The impact of Endurance Exercise and Carbohydrate feeding on hepatic enzymes in Ultra-endurance athletes.

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Abstract

Introduction: Ultra-endurance sports, such as ultramarathons, ultra-triathlons, and ultra-distance cycling, present extreme physiological and psychological demands, testing athletes' limits over prolonged periods of high-intensity exertion. From 1996 to 2018, global ultramarathon participation surged by 1,676%, reflecting the growing interest in these challenging events. Carbohydrates are the primary source of energy for ultra-endurance athletes, and carbohydrates with different glycaemic indices (GI) have markedly different effects on energy supply and liver function markers such as ALT and AST. High-GI carbohydrates, like maltodextrin, provide rapid energy boosts but can increase metabolic strain on the liver due to blood sugar fluctuations. In contrast, low-GI carbohydrates, such as isomaltulose, offer a slower, more stable release of energy, potentially reducing liver stress and improving endurance. Research indicates that prolonged high-intensity exercise increases liver enzyme levels, such as the key liver biomarkers alanine aminotransferase (ALT) and aspartate aminotransferase (AST), suggesting an increased burden on the liver. This study aims to examine the effects of different GI carbohydrates on liver function and metabolic recovery in ultra-endurance athletes following an acute endurance run and over a 28-day period of carbohydrate feeding. The findings provide insights into nutritional strategies for reducing liver strain, optimising performance, and enhancing recovery in ultra-endurance sports.

Methods & Materials: This study employed a randomised crossover design to evaluate the impact of different dietary interventions on ultra-endurance athletes' performance. After obtaining ethical approval from the Swansea University Ethics Committee and informed consent from all participants, nine healthy adults aged 34 to 52 from local sports clubs participated in a 77-day study. The study consisted of two consecutive 28-day dietary interventions: one involving a low glycaemic index (GI) diet and the other a high GI diet, with a 14-day washout period in between. On testing days, participants arrived at the laboratory in a fasted state for baseline data collection, followed by a 3-hour outdoor endurance run at 70% of their maximum heart rate. After the run, participants returned to the laboratory for a 3-hour rest and carbohydrate refeeding, then completed a treadmill endurance run at approximately 74% of their maximal oxygen uptake ($\dot{V}O_{2max}$) until exhaustion. A carbohydrate-rich meal was provided post-activity. Data collected included anthropometric measurements, continuous heart rate monitoring, respiratory gas exchange via the Metamax 3B device, and glucose and lactate concentrations from capillary and venous blood samples taken pre- and post-exercise. All data were analysed for normality and subjected to repeated measures analysis of variance (ANOVA) and paired-sample t-tests using IBM SPSS statistical software.

Results: Following a 28-day dietary intervention with two different glycaemic index (GI) carbohydrates, isomaltulose (low GI) and maltodextrin (high GI), the effects on participants' body weight, BMI, body fat percentage, and lean body mass (LBM) were analysed. Statistical analysis revealed no significant differences either pre- and post-intervention or between the two carbohydrate diets. In the 3-hour fixed-intensity outdoor running test, conducted in a fasted state, no significant differences were observed in running performance (including running distance, heart rate, percentage of maximum heart rate, and speed) before and after the interventions or between the two diets. Liver function biomarkers, including ALT, AST, GGT, and total bilirubin, showed no significant differences between the two dietary interventions, although AST levels significantly increased after both diets. Additionally, no significant changes were observed in blood glucose and lactate levels. The exercise performance test to exhaustion (measuring running time, $\dot{V}O_2$, $\dot{V}CO_2$, and heart rate) also revealed no significant differences. Overall, the 28-day intervention with different GI carbohydrates had no significant effect on participants' anthropometric data, liver function biomarkers, blood glucose, blood lactate, or exercise performance.

Conclusion: This study systematically investigated the effects of different GI carbohydrates—isomaltulose (low GI) and maltodextrin (high GI)—on liver function and metabolic recovery in ultra-endurance athletes following an acute endurance run and a 28-day period of carbohydrate feeding. The results indicated that neither carbohydrate diet had a significant impact on key metabolic indicators such as blood glucose, lactate, ALT, GGT, or total bilirubin, nor did it result in notable changes in body composition or athletic performance. However, a slight but significant increase in AST levels was observed in both dietary groups, suggesting a mild degree of liver or muscle stress potentially related to post-exercise muscle micro-damage and its subsequent repair process. The study suggests that carbohydrate feeding does not affect liver enzymes in ultra-endurance athletes, though the observed rise in AST warrants further investigation to determine whether it is attributable to the different GI carbohydrates. Longer-term studies, controlling for exercise-induced liver stress, are needed to gain a more comprehensive understanding of the impact of carbohydrate intake on liver enzymes and metabolism in ultra-endurance athletes, in order to inform more personalised nutritional and recovery strategies.

Declarations

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

Signed.....Ruiyang Xia.....

Date.....24/09/2024.....

This thesis is the result of my own investigations, except where otherwise stated. Other sources are acknowledged by footnotes giving explicit references. A bibliography is appended.

Signed.....Ruiyang Xia.....

Date.....24/09/2024.....

I hereby give consent for my thesis, if accepted, to be available for photocopying and for interlibrary loan, and for the title and summary to be made available to outside organisations.

Signed.....Ruiyang Xia.....

Date.....24/09/2024.....

The University's ethical procedures have been followed and, where appropriate, that ethical approval has been granted.

Signed.....Ruiyang Xia.....

Date.....24/09/2024.....

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Acknowledgements

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List of Tables & Illustrations

Abbreviations

10IMT - Deca IRONMAN® Triathlon

2IMT - Double IRONMAN® Triathlon

3IMT - Triple IRONMAN® Triathlon

ACSM - American College of Sports Medicine

ALP - Alkaline Phosphatase

ALT - Alanine Aminotransferase

AST - Aspartate Transaminase

ATP - Adenosine Triphosphate

AUC - Area Under the Curve

BMI - Body Mass Index

CECS - Chronic Exertional Compartment Syndrome

CHO - Carbohydrate

CK - Creatine Kinase (mentioned indirectly as a biomarker)

EAH - Exercise-Associated Hyponatremia

ECG - Electrocardiogram

EDTA - Ethylenediaminetetraacetic Acid

EE - Energy Expenditure

EI - Energy Intake

ELISA - Enzyme-Linked Immunosorbent Assay

GGT - Gamma-Glutamyl Transferase

GI - Glycemic Index

GL - Glycemic Load

GLP-1 - Glucagon-Like Peptide-1

HGI - High Glycemic Index

HR - Heart Rate

HR_{peak} - Peak Heart Rate

IAU - International Association of Ultrarunners

IMT - IRONMAN® Triathlon

IRONMAN® - Brand name of the Ironman Triathlon series.

