	PRO	0.6 ± 0	1.2 ± 0	1.4±0	1.6±0.1	1.9±0.1	2±0.1	1.1 ± 0
	CHO	0.8 ± 0.1	1.2 ± 0.1	1.5±0.1	1.7±0.1	1.9±0.1	2.1±0.1	1.2 ± 0.1
	MIX	0.5 ± 0	1.2 ± 0.1	1.4±0.1	1.7±0.1	1.9±0.1	2±0.2	1.4 ± 0.2
Cardiovascular System								
SV (ml)	PRO	79.5 ± 3.4	96.4 ± 5.5	100.1 ± 5.4	104.4 ± 6.7	106.2 ± 4.9	108.2 ± 5.2	99.1 ± 4.1
	CHO	78.6 ± 4.3	94.2 ± 5.3	98.4 ± 5.9	100.6 ± 6.4	106 ± 4.2	104.2 ± 4.8	95.3 ± 3.4
	MIX	77.9 ± 3.8	93 ± 5.5	98.1 ± 7	98.8 ± 7	102.3 ± 6.4	109.2 ± 4.8	98.5 ± 6.9
HR ($bpm \cdot min^{-1}$)	PRO	81.6 ± 3.7	109.5 ± 4.2	125.1 ± 4.7	141.9 ± 6.3	158.6 ± 4.9	179.1 ± 2.5	122.9 ± 4.2
	CHO	81.8 ± 3.9	111 ± 4.8	126.8 ± 4.6	148.7 ± 4.4	164.5 ± 2.7	177.8 ± 3.1	123.2 ± 4.6
	MIX	82.1 ± 4.2	112.1 ± 7	125.4 ± 6.1	146.6 ± 5.9	164.4 ± 4.6	182 ± 3.7	126.8 ± 3.8
CO ($L \cdot min^{-1}$)	PRO	6.2 ± 0.3	10 ± 0.6	11.9 ± 0.6	14.1 ± 0.9	15.8 ± 0.9	18.2 ± 1.3	11.8 ± 0.6
	CHO	6.5 ± 0.4	10.5 ± 0.6	12.6 ± 0.7	14.8 ± 0.8	17.1 ± 0.6	18.5 ± 0.7	12.3 ± 0.7
	MIX	6.2 ± 0.2	10 ± 0.3	11.7 ± 0.6	13.6 ± 0.6	16.6 ± 0.7	19.2 ± 0.7	12.3 ± 0.7
CI ($L \cdot min \cdot m^2$)	PRO	3.5 ± 0.2	5.7 ± 0.4	6.8 ± 0.4	8 ± 0.5	9 ± 0.5	10.3 ± 0.7	6.7 ± 0.4
	CHO	3.7 ± 0.3	6.1 ± 0.3	7.2 ± 0.4	8.5 ± 0.5	9.5 ± 0.5	10.2 ± 0.6	6.9 ± 0.5
	MIX	3.6 ± 0.2	5.8 ± 0.3	6.8 ± 0.5	7.9 ± 0.5	9.6 ± 0.6	10.7 ± 0.7	7 ± 0.3
SVR ($dyn \cdot s \cdot cm^{-5}$)	PRO	1157.9 ± 54	758.3 ± 59.9	628 ± 48.6	543 ± 50.1	472.1 ± 37.5	418.3 ± 36.4	631 ± 45.7
	CHO	1167.4 ± 85	714 ± 66.3	600.8 ± 54.4	524.5 ± 51.6	439.8 ± 22.5	396.3 ± 25.9	606.3 ± 60.9
	MIX	1228.1 ± 64	787.5 ± 38.1	652.7 ± 44.5	554.8 ± 45.1	473.5 ± 29.9	427.2 ± 18.5	627.5 ± 48.7
EDV (ml)	PRO	119.7 ± 6.3	134.2 ± 9	138.3 ± 6.4	138.7 ± 8.8	141.2 ± 7.8	141.3 ± 8.8	139.6 ± 8.8
	CHO	117.4 ± 5.4	128.6 ± 5.7	132.6 ± 5.9	135.4 ± 5.3	142.7 ± 9.6	141.4 ± 6	132.4 ± 6.9
	MIX	115.7 ± 5.3	132.2 ± 7.2	135.2 ± 8	132.8 ± 7.4	139.4 ± 7	144.3 ± 5.7	138 ± 6.6

