THE EFFECTS OF CO-INGESTION OF HIGHLY BRANCHED CYCLIC DEXTRIN AND DIETARY NITRATE ON PHYSIOLOGICAL RESPONSES AND ENDURANCE CAPACITY IN RECREATIONAL ENDURANCE RUNNERS

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ผลของการบริโภคไฮลี่บรานซ์ไซคลิกเด็กซ์ตรินร่วมกับไนเตรทที่มีต่อการตอบสนองทางสรีรวิทยาและความสามารถด้านความอดทนในนักกีฬาวิ่งระยะไกลเพื่อการนันทนาการ

นายทรงทรรศน์ จินาพงศ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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ทรงทรรศน์ จินาพงศ์ : ผลของการบริโภคไฮลี่บรานซ์ไซคลิกเด็กซ์ตรินร่วมกับไนเตรทที่มีต่อการตอบสนองทางสรีรวิทยาและความสามารถด้านความอดทนในนักกีฬาวิ่งระยะไกลเพื่อการนันทนาการ. (THE EFFECTS OF CO-INGESTION OF HIGHLY BRANCHED CYCLIC DEXTRIN AND DIETARY NITRATE ON PHYSIOLOGICAL RESPONSES AND ENDURANCE CAPACITY IN RECREATIONAL ENDURANCE RUNNERS) อ.ที่ปรึกษาหลัก : ผศ. ดร.ณัติพร นกแก้ว, อ.ที่ปรึกษาร่วม : รศ. ดร.เจสัน ลี ไค เหว่ย,ผศ. ดร.กฤติยา เขื่อนเพชร

การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาผลของการบริโภคไฮลี่บรานซ์ไซคลิกเด็กซ์ตรินร่วมกับไนเตรทต่อการตอบสนองทางสรีรวิทยาและความสามารถด้านความอดทนของนักวิ่งระยะไกลเพื่อการนันทนาการเปรียบเทียบกับการบริโภคไฮลี่บรานซ์ไซคลิกเด็กซ์ตริน มอลโตเด็กซ์ตรินร่วมกับไนเตรท และมอลโตเด็กซ์ตรินระหว่างการวิ่งที่มีความหนักสูงเป็นเวลานาน การศึกษาเป็นการวิจัยเชิงทดลองข้ามกลุ่มแบบสุ่มปกปิดสองทาง กลุ่มตัวอย่างเป็นนักวิ่งมาราธอน เพศชาย จำนวน 9 คน (อายุ 35.00 ± 4.00 ปี) โดยสุ่มลำดับให้ได้รับไฮลี่บรานซ์ไซคลิกเด็กซ์ตริน 1.5 กรัมต่อน้ำหนักตัว 1 กิโลกรัมและไนเตรท (NO_3^-) จากผงสกัดบีทรูทปริมาณ 500 มก. (~8.00 มิลลิโมล) (HBCD+NO_3^-), หรือไฮลี่บรานซ์ไซคลิกเด็กซ์ตริน 1.5 กรัมต่อน้ำหนักตัว 1 กิโลกรัม (HBCD), หรือ มอลโตเด็กซ์ตริน 1.5 กรัมต่อน้ำหนักตัว 1 กิโลกรัมและ ไนเตรท (NO_3^-) จากผงสกัดบีทรูทปริมาณ 500 มก. (MD+NO_3^-) หรือมอลโตเด็กซ์ตริน 1.5 กรัมต่อน้ำหนักตัว 1 กิโลกรัม (MD) เป็นระยะเวลา 60 นาทีก่อนการทดสอบประสิทธิภาพการวิ่งตามด้วยการวิ่งด้วยความเร็วเทียบเท่าร้อยละ 70 ของอัตราการใช้ออกซิเจนสูงสุด ระยะเวลา 60 นาที โดยวัดอัตราการออกซิเดชันของสารอาหารที่ให้พลังงานตั้งต้น ปริมาณออกซิเจนในกล้ามเนื้อ ต้นทุนการใช้ออกซิเจน ระดับกลูโคสในเลือด ระดับอินซูลินในเลือด ระดับแลคเตทในเลือด รวมไปถึงการรับรู้อาการทางระบบทางเดินอาหารและอัตราการรับรู้ความเหนื่อย ผลการวิจัยพบว่าไม่มีความแตกต่างอย่างมีนัยสำคัญในระดับอินซูลินในเลือด ระดับกลูโคสในเลือด ระดับแลคเตทในเลือด ต้นทุนการใช้ออกซิเจน ปริมาณออกซิเจนในกล้ามเนื้อ ระหว่างการบริโภคเครื่องดื่มในเงื่อนไข HBCD+NO_3^-, HBCD, MD+NO_3^- และ MD อย่างไรก็ตาม พบว่าการบริโภค HBCD มีผลต่อการเพิ่มขึ้นของอัตราการออกซิเดชันของไขมันและอัตราส่วนการแลกเปลี่ยนก๊าซ (RER) ที่ต่ำกว่าเมื่อเทียบกับ HBCD+NO_3^-, MD+NO_3^- หรือ MD ซึ่งบ่งชี้ว่าการบริโภค HBCD อาจมีผลต่อการใช้พลังงานจากไขมันเพิ่มขึ้นและส่งผลต่อค่า RER ที่ต่ำลงเมื่อเปรียบเทียบกับการกิน HBCD+NO_3^- หรือคาร์โบไฮเดรตจากแหล่งอื่น.

สาขาวิชา	วิทยาศาสตร์การกีฬาและการออกกำลังกาย	ลายมือชื่อนิสิต .....
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Songdhasn Chinapong : THE EFFECTS OF CO-INGESTION OF HIGHLY BRANCHED CYCLIC DEXTRIN AND DIETARY NITRATE ON PHYSIOLOGICAL RESPONSES AND ENDURANCE CAPACITY IN RECREATIONAL ENDURANCE RUNNERS. Advisor: Asst. Prof. NATTIPORN NOKKAEW, Ph.D. Co-advisor: Assoc. Prof. JASON, LEE KAI WEI, Ph.D.,Asst. Prof. Krittiya Khuenpet, Ph.D.

This study aimed to investigate the effects of co-ingestion of HBCD and dietary nitrate on physiological responses and endurance capacity in recreational endurance runners compared with an isocaloric HBCD beverage, a maltodextrin-dietary nitrate beverage, and a maltodextrin beverage during high-intensity prolong running. Nine male marathon runners (age 35.00 ± 4.00 years) participated in a double-blind crossover design, where they were randomly assigned to receive either the co-ingesting 1.5 g HBCD·kg^{-1} BM and 500 mg dietary NO_3^- (~8.00 mmol NO_3^-) beverages (HBCD+NO_3^-), the ingesting 1.5 g HBCD·kg^{-1} BM (HBCD), the co-ingesting 1.5 g MD·kg^{-1} BM and 500 mg dietary NO_3^- (~8.00 mmol NO_3^-) beverages (MD+NO_3^-) or the ingesting 1.5 g MD·kg^{-1} BM beverage (MD) 60 min prior to the running economy test following by a 60-min constant load running at a speed equivalent to 70% of $\dot{V}O_2$peak. Measurements of substrate oxidation, muscle oxygenation, and oxygen cost were taken to assess running economy as well as serum glucose concentration, serum insulin concentration and blood lactate concentration, gastrointestinal symptoms, and rate of perceived exertion (RPE). The results showed no significant differences in the serum insulin concentration, serum glucose concentration, blood lactate concentration, oxygen cost, muscle oxygenation, among the HBCD+NO_3^-, HBCD, MD+NO_3^-, and MD conditions. However, it was found that the ingesting HBCD it had increase fat oxidation rate and lower the RER compared to the HBCD+NO_3^-, the MD+NO_3^-, or MD. This indicate that the ingesting HBCD may modulate fat metabolism and affect the lower RER compared to the co-ingesting of HBCD+NO_3^- or other CHO sources.

Field of Study:	Sports and Exercise Science	Student's Signature .....
Academic Year:	2022	Advisor's Signature .....
		Co-advisor's Signature .....
		Co-advisor's Signature .....

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INTRODUCTION

Background and rationale

Long-distance running or endurance running events have become popular sports. The number of participants has increased nationally and globally, as evidenced by the participation of amateurs in hundreds of organized marathon events worldwide (1) and the 11.96 million recreational endurance runners in Thailand in 2016 (2). Worldwide marathon statistics reveal that Thailand is a male-dominated marathon nation, with 77% of participants being male and 23% female (1). The number of participants in endurance running events demonstrates that not only elite runners but also recreational runners take part in these events. The two largest age groups of runners, 30-39 and 40-49, constitute approximately 60% of all marathon participants, and this percentage remains consistent for both men and women (3). Most endurance runners strive to achieve a specific finish time and placement within their gender and age group. In 2018, the average finish time for a marathon, covering the official distance of 42.195 km, was 4:29:53 worldwide (1). Additionally, half-marathon events are gaining popularity among male runners aged 30-39, with an average finish time of around 02:05:05 for a race covering 21.0975 km (4).

Endurance running performance is influenced by various factors that interact in a complex manner. One crucial factor is maximal oxygen uptake ($\dot{V}O_2max$), which directly relates to the rate at which ATP generation can be sustained by a runner during a distance race. This value is determined by the runner's $\dot{V}O_2max$ and the percentage of $\dot{V}O_2max$ they can maintain (5). For example, elite marathon runners finish in times between 2:05:00 and 2:20:00 at speeds requiring between 80–90% of $\dot{V}O_2max$, sub-elite marathon runners will run at 70–75% of $\dot{V}O_2max$ for approximately finishing in time 2:45:00, and recreational marathon runners run at 60–65% of $\dot{V}O_2max$ for 3:45:00 (6). Although a high $\dot{V}O_2max$ is required for high-level competition in long-distance races, a high anaerobic threshold and a good running economy are determinative in runners with similar $\dot{V}O_2max$ (7, 8).

Moreover, the combination of running economy and $\dot{V}O_2$max could be a good predictor of males' endurance running performance (8).

There are several factors that can limit endurance running performance, including hypoglycaemia and glycogen depletion, muscle acidosis, hyperthermia, and dehydration. The maximum running pace achievable appears to be controlled by the rate of aerobic metabolism of a limited amount of muscle glycogen and blood glucose, as well as the ability to maintain a velocity without developing hyperthermia. If runners were to increase their pace slightly, they would experience premature fatigue, likely due to accelerated glycogenolysis (9, 10). This fatigue could be characterized by acidosis and eventual depletion of glycogen in the readily recruitable motor units of the running musculature.

Muscle glycogen and blood glucose are the primary substrates utilized by the working muscles during exercise. In endurance running lasting approximately 2-4 hours, the major fuels consumed are carbohydrates (CHO), stored as glycogen in the muscles and liver, and fats, stored in muscle and adipose tissue. The relative amounts of CHO and fats oxidized during endurance running are influenced by exercise intensity and dietary intake before (11). During moderate to high-intensity running, fat oxidation alone is insufficient to meet the muscular ATP demands. Therefore, all marathon runners rely on CHO fuelling. This is supported by the average respiratory exchange ratio (RER) of approximately 0.90 observed in recreational runners during the latter half of a marathon (6). The depletion of muscle glycogen and reduction in blood glucose concentrations can occur after as little as 90 minutes of running. This depletion is associated with physical fatigue experienced by athletes during prolonged exercise (12). A significant proportion of marathon runners, more than two-fifths, experience severe depletion of their physiological CHO reserves during the race. This phenomenon, commonly referred to as "hitting the wall," can severely limit their performance. Additionally, a small percentage, approximately 1-2% of runners, may even drop out of the race before reaching the finish line (13). For this reason, it has been proposed to CHO intake approximately 30-60 $g \cdot h^{-1}$ and administer sports drinks during high-intensity exercise. This helps prevent muscle glycogen depletion and maintain hydration. Several studies have confirmed that

consuming CHO-containing beverages during endurance exercise can delay the onset of fatigue. However, it is important to note that the consumption of highly concentrated sports drinks, especially those with high CHO concentrations or large amounts of CHO, may exceed the intestinal capacity for CHO absorption. This can lead to gastrointestinal distress during prolonged running (14). Gastrointestinal complaints are extremely common during exercise among long-distance runners, triathletes, and athletes participating in other forms of intense, prolonged exercise. Studies indicate that the prevalence of these complaints can range from 10% to 95%, depending on factors such as the specific event, environmental conditions, and the methods used to assess gastrointestinal distress (15, 16). Although most runners typically encounter mild symptoms like nausea and dizziness, a small portion of runners may experience more severe complications such as stomach and intestinal cramps, vomiting, and diarrhoea. These more serious symptoms can greatly hinder running performance (17). Consuming a highly concentrated CHO solution or one with a high osmolality can result in gastrointestinal discomfort (15). The composition of CHO plays a critical role in determining osmolality, which directly impacts the likelihood of experiencing gastrointestinal discomfort. Therefore, athletes need to find the appropriate balance between consuming an adequate amount of CHO for extra energy without going overboard and increasing the risk of gastrointestinal discomfort. Additionally, the selection and availability of different CHO sources are crucial in developing new beverages or sports drinks that contain CHO. Food technologists often work with combinations of sugars to manipulate their products, aiming to create formulations that maintain optimal osmolality, total energy content, and taste (18).

Highly branched cyclic dextrin (HBCD) is an innovative polysaccharide, specifically a type of maltodextrin, derived from waxy corn starch through the use of a branching enzyme. It possesses an average molecular weight (MW) of 400,000 and demonstrates a narrow size distribution. Including HBCD as a CHO ingredient in sports drinks is supported by empirical evidence. HBCD exhibits high solubility, low viscosity, and resistance to retrogradation, making it an excellent choice for formulating sports

drinks (19). When compared to drinks containing glucose and standard dextrin, beverages with HBCD have a shorter gastric emptying time. This is attributed to the lower osmotic pressure of HBCD, resulting in a reduced risk of gastrointestinal issues during exercise. Studies have demonstrated that administering 0.5 $g \cdot kg^{-1}$ body weight (BW) of HBCD to mice and 1.5 $g \cdot kg^{-1}$ BW to humans can enhance exercise endurance. However, commercial sports drinks typically have a low CHO concentration, usually below 10%, to facilitate gastric emptying and minimize gastrointestinal discomfort during exercise. Consequently, the amount of CHO that can be consumed from commercial sports drinks during exercise is limited (19-21).

Notably, administering HBCD to elite athletes was found to increase the time to fatigue compared to glucose and control trials (22). In healthy volunteers, the ingestion of 15 g of HBCD resulted in a lower perceived exertion during endurance exercise (23). Some studies have shown that ingesting HBCD before exercise tends to increase blood glucose concentration, while also shortening gastric emptying time and maintaining stable blood insulin levels. This provides an additional exogenous energy source and enhances time to exhaustion, particularly during high-intensity exercise (lasting approximately 8 minutes at 90% $\dot{V}O_2max$) (20-24). The study of Takii et al. (19) suggests that the HBCD may be a useful ingredient in sports drinks due to its low osmotic characteristics than commercially available dextrin. To our knowledge, there is limited information available on the effects of HBCD ingestion on endurance capacity in endurance athletes who run for 2-4 hours at a moderate to high intensity compared with maltodextrin ingestion. One objective of this study will be to investigate the effects of ingesting HBCD, a type of CHO, on physiological responses and endurance capacity in recreational endurance runners compared to ingesting maltodextrin. Our hypothesis is that consuming HBCD will result in improved endurance capacity compared to an isocaloric maltodextrin beverage. We expect this improvement to be due to higher rates of total CHO utilization, while ensuring that gut comfort is not adversely affected.

During high intensity during endurance running, oxidative phosphorylation is declined as the O_2 supply is inadequate, and dependence on the anaerobic metabolic

pathway of ATP regeneration is preferred. These phenomena affect exercise-induced hypoxemia inducing the muscles to become hypoxic and acidic. This impairment in homeostasis may also disturb the functioning of the NOS-dependent pathway. Therefore, the reliance on the substitutional $NO_3^- - NO_2^- - NO$ pathway independent of the O_2 supply is increased (25). Another functional supplementation to enhance endurance performance that has received widespread attention is nitrate (NO_3^-), a precursor for nitric oxide (NO), since long-duration high-intensity exercise and intermittent high-intensity exercise (26).

Dietary nitrate supplementation increases circulating plasma nitrite concentration $[NO_2^-]$ and subsequently nitric oxide (NO). Nitric oxide is a potent signalling molecule that plays a key role in vasodilation by relaxing the smooth muscle and subsequently improving blood circulation. This process is promoted in conditions of low partial pressure oxygen and acid-base imbalance or lactate acid accumulation that may exist in skeletal muscle during endurance racing that limits physical capacity (27), enabling NO to be produced where it is most required (28-31). Interestingly, nitrate supplementation also reduces the oxygen cost of submaximal exercise and can, in some circumstances, enhance exercise tolerance and performance (28). Acute nitrate supplementation can improve exercise economy and endurance performance in moderately trained subjects, but these effects are less likely in well-trained athletes (30).

There is a growing interest in exploring alternative approaches to enhance endurance performance through nutrition, particularly strategies that go beyond the benefits provided by immediate CHO supplementation alone. One area of interest is the potential synergistic effects of combining CHO with other reasonable ergogenic substances to further enhance performance. Among these substances, dietary nitrate is considered an attractive candidate for combination with CHO (32). Furthermore, dietary nitrate is believed to produce its effects through mechanisms that differ from those of CHO. Numerous studies indicate that it can decrease the amount of O_2 required during exercise by improving the efficiency of skeletal muscle contraction or mitochondrial oxidative phosphorylation. This reduction in O_2 demand leads to a decrease in exercise

intensity for a given level of total work output, thereby improving exercise tolerance or the ability to sustain a higher pace for longer periods (33). While there has been limited research on the interaction between dietary nitrate and glucose/glycogen metabolism during exercise, a study by Betteridge et al. (34) investigated the effects of consuming beetroot juice (containing approximately 8 mmol of NO_3^-) before a 60-minute prolonged exercise session at 65% of $\dot{V}O_2$max. During this exercise, glucose was infused to assess its metabolic fate. The findings from this study revealed no significant impact of beetroot juice ingestion on $\dot{V}O_2$ consumption, glucose disposal, or muscle metabolites during submaximal exercise.

At present, no research has determined whether the improved exercise efficiency associated with dietary nitrate is linked to potentially beneficial effects that could support the synergistic enhancement of exogenous CHO metabolism or glycogen storage, despite the logical reasoning and intriguing mechanistic evidence for potential additive or synergistic effects. The main objective of the present study is to investigate the acute effects of co-ingesting HBCD and dietary nitrate on physiological responses and endurance capacity in recreational endurance runners, comparing it with the ingestion of an isocaloric maltodextrin beverage. The hypothesis of this study is that administering a single dose of the HBCD-dietary nitrate beverage will result in different physiological responses compared to ingesting isocaloric CHO beverage without dietary nitrate. This difference in physiological responses is expected to enhance endurance capacity in recreational endurance runners by providing an additional exogenous energy source and reducing $\dot{V}O_2$ consumption for a given workload. As a result, more efficient and economical energy production mechanisms are anticipated.

The purposes of this study

1. To investigate the effects of co-ingestion of HBCD and dietary nitrate on physiological responses during high-intensity prolong running in recreational endurance runners compared with an isocaloric HBCD beverage, a maltodextrin-dietary nitrate beverage, and a maltodextrin beverage.

2. To investigate the effects of co-ingestion of HBCD and dietary nitrate on endurance capacity during high-intensity prolong running in recreational endurance runners compared with an isocaloric HBCD beverage, a maltodextrin-dietary nitrate beverage, and a maltodextrin beverage.

Research questions

RQ1: Whether the effects of co-ingestion of the HBCD-dietary nitrate beverage, an isocaloric HBCD beverage, a maltodextrin-dietary nitrate beverage, and a maltodextrin beverage. can affect the different physiological responses in recreational endurance runners?

RQ2: Whether the effects of the HBCD-dietary nitrate beverage can improve endurance capacity in recreational endurance runners than an isocaloric HBCD beverage, a maltodextrin-dietary nitrate beverage, and a maltodextrin beverage?

Research hypothesis

H1: An acute single dose of the HBCD-dietary nitrate beverage would affect the difference physiological responses in recreational endurance runners compared with an isocaloric HBCD beverage, a maltodextrin-dietary nitrate beverage, and a maltodextrin beverage.

H2: An acute single dose of the HBCD-dietary nitrate beverage would improve endurance capacity in recreational endurance runners by reducing $\dot{V}O_2$ for a given workload than an isocaloric HBCD beverage, a maltodextrin-dietary nitrate beverage, and a maltodextrin beverage.

Scope of research

The study aimed to examine the enhancement of endurance capacity in recreational endurance runners through the use of the HBCD-dietary nitrate beverage. The beverage was formulated using HBCD powder and beetroot extract 10:1 powder.

Participants

The study recruited twelve male recreational endurance runners, aged 30-39 years, from various running clubs in the Bangkok metropolis. All participants were required to consume four different beverages in a random sequence, with a 2-week washout period between each beverage. This was done to investigate the endurance capacity of the runners. The four beverages used in the study were as follows:

1. The co-ingestion of HBCD and dietary nitrate (HBCD+NO_3^-): 1.5 g HBCD·kg^{-1} body mass with 500 mg of beetroot extract 10:1 powder (providing ~8.00 mmol of NO_3^-).

2. The ingestion of HBCD (HBCD): 1.5 g HBCD·kg^{-1} body mass.

3. The co-ingestion of maltodextrin and dietary nitrate (MD+NO_3^-): 1.5 g maltodextrin·kg^{-1} body mass with 500 mg of beetroot extract 10:1 powder (providing ~8.00 mmol NO_3^-).

4. The ingestion of maltodextrin (MD): 1.5 g maltodextrin·kg^{-1} body mass (equivalent to commercial sports drinks).

The variables employed in the study include:

1. Independent variables are (1) the co-ingestion of HBCD and dietary nitrate, (2) the ingestion of HBCD, (3) the co-ingestion of maltodextrin and dietary nitrate, and (4) the ingestion of maltodextrin

2. Dependent variables are as follows:

- Physiological variables: $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_E$, RER, oxygen cost, total CHO oxidation, fat oxidation, and muscle oxygenation
- Biochemistry variables: blood lactate, serum glucose concentration, plasma nitrate/nitrite concentration, and serum insulin concentration
- Psychometric variables: RPE and gastrointestinal symptom

Operational definition

Highly branched cyclic dextrin (HBCD) is a type of maltodextrin produced from waxy corn starch by the cyclization of a branching enzyme with an average molecular weight of 400,000 with a narrow size distribution, high solubility in water contributes little to osmotic pressure and has a relatively low propensity for retrogradation.

Dietary nitrate is a 500 mg of beetroot extract 10: 1 powder providing ~8.0 mmol of NO_3^-.

The co-ingestion of HBCD and dietary nitrate is a 500 mL solution containing 1.5 g HBCD·kg^{-1} body mass and a 500 mg of beetroot extract 10:1 power providing ~8.00 mmol NO_3^-.

Recreational endurance runner is male endurance runners aged 30-39 years who are normally running with the shoe. Participants must have trained for at least *3* occasions each week, typical weekly training mileage at least 30 km per week, as well as an aerobic capacity baseline ($\dot{V}O_2$peak) value > 45 mL·kg^{-1}·min^{-1}

Physiological responses are the physiological variables reactions to the ingestion of experimental beverage include oxygen uptake, carbon dioxide production, minute ventilation, respiratory exchange ratio, total carbohydrate oxidation rate, fat oxidation rate, and the change in muscle oxygenation that obtained during the running economy and constant load running test.

Endurance capacity is physiological components include runner's oxygen uptake and running economy (oxygen cost) that obtained during the running economy and constant load running test.

Oxygen uptake ($\dot{V}O_2$) is the volume of oxygen consumption body per unit time during exercise that determined using a portable metabolic analyser for breath-by-breath analysis during the running economy and constant load running test.

Carbon dioxide production ($\dot{V}CO_2$) is the volume of carbon dioxide exhaled from the body per unit time during exercise that determined using a portable metabolic analyser for breath-by-breath analysis during the running economy and constant load running test.

Minute ventilation ($\dot{V}E$) is the volume of air breathed per minute during exercise that determined using a portable metabolic analyser for breath-by-breath analysis during the running economy and constant load running test.

Respiratory exchange ratio (RER) is the ratio of $\dot{V}CO_2/\dot{V}O_2$ during exercise that determined using a portable metabolic analyser for breath-by-breath analysis during the running economy and constant load running test.

Running economy is the oxygen cost for a given velocity of submaximal running and is determined by measuring the steady-state $\dot{V}O_2$ during the submaximal incremental test. Running economy was calculated as the oxygen cost (EO_2) using the average $\dot{V}O_2$ ($mL \cdot kg^{-1} \cdot min^{-1}$) over the 2-minutes steady-state period, and the running speed ($m \cdot min^{-1}$)

Total carbohydrate oxidation rate is the rate of CHO substrate oxidation from $\dot{V}O_2$ and $\dot{V}CO_2$ during the running economy and constant load running test and calculates by $4.210\ \dot{V}CO_2 - 2.962\ \dot{V}O_2$.

Fat oxidation rate is the rate of fat substrate oxidation from $\dot{V}O_2$ and $\dot{V}CO_2$ during the running economy and constant load running test and calculates by $1.695\ \dot{V}CO_2 - 1.701\ \dot{V}O_2$.

Blood lactate concentration is a product of muscle metabolism and accumulates in muscle and blood before and during the running economy and constant load running test.

Serum glucose concentration is the amount of glucose in the serum before and during the running economy and constant load running test.

Plasma nitrate/nitrite concentration is a biomarker of nitrite concentration in the blood before beverage ingestion, after 30-min beverage ingestion, before exercise test and post-exercise test.

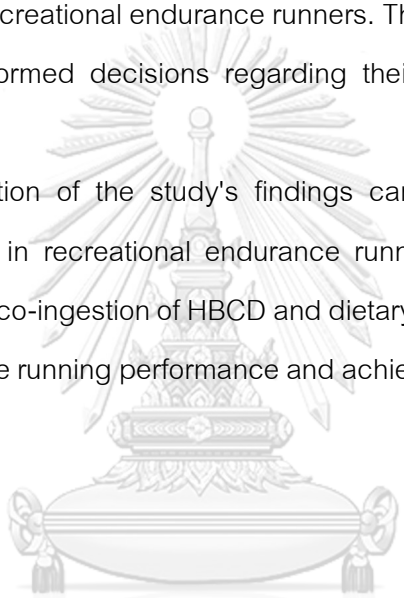
Muscle oxygenation is the level of oxygen availability in the oxyhaemoglobin and myoglobin within the skeletal muscle, determined using near-infrared spectroscopy (NIRS).

Expected benefits and applications

1. The study expects to identify whether the co-ingestion of HBCD and dietary nitrate can lead to improved endurance capacity in recreational endurance runners. This information can be valuable for designing effective nutrition strategies to enhance endurance running performance.

2. Understanding the effects of HBCD and dietary nitrate on endurance capacity, the study's results can contribute to the development of evidence-based nutrition strategies for recreational endurance runners. This knowledge can help athletes and coaches make informed decisions regarding their dietary choices to maximize performance.

3. The application of the study's findings can potentially lead to improved performance outcomes in recreational endurance runners. By implementing nutrition strategies based on the co-ingestion of HBCD and dietary nitrate, athletes may be able to enhance their endurance running performance and achieve better results in their training and competitions.



CHAPTER II
REVIEW OF LITERATURE

Marathon and half marathon

Long-distance running or endurance running events has become a popular sport. One of the most massive mass participation sports is the marathon, the longest-running road race on the Olympic program since 1896 though the distance did not become standardized until an official at 42.195 km (26 mi 385 yds) distance in 1921. The event was instituted in remembrance of the mythological run of the Greek soldier Pheidippides, a messenger from the Battle of Marathon to Athens, who reported the victory (35).

The important for most marathon runners are finishing time and placement within their specific gender and age group, though some runners simply want to finish. The worldwide growth from 2008 to 2018 was +49.43%. Women pick up faster than men with a growth of +56.83%, while men's participation rate has increased +46.91% (1). The current marathon world record time which officially recognized by the World Athletics (formerly known as the International Association of Athletics Federations; IAAF) for men over the distance is 2:01:39, placed in the Berlin Marathon by Eliud Kipchoge of Kenya on 16 September 2018 (36), while the world record for women was set by Brigid Kosgei of Kenya in the Chicago Marathon on 13 October 2019, in 2:14:04 (37).

Table 1 The current marathon world record time (officially by World Athletics)

Type	Mark	Competitor
MEN [36, 37]		
World Records	2:01:39	Eliud KIPCHOGE, Kenya
World Leading 2020	2:04:15	Birhanu LEGESE, Ethiopia
World Championships in Athletics Records	2:06:54	Abel KIRUI, Kenya
Olympic Games Records	2:06:32	Samuel Kamau WANJIRU, Kenya
Area Records – Asia	2:04:43	El Hassan EL ABBASSI, Bahrain
World U20 Leading 2020	2:29:45	Yegor VINOGRADOV, Russia
WOMEN [36, 37]		
World Records	2:14:04	Brigid KOSGEI, Kenya
World Leading 2020	2:17:45	Lonah Chemtai SALPETER, Israel
World Championships in Athletics Records	2:20:57	Paula RADCLIFFE, Great Britain
Olympic Games Records	2:23:07	Tiki GELANA, Ethiopia
Area Records - Asia	2:19:12	Mizuki NOGUCHI, Japan
World U20 Leading 2020	2:56:23	Megan DEMPSTER, South Africa

Note. From "Men senior outdoor records (2020)" (36) and "Women senior outdoor records (2020)" (37)

At present more than 800 marathons are held throughout the world annually, with most competitors being recreational athletes as larger marathons can have tens of thousands of participants. The most significant two age groups of runners, 40-49 and 30-39, make up about 60% of all marathon's participants, and this percentage is maintained for both men and women (3). In 2016, the trend of running in Thailand was considered the most popular. As can be seen from the statistics of running exercisers in Thailand, there were 5.5 million runners in 2011 and increased to 11.96 million runners in 2016 divided into a female, 7.4 million, and male, 4.56 million people, if classified by age, it was found that working age was the most number 6.88 million (57.5%), followed by the older age of 2.49 million (20.8%), teenagers of 1.9 million (16%) and childhood, about 700 thousand people (5.7%) (2).

Worldwide marathon statistics, analyse from 3,446 unique races worldwide, with 32,335 events in total over the 11 years between 2008 and 2018 with a total of 19,614,975 participants, show that Thailand is a male-dominated marathon nation with a proportion of male and female marathon runners are 77% and 23%, respectively (1). The world's average finish time of a marathon was 4:29:53 in 2018. The Swiss are also the world's fastest marathon nation. In 2018, Switzerland is first (3:50:37), second on the list the Netherlands (3:52:10) and third Spain (3:52:25). The slowest nations are the Philippines (5:25:35) followed by India (5:05:21), and Mexico (4:53:11) (Table 2).

Table 2 The world's fastest marathon nations

Position	Nation	Average finish time
1	Switzerland	3:50:37
2	The Netherlands	3:52:10
3	Spain	3:52:25
4	Portugal	3:59:12
5	Norway	4:01:06
6	Slovenia	4:02:51
7	Iceland	4:03:48
8	Italy	4:07:20
9	Russia	4:07:46
10	Canada	4:07:57
…		
26	Thailand	4:51:38

Note. From "Marathon Statistics 2019 Worldwide (2020)" (1)

The half marathon is a road running event that covers a distance of 21.0975 km, which is half the distance of a full marathon. This shorter distance has contributed to an increase in the number of participants and finishers. During the 1950s, with the rise in recreational running, race organizers sought to provide an alternative to the standard marathon, giving rise to the concept of the half marathon. Although the half marathon is not included in the World Championships or Olympic programs, it gained its own championship status in 1992 with the establishment of the IAAF World Half Marathon Championships. Over time, the popularity of the half marathon has continued to grow, making it one of the most renowned road running events (38). There were around 482,000 finishers in 2011 and increased to 2 million finishers in 2014 in the United States (8). Not only United State but also Thailand, such as in the Bangsaen21 Half Marathon event, have remarkably increased the number of finishers from 699 in 2015 to 7,421 in 2020 (39). These increased participation levels have led to an increased range of abilities in runners participating, from recreational or amateur to elite levels (8). The current half marathon world record time which officially recognized by the World Athletics for men over the distance is 57:32, by Kibiwott Kandi (40), while the world record for women was set by Peres Jepchirchi, in 1:05:16 (41).

Given the heterogeneity among runners, segmenting them into groups to understand their attitudes, interests, and opinions (AOIs) is useful and appealing. The findings of Janssen et al. (42) were identified the runners to four types: type I casual individual, type II social competitive, type III individual competitive, and type IV devoted.

Type I: Casual Individual Runners

Compared to other types, type I runners identified with running the least and were the most susceptible to quitting the sport for individual reasons, thus they also scored low on competitiveness. Type I runners were classified as casual individual runners, and the socio-demographics showed that this group consisted of relatively more women, runners <35 years of age, runners with higher education, and students compared with the other types. Considering the habits of runners, the analysis showed that this group comprised relatively more 5 km and 10 km runners, more runners for whom running was not their

main sport, more inexperienced runners, and more runners who trained less frequently, participated in fewer events than others, and ran more individually compared to other types.

Type II: Social Competitive Runners

Type II runners were characterized as competitive and were the most susceptible to quitting in general, especially for social reasons. The authors (42) referred to them as social competitive runners, and this was not a group that stood out (scoring highest or lowest of all types) in terms of socio-demographic. An analysis of their running habits showed that the type II group included relatively more 5 km and 10 km runners (as noted for type I). The social competitive runners group scored relatively higher (compared to individual competitive and devoted runners) regarding runners for whom running was not their main sport, less experienced runners, and runners who trained less frequently and participated in fewer events than others, while casual individual runners scored even higher on these items. For running context, social competitive runners scored the lowest on running individually, but had the highest scores for running with friends, colleagues, small groups, and clubs.

Type III: Individual Competitive Runners

Individual competitive runners, classified as type III, were distinguished by their competitive nature and demonstrated a reduced inclination to quit either individually or within a social context. In contrast to the previous group discussed, they displayed favourable scores in areas such as perceived benefits of running and a strong identification with the activity. Notably, the gender distribution within this group differed significantly compared to the other groups, with a higher representation of male runners among the type III category. Additionally, this group comprised the largest number of participants with lower or moderate levels of education, while having the fewest number of students among the four types.

Regarding running habits, individual competitive runners scored high on running as the main sport, long training distances, frequent training sessions, and participating in five or more events annually. While these habits did not differ from devoted runners, the

individual competitive group ran individually more than either devoted or social competitive runners.