LBM - Lean Body Mass

LDH - Lactate Dehydrogenase

LGI - Low Glycemic Index

MAO - Monoamine Oxidase

MSKI - Musculoskeletal Injuries

MTSS - Medial Tibial Stress Syndrome

NADH - Nicotinamide Adenine Dinucleotide (reduced form)

NAFLD - Non-Alcoholic Fatty Liver Disease

NYHA - New York Heart Association

PBP - Paris-Brest-Paris

PUFA - Polyunsaturated Fatty Acids

RAAM - Race Across America

ROS - Reactive Oxygen Species

RPE - Rating of Perceived Exertion

SFA - Saturated Fatty Acids

SST - Serum Separator Tube

TBIL - Total Bilirubin

TCA cycle - Tricarboxylic Acid Cycle (also known as the Krebs cycle or Citric Acid Cycle)

UER - Ultra-Endurance Running

$\dot{V}O_{2max}$ - Maximum Oxygen Uptake

$\dot{V}O_{2peak}$ - Peak Oxygen Uptake

$\dot{V}CO_2$ – Volume of expired carbon dioxide

CHAPTER 1–
Literature Review

1.1 Introduction to Ultra-Endurance Sports

1.1.1 Ultra-Endurance Sports

Definition of Ultra-Endurance Sports

Ultra-endurance sports, lasting over six hours, challenge athletes to complete significantly longer distances than typical endurance events (1, 2). Sports such as Ultraman triathlons, ultra-marathons, and ultra-distance cycling examine physiological adaptability, nutritional strategies, recovery processes, and psychological resilience in extreme conditions. Long-duration endurance activities require the integrated functioning of various physiological mechanisms, complemented by physical fitness, nutrition, and psychological factors, rather than relying on a single factor (3, 4). In essence, ultra-endurance sports test both physical and psychological limits, demonstrating athletes' advanced self-management capabilities under extreme conditions. (5).

Types of Ultra Endurance Sports

Ultramarathon: Ultramarathons are races that usually exceed a standard marathon length of 42.195 kilometers (26.2 miles), with distances starting from 50 kilometers. Race lengths vary up to 1,000 kilometers (3,100 miles) (6), ranging from single-day events testing speed and endurance to multi-day events that evaluate the limits of stamina and recovery. Participants need adequate rest between segments for ongoing challenges. Time-limited and stage races assess athletes' capacity to achieve maximum distances within predefined periods or segmented stages, requiring strategic planning and effective self-pacing. Some events also offer flexible timing or self-determined race lengths, focusing on personal achievement rather than competition

(7). The diverse durations of ultramarathons increase the sport's inclusivity, allowing participants, from elite to amateur, and across genders, to select challenges that match their objectives (8).

Ultramarathon environments range from urban streets to natural settings, each posing distinct challenges. Urban courses utilize existing infrastructure with artificial obstacles, while more remote terrains like mountains, hills, and deserts offer natural difficulties including uneven surfaces, elevation changes, and severe weather. These conditions require runners to exhibit exceptional adaptability, endurance, and efficiency. Additionally, forest and cross-country events demand heightened adaptability and quicker reactions, whereas icy conditions challenge stability and endurance. Transnational races necessitate adaptation to varied terrains and weather, reflecting the demands of long-distance travel. Collectively, these diverse settings test ultramarathon participants' physical and adaptive capabilities (7, 9, 10, 11, 12).

Ultra-triathlon: Ultra-triathlons extend well beyond standard triathlon distances, starting from IRONMAN® distances of 3.8 kilometers swimming, 180 kilometers cycling, and a 42.195-kilometer run. From 1985 to 2014, this sport has developed from the double IRONMAN® (7.6 kilometers swimming, 360 kilometers cycling, 84.4 kilometers running) to the deca IRONMAN® (38 kilometers swimming, 1800 kilometers cycling, 422 kilometers running), and further to more rigorous formats such as the double deca IRONMAN® (76 kilometers swimming, 3600 kilometers cycling, 844 kilometers running) and the triple deca IRONMAN® (114 kilometers swimming, 5400 kilometers cycling, 1266 kilometers running) (13).

Ultra-triathlons typically start early to capitalize on cooler morning temperatures for swimming and cycling, mitigating the effects of afternoon heat. As the day progresses, varying weather and rising temperatures test the participants' fitness and adaptability. The swim occurs in open water, facing challenges from currents and temperature, while cycling and running traverse diverse terrains like urban paths and rural hills, assessing athletes' speed, control, and resilience. Race environments range

from tropical to cold and from sea level to high altitude, requiring extensive adaptation. Over three days, ultra-triathletes encounter comprehensive physical, strategic, and psychological demands (14, 15). These competitions evaluate athletes' physical strength, speed, endurance, strategy, and psychological resilience. Success in longer triathlon events heavily relies on previous racing experience, such as the number of races completed and past achievements. Notably, personal bests in shorter events are strong predictors of performance in longer races, demonstrated by a significant correlation between IRONMAN® best times and triple IRONMAN® results (16).

Ultra-Distance Cycling: These races present significant physical and endurance challenges, covering extensive distances from hundreds to thousands of kilometers, sometimes spanning continents, across varied terrains like mountains, deserts, and urban areas. Effective energy management and adaptability to varying climatic conditions are crucial for cyclists. In self-supported events, competitors are responsible for their own sustenance and repairs, whereas supported races provide logistical and technical assistance (17).

Events like the Race Across America (RAAM) demand cyclists cover over 3000 miles in 12 days, about 250 miles daily (18), while the Paris-Brest-Paris (PBP) requires 1200 kilometers within 90 hours, emphasizing the need for effective time and sleep management (19). Similarly, the Tour Divide and the Indian Pacific Wheel Race test cyclists' ability to navigate 2700 miles of mountainous terrain and the Australian continent without support vehicles, facing extreme weather and challenging conditions (20). Cyclists undergo rigorous training to improve endurance, strength, and speed, while developing advanced expertise in nutrition, hydration, and equipment maintenance to sustain optimal performance during long-distance events (20,21).

The global popularity of ultra-endurance sports

In recent years, the popularity of ultra-endurance sports has steadily increased globally. According to a report jointly published by RunRepeat and the International Association of Ultrarunners (IAU), research from 1996 to 2018, covering over 5 million race results across 15,451 events, has revealed an explosive growth in ultramarathon participation worldwide. Over 23 years, participation increased by 1,676%, with a 345% rise in the last decade alone, bringing the annual participant number to 329,584. Notably, since 2009, the growth rate of ultramarathons has surpassed that of traditional marathons. Additionally, the frequency of participation in ultramarathon events has also risen; in 1996, only 14% of athletes participated in more than one event per year, which increased to 41% by 2018, with a total annual participation of 611,098 instances. Simultaneously, the number of events has also been increasing, indicating not only more participants but also more racing opportunities (22). The diversity and challenge of the events are key factors attracting ultra-endurance marathon runners. From single-stage races like the "Western States 100 Mile Endurance Run" to multi-stage events like the "Marathon des Sables," ultramarathons have showcased their unique allure and challenges, successfully attracting athletes of various ages and genders (23, 24). This trend is not only reflected in the increase in participant numbers but also in the global spread of ultramarathon events across continents. Data from 2010 further emphasized this, noting that the number of people completing 161-kilometer ultramarathon races has grown exponentially over the past thirty years, further confirming the widespread appeal of ultra-endurance events (25).