EF (%)	PRO	66.9 ± 1.2	72.8 ± 2.2	74.2 ± 2.3	75.6 ± 2.2	75.6 ± 1.9	77.4 ± 1.8	73.4 ± 2.5
	CHO	68.9 ± 2.4	74.3 ± 2.9	75.7 ± 2.9	73.8 ± 3.4	74.7 ± 1.3	75.2 ± 2.6	75.2 ± 2.6
	MIX	68.2 ± 1.9	71.5 ± 1.4	73.3 ± 1.9	74.6 ± 1.9	74 ± 1.7	77 ± 1.6	71.5 ± 2.3

All values are means ± SEM. **Abbreviations: SV** = Stroke Volume; **HR** = Heart Rate; **CO** = Cardiac Output; **CI** = Cardiac Index; **SVR** = Systemic Vascular Resistance; **EDV** = End-Systolic Volume; **EF** = Ejection Fraction; **V_E** = Minute Ventilation; **RER** = Respiratory Exchange Ratio; **BF** = Breath Frequency; **EE** = Energy Expenditure; **VTin** = Inspiratory Tidal Volume; **TTE** = Time To Exhaustion; **RPE** = Rating of Perceived Exertion.

Changes in Blood Pressures

The participants' SBP showed an increase with exercise intensity; whereas, DBP exhibited quite the steady characteristics (Figure 1). No significant differences of SBP were found among trails at rest, during the warm-up, the exercises, and the cool down. DBP in PRO showed remarkable drop during the warm-up, the three stages, and the exhaustive exercises, compared to PRO-CHO ($P < 0.05$) and PRO-MIX ($P < 0.05$) and even lower than the resting value during the cool down ($P < 0.05$).

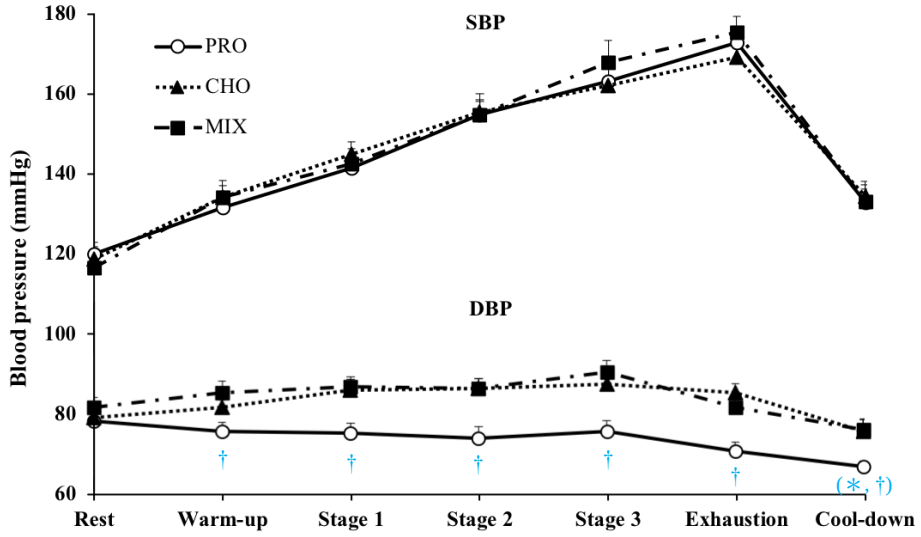

Figure 1. Change of Systolic Blood Pressure and Diastolic Blood Pressure during the Incremental Cycling Test of Rest, Warm-Up, Incremental Stages and 5 minutes after Cool-Down Exercise. Means ± SEM. *Significant from its corresponding baselines ($P < 0.05$); †significant difference between PRO vs. CHO and MIX.

