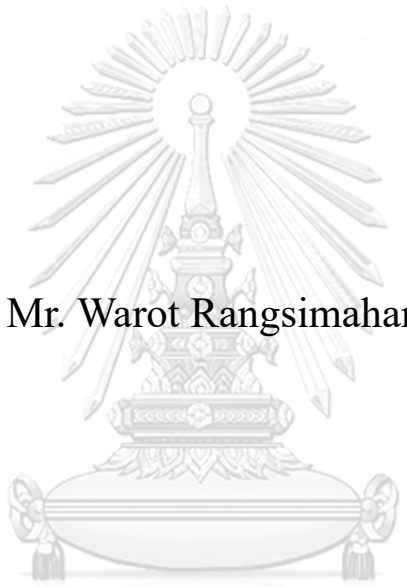
The effect of water temperature and carbohydrate drink on intestinal epithelial injury, endotoxemia, splanchnic perfusion, and core temperature, during running in the heat.

Mr. Warot Rangsimahariwong



A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Sports Medicine
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ผลของอุณหภูมิน้ำดื่ม และ เครื่องดื่มคาร์โบไฮเดรต ต่อการบาดเจ็บของลำไส้ ภาวะเอนโดทอกซีเมีย การไหลเวียนของเลือดที่ทางเดินอาหาร และอุณหภูมิแกนกลางลำตัว ในขณะวิ่งท่ามกลางความร้อน

นายวรทย์ รังสิมาหริวงศ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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Thesis Title	The effect of water temperature and carbohydrate drink on intestinal epithelial injury, endotoxemia, splanchnic perfusion, and core temperature, during running in the heat.
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Field of Study	Sports Medicine
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วรทย์ รังสิมาหริวงศ์ : ผลของอุณหภูมิน้ำดื่ม และ เครื่องดื่มคาร์โบไฮเดรต ต่อการบาดเจ็บของลำไส้ ภาวะเอนโดทอกซีเมีย การไหลเวียนของเลือดที่ทางเดินอาหาร และอุณหภูมิแกนกลางลำตัว ในขณะวิ่งท่ามกลางความร้อน. (The effect of water temperature and carbohydrate drink on intestinal epithelial injury, endotoxemia, splanchnic perfusion, and core temperature, during running in the heat.) อ.ที่ปรึกษาหลัก : รศ. ดร.พญ.อรอนงค์ กุละพัฒน์, อ.ที่ปรึกษาร่วม : ผศ. ดร.ณัฐธยาน์ ช่วยเพ็ญ

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Warot Rangsimahariwong : The effect of water temperature and carbohydrate drink on intestinal epithelial injury, endotoxemia, splanchnic perfusion, and core temperature, during running in the heat.. Advisor: Assoc. Prof. ONANONG KULAPUTANA, M.D.,Ph.D. Co-advisor: Asst. Prof. NATTHAYA CHUAYPEN, Ph.D.

Background: Prolonged exercise can result in splanchnic hypoperfusion and elevated core body temperature (Tcore), which can contribute to gastrointestinal injury and endotoxemia. The effects of fluid temperature and carbohydrate consumption during exercise on these variables are less clear. Objectives: The study aimed to determine the effects of the temperature of water and carbohydrate drinks on intestinal epithelial injury, endotoxemia, splanchnic perfusion, and Tcore during 60-minute of moderate-intensity running. Methods: Ten participants completed four 60-minute running trials at 70% VO_{2max} with different types of hydration: no water (NW), ambient temperature water (ATW), cold water (CW), and cold carbohydrate water (CW+CHO). Doppler ultrasound of the superior mesenteric artery (SMA) and portal vein (PV) and blood for intestinal fatty acid binding protein (I-FABP) and lipopolysaccharide (LPS) levels were assessed before and after exercise. Tcore was continuously monitored during exercise. Results: I-FABP significantly increased in all trials (NW: 1045.85 ± 1571.73 pg/mL; ATW: 1940.83 ± 910.65 pg/mL; CW: 1567.87 ± 1069.36 pg/mL; and CW+CHO: 779.92 ± 654.40 pg/mL; $p < 0.05$ for all). LPS showed no significant differences within each trial and between all trials ($p > 0.05$ for all). All fluid replacement trials exhibited a significant reduction in splanchnic hypoperfusion in both SMA and PV compared to NW ($p < 0.05$ for all). NW showed the highest increase in Tcore at 60-minute (38.6 ± 0.3 ^{o}C), while fluid replacement trials (ATW: 38.43 ± 0.33 ^{o}C, CW: 38.33 ± 0.20 ^{o}C, and CHO + CW: 38.27 ± 0.50 ^{o}C) had a blunted increase compared to NW ($p = 0.002$; $p < 0.001$; and $p < 0.001$; respectively). Conclusion: Running with or without fluid replacements resulted in intestinal epithelial injury. The cold carbohydrate drink was the most effective in reducing splanchnic hypoperfusion. All water replacement conditions help attenuate the rise in Tcore without changes in endotoxemia.

Field of Study:	Sports Medicine	Student's Signature .....
Academic Year:	2023	Advisor's Signature .....
		Co-advisor's Signature .....

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Warot Rangsimahariwong

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CHAPTER 1 INTRODUCTION

Numerous sporting activities and events are frequently organized in hot and humid environments, particularly running events in Thailand. Thailand experiences an average temperature of 28.1°C with relative humidity (RH) ranging from 64% to 80% (Thailand Meteorological Department, 2021). Engaging in prolonged exercise under such conditions can pose significant physiological challenges (1). These challenges include the redistribution of blood flow, driven by sympathetic responses to working muscles, heart, lungs, and skin (2). Additionally, there is an increase in tissue metabolic rate, leading to thermoregulatory strain (3) and elevated core body temperature (4). These physiological changes can potentially compromise intestinal function by inducing damage to epithelial cells (5), increasing permeability, and facilitating bacterial translocation (endotoxemia)(6), thereby triggering a state of low-grade systemic inflammation (7). Such effects may disrupt gastrointestinal (GI) homeostasis and overall health (8). It has been reported that up to 70% of individuals engaging in strenuous exercise experience GI issues, which may even lead to cessation of sports participation (9, 10).

Exercise-induced splanchnic hypoperfusion is well documented as the main pathophysiological mechanism for intestinal ischemia that induces epithelial damage (5, 6), particularly when participants are hypohydrated (11). Rehrer et al. (12), previously demonstrated that portal blood flow decreases by 20% within 10 minutes and by 80% after 1 hour of cycling at 70% of maximal power output (Wmax). Further supporting evidence by van Wijck et al. (13) revealed significant GI hypoperfusion during the initial 10 minutes of cycling at 70% Wmax, resulting in intestinal ischemia and subsequent intestinal injury as indicated by elevated plasma intestinal fatty acid-binding protein (I-FABP) levels. The occurrence of acute local ischemia during exercise has been found to disrupt and dysregulate tight junction proteins, leading to increased permeability and facilitating the translocation of toxins, antigens, and bacteria (such as lipopolysaccharide, LPS) from the intestinal lumen into the bloodstream (endotoxemia). This process may induce both local and systemic inflammatory responses (1, 14). However, the investigation of exercise-induced splanchnic hypoperfusion in relation to markers of GI injury and endotoxemia remains limited in the existing literature.

The increased metabolic rate experienced during long-distance running can induce heat strain on the body, resulting in a consistent increase in tissue temperature within the small and large intestines. This elevation in tissue temperature, assessed through core body temperature (Tcore) using rectal probes (15), can compromise the integrity of the GI barrier, leading to heightened intestinal permeability (14). Previous studies conducted by Pals and colleagues (16) illustrated a direct relationship between Tcore and exercise intensity, coupled with an increase in body mass loss. When the environmental temperature exceeds 23°C, it exacerbates markers associated with gut damage (1). Additionally, there is evidence that both Tcore and a marker of GI injury (I-FABP) increase in response to heat exposure during exercise in various environmental conditions (17). The elevated temperatures in hot conditions cause blood flow to redirect towards the skin for sweating in an attempt to cool down the body, which disrupts splanchnic perfusion (1). Pre-exercise hypohydration and fluid

loss during exercise contribute to dehydration, worsening splanchnic hypoperfusion, and increasing permeability (18). However, there is limited available data regarding the impact of exertional heat stress on intestinal permeability, emphasizing the importance of exploring strategies to minimize the rise in Tcore and exercise-induced intestinal injury.

The replacement of fluids during prolonged exercise in hot conditions has demonstrated its advantages in mitigating dehydration and tempering the increase in Tcore (19). Pre-exercise consumption of cold beverages, such as an ice slurry at -1.4°C, in hot conditions, has proven effective in reducing Tcore by up to 0.5°C, enhancing perceived exertion, improving thermal comfort, and enhancing endurance exercise performance (20). Ingesting cold fluids represents a practical approach to reducing Tcore and minimizing gastrointestinal (GI) disturbances. Notably, a study by Snipe RMJ and Costa RJS (17) examined the impact of cold and cool water replacement (ice-cold at 0°C and cool at 7°C) during exertional heat stress and found that it attenuates thermoregulatory strain and reduces intestinal injury (I-FABP reduction) in comparison to consuming water at room temperature (22°C). However, the distinct effects of no water intake and the consumption of cold fluids during prolonged exercise in a hot environment, as they relate to splanchnic perfusion, core body temperature, and their influence on intestinal epithelial injury, have not been adequately studied and constitute a significant area for further research and investigation.

Carbohydrate (CHO) consumption before and during exercise is widely recommended due to its well-established positive effects on endurance capacity and overall performance (21). The ingestion of CHO during exercise may help alleviate exercise-induced splanchnic hypoperfusion and GI disruptions by increasing splanchnic perfusion during GI digestion and nutrient absorption (22, 23). The research by Rehrer et al.(24) has demonstrated that CHO ingestion effectively sustains portal vein blood flow during cycling exercise. Another study showed that a carbohydrate drink was efficient in reducing epithelial injury, decreasing small intestinal permeability, enhancing the clearance of endotoxins, and lowering physical strain during a 2-hour run at 60% of VO_{2max} in hot conditions (35 °C) when compared to water consumption alone(25). Nevertheless, there is an essential need for further research to investigate the practical implications of consuming cold CHO drinks compared to abstaining from water intake and consuming room temperature water during extended exercise in a hot and humid environment like Thailand. This research should delve into their practical effects on intestinal injury, core body temperature, and splanchnic perfusion.

The elevation in body temperature and concurrent splanchnic hypoperfusion experienced during strenuous exercise in hot conditions carries significant implications for GI integrity, function, and systemic responses. In light of these effects, this study seeks to explore the consequences of water temperature and carbohydrate-based drinks on intestinal epithelial injury, endotoxemia, splanchnic perfusion, and core temperature during continuous running in hot environmental conditions.