Type IV: Devoted Runners

Type IV runners, similar to type III runners, exhibited a strong inclination towards running and identified closely with the activity. They displayed a lower likelihood of quitting, both individually and within a social context. However, in terms of competitiveness, they ranked lower compared to other types. In light of these characteristics, we classified them as devoted runners. Among the various groups, this category consisted of the highest number of runners aged 45 and above, individuals with lower or moderate education levels, and a significant proportion of part-time employees. Devoted runners demonstrated a strong preference for running as their primary sport, engaged in extensive training distances, maintained frequent training sessions, and participated in five or more annual events, mirroring the traits of type III runners. Notably, devoted runners represented the most experienced group and, alongside socially competitive runners, constituted the largest contingent of club runners.

The recreational endurance runner participated in this study presume that they are Type III runner according to Janssen et al. (42) with runners aged 30-39 years who are normally running with the shoe. Participants must have trained for at least 4 occasions each week, typical weekly training mileage at least 30 km per week.

Exercise energy metabolism

The energy demand of exercise varies depending on the intensity and duration of the activity. Different energy systems, as well as combinations of these systems, are utilized to maintain an adequate concentration of adenosine triphosphate (ATP) within the working skeletal muscles. The contraction process in muscles primarily relies on ATP hydrolysis by actomyosin ATPase. However, other ATPases such as Ca_2^+ ATPase and Na^+/K^+-ATPase also play roles in the overall excitation-contraction coupling process. Therefore, it is crucial to maintain and protect cellular ATP concentration in order to delay the onset of muscle fatigue and ensure proper functioning throughout the entire contractile process (43).

The dephosphorylation of ATP releases energy, converting it into ADP. This ADP can be rephosphorylated back into ATP through the processes of respiration. ATP is a universal energy transducer found in all living cells. Glucose is the primary metabolic fuel, and it undergoes three stages of oxidation to produce carbon dioxide, water, and ATP as captured energy. This process can be summarized by the following equation:

$$\textit{Glucose} + \textit{Oxygen} \longrightarrow \textit{Carbon Dioxide} + \textit{Water} + \textit{ATP}$$

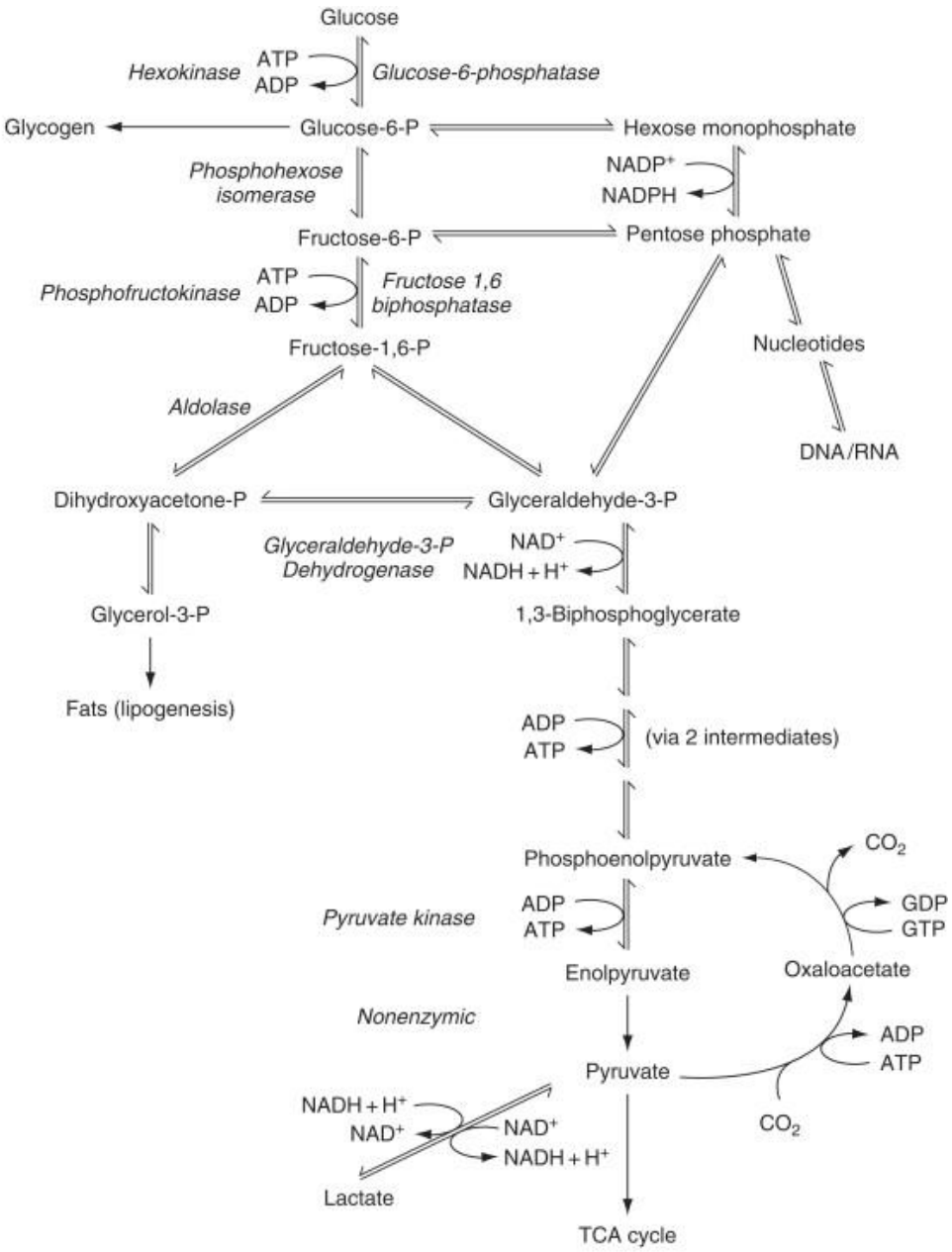
$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + ATP$$

In the initial steps of glycolysis and the tricarboxylic acid cycle, glucose and other metabolic fuels undergo oxidation, which is linked to the chemical reduction of coenzymes such as NAD+ (nicotinamide adenine dinucleotide), FAD (flavin adenine dinucleotide), and FMN (flavin mononucleotide). ATP is then synthesized from ADP and phosphate, utilizing the energy released during the oxidation and recycling of these reduced coenzymes. This final stage of oxidative phosphorylation occurs through the hydrogen electron transfer chain. As a result, the oxidation of metabolic fuels is closely connected to energy consumption and the production of ADP from ATP in energy-consuming processes (44).

The three principal stages in the production of ATP from glucose including (44);

1. Glycolysis

Glucose is the primary substrate for glycolysis. Other monosaccharide such as fructose and galactose can be supplied into glycolysis at different points and then metabolized in the same way as glucose to pyruvic acid. Glycolysis does not require oxygen and is essential for the direct production of ATP when oxygen is limiting (i.e., in rapidly contracting muscle)—glycolysis results in breaking glucose into two pyruvic acid molecules, which in the cytoplasmic solution becomes pyruvate. Pyruvate can enter the mitochondrion and be metabolized by oxidative decarboxylation to CO_2. If oxygen is unavailable, it can be further metabolized to lactic acid resulting in the regeneration of nicotinamide adenine dinucleotide (NAD^+) from $NADH+H^+$, allowing glycolysis to continue in the inadequacy of oxygen. Red blood cells can only metabolize glucose or another monosaccharide because red blood cells lack mitochondria, and therefore glycolysis is the only source of energy metabolism. Red cells produce lactate that is excreted into the blood. Lactate is primarily metabolized back to pyruvate in the liver, where it is mostly used to synthesize glucose (gluconeogenesis), which is essentially the reverse of glycolysis, except for the irreversible reaction of phosphoenolpyruvate (PEP) to pyruvate. Hence in the liver, pyruvate is converted back to PEP via oxaloacetate. This cycle of lactate and pyruvate is known as the Cori cycle. Glycolysis and its interactions with other metabolic pathways have shown in Figure 1.



net = 2 NADH = 6 ATP + net 2 ATP

Figure 1 Glycolysis and its interactions with other metabolic pathways

Source for Figure 1 (44)

2. Krebs's cycle (TCA cycle)

Tricarboxylic Acid Cycle (also known as the TCA Cycle, Citric Acid Cycle, or Krebs's Cycle) is located in the mitochondrial matrix and is a common metabolic pathway for all fuels and is responsible for the production of the majority of the reduced coenzymes used for the generation of ATP in the electron transfer chain. It also plays a central role in the interconversion of fuels and metabolites—the TCA cycle associates in gluconeogenesis from amino acids and lactate during fasting between meals and longer-term starvation. TCA cycle intermediates are the source of most of the nonessential amino acids such as aspartate and glutamate. It is also involved in the conversion of carbohydrates to fat for storage after a CHO-rich meal.

Pyruvate from glycolysis is oxidatively decarboxylated to acetyl-CoA in the mitochondria, catalysed by the multienzyme complex, pyruvate dehydrogenase, and the coenzyme A:

$$Pyruvate + coenzyme\ A + NAD^{+} \rightarrow acetyl\text{-}CoA + CO_2 + NADH + H^{+}$$

Acetyl-CoA can be produced from pyruvate and fatty acids released from fat stores and from amino acids released from proteolysis of protein tissue, which can be converted to acetyl-CoA TCA cycle intermediates.

In the electron transfer chain (oxidative phosphorylation), each $NAD+H^{+}$ yields approximately 3 ATP, and FADH2 yields 2 ATP. Thus, each TCA cycle rotation produces approximately 12 ATP. As two molecules of acetyl-CoA are formed from one glucose molecule, the TCA cycle rotates twice for each glucose response molecule, producing a net of 24 ATP. The oxidative decarboxylation of pyruvate and the tricarboxylic acid cycle have shown in Figure 2.

3. The electron transfer chain (oxidative phosphorylation)

Oxidative phosphorylation, which is conceptually simple and mechanistically complex, occurs in the crista of mitochondria. The hydrogen accepted by NAD^+ and FAD during glycolysis, and the TCA cycle is oxidized to water by molecular oxygen accompanying ADP⟶ATP phosphorylation. For example, oxidative phosphorylation generates 26 of the 30 molecules of ATP that are formed when glucose is completely oxidized to CO_2 and H_2O. The flow of electrons from NADH or $FADH_2$ to O_2 through protein complexes located in the mitochondrial inner membrane leads to the pumping of protons out of the mitochondrial matrix. The resulting uneven distribution of protons generates a pH gradient and a transmembrane electrical potential that creates a proton-motive force. ATP is synthesized when protons flow back to the mitochondrial matrix through an enzyme complex. Thus, the oxidation of fuels and the phosphorylation of ADP is coupled by a proton gradient across the inner mitochondrial membrane.

Oxidative phosphorylation is the culmination of a series of energy transformations called "cellular respiration" or "simply respiration" in its entirety. First, carbon fuels are oxidized in the citric acid cycle to yield electrons with high transfer potential. Then, this electron-motive force is converted into a proton-motive force, and, finally, the proton-motive force is converted into phosphoryl transfer potential. The conversion of electron-motive force into proton-motive force is carried out by three electron-driven proton pumps—NADH-Q oxidoreductase, Q-cytochrome c oxidoreductase, and cytochrome c oxidase. These large transmembrane complexes contain multiple oxidation-reduction centres, including quinones, flavins, iron-sulfur clusters, hemes, and copper ions. The final phase of oxidative phosphorylation is carried out by ATP synthase, an ATP-synthesizing assembly driven by the flow of protons back into the mitochondrial matrix. Components of this remarkable enzyme rotate as part of its catalytic mechanism. Oxidative phosphorylation vividly shows that proton gradients are an interconvertible currency of free energy in biological systems (45). Overview of the electron transfer chain have shown in Figure 3.

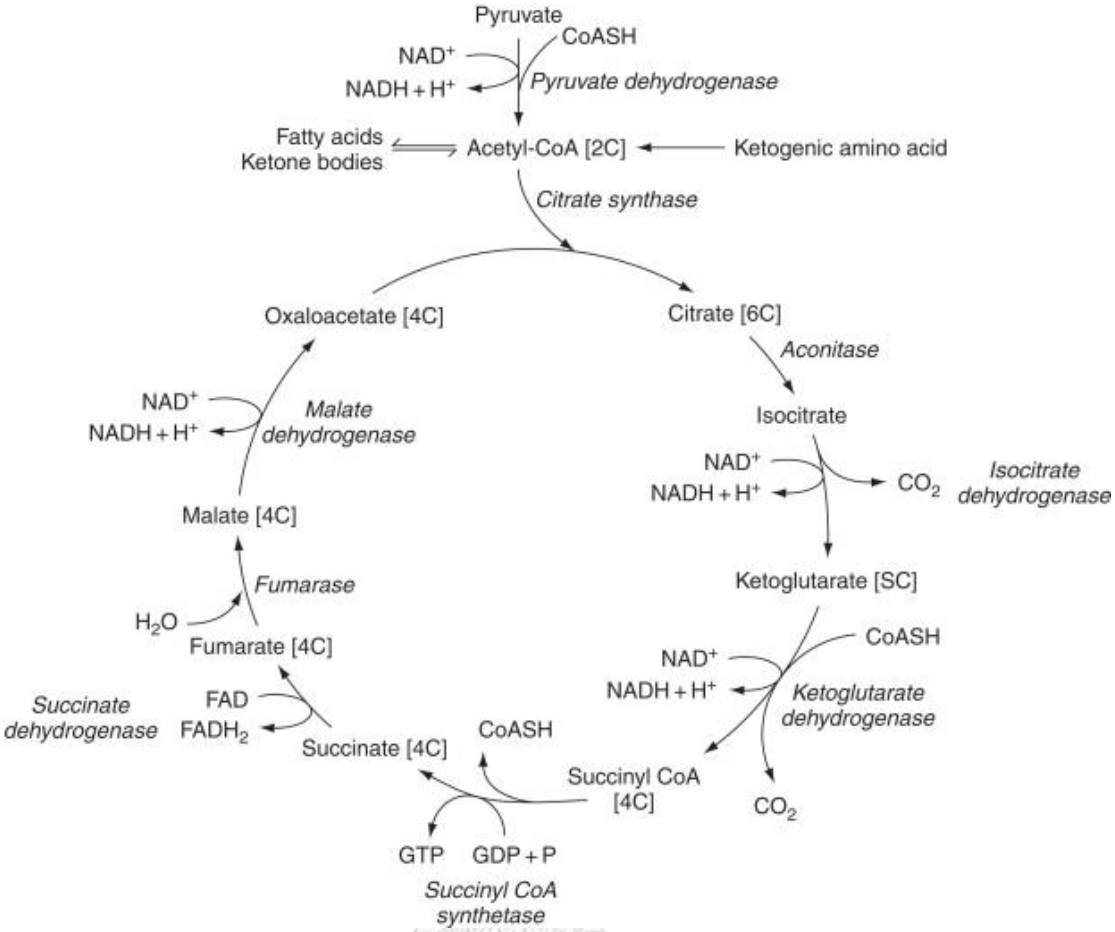


Figure 2 The oxidative decarboxylation of pyruvate and the tricarboxylic acid cycle

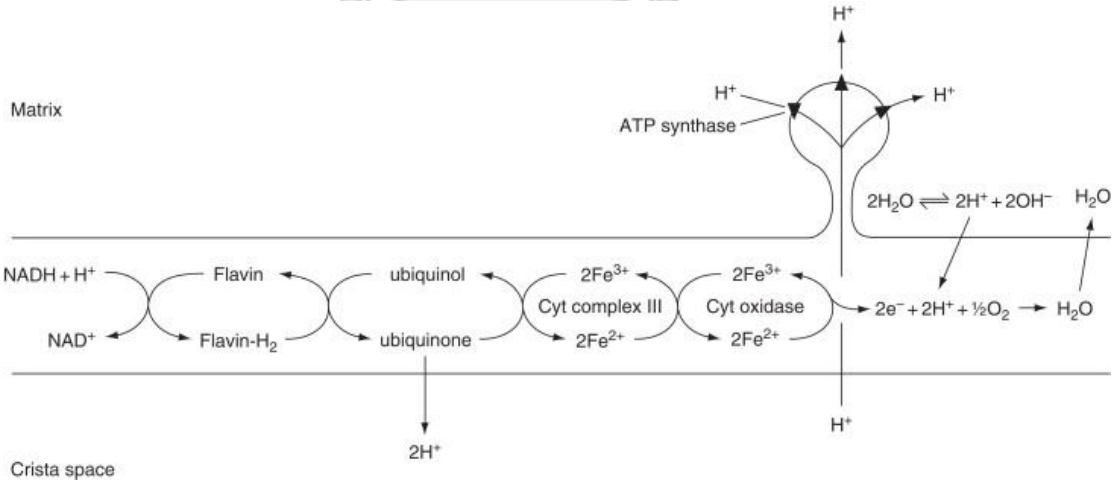


Figure 3 Overview of the electron transfer chain

Source for Figure 2,3 (44)

In conclusion, once CHO are digested into monosaccharides, predominantly glucose, there are three main stages involved in ATP production from glucose:

1. Glycolysis takes place in the cytoplasm and does not require oxygen. During this process, ATP, pyruvate, and NADH are produced.

2. Pyruvate, the end product of glycolysis, enters the mitochondrion and undergoes oxidative decarboxylation. In the presence of oxygen, pyruvate is further metabolized to CO_2. However, if oxygen is not available, pyruvate can be converted to lactic acid, which allows for the regeneration of NAD^+ from $NADH+H^+$. This regeneration of NAD^+ is important as it enables glycolysis to continue even in the absence of oxygen.

3. The electron transfer chain, also known as oxidative phosphorylation, occurs in the mitochondrial cristae and involves the primary particles. During this stage, the hydrogen molecules that were accepted by NAD^+ and FAD in glycolysis and the tricarboxylic acid (TCA) cycle are oxidized to water by molecular oxygen. This process is accompanied by the phosphorylation of ADP to ATP, resulting in the production of energy-rich ATP molecules.

Overall, these three consecutive stages ensure the efficient production of ATP from glucose, providing the necessary energy for cellular processes.

4. Energy metabolism of fats (triacylglycerides)

Fats (triacylglycerides) are stored mainly in adipose tissue. The diet supplies most fatty acids, but many tissues can *de novo* synthesis, including the liver, brain, kidney, mammary glands, and adipose tissue. The *de novo* synthesis of fatty acids occurs in conditions of excess energy intake (44).

Fat may be metabolized by mobilizing intramuscular triglyceride stores or through oxidation of plasma-derived fatty acids obtained from peripheral adipose tissue stores or dietary fat intake. The main difference between fat and carbohydrate storage is the size of the available storage. Most adult humans have sufficient adipose tissue stores to provide an almost unlimited supply of substrate for ATP re-synthesis during exercise and could theoretically sustain over 120 hours of continuous effort. This is not possible as substrates are not used in isolation, and other factors would act to limit fat oxidation. It is

now widely acknowledged that the key factors limiting fatty acid oxidation in skeletal muscle largely relate to three components (43):

1) ability to transport fatty acids into muscle via passive diffusion as well as facilitated transport via fatty acid-binding proteins (FABPpm) and carrier proteins such as fatty acid translocase (FAT/CD36), fatty acid transport proteins (FATPs) or cytosolic FABP (FABPc).

2) transfer of activated fatty acids, derived from plasma or intramuscular triglyceride stores, across the mitochondrial membranes via carnitine palmitoyl transferase I and II (CPT I/II) with regulatory involvement from FAT/CD36 and malonyl-CoA; and

3) enzymatic regulation of β-oxidation through the mitochondrial capacity for fatty acid oxidation typically assessed using the maximal activity of l-β-hydroxy acyl CoA dehydrogenase (β-HAD).

All these components can change to improve fatty acid metabolism in trained individuals. However, other factors that will influence fat oxidation in exercises, such as substrate availability (CHO ingestion will blunt fatty acid mobilization and oxidation) and hormonal control. Thus, the intensity of exercise and duration are also critical in determining rates of whole-body fatty acid oxidation. Metabolism of activated long-chain fatty acids within the mitochondrion involves cleaving 2-carbon units off the chain on each pass through the β-oxidation pathway. Thus, a 16-carbon palmitate chain produces eight 2-carbon acetyl-CoA units for oxidation in the TCA cycle and respiratory chain (43).

Both fat and CHO serve as crucial fuel sources for aerobic exercise. However, the relative proportions of CHO and fat that are oxidized can vary depending on the exercise intensity and metabolic demands. This means that there can be reciprocal shifts in the utilization of CHO and fat as fuel sources. The balance between CHO and fatty acid oxidation is influenced by the metabolic conditions both inside and outside the cells. The availability of substrates, such as glucose and fatty acids, as well as the specific metabolic environment, play a role in determining which fuel source is primarily utilized (46).

The substrate availability refers to the presence and accessibility of glucose and fatty acids in the body. The amount and availability of these substrates can impact the preferential utilization of CHO or fat as fuel sources during exercise. Additionally, the intensity and duration of the exercise also influence the metabolic environment. Higher intensity exercises tend to rely more on CHO oxidation, while lower intensity exercises can rely more on fat oxidation.

In summary, the interplay between CHO and fatty acid oxidation during aerobic exercise is influenced by factors such as substrate availability, exercise intensity, and exercise duration. These factors contribute to the dynamic metabolic environment that determines the proportions of CHO and fat oxidized during exercise.

Substrate demand during endurance running

Energy production during prolonged running is almost exclusively aerobic. The primary energy substrates utilized during endurance running are CHO originating from intramuscular glycogen stores and plasma glucose, which is maintained by liver glycogen as well ingested dietary CHO, and fatty acids originating from intramuscular triglycerides and plasma free fatty acids, which are mainly derived from adipose tissue (6). A more sustained glucose availability could supply CHO to maintain the energy substrates' oxidation when the glycogen stores have been depleted; however, CHO storage is limited, the depletion of muscle glycogen and plasma glucose can occur after as little as 90-min of running, which is associated with the onset of fatigue and the need to reduce the running pace (12, 13).

Physiological aspects of endurance running

There are numerous physiological and biological components limiting the performance a during 42.195 km-marathon race (Figure 4). The successful physiological capacity for marathon endurance performance is believed to be influenced by essential physiological components. These key components encompass both aerobic and anaerobic energy production, which are reflected in the runner's $\dot{V}O_2$peak. This measurement indicates the ability to transport substantial amounts of oxygen to the muscles and the muscles' capacity to effectively utilize oxygen during prolonged running. Additionally, other critical factors include the velocity at lactate threshold (vLT) and excellent running economy (RE), which enable the efficient utilization of oxygen while maintaining a marathon pace (47, 48). Moreover, it also includes biological attributes limiting marathon performance—the proportion of slow-twitch muscle fibres that are high-genetically determined influences other physiological characteristics. High glycogen storage and well-developed fat utilization enable athletes to store enough glycogen in muscles and liver to run hard for 42.195 km and enable muscles to rely more on fuel from fat (47).

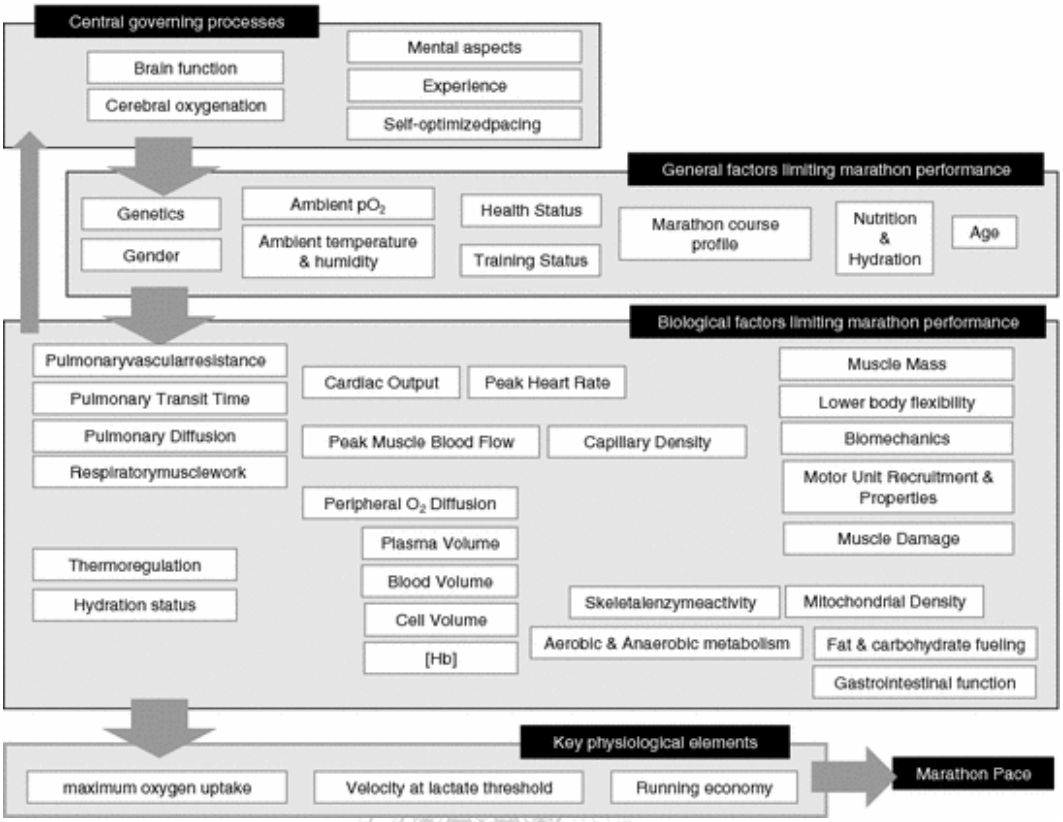


Figure 4 Physiological, biological, and other components limiting the marathon performance

Source for Figure 4 (48)

1. Maximum oxygen uptake; $\dot{V}O_2$peak

Successful marathon runners have high $\dot{V}O_2$peak values, which is defined as the maximum amount of oxygen taken up, and all tissue consumed during exhaustive exercise. Elite marathon runners hold a $\dot{V}O_2$peak of > 80 $mL \cdot kg^{-1} \cdot min^{-1}$ and > 75 $mL \cdot kg^{-1} \cdot min^{-1}$ (48). That demonstrates the ability to transport large amounts of blood (i.e., high cardiac output and total body haemoglobin), distribute blood (i.e., by muscle blood flow), and extract and utilize oxygen within the muscle cell (47, 49).

$\dot{V}O_2$peak is directly linked to the rate of ATP generation that can be maintained during a long-distance aerobic exercise, even though distance races are not run at 100% $\dot{V}O_2$peak. The ATP production rate is dependent on the $\dot{V}O_2$ ($mL \cdot kg^{-1} \cdot min^{-1}$) that can be maintained during the run, which is determined by the runner's $\dot{V}O_2$peak and the percent

of $\dot{V}O_2$peak at which the runners can perform (49). For example; during an official distance of 42.195 km race, elite marathon runners run at speeds requiring between 80–90% of $\dot{V}O_2$peak and finish in times between 2:05:00 and 2:20:00 (6), sub-elite marathon runners will run at 70–75% of $\dot{V}O_2$peak for approximately finishing in time 2:45:00, and another slower, recreational marathoner run at 60–65% of $\dot{V}O_2$peak for 3:45:00 (6, 50). In this way, $\dot{V}O_2$peak sets the upper limit for energy production in endurance performances but does not determine the final marathon performance. Significant variations in $\dot{V}O_2$peak have been observed among runners with a similar performance level, indicating that other components represent an essential role in inter-individual performance variability. The oxygen cost of running and lactate threshold are additional factors that significantly impact marathon performance. Essentially, $\dot{V}O_2$peak and lactate threshold play a crucial role in determining the duration for which aerobic and anaerobic processes can be sustained during a marathon. On the other hand, the runner's economy, or running efficiency, governs the marathon velocity that can be achieved while minimizing energy consumption. Together, these factors play a vital role in determining an athlete's performance during a marathon (10, 48, 49).

2. Velocity at the lactate threshold; V_{LT}

The lactate threshold (LT) used synonymously with the anaerobic threshold (AT), and the ventilatory threshold (VT) has been associated with the intensity of exercise above which lactate levels rise, and ventilation increases disproportionately with oxygen consumption. The lactate threshold - is determined using invasive procedures, as a blood sample is required- can be explained as the point when lactate production overcomes lactate removal or consumption, and the ventilatory threshold - is often used to estimate when lactate threshold occurs - is the inflection point when a person starts breathing heavily. An increase in anaerobic metabolism often limits aerobic performance because not as much ATP is created to fuel the body. The lactate threshold and ventilatory threshold have a reasonable correlation and generally occur simultaneously during an exercise.

The velocity at the lactate threshold (V_{LT}) is firmly associated with marathon performance. The V_{LT} and fractional utilization of $\dot{V}O_2$peak at V_{LT} (%$\dot{V}O_2$peak) are considered beneficial indicators of endurance performance between individuals of different ages, genders, and disciplines (51). The average runner's lactate threshold occurs at about 75 to 80 % of $\dot{V}O_2$peak, while successful marathon runners generally have lactate thresholds of 84 to 88 % of $\dot{V}O_2$peak; elite marathon runners tend to have lactate thresholds of about 88 to 91 % of $\dot{V}O_2$peak. That indicates that elite marathon runners can utilize a larger proportion of $\dot{V}O_2$peak before accumulating lactate in muscles and blood (47, 48).

3. Running economy; RE

Running economy (RE) represents a complex interplay of physiological and biomechanical factors that are typically defined as the energy demand for a given velocity of submaximal running and expressed as the submaximal $\dot{V}O_2$ at a given running velocity (52). This can be shown by plotting $\dot{V}O_2$ ($mL \cdot kg^{-1} \cdot min^{-1}$) versus running velocity ($m \cdot min^{-1}$) or by merely expressing the economy as the energy required per unit mass to cover a horizontal distance ($mLO_2 \cdot kg^{-1} \cdot km^{-1}$)(49). Marathon runners with similar $\dot{V}O_2$peak may perform differently during a marathon, depending on their economy of running. Well-trained runners display lower submaximal $\dot{V}O_2$ at a given intensity when compared to less-trained runners (48). Elite-male runners have been shown to have a $\dot{V}O_2$peak 83 $mL \cdot kg^{-1} \cdot min^{-1}$ need at 16 $km^{-1} \cdot h^{-1}$ approximately 40 $mL \cdot kg^{-1} \cdot min^{-1}$(53).

Achieving optimal performance in endurance running events necessitates the efficient utilization of available energy resources. This entails having a substantial capacity to supply energy to the working muscles, as well as the ability to sustain a high intensity or faster pace without negatively impacting overall energy consumption. The concept of "efficiency" in running refers to the relationship between work performed and energy expended. For distance runners, it is desirable to minimize or eliminate unnecessary or counterproductive muscular movements. However, it is important to note that the terms "efficient" and "efficiency" should not be used to directly associate energy demands with running velocity, as running velocity represents only a fraction of the total

work performed by the body during locomotion. In the literature, alternative terms such as "cost," "oxygen cost," "energy cost," and "requirement" have been utilized to describe the relationship between running velocity and $\dot{V}O_2$ consumption. These terms serve as additional descriptors to capture the connection between the speed of running and the amount of oxygen utilized during exercise. However, the term "running economy" remains the most suitable and widely accepted term to denote the interplay between running speed and energy expenditure, providing a comprehensive understanding of the efficiency of energy utilization in endurance running (54).

For endurance athletes, especially distance runners, running economy (RE) is regarded as a crucial physiological indicator. Running economy encompasses a multifaceted interaction of physiological and biomechanical factors, which is typically defined as the energy demand associated with a specific submaximal running velocity. It is commonly quantified by measuring submaximal $\dot{V}O_2$ at a given running velocity (55). This relationship can be visualized by plotting oxygen uptake ($ml \cdot min^{-1} \cdot kg^{-1}$) against running velocity ($m \cdot min^{-1}$) or by expressing running economy as the energy required per unit mass to cover a horizontal distance ($mlO_2 \cdot kg^{-1} \cdot km^{-1}$) (56). Running economy plays a crucial role in marathon performance, as it can determine the outcomes of runners with similar $\dot{V}O_2$peak levels. In essence, runners with superior running economy are able to use less oxygen compared to those with inferior running economy while maintaining the same steady-state pace. This indicates that the efficient utilization of oxygen and energy resources is a determining factor in marathon success. By optimizing their running economy, athletes can enhance their ability to sustain a given pace and improve overall performance in endurance events (48). Previous studies have indicated that running economy can differ by up to 30% among trained runners who possess similar $\dot{V}O_2$max levels (55). Well-trained runners tend to demonstrate lower submaximal oxygen uptake at a given intensity compared to less-trained runners (48). In the case of elite male runners, their $\dot{V}O_2$peak has been reported to be approximately 83 $ml \cdot min^{-1} \cdot kg^{-1}$ at a speed of 16 $km \cdot h^{-1}$, equating to an energy requirement of approximately 40 $ml \cdot min^{-1} \cdot km^{-1}$ (53).

Running economy has most commonly been expressed in endurance runner studies as the $\dot{V}O_2$ per unit time, the oxygen cost (EO_2), and the energy cost (E_{aer}) (57).

1) The $\dot{V}O_2$ per unit time ($mL \cdot kg^{-1} \cdot min^{-1}$) was calculated as the average $\dot{V}O_2$ ($mL \cdot kg^{-1} \cdot min^{-1}$) over the steady-state period and the participant's body mass (BM) (58):

$$\dot{V}O_2(mL \cdot kg^{-1} \cdot min^{-1}) = \frac{\dot{V}O_2(mL \cdot min^{-1})}{BM\ (kg)}$$

2) The oxygen cost ($mL \cdot kg^{-1} \cdot m^{-1}$) was calculated using the average $\dot{V}O_2$ ($mL \cdot kg^{-1} \cdot min^{-1}$) over the steady-state period, and the running speed ($m \cdot min^{-1}$) (59):

$$EO_2(mL \cdot kg^{-1} \cdot m^{-1}) = \frac{\dot{V}O_2(mL \cdot kg^{-1} \cdot min^{-1})}{speed\ (m \cdot min^{-1})}$$

3) The energy cost ($J \cdot kg^{-1} \cdot m^{-1}$) was calculated using the average $\dot{V}O_2$ ($L \cdot min^{-1}$) over the steady-state period, the kilojoule equivalent of the $\dot{V}O_2$ ($kJ \cdot L^{-1}$ O_2, with a calorie-to-kilojoule conversion factor of 4184) determined by the RER using non-protein respiratory quotient tables, and the running velocity ($m \cdot min^{-1}$) normalized to the participant's body mass (60):

$$E_{aer}(J \cdot kg^{-1} \cdot m^{-1}) = \frac{\dot{V}O_2(L \cdot min^{-1})\ x\ caloric\ equivalent\ (kCal \cdot L^{-1}) \div BM\ (kg)}{speed\ (m \cdot min^{-1})}$$

Beyond the typical endurance athlete preparation, one of the acute strategies to improve running economy is nutritional interventions that have received attention for reducing oxygen demand during exercise, most notably dietary nitrates (52).