Blood Glucose Levels

With normal ranges at rest, blood glucose (BG) levels after supplement ingestions showed a remarkable increase in CHO and MIX, with significant differences between CHO-MIX ($P < 0.05$) and CHO-PRO ($P < 0.05$) (Figure 2). Immediately after the exhaustive exercises, BG dropped significantly lower than the resting levels ($P < 0.05$) and after ingestion in all trails ($P < 0.05$). These mean supplements of 25 gm, regardless of whether it is PRO, CHO, or MIX are not suitable for exhaustive exercises. BG in PRO showed significantly lower ($P < 0.05$) than the resting levels even at 30 min after exercise, BG in CHO and MIX was significantly lower than after ingestion levels in both CHO ($P < 0.05$) and MIX ($P < 0.05$).

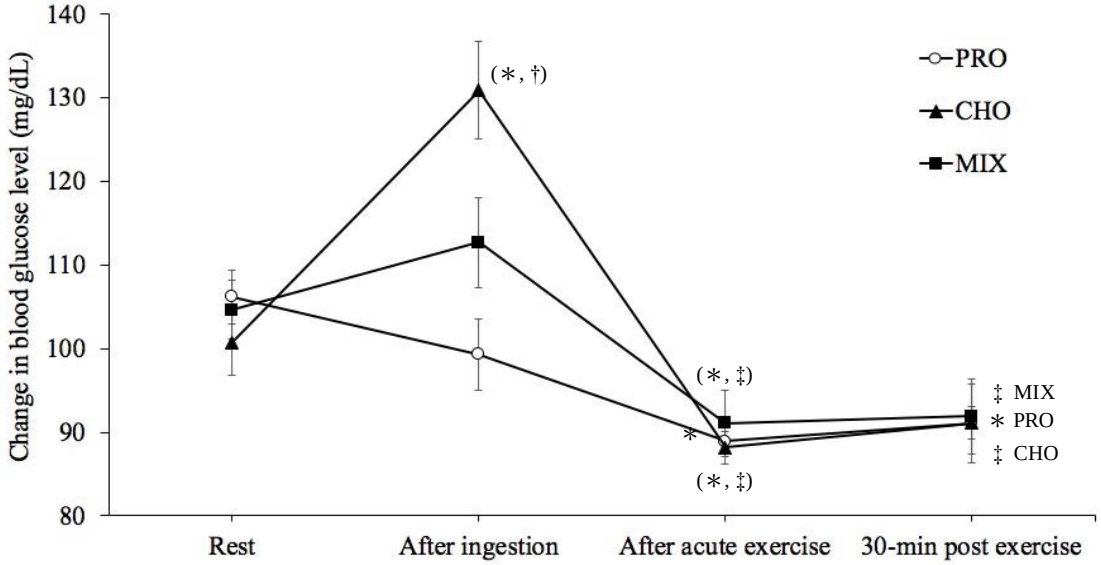

Figure 2. Blood Glucose Responses at Rest, Supplementation Ingestion and Exercise. Protein (PRO; open circles), Carbohydrate (CHO; filled triangles), Protein + Carbohydrate (MIX; filled squares). *Significantly different ($P < 0.05$) from rest; †significantly treatment different ($P < 0.05$); ‡significantly different ($P < 0.05$) from after ingestion. Values are expressed as mean ± SE.

Acute Effects of Isocaloric Drinks on Aerobic Capacity

Determinations of VO_2 peak were performed on 4 occasions: (a) at rest after inclusion (baseline, pre-ingestion as part of screening); and (b) after every 3 randomized acute supplements (Figure 3). Only the MIX showed significantly higher VO_2 peak than pre-ingestion value ($P < 0.05$) with no significant differences between the trails.