1.1 Research question:

Do intestinal epithelial injury, endotoxemia, splanchnic perfusion, and core temperature respond differently to room temperature water, cold temperature water, and cold carbohydrate (CHO) drink replacement during moderate continuous running in the heat?

1.2 Objectives

- To investigate the impact of cold water (CW) with a temperature range of 0 to 1°C in comparison to ambient temperature water (ATW) with a temperature range of 28 to 29°C on variables related to intestinal epithelial injury, endotoxemia, splanchnic perfusion, and core temperature during continuous running at a moderate intensity set at 70%VO2max. These experiments will be conducted within a warm environment-controlled room with a temperature range of 28 to 29°C and a relative humidity level of 60 to 70%.
- To assess and compare the effects of a cold carbohydrate drink (CW+CHO) with cold water (CW) during continuous running at a moderate intensity (70%VO2max) in the same warm environment-controlled room as described above. The focus is on variables related to intestinal epithelial injury, endotoxemia, and splanchnic perfusion.
- To examine the effects of fluid replacement using ambient temperature water (ATW), cold water (CW), and cold carbohydrate water (CW+CHO) in comparison to a no water replacement (NW) scenario on variables related to intestinal epithelial injury, endotoxemia, splanchnic perfusion, and core temperature during continuous running at a moderate intensity (70%VO2max) within the same warm environment-controlled room with a temperature range of 28 to 29°C and a relative humidity level of 60 to 70%.

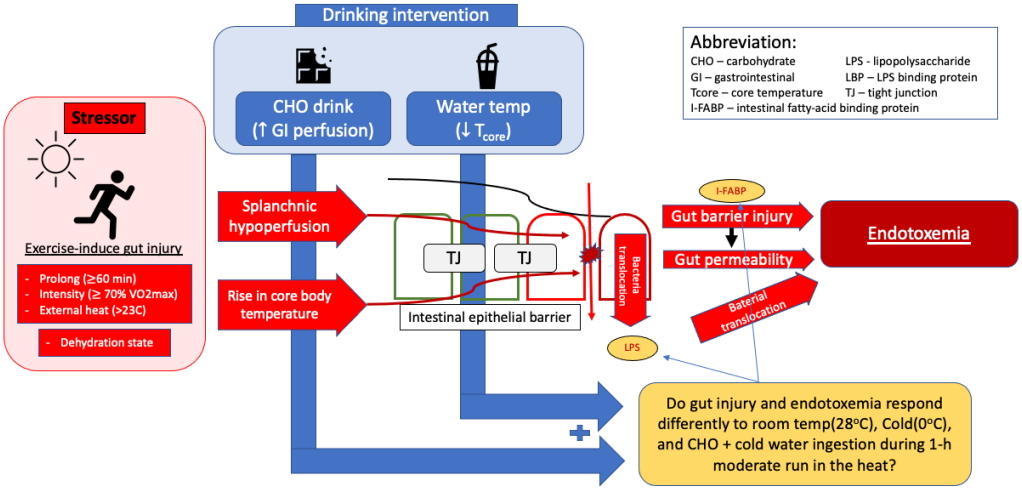
1.3 Hypothesis

- Cold water ingestion during continuous moderate-intensity run in the heat would have a superior counter effect on decreasing core temperature, intestinal epithelial injury, and endotoxemia, and improved splanchnic perfusion when compared with ambient temperature water.
- Cold CHO drink would increase splanchnic perfusion and subsequent in reducing intestinal epithelial injury and endotoxemia during 1-h running at moderate intensity compared with cold water ingestion alone.
- All fluid rehydration (water at both temperature and cold CHO drink) during 1-h running at moderate intensity would improve intestinal epithelial injury, splanchnic perfusion, endotoxemia, and decreasing core temperature when compared with no rehydration trial.

1.4 Keyword:

- Exertional heat stress
- Intestinal epithelial injury
- Intestinal permeability
- Splanchnic hypoperfusion
- Endotoxemia

1.5 Conceptual framework:



CHAPTER 2 LITERATURE REVIEW

2.1 Exercise-induced gastrointestinal injury (EIGI): etiology

The etiology of EIGI is a complex interplay of various factors, primarily involving two key components. Firstly, it is associated with alterations in the circulatory-gastrointestinal pathway, which results in the redistribution of blood flow towards the working muscles and the skin, consequently leading to a reduction in splanchnic perfusion. This sequence of events subsequently triggers ischemia-reperfusion injury, as elucidated by van Wijck et al.(5). Secondly, the dysregulation of gastrointestinal tight junctions due to hyperthermia plays a significant role in the development of EIGI, a phenomenon documented by Dokladny et al.(14).

2.1.1 Exercise-induced splanchnic hypoperfusion

At rest, splanchnic vascular beds typically receive around 20% of the total cardiac output and consume approximately 10-20% of the available oxygen (6). However, during exercise, the sympathetic nervous system becomes activated, instigating rapid physiological adaptations primarily mediated by catecholamines and neurotransmitters (26). Specifically, norepinephrine acts on α-adrenergic receptors located on the smooth muscles of splanchnic arterioles, causing vasoconstriction and subsequently reducing blood flow to splanchnic organs. Doppler ultrasonography (DU) serves as a valuable tool for measuring blood flow in the splanchnic vasculature due to its accessibility in clinic setting. Additionally, increased exercise intensity is associated with higher activity of α-adrenergic receptors on splanchnic vessels (6), particularly in the superior mesenteric artery (SMA), which leads to substantial increases in vascular resistance (27). Previous research has shown that during exercise, splanchnic blood flow decreases by approximately 43% at moderate exercise intensity (28) and further diminishes to 80% at maximal exercise intensity (29). Consequently, exercise intensity can induce changes in the gastrointestinal system by influencing tissue hypoperfusion.

Splanchnic hypoperfusion, a result of reduced blood flow to the gastrointestinal region, can lead to gastrointestinal ischemia. This ischemic condition, in turn, contributes to damage to the intestinal epithelium, involving various components like goblet cells responsible for mucus production, cells secreting antimicrobial proteins like Paneth cells, and tight junction proteins such as claudin and occludin. These tight junction proteins play a crucial role in forming a protective barrier that prevents the infiltration of pathogenic microorganisms into the systemic circulation (7). This barrier disruption, often termed hyperpermeability, can permit the passage of endotoxins like lipopolysaccharide (LPS) found on the surface of gram-negative bacteria (30), ultimately compromising the absorption of nutrients in the intestines (7). Furthermore, following an exercise session, the restoration of splanchnic circulation can lead to reperfusion injury. This injury triggers local inflammation and the generation of reactive oxygen species, which may exacerbate damage to the intestinal epithelial barrier (31).

Exercise-induced splanchnic hypoperfusion emerges during the early stages of physical activity, leading to the subsequent development of intestinal epithelial

damage. Notably, van Wijck and colleagues (13) highlighted that just one hour of moderate exercise triggers a rapid onset of hypoperfusion, which is significantly correlated with elevated plasma intestinal fatty acid-binding protein (I-FABP) within a mere 10 minutes. Interestingly, these elevated I-FABP levels return to baseline within an hour after exercise cessation, underscoring the gut's resilience in tolerating a temporary reduction in blood flow during one hour of exercise (13). Human ischemia-reperfusion models have further shown that the epithelial lining of the small intestine can recover from brief periods (as short as 30 minutes) of ischemic damage within 2 hours post-reperfusion (32). Therefore, it appears that relatively shorter durations of moderate-intensity exercise induce recoverable and manageable damage to the gastrointestinal tract, resulting in milder damage and less pronounced inflammatory responses. Ribeiro and colleagues(8) also pointed out that the risk of injury and permeability is elevated in aerobic exercises lasting one hour or longer, conducted at ≥70% VO_{2max}, especially in hot conditions, and is influenced by various factors like nutrition, hydration, and exercise timing.

Intestinal Permeability: The intestinal barrier must maintain a semi-permeable nature to facilitate the selective passage of vital dietary nutrients, electrolytes, and water from the intestinal lumen into the circulatory system (33). However, several circumstances can disrupt the integrity of the intestinal tight junctions, resulting in increased permeability. These conditions include exercising in high temperatures (34) and engaging in high-intensity physical activities (35). Moreover, factors such as the use of non-steroidal anti-inflammatory drugs (NSAIDs) for pain relief (18), excessive alcohol consumption (36), and psychological stress can also contribute to heightened intestinal permeability (37). It's important to note that not all forms of exercise lead to gut injury and hyperpermeability. Still, a consistent observation is that when exercise-induced core body temperature surpasses 39°C, an increase in intestinal permeability is often noted (34).

Endotoxemia: Endotoxin refers to a type of LPS located on the outer surface of gram-negative bacteria. Elevated intestinal permeability paves the way for LPS to enter the bloodstream, a phenomenon documented in cases of 1-h duration exercise (38) and exercise performed in hot environments (39). The translocation of endotoxin results in the detection of LPS in both the portal and systemic circulations. Endotoxemia can incite a cytokine response, triggering local and systemic inflammation (40), and ultimately affecting performance and overall health status(8).

2.1.2 Exertional heat stress (EHS)

Intensive physical exercise can result in metabolic heat production levels 15 to 20 times higher than those at rest (41). When heat dissipation mechanisms are not adequately activated during physical exertion, Tcore can rise by approximately 1°C every 5 minutes (3). In a moderate environment with conditions set at 22°C and 50% relative humidity, a dose-response relationship has been established between exercise intensity levels (40%, 60%, and 80% VO_{2max}) over a 60-minute duration and increased small intestinal permeability. This relationship correlates with the final rectal temperatures (38.0°C, 38.7°C, and 39.6°C, respectively) and the associated body mass loss (0.6%, 1.2%, and 1.9%, respectively), which escalate proportionally

with exercise intensity (16). An elevation in tissue temperature within both the small and large intestinal segments can be predicted by assessing Tcore in the distal colon (15). Previous cell culture studies have indicated that even a modest 1.3°C increase in temperature can rapidly disrupt gastrointestinal (GI) barrier proteins (42). However, the precise mechanistic pathways leading to hyperthermia and the consequent loss of GI barrier integrity remain relatively underexplored. Ethical considerations have limited research involving the induction of severe hyperthermia (>40°C) in human subjects for assessing GI barrier integrity. Nonetheless, a systematic review conducted by Pires and colleagues (34) concluded that exercise-induced hyperthermia exceeding 39°C consistently correlated with heightened intestinal injury and permeability.