Ultra-triathlon events have also become increasingly popular, especially among triathlon races. Since the introduction of the ultra-triathlon, which exceeds the distance of traditional IRONMAN® races in 1985, interest in these events has steadily grown. For example, the inaugural 2IMT event held in Huntsville, USA, in 1985 had 23 participants, indicating the initial popularity of this format. Over the following years, 3IMT and even longer distance triathlon events like 4IMT began to appear,

further demonstrating athletes' and spectators' interest in more challenging ultra-endurance events. Although the number of participants in these ultra-triathlons is relatively low compared to traditional triathlons, the fact that these events are held, and the enthusiasm of the participants reflect a trend of not only stable but growing interest in ultra-endurance events. Despite limited participation numbers, this is not due to a lack of interest but because these events demand even higher physical and mental endurance from competitors (26). From 1985 to 2014, the development of these events from the Double Iron to the Deca Iron, Double Deca Iron, and even Triple Deca Iron ultra-triathlons show the trend of diversifying development in ultra-endurance events and participants' pursuit of higher challenges. These events not only confirm the continuing growth in interest in ultra-triathlons but also showcase people's relentless pursuit of surpassing personal limits (16). From 1985 to 2009, a total of 85 2IMT events, 48 3IMT, and 10 10IMT events were held globally, with total participant numbers of 1333, 796, and 127, respectively. Notably, female participation in marathons and ultramarathons has grown significantly. Although female athletes' performances have improved, a performance gap between genders persists (26).

In addition to ultramarathons and ultra-triathlons, ultra-distance cycling events have also garnered global attention and participation, such as races exceeding 100 miles. These events can be either time-limited (like 6, 12, or 24-hour races) or distance-limited (like 100, 200, 400, 500 miles). The popularity of these races and the changing gender disparities reflect the dynamic development and ongoing progress within the ultra-endurance cycling discipline. Over time, the performance of female ultra-distance cyclists has notably improved, especially in the past 20 years. For instance, women's performance in 24-hour races has improved by 21.9%, compared to a 15.6% improvement for men, indicating significant progress for female athletes in ultra-endurance cycling competitions. Additionally, with more women and men participating in these events, the global popularity of ultra-endurance cycling races is expected to continue growing.

Ultra-endurance cycling has increasingly captured the attention and participation of many. Notably, ultra-distance cyclists participate in extreme events like the RAAM, a 5000-kilometer challenge that represents the pinnacle of ultra-endurance cycling competitions. While participation and performance trends in ultra-endurance running and triathlon have been extensively studied, data on ultra-endurance cycling events are relatively scarce. However, the trends suggest that with increasing participation from both women and men, the popularity of ultra-endurance cycling races worldwide is expected to continue rising. This growth signifies a broader acceptance and interest in pushing the boundaries of human endurance and performance in cycling (27, 28).

Participants in ultra-endurance sports

Comparing marathon runners to ultramarathon runners reveals significant differences in training, race experience, and physical composition. Typically, ultramarathon runners have completed at least one marathon prior to their first ultramarathon and often participate in additional marathons, demonstrating extensive race experience. Ultramarathon runners typically exhibit a slimmer physique than marathon runners, characterized by less skin fold thickness and smaller upper arm and thigh circumferences, but larger calf circumferences, likely adaptations to longer distance training. Their training involves longer runs at slower speeds, and they generally accumulate more weekly mileage and training time compared to marathon runners. This training pattern, indicative of ultramarathon runners' higher pain tolerance and preparation for extended distances, leads to a leaner physique as training intensity increases (29). Research comparing 100-kilometer ultramarathon runners with marathon runners shows that ultramarathoners' body compositions are better adapted for endurance, with a focus on increased running volume, whereas marathon training prioritizes speed enhancement. In 100-kilometer ultramarathons, age, weight, and body fat percentage show a positive correlation with performance, while weekly running mileage negatively correlates, underscoring the importance of running volume (30). Conversely, for marathon runners, body fat percentage has a positive

correlation with race times, and training speed shows a negative correlation, reflecting the influence of training pace on marathon outcomes (30). In triathlons, particularly at the IRONMAN® World Championship in Hawaii, which originally saw American dominance, European athletes tend to favour and excel in ultra-triathlons such as double and triple Ironman events. Ultra-triathletes generally engage in higher training volumes at lower intensities, whereas Ironman athletes concentrate more on training speed and efficiency. Pacing, nutrition, and hydration management are crucial for maintaining energy and hydration balance in long-distance races. Research indicates that while maximum oxygen uptake is vital for Ironman performance, ultra-triathletes must manage physical and muscular fatigue more effectively (31).

In ultra-endurance sports, gender differences in performance and participation have evolved significantly. Since the late 1970s, women's participation has risen from nearly none to approximately 20% by 2004, stabilizing at 10-20% in recent years. Despite early forecasts suggesting women might outperform men in endurance by the late 1990s, these predictions did not materialize. The performance gap remains, largely attributed to men's higher maximal aerobic capacity, with men being about 12.4% faster on average (32). In recent years, women have increasingly closed the performance gap in ultra-endurance sports, sometimes outperforming men in later race stages. This trend is due to a combination of physiological and psychological factors. Psychologically, women often pursue intrinsic motivations like health, leading to more frequent experiences of flow states during races. Physiologically, women typically utilize fat as an energy source, influenced by estrogen, while men predominantly use carbohydrates and benefit from higher maximum oxygen uptake ($\dot{V}O_2max$) due to greater muscle mass and hemoglobin levels. Sex hormones, especially estrogen and progesterone, affect various aspects of athletic performance in women, including musculoskeletal, metabolic, cellular functions, and gastrointestinal health. In ultra-endurance sports, where gastrointestinal discomfort is common, women may be more prone to these issues. However, by adapting gut training

strategies, female athletes like males can improve their digestive processes and tolerance during extended activities. The success in ultra-endurance events is significantly influenced by psychological attributes and the need for athletes to adapt to varied sports modalities, distances, and terrains. Performance disparities also relate to the participants' age and gender distribution, with gender differences stabilizing in longer races but narrowing in shorter ones. Additionally, fluctuations in estrogen and progesterone during the menstrual cycle, alongside factors like low energy availability and nutritional status, can impact female athletes' performance, complicating gender-related performance analysis (33).

From 1996 to 2010, in the Swiss "Zurich 12-Hour Swim", while male participation increased, female participation stayed constant, and performance differences varied by age group. Notably, males under 19 years old swam further than females, but in older age groups, gender differences in performance were not significant. Despite variances in some age groups, the annual top performances of male and female athletes in indoor ultra-endurance swimming events have shown no significant differences over the years, indicating that both genders can achieve comparable elite results (34). Research on gender differences in triathlon and ultra-triathlon races reveals that while women significantly improve in the running segment of short-distance triathlons compared to men, in longer events like the Hawaiian IRONMAN® and double IRONMAN®, although disparities in swimming and cycling are stable, differences in running and overall race times have progressively narrowed. In triple IRONMAN® ultra-triathlons, gender differences have widened, suggesting variable trends in performance over time that could see future narrowing or even female superiority. Studies in ultra-endurance cycling show that although men outpace women in shorter distances like 100 and 200 miles, the gender gap diminishes in longer races of 400 and 500 miles and lessens further with increasing participant age. Studies indicate that gender differences in ultra-endurance sports vary by race length, and performance gaps between genders decrease with age and longer distances, suggesting that older

women and those in extended races can compete closely with male athletes (35, 36, 37, 38).

In ultra-endurance sports, participants vary widely in age, with the average age of ultramarathon runners at 44.5 years, predominantly married males with at least a bachelor's degree. These athletes typically possess about 7 years of running experience prior to their first ultra-endurance event. Top performers in ultra-endurance events are typically aged 25 to 44 years old, with peak performance ages in 24-hour ultramarathons occurring in the late thirties to early forties and around 32 to 33 years for triathletes. Despite the physiological decline with age, these trends indicate that athletes can maintain competitive levels in ultra-endurance sports well into older age (30).