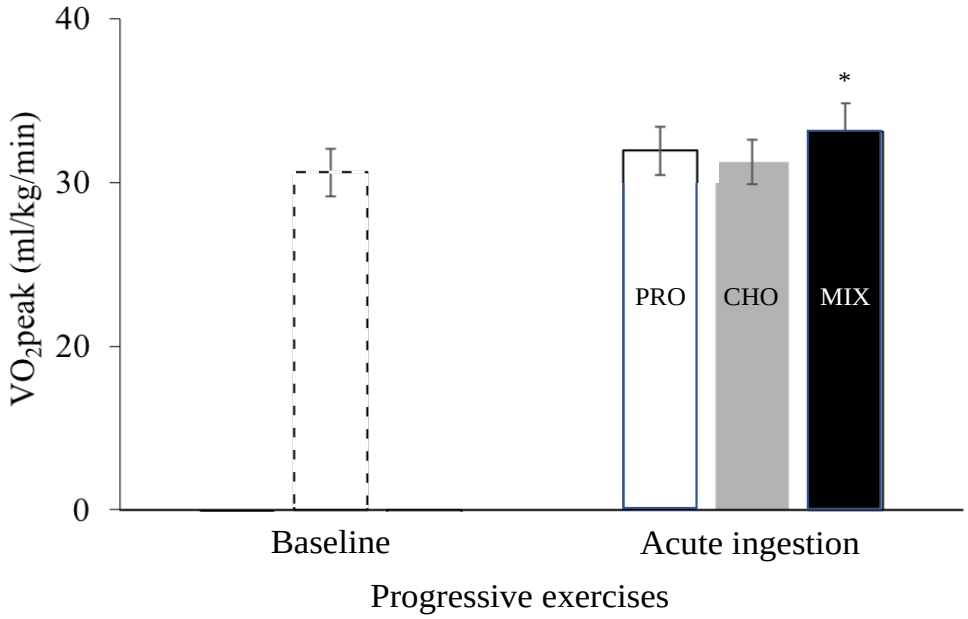

Figure 3. Change in VO_2 peak from Progressive Cycle Exercises Until Exhaustion Obtained from Baseline (after inclusion, pre-ingestion) and After Acute Randomized Isocaloric Supplementations of PRO (whey protein), CHO (carbohydrate), and MIX (protein + carbohydrate) Trails. Values are expressed as mean ± SE. *Significant time different ($P < 0.05$).

DISCUSSION

In the present study, the three daily macronutrient supplementations were similar and in normal ranges of young Thai males. Similar dietary intakes during three randomized trials, in particular for macronutrients (Table 2) and the USG (Table 1) reveal that any physiologic changes during the tests should likely be derived from exogenous supplements.

This study does not support beneficial effects of acute loading of carbohydrate or protein alone or a mixture on respiratory functions and metabolism during exercise in sedentary young adults, that is, apart from the hemodynamic response in that PRO induced a lower diastolic blood pressure. As to the performance aspect, the isocaloric mixture of CHO and PRO resulted in a higher VO_2 peak in comparison to the carbohydrate or protein alone.

In the present study, changes of blood glucose level at post-ingestion depend on the types of macronutrients being consumed. Healthy individuals who consumed 25 g CHO (maltodextrin) can expect it to be absorb into the bloodstream and enhanced to ~ 40 $mg \cdot dL^{-1}$ in 30 to 45 minutes (2). Moreover, ingestion of little amount of carbohydrate could raise blood glucose of 15 to 20 $mg \cdot dL^{-1}$ (26). However, people with diabetes or other medical conditions that affect blood sugar regulation may experience different responses. In this study however, there was no significant change in blood glucose while ingesting CHO combined with protein powder. The combination may affect insulin secretion. Yet, previous evidence indicated that insulin could be rapidly elevated at 15 to 60 min following whey protein ingestion (3). Although we did not measure insulin concentration, some previous studies point out that adding whey protein to carbohydrate could induce higher insulin responses than CHO alone (34), and its potentially reduced the glycemic levels due to the increased insulinemic, which mediated in part by enhanced glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1) concentration and dipeptidyl peptidase IV (DPP-IV) reduction (27). On the other hand, in a previous study with female athletes, it was reported that the supplement of low CHO and PRO could have increased glucose clearance from the blood at a greater rate than CHO alone, thus resulting in lower blood glucose levels and an increase in exogenous CHO availability to the working muscle. However, it is also possible that the lower plasma glucose values of the CHO + PRO treatment were associated with its lower CHO concentration. The CHO + PRO treatment delivered CHO at a rate of 24.75 g $CHO \cdot h^{-1}$ (0.413 g $CHO \cdot min^{-1}$), in comparison to 49.5 g $CHO \cdot h^{-1}$ (0.83 g $CHO \cdot min^{-1}$) in the CHO treatment (21).