Limitations to endurance running performance

During endurance running and prolonged endurance exercise, the delivery of oxygen to the working muscles can be constrained by various central factors. These factors include cardiac dimensions (related to the heart's pumping capacity), pulmonary diffusion (the efficiency of oxygen uptake in the lungs), and blood oxygen-carrying capacity (which depends on factors like haemoglobin levels and red blood cell count). These central factors play a crucial role in determining the overall oxygen delivery to the muscles and have a significant impact on an athlete's performance during prolonged endurance activities.

1. Cardiac dimensions

Endurance athletes' hearts adapt to training with a reduction in their resting bradycardia resulting in heart rates <50 bpm. Such a reduction is due to enhanced parasympathetic and reduced sympathetic activity. The reduction in resting heart rate following endurance training allows athletes to perform at a lower heart rate with equal running velocity than pre-training (48).

2. Pulmonary diffusion

The runner's large cardiac output, that is, the volume of blood being pumped over time, permits the transportation of more significant amounts of blood volume per heartbeat. However, the large blood transport may not allow the lungs to thoroughly saturate haemoglobin with oxygen since the blood's transit time within the lungs may be too short. Thus, well-trained athletes with large cardiac dimensions may show signs of "exercise-induced arterial hypoxemia" when commencing heavy exercise (61).

3. Blood oxygen carrying capacity

At the onset of endurance exercise, the vascular system undergoes changes to redirect blood flow within and between muscles, prioritizing metabolically active skeletal muscles to facilitate optimal oxygen extraction. This increased blood flow to the muscles is mainly facilitated by exercise training, which promotes higher blood volume and red blood cell count. However, dehydration can negatively impact blood volume, compromising heat dissipation and increasing thermal strain in marathon runners.

Maintaining proper hydration during a marathon, particularly in hot conditions, is crucial for effective temperature regulation and to support adequate blood pressure and cardiac output, ensuring optimal oxygen transport throughout the body. (48, 62).

The central factors may limit the oxygen delivery to the exercising muscles. However, several peripheral factors may also minimize endurance performance.

4. Substrate regulation during endurance running

Endurance running demands carbohydrate (CHO) reserves that can be performance-limiting, unlike fat reserves, because they are comparably small. For a marathon runner, fat oxidation during moderate or high-intensity running is insufficient to satisfy the muscular ATP demands, thereby favouring glycogen pathways. All marathon runners, independent of performance level, rely on CHO fuelling, as evidenced by an average respiratory exchange ratio (RER) of >0.90 in the last half of the marathon (6). Elite marathon runners run at speeds requiring between 80–90% of maximal oxygen uptake ($\dot{V}O_2$peak) and finish in times between 2:05:00 and 2:20:00, sub-elite marathon runners will run at 70–75% $\dot{V}O_2$peak for approximately finishing in time 2:45:00, and another slower, recreational marathoner run at 60–65% of $\dot{V}O_2$peak for 3:45:00. The two latter groups, marathon runners, all rely on CHO and fat during the marathon race, with the slower runners having an average respiratory exchange ratio (RER) of ≈ 0.90 in the last half of the marathon and the faster runners ≈ 0.95–0.97, it seems possible that these athletes could complete the marathon using only CHO as fuel (6). Running a marathon at the fastest speed possible, if runners were to set a slightly faster pace, they would fatigue prematurely, probably due to accelerated glycogenolysis. Elite marathon runners derive more than two-thirds of their energy from CHO stemming from muscle glycogen and, to a lesser extent, blood glucose oxidation (10). Running at 70–85% $\dot{V}O_2$peak cannot be maintained without sufficient CHO oxidation, and thus the severe lowering of muscle glycogen, often coupled with hypoglycaemia, results in the need to reduce the intensity to ≈ 40–60% $\dot{V}O_2$peak. This phenomenon has been termed ‘hitting the wall,’ and the subsequent velocity appears to be that which can be maintained mostly by oxidation of fat, blood glucose, and lactate (10).

At this point in the race, the depletion of muscle glycogen and muscle acidosis in the more easily recruited motor units of the running musculature could cause fatigue in marathon runners. If oxidation, primarily from carbohydrate, cannot be maintained at sufficiently high rates in enough muscle fibres, the pace must be slowed. Ingestion of CHO during exercise delays fatigue as exogenous glucose appears in the blood and helps maintain the rate of CHO oxidation (10).

Nutrition for endurance runners

1. Dietary Carbohydrates and endurance running performance

Carbohydrate (CHO) is an essential macronutrient and a crucial substrate for almost all metabolic processes. The body's storage of CHO, in the form of glycogen in the muscles and liver, is relatively limited. However, CHO plays a vital role in energy metabolism, particularly during exercise, as muscle glycogen depletion is associated with fatigue. Compared to fats, CHO can provide a higher amount of energy per unit of time and becomes increasingly important as exercise intensity rises, often at the expense of fat utilization. During low to moderate exercise intensities, the majority of energy is derived from the oxidative phosphorylation of acetyl-CoA obtained from both CHO and fat. As exercise intensity increases to high levels, the oxidation of CHO and fat alone cannot meet the energy demands. CHO can be metabolized both aerobically and anaerobically. At very high exercise intensities, glycogen is rapidly converted to pyruvate and lactate to provide energy to the working muscles (63). Glycogen stores are essential, and depletion of these stores needs to be prevented from reducing fatigue; a high dietary CHO intake can help restore muscle glycogen stores between repeated bouts of prolonged exercise and improve performance during subsequent exercise bouts. CHO ingestion effects on aerobic performance are dose-dependent, and performance benefits are maximized at CHO intake rates of 60–80 $g \cdot h^{-1}$ (64).

The joint position stands of the Academy of Nutrition and Dietetics (AND), Dietitians of Canada (DC), and the American College of Sports and Medicine (ACSM) recommends that moderate exercise (1 $h \cdot day^{-1}$) requires 5-7 $g \cdot kg^{-1} \cdot day^{-1}$ of CHO, while

moderate to high-intensity exercise (1-3 $h \cdot day^{-1}$) mandates 6-10 $g \cdot kg^{-1} \cdot day^{-1}$. The exercise with extreme levels of commitment to daily activity (4-5 h of moderate to high-intensity exercise every day) such as ultra-marathon athletes may need up to 8-12 $g \cdot kg^{-1} \cdot day^{-1}$. The International Society of Sports Nutrition (ISSN) recommends maximizing glycogen stores; athletes should employ an 8-12 $g \cdot kg^{-1} \cdot day^{-1}$ high CHO diet (65, 66).

CHO has the advantage of generating more ATP per volume of oxygen than fat primarily due to their chemical structure and the metabolic pathways involved in their breakdown (46), CHO metabolism has the advantage of generating ATP more quickly without oxygen (anaerobic glycolysis) and with oxygen (aerobic respiration) in situations with limited oxygen availability, such as intense exercise. Fat breakdown, on the other hand, relies on a continuous oxygen supply for efficient ATP production (67). However, the exhaustion of liver and muscle CHO stores is associated with fatigue, reduced work, and impaired cognitive performance. It is the often described feeling by athletes of "hitting the wall" or "bonking." Therefore, fuelling strategies both before and during the race/event have been developed. Glycogen depletion must not be the sole determiner of fatigue. Other CHO sources such as lactate utilization and other mechanisms such as the increased capability to oxidize fat are postulated to account for this effect (65).

2. Fuelling for endurance running competition

For events lasting >2.5 h, such as marathon running in recreational runners, higher CHO intakes of 60–70 $g \cdot h^{-1}$ and up to 90 $g \cdot h^{-1}$, if tolerable, are associated with enhanced marathon performance. This higher intake recommendation stems from research demonstrating that exogenous CHO oxidation peaks at a CHO ingestion rate of 1.0–1.1 $g \cdot min^{-1}$ due to the maximal GI absorption at this rate (68). Including multiple CHO sources (glucose/fructose mixtures) at higher ingestion rates of 1.8 $g \cdot min^{-1}$ can further increase oxidation up to 1.2–1.3 $g \cdot min^{-1}$ due to differential intestinal transport mechanisms, and these glucose/fructose combinations also improve GI tolerance (65, 68).

Marathon runners are typically provided with designated aid stations along the race route, where they can access customized drinks and foods without the need to carry them. Using CHO beverages during the race has several advantages, as they not only

provide fluid but also supply CHO and electrolytes, which facilitate fluid uptake and help maintain hydration. It is important to adjust the CHO concentration in the beverage to meet both fluid and CHO requirements. Ideally, the CHO concentration should fall within the range of 4-8% to avoid issues such as delayed gastric emptying and gastrointestinal complications, which are more likely to occur when the CHO concentration exceeds 8%. If additional CHO is required, athletes can supplement their intake with sports gels, sports bars, or confectionary products. It is highly recommended that athletes incorporate their race strategies into their marathon training. This allows them to familiarize themselves with the strategies and make necessary refinements and adjustments based on their individual needs and preferences (69, 70).

Nutrition strategies in endurance runners

In endurance running races, nutritional strategies aim to address the factors that contribute to fatigue and hinder performance. These factors encompass hyperthermia (elevated body temperature), dehydration, hypoglycaemia (low blood sugar), muscle glycogen depletion (reduced stored carbohydrate in muscles), electrolyte imbalances, and gastrointestinal distress. By effectively managing these aspects through appropriate nutrition, athletes can optimize their performance and mitigate the negative effects associated with these factors.

1. Hyperthermia

Hyperthermia can limit endurance running performance as it stresses the cardiovascular, central nervous and muscular systems (71). The level of bodily hyperthermia experienced during a marathon reflects the balance between heat production and heat dissipation. Heat is produced from the hydrolysis of ATP and the metabolic processes needed for oxidative ATP resynthesis (Figure 5). When running on level ground, little physical work is accomplished, and most of the metabolic energy, calculated using indirect calorimetry (i.e., $\dot{V}O_2$ and RER), is transferred to heat and released into the body. The critical implication of this is that individuals who have a superior running economy, which is a low $\dot{V}O_2$ for a given running velocity, will also

generate proportionally less heat. This should be a distinct advantage when competing in hot environments that limit the amount of heat dissipation, as has typically been the case during Olympic marathon competitions (10).

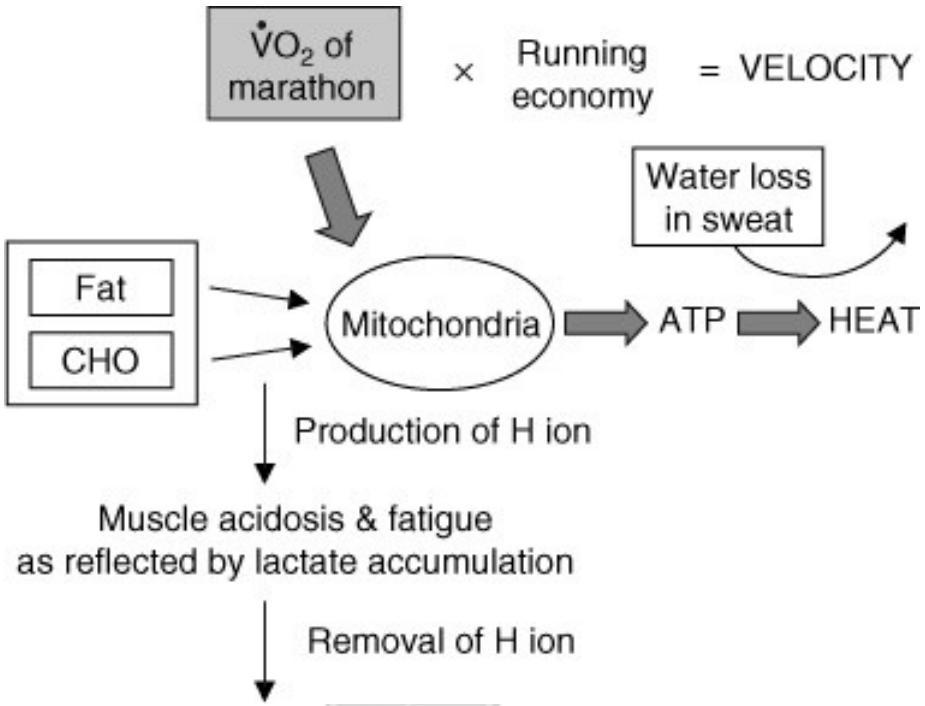


Figure 5 Concept of the physiological factors regulating marathon performance

Source for Figure 5 (10)

2. Dehydration

Depending on environmental conditions, hydration can become a performance-limiting factor during endurance running. Dehydration frequently occurs during prolonged exercise and can impair aerobic exercise performance (72). The primary mechanism for heat dissipation during a marathon, especially in warm environments, is cooling through sweat evaporation. Sweat loss that is not matched by fluid intake will produce dehydration. The major problem with dehydration is that it impairs heat dissipation due to reduced skin blood flow and reduced sweating rate. Pre-exercise dehydration could impede their $\dot{V}O_2$peak, $\dot{V}O_2$ at lactate threshold ($\dot{V}O_2$LT), and aerobic performance (73). When marathon running in a hot and humid environment, dehydration by 2% of bodyweight is hypothesized to raise the probability of impaired performance, hyperthermia, and heat illness. Documented fluid loss during marathon running and similar distance running

events is typically in the range of 0.7–1.8 $L \cdot h^{-1}$, but maybe as high as 3.7 $L \cdot h^{-1}$ in some individuals (62). The goal of pre-hydrating with beverages, in addition to regular meals and fluid intake, is to start the activity euhydrated and with normal plasma electrolyte levels, which can be achieved through consuming a fluid volume equivalent to 5–10 $mL \cdot kg^{-1}$BW in the 2 to 4 h before exercise to allow sufficient time for excess fluid to be voided and subsequent monitoring of the urinary response (74). According to the advice during exercise, the goal of drinking is to prevent excessively (>2% body weight loss from water deficit) dehydration and excessive changes in electrolyte balance to avert compromised performance (75).

3. Hypoglycaemia and muscle glycogen depletion

Endurance runners all rely on CHO and fat during the marathon race, with the slower runners having an average respiratory exchange ratio (RER) of ≈ 0.90 in the last half of the marathon and the faster runners, who are finishing in time 2:45:00, ≈ 0.95–0.97; it seems possible that these athletes could complete the marathon using only CHO as fuel (6). Primary fuels consumed during marathon running (≈ 2–4 hours) are CHO stored in the contracting muscle and the liver, and fat stored in muscle and adipose tissue. Muscle glycogen and blood glucose are the most important substrates for the contracting muscle. The depletion of muscle glycogen and reducing blood glucose concentrations are associated with athletes' physical fatigue during prolonged exercise (12).

Running a marathon at the fastest speed possible at intensities well below maximal oxygen consumption (e.g., 65–85% $\dot{V}O_2$peak) seems to be regulated by the rate of aerobic metabolism of a limited amount of CHO energy (i.e., muscle glycogen and blood glucose) and the velocity that can be maintained without developing hyperthermia. If marathon runners were to set a slightly faster pace for the 42.195 km distance, e.g., 5–10% than ideal (lactate threshold velocity), they would fatigue prematurely (i.e., after 5–10km), probably due to accelerated glycogenolysis (9, 10). This fatigue could be manifested by acidosis and eventual depletion of glycogen in the more easily recruited motor units of the running musculature.

Even when pacing is ideal and constant during the marathon, the sensation of effort needed for sufficient motor-unit recruitment increases, especially after running ≈ 25–35km, at this point in the race, muscle glycogen is low in many muscle fibres, particularly in the quickly recruited type I muscle fibres. If oxidation, primarily from CHO, cannot be maintained at sufficiently high rates in enough muscle fibres, the pace must be slowed. Ingestion of CHO during exercise delays fatigue as exogenous glucose appears in the blood and helps maintain the rate of CHO oxidation. The maintenance of blood glucose concentration by CHO ingestion and prevention of hypoglycaemia prevent neuroglycopenia and the central nervous system symptoms of fatigue that are sometimes manifested in a large catecholamine response and subsequent skin paleness associated with irritability, confusion and lethargy (10).

Ingestion of CHO during exercise delays the time of fatigue as exogenous glucose appears in the blood and helps maintain the rate of CHO oxidation. The maintenance of blood glucose concentration by CHO ingestion, and thus prevention of hypoglycaemia (10, 71). CHO ingestion effects on aerobic performance are dose-dependent, and performance benefits are maximized at CHO intake rates of 60–80 $g \cdot h^{-1}$. However, the utilization of exogenous CHO as metabolic fuel is limited by the ability to absorb CHO in the small intestine, and marathon runners should carefully choose CHO sources that allow them to maximize CHO's potential ingestion. When CHO is consumed from a single source, such as glucose or glucose polymers (e.g., maltodextrin), exogenous CHO oxidation is limited by the saturation of intestinal glucose transporters (SGLT-1), which occurs at around 60 $g \cdot h^{-1}$ during exercise (69, 70, 76).

4. Gastrointestinal distress

Gastrointestinal distress is a common issue experienced by some athletes during races. It can be caused by consuming highly concentrated sports drinks that contain a high concentration of CHO or consuming large amounts of CHO, exceeding the intestinal absorption capacity. This can lead to gastrointestinal discomfort during prolonged running (14). Research by Jeukendrup indicated that between 10 and 95% of marathon runners experience gastrointestinal distress during prolonged running (12). Most runners report mild symptoms such as nausea and dizziness, but a small percentage may experience more severe complications, including stomach and intestinal cramps, vomiting, and diarrhoea. These symptoms can significantly impair marathon performance. To minimize the risk of adverse gastrointestinal reactions, athletes should carefully choose their food and fluids during the race (17).

Highly branched cyclic dextrin (HBCD)

1. Definition and structure

Highly branched cyclic dextrin (HBCD) (Figure 6) is an innovative type of polysaccharide (maltodextrin) produced from waxy corn starch by the cyclization of a branching enzyme (BE, 1,4-α-D-glucan: 1,4-α-D-glucan 6-α-D-(1,4-α-D-glucano)-transferase, EC 2.41.18) with an average molecular weight of 400,000 with a narrow size distribution, high solubility in water contributes little to osmotic pressure and has a relatively low propensity for retrogradation than another commercially available dextrin, which mixtures with a much wider size distribution, containing glucose, maltose, and maltodextrin (19, 23).

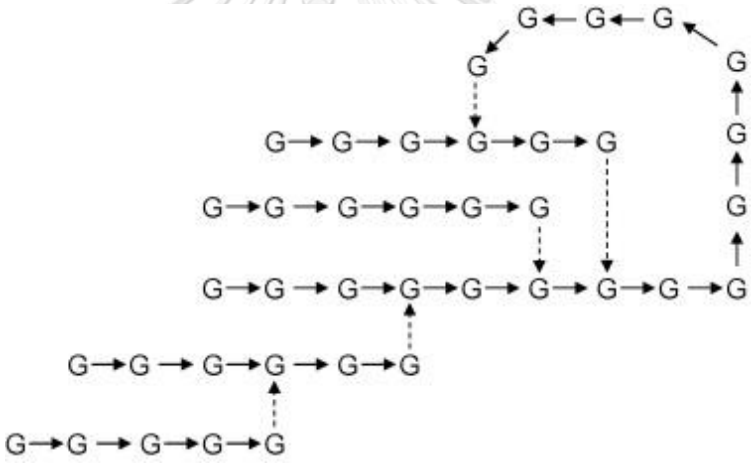


Figure 6 Molecular structure of highly-branched cyclic dextrin (HBCD)

Source for Figure 7 (77)

When consuming HBCD, it was transferred quickly from the stomach to the small intestine, and then it was assumed to have been gradually digested by α-amylase and α-glucosidase. HBCD is thought to have been absorbed more slowly than glucose in the small intestine. It is well-known that CHO's ingestion has a direct influence on blood glucose concentration and that it affects insulin secretion. Takii and colleagues' rodent study (20) showed that the resting postprandial blood glucose and insulin response in HBCD-ingested mice were lower than glucose-ingested mice. The ingestion of HBCD at a dose of 500 $mg \cdot kg^{-1}$ 10 min after beginning swimming, an early stage of prolonged exercise, can raise the postprandial blood glucose and insulin responses after HBCD ingestion during prolonged exercise, although the rise was significantly less than by glucose. HBCD is a large molecule and needs to be hydrolysed to glucose; when available glucose molecules are gradually produced from HBCD, glucose absorption takes a relatively long time and causes lower glycaemic and insulin responses. The low insulin responsiveness of HBCD might have had an influence on maintaining the blood glucose concentration and the energy substrates' oxidation in mice (20).

2. Optimal dosage, timing, and duration of HBCD

The recent study of Shikari et al. (22), administering 1.5 g $HBCD \cdot kg \cdot BW^{-1}$ to elite swimmers immediately before exercise, reportedly increases blood glucose concentration subsequently increases time to exhaustion. The other finding in the literature in relation to HBCD ingestion showed in Table 3.

3. HBCD and exercise performance

Most of the international publication reports confirmed the benefit of the HBCD ingestion in endurance exercise performance with shorter gastric emptying time. HBCD can improve endurance performance depending on three factors, including the fast gastric emptying rate, the gradual digestion in the small intestine, and the low postprandial blood glucose response (20). The results of previous study showed that the gastric emptying time after ingested HBCD-based sports drink tended to be faster than that of the glucose- and dextrin-based sports drink during prolonged exercise (19, 21). The blood glucose concentrations at time measured points were not significantly different

between HBCD and maltodextrin drinks but that at 30 min after ingestion tended to be higher in ingesting HBCD than maltodextrin (23). Together with Shikari et al., the results showed that the ingestion of HBCD resulted in increased time to fatigue, about 70% longer than that in the glucose and control trials. After 90% $\dot{V}O_2$max swimming in the HBCD trial, the higher lactate level suggested that the subjects oxidized more significant CHO amounts to yield energy following HBCD intake than glucose or water intake. Plasma glucose in the HBCD group was maintained at higher levels during pre-swimming cycles than in the glucose or water group (22). The suitable mechanism responsible for suppressing RPE elevation during exercise after ingesting HBCD was ambiguous, but it might be associated with the ability of HBCD to supply glucose energy for a more extended period and its shorter gastric emptying time.

Table 3 Overview of previous studies assessing the effects of HBCD on exercise performance and associated variable

Study	HBCD dose	Timing	Exercise	Finding
Takii et al. (1999) (20) *(Rodent study)*	500 mg·kg·BW^{-1}	10 min before, 10 min after, or 30 min after beginning swimming exercise	Swimming until exhaustion	↑ T_{ex} when consuming 10 min after beginning the exercise ↓ gastric emptying time ↑ blood glucose concentration ↓ insulin response
Takii et al. (2004) (21)	10% HBCD	10 min before exercise	30-min of low-intensity cycling exercise	↓ gastric emptying time ↓ RPE exercise performance?
Furuyashiki et al. (2014) (23)	15 g HBCD in 200mL distilled water	1 h after starting exercise	2 h of exercise; 30-min at 40% $\dot{V}O_2$max and then 90-min at 60% $\dot{V}O_2$max	↑ blood glucose concentration ↔ insulin concentration ↔ RPE exercise performance?
Suzuki et al. (2014) (24)	5% HBCD	Immediately before exercise	5 km of running, 40 km of cycling and 5 km of running	↑ plasma catecholamine concentration ↑ urinary cytokine concentration exercise performance?
Shikari et al. (2015) (22)	1.5 g HBCD·kg·BW^{-1}	Immediately before exercise	Intermittent swimming followed by swimming to exhaustion	↑ T_{ex} ↑ blood glucose concentration
Chuychai et al. (2022) (78)	1.5 g HBCD·kg·BW-1	30 min before TTE test	Running at the velocity at VT_2 until exhaustion	↑ T_{ex} ↓ Fluid loss

↑ = increase; ↓ = decrease; ↔ = no change; T_{ex} = Time to exhaustion

Nitric oxide and exercise performance

1. Nitric oxide

Nitric oxide (NO) is a signalling molecule that serves various functions within different parts of the human body, including skeletal muscle tissue (79). Some of these functions involve the regulation of muscle contractility, tissue blood flow, mitochondrial oxygen consumption, and glucose uptake (28). Nitric oxide (NO) plays a crucial role in signalling and physiological regulatory functions of the human body which are crucial to exercise economy and performance (i.e., vasodilatation, mitochondrial respiration, glucose, and calcium (Ca_2^+) homeostasis, skeletal muscle contractility and fatigue development). Because the NO molecule is highly unstable, there is a constant need for its regeneration (25). NO could only be produced through the oxygen-dependent pathway that requires the amino acid L-arginine to be metabolized by nitric oxide synthase (NOS) (80). The eNOS enzyme is the predominant NOS isoform in endothelial cells of blood vessels and therefore fulfils an important role in vascular health through local production of NO (81).

Nitric oxide, synthesized by endothelial NOS (eNOS), is involved in the regulation of arterial blood pressure and airway lumen diameter (82). Endothelial NO synthase (eNOS) is a membrane-bound isoform of the enzyme localized in the caveolae, small invaginations of plasma membrane containing transmembrane protein caveolin. eNOS is found in the lungs, trachea, alveolar and bronchial epithelial cells, alveolar macrophages, vascular smooth muscle cells, pulmonary endothelium, and endothelial cells of the blood vessels feeding the airways. This enzyme is present in the basal bodies of the cilia and increases the ciliary beat frequency. eNOS is a calcium-dependent isoform producing discrete NO quanta. The activity of eNOS is suppressed when the enzyme binds to caveolin in the endothelial cells. In the presence of the agonist induced Ca_2^+ currents, eNOS binds to calmodulin and dissociates from caveolin. Synthesis of NO by the complex of eNOS-calmodulin continues until the Ca_2^+ currents have decreased and the inhibitory eNOS-caveolin complex has formed (83).

2. NO Metabolism

Nitric oxide synthesis in the human body is carried out via two pathways: the NO synthase (NOS)-dependent pathway and nitrate–nitrite–NO (NO_3^-–NO_2^-–NO) pathway. Formation of NO via the NOS-dependent pathway is carried out through the utilisation of L-arginine and oxygen (O_2). Thus, this reaction relies on the delivery of O_2. Insufficient O_2 delivery during high-intensity exercise may cause this pathway to become dysfunctional. Therefore, the O_2-independent pathway can substitute NO production via the reduction of NO_3^- from the diet (e.g., green leafy vegetables, beetroot, radish). Nitrates are reduced by oral anaerobic bacteria to NO_2^-. Subsequently, part of the total NO_2^- is reduced to NO in the acidic environment of the stomach where it protects the organism from some pathogens. The rest of the NO_2^- is then transported via the upper gastrointestinal tract into the blood reaching its plasma peak level 2–3 h postprandially. The reduction of NO_2^- to NO substitutes the O_2-dependent NOS pathway in various tissues under hypoxic or acidic conditions (84). These are conditions that typically occur in working muscles during vigorous exercise (25, 85).

Nitrate supplementation

1. Definition and structure

Nitrate (NO_3^-) is a nitrogen oxyanion formed by the loss of a proton from nitric acid. Principal species present at pH 7.3. It is a nitrogen oxyanion, a member of reactive nitrogen species, and a monovalent inorganic anion. It is a conjugate base of nitric acid (86).

Dietary nitrate (NO_3^-), an inorganic polyatomic anion that exists naturally in the environment. Despite relatively weak evidence, it was considered nitrate (NO_3^-) and nitrite (NO_2^-) to be carcinogens for many years. Still, more recent evidence indicates that nitrate may be a critical bioactive component in salad vegetables, the consumption of which is encouraged to promote human health and is becoming in popularity as a sports nutrition supplement (87). Nitrate is a precursor for nitric oxide (NO), vasodilator, synthesis in hypoxic and acidic conditions during exercise. The primary dietary source of nitrate is

green leafy and root vegetables (Table 4). Vegetables with a very high nitrate concentration (>250 mg per 100 g) include celery, cress, chervil, lettuce, red beetroot, spinach, rocket (88).

Table 4 Classification of vegetables according to nitrate content

Nitrate Content (mg/100 g Fresh Weight)	Vegetable Varieties
Very low, <20	Artichoke, asparagus, broad bean, eggplant, garlic, onion, green bean, mushroom, pea, pepper, potato, summer squash, sweet potato, tomato, watermelon
Low, 20 to <50	Broccoli, carrot, cauliflower, cucumber, pumpkin, chicory
Middle, 50 to <100	Cabbage, dill, turnip, savoy cabbage
High, 100 to <250	Celeriac, Chinese cabbage, endive, fennel, kohlrabi, leek, parsley
Very high, >250	Celery, cress, chervil, lettuce, red beetroot, spinach, rocket

Source of Table 4 (88)

2. The NO_3^--NO_2^--NO pathway

Ingested inorganic NO_3^- is rapidly absorbed from the gut and passes into the systemic circulation with peak plasma [NO_3^-] observed ~60 minutes following NO_3^- ingestion. While ~60% of systemic NO_3^- is excreted in the urine, ~25% of NO_3^- passes into the enterosalivary circulation and is concentrated in saliva at least 10-fold. In the mouth, facultative anaerobic bacteria on the surface of the tongue reduce NO_3^- to NO_2^-. This NO_2^- is swallowed and reduced to NO and other reactive nitrogen intermediates within the acidic environment of the stomach. However, some NO_2^- is absorbed to increase circulating plasma [NO_2^-]. Therefore, dietary NO_3^- supplementation represents a practical method to increase the circulating plasma [NO_2^-]. This has been demonstrated in humans after pharmacological sodium nitrate ($NaNO_3^-$) and potassium nitrate (KNO_3^-) ingestion, as well as following NO_3^--rich beetroot juice ingestion. It is important to note, however, that the characteristic rise in plasma [NO_2^-] following an oral NO_3^- bolus is largely abolished

after antibacterial mouthwash treatment, indicating that the reduction of NO_3^- to NO_2^- in humans is critically dependent on the bacterial NO_3^- reductases (89).

The final step in the NO_3^--NO_2^--NO pathway is the one electron reduction of NO_2^- to NO. This NO_2^- reduction is catalysed by deoxyhaemoglobin, deoxymyoglobin, NOS, xanthine oxidase, aldehyde oxidase, cytochrome P-450 and the mitochondrial electron transfer complexes. This reaction is potentiated in hypoxic and acidic environments such as those which may be extant during exercise. The existence of an alternative NO generation pathway is important as it promotes NO synthesis under conditions that would otherwise limit the production of NO from NOS, including hypoxia and oxidative stress. Importantly, contracting skeletal muscles become hypoxic and produce reactive oxygen species at an elevated rate. This suggests that the NO_3^--NO_2^--NO pathway may be particularly important for NO production during exercise. This compensatory role of the NO_3^--NO_2^--NO pathway is supported by the observations that dietary supplementation with NO_2^- and NO_3^- restores tissue and plasma [NO_3^-] and [NO_2^-] (markers of NO synthesis). Therefore, the complementary nature of the NOS-NO and NO_3^--NO_2^--NO pathways ensures that NO synthesis can occur across a wide range of cellular O_2 tensions and redox states (89).

3. Mechanism

Once ingested, dietary nitrate is reduced to nitric oxide via the NO_3^--NO_2^--NO pathway, increasing the level of nitric oxide in the blood and tissues. The production of NO via the NOS pathway is inhibited under hypoxic conditions, whereas the $NO_3^- \longrightarrow NO_2^- \longrightarrow$ NO pathway is activated under hypoxic conditions. Once ingested, dietary NO_3^- is reduced to bioactive NO_2^- by commensal anaerobic bacteria found in the saliva and then further reduced to NO via multiple enzymatic and non-enzymatic pathways (90). Following oral consumption, ingested inorganic nitrate circulates in the plasma and has a half-life of about 5 h, a portion (~25 %) is taken up by the salivary glands and concentrated in the saliva. Nitrate is quickly absorbed in the duodenum and jejunum and is dispersed amongst the whole body. Commensal facultative anaerobic bacteria residing in the crypts on the surface of the tongue reduce nitrate (NO_3^-) to nitrite (NO_2^-). Some of the swallowed

nitrite is reduced to nitric oxide in the acidic environment of the stomach, but a substantial amount of nitrite enters the systemic circulation, elevating the plasma nitrite concentration. Plasma nitrate levels increase rapidly within 30 min after supplementation, reaching the peaks of nitrate and nitrite at 1–2 h. Two to three hours later, nitrite and nitrate gradually fall, returning to the baseline after approximately 24 h. The average life of plasma nitrate in humans is approximately 5 h, with a considerable decrease 4 h after ingestion. A variety of nitrite reductases, including deoxyhaemoglobin, oxymyoglobin, xanthine oxidase, and the mitochondrial respiratory complexes, can subsequently catalyse the one-electron reduction of nitrite to nitric oxide in the blood and other tissues. Nitric oxide is a potent signalling molecule that plays a key role in vasodilation by relaxing the smooth muscle and subsequently improving blood circulation. This process is promoted in conditions of low partial pressure oxygen and pH that may exist in skeletal muscle during exercise, enabling nitric oxide to be produced where it is most required (28-31).

4. Dietary nitrate and exercise performance

Nitric oxide bioavailability is enhanced by dietary nitrate-rich, e.g., beetroot juice, by altering nitrate to nitrite and conversion to nitric oxide. This may be more important in hypoxic situations, particularly during long-duration exercise. Moreover, beetroot juice has beneficial effects on submaximal exercise lasting 5-30 min (28, 91). During high-intensity exercise, oxidative phosphorylation is declined as the O_2 supply is inadequate, and dependence on the anaerobic metabolic pathway of ATP regeneration is preferred. Long-duration high-intensity exercise and intermittent high-intensity exercise affect exercise-induced hypoxemia inducing the muscles to become hypoxic and acidic. This impairment in homeostasis may also disturb the functioning of the NOS-dependent pathway. Therefore, the reliance on the substitutional NO_3^--NO_2^--NO pathway independent of the O_2 supply is increased (25).

Enhanced exercise performance after nitrate supplementation might be linked to improvements in skeletal muscle perfusion, metabolism, and/or contractile function. A recent examination into the dietary nitrates (NO_3^-) consumption-mainly administered via beetroot juice, and their role in vascular function has driven to it becoming progressively

more popular amongst athletes attempting to improve performance. The findings from the meta-analysis of McMahon and colleague's analysis highlight the positive ergogenic effect of dietary nitrate supplementation on endurance exercise capacity and showed a significantly greater effect size of the influence of nitrate supplement on time to exhaustion (TTE) when compared to placebo control. However, the small effects of dietary nitrate supplementation on time trial (TT) and graded-exercise test (GXT) performance were not statistically significant. (92). Other physiological processes that might be altered to provide an ergogenic effect due to nitrate ingestion include skeletal muscle force production (excitation-contraction coupling) autoregulation of blood flow, myocyte differentiation, respiration, and glucose homeostasis (79).