The thermoregulatory response to heat stress during exercise initiates autonomic thermo-effector mechanisms aimed at dissipating heat. Sweating and cutaneous vasodilation facilitate evaporative cooling, which aids in heat dissipation from the body. However, these processes result in fluid loss, decreased blood volume, and subsequent dehydration (43). Simultaneously, splanchnic and renal blood flow decrease while skin blood flow increases, serving as compensatory responses to maintain blood pressure (29, 44).

The combination of environmental heat stress, typically occurring in conditions exceeding 23°C (1), and high relative humidity (above 70%) (45), has an additive effect, leading to increased markers of gut damage compared to thermoneutral conditions. This phenomenon was observed in a prior study conducted by Snipe and colleagues(46), where ten athletes engaged in exercise of the same duration and intensity (2 hours on a treadmill at 60% VO_{2max}) under varying environmental conditions (22°C, 44%RH; 30°C, 35%RH; and 35°C, 26%RH). This study revealed a proportional increase in core body temperature (Tcore) corresponding to the external temperature (38.1°C, 38.4°C, and 39.6°C, respectively).

2.2 Intestinal Fatty-Acid Binding Protein (I-FABP)

I-FABP is a vital cytosolic protein with a molecular weight of 15 kDa, primarily synthesized by enterocytes located at the apices of intestinal villi. It plays a pivotal role in facilitating the uptake and intracellular transport of fatty acids within the epithelium of the small and large intestines. Under normal physiological conditions, I-FABP is not prominently present in the bloodstream. However, when the integrity of cell membranes is compromised due to factors such as ischemic injuries, it is released into the systemic circulation. Following epithelial injury, I-FABP has a relatively short half-life of approximately 11 minutes (47). The enterocytes, situated at the forefront of the intestinal lining, are particularly susceptible to damage in cases of ischemic intestinal injuries, rendering I-FABP a valuable diagnostic marker for acute intestinal ischemia (48). Under regular circumstances, baseline FABP levels are indicative of the normal turnover rate of enterocytes. Elevated levels, on the other hand, signify damage to the intestinal epithelial cells.

Recent research involving 61 healthy individuals established a reference value for I-FABP levels below 2.0 ng/mL (2000 pg/mL), irrespective of gender and age.(49) Importantly, mild abdominal discomfort or diarrhea preceding blood sampling did not

appear to affect I-FABP levels (50). Subsequently, a comprehensive analysis using data from 242 patients who underwent abdominal surgery was conducted, culminating in the identification of a cutoff level of 3.1 ng/mL(51). This threshold proved to be highly effective in distinguishing between cases of small bowel ischemia and non-ischemic conditions(51). Notably, the area under the receiver operating characteristic (ROC) curve was greater for serum I-FABP (0.792) in comparison to other diagnostic markers (51). Consequently, I-FABP is widely employed as a prominent biomarker for assessing intestinal epithelial injury and various conditions impacting the health of the small intestine (52).

2.3 Fluid and nutrition replacement

2.3.1 Fluid replacement

Adequate fluid intake during prolonged exercise in hot conditions is pivotal to counteract the effects of sweat loss. A body mass reduction exceeding 2-3% is associated with heightened fatigue perception, elevated core temperature, and diminished performance, particularly in hot weather (3). Fluid consumption helps combat dehydration and curtails the rise in core body temperature during warm and hot conditions (19). The American College of Sports Medicine (ACSM) issued a position statement in 2007 (53), advocating for athletes to drink ad libitum within the recommended range of 400-800 ml/h. However, it's essential to tailor hydration plans to individual athletes, considering factors like sweat rates, sweat sodium content, exercise intensity, heat generation, ambient temperature, body weight, kidney function, and other pertinent variables, to prevent both underhydration and overhydration. For example, elite runners, owing to their higher running speed and the increased duration between drink stations, face challenges in fluid and carbohydrate intake during races. A marathon runner might take around 15-18 minutes to reach each 5 km drink station, whereas racewalkers could take 8-10 minutes to reach each 2 km drink station (54).

2.3.2 Hypohydration

Recent evidence indicates that dehydration can negatively impact GI integrity and function, leading to varying degrees of GI symptoms in athletes. Elevated water loss through sweating during exercise can exacerbate heat stress (55). Inadequate replacement of lost body water can lead to a reduction in plasma volume (56), which further impairs splanchnic perfusion (57). A study that investigated pre-exercise dehydration followed by 90 minutes of cycling at 70% VO_{2max} found impaired gastric emptying and increased GI symptoms compared to the same exercise protocol under euhydrated conditions (58). Another study showed that running for one hour at 70% VO_{2max} without fluid intake led to increased gastroduodenal and intestinal permeability beyond resting levels (18), with a body mass loss of 1.5%. Conversely, maintaining hydration status (body mass loss of 0.6%) during 2 hours of running at 70% VO_{2max} at 24.7°C with 46% RH was more effective in attenuating markers of intestinal injury (I-FABP) compared to dehydration (body mass loss of 3.1%) (11). Interestingly, the severity of GI symptoms did not significantly differ between the dehydration and well-hydrated states. GI symptoms in the euhydration trial may be attributed to the forced drinking schedule, potentially causing gastric intolerance due

to excessive intragastric pressure, as opposed to ad libitum fluid intake, which would alleviate these detrimental gastric factors (59). Additionally, it is reported that many endurance athletes often avoid aggressive fluid replacement strategies to prevent the onset of GI symptoms during exercise (60). Therefore, evaluating fluid balance alongside the assessment of gastrointestinal tolerance to fluids may help identify individuals for whom ad libitum fluid consumption during endurance exercise may not be sufficient, potentially leading to greater loss of GI integrity and an increase in GI symptoms.

2.3.3 Water temperature

The ingestion of cold fluids before and during exercise in hot conditions may have a beneficial effect in mitigating GI disturbances. The internal cooling facilitated by cold fluid intake can help attenuate the increase in localized GI temperature and peripheral blood flow associated with exercise, thereby reducing the extent of gastrointestinal hypoperfusion (61). Studies examining pre- and per-exercise ice slurry ingestion have shown a reduction in core body temperature of up to 0.7°C during exercise in the heat (61). Another study by Snipe and Costa (17) found that ingesting ice-cold (0.4°C) and cool (7.3°C) water every 15 minutes during prolonged running in the heat (35°C) can attenuate thermoregulatory strain and potentially reduce intestinal injury (I-FABP),compared to ambient temperature water (22°C). This resulted in a slightly lower peak temperature in the cold and cool water groups (38.6°C) compared to the ambient water group (38.9°C). Cold and cool water ingestion also tended to reduce upper GI symptoms, particularly the urge to regurgitate. No differences were observed in the peak post-exercise cytokine profile (plasma IL-6, IL-1beta, TNF-alpha, IL-8, IL-10, and IL-1ra concentration) between the three groups. However, the trend of I-FABP concentration between pre- and post-exercise was observed to decrease more rapidly with cold and cool water ingestion compared to ambient temperature water. Moreover, measures such as rectal temperature, heart rate, and plasma I-FABP concentration were similar in the cold and cool water pre-cooling groups, suggesting that water temperatures between 0°C and 7°C may be equally effective in reducing thermoregulatory strain and intestinal injury. In practice, it may be more challenging to access and maintain ice-cold water at 0°C during exercise in the heat compared to cool water. Therefore, cool temperature water is often more practical for athletes during exercise in hot conditions.

2.3.4 Carbohydrate (CHO)

Endurance athletes rely on CHO as their primary energy source during physical activity. The American College of Sports Medicine (ACSM)(53) recommends CHO intake based on exercise duration and intensity. For moderate exercise lasting around 1 hour per day, athletes are advised to consume 5-7 grams of CHO per kilogram of body weight daily. Longer or more intense exercise regimens (1-3 hours per day) require a higher intake of 6-10 grams of CHO per kilogram per day. Ultra-endurance athletes engaged in activities lasting 4-5 hours daily may even need up to 8-12 grams of CHO per kilogram per day (62). To optimize glycogen stores before exercise, it's recommended to follow a CHO-rich diet, providing 6-12

grams of CHO per kilogram in the 24-hour period leading up to the event, particularly for events lasting less than 90 minutes (62).

During training or competition sessions extending beyond 60 minutes, implementing CHO intake strategies to sustain blood glucose levels can be beneficial and thus, is recommended.(53). The recommended CHO consumption is 30-60 grams per hour for sporting events lasting 1-2.5 hours. It's ideal to consume a 6-8% CHO solution every 10-15 minutes to maximize glycogen sparing. Care should be taken regarding the concentration and quantity of CHO to balance the rate of GI absorption and the peaks of CHO oxidation (around 1 gram per minute or 60 grams per hour)(63). Excessive intake can hinder gastric emptying and lead to GI symptoms during exercise (64). Using amultiple CHO sources, such as glucose/fructose mixtures in a 2:1 ratio, at a higher ingestion rate of 1.8 grams per minute can further enhance oxidation rates up to 1.2-1.3 grams per minute due to differential intestinal transport mechanisms. These mixtures also tend to improve gut tolerance (65) Nevertheless, it's important to avoid hyperosmolar fluids, as they can cause abdominal distress and hyperosmolar diarrhea during exercise (66). Gut training for CHO fueling plans at different exercise durations and intensities is beneficial as it helps improve GI tolerability. Exercise and heat stress can affect gut function and lead to stress and sympathetic/parasympathetic imbalances.

In fact, regular ingestion of CHO drinks during exercise can positively impact splanchnic perfusion and help mitigate exercise-induced GI disturbances (23). Research has demonstrated that consuming CHO drinks during exercise aids in maintaining portal vein blood flow, preventing splanchnic hypoperfusion (24). Adequate blood flow to the gastrointestinal system is crucial for optimal nutrient digestion and absorption. Snipe et al. (25) found that consuming 15 grams of CHO before exercise and every 20 minutes (45 grams per hour) during a 2-hour run at 60% VO_{2max} in the heat (35°C) yielded several benefits. These include reduced epithelial injury (a lower increase in I-FABP), decreased small intestinal permeability, improved endotoxin clearance, and reduced physiological strain compared to water intake alone (25). These findings suggest that frequent ingestion of CHO drinks with well-tolerated concentrations before and during exercise in hot conditions can play a crucial role in preventing and mitigating exercise-induced GI stress without exacerbating GI symptoms. It's essential to determine a CHO concentration that suits the individual to optimize these benefits while minimizing potential negative effects on GI function.