Studies from 1992 to 2010 show a yearly increase in participants in Triple Iron and Deca Iron ultra-triathlons, with average annual finishers numbering 23±9 for Triple Iron and 11±9 for Deca Iron. The highest participation occurs in the 35-39 and 40-44 age groups, suggesting that longer races attract older athletes. While the age of race winners remained stable, the overall average age of participants, especially in Deca Iron events, showed an upward trend, indicating that these ultra-endurance events increasingly attract middle-aged and older athletes over time. For athletes aged 25 to 44, performance in Triple Iron and Deca Iron events showed no significant difference, demonstrating consistent high-level endurance across both formats. However, participants in Deca Iron events exhibited stable performances in swimming and cycling across a wider age range (25 to 54 years), suggesting adaptability to the disciplines and specific race characteristics (39, 40, 41).

1.1.2 Physiology of ultra-endurance sports participants

Energy balance in ultra-endurance sports

In ultra-endurance sports, due to their high intensity and prolonged duration, the energy demands on athletes are typically large. Athletes continually deplete their

bodily energy reserves to sustain muscle activity and other physiological functions. Muscle contractions sustained over long periods, aerobic processes, and an increased metabolic rate due to prolonged activity all contribute to these demands. To avoid overheating, the body also dissipates heat through sweating, further increasing the overall energy and hydration requirements in ultra-endurance sports. Previous studies have shown that energy expenditure (EE) during ultra-triathlon events can range between 8,500 and 11,500 kilocalories. According to prior research, the average EE for male athletes participating in such events is estimated at 10,036 kilocalories, while for female athletes, it is estimated at 8,550 kilocalories (42). One study provided detailed characteristics of energy and fluid intake, as well as estimated EE, for a group of male triathletes throughout an ultra-triathlon. The estimated EE was about 11,000 kilocalories (46 MJ), while energy intake (EI) was only about 3,600 kilocalories (15 MJ), leading to an energy deficit of nearly 70% (42). In 24-hour ultramarathons, the average daily EE of runners is about 6,300 kilocalories (30), and a 54-kilometre mountain ultramarathon can result in a negative energy balance of approximately 3,700 kilocalories (2).

Faced with such massive energy demands, ultra-endurance athletes not only need to maintain efficient energy output over long periods but also strive to balance energy supply and consumption throughout the activity, especially during high-intensity periods. However, due to various factors, athletes often struggle to avoid energy deficits. The nature of ultra-endurance sports dictates substantial energy consumption. Athletes continuously burn fats and carbohydrates to fuel their energy during competitions, and this sustained energy output is difficult to fully replenish through intake during the race. Prolonged activity can cause changes in gastrointestinal function, leading to reduced efficiency in food and fluid absorption, thus impacting effective energy replenishment. Increased exercise intensity can also cause gastrointestinal discomfort, limiting the athlete's ability to eat and drink, further exacerbating the energy gap. Additionally, prolonged physical exertion and

psychological stress can lead to a reduction in appetite during competitions, meaning that athletes may be unwilling or unable to consume sufficient food even when desperately needing energy replenishment. This natural physiological response, driven by hormonal level changes, significantly limits energy intake, increasing the risk of an energy deficit. Implementing nutrition supplementation strategies in ultra-endurance sports is challenging, such as finding appropriate points during the event for nutritional supplementation, selecting the right foods and drinks to maximise absorption and utilisation, and individual tolerance and preferences for different nutritional products, all impact energy replenishment. Therefore, without appropriate nutritional and race strategies, athletes are likely to experience weight loss, declines in muscle mass and fat mass, leading to decreased athletic performance (43, 42, 44, 45).

Fuel for Ultra-endurance exercise

Substrate Utilization in Ultra-Endurance Sports

Carbohydrates are the most critical energy source in ultra-endurance sports. Under typical conditions, the energy intake of ultra-endurance athletes primarily relies on carbohydrates (CHO), followed by fats and proteins. For instance, in a 100 km ultramarathon, runners derive as much as 88.6% of their energy intake from carbohydrates, only 6.7% from fats, and 4.7% from proteins. In longer challenges, such as a 1005 km ultramarathon run over nine days, the average daily intake of carbohydrates drops to 62%, while the intake of fats and proteins increases to 27% and 11% respectively. Despite this, ultramarathon athletes often struggle to meet the required carbohydrate intake levels (30). However, under specific environmental conditions, such as in extreme cold climates, athletes might adopt a relatively lower intake of carbohydrates. For example, in an 800 km ultra-endurance race in Antarctica, caloric intake comprised 23.7% from CHO, 60.6% from fats, and 15.7% from proteins (46). Carbohydrates can be converted into energy more rapidly by the body compared to fats and proteins, especially during high-intensity exercise. Glycogen stored in muscles and the liver provides a rapid source of energy during

exercise, effectively supporting sustained performance. When oxygen intake is constant, glycogen oxidation produces more energy compared to fat. This means that the body prefers using carbohydrates as the primary energy source when high-efficiency energy production is needed to maintain prolonged, high-intensity activity. Carbohydrate intake helps maintain stable blood sugar levels, crucial for sustaining the function of the brain and muscles. During prolonged activity, stable blood sugar levels help prevent energy depletion and cognitive decline. Adequate carbohydrate supplementation can delay the onset of fatigue and enhance an athlete's endurance. This is because carbohydrate intake can delay the depletion of muscle glycogen, which is considered one of the main causes of fatigue during prolonged exercise. However, the body’s glycogen stores are limited, particularly muscle and liver glycogen. In prolonged continuous exercise, especially without frequent carbohydrate replenishment, these reserves gradually deplete. Once glycogen is depleted, the body is forced to rely on other energy sources, namely fats and to some extent proteins, to meet energy demands. As exercise duration increases, the body gradually adapts and improves its efficiency of energy utilization, increasingly using fats as the primary energy source. Fat oxidation provides more energy (about 9 kcal per gram of fat compared to about 4 kcal per gram of carbohydrates), although at a slower rate. Fat becomes a more significant energy source during prolonged low to moderate intensity exercise. While proteins are primarily used for muscle repair and rebuilding, in ultra-long endurance activities, especially when carbohydrate and fat energy supplies are insufficient to sustain continuous energy demands, the body may also start oxidizing proteins to obtain energy. Moreover, prolonged activity can lead to muscle damage, necessitating increased protein intake for muscle repair and rebuilding. Prolonged exercise promotes metabolic adaptations, increasing reliance on fat oxidation while reducing dependence on quickly depleting glycogen reserves. These adaptations help athletes maintain energy levels during long-term exercise (47, 48, 49, 50).

Metabolism of Carbohydrates for Ultra-endurance exercise

There are significant differences in carbohydrate (CHO) intake rates among athletes in different endurance events (51, 52). In triathlon events (including IM Hawaii, IM GER, and IM 70.3), the average CHO intake rates are 62 ± 26, 71 ± 25, and 65 ± 25 grams per hour, respectively, with no significant differences between these events. However, within the stages of a triathlon, CHO intake is significantly higher during the cycling stage compared to the swimming and running stages. In standalone cycling events, the average CHO intake rate is 53 ± 22 grams per hour, which is lower than in triathlons but higher than in marathons. Marathon events have the lowest average CHO intake rate of 35 ± 26 grams per hour, significantly lower than in cycling events and triathlons (53).

The American College of Sports Medicine (ACSM) recommends an intake of 30 to 60 grams of carbohydrates per hour for endurance exercises lasting more than one hour (54). However, individual ultra-endurance athletes may exceed or fall short of this standard, indicating variability in CHO intake among ultra-endurance sports.