Related literature

1. The effects of HBCD ingestion on endurance exercise performance

There was no publication in Thai literature that conducted the effects of HBCD ingestion on any exercise performance. Most of the reports of international publication confirmed the benefit of the HBCD ingestion in endurance exercise performance with shorter gastric emptying time.

Takii et al. (1999) investigated the ergogenic effect in mice administering 500 mg/kg body weight HBCD at 10 min before, 10 min after, or 30 min after the beginning of exercise on the swimming endurance performance. The outcomes showed that HBCD ingestion during medium intensity continued exercise could enhance mice endurance performance, also ingestion 10 min after starting exercise was the best effect (20).

Takii et al. (2004) investigated the effects of HBCD-based sports drink ingestion (consumed at 10 min before exercise) on gastrointestinal disorders and fatigue during 30 min of low-intensity cycling exercise in untrained men. Three prototypes sports drink based on HBCD, dextrose equivalent 16 (DE16), and glucose were composed of 10% CHO, 1.4% fructose, 0.01% stevia, 0.2% ascorbic acid, 0.1% sodium ascorbate, 0.04% vitamin mixture, 0.02% sodium chloride, and 0.01% aroma. The results showed that the gastric emptying time after ingested HBCD-based sports drink tended to be faster than that of the glucose- and dextrin-based sports drink during prolonged exercise. RPE in the later stage of exercise tended to be lower with the HBCD-based sports drink, and this implied that participants perceived little fatigue after ingesting the HBCD-based sports drink (21).

Takii et al. (2005) employing ultrasonography techniques for examined gastric emptying rates for HBCD and other carbohydrates (CHO) solutions such as glucose, maltose, sucrose, and commercially available dextrin. The results showed that sports drink based on 10% HBCD adjusted to 150 mOsm by adding various minerals, vitamins, and organic acids were emptied faster than a 10% HBCD solution or a sports drink based on 10 % commercially available dextrin, which has a higher osmotic pressure (19).

Furuyashiki et al. (2014) compared the effect of relatively low doses of HBCD (15 g) with that of maltodextrin (dissolved in 200-mL distilled water) during endurance exercise on the rating of perceived exertion (RPE) and blood components associated with energy metabolism in a crossover, double-blind study of healthy volunteers. All participants exercising on the bicycle ergometer at 40% $\dot{V}O_2$max for 30 min and then at 60% $\dot{V}O_2$max for 90 min (total 2 h of exercise) and consumed one of the drinks at 1 h after starting exercise. The results showed that the RPE increased during exercise and its increase was significantly less at 30 and 60 min after ingesting HBCD than maltodextrin. Both drinks increased blood glucose concentrations, which showed a peak at 15 min after consuming both drinks. The blood glucose concentrations at time measured points were not significantly different between two drinks but that at 30 min after ingestion tended to be higher in ingesting HBCD than maltodextrin. Blood lactic acid, non-esterified fatty acids (NEFA), and ketone bodies concentration after consuming both drinks did not look different at any time measured point, and neither did changes in blood insulin concentrations (23).

Suzuki et al. (2014) adduce that the alteration in immune function following exhaustive exercise may lead to flu-like symptoms associated with systemic cytokine release, then compared the effects of HBCD drink with a glucose-based control drink on immunoendocrine responses to endurance exercise. Well-trained male triathletes consumed 5% HBCD or 5% glucose, 0.003% acesulfame potassium, and 0.1% flavor, which was the same in fluid volume and calorie content. The results of this study demonstrated that the HBCD-based drink alleviated plasma catecholamine concentration and urinary cytokine concentration, such as IL-8 (pro-inflammatory cytokine), IL-10 (anti-inflammatory cytokine), and IL-12p40, compared with the drink containing the same amount of glucose, suggesting a modification of the immune system (24).

Shikari et al. (2015) investigated the HBCD administration's effects on swimming endurance performance. Elite swimmer received either HBCD, glucose (1.5 g carbohydrate/kg body weight) or water and immediately swimming exercise (10 cycles of intermittent swimming consisting of 5 min of swimming at 75% maximal aerobic capacity

($\dot{V}O_2$max) followed by 3 min of rest, and subsequent swimming at 90% $\dot{V}O_2$max) until exhaustion. The ingestion of HBCD resulted in increased time to fatigue, about 70% longer than that in the glucose and control trials. After 90% $\dot{V}O_2$max swimming in the HBCD trial, the higher lactate level suggested that the subjects oxidized more significant CHO amounts to yield energy following HBCD intake than glucose or water intake. Plasma glucose in the HBCD group was maintained at higher levels during pre-swimming cycles than in the glucose or water group (22).

The main finding of the Chuychai et al. (2022) study suggests that consuming 500 mL of HBCD at a dosage of 1.5 $g \cdot kg^{-1}$ BW, 30 minutes before exercise, may offer distinct advantages over an isocaloric glucose beverage in terms of maintaining fluid balance and enhancing endurance performance in male marathon runners. Notably, there were no significant differences observed in the interaction effect of blood glucose and lactate concentration, heart rate, and rating of perceived exertion (RPE) between the HBCD and glucose (GLUC) groups during the trials. However, the HBCD group demonstrated a significant improvement in the time to exhaustion running test, indicating increased endurance capacity, and also exhibited a reduction in body fluid loss when compared to the GLUC group. These findings suggest that HBCD supplementation may provide specific benefits in terms of prolonged exercise performance and fluid regulation, potentially making it a favourable choice for marathon runners seeking improved endurance and hydration management (78).

From the above, the enhancement of endurance performance by HBCD ingestion depending on the fast gastric emptying rate, the gradual digestion in the small intestine, and the low postprandial blood glucose response. It can be concluded that the benefit of HBCD ingestion before exercise tended to increase blood glucose concentration with shorter gastric emptying time and no change in blood insulin concentration result in enhancing time to exhaustion consequently.

2. The effects of nitrate supplement on endurance exercise performance

Larsen et al. (2007) observe the benefits of dietary NO_3^- ingestion on exercise for the first time and incited further studies investigating dietary NO_3^- supplementation on exercise performance. The researchers examined the effect of dietary nitrate on physiological and metabolic parameters during exercise. Nine well-trained male subjects performed progressive work rate cycling after chronic sodium NO_3^- supplementation (0.1 $mmol \cdot kg^{-1} \cdot day^{-1}$ for 3 days). The results showed that the oxygen cost at submaximal exercise was reduced after nitrate supplementation without increased blood lactate concentration, resulting in enhanced exercise efficiency (93).

Bescós et al. (2011) evaluate the effect of a single dose of nitrate given before cycling exercise on the cardiorespiratory and metabolic response in endurance athletes at different intensities, as well as investigated the influence of nitrate supplementation on plasma levels of nitrate and nitrite over time. Male cyclists and triathletes were randomly assigned in a double-blind crossover design to receive a single dose of either sodium nitrate (10 $mg \cdot kg^{-1}$ of body mass) or the placebo (sodium chloride) dissolved in 250 mL of water 3 h before the test. Subjects then performed a cycle ergometer test that consisted of four 6-min submaximal workloads, corresponding to 2.0, 2.5, 3.0, and 3.5 $W \cdot kg^{-1}$ of body mass, interspersed with 3 min of passive recovery. After a 5-min recovery period, subjects performed one incremental exercise test until exhaustion. The outcomes showed that plasma nitrate and nitrite were significantly higher 3 h after supplementation than after the placebo at resting conditions. Nitrate supplementation significantly reduced $\dot{V}O_2$peak and the ratio between $\dot{V}O_2$ and power at maximal intensity. This reduction of $\dot{V}O_2$ occurred without changes in the time to exhaustion or in the maximal power. These imply that a single oral dose of inorganic nitrate acutely reduces $\dot{V}O_2$peak without compromising the maximal exercise performance (94).

Lansley et al. (2011) investigated the effects of acute dietary nitrate supplementation (0.5 L of beetroot juice: containing approximately 6.2 mmol of nitrate) 2.5 h before the completion of a 4- and 16.1-km cycling time trials (TT) on power output (PO), $\dot{V}O_2$, and time trial performance. The plasma nitrite concentration [NO_2^-] (a

biomarker of nitric oxide availability) increasing by 138% 2.5 h after beetroot juice ingestion. The $\dot{V}O_2$ values during the time trials were not significantly different between the beetroot juice and placebo conditions at any elapsed distance, but beetroot juice ingestion significantly increased mean power output during the 4-km and 16.1-km time trials test. Consequently, beetroot juice ingestion improved 4-km performance by 2.8% and 16.1-km performance by 2.7%. The results suggest that acute dietary nitrate supplementation with 0.5 L of beetroot juice supplementation improves cycling economy, as demonstrated by a higher power output for the same $\dot{V}O_2$ and enhances both 4- and 16.1-km cycling time trials performance (95).

Cermak et al. (2012) investigated the impact of ingesting a single bolus of concentrated nitrate-rich beetroot juice on subsequent 1-hr time-trial performance in well-trained cyclists. 20 trained male cyclists ingested 140 ml of concentrated beetroot juice (8.7 mmol NO_3^-) with breakfast 2.5 h before an ~1-h cycling time trial. The results showed that plasma nitrite concentration was higher in beetroot ingestion than placebo before the onset of the time trial, but subsequent time-trial performance, power output, and heart rate did not differ between beetroot ingestion and placebo treatments. The findings seem to be in contrast to the study of Lansley and colleague[95] that reported significant performance improvements in a 4- and 16.1-km time trial after the ingestion of a single 500-ml bolus of beetroot juice (~6.2 mmol NO_3^-) 2.5 h before the onset of exercise (96).

Wilkerson et al. (2012) investigate whether dietary nitrate supplementation might alter the physiological responses and performance of well-trained cyclists during a self-paced bout of long duration endurance exercise- 50 miles time trial test, is the first study to investigate the effects of dietary nitrate supplementation on performance in self-paced athletic events lasting longer than ~30 min. Well-trained male cyclists asked to drink 500 mL of either organic beetroot juice containing ~6.2 mmol of NO_3^- or placebo approximately 2.5 h before 50 miles time trial test. The results showed that beetroot juice ingestion significantly elevated plasma nitrite [NO_2^-] and reduced completion time for the 50-miles time trial by 0.8%, which was not statistically significant. This demonstrates that acute

dietary supplementation with beetroot juice did not significantly improve 50-miles time trial performance in well-trained cyclists (97).

Handzlik et al. (2013) evaluate potential additive effects of acute pre-exercise beetroot juice and caffeine supplementation on time to exhaustion (TTE) during cycling at 80% $\dot{V}O_2$max in healthy, well-trained male participants, and determine if nitrate's effects on the oxygen cost of exercise are influenced by caffeine co-ingestion. Well-trained male participants performed four trials on different occasions following pre-exercise ingestion of placebo (PLA), PLA plus 5 mg/kg caffeine (PLA+C), beetroot juice providing 8 mmol of nitrate (BR), and beetroot juice plus caffeine (BR+C). Following another 75 min, rest period subjects performed 30 min cycling at 60% $\dot{V}O_2$max followed by a time to exhaustion (TTE), defined as an inability to maintain pedalling cadence, the trial at 80% $\dot{V}O_2$max. Without the percentage NO_3^-/NO_2^- change reports, the major finding of this study was 18% and 27% non-significant improvement in time to exhaustion at 80% $\dot{V}O_2$max on BR+C compared to BR and PLA+C, respectively. These results imply that the acute pre-exercise beetroot juice co-ingestion with caffeine likely has additive effects on exercise performance compared with either beetroot or caffeine alone (98).

Wylie et al. (2013) investigated the influence of acute nitrate doses of 4.2, 8.4, and 16.8 mmol consumed in 70-, 140-, and 280-ml concentrated beetroot juice on plasma nitrate concentration [NO_3^-] and [NO_2] and blood pressure over a 24-h period and investigated the physiological responses to step transitions to moderate- and severe-intensity exercise, 2.5 h post-ingestion of the same nitrate doses. Subjects completed moderate-intensity and severe-intensity cycle exercise tests, 2.5 h post-ingestion of 70-, 140-, and 280-ml beetroot juice or placebo. Following acute beetroot juice ingestion, plasma [NO_2^-] increased in a dose-dependent manner, with the peak changes occurring at approximately 2–3 h. Compared with placebo, 70 ml beetroot juice did not alter the physiological responses to exercise. However, 140- and 280-ml beetroot juice reduced the steady-state oxygen (O_2) uptake during moderate-intensity exercise by 1.7% and 3.0%, whereas time to fatigue was extended by 14% and 12%, respectively, compared

with placebo. The results indicate that whereas plasma [NO_2^-] and the O_2 cost of moderate-intensity exercise are altered dose dependently with NO_3^--rich beetroot juice, there is no additional improvement in exercise tolerance after ingesting beetroot juice containing 16.8 compared with 8.4 mmol NO_3^- (99).

Trexler et al. (2014) investigate the acute effects of pomegranate extract on blood flow, vessel diameter, and exercise performance in active individuals. Subjects were randomly assigned to ingested either a placebo or a 1000 mg of pomegranate extract and performed three treadmill runs to exhaustion (TTE) at 90%, 100%, and 110%PV. Blood flow was assessed immediately after each exercise bout and 30 minutes post-exercise. After a 7-10-day washout, participants repeated the same procedures, ingesting the opposite supplement. Blood flow was significantly augmented 30 min post-ingestion with pomegranate extract in comparison to placebo. Vessel diameter was significantly larger 30 min post-exercise with pomegranate extract. Ingestion of the pomegranate extract was found to significantly augment time to exhaustion (TTE) at 90% and 100% peak velocity (PV). Acute ingestion of pomegranate extract 30 min prior to exercise may enhance vessel diameter, blood flow, and delay fatigue during exercise. Results of the current study indicate that pomegranate extract is ergogenic for intermittent running, eliciting beneficial effects on blood flow (100).

Glaister et al. (2015) examines the acute supplementation effects of dietary nitrate, caffeine, and their combination on subsequent 20-km time trial performance in well-trained athletes. Well-trained, competitive, female cyclists and triathletes consumed a 70-ml dose of concentrated beetroot juice containing either 0.45 g of dietary nitrate or placebo 2.5 hours before trial, and one hour before each trial, subjects consumed a capsule containing either 5 $mg \cdot kg^{-1}$ of caffeine or maltodextrin (placebo). The results showed a significant effect of caffeine, but no effect of nitrate, compared with the placebo. Moreover, although performance after caffeine + nitrate was not significantly different from

the caffeine only condition, it was not significantly different from the nitrate and placebo conditions (101).

Garnacho-Castaño et al. (2018) assess the effects of acute beetroot juice supplementation on endurance exercise performance and cardio-ventilatory responses in well-trained triathletes during a cycle ergometer test conducted at a load intensity equivalent to the first and second ventilatory threshold. Well-trained triathletes were assigned in a randomized, double-blind, crossover design to receive 70 ml of beetroot juice (contained 6.5 mmol NO_3^-) or placebo. Three hours after taking the supplement, participants completed an endurance test on a 30-min exercise on a cycle ergometer at a constant work rate corresponding to the first ventilatory threshold (VT_1) and a 15-min time trial at the second ventilatory threshold (VT_2). The outcomes showed that no significant interaction effect was observed on any of the cardio-ventilatory variables, efficiency/economy, VT_2 time trial, energy expenditure, carbohydrate oxidation, and fat oxidation (102).

In conclusion, the benefits of dietary nitrate ingestion for endurance performance tended to improve blood flow during exercise, resulting in reduced oxygen cost at a submaximal exercise without increasing blood lactate concentration, resulting in enhanced exercise efficiency: enhanced time trial performance and time to exhaustion in endurance athletes consequently.

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In conclusion, the benefits of dietary nitrate ingestion for endurance performance tended to improve blood flow during exercise, resulting in reduced oxygen cost at a submaximal exercise without increasing blood lactate concentration, resulting in enhanced exercise efficiency: enhanced time trial performance and time to exhaustion in endurance athletes consequently.

Conceptual framework

From the above-mentioned literature review, this study is obtained a possible course of action in figure

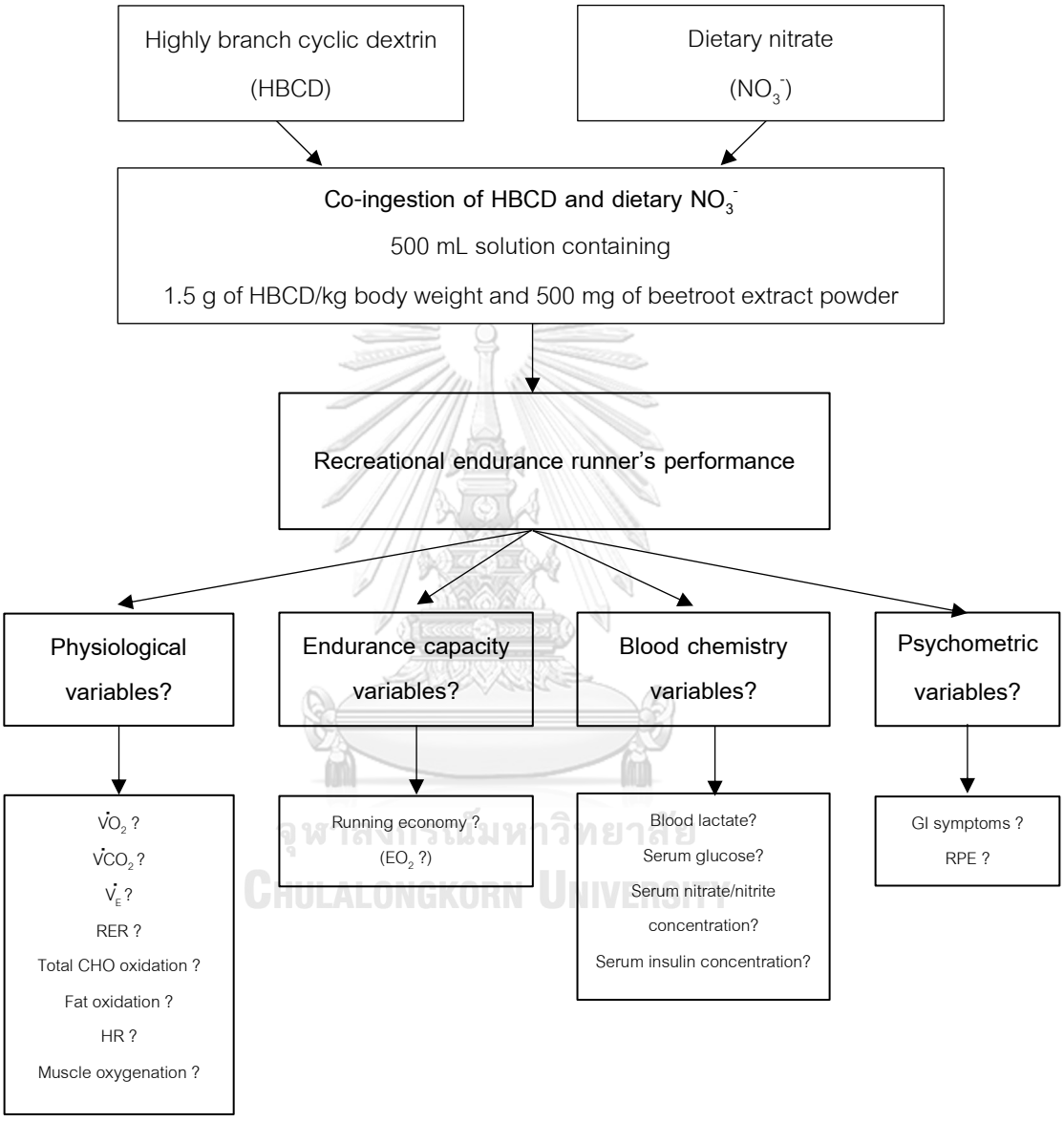

Figure 7 Conceptual framework

CHAPTER III
METHODOLOGY

This randomized, double-blind, placebo-controlled cross-over trial aimed to investigate the effects of the co-ingestion of HBCD and dietary nitrate on physiological responses and endurance capacity in recreational endurance runners. An experiment was conducted at the Exercise and Sports Performance Laboratory (ESPL), Faculty of Sports Science, Chulalongkorn University. The Research Ethics Review Committee for Research involving Human Research Participants, Group I, Chulalongkorn University, Thailand, approved the study and it was carried out in accordance with the Belmont Report of 1979, Declaration of Helsinki of 2013, Council for International Organizations of Medical Science (CIOM) of 2016, Standards of Research Ethics Committee (SREC) of 2017, and National Policy and guidelines for Human Research of 2015 (COA No. 125/2022).

Sample group

An a *priori* power analysis (G*Power 3.1) was conducted to determine the required number of participants for this study. Based on the effect of HBCD on endurance performance reported in the previous work of Shiraki et al. (22), an alpha level of 0.05 and a statistical power of 95% were used. The analysis determined that a minimum of 8 participants in each experimental condition was required. However, to account for potential participant dropouts during the study, the number of participants was increased to 12 in each experimental condition.

Twelve male recreational endurance runners aged 30-39 years were recruited from various running clubs in the Bangkok metropolis. All participants were required to take part in this study in order to detect differences between the four experimental conditions: (1) the co-ingestion of 1.5 g HBCD · kg^{-1} BM and 500 mg of beetroot extract powder (~8.00 mmol dietary NO_3^-) (HBCD+NO_3^-), (2) the ingestion of 1.5 g HBCD·kg^{-1} BM (HBCD), (3) the co-ingestion of 1.5 g maltodextrin·kg^{-1} BM and 500 mg of beetroot extract

powder (~8.00 mmol dietary NO_3^-) (MD+NO_3^-), and (4) the ingestion of 1.5 g maltodextrin·kg^{-1} BM (MD). The experimental conditions were administered in a random sequence, with a 2-week washout period between each condition.

Inclusion criteria

1. Participants were required to meet specific criteria to be classified as "recreational endurance runners." These criteria included training at least three times per week, covering a training distance of at least 30 km per week, and having a baseline aerobic capacity ($\dot{V}O_2$peak) value greater than 45 mL·kg^{-1}·min^{-1} (103).

2. All participants were not participating in any experimentation and/or endurance road race at least 4 weeks before the study begins and during the data collection in this study.

3. Prior to participation, all participants underwent screening using the 2021 physical activity readiness questionnaire (PAR-Q+) and a general health questionnaire. Participants were required to be free from cardiovascular, respiratory, metabolic, neurological, or orthopedic disorders that could affect their running performance. The approval of an attending physician was obtained to confirm their suitability for the study.

4. All participants were non-smoker and without any nutritional supplements taken in the three months before the study outset (e.g., nitrate-rich foods, caffeine, β-alanine, creatinine, sodium bicarbonate, glutamine, etc.)

5. Each participant received an information sheet outlining the details of the research and their rights as a research participant. They were required to read the information and provide their informed consent by signing the consent form.

Exclusion criteria

1. The participants expressed their unwillingness to continue participating in the study.

2. The participants are not completed all of condition in the study.

Experimental instruments

1. The following instruments were used in the selection of participants:
    1.1 The 2021 Physical Activity Readiness Questionnaire (PAR-Q+)
    1.2 The general health questionnaire
    1.3 The general characteristic of participant sheet
    1.4 The informed consent forms
2. The following instruments were used in the beverage preparation:
    2.1 Highly branched cyclic dextrin
        (Cluster Dextrin™, Glico Nutrition Co., Ltd)
    2.2 Maltodextrin (Food grade, Krungthepchemi Co., Ltd, Thailand)
    2.3 Beetroot extract 10:1 powder (Feaga Life®, Thailand)
    2.4 Stevioside (Festa® Stevia Sweetener, Thailand)
    2.5 Water
    2.6 Food processor (Kitchen Aid®, USA)
    2.7 Bottle
    2.8 Refrigerator
3. The following instruments were used for measuring physiological data variables:
    3.1 A body composition analyzer (ACCUNIQ BC510, Korea)
    3.2 An automated sphygmomanometer (Omron, UK)
    3.3 A heart rate sensor (Polar, Finland)
    3.4 A motorized treadmill (h/p/cosmos®, Germany)
    3.5 A portable metabolic analyzer equipped with a gas analysis system software (Cortex Metamax 3B, Germany)
    3.6 A portable near-infrared spectroscopy (NIRS) (Portamon, The Netherlands)

4. The following instruments were used for measuring blood chemical data variables:
    4.1 A laboratory centrifuges
    4.2 The freezer -40°C
    4.3 The blood collected tube
    4.4 A commercial nitrite assay kit utilizes the Griess Reagent (Sigma-Aldrich, USA)
    4.5 A Corning® Spin-X® UF concentrator
    4.6 A spectrophotometric multiwell-plate reader
    4.7 A clear flat-bottom 96-well plates
    4.8 A handheld lactate analyzer (Nova Biomedical, USA)
5. The following parameters were sent to the Health Sciences Service Unit, Faculty of Allied Health Sciences, Chulalongkorn University, for analysis:
    5.1 Serum glucose concentration
    5.2 Serum insulin concentration
6. The following instruments were used for measuring psychometric variables:
    6.1 The RPE 6-20 scales
    6.2 The validated gastrointestinal symptom questionnaire

Study design

This study employed a double-blind, randomized (counter-balanced), cross-over design, and the experiment took place at the Exercise and Sports Performance Laboratory (ESPL), the Faculty of Sports Science, Chulalongkorn University, Thailand. In this experiment, both the participants and the researcher were unaware of which participants were receiving any of the experimental drinks until the experiment was concluded, with the exception of an independent food scientist who prepared the beverages outside the experimental site. Each participant visited the laboratory five times over the course of five weeks. During the initial visit, participants performed an exhaustive ramp incremental test on a motorized treadmill (h/p/cosmos®, Germany) to determine the $\dot{V}O_2$peak, the first ventilatory threshold (VT_1), and the second ventilatory threshold (VT_2) in a moderate environment room maintained at a temperature of 21-22°C and relative humidity of 55-60%. Participants were instructed to abstain from engaging in any physical activity and consuming caffeinated beverages and alcohol prior to the performance tests.

Participants were randomly assigned to consume (1) the HBCD-dietary nitrate beverage, the co-ingestion of 1.5 g HBCD·kg^{-1} BM and 500 mg of beetroot extract powder (~8.00 mmol dietary NO_3^-) (HBCD+NO_3^-), (2) the HBCD beverage, the ingestion of 1.5 g HBCD·kg^{-1} BM (HBCD), (3) the maltodextrin-dietary nitrate beverage, the co-ingestion of 1.5 g MD·kg^{-1} BM and 500 mg of beetroot extract powder (~8.00 mmol dietary NO_3^-) (MD+NO_3^-), or (4) the maltodextrin beverage, the ingestion of 1.5 g MD·kg^{-1} BM (MD). Prior to consuming the first beverage, participants were asked to complete a three-day food diary and physical activity diary. They were instructed to replicate the food and beverage consumption and physical activity recorded in the diary before each visit. The schematic overview of the experimental protocol is outlined in Figure 7.

During the COVID-19 pandemic, in accordance with the Chulalongkorn University Announcement, the researchers took necessary precautions to prevent, avoid, and minimize the spread of COVID-19. These measures included temperature measurements, screening for illness, mandatory mask-wearing, and maintaining social distancing among

individuals utilizing the space. Additionally, regular cleaning of the area was conducted, and an ample supply of sanitizing gel/alcohol was provided for the participants.

Visit 1: Baseline measures

During the first visit, all participants were provided with comprehensive information regarding the experimental procedures, associated risks, and the rationale of the study. They were given the opportunity to review this information and provide their written consent before participating in the study. Inclusion criteria for participants consisted of being nonsmokers, currently in good health, and engaging in training activities at least three times per week, with a minimum training distance of 30 km per week. Prior to participation, all participants underwent screening using the 2021 Physical Activity Readiness Questionnaire (PAR-Q+), and a general health questionnaire. It was ensured that participants were free from cardiovascular, respiratory, metabolic, neurological, or orthopedic disorders that could potentially impact their running performance, as determined by the attending physician's approval.

Anthropometric measurements

Anthropometric data was collected from participants during their first visit to the laboratory. Upon arrival, participants were instructed to remove any heavy clothing and footwear, and they were provided with lightweight shorts to wear during the measurements. Whole body composition was assessed using bioelectrical impedance analysis (ACCUNIQ BC510, Korea). Participants were measured while in a state of euhydration, after voiding their bladder. There were no requirements for fasting or any restrictions on their regular activities prior to the measurements.

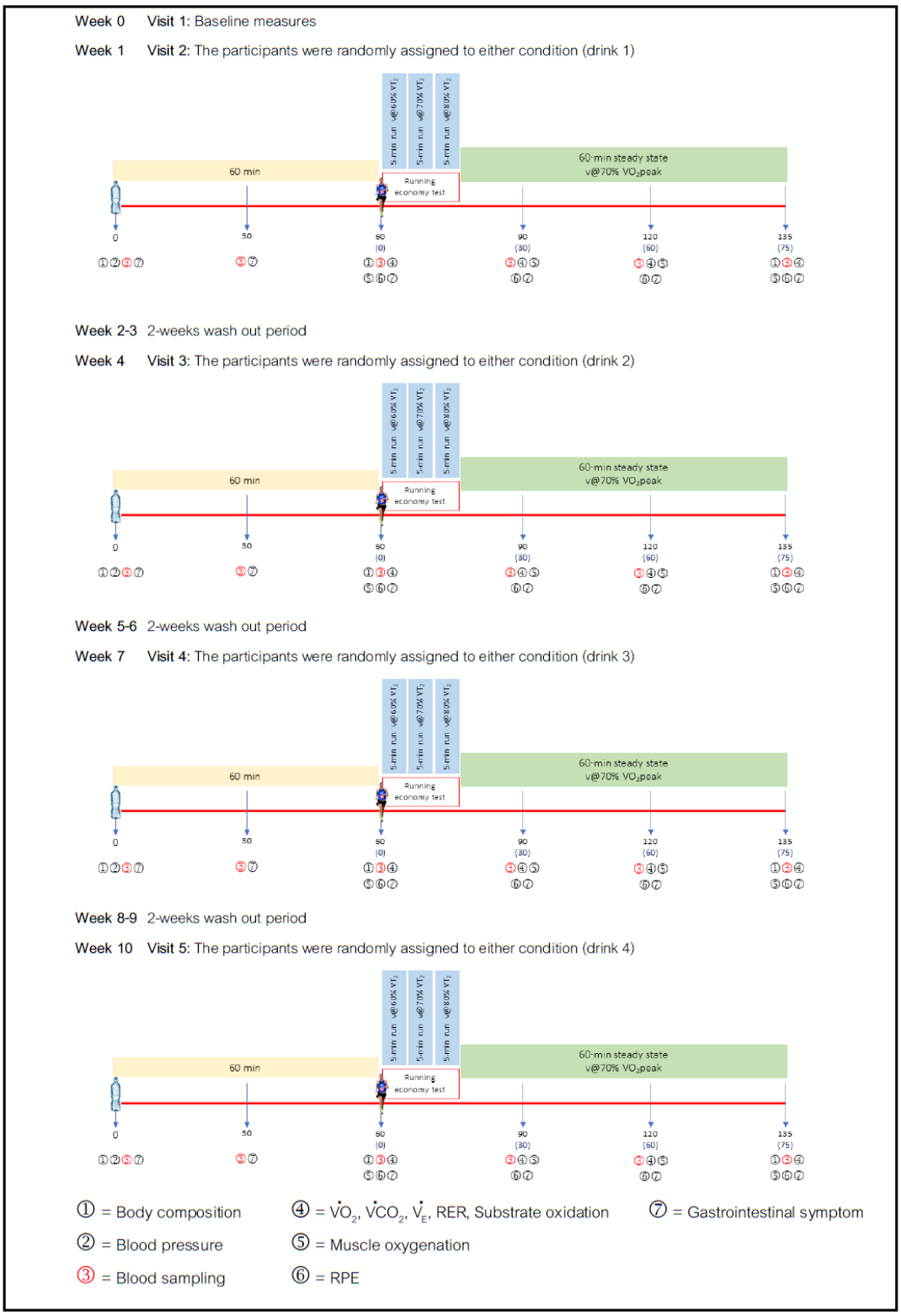

Figure 8 Schematic overview of the laboratory protocol

Blood pressure

Participants were instructed to rest and relax for a period of 10 minutes in a room with controlled temperature and minimal noise. Blood pressure measurements of the brachial artery were obtained using an automated sphygmomanometer (Omron, UK). The measurements were recorded to the nearest unit of systolic and diastolic pressure (mmHg). Triplicate measurements were taken after the participant had been seated for 10 minutes. The mean of the final two measurements was recorded for further analysis.

Heart rate

Heart rate was measured during exercise using a telemetry device, specifically a heart rate sensor with a chest strap (Polar, Finland). The device transmitted heart rate data as waveforms to the receiver on the gas analysis system software (Cortex Metamax 3B, Germany). This allowed for the continuous recording of heart rate, expressed in beats per minute (bpm).

Peak oxygen uptake

$\dot{V}O_2$peak was determined on a motorized treadmill (h/p/cosmos®, Germany) in a temperature-controlled laboratory. Participants underwent a 3-minute warm-up on the motorized treadmill, set at a speed of 4.5 $km \cdot h^{-1}$ followed by running at an initial velocity of 4.95 $km \cdot h^{-1}$ for 1 minute. The speed was then increased by 0.65 $km \cdot h^{-1}$ every 1 minute, along with a 0.4% increase in treadmill inclination every 1 minute, until the participants reached volitional exhaustion (104). Oxygen uptake ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), minute ventilation ($\dot{V}E$), Ventilation threshold (VT), and respiratory exchange ratio (RER) was determined using a portable metabolic analyzer (Cortex Metalyzer, Germany) for breath-by-breath analysis, $\dot{V}O_2$peak and the corresponding velocity (v$\dot{V}O_2$peak) were determined based on the highest 60-sec average (105). The criteria for determining exhaustion included a heart rate exceeding 90% of Tanaka's age-

predicted maximal heart rate equation (APMHR; 208-0.7 x age) (106), a respiratory exchange ratio (RER) of ≥ 1.10-1.15, and a rating of perceived exertion (RPE) above 18 (extremely hard) (107).

Visit 2-5: Beverage ingestion visits

Approximately one week after the incremental exercise test conducted during the first visit, each participant was scheduled to visit the laboratory early in the morning following an 8-hour fast (water consumption only permitted). Participants were instructed to maintain a record of their food and beverage intake as well as their physical activity for three days leading up to the laboratory visit. They were also asked to replicate their food and beverage consumption from the day before each laboratory visit.

To ensure consistent conditions, participants were requested to abstain from engaging in vigorous physical activity and consuming any pre-workout supplements for 24 hours prior to the visit. Furthermore, participants were informed about foods known to be rich in nitrate, such as celery, cress, chervil, lettuce, red beetroot, spinach, rocket, and cured meats, and were instructed to avoid these foods within 24 hours before each beverage ingestion visit.