CHAPTER 3 EXPERIMENTAL

3.1 Methodology

This is an experimental design study with four exercise trials; no water (NW) run, an ambient temperature water (28-29°C water, ATW) run, a cold water (0-1°C water; CW) run, and a glucose powder mixed with cold water (CW+CHO) run. These trials were spaced at least 48 hours apart and consisted of 60 minutes of treadmill running at a speed set to achieve 70% of maximum oxygen consumption (VO_{2max}). The exercise sessions were conducted in an environmental room maintained at approximately 28-29°C with a relative humidity (RH) of around 60-70% and were located in the sports and exercise medicine laboratory at the Faculty of Medicine, Chulalongkorn University, Thailand. Blood samples were collected before and immediately after each running trial, and Doppler ultrasound was used to measure superior mesenteric artery (SMA) and portal vein blood flow before and immediately after each running session. Participants were informed about the study's benefits and potential risks and provided written informed consent before participating. The study protocol received approval from the Ethics Committee of the Faculty of Medicine, Chulalongkorn University (COA no. 887/2022).

3.1.1 Variables

Independent variable: Types of fluid replacement
Dependent variable: Gut injury marker (plasma I-FABP); Bacterial translocation, Endotoxemia (serum LPS); Splanchnic perfusion (SMA and PV); Core body temperature (rectal temperature)

3.1.2 Target Population

Healthy participants aged between 18 and 45 years.

3.1.2.1 Inclusion criteria

- Male and female participants
- Healthy (defined as not having health problems, including cardiovascular disease, diabetes mellitus, chronic kidney disease, and GI issues).
- Regular physical activity (defined as participating in exercise session at least three days per week).
- Running as the primary form of regular exercise

3.1.2.2 Exclusion criteria

- Those who cannot complete the study experimental protocols.
- Having previous GI issues; inflammatory bowel disease (Ulcerative colitis, Crohn's disease), GI surgery, severe GI complaints during daily activities (such as gastric pain, active diarrhea, nausea, and vomiting)

- Having a disability or previous health condition(s) that influence(s) exercise capacity or thermoregulation, such as acute infection/injury, or obesity (BMI > 30 kg/m2).
- Having chronic disease (cardiovascular disease, diabetes mellitus, and chronic kidney disease)
- History of heat stroke or heat illness
- Use of certain medications, such as NSAIDs (within 24 hours) and antimicrobial drugs (2 weeks prior)
- Dietary supplement (prebiotic, probiotic, bovine colostrum, curcumin, dietary nitrate, glutamine, L-citrulline, L-arginine, or other gut promotion) within 24 hours
- Smoking
- Pregnancy and lactating

3.1.2.3 Sample size

Based on a standard deviation of 118 pg mL^{-1} for post-exercise plasma I-FABP concentration of the previous study (13),using a standard alpha value (0.01) and beta value (0.1) to achieve the power of 90%, a sample size of n = 8 was calculated to detecting a mean of the differences of 150 increase in pre-post exercise plasma I-FABP concentration, the sample size is determined by paired t-test analysis (67).

Formula:

$$n = \left[\frac{(z_{1-\alpha} + z_{1-\beta})\sigma_d}{\Delta} \right]^2$$

$$n = \frac{[((2.576 + 0.9)118)]^2}{150}$$

Sample size = 7.48 => 8

Sample size (dropout 20%) = 10

After adding a 20% dropout rate and readjusting the number of participants, the lowest **sample size of n = 10** is required. This number of participants also matched with previous work (13, 17).

3.2 Study Procedure

The study included participants who had provided written informed consent. These participants were required to make five visits to the sports medicine laboratory, all at approximately the same time of day. Each visit adhered to a specific protocol. Participants received instructions to abstain from engaging in any physical exercise and from consuming alcohol during the 24 hours preceding each experimental trial. Moreover, they had to do a minimum 8-hour fasting period before the exercise trial,

with the allowance of water intake during this fasting window. To maintain proper hydration, participants were advised to consume an adequate volume of fluids in the 24 hours leading up to each visit. Furthermore, within a timeframe of 30 minutes to 1 hour before the exercise trial, participants were directed to ingest room temperature water (450-500 ml at 28-29°C) to facilitate optimal hydration.

3.2.1 Screening and Maximum oxygen consumption (VO_{2max}) test

Participants underwent a preliminary screening and testing session, which included an assessment of their exercise training history, completion of the exercise-associated gastrointestinal symptoms questionnaire (68), a brief physical examination by a physician, and measurement of body composition including weight, height, and percentage of body fat, utilizing the InBody770 model (BPM040S12FXX). Additionally, a maximal aerobic capacity test (VO_{2max}) following the modified Balke protocol (69), on a motorized treadmill (Nautilus SportseriesT518). Subsequently, participants were provided with a recovery period. After the recovery, the treadmill speed at 70% of their VO_{2max} was determined, with a minimum duration of 10 minutes to obtain an average running speed. Subsequently, this established speed will be applied as the designated running protocol speed in the subsequent exercise trials.

3.2.2 Running trials

Participants arrived at the laboratory in the morning (from 6 a.m. to 10 a.m.) and undergo a 20-minute rest period. Prior to body mass measurement in a private room, participants did a self-collect urine in a urine cup (further details in the **Urine specific gravity** section), and blood samples were taken (further details in the **Blood sampling and Biomarker Assays** section). The running protocol was designed to maintain a constant speed, corresponding to 70% VO_{2max}. All subjects were required to maintain this speed for 60 minutes. In the trials involving water replacement (ATW, CW, and CW+CHO), the water bottles were prepared in equal portions and dispensed every 10 minutes, with the serving temperature adjusted accordingly (additional information in the **Drink Preparation** section).

Throughout the 60 minutes running session, the rate of perceived exertion (RPE) based on Borg's scale (70), core temperature (Tcore), and heart rate (HR) monitored by a Polar device were assessed every 10 minutes. Each participant underwent four trials: NW then ATW, CW, and CW+CHO. Immediately after exercise, participants had the Tcore monitoring device removed and exited the treadmills to assume a supine position for blood collection and a Doppler ultrasound assessment (as detailed in the ultrasound data collection section). Subsequently, participants towel-dried, removed rectal probe, urination, and underwent a semi-naked body mass measurement. The body mass loss was calculated by subtracting the pre-exercise body mass from the post-exercise body mass. The formula is as follows: Body mass lost = body mass pre – (body mass post + total volume of water consumed). The urine volume did not use to calculated in this study.

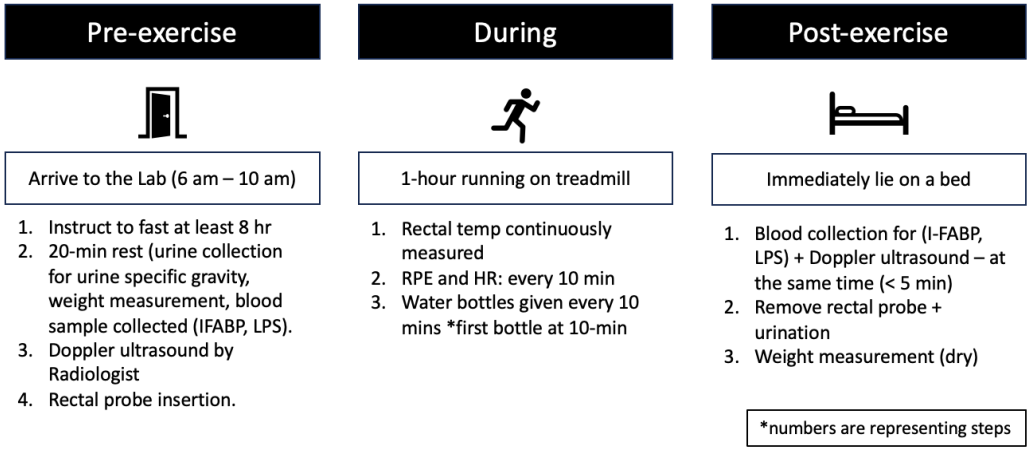


Figure 1. Running Experiments Procedure.

3.2.3 Drink Preparation

The total volume of fluids provided to each participant during the exercise trials, starting from the NW visit, was adjusted based on their body mass loss. The fluid intake was divided into six equal portions, with participants consuming one portion every 10 minutes during the exercise session. The specific procedures for preparing the beverages are detailed below.

- Ambient temperature water (ATW) - Water bottles were stored in a temperature-controlled room maintained at 28-29°C.
- Cold water (CW) - Water bottles were cooled in an ice-filled container, reaching a temperature of 0-1°C.
- Cold carbohydrate water (CW+CHO) - A solution containing 60 grams of glucose powder (Bangkok Chemical, FDA 10-1-23362-5-0029) and water was prepared, ensuring that the concentration does not exceed 8% to prevent gastrointestinal discomfort, as previously described(63). This mixture was then chilled in an ice-filled container, maintaining a temperature of 0-1°C. Water temperature was verified using a portable thermometer (EMT-100, Etekcity) to ensure the correct temperature before participants' consumption.

  All the cold fluids were placed in an ice bucket and measured to provide the appropriate amount in a bottle for consumption within a 1-minute timeframe at each designated time point.

3.2.4 Blood sampling and biomarker assays

Blood samples were obtained through venipuncture, drawing approximately 11 mL in total (5 mL in an EDTA tube for plasma I-FABP and 5 mL in a clot blood tube for serum LPS). This blood collection procedure was conducted twice, once before the exercise and immediately after the exercise, for each of the four exercise trials. Subsequently, 1 mL of the blood sample was reserved for measuring hematocrit, while the remaining sample was subjected to centrifugation to separate the

red blood cells from the plasma and serum. These separated components are then aliquoted and stored at -80°C for subsequent analysis and interpretation.

The serum levels of lipopolysaccharides and plasma levels of human fatty acid binding protein 2 (FABP2) were measured using the commercially available enzyme-linked immunosorbent assay (ELISA) kits from Abbexa LTD, UK (cat no. abx 150357), and the QuantikineTM human FABP2/I-FABP from R&D Systems, USA (cat no. DFBP20) in accordance with the manufacturer's instructions, respectively.

Briefly, the plasma samples were loaded into each well for LPS and plasma were diluted at a ratio of 1:2 for FABP2 prior to adding to the well. The concentrations of LPS and FABP2 levels were calculated using their standard curves. The minimum of detectable values ranged from 12.35-1,000 ng/ml for LPS and 2.12-6.21 pg/ml for FABP2. The absorbance in triplicate was measured for the determination of both LPS and FABP2 concentrations.

3.2.5 Urine-specific gravity

The measurement of urine-specific gravity serves as an indicator of hydration status. For this study, a defined normal range for urine-specific gravity was set at 1.005 to 1.030. In cases where a participant's urine specific gravity exceeded 1.030, they were provided with instructions to consume 500 ml of water and wait for 30 minutes before undergoing the test to ensure adequate hydration. Subsequently, urine samples were meticulously examined using a refractometer to precisely determine the specific gravity. This standardized procedure was implemented to consistently gather and evaluate urine-specific gravity values falling within the predefined normal range.