Carbohydrates are a quick and efficient source of energy. The demand for carbohydrates may vary with different exercise intensities. During long-duration exercise (over two hours) and short-duration, high-intensity exercise, carbohydrates significantly enhance performance, albeit through different mechanisms.

In prolonged exercise, muscle glycogen directly fuels muscle activity, while liver glycogen helps maintain blood glucose levels. During exercise, muscle glycogen is broken down through glycolysis, producing ATP and NADH for rapid energy. Under aerobic conditions, pyruvate is further metabolised in the mitochondria via the citric acid cycle (TCA cycle) to generate substantial ATP (55, 56). Concurrently, when blood glucose levels drop, the liver compensates through gluconeogenesis and glycogenolysis to replenish blood glucose, preventing fatigue and performance decline due to hypoglycaemia (56). To support extended high-intensity exercise and prevent fatigue, athletes typically supplement with exogenous carbohydrates through food and drinks. These carbohydrates not only provide energy but may also influence

the central nervous system, improving mood and perceived effort, thereby enhancing performance (57). In short-duration, high-intensity exercise, although energy is primarily supplied by anaerobic metabolism, carbohydrate intake can still significantly improve performance. This is achieved by activating the central nervous system, directly supplying energy to muscles, and delaying the onset of fatigue, helping athletes sustain higher performance for longer durations (58, 59, 60, 61).

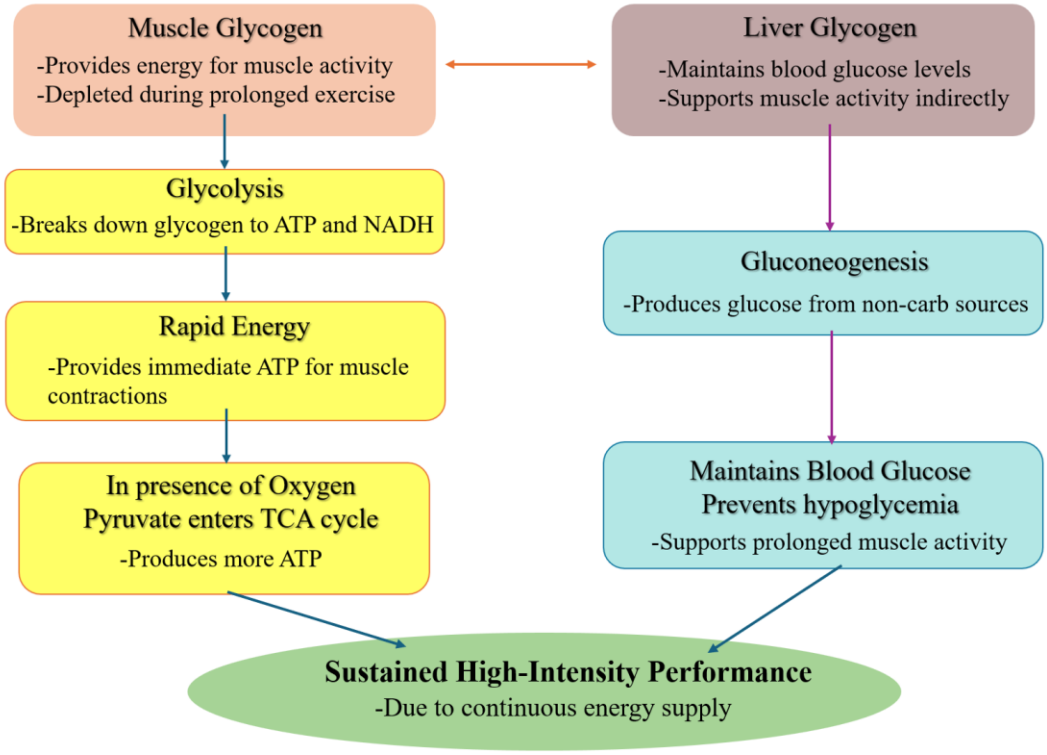

Figure 1. Energy Utilization and Glycogen Metabolism During Endurance Exercise

Intake of Vitamins and Minerals

Ultra-endurance sports, accompanied by high energy consumption, often make it difficult for athletes to meet their substantial energy and nutrient needs through regular diets, especially during competitions where sufficient food intake to replenish energy and nutrients can be challenging. Additionally, the prolonged duration of races or training limits food choices, with athletes likely opting for foods that are easy to carry and digest, restricting the opportunity to obtain a comprehensive range of

nutrients from a varied diet. Extensive sweating not only leads to water loss but can also result in the depletion of minerals such as sodium, potassium, and magnesium, which, if not replenished timely, can lead to deficiencies. Prolonged high-intensity exercise may cause changes in hormone levels, such as an increase in glucagon-like peptide-1 (GLP-1), which may suppress appetite, further reducing food intake during exercise. Moreover, some ultra-endurance athletes may lack targeted nutritional knowledge or guidance, failing to effectively plan their diets to ensure adequate intake of vitamins and minerals. Previous studies indicate that athletes widely use vitamin and mineral supplements during training and competitions to meet specific nutritional needs or to prevent/reverse common nutrient deficiencies found in athletes. While the intake of vitamins C and E may impair training adaptations, these supplements are very popular among long-distance triathletes, primarily used to reduce the risk of colds (46). In a 2006 Deutschlandlauf (Germany Cross-Country Race), despite vitamin-supplemented athletes finishing 7.8 hours faster and those taking minerals 13.7 hours faster than those who did not supplement, there was no significant performance difference between athletes who consumed these supplements and those who did not (62). However, vitamin supplementation can reduce excessive inflammatory responses in athletes, which limit performance. Exercise-induced excessive inflammation can damage skeletal muscle and affect insulin signalling, even inducing central nervous system inflammation, leading to motor coordination issues. This explains why many endurance athletes use non-steroidal anti-inflammatory drugs, although they may be ineffective or harmful. Studies show that vitamin D has anti-inflammatory effects, reducing the synthesis of tumor necrosis factor-alpha and interleukin-6, and by upregulating nuclear factor kappa B inhibitors, it reduces inflammation in macrophages (63). Vitamin D supplementation has shown significant anti-inflammatory effects against high-intensity exercise-induced inflammation in animal models. Although studies on the anti-inflammatory effects of vitamin D on human ultra-marathon runners are sparse, previous research indicated that runners who received a high dose of vitamin D before running experienced

significantly reduced inflammation induced by ultra-marathon running (64).

Iron is a crucial trace mineral important for the transport of oxygen and many key enzyme systems in energy metabolism. In high-intensity and prolonged exercise, the demand for iron increases, while losses through sweat and damage to the intestines during exercise can lead to iron depletion, increasing the risk of iron deficiency. Ultra-endurance athletes, due to sustained high-intensity physical activity, may experience an increase in plasma volume, diluting the concentration of haemoglobin in the blood, presenting a phenomenon known as sports anaemia. Although this condition does not directly impair performance, reduced haemoglobin levels may affect the efficiency of oxygen transport, potentially impacting long-term endurance. Excessive iron intake should also be avoided, as iron overload can lead to a range of health issues, including hemochromatosis, a condition where excess iron accumulation damages organs. Therefore, appropriate blood testing to monitor iron levels is advisable when considering iron supplements (65, 47).