The present study indicates that cardiac control is dependent on exercise intensity (Figure 3), which can be effectively done via the rhythm (HR) rather than volume (SV) regulation. Thus, 3 isocaloric supplements play no significant role on cardiac inotropic autoregulation of either at rest, during, or after exercise. Apart from those intrinsic regulatory control mechanisms of the heart, extrinsic factors also play important role. Vasodilation is remarkably presented in this study. An elevation in skeletal muscle blood flow is generally considered to be mediated by the release of chemicals metabolites and other local vasoactive compounds that trigger the vasodilation. Moreover, the vascular endothelium cell plays a key modulate role on vascular tone by numerous endothelium-derived vasoactive factors. Previous studies revealed that exercise enhances shear stress or receptor-mediated agonists such as Acetylcholine (Ach) causing endothelium vasodilation. That underlying pathway is through the enhancing expression of endothelium nitric oxide synthase (eNOS), which synthesizes nitric oxide (NO)

from amino acid L-arginine oxidation. NO is a gaseous molecule that diffuses to the underlying vascular smooth muscle cells, resulting in vasodilation (6,17,33).

On the other, by consuming whey protein that contains several amino acid and peptide molecules, it has been shown to play a significant role in decreasing blood pressure by inhibiting angiotensin converting enzyme (ACE) that stimulates vasocontraction via the renin-angiotensin system or increased nitric oxide–mediated vasodilation (9). According to our result, diastolic blood pressure was decreased only during the whey treatment of which the value even approached hypotension. The exact mechanism behind post-exercise hypotension (PEH) is not fully understood, but it is thought to be related to a combination of factors, including a decrease in sympathetic nervous system activity and an increase in the production of vasodilators such as nitric oxide. During exercise, sympathetic nervous system activity increases, leading to an increase in heart rate and blood pressure. After exercise, sympathetic activity decreases, which can cause a temporary decrease in blood pressure. At the same time, the production of vasodilators such as nitric oxide increases, which can also contribute to a decrease in blood pressure. Moreover, increased carbon dioxide concentration and activated histamine receptor on endothelial cells and vascular smooth muscle contribute to vasodilation and blood flow resistance that results in a decrease in blood pressure (18).

The present study indicates the increase in SBP with essentially an unchanged DBP even at exhaustive exercise. Previous studies have identified similar outcomes from submaximal exercise (10,35). Systolic blood pressure during dynamic, isotonic exercise is expected to rise according to the increasing workload while diastolic blood pressure in such conditions usually remains unchanged or may decrease insignificantly (35). Accordingly, at high dynamic exercise in hypertensive conditions, the maximum value of SBP can increase up to 250 mmHg and that of diastolic pressure up to 110 mmHg (19). The steady increase in both SBP and DBP is a very useful safety indicator to estimate for mean arterial blood pressure (MABP), which is a function of left ventricular contractility, heart rate, and vascular resistance, and elasticity averaged over time (30).

It is quite clear that blood pressure responses are intensity-dependent. Theoretically, SBP responses are dependent on cardiac contractility, which pumps a certain amount of cardiac output into the aorta and large arteries known as the afterload of the heart. According to Poiseuille’s law on fluid dynamics, the velocity of the steady flow of a fluid through a narrow tube (such as a blood vessel or a catheter) varies directly with the pressure and the fourth power of the radius of the tube and inversely with the length of the tube and the coefficient of viscosity.

CONCLUSIONS

The findings indicate that isocaloric supplements of macronutrients prior to exercises do not alter most of physiologic functions of cardiorespiratory and metabolic systems. Amino acids play a role in regulating diastolic blood pressure via vasodilation and a mixture of amino acids-carbohydrate plays a role on higher aerobic capacity. Supplements of macronutrients may possibly be used in hypertension. Further investigations of the clinical aspect of hypertension or long-term effects of isocaloric supplements of macronutrients are needed.

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Address for correspondence: Chen Pei-Ni, College of Sports Science and Technology, Mahidol University, Salaya, Nakhonpathom, Thailand, 73170, Email: chenpeini.benny@gmail.com

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