3.2.6 Hematocrit

In this study, we obtained a blood sample from the participants before and after exercise, and subsequent centrifugation was performed to separate red blood cells from the plasma. The hematocrit level was then determined by assessing the length of the red blood cell column in relation to the total blood column length, with results interpreted using established reference ranges. Commonly, alterations in plasma volume occur due to exercise-induced hemoconcentration. To quantify this change, we applied a well-established equation (71), enabling the calculation of the percentage change in plasma volume: [100/(100 – hematocrit pre-exercise values)] x [100(hematocrit pre-exercise values – hematocrit post-exercise values)/hematocrit post-exercise value]. This percentage change in plasma volume was subsequently employed to derive corrected values for plasma using the formula: Plasma volume post-exercise values corrected = (Plasma volume post-exercise values uncorrected x (1 + percentage change in plasma volume)(72).

3.2.7 Questionnaire

The investigator was administered an exercise-associated GI symptoms questionnaire before, during, and after each running trial. Severe GI symptoms were employed to delay trials if they occurred before initiation, to halt the trial in the event of severe symptoms during the exercise, and to assess impaired exercise performance or even lead to withdrawal(68). The data collected include inquiries about exercise-associated gastrointestinal symptoms presented in the Thai language.

3.2.8 Laboratory condition

The temperature and humidity in the experimental room are meticulously monitored and managed. A fan, positioned approximately 2 feet in front of the treadmills, serves two purposes: simulating outdoor airflow and aiding in expelling excess heat when the room's temperature increases. Heaters are employed to adjust the room's temperature, and humidity is regulated by introducing water fog to elevate humidity levels. The room was, on average, maintained at a temperature of 28.0°C (with a range of 28.0 to 29.0°C), and the relative humidity was carefully maintained within the range of 60% to 70%.

3.2.9 Ultrasonographic data collection

To visualize and assess blood vessel flow velocity and diameter, a transcutaneous ultrasound duplex system (Vivid IQ, GE Healthcare) was employed. This system includes a real-time sector scanner (3.5 MHz) and a pulsed Doppler flowmeter (3.0 MHz). The angle between the incident ultrasound beam and the long axis of the vessel was approximately 60^{o}, determined using B-mode imaging, as previously described (73). Participants assumed a supine posture and were instructed to briefly suspend their breath to minimize potential disruptions caused by breathing movements. Doppler ultrasound scans were performed both before and immediately after each running trial, with a maximum time of 5 minutes allowed for three to five scans.

The superior mesenteric artery (SMA) was visualized in the sagittal plane, with the sample volume (2 mm) positioned just distal to its origin from the abdominal aorta, ensuring differentiation from the celiac artery via Doppler waveforms, as described in previous research (74). Furthermore, a longitudinal image of the portal vein was acquired through a subcostal approach. The sample volume cursor was placed at the central part of the vein, between the confluence of the splenic and superior mesenteric veins, and the division into left and right hepatic branches. This technique allowed for a precise assessment of portal vein hemodynamics.

The machine's auto calculation of time-averaged mean velocity (TAMV) involved averaging values from 2 - 3 consecutive cardiac cycles (in cases where the waveform was not fully formed in all three consecutive cardiac cycles, we specifically opted for two). Blood flow was determined using the TAMV and the vessel diameter (d) during systole, identified via the electrocardiogram wave. The formula $TAMV \times \pi \times 4 \times d^2$ was employed for this calculation. To ensure consistent measurements across all images in SMA, the diameter and velocity were obtained at the origin of the T wave. This choice was made based on the physiological understanding that the systolic flow velocity occurs with a delay from the QRS complex, attributed to the transmission from the heart to the blood vessels. We observed that this aligned well with the peak velocity point thus served as a reference point for diameter and velocity flow measurements. In PV, as its pulsation is not similar to the arterial pattern, the marked point at the R wave of the EKG was used. An electronic caliper was positioned near the sample volume cursor, and a manual line was drawn along the vessel's length. The diameter measurements were then manually obtained and oriented

perpendicularly to the vessel wall. This process was repeated three times in each cardiac cycle and across three cardiac cycles to derive an average measurement value.

All Doppler ultrasound measurements were conducted by a single radiologist, and subsequent analyses were performed by the author of this study. Due to the identification requirements of the test, there was no blinding of subjects and experiments. To assess measurement reliability, reproducibility tests were conducted using the Intraclass Correlation Coefficient (ICC). All tests were recorded as video files in duplex mode, incorporating baseline values from all participants for subsequent analysis. For SMA measurements, the diameter exhibited good reproducibility (ICC: 0.910), and the flow velocity showed satisfactory reproducibility (ICC: 0.829). In PV measurements, the diameter demonstrated good reproducibility (ICC: 0.899), while the flow velocity showed moderate reproducibility (ICC: 0.798).

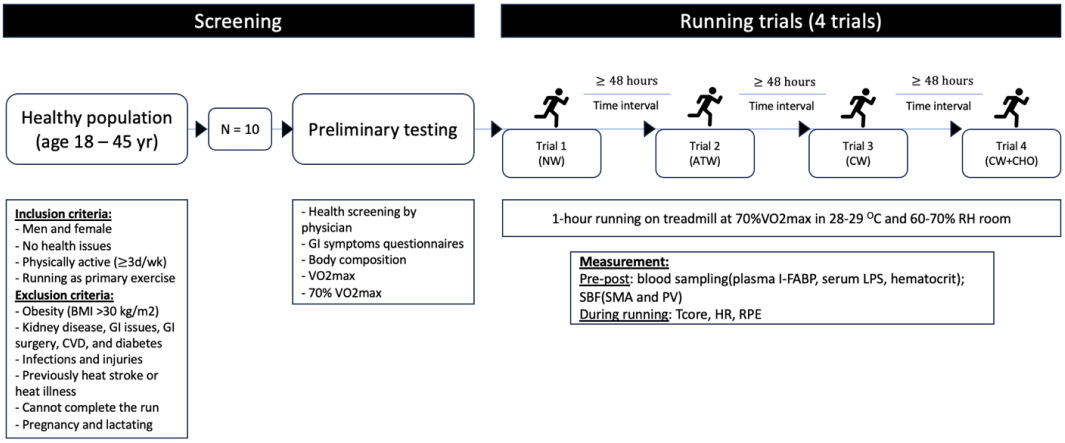
3.2.10 Core body temperature (Tcore)

Tcore measurement was conducted to assess how exercise and fluid replenishment affect the accumulation of body heat. A rectal thermocouple (YSI 400 series, with an interchangeability tolerance of ± 0.1°C) was inserted to a depth of 10 cm beyond the external anal sphincter. Continuous monitoring of Tcore was carried out, and temperature readings were documented every 1 minutes over the course of the 60-minute running session.

3.2.11 Materials and Equipment

- Motorized treadmill (Nautilus)
- Bio-Electrical Impedance Analysis (Inbody 770)
- Water, Glucose powder (Bangkok Chemical, FDA 10-1-23362-5-0029), and water container
- Room thermometer and humidity sensor (Smart Sensor, AR807), Food thermometer (Etekcity EMT-100, USA), rectal thermometer (YSI Incorporated 400 series, USA)
- Gas analyzer (Jaeger, Oxycon mobile, Germany) and tools
- Rating of Perceived, Borg scale (6 – 20)
- Heart rate monitor, chest strap (Polar)
- Blood collection tools
- ELISA test kits; I-FABP (R&D Systems, USA), LPS (Abbexa, UK)
- Ultrasound (Vivid IQ, GE Healthcare)
- Computer/Notebook and document and analyzing software.

3.3 Study overview



3.4 Statistical Analysis

Descriptive analysis was employed to present general information, including age, gender, weight, height, percentage of body fat, and VO2max, with mean values accompanied by standard deviations for normally distributed data. The normal distribution of the data was assessed through the calculation of skewness and kurtosis coefficients. The paired sample t-test was used to compare the mean changes in I-FABP, LPS, and splanchnic blood flow at 60 minutes to the baseline value (post-pre). All variables, including I-FABP, LPS, Tcore, and splanchnic blood flow (SMA and PV blood flow, diameter, and flow velocity), underwent analysis using a linear mixed-effects model for repeated measurements. This model considered changes in outcomes at various time points, comparing mean changes (with standard error (SE) as variability) for each trial; and mean change difference with 95% confidential interval (CI) in Tcore variables. Statistical analyses were conducted using STATA (Stata/SE 17.0), with statistical significance defined as a P-value of ≤ 0.05.

CHAPTER 4 RESULTS

4.1 Participants

A total of 10 participants (7 males and 3 females) were included in this study, with one participant excluded due to the inability to ingest water as instructed. The baseline characteristics of these 10 participants are detailed in Table 1. The median time interval between all the trials was 11.3 days, ranging from 2 to 56 days. The mean intervals between specific trials were as follows: NW and ATW (14.3 ± 16.8 days), ATW and CW (7.5 ± 6.3 days), CW and CW+CHO (8 ± 9.7 days). The majority of the time intervals between trials (20 out of 30) occurred every 2-7 days, except for one instance with a 56-day gap due to personal reasons. All participants reported no gastrointestinal (GI) symptoms such as diarrhea and infection before any of the running trials. Additionally, no participants reported severe GI symptoms, only mild symptoms (such as bloating, regurgitation) after water ingestion during running.

4.2 Laboratory conditions

The laboratory conditions were controlled and no difference between the 4 trials was observed (NW: 28.3 ± 0.2, RH 63.1 ± 2%; ATW: 28.4 ± 0.2, RH 62.7 ± 1.5%; CW: 28.4 ± 0.1, RH 63.5 ± 1.4%; CW+CHO: 28.3 ± 0.1, RH 63.0 ± 1.3%; p = 0.625).

Table 1. Demographic and Characteristics of the Participants at baseline (N = 10)

Characteristics	
Age (years)	31.5 ± 9.1
Maximum oxygen capacity (ml/kg/min)	50.3 ± 3.5
Maximum heart rate (beats per minute)	183.1 ± 8.9
70% VO_{2max} running speed on treadmill (km/h)	9.9 ± 1.1
Body mass (kg)	61.8 ± 11.0
BMI (kg/m^2)	21.8 ± 2.7

Data, obtained during the preliminary visit, are presented as mean ± standard deviation.

4.3 Body mass loss, fluid volume replacement, and urine specific gravity

The average body mass loss varied among the different exercise trials following a 60-minute running session. In the NW trial, the average body mass loss was -1.3 ± 0.2 kg (approximately ~2.1% dehydration), while in the ATW, CW, and CW+CHO trials, the average body mass losses were -0.2 ± 0.2 kg, -0.1 ± 0.2 kg, and -0.2 ± 0.2 kg, respectively. The body mass loss was significantly different between the NW and the other fluid replacement trials ($p < 0.001$). However, there were no significant differences of the change in body mass loss after exercise observed among the fluid replacement trials (ATW, CW, and CW+CHO, p = 1.000). The average fluid replacement volume for ATW was 1287 ± 247 ml; CW was 1278 ± 243 ml; and CW+CHO was 1281 ± 239 ml with an average CHO content of 4.68 percent.