Water and Electrolyte-Related Issues in Ultra-Endurance Sports

In ultra-endurance sports, dehydration and fluid overload are significant challenges. Dehydration not only reduces aerobic performance but also leads to increases in body temperature and heart rate, and a heightened reliance on carbohydrates as an energy source. Appropriate fluid replacement helps prevent excessive dehydration, and adding sodium (Na+) to sports drinks can increase thirst sensation, reduce fluid loss, and prevent exercise-associated hyponatremia (EAH). However, excessive fluid intake can lead to weight gain and a decrease in plasma sodium concentration, increasing the risk of EAH (47). For instance, in the Deca Iron ultra-triathlon, participants may gain up to 8 kilograms in the first three days, indicating fluid overload. Post-race weight gain is often accompanied by increased skinfold thickness and limb circumference, changes that are more common in hot weather conditions (66).

As exercise intensity increases, so do metabolic demands and heat production, leading to increased blood circulation and redistribution of blood flow. In particular, blood flow increases to muscles and skin while decreasing to abdominal viscera. The production of sweat increases, and evaporation becomes the primary means of heat dissipation. Significant sweating can substantially reduce body water content. If these losses are not offset by appropriate fluid replacement, it will lead to a decrease in plasma volume and stroke volume, an increase in heart rate, a decrease in cardiac output, and an increase in core temperature. Dehydration also alters substrate metabolism, increasing reliance on glycogen breakdown and anaerobic metabolism. However, the availability of carbohydrate stores is not a direct cause of dehydration-related fatigue but is due to the heat produced. Environmental conditions, type of sport, intensity, and duration affect the requirements for fluid and carbohydrate replenishment. Sweating not only leads to water loss but also to the loss of electrolytes, especially sodium and potassium. Sodium is crucial for maintaining extracellular fluid balance, and its deficiency is associated with prolonged exercise and substantial intake of low-sodium fluids. Therefore, fluid and electrolyte replacement during exercise must be carefully balanced to avoid issues such as dehydration and EAH, optimizing performance and health. Supplementation of sodium and potassium is vital for restoring intra- and extracellular fluid balance and related performance impacts, but supplementation strategies should be adjusted based on individual circumstances and environmental conditions (47, 67, 68).

Ultra-endurance sports and injuries

Hyponatremia

Exercise-associated hyponatremia (EAH) in ultra-endurance sports is a condition characterized by abnormally low serum sodium levels resulting from excessive fluid intake or inadequate electrolyte replenishment. The occurrence of EAH is not only associated with overhydration but also closely linked to sodium losses through prolonged intense exercise-induced sweating. EAH can lead to severe health

complications, including hyponatremic encephalopathy and swelling of the hands and feet (69, 70, 71). Factors such as environmental conditions, race duration, gender differences, and the type of event may influence the incidence of EAH (69). For instance, EAH is more prevalent in extreme cold or hot environments, while it is relatively rare in temperate climates (73, 74). The incidence of EAH is higher in events such as Ironman triathlons, triple Iron ultra-triathlons, ultra-endurance swimming, and ultramarathons exceeding 161 kilometers, whereas it is lower among cyclists (72, 70). Additionally, EAH tends to occur more frequently in female athletes, possibly due to lower body weight and relatively higher fluid intake. Risk factors for EAH include weight gain, race durations exceeding four hours, and extreme body mass index (BMI) values, rather than gender alone. While EAH incidence may also be higher in male athletes under specific conditions, such as extreme endurance events, female athletes tend to be more sensitive to fluid management and fluid balance due to physiological and anatomical differences. In emergency situations, managing EAH typically requires rapid adjustment of fluid and sodium levels to prevent severe health consequences (69).

Musculoskeletal Injuries

Musculoskeletal injuries (MSKI) are common in UER and predominantly affect the lower limbs, with most being overuse injuries. These injuries vary between races and training, with multi-stage events primarily affecting the lower legs, feet, and knees, and time-based events impacting the ankles, Achilles tendons, and knees (1). In short-distance continuous UER events, particularly those held outdoors, MSKI incidence is highest, mainly affecting the feet and ankles, while long-distance continuous UER events impact the knees and ankles. In training, common issues include back, knee, and bone stress injuries. Specifically, hip injuries occur at a rate of about 3.8% in multi-day UER events, with the iliotibial band being the most affected structure. The knee is one of the most frequently injured areas, with an incidence rate between 13.1% and 31.3% during races. The lower leg also often suffers injuries, with

incidence rates ranging from 14% to 35%, including medial tibial stress syndrome (MTSS) and chronic exertional compartment syndrome (CECS). The ankle is another common site of injury, with race incidences ranging from 16.8% to 36%. Foot injuries are also very prevalent in UER, with occurrence rates between 6.3% and 12.6%. In the 1005 km Sydney to Melbourne ultramarathon, 64 injuries were found among 32 runners, with the most common sites being the knees (31.3%) and ankles (28.1%). The most frequent single diagnosis was patellofemoral pain syndrome, followed by Achilles tendinitis and medial tibial stress syndrome (75, 76).

Organ Damage in Ultra-Endurance Sports

In extreme endurance events like ultramarathons, athletes frequently experience short-term impacts on critical organs such as the heart, liver, and kidneys, which generally recover quickly after the race ends. The heart is a primary focus in ultramarathons due to increased biomarkers post-race, such as creatine kinase, creatine kinase-MB, troponin I, and N-terminal pro b-type natriuretic peptide, indicating potential cardiac damage. These changes are usually temporary, suggesting potential cardiac impacts might not have long-term functional consequences. Importantly, the pace and length of the event can affect the degree of changes in cardiac damage markers. Echocardiographic studies have also observed post-race reductions in left and right ventricular function, although these changes are transient.

Ultramarathons can cause short-term liver damage, evident from increases in liver biomarkers such as gamma-glutamyl transferase, alanine transaminase, aspartate transaminase, alkaline phosphatase, and bilirubin. These biomarker levels typically normalize within days after the race, indicating that liver damage is usually temporary, and severe liver injury is rare in ultramarathons (79, 80, 81). Kidney damage is common among ultramarathon runners, with nearly half of all participants potentially experiencing acute kidney injury. Pathophysiological mechanisms for kidney damage include the release of muscle proteins such as myoglobin, dehydration, and heat effects. Normal kidney function markers include creatinine

levels of 0.6 to 1.2 mg/dL for men and 0.5 to 1.1 mg/dL for women, and urea levels of 7 to 20 mg/dL. During races, elevated levels of creatinine, urea, uric acid, and electrolytes indicate impaired function, though these damages typically recover within days post-race, with severe kidney damage being rare (1, 30).

Gastrointestinal Issues

The digestive system is significantly challenged in extreme endurance events like ultramarathons, where continuous nutritional intake is crucial to meet high energy demands. Unfortunately, ultramarathon runners often encounter gastrointestinal issues, including gastrointestinal bleeding, occult bleeding, and lower gastrointestinal symptoms. In some races, up to 80% of athletes report digestive issues, with nausea being the most commonly mentioned problem. These symptoms may be due to physiological changes, mechanical factors, and improper intake of fluids and food. Particularly, increased sympathetic nervous system activity induced by exercise leads to blood diversion from visceral organs to muscles and skin, reducing visceral blood flow during maximal exercise. This could result in slowed gastrointestinal motility, reduced intestinal absorption, bacterial translocation, increased intestinal permeability, and ultimately gastrointestinal symptoms. Running is more likely to cause gastrointestinal discomfort than cycling or swimming, especially during the running stage of a triathlon. Changes in oesophageal motility and endotoxemia during races have also been observed. Studies suggest that the occurrence of digestive issues is related to the athlete's volume and duration of training, with those having less training and shorter training durations more prone to digestive problems. Additionally, increases in endotoxins and pro-inflammatory cytokines lead to a counter-regulatory anti-inflammatory response, further exacerbating digestive system issues (77, 78, 30).