To maintain the natural oral microbiota, participants were asked to refrain from flossing and using mouthwash for 18 hours prior to each test (108).

In this experiment, neither the participants nor the researchers were aware of which participants were assigned to each experimental drink until the conclusion of the study. Only the independent food scientist, who prepared the beverages outside the experimental site, had knowledge of the drink assignments.

During the first beverage ingestion visit, participants were randomly assigned in a double-blind crossover design to consume one of four beverage conditions: (1) the co-ingestion of HBCD and dietary nitrate beverage (HBCD+NO_3^-), (2) the ingestion of HBCD

beverage (HBCD), (3) the co-ingestion of maltodextrin and dietary nitrate beverage ($MD+NO_3^-$), or (4) the ingestion of maltodextrin beverage (MD). Participants were instructed to consume the assigned beverage within a 10-minute timeframe before commencing the running economy and time to exhaustion performance tests. A minimum of a 2-week washout period was implemented between each beverage condition.

Beverage preparation

The beverages were prepared by independent food scientists who were not directly involved in the experiment. The preparation took place at the pantry located outside the experimental site, approximately 30 minutes before the start of each experiment.

1. The co-ingestion of HBCD and dietary nitrate beverage was prepared by dissolving 1.5 g of HBCD·kg^{-1} of body mass (BM) and 500 mg of beetroot extract 10:1 powder (Feaga Life®, Thailand) in 500 ml of distilled water at a temperature of 4°C, the temperature that reduces the sweet taste (109). The mixture was then processed using a food processor to ensure a homogeneous texture. The prepared beverage was then bottled and stored at a temperature of 4°C until it was ready for use.

2. The ingestion of HBCD beverage was prepared by dissolving 1.5 g of HBCD·kg^{-1} of body mass (BM) in 500 ml of distilled water at a temperature of 4°C. The mixture was then processed using a food processor to ensure a homogeneous texture. The prepared beverage was then bottled and stored at a temperature of 4°C until it was ready for use.

3. The co-ingestion of maltodextrin and dietary nitrate beverage was prepared by dissolving 1.5 g of maltodextrin·kg^{-1} of body mass (BM) and 500 mg of beetroot extract 10:1 powder (Feaga Life®, Thailand) in 500 ml of distilled water at a temperature of 4°C. The mixture was then processed using a food processor to ensure a

homogeneous texture. The prepared beverage was then bottled and stored at a temperature of 4°C until it was ready for use.

4. The ingestion of maltodextrin beverage was prepared by dissolving 1.5 g of maltodextrin·kg^{-1} of body mass (BM) in 500 ml of distilled water at a temperature of 4°C. The mixture was then processed using a food processor to ensure a homogeneous texture. The prepared beverage was then bottled and stored at a temperature of 4°C until it was ready for use.

To maintain consistent flavors between the maltodextrin and HBCD drinks, flavor masking techniques were employed by the researchers. This was necessary to minimize any potential bias that could impact the experiment's outcomes. The researchers opted to use steviol glycosides or stevia as sweeteners in all drink variants, ensuring a similar level of sweetness. The specific methods for achieving this uniform sweetening will be implemented according to established procedures.

- The researchers established a standard sweetness level for the beverages as the maltodextrin and HBCD drinks were not equally sweet. This standard was determined to match the sweetness of a glucose solution containing 1.5 g·kg^{-1} BM, considering that the subjects in the study had an average weight of 60 kg, for example. This standard sweetness value served as a reference point for adjusting the sweetness of both drinks equally.

$$\text{Standard sweetness} = \text{glucose sweetness x amount x body mass}$$
$$= 0.7 \text{ x } 1.5 \text{ x } 60 = 63$$

- To modulate the taste of the maltodextrin solution, a sweetener is necessary to achieve the desired level of sweetness based on the specified standard. In this case, steviol glycosides were utilized as the sweetener due to their similar taste profile to glucose and their lack of caloric content. The amount of steviol glycoside to be added is calculated using the following equation:

$$\text{Stevia sweetness} = \text{Standard sweetness - Maltodextrin sweetness}$$
$$\text{Consumption x sweetness} = \text{Standard sweetness} - \text{Maltodextrin sweetness}$$
$$\text{Consumption x } 300 = 63 - (0.1 \text{ x } 1.5 \text{ x } 60)$$
$$\text{Dosage} = 0.18 \text{ grams} = 180 \text{ milligrams}$$

- To adjust the taste of HBCD drinks, the sweetness will be derived solely from steviol glycosides since HBCD itself does not possess a naturally sweet taste. The amount of steviol glycoside to be added can be calculated using the following equation:

$$\text{Stevia sweetness level} = \text{standard sweetness}$$
$$\text{Consumption x } 300 = 63$$
$$\text{Dosage} = 0.21 \text{ grams} = 210 \text{ milligrams}$$

After the preparation of the beverages, food scientists conducted assessments of the physical quality of the drinks, including factors such as color, texture, viscosity, and cleanliness. A process flowchart detailing the preparation of the experimental beverages was provided in Figure 8.

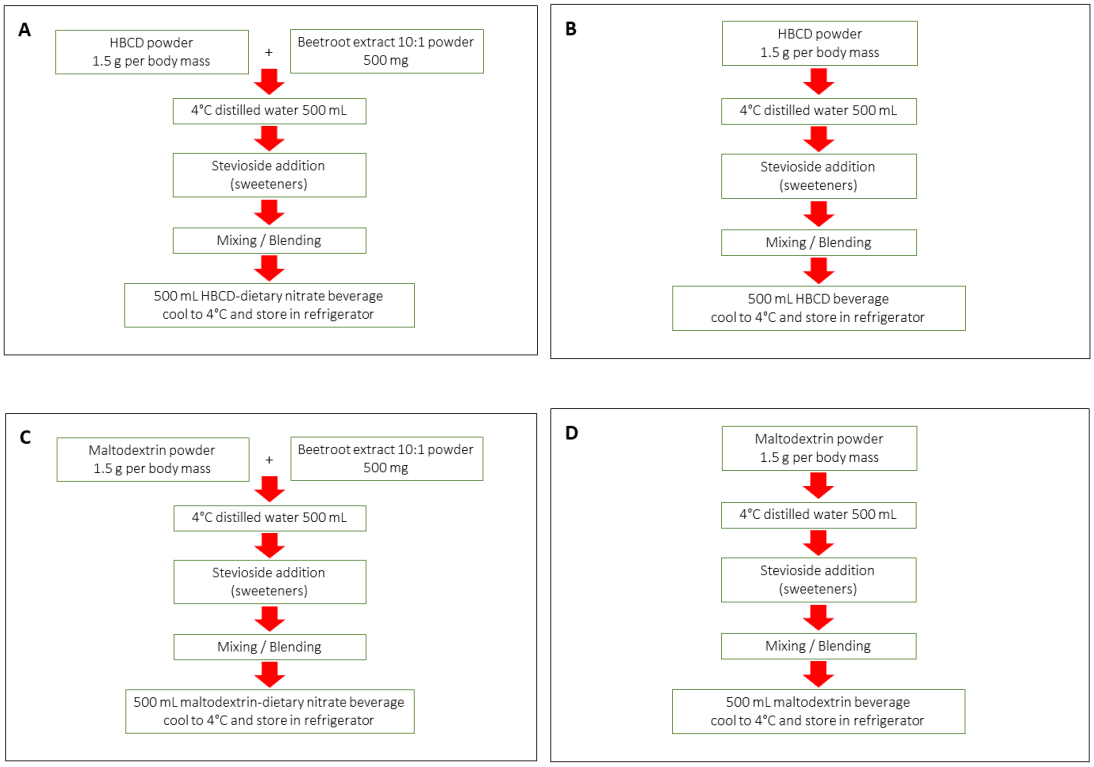

Figure 9 Process flowchart for preparation of experimental beverage (a) the HBCD+NO_3^- beverage preparation, (b) the HBCD beverage preparation, (c) the MD+NO_3^- beverage preparation, and (d) the MD beverage preparation.

Anthropometric measurements

Anthropometric measurements were conducted to assess the participants' body composition. Bioelectrical impedance analysis was used to collect the data. Measurements were taken at before and the end of running test. A digital body composition analyzer (ACCUNIQ BC510, Korea) was used to ensure accurate and consistent measurements throughout the study period. The purpose of these measurements was to monitor and ensure that there were no significant changes in body composition over the course of the study.

Blood collection and analysis

Participants were instructed to fast overnight for a duration of 12 hours prior to the provision of a venous blood sample. A medical laboratory technologist collected all the blood samples in this experiment, which were later analyzed at the Health Sciences Service Unit, Faculty of Allied Health Sciences, Chulalongkorn University. During each blood collection, three milliliters from antecubital vein blood were drawn from the participants. Samples were collected for both serum and plasma at six designated time points throughout the study. These time points included: (1) baseline, which was before the participants consumed the beverage (BL), (2) 30 minutes after beverage ingestion (30 min POST), (3) 60 minutes after beverage ingestion (60 min POST), which is the same point as before the exercise test (PRE-EX), (4) 30 minutes into the running test (30 min RUN), (5) 60 minutes into the running test (60 min RUN), and (6) immediately after the completion of the exercise test (POST-EX).

The prominent antecubital forearm vein area was disinfected by wiping with an alcohol pad saturated with 70% ethyl alcohol from 3M (Thailand). A total of nine mL of blood was drawn from the vein. The blood sample was divided into three portions: two mL for glucose determination in sodium fluoride (NaF) tubes, six mL for insulin and nitrate/nitrite analysis in serum separation gel tubes (SSGT), and the remaining portion for blood lactate analysis. Following centrifugation at 3,000 rpm at 25 °C for 10 minutes, the tubes were used to separate the plasma. The separated plasma samples were then frozen at -80 °C until they were ready for further analysis, including the determination of serum glucose concentration, serum insulin concentration, and serum levels of nitrate/nitrite concentration.

Blood lactate concentration was determined from fresh venous whole blood using a handheld lactate analyzer (Nova Biomedical, USA) immediately after blood collection.

The serum glucose concentration was determined by the Hexokinase/G-6-PDH method using Architect Analyzer (Abbott Laboratories, Abbott Park, IL). The serum insulin concentration was analyzed using the electrochemiluminescence immunoassay

method (ECLIA) kits (Roche, US) at the Health Sciences Service Unit, Faculty of Allied Health Sciences, Chulalongkorn University.

To evaluate the concentration of nitrate/nitrite ([NO_x]), the serum was subjected to treatment with 10 mM vanadium chloride (VCl_3) at 45°C for 30 minutes in the absence of light. This conversion process aimed to convert nitrate to nitrite, which is represented as [NO_x]. The total [NO_x] was analyzed using commercial nitrite assay kits following the instructions provided by the manufacturer (Sigma-Aldrich, USA). The Griess Reagents method, a well-established protocol for nitrite estimation, was utilized. This method involved the use of sulfanilamide (SUL) and N-1-naphthyl ethylenediamine (NED). In this assay, nitrite was initially reduced to nitrogen oxide by SUL. Subsequently, the nitrogen oxide reacted with NED, resulting in the formation of a pink azo dye product. The concentration of this product was quantified by measuring its absorbance at 540 nm using a microplate reader. The absorbance response increased in relation to the concentration of [NO_x]. All measurements were performed in duplicate, and the results were interpreted based on the NO_x calibration curve.

Running economy and constant load running test

After consuming the experimental beverage, the participants underwent a submaximal incremental test to measure running economy (RE) at various speeds on a motorized treadmill (h/p/cosmos®, Germany) in a moderate environmental setting (22°C and 60% RH). The participants' heart rates were monitored using integrated heart rate monitoring equipment (Polar, Finland). The study protocol began with a 3-minute resting period to establish baseline data. This was followed by a 5-minute warm-up phase at a velocity of 4.0 km·h-1. Subsequently, three consecutive steady-state intervals of 5 minutes each were performed, without any intervening recovery periods. After the 15-minute running economy test, the running speed was increased to the initial velocity that corresponded to 70% of $\dot{V}O_2$peak. This velocity was maintained for a duration of 60 minutes to observe the physiological responses following the ingestion of the beverage over the time frame of 60 minutes during high-intensity prolonged running. To ensure that the study results were not affected by training or competitions, participants did not participate in any experimentation or endurance road races during the data collection period. They completed four beverage ingestion visits, with a 2-week interval between each visit. The running economy and constant load running test were repeated at the same time of day to minimize the impact of diurnal variation throughout the duration of the study.

Gas analysis and substrate oxidation

During the running economy and a 60-minutes constant load running test, oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), minute ventilation ($\dot{V}_E$), and respiratory exchange ratio (RER) were measured breath by breath by a portable metabolic analyzer (Cortex Metamax 3B, Germany). Running economy (RE) is determined by measuring the steady-state $\dot{V}O_2$, the physiological steady state was defined as the last 2 minutes of each stage with an increase of less than 100 mL of $\dot{V}O_2$, RER < 1.0 to assume a negligible anaerobic contribution to energy expenditure, and the period with the lowest $\dot{V}O_2$ standard deviation (57). RE was calculated as the oxygen cost (EO_2) using the average $\dot{V}O_2$ ($mL \cdot kg^{-1} \cdot min^{-1}$) over the 2-minutes steady-state period, and the running speed ($m \cdot min^{-1}$):

$$EO_2(mL \cdot kg^{-1} \cdot m^{-1}) = \frac{\dot{V}O_2(mL \cdot kg^{-1} \cdot min^{-1})}{speed\ (m \cdot min^{-1})}$$

Total CHO and fat oxidation rates were calculated based on those proposed by Jeukendrup and Wallis (2005), where $\dot{V}CO_2$ and $\dot{V}O_2$ will be measured in liters per min. Protein oxidation will be considered as negligible (110):

$$Total\ CHO\ oxidation\ rate\ (g \cdot min^{-1}) = 4.210\,\dot{V}CO_2 - 2.962\,\dot{V}O_2$$
$$Fat\ oxidation\ rate\ (g \cdot min^{-1}) = 1.695\,\dot{V}CO_2 - 1.701\,\dot{V}O_2$$

Muscle oxygenation

During the assessment of running economy and a 60-minute constant load running test, the portable near-infrared spectroscopy (NIRS) was employed to continuously monitor muscle oxygenation. The NIRS device (Portamon, Artinis, Medical System, Gelderland, The Netherlands) was positioned on the belly of the vastus lateralis muscle of the dominant leg, specifically about 10-12 centimetres above the knee joint along the thigh's vertical axis (111). To ensure the optical sensor and detector remained stationary in relation to the skin, the device was affixed with sports adhesive tape and subsequently enveloped with a black elastic bandage around the leg to eliminate any influence from external light sources. To ensure precise repositioning of the NIRS device at the same location for each trial, the position was marked using an indelible pen and photographed. Muscle oxygenation was evaluated by calculating the difference (Δ) to assess utilization. The approach considered individual variations in muscle oxygenation values at rest, as well as the maximum capacity of athletes to utilize oxygen in their muscles. Tissue oxygen saturation (TSI) was expressed in percentage, while oxyhemoglobin (O_2Hb), deoxyhemoglobin (HHb), and total hemoglobin (tHb) were expressed in micromolar (μM). The changes in TSI (ΔTSI), O_2Hb (ΔO_2Hb), HHb (ΔHHb), and tHb (ΔtHb) were calculated by subtracting the mean value of the last 3 min of each run from the baseline value at the specified time point during the 3-min resting period in each trial. The reliability of NIRS data was confirmed by the Cronbach alpha value of .807, indicating strong reliability (112).

RPE and gastrointestinal symptoms outcomes

The rate of perceived exertion (RPE) was measured at before exercise and every 30-minute intervals during the exercise test using the RPE 6-20 scale. Participants were asked to rate their perceived level of exertion on a scale ranging from 6 to 20, with higher values indicating a greater perceived effort or intensity of the exercise. The RPE was assessed regularly throughout the duration of the exercise test to track changes in perceived exertion over time (113) and the gastrointestinal symptoms were evaluated using a modified visual analogue scale (114) consisting of a 100-mm continuous line anchored by "no pain" (0 mm) and "very, very painful" (100 mm). The assessment was conducted for six symptoms, namely nausea, vomiting, stomach fullness, abdominal pain, heartburn, and bloating, at four specific time points: baseline, which was before the participants consumed the beverage (BL), 30 minutes after beverage ingestion (30 min POST), prior to the exercise test (PRE-EX), and immediately after the completion of the exercise test (POST-EX). Participants indicated the severity of each symptom by marking a point on the line corresponding to their perceived level of discomfort or pain.

Data analysis

An a priori power calculation (G*power 3) was used to determine the required number of participants for this study, with an alpha level of 0.05 and a statistical power of 95%. The data, including anthropometric data, physiological variables data, blood chemistry variables data, physical performance data, and psychological variables data, were expressed as the mean ± standard error of the mean (SEM) unless otherwise specified. The normal distribution of the variables was assessed using the Shapiro-Wilk test. Data were analyzed using repeated measures ANOVA (condition x time point), followed by the Bonferroni correction for post hoc tests, using IBM SPSS Statistics software, version 28.0 for MacOS (IBM Corp., Armonk, NY). Effect sizes for main effects of the ANOVA was reported as partial η^2. A significance level of $p < 0.05$ was considered statistically significant.

CHAPTER IV
RESULTS

Part I Characteristics of the participants

This study followed the CONSORT guidelines for reporting randomized trials. Initially, a total of 12 male recreational endurance runners were assigned to the study. However, during the intervention, 2 participants dropped out due to COVID-19 illness, and 1 participant dropped out due to injuries. As a result, a total of 9 participants were included in the final analysis. The study flowchart was presented in Figure 9, illustrating the continuation of participants throughout the study. The baseline characteristics of all participants who received the assigned intervention are summarized in Table 5.

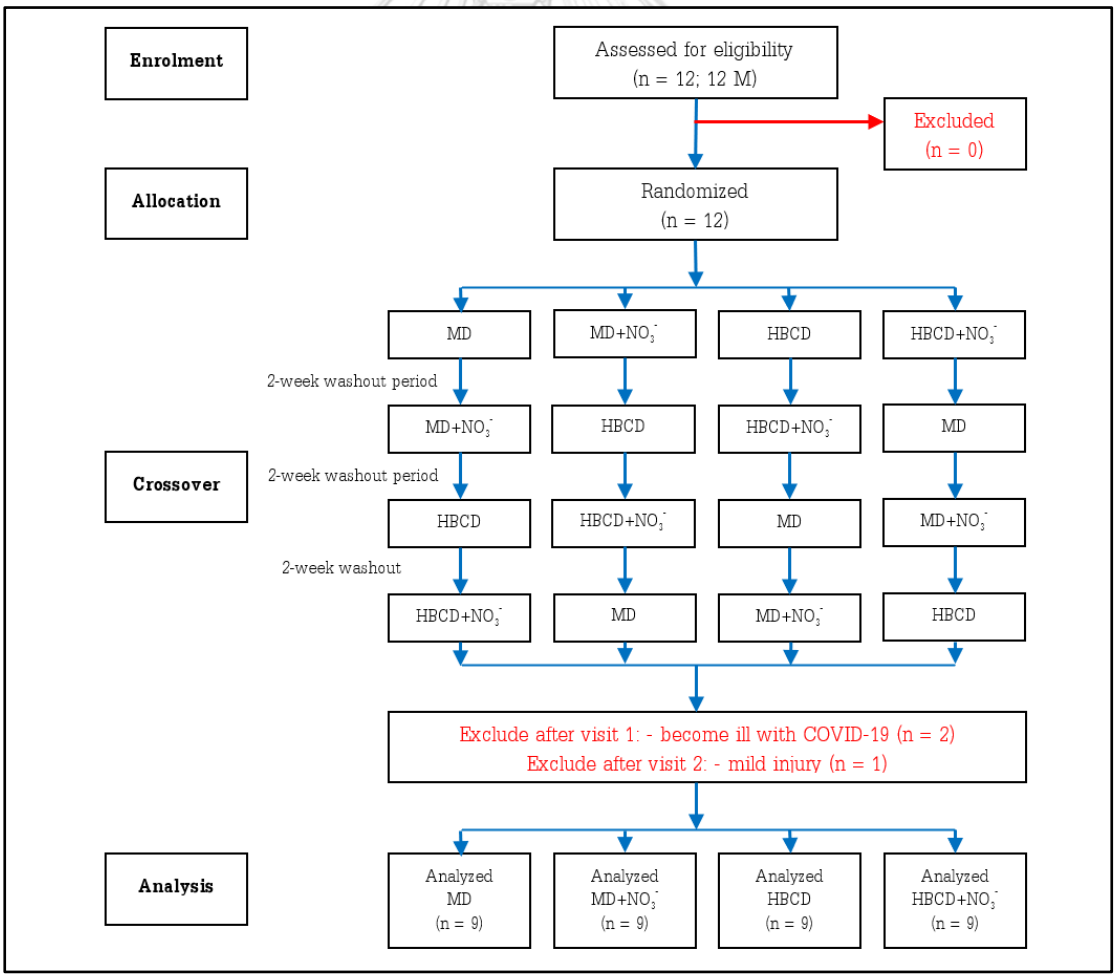

Figure 10 CONSORT flow diagram of the study

Table 5 Baseline characteristics of all participants (n =9)

	Mean ± S.D.
Age (years)	35.00 ± 4.00
Body mass (kg)	68.60 ± 6.94
Height (cm)	174.00 ± 4.64
$\dot{V}O_2$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	54.67 ± 4.12
Running experience (years)	5.00 ± 3.00
Experience in participating in marathon races (time)	8.00 ± 7.00
Average running distance per week (km)	55.00 ± 20.70
Frequency of running training per week (time)	5.00 ± 1.00

Part II Respiratory gas exchange and substrate oxidation data

Oxygen consumption

Table 6 The comparison of oxygen consumption data among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9)

		$\dot{V}O_2$ ($mL \cdot kg^{-1} \cdot min^{-1}$)			
		MD	MD+NO_3^-	HBCD	HBCD+NO_3^-
PRE-EX (REST)		0.43 ± 0.01	0.44 ± 0.02	0.45 ± 0.02	0.43 ± 0.04
RE	STAGE I	2.16 ± 0.08*	2.19 ± 0.14*	2.20 ± 0.10*	2.14 ± 0.08*
	STAGE II	2.44 ± 0.11*	2.54 ± 0.15*	2.54 ± 0.11*	2.50 ± 0.10*
	STAGE III	2.71 ± 0.10* **	2.79 ± 0.15* **	2.87 ± 0.12* **	2.72 ± 0.13* **
30 min RUN		2.79 ± 0.12* **	2.90 ± 0.16* **	2.97 ± 0.14* **	2.92 ± 0.10* **
60 min RUN		2.84 ± 0.13* **	2.95 ± 0.16* **	3.10 ± 0.15* **	2.91 ± 0.12* **
POST-EX		2.84 ± 0.13* **	2.93 ± 0.16* **	3.07 ± 0.13* **	2.93 ± 0.12* **

Data were expressed in mean ± SEM

* Denotes significant higher compared with PRE-EX within condition ($p < 0.05$)

** Denotes significant higher compared with STAGE I within condition ($p < 0.05$)

$\dot{V}O_2$ tended to increase over the duration of the trial, with the HBCD condition showing a higher rate of increase compared to MD+NO_3^-, HBCD+NO_3^-, and MD, respectively (Figure 10). There was a significant effect of time point for mean $\dot{V}O_2$ ($p < 0.001$, partial $\eta^2 = 0.865$). However, there was no significant main effect for condition for mean $\dot{V}O_2$ among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions ($p = 0.158$, partial $\eta^2 = 0.023$) and no significant condition x time point interaction effect ($p = 1.000$, partial $\eta^2 = 0.011$).

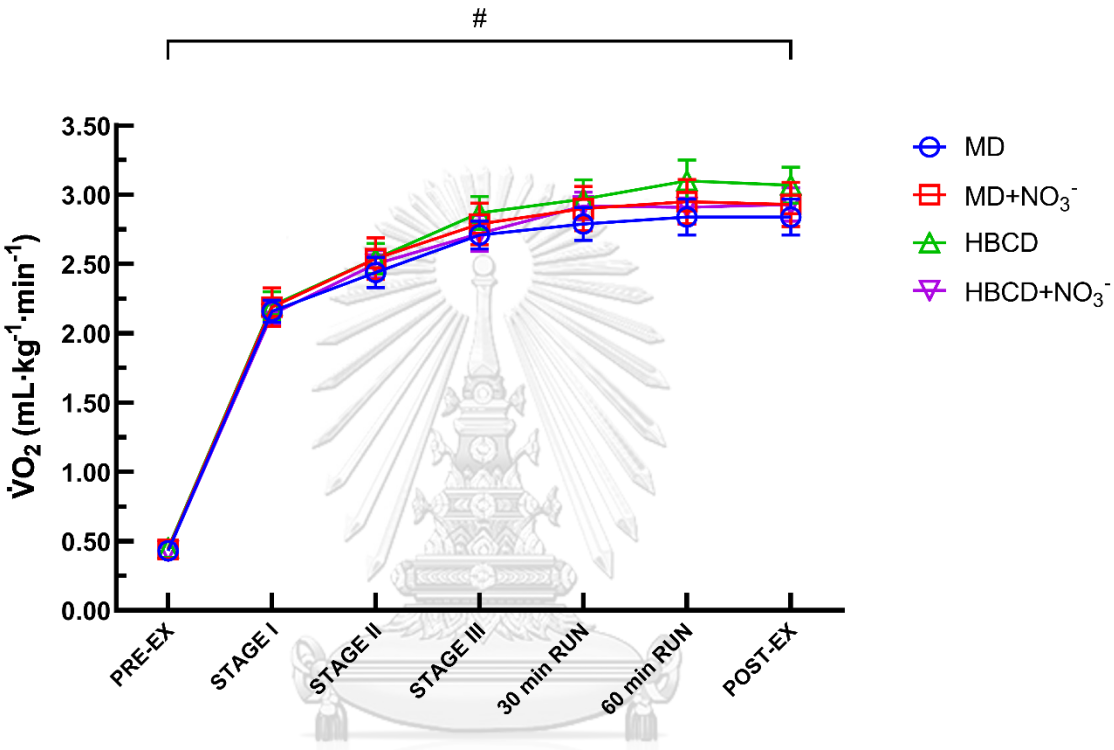

Figure 11 Mean ± SEM of oxygen consumption response among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9).

 # Denotes significant differences over time ($p < 0.05$).

Carbon dioxide production

Table 7 The comparison of carbon dioxide production data among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9)

		$\dot{V}CO_2$ ($mL \cdot kg^{-1} \cdot min^{-1}$)			
		MD	MD+NO_3^-	HBCD	HBCD+NO_3^-
PRE-EX (REST)		0.40 ± 0.02	0.40 ± 0.03	0.41 ± 0.03	0.39 ± 0.03
RE	STAGE I	1.99 ± 0.08*	2.00 ± 0.14*	1.99 ± 0.09*	1.99 ± 0.08*
	STAGE II	2.31 ± 0.11*	2.39 ± 0.16*	2.36 ± 0.10*	2.38 ± 0.10*
	STAGE III	2.59 ± 0.11* **	2.64 ± 0.16* **	2.68 ± 0.12* **	2.61 ± 0.13* **
30 min RUN		2.64 ± 0.11* **	2.72 ± 0.16* **	2.74 ± 0.12* **	2.77 ± 0.10* **
60 min RUN		2.68 ± 0.13* **	2.77 ± 0.16* **	2.87 ± 0.13* **	2.76 ± 0.12* **
POST-EX		2.66 ± 0.12* **	2.75 ± 0.17* **	2.81 ± 0.12* **	2.76 ± 0.13* **

Data were expressed in mean ± SEM

* Denotes significant higher compared with PRE-EX within condition ($p < 0.05$)

** Denotes significant higher compared with STAGE I within condition ($p < 0.05$)

$\dot{V}CO_2$ tended to increase over the duration of the trial, with the HBCD condition showing a higher rate of increase compared to MD+NO_3^-, HBCD+NO_3^-, and MD, respectively (Figure 11). There was a significant effect of time point for mean $\dot{V}CO_2$ ($p < 0.001$, partial $\eta^2 = 0.851$). However, there was no significant main effect for condition for mean $\dot{V}CO_2$ among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions ($p = 0.569$, partial $\eta^2 = 0.003$) and no significant condition x time point interaction effect ($p = 1.000$, partial $\eta^2 = 0.007$).

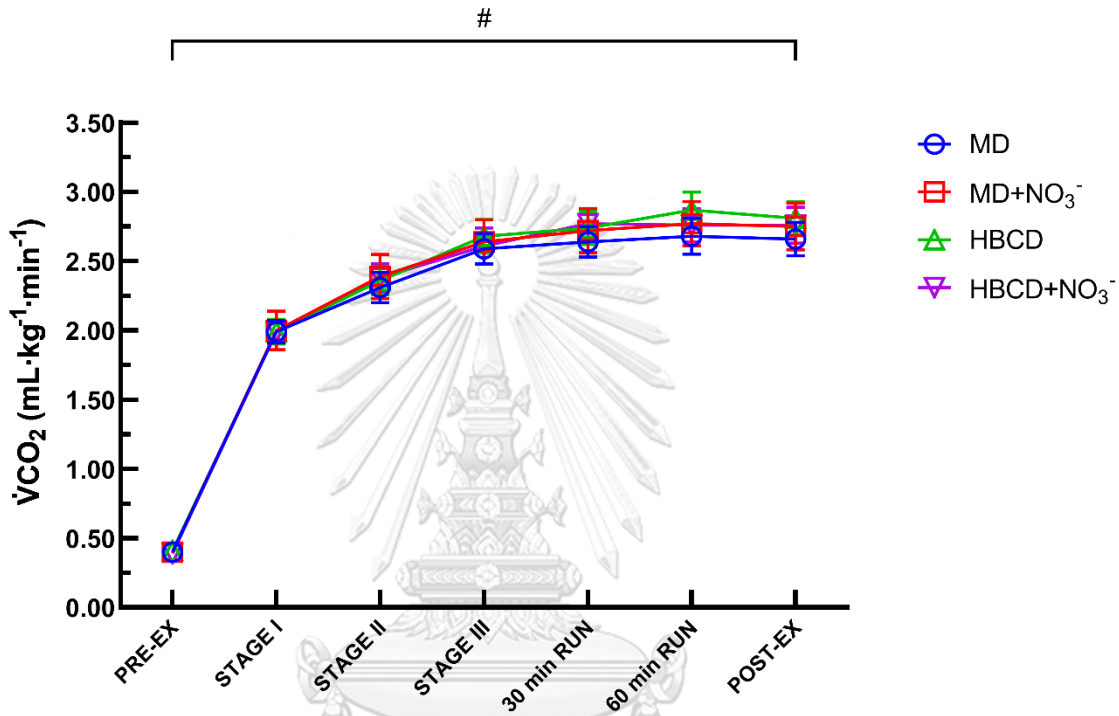

Figure 12 Mean ± SEM of carbon dioxide production response among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9).

# Denotes significant differences over time ($p < 0.05$).

Minute ventilation

Table 8 The comparison of minute ventilation data among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9)

		$\dot{V}E$ ($L \cdot min^{-1}$)			
		MD	MD+NO_3^-	HBCD	HBCD+NO_3^-
PRE-EX (REST)		14.67 ± 1.31	14.09 ± 1.39	14.74 ± 1.13	13.99 ± 1.32
RE	STAGE I	54.33 ± 4.39*	55.59 ± 4.82*	56.18 ± 3.93*	55.84 ± 3.02*
	STAGE II	63.57 ± 4.82*	66.64 ± 5.11*	66.71 ± 3.89*	66.14 ± 3.32*
	STAGE III	71.77 ± 5.51*	75.87 ± 5.10*	76.57 ± 4.30*	72.54 ± 3.80*
30 min RUN		75.47 ± 6.67* **	79.59 ± 5.67* **	82.33 ± 4.68* **	79.41 ± 4.23* **
60 min RUN		79.14 ± 7.32* **	83.26 ± 5.83* **	89.28 ± 5.69* **	82.73 ± 5.33* **
POST-EX		79.99 ± 7.61* **	87.22 ± 6.02* **	88.49 ± 5.07* **	86.62 ± 4.97* **

Data were expressed in mean ± SEM

* Denotes significant higher compared with PRE-EX within condition ($p < 0.05$)

** Denotes significant higher compared with STAGE I within condition ($p < 0.05$)

There was a significant effect of time point for mean $\dot{V}E$ ($p < 0.001$, partial $\eta^2 = 0.741$). However, there was no significant main effect for condition for mean $\dot{V}E$ among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions ($p = 0.265$, partial $\eta^2 = 0.018$) and no significant condition x time point interaction effect ($p = 1.000$, partial $\eta^2 = 0.010$).

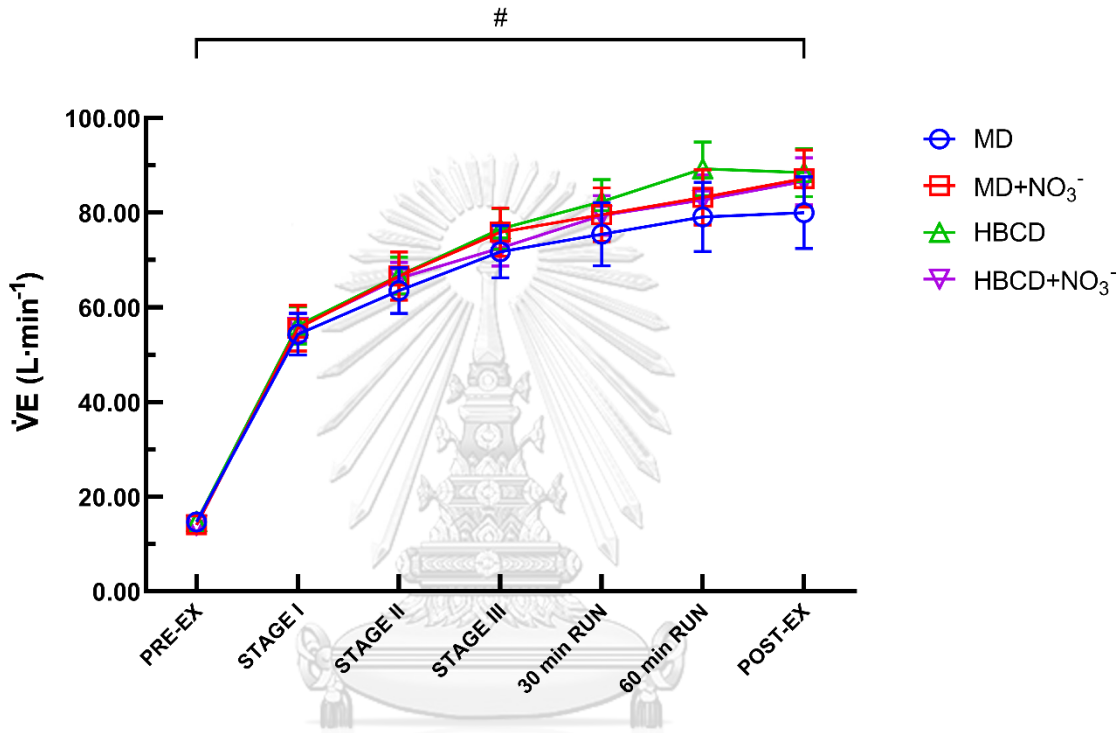

Figure 13 Mean ± SEM of minute ventilation response among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9).