Furthermore, there were no statistically significant differences in the baseline urine specific gravity among all four trials (NW: 1.012 ± 0.008; ATW: 1.012 ± 0.006; CW: 1.013 ± 0.009; CW+CHO: 1.014 ± 0.008; p = 0.911). Notably, none of the subjects exhibited dehydration or a urine specific gravity exceeding 1.030 before the initiation of the exercise sessions. These findings indicate that adequate hydration levels were maintained before the commencement of the exercise trials.

4.4 Blood test

4.4.1 Hematocrit for hemoconcentration

The baseline hematocrit level was recorded at 42 . 3 ± 2. 6 %, with no significant differences observed between the different trials (p = 0.926). The change in hematocrit pre- and post-exercise are as follows: NW: 0.5 ± 1.0%; ATW: -0 . 6 ± 0 . 5 %; CW: 0.0 ± 0.9%; CW+CHO: 0.1 ± 0.6%; Individual changes fall within the range of -1.0% to 2.0%. Additionally, the calculation of plasma I-FABP and serum LPS values was adjusted for hemoconcentration change after exercise.

4.4.2 Intestinal injury (plasma I-FABP)

The ELISA standard curve for I-FABP yielded a value of 0.9987. Plasma I-FABP levels were measured before and immediately after running in each trial and are presented as mean ± standard deviation in Table 2. One female participant displayed an unusually high I-FABP level for one occasion (7,054.167 pg/mL) compared to the others (average I-FABP level across all pre-exercise trials: 1936.57 ± 1148.60 pg/mL), which was considered an outlier. Thus, her I-FABP values from all trials were excluded from the analysis. Baseline plasma I-FABP levels did not differ significantly among the four trials (p = 0.434). The change in I-FABP (post – pre) values was analyzed and presented in Table 2, indicating a significant post-exercise change in I-FABP values across all trials (NW: 1045.95 ± 395.55 pg/mL, p = 0.002; ATW: 1940.83 ± 395.55 pg/mL, p = <0.001; CW: 1567.87 ± 395.55 pg/mL, p < 0.001; and CW+ CHO: 779.92 ± 395.55 pg/mL, p = 0.019). There was a significant difference in change from NW in ATW (894.88 ± 395.55 pg/mL, p = 0.024), but not in CW (521.92 ± 395.55 (SE) pg/mL, p = 0.187) and CW+CHO (-266.03 ± 395.55 (SE) pg/mL, p = 0.501). The change of I-FABP was significantly reduced in CW+CHO when compared with ATW (-1160.91 ± 395.55 (SE) pg/mL, p = 0.003) and to CW (-787.95 ± 395.55 (SE) pg/mL, p = 0.046). However, there were no significant differences in the change of I-FABP levels between CW and ATW (-372.96 ± 395.55 (SE) pg/mL, p = 0.346).

Table 2. The plasma I-FABP values (pre-post) and comparison of change within trials, and different in change between all running trials (NW, ATW, CW, and CW+CHO). (n = 9)

Outcome	**NW**		**ATW**		**CW**		**CW+CHO**	
	Mean (± SD)		Mean (± SD)		Mean (± SD)		Mean (± SD)	
I-FABP (pg/mL)								
Pre	1862.90	± 942.65	1568.98	± 783.19	2454.17	± 1627.75	1860.25	± 1074.36
Post	2908.85	± 1707.37*	3509.81	± 1391.12**	4022.04	± 1693.13**	2640.17	± 1322.64*
Change	1045.85	± 1571.73	1940.83	± 910.65$^{\#}$	1567.87	± 1069.36	779.92	± 654.40$^{\$\&}$

Abbreviation: NW, no water; ATW, ambient temperature water; CW, cold water; CW+CHO, cold water with carbohydrate. Data are presented as mean ± standard deviation. Analyses were conducted with the use of a linear mixed-effects model adjusted for baseline value. * Significant level $p < 0.05$; ** Significant level $p < 0.001$; # Significant level $p < 0.05$ when compared to NW; $ Significant level $p < 0.05$ when compared to ATW; & Significant level $p < 0.05$ when compared to CW. Note: the change difference between trial values was not present here.

4.4.3 Endotoxemia (Serum LPS)

The ELISA standard curve yielded a value of 0.9302. Serum LPS levels were measured before and immediately after running in each trial and are presented as mean ± standard deviation in Table 3. However, one female participant (different from the participant that had an outlier I-FABP) had a recorded LPS level below the detectable limit of the assay kit on 3 samples and was consequently excluded from the analysis. The analysis revealed no significant differences in baseline serum LPS levels among the four trials ($p = 0.994$). The change in LPS (post-pre) values are detailed in Table 3, indicating a non-significant post-exercise increase in LPS values in all trials (NW: 7.19 ± 62.60 ng/mL, $p = 0.593$; ATW: 0.03 ± 42.62 ng/mL, $p = 0.998$; CW: 8.44 ± 25.94 ng/mL, $p = 0.530$; and CW+CHO: 0.05 ± 41.91 ng/mL, $p = 0.997$). Furthermore, the mean change between the trials did not show any significant differences ($p = 0.965$).

Table 3. The serum LPS values (pre-post) and comparison of change within trials, and different in change between all running trials (NW, ATW, CW, and CW+CHO). (n = 9)

Outcome	NW	ATW	CW	CW+CHO
	Mean (± SD)	Mean (± SD)	Mean (± SD)	Mean (± SD)
LPS (ng/mL)				
Pre	254.74 ± 85.83	251.45 ± 45.50	251.55 ± 60.80	259.28 ± 78.86
Post	261.93 ± 85.83	251.49 ± 68.46	260.00 ± 62.75	260.00 ± 76.58
Change	7.19 ± 62.60	0.03 ± 42.62	8.44 ± 25.94	0.05 ± 41.91

Abbreviation: NW, no water; ATW, ambient temperature water; CW, cold water; CW+CHO, cold water with carbohydrate. Data are presented as mean ± standard deviation. Analyses were conducted with the use of a linear mixed-effects model adjusted for baseline value.

4.5 Splanchnic blood flow

4.5.1 Superior Mesenteric Artery (SMA)

The SMA volume flow, diameter, and flow velocity measured before and immediately after running in each trial are presented in Table 4. Baseline SMA volume flow did not exhibit significant differences among the four trials ($p = 0.794$). The change (post - pre) in SMA volume flow showed a significant reduction in NW (-149.84 ± 97.00 mL/min, $p < 0.001$), while the CW+CHO trial displayed a significant increase in SMA volume flow (259.61 ± 145.53 mL/min, $p < 0.001$). In contrast, the ATW and CW trials did not exhibit significant changes within the trials (-59.22 ± 60.63 mL/min, $p = 0.057$, 10.56 ± 112.05 mL/min, $p = 0.734$, respectively). Table 4. Additionally, all water replacement (ATW, CW, and CW+CHO) significantly reduced the splanchnic hypoperfusion when compared to NW (ATW: 90.62 ± 43.66 (SE) mL/min, $p = 0.038$; CW: 160.40 ± 43.66 (SE) mL/min, $p < 0.001$; and CW+CHO: 409.45 ± 43.66 (SE) mL/min, $p < 0.001$). The CW+CHO trial shows a significant

increase in SMA volume flow when compared to ATW and CW (318.84 ± 43.66 (SE) mL/min, $p < 0.001$; 249.05 ± 43.66 (SE) mL/min, $p < 0.001$, respectively). However, there were no significant differences in SMA blood flow between ATW and CW (69.78 ± 43.66 (SE) mL/min, $p = 0.110$).

The baseline SMA diameter shows no significant difference among the four trials ($p = 0.992$). The change in SMA diameter indicated a significant reduction in NW (-0.08 ± 0.05 cm, $p < 0.001$), ATW (-0.02 ± 0.03 cm, $p = 0.023$), and CW (-0.04 ± 0.04 cm, $p < 0.001$). Conversely, CW+CHO shows no significant difference in SMA diameter (0.01 ± 0.01cm, p =). Table 4. Furthermore, all water replacement trial shows a significant increase in SMA diameter (ATW: 0.06 ± 0.01 (SE) cm, $p < 0.001$; CW: 0.04 ± 0.01 (SE) cm, $p = 0.001$; and CW+CHO: 0.09 ± 0.01 (SE) cm, $p < 0.001$) when compared to NW. Additionally, the CW+CHO exhibited a significant increase in SMA diameter when compared to ATW and CW (0.03 ± 0.01 (SE) cm, $p = 0.016$; and 0.05 ± 0.01 (SE) cm, $p < 0.001$). There is no significant difference in SMA diameter between ATW and CW (-0.02 ± 0.01 (SE) cm, $p = 0.126$). Table 4.

Baseline SMA flow velocity shows no significant difference among all four trials ($p = 0.506$). The change in SMA flow velocity from baseline indicated a significant increase in CW (5.06 ± 6.64 cm/s, $p = 0.014$) and CW+CHO (16.13 ± 8.22 cm/s, $p < 0.001$). While there were no significant differences in NW (-3.23 ± 4.86 cm/s, $p = 0.116$), and ATW (-2.78 ± 7.73 cm/s, $p = 0.175$). Furthermore, the cold temperature water exhibits a significant increase in SMA flow velocity (CW: 8.29 ± 2.90 (SE) cm/s, $p = 0.004$; and CW+CHO: 19.37 ± 2.90 (SE) cm/s, $p < 0.001$) when compared to NW. Conversely, ATW did not exhibit a significant difference (0.45 ± 2.90 (SE) cm/s, $p = 0.877$). Table 4.