1.2. Carbohydrate feeding and Carbohydrate Glycemic Index (GI)

1.2.1 Methods and Classification of GI Measurement

The Glycemic Index (GI) of a carbohydrate is determined by comparing the blood sugar response induced by a specific food to the response induced by an equal amount (typically 50 grams) of pure glucose, used to measure the impact of foods on blood sugar levels. Foods are classified based on their GI value into three categories: low GI (55 or below), medium GI (56 to 69), and high GI (70 and above). To measure GI, healthy adults are typically used as test subjects, with pure glucose (assigned a GI value of 100) or white bread used as the reference food. The test dose is based on 50 grams of digestible carbohydrates, but for foods low in carbohydrates, the test dose can be reduced to 25 grams. Blood sugar levels are measured at specific time points after consuming the test and reference foods (0, 15, 30, 45, 60, 90, and 120 minutes). The incremental area under the blood sugar response curve (AUC) is calculated, ignoring areas below the baseline. The GI value is then calculated by comparing the AUC of the test food with the AUC of the reference food. This process requires participants to follow specific dietary and lifestyle guidelines before testing to ensure the accuracy and consistency of the results (104). For ultra-endurance athletes, the application of the GI and food choices are particularly crucial given their substantial EE. They need to support prolonged, high-intensity training and competition through a well-planned diet. Understanding the GI values of foods helps these athletes manage their energy supply more effectively, optimize performance, and maintain good health (105).

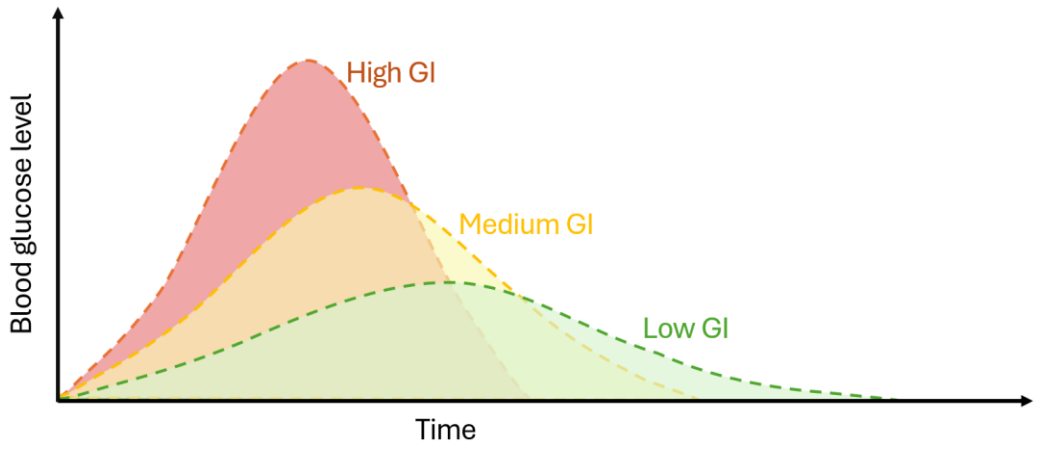

Figure 2. Comparison of Blood Glucose Levels Based on Different GI Values

1.2.2 Characterisation and physiological effects of Maltodextrin and Isomaltulose

Characteristics and Physiological Effects of Maltodextrin

Maltodextrin is a polysaccharide made up of multiple glucose units, produced by the partial hydrolysis of starch. It has a high GI, typically ranging from 85 to 105, allowing it to be quickly broken down into glucose during digestion, providing immediate energy. This characteristic makes it an ideal energy supplement for athletes engaged in high-intensity activities such as running and cycling, where endurance is demanded. Due to its high GI, maltodextrin can rapidly increase blood sugar levels after ingestion, aiding in quick energy recovery. It is easily digestible and commonly used in pre- and post-exercise recovery drinks to effectively promote rapid recovery and muscle glycogen resynthesis, thus alleviating exercise-induced fatigue. Therefore, maltodextrin is not only a key component in sports nutrition products but also an essential part of athletes' energy management and performance optimization strategies (85, 86).

As a high-GI carbohydrate, maltodextrin's rapid digestion and absorption can cause a quick rise and subsequent drop in blood sugar and insulin levels, leading to a brief surge in energy followed by a depletion of energy and a sensation of hunger. This rapid glycemic response may be disadvantageous for individuals managing weight and blood sugar levels, but appropriate in situations requiring quick energy replenishment, such as sprinting or intense training. However, reliance on high-GI foods can lead to energy fluctuations and frequent hunger, which are not conducive to long-term health and weight management. Although high-GI carbohydrates have their advantages in certain sports scenarios, they should be consumed in moderation in a regular diet to maintain a more stable energy level and overall health (105, 106, 107).

Characteristics and Physiological Effects of Isomaltulose

Isomaltulose is a low-GI carbohydrate, composed of several glucose units linked in a specific way that slows its breakdown in the human body, thus providing a more stable release of energy. Its GI is typically below 35, making isomaltulose particularly valuable in sports nutrition as it provides sustained energy support without causing drastic fluctuations in blood sugar levels. The slow digestion and absorption rate means that it gradually releases glucose into the bloodstream, leading to a slow and steady rise in blood sugar and insulin levels. This mild glycemic response helps maintain energy levels and satiety over an extended period, preventing severe fluctuations in blood sugar levels, thus benefiting weight control, reducing hunger sensations, and improving insulin sensitivity.

Additionally, the slow-release energy properties of isomaltulose help athletes maintain prolonged energy output during training or competition, reducing fatigue. This steady energy release is crucial for athletes who need to perform at a high level over long periods and can be an important part of their dietary plans. Isomaltulose's low-GI property also promotes the utilization of fat as an energy source, reducing reliance on muscle glycogen, which is particularly appealing to athletes who wish to optimize energy use and extend their activity duration. Its slow absorption also helps alleviate gastrointestinal discomfort during exercise, making it an ideal energy supplement for endurance sports such as long-distance running, cycling, and triathlons (87, 88, 89).

A study examined the impact of a low GI diet on ultra-endurance athletes. Compared to a high GI diet, consuming a low GI meal two hours before exercise not only significantly enhanced performance in a 21-kilometre run by up to 2.8% but also affected the athletes' energy metabolism during the activity. Specifically, the low GI diet led to a shift from relying on carbohydrates to using more fats as an energy source while maintaining more stable blood sugar levels, thus reducing fluctuations in blood glucose and the rate of EE during exercise. Additionally, the study observed that in the later stages of activity, a low GI diet promoted higher blood glucose concentrations and a lower rate of carbohydrate oxidation, suggesting that low GI

foods might help athletes maintain better energy levels over prolonged physical activity and delay the onset of fatigue (108).