# Denotes significant differences over time ($p < 0.05$).

Respiratory exchange ratio

Table 9 The comparison of respiratory exchange ratio data among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9)

		RER ($\dot{V}CO_2$/ $\dot{V}O_2$)			
		MD	MD+NO_3^-	HBCD	HBCD+NO_3^-
PRE-EX (REST)		0.93 ± 0.01	0.92 ± 0.02	0.91 ± 0.02	0.91 ± 0.01
RE	STAGE I	0.92 ± 0.01	0.92 ± 0.02	0.90 ± 0.02	0.93 ± 0.01
	STAGE II	0.95 ± 0.01	0.91 ± 0.01	0.93 ± 0.02	0.95 ± 0.01
	STAGE III	0.96 ± 0.01	0.94 ± 0.01	0.94 ± 0.02	0.96 ± 0.01
30 min RUN		0.95 ± 0.01	0.95 ± 0.01	0.92 ± 0.02	0.95 ± 0.01
60 min RUN		0.94 ± 0.01	0.94 ± 0.01	0.93 ± 0.02	0.95 ± 0.01
POST-EX		0.94 ± 0.01	0.94 ± 0.01	0.92 ± 0.02	0.94 ± 0.01

Data were expressed in mean ± SEM

There was a significant main effect for condition for mean RER among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (p = 0.013, partial η^2 = 0.047), with a significant higher in the MD condition than HBCD condition throughout the trend line (p = 0.032), as well as a significant higher in the HBCD+NO_3^- condition than the HBCD condition throughout the trend line (p = 0.028) (Figure 13). There was also a significant effect of time point for mean RER (p < 0.001, partial η^2 = 0.099). However, there was no significant condition x time point interaction effect (p = 1.000, partial η^2 = 0.013). When pairwise comparisons were conducted between time points, no statistically significant difference was found for mean RER.

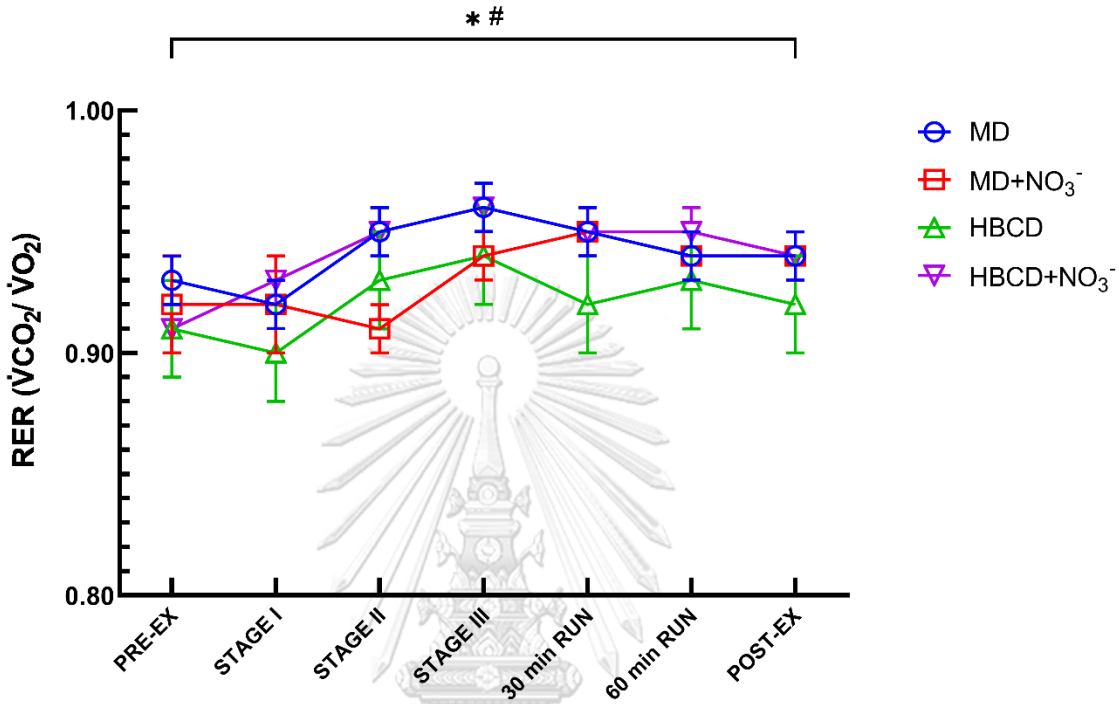

Figure 14 Mean ± SEM of respiratory exchange ratio response among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9).

* Denotes significant differences between condition, # significant differences over time ($p < 0.05$).

Total carbohydrate oxidation rate

Table 10 The comparison of total CHO oxidation rate data among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9)

		Total CHO oxidation rate ($g \cdot min^{-1}$)			
		MD	MD+NO_3^-	HBCD	HBCD+NO_3^-
PRE-EX (REST)		0.41 ± 0.03	0.40 ± 0.05	0.40 ± 0.05	0.38 ± 0.04
RE	STAGE I	1.98 ± 0.13*	1.91 ± 0.19*	1.84 ± 0.16*	2.01 ± 0.11*
	STAGE II	2.49 ± 0.16*	2.57 ± 0.23*	2.42 ± 0.18*	2.62 ± 0.15*
	STAGE III	2.88 ± 0.17* **	2.88 ± 0.23* **	2.79 ± 0.25* **	2.92 ± 0.20* **
30 min RUN		2.83 ± 0.16* **	2.86 ± 0.23* **	2.72 ± 0.23* **	3.02 ± 0.18* **
60 min RUN		2.87 ± 0.17* **	2.93 ± 0.22* **	2.89 ± 0.24* **	3.02 ± 0.19* **
POST-EX		2.78 ± 0.16* **	2.93 ± 0.23* **	2.77 ± 0.24* **	2.96 ± 0.22* **

Data were expressed in mean ± SEM

* Denotes significant higher compared with PRE-EX within condition ($p < 0.05$)

** Denotes significant higher compared with STAGE I within condition ($p < 0.05$)

Total CHO oxidation rate increase over the duration of the trial then there was a steady trend with the HBCD+NO_3^- condition showing a higher compared to MD+NO_3^-, MD, and HBCD, respectively (Figure 14). There was a significant effect of time point for mean total CHO oxidation rate ($p < 0.001$, partial $\eta^2 = 0.733$). However, there was no significant main effect for condition for mean total CHO oxidation rate among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions ($p = 0.442$, partial $\eta^2 = 0.012$) and no significant condition x time point interaction effect ($p = 1.000$, partial $\eta^2 = 0.006$).

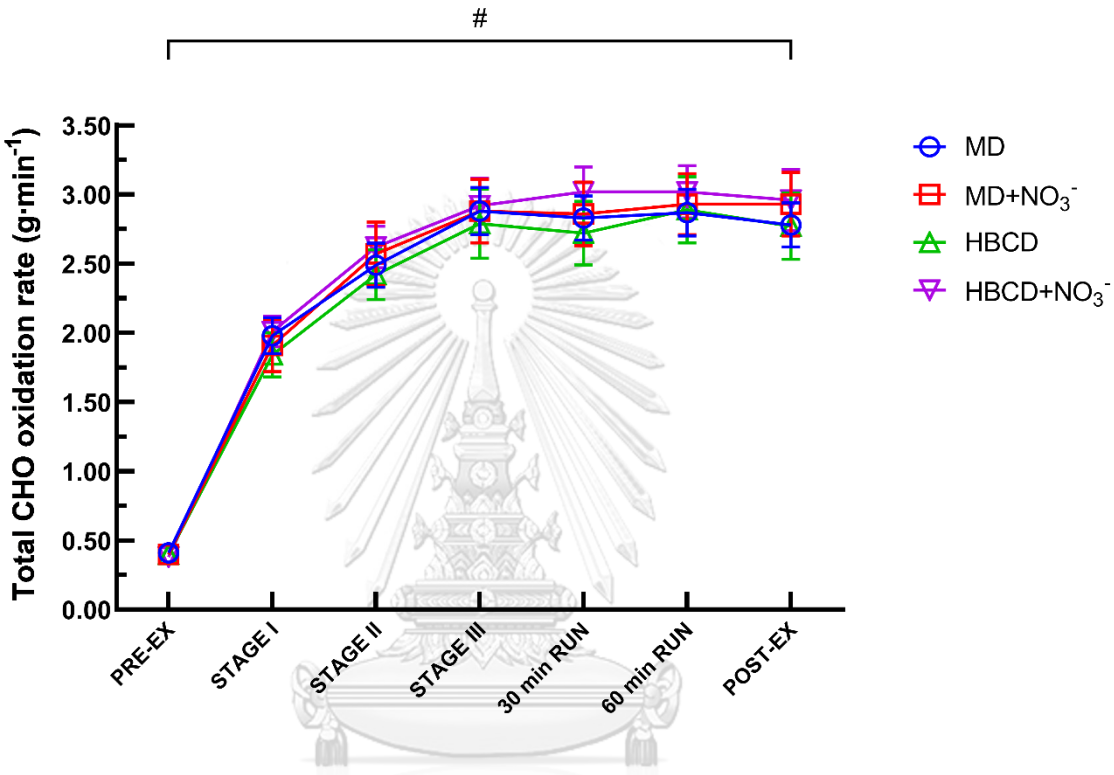

Figure 15 Mean ± SEM of total CHO oxidation rate response among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9).

# Denotes significant differences over time ($p < 0.05$).

Fat oxidation rate

Table 11 The comparison of fat oxidation rate data among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9)

		Fat oxidation rate ($g \cdot min^{-1}$)			
		MD	MD+NO_3^-	HBCD	HBCD+NO_3^-
PRE-EX (REST)		0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.02	0.06 ± 0.01
RE	STAGE I	0.27 ± 0.04	0.32 ± 0.03*	0.35 ± 0.07*	0.26 ± 0.03
	STAGE II	0.21 ± 0.03	0.23 ± 0.03	0.29 ± 0.08	0.19 ± 0.03
	STAGE III	0.18 ± 0.03	0.23 ± 0.03	0.30 ± 0.10*	0.17 ± 0.05
30 min RUN		0.25 ± 0.05	0.29 ± 0.03	0.38 ± 0.10*	0.23 ± 0.04
60 min RUN		0.26 ± 0.05	0.29 ± 0.03	0.38 ± 0.10*	0.23 ± 0.04
POST-EX		0.29 ± 0.05*	0.27 ± 0.04	0.41 ± 0.11*	0.26 ± 0.05

Data were expressed in mean ± SEM

* Denotes significant higher compared with PRE-EX within condition ($p < 0.05$)

There was a significant main effect for condition for mean fat oxidation rate among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions ($p = 0.001$, partial $\eta^2 = 0.070$), with a significant higher in HBCD condition than the MD condition throughout the line ($p = 0.008$), as well as a significant higher in the HBCD condition than the HBCD+NO_3^- condition ($p = 0.001$) (Figure 15). There was also a significant effect of time point for mean fat oxidation rate ($p < 0.001$, partial $\eta^2 = 0.218$). However, there was no significant condition x time point interaction effect ($p = 1.000$, partial $\eta^2 = 0.017$).

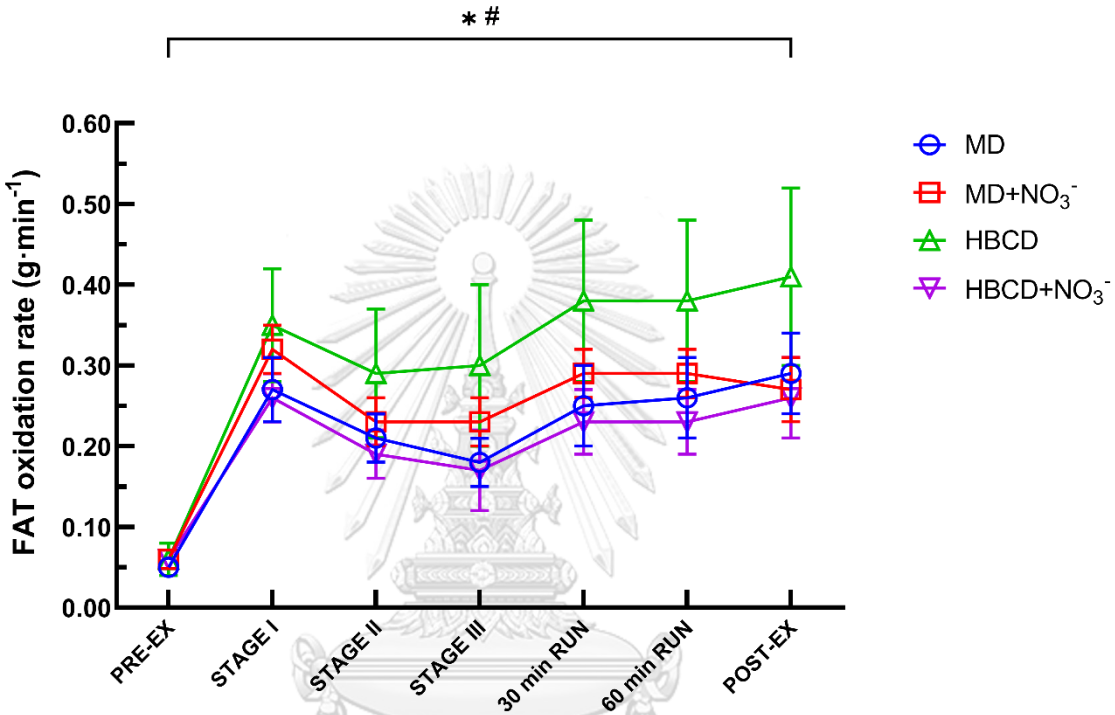

Figure 16 Mean ± SEM of fat oxidation rate response among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9).

* Denotes significant differences between condition, # significant differences over time ($p < 0.05$).

Part III Running economy data

Oxygen cost

Table 12 The comparison of oxygen cost data among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9)

	EO_2 ($mL \cdot kg^{-1} \cdot m^{-1}$)			
	MD	MD+NO_3^-	HBCD	HBCD+NO_3^-
STAGE I	0.235 ± 0.006	0.239 ± 0.013	0.239 ± 0.007	0.233 ± 0.005
STAGE II	0.228 ± 0.007	0.238 ± 0.012	0.237 ± 0.007	0.234 ± 0.007
STAGE III	0.229 ± 0.005	0.236 ± 0.011	0.243 ± 0.007	0.230 ± 0.010
Steady state	0.229 ± 0.008	0.235 ± 0.010	0.247 ± 0.009	0.236 ± 0.007

Data were expressed in mean ± SEM

There was no significant main effect for condition for mean EO_2 among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (p = 0.274, partial η^2 = 0.030). There was also no significant effect of time point for mean EO_2 (p = 0.970, partial η^2 = 0.002) and condition x time point interaction effect (p = 0.999, partial η^2 = 0.010) (Figure 16).

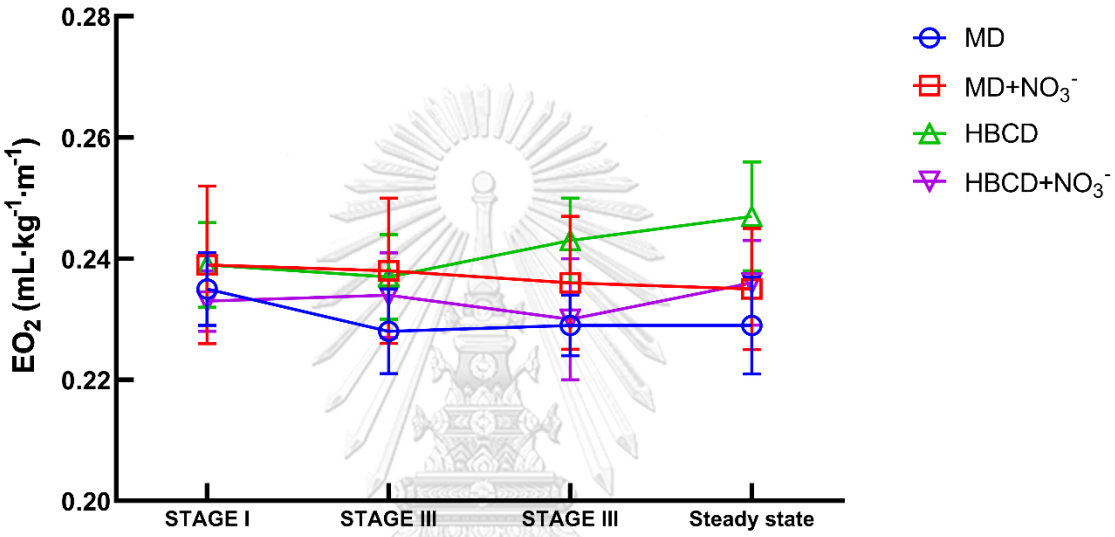

Figure 17 Mean ± SEM of oxygen cost response during running economy test among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9).

Part IV Muscle oxygenation data

The changes in tissue saturation index

Table 13 The comparison of the changes in tissue saturation index among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9)

	ΔTSI (%)			
	MD	MD+NO_3^-	HBCD	HBCD+NO_3^-
STAGE I	-3.17 ± 2.43	-2.19 ± 2.18	-2.68 ± 1.39	-2.78 ± 0.86
STAGE II	-3.92 ± 2.74	-3.85 ± 2.84	-5.03 ± 1.27	-5.01 ± 1.50
STAGE III	-3.27 ± 2.41	-5.26 ± 3.16	-7.18 ± 1.40	-7.40 ± 1.76
Steady state	-6.59 ± 3.37	-7.09 ± 3.56	-10.32 ± 1.83	-10.47 ± 2.02

Data were expressed in mean ± SEM

There was no significant main effect for condition for mean ΔTSI among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (p = 0.310, partial η^2 = 0.028). There was a significant effect of time point for mean ΔTSI (p = 0.008, partial η^2 = 0.087), when pairwise comparisons were conducted between time points, no statistically significant difference was found for mean ΔTSI. However, there was no significant condition x time point interaction effect (p = 0.998, partial η^2 = 0.011) (Figure 17).

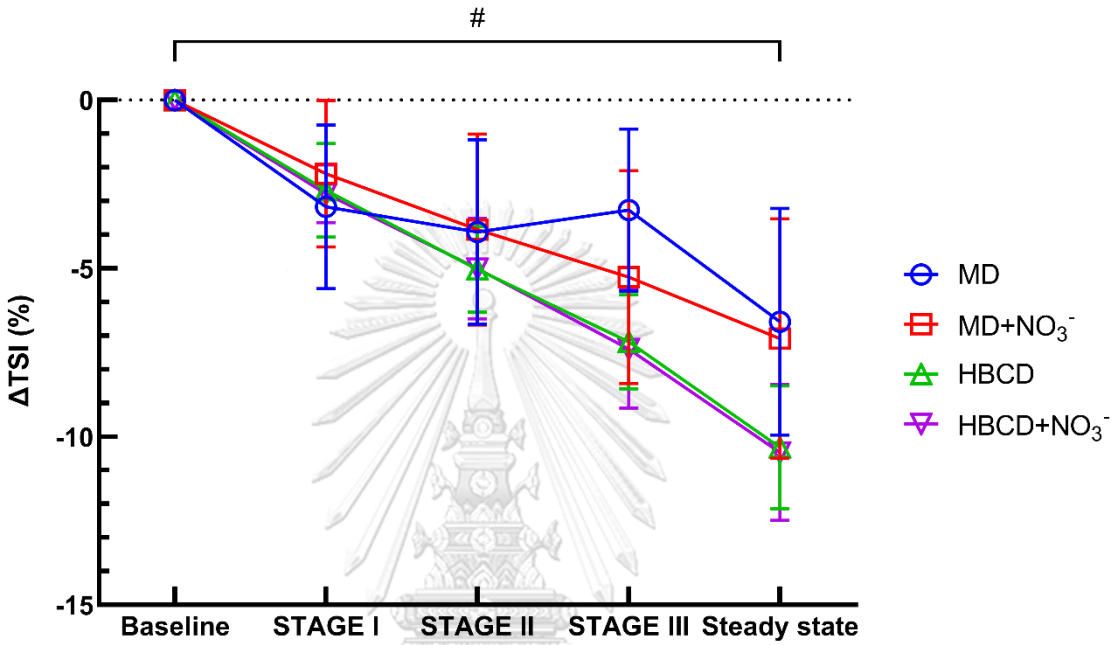

Figure 18 Mean ± SEM of the changes in tissue saturation index during running economy test among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9).

# Denotes significant differences over time ($p < 0.05$).

The changes in oxyhaemoglobin

Table 14 The comparison of the changes in oxyhaemoglobin among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9)

	ΔO_2Hb (μM)			
	MD	MD+NO_3^-	HBCD	HBCD+NO_3^-
STAGE I	-4.10 ± 0.87	-3.81 ± 1.09	-3.57 ± 1.14	-3.40 ± 0.93
STAGE II	-4.19 ± 2.52	-1.99 ± 0.93	-3.92 ± 2.20	-2.56 ± 1.10
STAGE III	-4.03 ± 3.39	-2.79 ± 1.14	-4.67 ± 2.70	-2.85 ± 1.29
Steady state	-5.29 ± 3.39	-3.46 ± 2.04	-4.68 ± 2.98	-3.31 ± 1.29

Data were expressed in mean ± SEM

There was no significant main effect for condition for mean ΔO_2Hb among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (p = 0.172, partial η^2 = 0.038). There was also no significant effect of time point for mean ΔO_2Hb (p = 0.758, partial η^2 = 0.009) and condition x time point interaction effect (p = 1.000, partial η^2 = 0.003) (Figure 18).

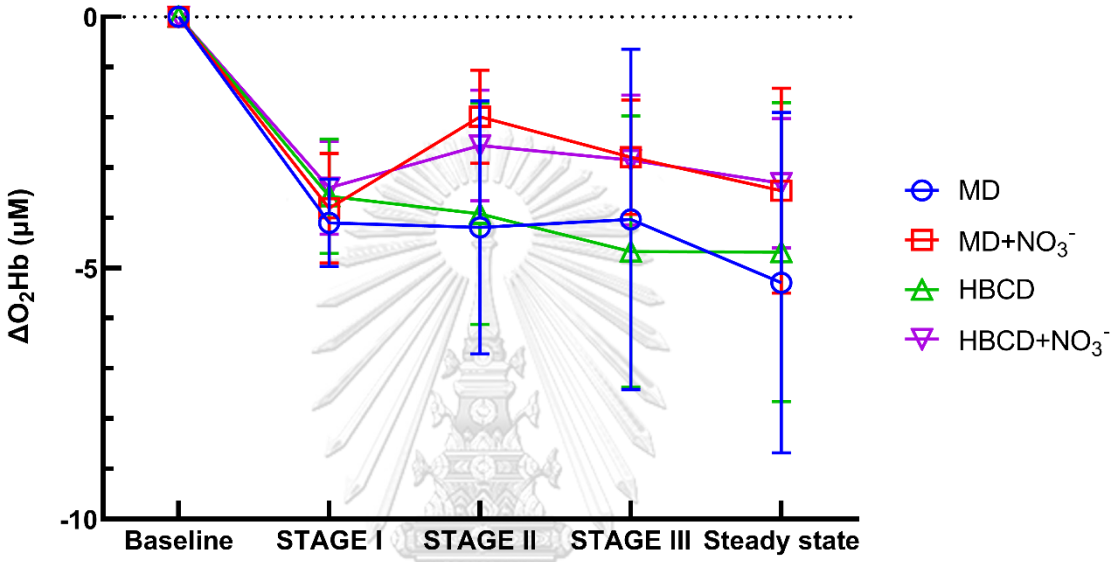

Figure 19 Mean ± SEM of the changes in oxyhaemoglobin during running economy test among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9).

The changes in deoxyhaemoglobin

Table 15 The comparison of the changes in deoxyhaemoglobin among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9)

	ΔHHb (μM)			
	MD	MD+NO_3^-	HBCD	HBCD+NO_3^-
STAGE I	1.42 ± 1.64	1.28 ± 1.26	1.05 ± 1.01	1.19 ± 1.01
STAGE II	0.92 ± 2.32	2.70 ± 2.22	1.41 ± 1.18	2.63 ± 1.32
STAGE III	0.77 ± 2.20	3.37 ± 2.80	3.11 ± 1.50	4.25 ± 1.47
Steady state	2.68 ± 2.65	5.02 ± 3.27	6.22 ± 1.97	6.93 ± 2.03

Data were expressed in mean ± SEM

There was no significant main effect for condition for mean ΔHHb between the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (p = 0.294, partial η^2 = 0.029). There was a significant effect of time point for mean ΔHHb (p = 0.009, partial η^2 = 0.085), when pairwise comparisons were conducted between time points, no statistically significant difference was found for mean ΔHHb. However, there was no significant condition x time point interaction effect (p = 0.997, partial η^2 = 0.012) (Figure 19).

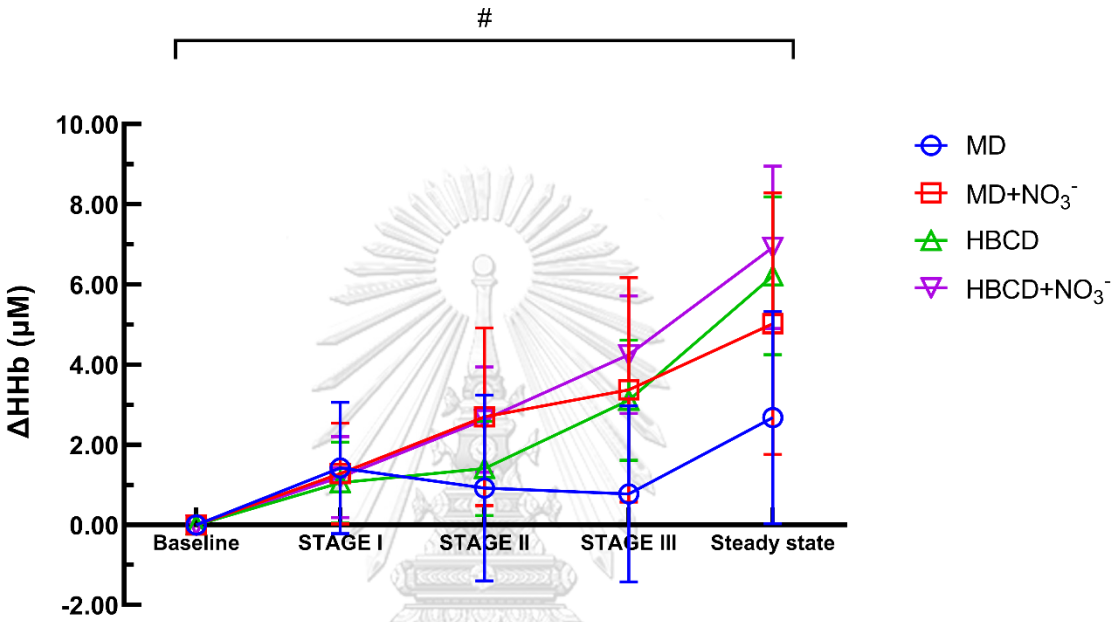

Figure 20 Mean ± SEM of the changes in deoxyhaemoglobin during running economy test among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9).

# Denotes significant differences over time ($p < 0.05$).

The changes in total haemoglobin

Table 16 The comparison of the changes in total haemoglobin among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9)

	ΔtHb (μM)			
	MD	MD+NO_3^-	HBCD	HBCD+NO_3^-
STAGE I	-4.91 ± 1.22	-3.03 ± 1.38	-3.64 ± 1.10	-2.97 ± 1.16
STAGE II	-3.27 ± 2.60	-0.80 ± 2.21	-2.51 ± 2.22	2.57 ± 1.65
STAGE III	-3.26 ± 4.04	-1.56 ± 2.91	-1.55 ± 2.59	4.20 ± 1.96
Steady state	-2.60 ± 4.70	-2.76 ± 3.35	1.53 ± 2.82	6.55 ± 2.35

Data were expressed in mean ± SEM

There was no significant main effect for condition for mean ΔtHb among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (p = 0.073, partial η^2 = 0.053). There was also no significant effect of time point for mean ΔtHb (p = 0.270, partial η^2 = 0.030) and condition x time point interaction effect (p = 0.982, partial η^2 = 0.018) (Figure 20).

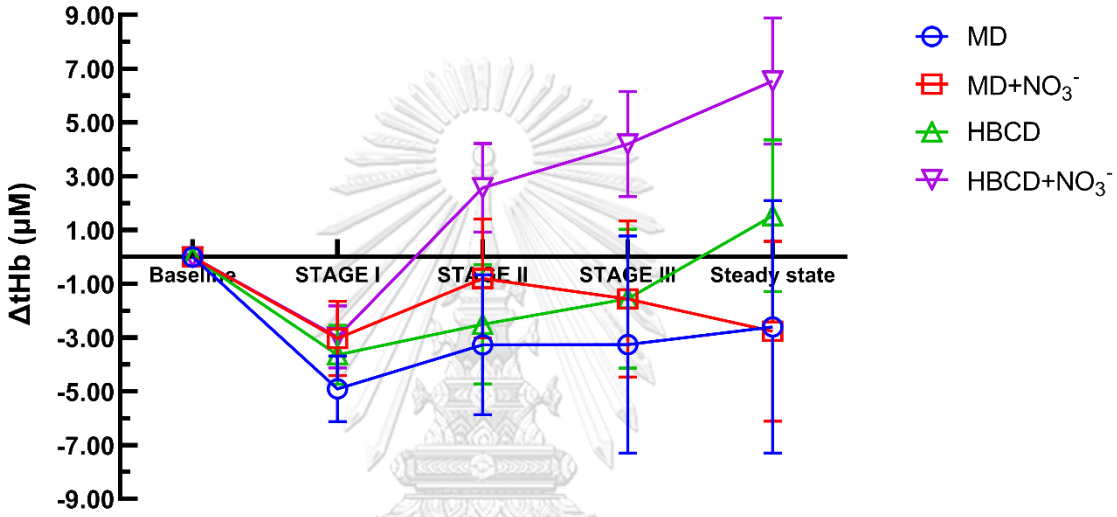

Figure 21 Mean ± SEM of the changes in total haemoglobin during running economy test among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9).

# Denotes significant differences over time ($p < 0.05$).

Part V Blood biochemistry variables data

Serum nitrate/nitrite concentration

Table 17 The comparison of serum nitrate/nitrite concentration data among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9)

	Serum nitrate/nitrite concentration (μM)			
	MD	MD+NO_3^-	HBCD	HBCD+NO_3^-
Baseline	6.59 ± 0.56	7.76 ± 0.56	7.45 ± 0.95	7.82 ± 0.88
30 min POST	6.79 ± 0.63	8.28 ± 0.50	7.58 ± 0.94	8.61 ± 0.84
PRE-EX	6.97 ± 0.64	8.59 ± 0.49	7.51 ± 0.93	8.98 ± 0.76
30 min RUN	7.45 ± 0.65	9.83 ± 0.55	7.58 ± 0.91	9.85 ± 0.81
60 min RUN	7.73 ± 0.58	10.69 ± 0.51	8.06 ± 0.90	11.04 ± 0.79
POST-EX	7.90 ± 0.42	11.00 ± 0.60	8.34 ± 1.05	11.08 ± 0.97

Data were expressed in mean ± SEM

There was a significant main effect for condition for mean serum nitrate/nitrite concentration among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions ($p < 0.001$, partial $\eta^2 = 0.193$), with a significant higher in the MD+NO_3^- condition than the MD condition ($p < 0.001$), the HBCD+NO_3^- condition higher than the MD condition ($p < 0.001$), as well as the MD+NO_3^- condition higher than the HBCD condition throughout the trend line ($p = 0.002$) (Figure 21). There was also a significant effect of time point for mean serum nitrate/nitrite concentration ($p < 0.001$, partial $\eta^2 = 0.137$). However, there was no significant condition x time point interaction effect ($p = 0.845$, partial $\eta^2 = 0.047$).

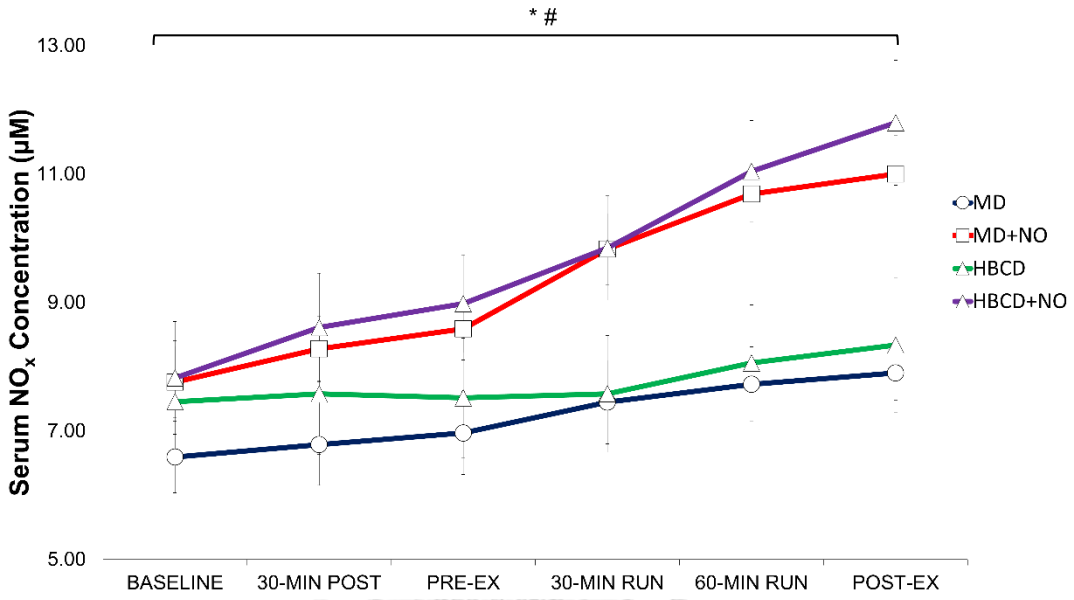

Figure 22 Mean ± SEM of serum nitrate/nitrite concentration response among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9).

* Denotes significant differences between condition, # significant differences over time ($p < 0.05$).