Table 4. The SMA (pre–post) values and comparison of change within trials, and different in change between all running trials (NW, ATW, CW, and CW+CHO). (n = 10)

Outcome	NW		ATW		CW		CW+CHO	
	Mean (± SD)		Mean (± SD)		Mean (± SD)		Mean (± SD)	
SMA volume flow (mL/min)								
Pre	431.11	± 135.59	447.83	± 148.50	399.36	± 38.09	400.94	± 130.84
Post	281.27	± 75.52**	388.60	± 120.53*	409.92	± 107.89	660.55	± 246.14**
Change	-149.84	± 97.00	-59.22	± 60.63$^{\#}$	10.56	± 112.05$^{\#\#}$	259.61	± 145.53$^{\#\#\$\$\&\&}$
SMA diameter (cm)								
Pre	0.56	± 0.09	0.55	± 0.06	0.55	± 0.06	0.55	± 0.07
Post	0.48	± 0.05**	0.53	± 0.08*	0.51	± 0.8**	0.56	± 0.07
Change	-0.08	± 0.05	-0.02	± 0.03$^{\#\#}$	-0.04	± 0.04$^{\#}$	0.01	± 0.01$^{\#\#\$\&\&}$
SMA flow velocity (cm/s)								
Pre	29.27	± 5.96	30.95	± 4.97	27.84	± 5.55	27.49	± 5.83
Post	26.04	± 4.83	28.16	± 5.57	32.90	± 5.13*	43.63	± 12.35**
Change	-3.23	± 4.86	-2.78	± 7.73	5.06	± 6.64$^{\#\$}$	16.13	± 8.22$^{\#\#\$\$\&\&}$

Abbreviation: NW, no water; ATW, ambient temperature water; CW, cold water; CW+CHO, cold water with carbohydrate. Data are presented as mean ± standard deviation. Analyses were conducted with the use of a linear mixed-effects model adjusted for baseline value. * Significant level $p < 0.05$ when compared to pre; ** Significant level $p < 0.001$ when compared to pre; $^{\#}$ Significant level $p < 0.05$ when compared to NW; $^{\#\#}$ Significant level $p < 0.001$ when compared to NW; $^{\$}$ Significant level $p < 0.05$ when compared to ATW; $^{\$\$}$ Significant level $p < 0.001$ when compared to ATW; $^{\&}$ Significant level $p < 0.05$ when compared to CW; $^{\&\&}$ Significant level $p < 0.001$ when compared to CW.

4.5.2 Portal vein (PV)

All the PV volume flow, diameter, and flow velocity measured before and immediately after running in each trial are presented in Table 5. There were no significant differences in the baseline PV flow among the four trials ($p = 0.728$). However, the NW (-286.74 ± 145.45 mL/min, $p < 0.001$), ATW (-138.97 ± 159.38 mL/min, $p < 0.001$), and CW (-72.46 ± 89.13 mL/min, $p = 0.040$) trials experienced a significant reduction in PV volume flow, while CW+CHO trial displayed a significant increase (214.22 ± 149.81 mL/min, $p < 0.001$). Table 5. All the water replacement trials show a significantly increase in PV volume flow (ATW: 147.77 ± 48.01 (SE) mL/min, $p = 0.002$; CW: 214.28 ± 48.01 (SE) mL/min, $p < 0.001$; CW+CHO: 500.96 ± 48.01 (SE) mL/min, $p < 0.001$) when compared to NW. Moreover, the CW+CHO significantly increased PV volume flow when compared to ATW (353.19 ± 48.01 (SE) mL/min, $p < 0.001$) and CW (286.69 ± 48.01 (SE) mL/min, $p < 0.001$). However, there is no significant difference between ATW and CW (66.50 ± 48.01 (SE) mL/min, $p = 0.166$).

Baseline PV diameter shows no significant difference between the four trials ($p = 990$). The NW revealed significant reductions in PV diameter (-0.16 ± 0.12 cm, $p < 0.001$), whereas the CW+CHO trial exhibited a notable increase (0.08 ± 0.08 cm, $p = 0.004$). In contrast, the ATW and CW trials showed no statistically significant changes in PV diameter (-0.01 ± 0.07 cm, $p = 0.754$; and 0.02 ± 0.06 cm, $p = 0.552$, respectively). Table 5. All water replacement trials exhibit a significant increase in PV diameter (ATW: 0.15 ± 0.04 (SE) cm, $p < 0.001$; CW: 0.17 ± 0.04 (SE) cm, $p < 0.001$; and CW+CHO: 0.23 ± 0.04 (SE) cm, $p < 0.001$). Moreover, the CW+CHO significantly showed a difference in SMA diameter to ATW (0.83 ± 0.04 (SE) cm, $p = 0.022$. There were no significant difference between ATW and CW, also CW+CHO and CW ($p = 0.521$ and $p = 0.101$, respectively.

There were no significant differences in baseline PV flow velocity ($p = 0.614$). Furthermore, the change in PV flow velocity exhibited a statistically significant decrease in the NW (-2.70 ± 4.26 cm/s, $p = 0.005$) and ATW (-3.82 ± 3.87 cm/s, $p < 0.001$) from baseline. CW did not reach a significant difference (-1.76 ± 1.51 cm/s, $p = 0.069$) The CW+CHO trial displayed a statistically significant increase in PV blood flow velocity (2.32 ± 3.82 cm/s, $p = 0.017$). Table 5. There was a significant difference in change of velocity flow between CW+CHO and NW (5.02 ± 1.34 (SE) cm/s, $p < 0.001$), but no significant in ATW and CW ($p = 0.124$).

Table 5. The PV (pre–post) values and comparison of change within trials, and different in change between all running trials (NW, ATW, CW, and CW+CHO). (n = 10)

Outcome	NW		ATW		CW		CW+CHO	
	Mean (± SD)		Mean (± SD)		Mean (± SD)		Mean (± SD)	
PV volume flow (mL/min)								
Pre	613.43	± 146.93	665.16	± 153.54	601.80	± 122.33	597.14	± 171.51
Post	326.69	± 61.39**	526.20	± 147.68**	529.34	± 104.06*	811.37	± 248.61**
Change	-286.74	± 145.45	-138.97	± 159.38$^{\#}$	-72.46	± 89.13$^{\#\#}$	214.22	± 149.81$^{\#\#\$\$\&\&}$
PV diameter (cm)								
Pre	0.98	± 0.17	0.98	± 0.12	0.98	± 0.11	0.96	± 0.12
Post	0.83	± 0.15**	0.97	± 0.15	0.99	± 0.12	1.04	± 0.09*
Change	-0.16	± 0.12	-0.01	± 0.07$^{\#\#}$	0.02	± 0.06$^{\#\#}$	0.08	± 0.08$^{\#\#\$}$
PV velocity flow (cm/s)								
Pre	13.59	± 2.09	14.79	± 2.10	13.42	± 2.59	13.74	± 3.36
Post	10.89	± 4.38*	10.97	± 3.85**	11.66	± 2.99	16.07	± 5.31*
Change	-2.70	± 4.26	-3.82	± 3.87	-1.76	± 1.51	2.32	± 3.82$^{\#\#\$\$\&\&}$

Abbreviation: NW, no water; ATW, ambient temperature water; CW, cold water; CW+CHO, cold water with carbohydrate. Data are presented as mean ± standard deviation. Analyses were conducted with the use of a linear mixed-effects model adjusted for baseline value. * Significant level $p < 0.05$ when compared to pre; ** Significant level $p < 0.001$ when compared to pre; $^{\#}$ Significant level $p < 0.05$ when compared to NW; $^{\#\#}$ Significant level $p < 0.001$ when compared to NW; $^{\$}$ Significant level $p < 0.05$ when compared to ATW; $^{\$\$}$ Significant level $p < 0.001$ when compared to ATW; $^{\&}$ Significant level $p < 0.05$ when compared to CW; $^{\&\&}$ Significant level $p < 0.001$ when compared to CW.

4.6 Core body temperature (Tcore)

No significant differences were observed in baseline Tcore among the four trials ($p = 0.178$), as presented in Table 6. However, after 10-minute of running, a significant increase in Tcore was evident in all trials ($p < 0.001$). Table 7. Notably, when comparing Tcore at 40-minute of running to the NW trial, both the CW and CW+CHO trials showed a significant difference in the rate of Tcore increase over time. Specifically, the CW trial demonstrated a difference in rate of Tcore increased by 0.34 °C ($p = 0.002$), and CW+CHO significantly different in rate of Tcore raised by 0.24 °C ($p = 0.031$), compared to the NW trial, as shown in Table 7. At the 60-minute mark, all of the fluid replacement trials (ATW, CW, and CW+CHO) exhibited a significant difference in Tcore increase (0.35 °C, $p = 0.002$; 0.57 °C, $p < 0.001$; 0.46 °C, $p < 0.001$; respectively) compared to the NW trial.

Table 6. The core body temperature (Tcore) in all running trials. (n = 10)

Time (min)	NW	ATW	CW	CW+CHO
Baseline	36.47 ± 0.21	36.62 ± 0.25	36.74 ± 0.32	36.57 ± 0.28
10-minute	37.15 ± 0.28	37.27 ± 0.32	37.41 ± 0.25	37.27 ± 0.29
20-minute	37.77 ± 0.29	37.86 ± 0.25	37.94 ± 0.26	37.78 ± 0.35
30-minute	38.10 ± 0.28	38.14 ± 0.37	38.21 ± 0.21	38.07 ± 0.41
40-minute	38.35 ± 0.31	38.30 ± 0.32	38.28 ± 0.23	38.21 ± 0.45
50-minute	38.49 ± 0.30	38.41 ± 0.35	38.33 ± 0.20	38.29 ± 0.47
60-minute	38.63 ± 0.31	38.43 ± 0.33	38.33 ± 0.20	38.27 ± 0.50

Abbreviation: NW, no water; ATW, ambient temperature water; CW, cold water; CW+CHO, cold water with carbohydrate. Data are presented as mean ± standard deviation.

Table 7. The comparison of differences in Tcore change at 10, 20, 30, 40, 50, and 60-minute among all running trials (NW, ATW, CW, and CW+CHO). (n = 10)

Time (min)	NW		ATW		CW		CW + CHO	
	Mean (95% CI)		Mean (95% CI)		Mean (95% CI)		Mean (95% CI)	
10 min								
Δ from baseline	0.68	(0.52, 0.84)**	0.65	(0.49, 0.81)**	0.67	(0.51, 0.83)**	0.70	(0.54, 0.86)**
Difference from NW		Reference	-0.03	(-0.25, 0.19)	-0.01	(-0.23, 0.21)	0.02	(-0.2, 0.24)
20 min								
Δ from baseline	1.30	(1.13, 1.47)**	1.24	(1.07, 1.41)**	1.20	(1.03, 1.37)**	1.21	(1.04, 1.38)**
Difference from NW		Reference	-0.06	(-0.28, 0.16)	-0.10	(-0.32, 0.12)	-0.09	(-0.31, 0.13)
30 min								
Δ from baseline	1.63	(1.44, 1.82)**	1.52	(1.33, 1.71)**	1.47	(1.28, 1.66)**	1.50	(1.31, 1.69)**
Difference from NW		Reference	-0.11	(-0.33, 0.11)	-0.16	(-0.38, 0.06)	-0.13	(-0.35, 0.09)
40 min								
Δ from baseline	1.88	(1.67, 2.09)**	1.68	(1.47, 1.89)**	1.54	(1.33, 1.75)**	1.64	(1.43, 1.85)**
Difference from NW		Reference	-0.20	(-0.42, 0.02)	-0.34	(-0.56, -0.12)#	-0.24	(-0.46, -0.02)#
50 min								
Δ from baseline	2.02	(1.79, 2.25)**	1.79	(1.56, 2.02)**	1.59	(1.36, 1.82)**	1.72	(1.49, 1.95)**
Difference from NW		Reference	-0.23	(-0.45, -0.01)#	-0.43	(-0.65, -0.21)##	-0.30	(-0.52, -0.08)#
60 min								
Δ from baseline	2.16	(1.9, 2.42)**	1.81	(1.55, 2.07)**	1.59	(1.33, 1.85)**	1.70	(1.44, 1.96)**
Difference from NW		Reference	-0.35	(-0.57, -0.13)#	-0.57	(-0.79, -0.35)##	-0.46	(-0.68, -0.24)##

Abbreviation: CI, confident interval; NW, no water; ATW, ambient temperature water; CW, cold water; CW+CHO, cold water with carbohydrate. Symbol: Δ, absolute change. Analyses were conducted with the use of a linear mixed-effects model adjusted for baseline value. * Significant level $p < 0.05$; ** Significant level $p < 0.001$; # Significant level $p < 0.05$ when compared to NW; ## Significant level $p < 0.001$ when compared to NW.

CHAPTER 5 DISCUSSION and CONCLUSION

In this study, our primary objective was to assess the impact of different fluid replacements on intestinal epithelial injury, endotoxemia, splanchnic blood flow, and core temperature in healthy participants engaged in 60-minute running at 70% VO_{2max}. Despite observing a reduction in splanchnic hypoperfusion and mitigation of the rising in Tcore across all fluid replacement trials (ATW, CW, and CW+CHO), no reduction in intestinal epithelial injury was identified when compared to the NW trial. Notably, CHO drinks demonstrated the most pronounced reduction in splanchnic hypoperfusion and intestinal injury when compared with fluid replacement alone (ATW and CW). Conversely, no substantial differences were noted in LPS, either induced by the exercise with or without fluid replacement. These findings highlight the importance of CHO intake during prolonged running.

Prolonged exercise is known for splanchnic hypoperfusion and resultant intestinal injury (7, 13). Our study observed a substantial increase in plasma I-FABP levels across all running trials, indicating the emergence of intestinal injury during the moderate-intensity exercise routines implemented. Notably, the observed plasma I-FABP concentrations in our study appear to surpass those documented in previous research(11, 17), emphasizing the significant variability in baseline I-FABP concentrations is attributed to diverse visit intervals (ranging from every 2 days to more than a month apart), dietary variations (high-fat diet can cause higher I-FABP expression)(75), and health status at the time of each visit. Unexpectedly, the NW trial showed lower changes in plasma I-FABP levels compared to the fluid replacement trials (ATW and CW). Contrasts to prior study (11), our findings indicate that ATW and CW, while improving splanchnic hypoperfusion, correlated with higher change values in intestinal epithelial injury than the NW trial. The study highlights potential variations in baseline I-FABP values due to insufficient intervals between visits for complete epithelial cell restoration (3-5 days) (76). The use of SMA in the systolic phase may limit the perspective on overall blood flow. Despite improvements in SMA with fluid replacement trials, they paradoxically correlated with higher changes in intestinal injury compared to NW. Fasting status and the absence of a caloric breakfast, unlike previous research (11), may contribute to these disparities. Despite these complexities, our data suggest that the carbohydrate drink demonstrated efficacy in reducing plasma I-FABP increment compared to ATW and CW, although it does not outperform NW in this regard. Caution is warranted in interpreting plasma I-FABP changes after water replacement exercises, complicating direct comparisons across studies.

In previous studies(40, 77), elevated blood LPS activity, often associated with intestinal epithelial injury and increased intestinal permeability, has been observed. In the present study, we observed that repeated ATW and CW, along with CW+CHO during prolonged running, did not result in a change in serum LPS. Notably, even in the NW trial, the LPS marker did not significantly increase from baseline. Our results align with prior studies (78, 79), reporting unchanged serum LPS concentrations following intense exercise. Despite the greater dehydration observed in our NW trial compared to previous studies(18) (2.1% vs. 1.5%, respectively), we did not observe an elevation in LPS levels. This implied that dehydration at this level does not elevate

endotoxemia levels unless Tcore exceeds a certain threshold, as previously described, exceeding 39°C(34), a condition that was not achieved in any of the running trials in our study. Previous studies(80) have found that LPS rapidly disappears from circulation, with a half-life of 2-4 minutes in mice, and the liver eliminates about three quarters of LPS from blood circulation, potentially explaining our findings. Other factors, such as dysbiosis from a high-fat diet (81), may contribute, although this falls beyond our current research focus. Importantly, this conclusion gains further support from the absence of increased LPS markers during 60 minutes of moderate-intensity running and in response to fluid replacement within this research environment.

In the NW trial, the degree of dehydration reached an average level of 2.1%. Conversely, in the fluid replacement trials, we successfully achieved proper fluid balance, with no significant body mass loss observed. Our finding also indicated a significant enhancement in splanchnic perfusion across all fluid replacement trials (ATW, CW, and CW+CHO) compared to the NW trial. Furthermore, our study offers insight into the mechanisms underlying this improvement, particularly concerning the modulation of SMA and PV blood flow via alterations in diameter and velocity. Specifically, both SMA and PV diameters exhibited significant dilation in response to all fluid replacement trials when contrasted with the NW trial. Regarding blood flow velocity, a significant increase was observed in SMA in the CW and CW+CHO trials and in PV in the CW+CHO trial. Moreover, CW+CHO outperformed ATW and CW in reducing splanchnic hypoperfusion, potentially mediated by nitric oxide (NO)-induced glucose-induced vasodilation (82). In practical terms for endurance athletes, our study demonstrates the potential benefits of cold carbohydrate water ingestion during prolonged exercise. This approach effectively mitigates splanchnic hypoperfusion, helps prevent dehydration, and mitigates the risk of intestinal injury when compared to fluid replacement alone. However, it's important to note that the rehydration effect of CHO drinks does not appear to differ significantly in the absence of fluid replacement.

As suggested by previous research(18), fluid restriction during a 60-minute running session has the potential to exacerbate GI dysfunction by diminishing GI blood flow and contributing to hyperthermia. The decrease in blood flow to the GI tract during exercise can be a contributing factor to elevated temperatures within the abdominal cavity, resulting from reduced heat dissipation(18). In our study, a notable deviation in the rate of Tcore increase became apparent after 40 minutes of running in the cold fluid replacement trials (CW and CW+CHO) and after 50 minutes in the ATW trial, compared to NW. It is plausible that NW may result in a more rapid elevation of Tcore over a longer duration of running compared to all fluid replacement trials, particularly cold water. Therefore, the consumption of water, even at ambient temperature, may effectively curtail the rise in Tcore and potentially alleviate the risk of exertional heat stress associated with prolonged running exercise. Additionally, in this study, we emphasized the differential effects of water temperature and carbohydrates. While they significantly reduce the increase in Tcore when compared to NW, they do not have a corresponding effect on reducing intestinal injury.

Limitation

The study has several limitations that warrant consideration. Firstly, the convenience of participants selecting their visit times, despite the study intervals being communicated during informed consent, resulted in varying intervals between visits. This variability could potentially influence the measurement of the intestinal injury marker. Secondly, the study did not assess or control diet types before each trial, which might have influenced intestinal sensitivity to certain foods, particularly high-fat diets, affecting the results of the I-FABP assessment. Additionally, the sensitivity of the ELISA kit for I-FABP and LPS, dependent on the manufacturer, might introduce variability from a singular perspective. The non-randomized nature of the exercise trial could result in a sequential effect. Considering the rapid clearance of LPS in the liver, examining other inflammation markers and liver function tests might provide additional insights. The assessment of splanchnic blood flow was confined to baseline and immediately at the end of exercise within a 5-minute window, potentially overlooking hemodynamic changes throughout the entire exercise duration. Furthermore, the study focused on specific exercise duration and intensity, potentially limiting the generalizability of findings, especially regarding endotoxemia markers, to more prolonged and intense exercise scenarios. These limitations highlight the necessity for further research, including the exploration of inflammation markers to understand the response of different fluid replacement types to intestinal injury and systemic inflammation. Future studies may benefit from ensuring more consistent exercise intervals, allowing for proper recovery of intestinal epithelial protein turnover.

Conclusion

While all fluid replacements during a 60-minute run at moderate intensity show an improvement in splanchnic hypoperfusion, they lack efficacy in mitigating intestinal epithelial injury compared to running without water intake. However, carbohydrate water substantially contributes to reducing splanchnic hypoperfusion and intestinal injury when compared to fluid intake alone. Notably, endotoxemia does not occur with the study's exercise duration and intensity, along with fluid replacement. Moreover, the enhancement in splanchnic perfusion from fluid replacement is primarily achieved through the modulation of the diameter of the SMA and PV. Additionally, the cooling effect of water helps attenuate the rise in core body temperature, especially noticeable after 40 minutes of exercise.

Conflict of interest

The authors, hereby, declare no conflict of interest.

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APPENDICES

แบบบันทึกข้อมูล

ตารางบันทึกข้อมูลขณะออกกำลังกาย

	0	10	20	30	40	50	60
HR							
RPE							

Core body temperature measurement (unit: Celsius)

	1	2	3	4	5	6	7	8	9	10
Rectal										
	11	12	13	14	15	16	17	18	19	20
Rectal										
	21	22	23	24	25	26	27	28	29	30
Rectal										
	31	32	33	34	35	36	37	38	39	40
Rectal										
	41	42	43	44	45	46	47	48	49	50
Rectal										
	51	52	53	54	55	56	57	58	59	60
Rectal										

อุณหภูมิในห้องทดลอง

เวลา(นาที)	0	10	20	30	40	50	60
องศา (°C)							
ความชื้น %							

น้ำหนักตัว และปริมาณน้ำดื่ม

	NW		Trail 1 ( )		Trail 2 ( )		Trail 3 ( )	
	ก่อน	หลัง	ก่อน	หลัง	ก่อน	หลัง	ก่อน	หลัง
น้ำหนักตัว								
ส่วนต่าง น้ำหนักตัว								
ปริมาณน้ำที่ดื่ม	Total							

ผลการเก็บตัวอย่าง

	NW		Trail 1 ( )		Trail 2 ( )		Trail 3 ( )	
	ก่อน	หลัง	ก่อน	หลัง	ก่อน	หลัง	ก่อน	หลัง
Hct								
Urine sp.gr.								
LPS								
I-FABP								

VITA

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