Serum glucose concentration

Table 18 The comparison of serum glucose concentration data among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9)

	Serum glucose concentration ($mg \cdot dL^{-1}$)			
	MD	MD+NO_3^-	HBCD	HBCD+NO_3^-
Baseline	78.89 ± 2.08	80.11 ± 3.95	70.33 ± 5.22	74.89 ± 3.89
30 min POST	114.67 ± 9.36*	115.78 ± 7.64*	109.89 ± 8.43*	117.67 ± 7.74*
PRE-EX	82.67 ± 7.19**	89.11 ± 7.51	106.44 ± 11.65*	71.33 ± 7.69**
30 min RUN	73.33 ± 3.24**	95.44 ± 6.59	76.56 ± 4.53** #	79.33 ± 3.45**
60 min RUN	96.00 ± 5.47	98.22 ± 7.24	89.89 ± 5.63	96.78 ± 4.23
POST-EX	94.89 ± 5.91	99.11 ± 5.06	91.22 ± 6.05	95.33 ± 6.31

Data were expressed in mean ± SEM

* Denotes significant different compared with baseline within condition ($p < 0.05$)

** Denotes significant different compared with 30 min POST within condition ($p < 0.05$)

# Denotes significant different compared with PRE-EX within condition ($p < 0.05$)

There was no significant main effect for condition for mean serum glucose concentration among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions ($p = 0.221$, partial $\eta^2 = 0.023$). There was a significant effect of time point for mean serum glucose concentration ($p < 0.001$, partial $\eta^2 = 0.315$). However, there was no significant condition x time point interaction effect ($p = 0.121$, partial $\eta^2 = 0.103$) (Figure 21).

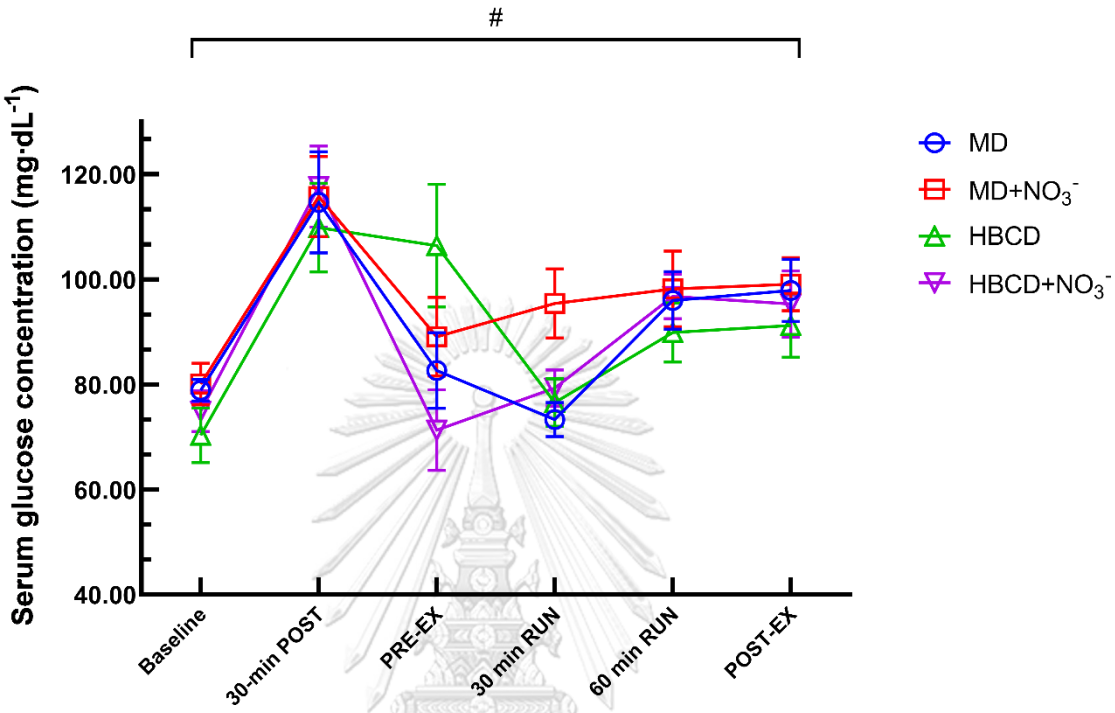

Figure 23 Mean ± SEM of serum glucose concentration response among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9).

# Denotes significant differences over time ($p < 0.05$).

Serum insulin concentration

Table 19 The comparison of serum insulin concentration data among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9)

	Serum insulin concentration ($\mu U \cdot mL^{-1}$)			
	MD	MD+NO_3^-	HBCD	HBCD+NO_3^-
Baseline	7.04 ± 1.09	7.57 ± 1.90	6.06 ± 0.83	6.58 ± 1.20
30 min POST	40.48 ± 6.10*	48.86 ± 9.24*	44.08 ± 7.67*	48.31 ± 6.00*
PRE-EX	29.16 ± 4.02*	22.09 ± 3.07**	36.26 ± 5.66*	25.14 ± 3.80* **
30 min RUN	4.17 ± 0.34** #	6.33 ± 1.54** #	4.97 ± 0.43** #	4.04 ± 0.34** #
60 min RUN	4.53 ± 0.97** #	4.37 ± 1.06** #	4.70 ± 0.94** #	3.94 ± 0.62** #
POST-EX	3.94 ± 0.84** #	3.37 ± 0.67** #	3.41 ± 0.31** #	3.46 ± 0.77** #

Data were expressed in mean ± SEM

* Denotes significant different compared with baseline within condition ($p < 0.05$)

** Denotes significant different compared with 30 min POST within condition ($p < 0.05$)

# Denotes significant different compared with PRE-EX within condition ($p < 0.05$)

There was no significant main effect for condition for mean serum insulin concentration among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions ($p = 0.860$, partial $\eta^2 = 0.004$). There was a significant effect of time point for mean serum insulin concentration ($p < 0.001$, partial $\eta^2 = 0.712$). However, there was no significant condition x time point interaction effect ($p = 0.666$, partial $\eta^2 = 0.060$) (Figure 22).

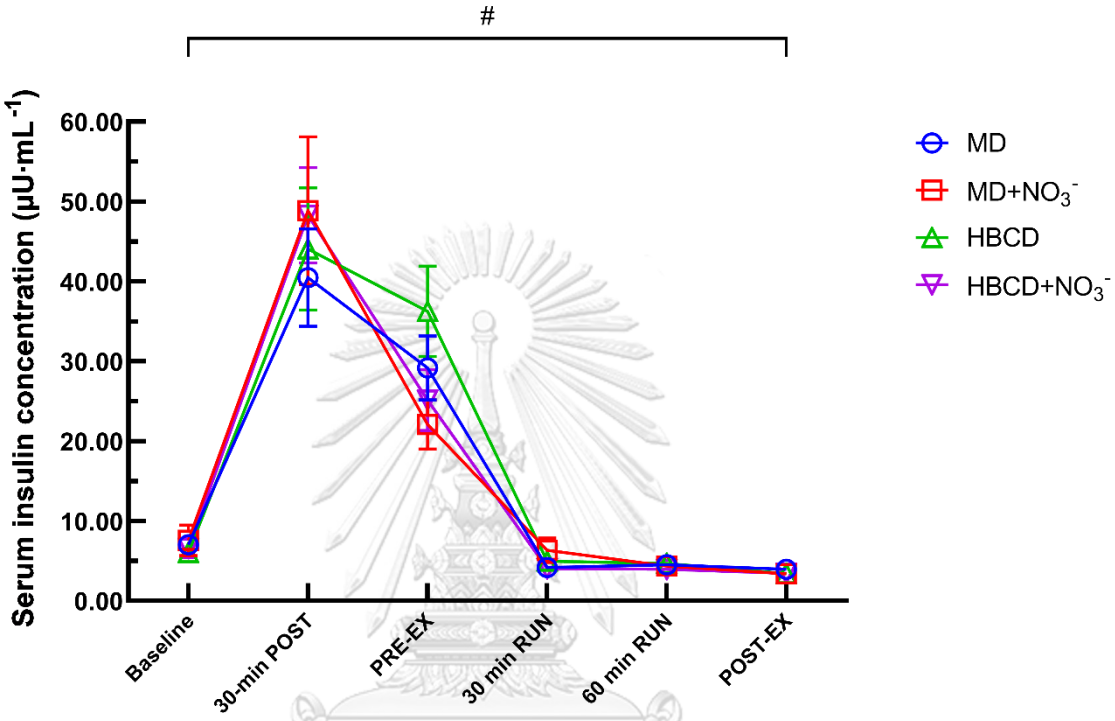

Figure 24 Mean ± SEM of serum insulin concentration response among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9).

# Denotes significant differences over time ($p < 0.05$).

Blood lactate concentration

Table 20 The comparison of blood lactate concentration data among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9)

	Blood lactate concentration ($mmol \cdot L^{-1}$)			
	MD	MD+NO_3^-	HBCD	HBCD+NO_3^-
Baseline	1.02 ± 0.24	0.98 ± 0.18	1.01 ± 0.24	0.71 ± 0.09
30 min POST	0.93 ± 0.13	0.99 ± 0.13	0.90 ± 0.11	1.12 ± 0.13
PRE-EX	1.30 ± 0.34	1.27 ± 0.19	1.34 ± 0.18	1.11 ± 0.13
30 min RUN	2.18 ± 0.28* **	2.18 ± 0.28* **	2.34 ± 0.31* **	2.22 ± 0.21* ** #
60 min RUN	2.16 ± 0.23* **	2.12 ± 0.26* **	2.36 ± 0.34* ** #	1.98 ± 0.27*
POST-EX	2.03 ± 0.21* **	1.89 ± 0.26	2.12 ± 0.30* **	2.14 ± 0.41* ** #

Data were expressed in mean ± SEM

* Denotes significant different compared with baseline within condition ($p < 0.05$)

** Denotes significant different compared with 30 min POST within condition ($p < 0.05$)

# Denotes significant different compared with PRE-EX within condition ($p < 0.05$)

There was no significant main effect for condition for mean blood lactate concentration among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions ($p = 0.795$, partial $\eta^2 = 0.005$). There was a significant effect of time point for mean blood lactate concentration ($p < 0.001$, partial $\eta^2 = 0.399$). However, there was no significant condition x time point interaction effect ($p = 0.999$, partial $\eta^2 = 0.017$) (Figure 23).

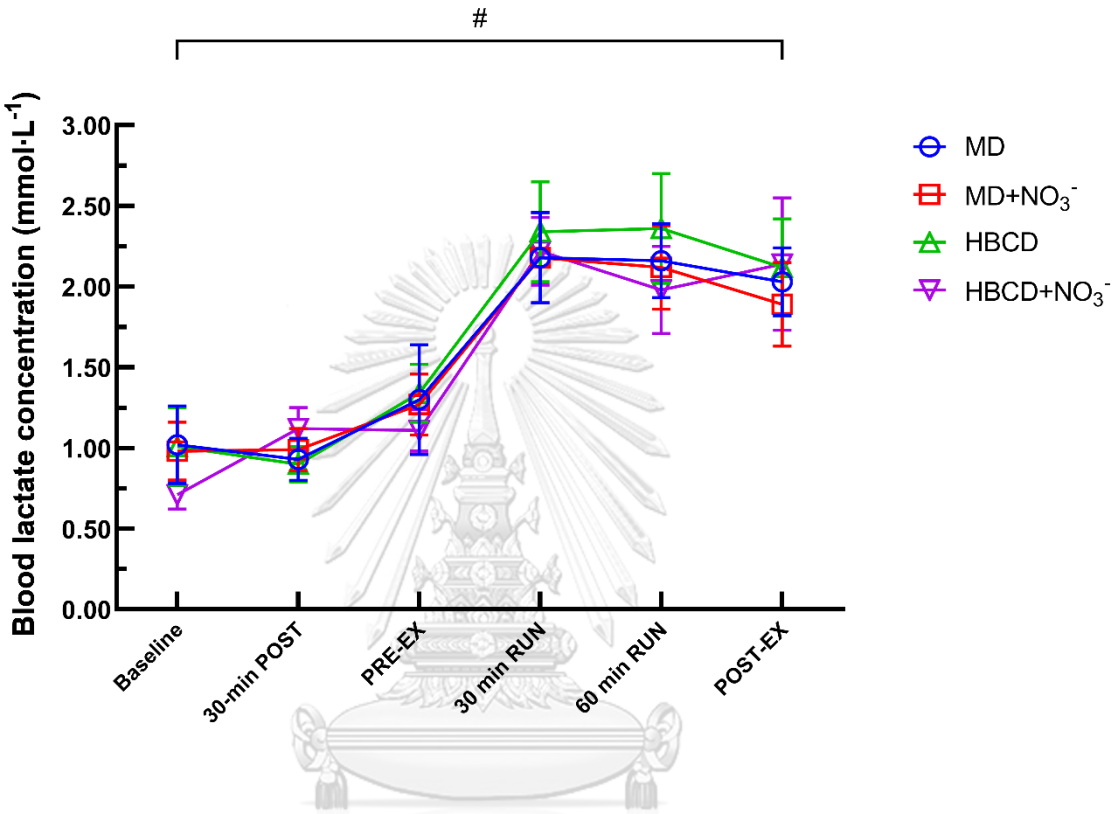

Figure 25 Mean ± SEM of blood lactate concentration response among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9).

# Denotes significant differences over time ($p < 0.05$).

Part VI Heart rate variables data

Heart rate

Table 21 The comparison of the heart rate response among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9)

		Heart rate (bpm)			
		MD	MD+NO_3^-	HBCD	HBCD+NO_3^-
PRE-EX		70.22 ± 3.60	77.22 ± 2.56	74.78 ± 4.70	71.56 ± 2.54
RE	STAGE I	115.33 ± 3.55*	115.00 ± 4.0*	106.22 ± 7.26*	118.22 ± 5.73*
	STAGE II	132.56 ± 4.62*	131.00 ± 5.85*	130.78 ± 4.86*	128.67 ± 3.24*
	STAGE III	141.00 ± 4.56* **	144.67 ± 4.45* **	137.44 ± 5.37* **	140.56 ± 3.44* **
30 min RUN		147.11 ± 4.89* **	151.44 ± 4.38* **	141.67 ± 7.29* **	146.33 ± 4.65* **
60 min RUN		153.56 ± 5.18* **	157.33 ± 5.28* ** #	149.56 ± 7.94* **	150.44 ± 4.55* **
POST-EX		152.67 ± 5.74* **	158.00 ± 5.30* ** #	148.22 ± 8.09* **	151.00 ± 3.42 * ** #

Data were expressed in mean ± SEM

* Denotes significant different compared with PRE-EX within condition ($p < 0.05$)

** Denotes significant different compared with STAGE I within condition ($p < 0.05$)

# Denotes significant different compared with STAGE II within condition ($p < 0.05$)

Heart rate tended to increase over the duration of the trial, with the MD+NO_3^- condition showing a higher rate of increase compared to MD, HBCD+NO_3^- and HBCD, respectively. There was a significant effect of time point for mean heart rate ($p < 0.001$, partial $\eta^2 = 0.770$). However, there was no significant main effect for condition for mean heart rate among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions ($p = 0.118$, partial $\eta^2 = 0.026$) and no significant condition x time point interaction effect ($p = 0.999$, partial $\eta^2 = 0.021$).

Part VII Psychometric variables data

Rate of perceived exertion

Table 22 The comparison of the rate of perceived exertion among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9)

		RPE (score)			
		MD	MD+NO_3^-	HBCD	HBCD+NO_3^-
PRE-EX		6.1 ± 0.1	6.1 ± 0.1	6.1 ± 0.1	6.1 ± 0.1
RE	STAGE I	8.3 ± 0.4	8.2 ± 0.5	9.1 ± 0.7	8.0 ± 0.4
	STAGE II	10.3 ± 0.6	10.1 ± 0.8	10.4 ± 0.8	9.3 ± 0.5
	STAGE III	11.3 ± 0.7	11.7 ± 0.8	11.8 ± 0.8	10.6 ± 0.5
30 min RUN		13.1 ± 0.7	13.0 ± 0.7	12.6 ± 0.8	11.8 ± 0.6
60 min RUN		14.2 ± 0.6	14.1 ± 0.6	13.7 ± 0.8	13.0 ± 0.8
POST-EX		14.9 ± 0.6	14.4 ± 0.6	14.7 ± 0.7	14.1 ± 0.7

Data were expressed in mean ± SEM

RPE score tended to increase over the duration of the trial, with the MD condition showing a higher rate of increase compared to MD+NO_3^-, HBCD, and HBCD+NO_3^-, respectively (Figure 24). There was a significant effect of time point for mean RPE score ($p < 0.001$, partial $\eta^2 = 0.692$). However, there was no significant main effect for condition for mean RPE score among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions ($p = 0.079$, partial $\eta^2 = 0.030$) and no significant condition x time point interaction effect ($p = 1.000$, partial $\eta^2 = 0.018$).

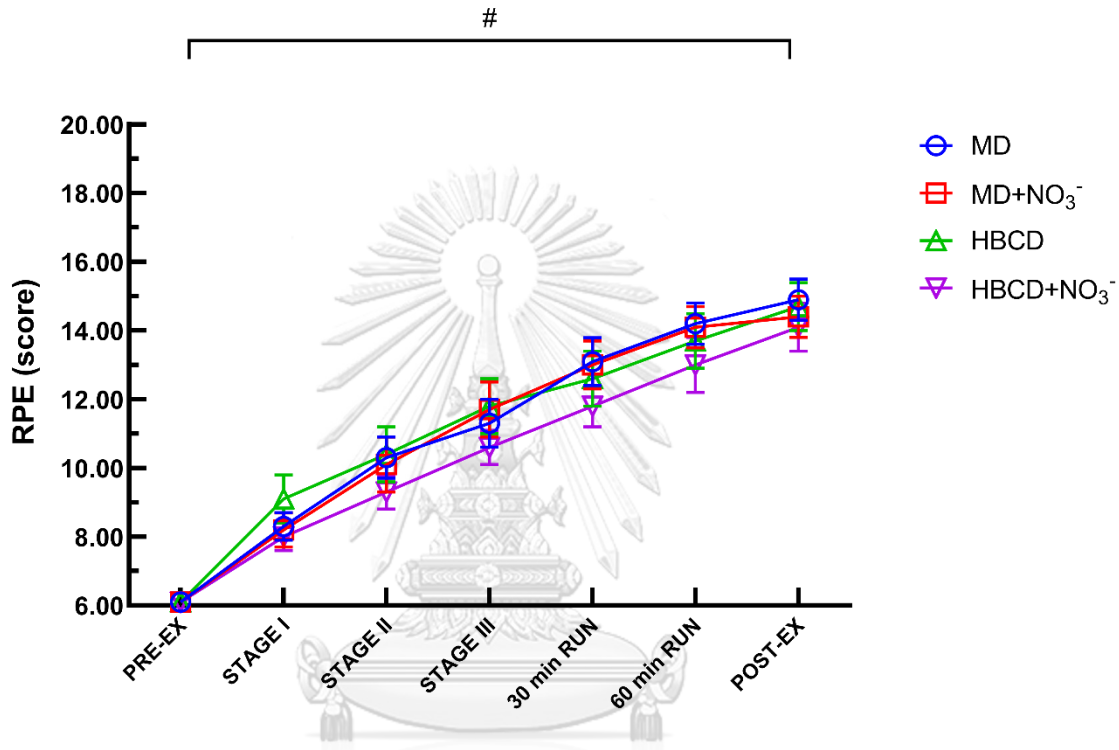

Figure 26 Mean ± SEM of RPE score among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9).

# Denotes significant differences over time ($p < 0.05$).

Gastrointestinal symptoms: Nausea

Table 23 The comparison of visual analogue scale score for nausea among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9)

	Visual analog scale score for nausea (mm)			
	MD	MD+NO_3^-	HBCD	HBCD+NO_3^-
Baseline	0.3 ± 0.2	0.3 ± 0.2	0.4 ± 0.3	0.4 ± 0.3
30 min POST	0.8 ± 0.5	0.4 ± 0.3	1.7 ± 1.0	1.7 ± 1.0
PRE-EX	1.7 ± 0.8	1.4 ± 0.7	1.7 ± 0.8	1.4 ± 0.7
POST-EX	1.7 ± 0.8	1.3 ± 0.7	1.7 ± 0.8	1.4 ± 0.7

Data were expressed in mean ± SEM

There was no significant main effect for condition for mean visual analogue scale score of nausea among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions ($p = 0.814$, partial $\eta^2 = 0.007$). There was also no significant effect of time point for mean visual analogue scale score of nausea ($p = 0.086$, partial $\eta^2 = 0.050$), and no significant condition x time point interaction effect ($p = 0.996$, partial $\eta^2 = 0.012$).

Gastrointestinal symptoms: Vomiting

Table 24 The comparison of visual analogue scale score for vomiting among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9)

	Visual analog scale score for vomiting (mm)			
	MD	MD+NO_3^-	HBCD	HBCD+NO_3^-
Baseline	0.3 ± 0.2	0.1 ± 0.1	0.8 ± 0.4	0.4 ± 0.3
30 min POST	0.8 ± 0.5	0.4 ± 0.3	0.9 ± 0.4	0.6 ± 0.4
PRE-EX	1.0 ± 0.6	0.7 ± 0.7	1.7 ± 0.8	0.8 ± 0.5
POST-EX	1.0 ± 0.6	0.4 ± 0.4	1.7 ± 0.8	0.8 ± 0.5

Data were expressed in mean ± SEM

There was no significant main effect for condition for mean visual analogue scale score of vomiting among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (p = 0.191, partial η^2 = 0.036). There was also no significant effect of time point for mean visual analogue scale score of vomiting (p = 0.284, partial η^2 = 0.029), and no significant condition x time point interaction effect (p = 1.000, partial η^2 = 0.006).

Gastrointestinal symptoms: Stomach fullness

Table 25 The comparison of visual analogue scale score for stomach fullness among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9)

	Visual analog scale score for stomach fullness (mm)			
	MD	MD+NO_3^-	HBCD	HBCD+NO_3^-
Baseline	0.9 ± 0.5	0.7 ± 0.5	0.4 ± 0.3	0.4 ± 0.3
30 min POST	5.9 ± 4.2	3.6 ± 2.2	0.6 ± 0.4	0.6 ± 0.4
PRE-EX	4.3 ± 1.9	3.3 ± 2.1	2.3 ± 0.9	1.4 ± 0.7
POST-EX	4.3 ± 1.9	1.3 ± 0.7	2.8 ± 0.9	1.4 ± 0.7

Data were expressed in mean ± SEM

There was no significant main effect for condition for mean visual analogue scale score of stomach fullness among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (p = 0.077, partial η^2 = 0.052). There was also no significant effect of time point for mean visual analogue scale score of stomach fullness (p = 0.193, partial η^2 = 0.036), and no significant condition x time point interaction effect (p = 0.875, partial η^2 = 0.034).

Gastrointestinal symptoms: Abdominal pain

Table 26 The comparison of visual analogue scale score for abdominal pain among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9)

	Visual analog scale score for abdominal pain (mm)			
	MD	MD+NO_3^-	HBCD	HBCD+NO_3^-
Baseline	0.3 ± 0.2	0.3 ± 0.2	0.4 ± 0.3	0.6 ± 0.4
30 min POST	0.8 ± 0.5	2.4 ± 2.1	0.6 ± 0.4	0.7 ± 0.4
PRE-EX	1.1 ± 0.7	2.1 ± 1.6	1.2 ± 0.6	0.9 ± 0.6
POST-EX	1.1 ± 0.7	1.4 ± 1.0	1.2 ± 0.6	0.9 ± 0.6

Data were expressed in mean ± SEM

There was no significant main effect for condition for mean visual analogue scale score of abdominal pain among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (p = 0.516, partial η^2 = 0.018). There was also no significant effect of time point for mean visual analogue scale score of abdominal pain (p = 0.477, partial η^2 = 0.019), and no significant condition x time point interaction effect (p = 0.989, partial η^2 = 0.016).

Gastrointestinal symptoms: Heartburn

Table 27 The comparison of visual analogue scale score for heartburn among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9)

	Visual analog scale score for heartburn (mm)			
	MD	MD+NO_3^-	HBCD	HBCD+NO_3^-
Baseline	0.3 ± 0.2	0.1 ± 0.1	0.4 ± 0.3	0.6 ± 0.4
30 min POST	1.8 ± 1.1	0.4 ± 0.3	0.6 ± 0.4	0.7 ± 0.4
PRE-EX	1.1 ± 0.7	1.6 ± 1.1	1.3 ± 0.6	0.9 ± 0.6
POST-EX	1.1 ± 0.7	0.7 ± 0.4	1.3 ± 0.6	0.9 ± 0.6

Data were expressed in mean ± SEM

There was no significant main effect for condition for mean visual analogue scale score of heartburn among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (p = 0.819, partial η^2 = 0.007). There was also no significant effect of time point for mean visual analogue scale score of heartburn (p = 0.267, partial η^2 = 0.030), and no significant condition x time point interaction effect (p = 0.942, partial η^2 = 0.026).

Gastrointestinal symptoms: Bloating

Table 28 The comparison of visual analogue scale score for bloating among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (n = 9)

	Visual analog scale score for bloating (mm)			
	MD	MD+NO_3^-	HBCD	HBCD+NO_3^-
Baseline	0.3 ± 0.2	0.3 ± 0.2	0.4 ± 0.3	0.7 ± 0.5
30 min POST	0.8 ± 0.5	1.6 ± 1.0	0.6 ± 0.4	0.8 ± 0.5
PRE-EX	1.1 ± 0.7	1.6 ± 1.1	1.2 ± 0.6	1.0 ± 0.6
POST-EX	1.1 ± 0.7	0.7 ± 0.4	1.2 ± 0.6	1.0 ± 0.6

Data were expressed in mean ± SEM

There was no significant main effect for condition for mean visual analogue scale score of bloating among the MD, MD+NO_3^-, HBCD, and HBCD+NO_3^- conditions (p = 0.974, partial η^2 = 0.002). There was also no significant effect of time point for mean visual analogue scale score of bloating (p = 0.402, partial η^2 = 0.023), and no significant condition x time point interaction effect (p = 0.990, partial η^2 = 0.016).

CHAPTER V
DISCUSSION AND CONCLUSION

The aim of this study was to examine the effects of co-ingesting highly branched cyclic dextrin (HBCD) and dietary nitrate on physiological responses and endurance capacity in recreational endurance runners. The researchers compared the outcomes with those of an isocaloric HBCD beverage, a maltodextrin-dietary nitrate beverage, and a maltodextrin beverage. The study focused on recreational endurance runners and aimed to determine how the combination of HBCD and dietary nitrate influenced various physiological responses during exercise. Additionally, the researchers sought to evaluate the effects of this combination on endurance capacity, comparing it to the effects of consuming an isocaloric HBCD beverage, a maltodextrin-dietary nitrate beverage, or a maltodextrin beverage alone.

No differences were found among beverage conditions in the responses of $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}E$, total CHO oxidation rate, oxygen cost, changes in muscle oxygenation parameters, serum glucose concentration, serum insulin concentration, and blood lactate concentration during high-intensity prolonged running. However, only HBCD ingestion led to an increase in fat oxidation rate and, consequently, a lower respiratory exchange ratio (RER) throughout the exercise compared to maltodextrin and co-ingesting HBCD with dietary nitrate. Additionally, subjective psychometric measures of rating of perceived exertion (RPE) and gastrointestinal discomfort did not differ among the conditions.

The findings of the study indicate that the co-ingestion of HBCD with dietary NO_3^- did not have a significant impact on serum insulin concentration, serum glucose concentration, or blood lactate concentration when compared to the consumption of HBCD alone, co-ingesting maltodextrin with dietary nitrate (MD+NO_3^-), or maltodextrin alone (MD). These results suggest that the combination of HBCD and dietary NO_3^- does not induce discernible metabolic effects on these particular parameters. The role of blood glucose levels in exercise, particularly after prolonged workouts, is crucial. Consumption of CHO is essential to maintain adequate blood glucose concentrations and prevent

hypoglycaemia. It is well-established that CHO intake directly affects blood glucose levels and insulin secretion. HBCD, a large molecule that needs to be hydrolysed into glucose, is absorbed gradually, leading to lower glycaemic and insulin responses compared to monosaccharides. The decreased insulin response associated with HBCD may help in maintaining stable blood glucose levels and utilizing energy substrates efficiently (20). Supporting previous studies, the administration of 500 mg HBCD·kg^{-1}body weight during an endurance swimming protocol in mice resulted in improved endurance. This enhancement in endurance was accompanied by the maintenance of energy substrate oxidation during exercise. Furthermore, HBCD ingestion led to lower insulin responsiveness, resulting in a slower and smaller increase in postprandial blood glucose levels compared to glucose (monosaccharide) (20). However, when comparing the effects of HBCD and maltodextrin, both polysaccharides, no significant differences were observed in blood glucose concentrations at the measured time points between the HBCD and maltodextrin drinks (23). Interestingly, the study found that the differences in serum glucose concentrations between conditions were no evident during high-intensity prolong exercise. Similarly, there were no significant variations in serum insulin concentrations observed in this study. These findings align with a study by Chuychai et al. (78), which demonstrated that HBCD ingestion did not significantly differ from glucose in terms of their impact on blood glucose levels throughout the trial period, indicating no significant differences in serum glucose concentration and insulin responsiveness between MD and HBCD, both being polysaccharides; however, contrasting results were observed in the comparison between MD (polysaccharides) and glucose (monosaccharide), suggesting that the variations in their structural composition may contribute to these contrasting outcomes. However, further research is required to directly compare the effects of MD and HBCD with those of glucose in order to obtain a more comprehensive understanding of the variations and similarities in terms of serum glucose concentration and insulin responsiveness. Conducting such investigations would provide valuable insights into the specific impact of these polysaccharides on glucose metabolism and insulin regulation during exercise. Furthermore, the research on the

combined consumption of CHO with dietary NO_3^- remains limited, despite the logical and plausible nature of this combination. Only one study, conducted by Betteridge et al. (2016), has investigated the potential interaction between dietary nitrate and glucose/glycogen metabolism during exercise. In this study, healthy recreationally active males were given beetroot juice containing approximately 8 mmol of NO_3^- before participating in a 60-minute prolonged exercise session on an ergometer at 65% of $\dot{V}O_2$max. During the exercise, glucose was infused to measure its metabolic fate. The results of this study indicated that the ingestion of beetroot juice had no effect on $\dot{V}O_2$, glucose disposal, or muscle metabolites during submaximal exercise. Furthermore, the study also reported no impact of beetroot juice on the efficiency of oxygen consumption during exercise (115).

Acute ingestion of 5.2 – 16.8 mmol of dietary nitrate (NO_3^-) within a timeframe of 1 to 3 hours has been shown to elevate plasma nitrite concentration ($[NO_2^-]$). This increase in $[NO_2^-]$ has been associated with potential reductions in the oxygen cost (EO_2), running economy indicator, of submaximal exercise (116) and improvements in exercise tolerance (117) in certain studies. The immediate effects of dietary nitrate are believed to be mediated through the elevation of $[NO_2^-]$) (99, 118). The previous study revealed that the consumption of 6 mmol of dietary nitrate resulted in an increase in plasma concentrations of both plasma nitrate ($[NO_3^-]$) and nitrite ($[NO_2^-]$). This increase in $[NO_3^-]$ and $[NO_2^-]$ levels was associated with a noticeable trend towards a reduction in the oxygen cost (EO_2) of exercise following acute ingestion of dietary nitrate after a 2-hour period (119). One of the hypotheses proposed in this study is that the consumption of a single acute dose (1 hour) of the co-ingestion of HBCD and dietary nitrate (~8.0 mmol NO_3^-) beverage would lead to an enhancement in endurance capacity among recreational endurance runners. This anticipated improvement is based on the notion that the combination of HBCD and dietary nitrate would provide an additional exogenous energy source and reduce the $\dot{V}O_2$ required for a given workload. The study specifically investigated this hypothesis by conducting a 60-minute constant workload running session at a velocity equivalent to 70% of the $\dot{V}O_2$peak. The researchers aimed to examine whether a reduction in $\dot{V}O_2$ uptake

would indicate a more efficient utilization of energy resources during exercise. They expected that this enhanced efficiency would result in improved performance and the ability to cover longer distances with reduced effort. Contrary to the researchers' expectations, the study's findings indicated that the co-ingestion of HBCD+NO_3^- did not have a significant impact on $\dot{V}O_2$ uptake, $\dot{V}CO_2$ production, and oxygen cost (EO_2) during high-intensity prolonged running. These results were observed when comparing the effects of HBCD+NO_3^- to those of HBCD alone, MD+NO_3^-, and MD. Therefore, it can be concluded that the consumption of HBCD and dietary nitrate together does not lead to a distinct alteration in energy expenditure during exercise compared to the other conditions. This was surprising considering that a previous study had reported lower $\dot{V}O_2$ during exercise after the administration of an acute dose of dietary NO_3^-. The primary influence of dietary NO_3^- supplementation on the cardiovascular system is closely associated with the vasodilator properties of nitric oxide (NO). The vasodilation induced by NO leads to an increase in the proportion of oxygenated haemoglobin (O_2Hb) within the muscle and a reduction in the overall rate of $\dot{V}O_2$ uptake by the body (120). The previous studies have indicated that acute consumption of dietary NO_3^- can have positive effects on various parameters of endurance performance. RE, for example, is a parameter that reflects the relationship between $\dot{V}O_2$, power generated, and the distance covered by endurance runners (121). The mentioned study demonstrated that the $\dot{V}O_2$ uptake during endurance exercise predominantly requiring aerobic energy production, following the acute ingestion of approximately 4.8 mmol of dietary NO_3^-, was significantly lower compared to the placebo group. This reduction in $\dot{V}O_2$ led to an improvement in exercise economy, which aligns with the findings of an earlier study, where a reduction in $\dot{V}O_2$ (approximately 3%) during endurance exercise after the acute ingestion of 5.0 mmol of dietary NO_3^- (122). While there were no significant additional effects for condition on RE during high-intensity prolonged running following NO_3^- co-ingestion with MD in the current study, which is consistent with previous research with moderate-trained runners (123).

From the results of this study, the lack of significant improvements in RE suggests that acute NO_3^- co-ingestion with MD may not be an effective strategy for enhancing

running economy in recreational male marathon runners. However, several factors contribute to the absence of improvements in oxygen cost and muscle oxygenation following a single dose of dietary NO_3^- supplementation. Firstly, the timing of nitrate consumption in relation to exercise is crucial. Research suggests that prolonged or repeated nitrate supplementation over time yields more noticeable benefits compared to a single dose, allowing the body to maximize its effects (26, 119). Secondly, individual variations in NO_3^- metabolism and response to supplementation influence the outcomes. Some individuals may not respond as effectively to NO_3^- supplementation due to genetic differences, baseline NO levels, or gut microbiota composition (124). Furthermore, the study design and participant characteristics also impact the results. Factors such as the specific dosage and duration of NO_3^- supplementation, as well as the fitness level and training status of the participants, can influence the outcomes (125). High-intensity exercise is a complex physiological challenge, and the effects of NO_3^- supplementation may vary based on exercise type and duration.

The study provided insights into the effects of co-ingesting HBCD with dietary NO_3^- and in HBCD alone, MD+NO_3^-, or MD alone on fat utilization and the respiratory exchange ratio (RER). The respiratory exchange ratio (RER) is a metric that compares the amount of $\dot{V}CO_2$ produced to the amount of $\dot{V}O_2$ consumed during metabolism, offering information about the predominant fuel source used for energy production. A lower RER indicates a higher reliance on fat oxidation, whereas a higher RER indicates a preference for carbohydrate metabolism (126). The lower RER observed in the condition of ingesting HBCD in this study suggests an increased utilization of fats as an energy substrate. This finding can be attributed to the characteristics of HBCD, which are complex carbohydrates requiring hydrolysis before glucose absorption (77). The gradual absorption of glucose from these sources can lead to a sustained supply of fatty acids as an alternative fuel source, thereby resulting in a lower RER. The results of this study showed that HBCD ingestion alone resulted in distinct effects on fat utilization and the RER compared to the other conditions. However, there were no significant differences

observed in total CHO oxidation rate among the different treatments. These findings indicate that the co-ingestion of HBCD with dietary NO_3^- may not have a substantial impact on the body's utilization of fats during exercise or the modification of RER. This suggests that the metabolic response to HBCD, in terms of fat utilization and respiratory parameters, may differ when combined with other substances such as NO_3^- or MD. However, it is important to note that the oxidation of carbohydrates remained consistent across all conditions. These results highlight the need for further research to explore the underlying mechanisms and potential implications of combining HBCD with other substances on fat metabolism and respiratory responses during exercise. By gaining a deeper understanding of these interactions, we can better optimize nutritional strategies for individuals seeking to enhance their exercise performance and metabolic responses.

Muscle oxygenation plays a vital role in determining muscle function and endurance capacity during exercise. The study examined the effects of consuming HBCD+NO_3^- on the levels of oxyhemoglobin (ΔO_2Hb), deoxyhemoglobin (ΔHHb), total hemoglobin (ΔtHb), and tissue oxygen saturation (ΔTSI) in the vastus lateralis muscle during running economy and a 60-minute constant load running session using near-infrared spectroscopy (NIRS), a non-invasive method commonly employed in both laboratory and sports settings to assess skeletal muscle oxygenation and hemodynamics (127). By analyzing the absorption of reflected light, NIRS enables the quantification of oxygenated hemoglobin and myoglobin (O_2Hb), deoxygenated hemoglobin and myoglobin (HHb), and tissue saturation index (TSI) within the active muscles during exercise. The changes observed in the NIRS signal provide valuable insights into the dynamic equilibrium between oxygen transport and utilization in muscle tissues (128).

The average ΔTSI exhibited a notable decrease, and the ΔO_2Hb showed a tendency to decrease throughout the trial, indicating an increased demand for oxygen in relation to its supply in the active muscle tissue (the vastus lateralis). The results showed that there were no significant differences in these parameters when HBCD+NO_3^- was

consumed compared to when HBCD was consumed alone, along with MD+NO_3^-, or MD. These findings suggest that the co-ingestion of CHO with NO_3^- in the form of both MD and HBCD does not have a noticeable impact on the ability of blood to carry oxygen, as indicated by changes in hemoglobin concentrations (ΔO_2Hb, ΔHHb, ΔtHb), as well as tissue oxygen saturation (ΔTSI). This suggests that the oxygen-carrying capacity of the blood and tissue oxygenation remain relatively unaffected by the combined consumption of CHO and NO_3^- in these forms. Furthermore, our findings revealed that the average ΔHHb, serving as a proxy measure of oxygen extraction, and the ΔtHb, which provides an estimate of local blood flow, both exhibited a tendency to increase during all conditions. However, no statistically significant difference was observed between the conditions, indicating a similar response in terms of oxygen extraction and local blood flow regardless of the co-ingestion of dietary NO_3^-. The results of this study are consistent with previous research. The acute supplementation of NO_3^- via beetroot juice, with a dosage of approximately 18.6 mmol NO_3^-, did not lead to an improvement in severe-intensity exercise tolerance under normoxic conditions. However, an improvement was observed under hypoxic conditions, and this improvement exhibited a negative correlation with the quadriceps TSI. These findings indicate that individuals who experienced greater levels of quadriceps deoxygenation in a hypoxic environment demonstrated the most significant enhancement in exercise tolerance when NO_3^- was ingested (129).

From the result of this study provide valuable insights into the potential effects of CHO and NO_3^- co-ingestion on oxygen dynamics during exercise. While further research is needed to explore the underlying mechanisms and potential interactions, these findings suggest that the combined consumption of CHO and NO_3^- in the form of MD and HBCD may not significantly impact oxygen transport and tissue oxygenation.

The simultaneous ingestion of NO_3^- and HBCD or MD requires careful consideration of the disparate timing of their intake, as well as the timing of pre-exercise

meals. This consideration is important to optimize the potential synergistic effects of these dietary components on exercise performance and metabolic responses. Understanding the temporal aspects of dietary NO_3^- and CHO intake in relation to exercise sessions is essential for maximizing their benefits and minimizing any potential interference or conflicting effects. The effect of NO_3^- supplementation on exercise performance was found to vary depending on the timing of supplementation relative to the exercise session. A meta-analysis indicated that the timing for NO_3^- supplementation ranged from 5 to 210 minutes before exercise, with the optimal timing identified as 2 to 3.5 hours prior to exercise (33). This timing was associated with the highest peak plasma concentration of NO_3^-, suggesting a potential link between supplementation timing and its ergogenic effects.

In contrast, guidelines promoting high CHO availability for optimizing performance before endurance events recommend consuming carbohydrates 1-4 hours prior to exercise (66, 74). Additionally, classic evidence further supports the idea that ingesting CHO at least 60 minutes before high-intensity prolonged exercise is considered appropriate and does not have an adverse effect on endurance performance (130, 131). Additionally, the unique characteristics of HBCD, such as its large molecule size and lower glycaemic and insulin responses, may require a different approach when co-ingesting it with dietary nitrate compared to other types of carbohydrates. The specific metabolic effects and potential interactions between HBCD and dietary nitrate need to be carefully examined and considered. Future research should focus on investigating the optimal strategies for co-ingestion of HBCD and dietary nitrate to maximize their potential benefits and enhance exercise performance. However, the available literature on the timing of combining NO_3^- with CHO remains limited. Further research is needed to determine the optimal duration of co-ingestion to maximize the potential benefits. Consequently, a comprehensive approach that considers the coordination of dietary NO_3^- and CHO intake, in addition to the timing of pre-exercise meals, is necessary to develop effective nutritional strategies for optimizing exercise performance.

Conclusions

The present study provided evidence that the acute co-ingestion of 500 ml beverage containing 1.5 g HBCD·kg^{-1} body mass with 500 mg NO_3^- (~8.00 mmol NO_3^-) via beetroot extract 10:1 powder 60 minutes before performed the high-intensity prolonged running, the results of this study reveald that:

Firstly, the study showed that there were no significant differences in the serum insulin concentration, serum glucose concentration, and blood lactate concentration when ingestion HBCD combined with NO_3^- compared to when HBCD was consumed alone, co-ingesting maltodextrin and dietary nitrate (MD+NO_3^-), or just maltodextrin (MD). This suggests that the combination of HBCD and dietary NO_3^- does not have a distinct effect on these metabolic parameters.

Secondly, the co-ingestion of HBCD+NO_3^- did not significantly affect the oxygen cost (EO_2), when compared to HBCD alone, MD+NO_3^-, or MD. This means that consuming HBCD and dietary NO_3^- together does not result in a different energy expenditure during exercise compared to the other conditions.

Thirdly, it was observed that HBCD increased the body's capacity to utilize fat as an energy source, as evidenced by a lower RER compared to conditions where HBCD was co-ingested with NO_3^-, co-ingested with MD+NO_3^-, or when MD alone was consumed. However, there were no notable differences in the total CHO oxidation rate. These findings indicate that the combination of HBCD may modulate fat metabolism and influence the RER differently compared to co-ingesting HBCD+NO_3^- or other sources of CHO.

Lastly, there were no significant differences in muscle oxygenation parameters when HBCD+NO_3^- was consumed compared to when HBCD was consumed alone, along with MD+NO_3^-, or MD. These findings suggest that the co-ingestion of CHO with NO_3^- in the form of both MD and HBCD does not have a noticeable impact on the ability of blood to carry oxygen, as indicated by changes in hemoglobin concentrations (ΔO_2Hb, ΔHHb, ΔtHb), as well as tissue oxygen saturation (ΔTSI).

Overall, these results emphasize the intricate effects of co-ingesting HBCD and dietary NO_3^- on diverse physiological parameters, such as fat oxidation, respiratory

exchange ratio, and hemoglobin concentrations. Further research is necessary to comprehensively comprehend the underlying mechanisms and ascertain the implications of these findings for athletic performance and metabolic responses during exercise.

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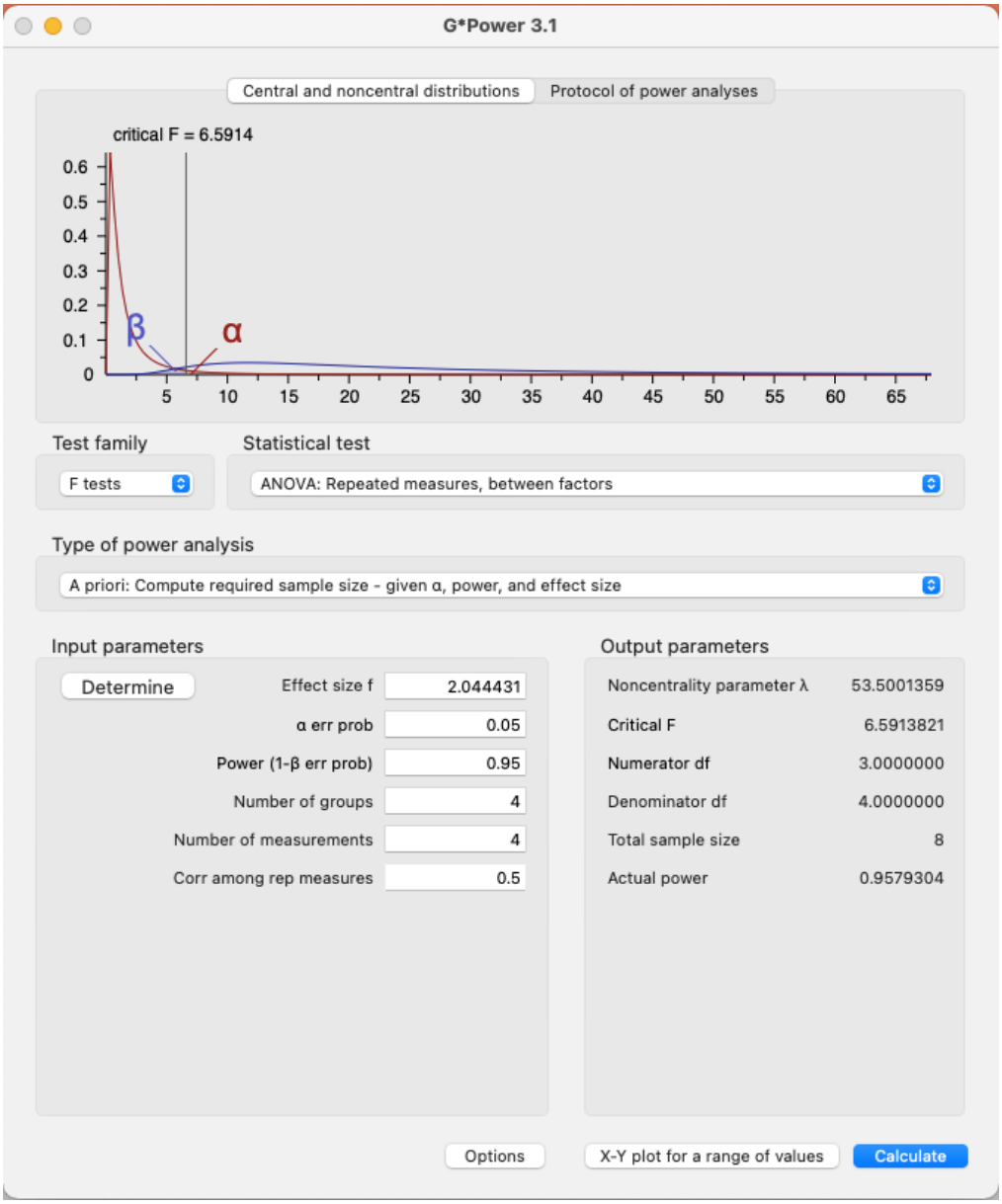
Appendix

Appendix I Power calculation for experimental



Appendix II List of experts assessing the content validity index of the protocol

รายนามผู้ทรงคุณวุฒิประเมินความเหมาะสมของโปรแกรมดำเนินการทดลอง

1. รองศาสตราจารย์ ดร.ราตรี เรืองไทย	คณะวิทยาศาสตร์การกีฬา มหาวิทยาลัยเกษตรศาสตร์
Associate Professor Ratree Ruangthai, Ph.D.	Faculty of Sports Science, Kasetsart University
2. อาจารย์ ดร.นุชรี เสนาคำ	คณะพลศึกษา มหาวิทยาลัยศรีนครินทรวิโรฒ
Nutcharee Senakham, Ph.D.	Faculty of Physical Education, Srinakharinwirot University
3. อาจารย์ ดร.คุณัญญา มาสดใส	คณะวิทยาศาสตร์การกีฬา จุฬาลงกรณ์มหาวิทยาลัย
Kunanya Masodsai, Ph.D.	Faculty of Sports Science, Chulalongkorn University
4. อาจารย์ ดร.สุทธิกร อาภานุกูล	คณะวิทยาศาสตร์การกีฬา จุฬาลงกรณ์มหาวิทยาลัย
Suttikorn Apanukul, Ph.D.	Faculty of Sports Science, Chulalongkorn University
5. อาจารย์ ดร.ชนวัฒน์ สรรพสิทธิ์	คณะวิทยาศาสตร์การกีฬา จุฬาลงกรณ์มหาวิทยาลัย
Acting Sub Lt. Chanawat Sanpasitt, Ph.D.	Faculty of Sports Science, Chulalongkorn University

Appendix III Ethics review: certificate of approval

AF 02-12

The Research Ethics Review Committee for Research Involving Human Research Participants, Group I, Chulalongkorn University
Jamjuree 1 Building, 2nd Floor, Phyathai Rd., Patumwan district, Bangkok 10330, Thailand,
Tel: 0-2218-3202, 0-2218-3049 E-mail: eccu@chula.ac.th

COA No.125/2022

Certificate of Approval

Study Title No. 195.1/64 : THE EFFECTS OF CO-INGESTION OF HIGHLY BRANCHED CYCLIC DEXTRIN AND DIETARY NITRATE ON PHYSIOLOGICAL RESPONSES AND ENDURANCE CAPACITY IN RECREATIONAL ENDURANCE RUNNER.

Principal Investigator : MR. SONGDHASN CHINAPONG

Place of Proposed Study/Institution: Faculty of Sport Science, Chulalongkorn University

The Research Ethics Review Committee for Research Involving Human Research Participants, Group I, Chulalongkorn University, Thailand, has approved constituted in accordance with Belmont Report 1979, Declaration of Helsinki 2013, Council for International Organizations of Medical Sciences (CIOM) 2016, Standards of Research Ethics Committee (SREC) 2017, and National Policy and guidelines for Human Research 2015. (Study I of research project)

Signature: Prida Tasanapradit
(Associate Prof. Prida Tasanapradit, M.D.)
Chairman

Signature: Raveenan Mingpakanee
(Assistant Prof. Raveenan Mingpakanee, Ph.D.)
Secretary

Date of Approval: 24 May 2022 Approval Expire date: 23 May 2023

The approval documents including;

1) Research proposal
2) Participant Information Sheet and Consent Form
3) Researcher
4) Questionnaires
5) Advertising leaflet

Protocol No. 195.1/64
Date of Approval 24 MAY 2022
Approval Expire Date 23 MAY 2023

The approved investigator must comply with the following conditions:

1. *It's unethical to collect data of research participants before the project has been approved by the committee.*
2. *The research/project activities must end on the approval expired date. To renew the approval, it can be applied one month prior to the expired date with submission of progress report.*
3. *Strictly conduct the research/project activities as written in the proposal.*
4. *Using only the documents that bearing the RECCU's seal of approval: research tools, information sheet, consent form, invitation letter for research participation (if applicable).*
5. *Report to the RECCU for any serious adverse events within 5 working days.*
6. *Report to the RECCU for any amendment of the research project prior to conduct the research activities.*
7. *Report to the RECCU for termination of the research project within 2 weeks with reasons.*
8. *Final report (AF 01-15) and abstract is required for a one year (or less) research/project and report within 30 days after the completion of the research/project.*
9. *Research project with several phases; approval will be approved phase by phase, progress report and relevant documents for the next phase must be submitted for review.*
10. *The committee reserves the right to site visit to follow up how the research project being conducted.*
11. *For external research proposal the dean or head of department oversees how the research being conducted.*

Appendix IV Screening form (in Thai)

แบบคัดกรองอาสาสมัครเข้าร่วมการวิจัย

อาสารสมัครร่วมการวิจัย รหัส..... (ผู้วิจัยกรอก)

วันที่.....

คำชี้แจง ให้อาสาสมัครเข้าร่วมการวิจัยทำสัญลักษณ์ ✓ ลงใน ☐ หรือเขียนข้อความลงในช่องว่างให้สมบูรณ์และครบถ้วน

1. อายุ _____ ปี
2. จำนวนวันในการออกกำลังกายหรือฝึกซ้อม _____ วัน/สัปดาห์
3. ระยะทางโดยรวมในการฝึกซ้อมต่อสัปดาห์ _____ กิโลเมตร
4. ในระยะเวลา 4 สัปดาห์ก่อนหน้านี้ ท่านได้เข้าร่วมการแข่งขันวิ่งระยะไกล ☐ ใช่ ☐ ไม่ใช่
5. ในระยะเวลา 4 สัปดาห์ก่อนหน้านี้ ท่านได้เข้าร่วมการทดลองอื่น ๆ ☐ ใช่ ☐ ไม่ใช่
6. ท่านมีแผนที่จะเข้าร่วมการแข่งขันวิ่งระยะไกลภายใน 4 เดือนนับจากนี้ ☐ ใช่ ☐ ไม่ใช่
7. ท่านต้องเข้าร่วมการทดลองอื่น ๆ ภายใน 4 เดือนนับจากนี้ ☐ ใช่ ☐ ไม่ใช่
8. ท่านต้องเข้าร่วมการทดลองอื่น ๆ ภายใน 4 เดือนนับจากนี้ ☐ ใช่ ☐ ไม่ใช่
9. ท่านมีปัญหาอย่างใดอย่างหนึ่งต่อไปนี้ *"มีปัญหาหรือโรคเกี่ยวกับระบบหัวใจและหลอดเลือด ระบบหายใจ ระบบเมตาบอลิซึม ระบบประสาท ระบบกระดูกและข้อต่อ ภายใต้การวินิจฉัยของแพทย์ว่าจะเป็นอุปสรรคต่อการออกกำลังกาย"* ☐ ใช่ ☐ ไม่ใช่
10. ในระยะเวลา 3 เดือนก่อนหน้านี้ท่านดื่มสุรา ☐ ใช่ ☐ ไม่ใช่
11. ในระยะเวลา 3 เดือนก่อนหน้านี้ท่านสูบบุหรี่ ☐ ใช่ ☐ ไม่ใช่
12. ในระยะเวลา 3 เดือนก่อนหน้านี้ท่านรับประทานผลิตภัณฑ์เสริมอาหารที่เสริมสร้างความสามารถในการออกกำลังกาย (ถ้าตอบใช่ โปรดระบุผลิตภัณฑ์_____) ☐ ใช่ ☐ ไม่ใช่

ส่วนของผู้วิจัยกรอก

ผลการทดสอบสมรรถภาพทางกาย

น้ำหนักตัว _____ กิโลกรัม

อัตราการใช้ออกซิเจนสูงสุด ($\dot{V}O_2peak$) _____ ml/kg/min

Appendix V The physical activity readiness questionnaire (in Thai)

แบบประเมินความพร้อมก่อนการออกกำลังกาย (2021 PAR-Q+) ฉบับภาษาไทย

มีหลักฐานยืนยันชัดเจนถึงประโยชน์ของการมีกิจกรรมทางกายต่อสุขภาพ คนส่วนใหญ่ควรมีกิจกรรมทางกายในทุกวันของสัปดาห์ การมีกิจกรรมทางกายมีความปลอดภัยสำหรับคนส่วนใหญ่ แบบสอบถามนี้จะให้ข้อมูลถึงความจำเป็นในการขอคำแนะนำเพิ่มเติมจากแพทย์หรือผู้เชี่ยวชาญด้านการออกกำลังกายก่อนที่จะมีกิจกรรมกายที่มีความหนักมากขึ้นจากเดิมที่เคยมีกิจกรรมทางกายหรือไม่

กรุณาอ่านคำถามทั้ง 7 ข้อด้านล่างนี้อย่างละเอียดและตอบตามความเป็นจริงว่า ใช่ หรือ ไม่ใช่	ใช่	ไม่ใช่
1. คุณเคยได้รับทราบจากแพทย์ว่า คุณเป็น ☐ โรคเกี่ยวกับหัวใจ **หรือ** ☐ ความดันโลหิตสูง	☐	☐
2. คุณรู้สึกเจ็บหน้าอกในขณะพัก **หรือ** ในขณะทำกิจวัตรประจำวัน **หรือ** ระหว่างออกกำลังกาย	☐	☐
3. คุณเคยสูญเสียการทรงตัวเนื่องจากเวียนศีรษะ **หรือ** หมดสติ ในรอบ 12 เดือนที่ผ่านมา (ในกรณีที่เวียนศีรษะเนื่องจากหายใจเร็วจากการออกกำลังกายอย่างหนัก ให้ตอบ **ไม่ใช่**)	☐	☐
4. คุณได้รับการวินิจฉัยว่าเป็นโรคเรื้อรังนอกเหนือจากโรคหัวใจและความดันโลหิตสูงหรือไม่ ถ้าตอบ **ใช่** โปรดระบุ _____	☐	☐
5. ปัจจุบัน คุณได้รับประทานยาที่รักษาโรคเรื้อรัง หรือไม่ ถ้าตอบ **ใช่** โปรดระบุเงื่อนไขและยาที่ได้รับ _____	☐	☐
6. ปัจจุบัน หรือในรอบ 12 เดือนที่ผ่านมา คุณมีปัญหาเกี่ยวกับกระดูก **หรือ** ข้อต่อ **หรือ** กล้ามเนื้อ **หรือ** เส้นเอ็น ซึ่งจะมีอาการแย่ลงเมื่อมีกิจกรรมทางกายเพิ่มขึ้น (ในกรณีที่เคยมีปัญหาดังกล่าว แต่ปัจจุบันภาวะนั้นไม่ได้เป็นอุปสรรคต่อการออกกำลังกายหรือกิจกรรมทางกาย ให้ตอบ **ไม่ใช่**) และโปรดระบุภาวะดังกล่าว _____	☐	☐
7. แพทย์เคยแจ้งให้คุณทราบว่า คุณควรได้รับคำแนะนำก่อนที่จะมีกิจกรรมทางกายหรือออกกำลังกาย	☐	☐

ถ้าตอบ **ไม่ใช่** ทุกข้อ ท่านสามารถที่จะมีกิจกรรมทางกายภายใต้ข้อปฏิบัติดังนี้

1. ให้เริ่มมีกิจกรรมทางกายมากที่เพิ่มขึ้น โดยค่อย ๆ เพิ่มความหนักของกิจกรรมทางกาย

2. ให้ท่านมีกิจกรรมทางกายให้สอดคล้องกับอายุ ตามแนวทางคำแนะนำของ International Physical Activity Guideline ท่านควรได้รับการประเมินสมรรถภาพทางกาย และประเมินสุขภาพหรือตรวจสุขภาพประจำปี

3. ถ้าท่านอายุมากกว่า 45 ปี และไม่ได้ฝึกซ้อมออกกำลังกายที่มีความหนักมาก่อน ให้ปรึกษาผู้เชี่ยวชาญด้านการออกกำลังกายก่อนไปเข้าร่วมกิจกรรมทางกายที่มีความหนัก

4. ถ้าท่านมีปัญหาเกี่ยวกับกิจกรรมทางกาย ให้ปรึกษาแพทย์ หรือผู้เชี่ยวชาญด้านทางออกกำลังกาย

ข้าพเจ้า ผู้ซึ่งลงนามในคำประกาศนี้ ได้อ่าน ทำความเข้าใจ โดยตอบคำถามทั้งหมดอย่างเต็มใจและตระหนักเป็นอย่าดีว่าคำประกาศนี้จะใช้ได้ภายใน 12 เดือน นับจากวันที่ได้ตอบแบบสอบถามและจะไม่มีผลในกรณีที่มีการเปลี่ยนแปลงของเงื่อนไข ข้าพเจ้ายินยอมที่จะให้ผู้จัด/ ศูนย์ฝึกการออกกำลังกาย ได้สำเนาเอกสารฉบับนี้เก็บไว้อีกฉบับ โดยผู้จัด/ ศูนย์ฝึกกิจกรรมทางกายต้องไม่นำข้อมูลไปเปิดเผยและปฏิบัติตามมาตรการรักษาความลับตามที่กฎหมายกำหนด

Appendix VI Food record (in Thai)

แบบบันทึกการบริโภคอาหาร

วันที่.....

ทำเครื่องหมาย x หน้าวันที่บันทึก

…. วันทำงานปกติ (วันจันทร์ - วันศุกร์) …. วันเสาร์ – อาทิตย์

…. วันหยุดนักขัตฤกษ์ …. วันพิเศษ (เช่น งานวันเกิด งานเลี้ยง)

คำแนะนำในการบันทึกการบริโภคอาหาร ในรอบ 3 วัน

1. บันทึกรายการอาหารมื้อหลักและอาหารว่างที่รับประทาน รวมถึงเครื่องดื่มทุกชนิด ทุกรายการใน 1 วัน จำนวน 3 วัน
2. กรณีรับประทานอาหารสำเร็จรูปเป็นจาน ให้บันทึกปริมาณของส่วนประกอบส่วนใหญ่ที่สังเกตเห็น เช่น ข้าวผัด ส่วนประกอบคือ ข้าวสวย 1 ถ้วย หมู 4 ชิ้น ไข่ 1 ฟอง ผักคะน้า 2 ช้อนโต๊ะ
3. ขนมขบเคี้ยว ให้ระบุยี่ห้อ และปริมาณที่รับประทาน (ถ้าเป็นไปได้ โปรดบันทึกภาพซองที่บรรจุให้ผู้วิจัย)
4. อาหารที่เป็นผลไม้ ให้ระบุเป็นจำนวนชิ้น จำนวนลูก และขนาด
5. บันทึกเครื่องดื่ม เช่น ชา กาแฟ น้ำหวาน น้ำอัดลม น้ำผลไม้ ในปริมาณที่ไม่รวมน้ำแข็ง

มื้ออาหาร/เวลา	รายการอาหาร	ส่วนประกอบ	น้ำหนัก (กรัม) หรือปริมาณ

Appendix VII Physical activity diary (in Thai)

แบบบันทึกกิจกรรมทางกาย

วันที่.....

ทำเครื่องหมาย x หน้าวันที่บันทึก

.... วันทำงานปกติ (วันจันทร์ - วันศุกร์) .... วันเสาร์ – อาทิตย์

.... วันหยุดนักขัตฤกษ์ .... วันพิเศษ (เช่น งานวันเกิด งานเลี้ยง)

เวลา	กิจกรรม	รวมระยะเวลา	ความหนักของกิจกรรม
เวลาตื่นนอน			
			☐ ความหนักเบา ☐ ความหนักปานกลาง ☐ ความหนักสูง
			☐ ความหนักเบา ☐ ความหนักปานกลาง ☐ ความหนักสูง
			☐ ความหนักเบา ☐ ความหนักปานกลาง ☐ ความหนักสูง
			☐ ความหนักเบา ☐ ความหนักปานกลาง ☐ ความหนักสูง
			☐ ความหนักเบา ☐ ความหนักปานกลาง ☐ ความหนักสูง
เวลาเข้านอน			

คำอธิบายความหนักของกิจกรรมทางกาย

กิจกรรมทางกายความหนักเบา คือ กิจกรรมที่ต้องนั่งหรือยืนนาน มีความรู้สึกเหนื่อยน้อยมาก เช่น กิจกรรมในสำนักงาน งานบ้านที่ยืนหรือนั่งทำ

กิจกรรมทางกายระดับปานกลาง คือ กิจกรรมที่ต้องใช้การออกแรงหรือออกกำลังกาย มีความรู้สึกเหนื่อยขึ้นจากขณะปกติ หายใจเร็วขึ้นพอควรแต่ไม่ถึงกับหอบ หรือหัวใจเต้นเร็วขึ้นเล็กน้อย เป็นระยะเวลา 10 นาทีขึ้นไป เช่น การเดินขึ้นบันได การก้าวเดินเร็ว ๆ การทำความสะอาดกวาดถูบริเวณบ้าน การปั่นจักรยานที่ความเร็ว 8-14 กม./ชม. เป็นต้น

กิจกรรมทางกายระดับหนัก คือ กิจกรรมที่ต้องใช้การออกแรงหรือออกกำลังกายอย่างหนัก จนทำให้ต้องหายใจแรงขึ้น หรือหัวใจเต้นเร็วขึ้นมาก เป็นระยะเวลา 10 นาทีขึ้นไป เช่น การแบกหรือถือของหนักมาก ๆ งานก่อสร้างที่หนัก การปั่นจักรยานที่ความเร็วมากกว่า 14 กม./ชม.

Appendix VIII General characteristic of participants (in Thai)

แบบสอบถามข้อมูลทั่วไปของผู้เข้าร่วมงานวิจัย

ส่วนที่ 1 คำถามทั่วไป

รหัส_____อายุ_____ปี

น้ำหนัก_____กิโลกรัม ส่วนสูง_____ เซนติเมตร มวลกล้ามเนื้อ _____

ประวัติการแพ้อาหาร __ ไม่มีประวัติ __ มีประวัติ ระบุ _____

ส่วนที่ 2 คำถามเกี่ยวกับประวัติการเข้าร่วมการแข่งขัน

1. ประสบการณ์ในการวิ่ง _____ ปี
2. ประสบการณ์ในการเข้าร่วมการแข่งขันมาราธอน _____ ครั้ง
3. ระยะเวลาเฉลี่ยในการฝึกซ้อมวิ่งต่อวัน _____ นาที
4. ระยะทางในการวิ่งโดยเฉลี่ย ต่อวัน _____ กิโลเมตร
5. ระยะทางในการวิ่งโดยเฉลี่ย ต่อสัปดาห์ _____ กิโลเมตร
6. ความบ่อยในการฝึกซ้อมวิ่ง สัปดาห์ละ _____ วัน
7. สถิติที่ดีที่สุดในการวิ่งมาราธอน 42.195 กิโลเมตร _____ ชั่วโมง
8. ระยะเวลาเฉลี่ยในการวิ่งมาราธอน 42.195 กิโลเมตร _____ ชั่วโมง

Appendix IX The gastrointestinal symptom questionnaire (in Thai)

แบบประเมินอาการแสดงของระบบทางเดินอาหาร

(Gastrointestinal Symptom questionnaire)

รหัสผู้เข้าร่วมการวิจัย_____วันที่_____

รหัสเครื่องดื่ม_____

ระดับอาการ (Visual analog scale)

ช่วงเวลา	คลื่นไส้, รู้สึกไม่สบายท้อง (Nausea)	อาเจียน (Vomiting)	แน่นท้อง (Stomach Fullness)	ปวดท้อง (Abdominal pain)	แสบร้อนกลางอก (Heartburn)	ท้องอืด (Bloating)
ก่อนดื่มเครื่องดื่ม						
หลังดื่ม 30 นาที						
หลังดื่ม 60 นาที (ก่อนวิ่ง)						
วิ่ง นาทีที่ 30						
วิ่ง นาทีที่ 60						
หลังวิ่งทันที						

คำอธิบาย

คลื่นไส้ หมายถึง ความรู้สึกปั่นป่วนในกระเพาะอาหารหรือลำไส้จนอยากจะอาเจียน

อาเจียน หมายถึง การขย้อยหรือสำรอกสิ่งที่กลืนลงไปแล้วออกมาทางปาก

แน่นท้อง หมายถึง อาการอึดอัดท้องเพราะกินอาหารมาก

ปวดท้อง หมายถึง รู้สึกเจ็บต่อเนื่องในบริเวณในบริเวณหนึ่งตั้งแต่ช่วงอกไปจนถึงขาหนีบ

แสบร้อนกลางอก หมายถึง รู้สึกแสบและร้อนที่ช่องท้องส่วนบนเคลื่อนขึ้นไปจนถึงหน้าอก

ท้องอืด หมายถึง ความรู้สึกแน่นท้องเนื่องจากมีแก๊สหรือลมในกระเพาะอาหาร

ที่มา: ดัดแปลงจาก Bovenschen et al.,2006

Appendix X Manufacturing attribute data of highly branched cyclic dextrin

Item	Value
Appearance	White powder
Dextrose equivalent	1.2
HBCD content	95.5
Loss on drying	4.5 %
Residue on ignition	0.003 %
Standard plate count	≤ 300 CFU/g
Coliforms	Negative
Yeasts	≤ 50 CFU/g
Molds	≤ 50 CFU/g

Appendix XI Experimental instruments

A body composition analyzer (InBody, USA)

Standard Outputs: Weight, Total Body Water, Dry Lean Mass, Lean Body Mass, Body Fat Mass, Skeletal Muscle Mass, Body Mass Index, Percent Body Fat, Segmental Lean Analysis, Body Composition History, Intracellular Water, Extracellular Water, ECW/TBW Analysis, Segmental Body Water Analysis, Segmental ECW/TBW Analysis

A motorized treadmill (h/p/cosmos®, Germany)

A near-infrared spectroscopy (PortarMon, The Netherlands)

A portable metabolic analyzer (Cortex Metalyzer, Germany)

Source: Stellenbosch University

(Available on http://www.sun.ac.za/english/faculty/science/CAF/running-vo2max)

VITA

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