1.2.3 The Application and Importance of GI in Sports Nutrition

The GI of pre-exercise meals directly impacts athletic performance. Research indicates that low GI foods provide a more sustained energy release, aiding in improved endurance performance. Compared to high GI foods, consuming low GI foods before exercise can reduce early exercise blood sugar peaks and decrease the risk of energy deficits from rapid blood sugar declines, thereby extending endurance and enhancing fat oxidation efficiency. This slow-release energy supply is particularly crucial for prolonged endurance activities such as long-distance running or cycling. The intake of moderate to high GI carbohydrates during exercise can quickly replenish energy, which is key for short-duration, high-intensity performance. During the recovery period after exercise, high GI foods can rapidly replenish muscle glycogen, facilitating quick recovery, though this may not be beneficial for long-duration endurance performance and fat oxidation. In contrast, consuming low GI foods post-exercise may help increase fat oxidation rates, benefiting subsequent endurance performance and weight management. The application of GI and Glycemic Load (GL) underscores the importance of personalised nutrition. Different athletes may respond differently to the same foods in terms of blood sugar response, influenced by various factors including individual metabolic differences, pre-exercise dietary status, and their training background (109).

The GI is a crucial measure for evaluating how carbohydrate-containing foods impact blood sugar response rates, and for athletes, making informed choices about foods with different GI values is vital for optimising performance and recovery. It is advised to consume low GI foods before exercise to reduce the risk of early exercise hyperglycaemia and hyperinsulinaemia while ensuring a continuous energy supply to avoid drastic drops in energy levels during exercise. During exercise, high GI foods are recommended to quickly replenish energy and maintain blood sugar levels, aiding

in sustaining performance. Post-exercise, high GI carbohydrates should be chosen to rapidly refill muscle glycogen reserves, helping to expedite the recovery process. The impact of GI is also influenced by the fibre, fat, and protein content of foods and the methods of food preparation and processing. Overall, by carefully selecting foods with varying GI values, athletes can optimise energy utilisation before, during, and after exercise, enhancing performance and speeding up recovery. GI serves as a reference tool, providing athletes with valuable information on how to choose carbohydrates that best meet their sports needs (110, 111).

1.3. Different GI Carbohydrates and Ultra-Endurance Exercise

In ultra-endurance sports, maintaining energy supply and optimising recovery are key to successfully completing races and training. This type of exercise, such as marathons, triathlons, or ultra-marathons, requires the body to sustain high levels of energy output over extended periods. In this context, understanding the impact of carbohydrates with different GI values on energy supply, fatigue management, recovery processes, and inflammation and immune responses is crucial. In ultra-endurance sports, energy management before and during the race is critical to an athlete's optimal performance. This process begins 48 to 72 hours before the event, when athletes maximise muscle glycogen stores by increasing carbohydrate intake—particularly selecting foods with medium to low GI values. This approach not only enhances endurance but also helps delay the onset of fatigue, allowing for sustained high performance during the race. The dietary arrangement in the few hours before the race is equally important, with medium to low GI carbohydrate foods, such as whole grain bread paired with protein and healthy fats, being ideal choices. This ensures a stable release of energy and reduces potential blood sugar fluctuations at the start of the race. During the race, timely supplementation with medium to high GI carbohydrates, such as energy gels or sports drinks, is crucial for maintaining blood sugar levels and extending performance. This strategy helps athletes maintain high

performance levels for extended periods and avoids the fatigue associated with rapid energy depletion, thereby sustaining optimal performance throughout the race (112).

During the recovery process following ultra-endurance activities, appropriate carbohydrate choices are essential for quickly replenishing muscle glycogen and initiating the body's repair mechanisms. The nutrition strategy for recovery is divided into two key periods: the immediate recovery phase and the extended recovery phase. The immediate recovery phase, within the first 30 minutes post-exercise, is widely recognised as a critical nutrition window. During this time, consuming high GI foods, such as white bread, specific energy bars, or fruit juice, can rapidly elevate blood sugar levels and stimulate insulin secretion. This insulin surge aids in accelerating muscle glycogen resynthesis and muscle protein repair, making it a crucial step in the recovery process. The primary goal during this phase is to leverage the rapid absorption properties of high GI foods to minimise recovery time and commence the muscle repair process. The extended recovery phase, spanning 24 to 48 hours post-exercise, is equally vital for athletes. During this period, athletes should continue to focus on balanced protein and carbohydrate intake to support ongoing recovery and muscle repair. Unlike the immediate recovery phase's need for high GI foods, athletes should balance the intake of high and low GI foods during the extended recovery phase to ensure stable blood sugar levels, avoiding fluctuations, while providing a continuous energy supply. This strategy helps optimise the recovery process, reduce muscle soreness and fatigue, and prepare for subsequent training or competition (113, 114, 115).

In ultra-endurance sports, gastrointestinal comfort is a crucial factor for athletes to maintain a stable energy supply and achieve optimal performance. Low GI foods release energy slowly during digestion, providing a more sustained energy supply, which is especially important during prolonged physical activity. These foods help to avoid dramatic fluctuations in blood sugar levels during a race, reducing hunger and the risk of gastrointestinal discomfort. For athletes who are sensitive to high GI foods,

consuming low GI foods can reduce the risk of experiencing bloating, diarrhoea, or other digestive issues during exercise. However, during specific stages of ultra-endurance events, particularly when rapid energy replenishment is needed, choosing medium to high GI foods may be more appropriate. These foods can be quickly digested and absorbed, rapidly increasing blood sugar and energy levels. This also means that careful selection of easily digestible, stomach-friendly high GI foods, as well as proper timing and quantity of intake, is essential to avoid potential digestive discomfort (116, 117).

1.4. Ultra-endurance sports and liver damage

1.4.1 Liver Enzymes as Indicators of Liver Stress or Damage

Liver stress enzymes are a crucial group of biochemical markers used to monitor and assess the stress state and damage level of the liver. These markers include alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT). Their levels in the blood can vary in response to different types of liver damage or stress, such as hepatitis, cirrhosis, drug or alcohol toxicity. ALT is primarily found in liver cells and is one of the most sensitive indicators of direct liver cell injury. When liver cells are damaged due to various causes, ALT is released from the cells into the blood, leading to elevated levels of ALT in the bloodstream. AST is also present in the liver but is less specific than ALT because it is also found in significant amounts in the heart, muscles, and kidneys. However, the combined measurement of AST and ALT can provide more comprehensive information about liver health. ALP is more widely distributed, found not only in the liver but also in bones, kidneys, and bile ducts. Elevated levels of ALP often indicate bile duct obstruction or certain liver diseases. Meanwhile, GGT is primarily associated with liver and bile duct diseases, and significant increases in GGT levels can provide important diagnostic clues, especially in cases of bile duct

damage or liver toxicity. By measuring these liver stress enzymes, athletes and physicians can assess the extent of liver damage and functionality, aiding in the diagnosis of liver diseases, monitoring disease progression, and evaluating treatment effectiveness. It's also important to note that elevated levels of liver enzymes are not always directly related to liver disease; they can also be influenced by other bodily conditions or factors (90, 91).

In conditions of prolonged, high-intensity, or extreme sports, the body's metabolic rate may increase significantly, placing additional strain on the liver and impacting liver function. Changes in liver stress enzyme levels can reflect the liver's physiological response to exercise stress and possible damage, which is important for monitoring athlete health and adjusting training. After intense physical activity, liver cells may experience temporary damage, increasing cell membrane permeability. Consequently, ALT (Alanine Aminotransferase) and AST (Aspartate Aminotransferase), enzymes primarily located within liver cells, are released into the bloodstream, leading to elevated serum levels. Normal ALT levels are generally about 7 to 56 units per litre, and AST levels are about 10 to 40 units per litre. An increase in these enzymes is typically seen as an indication of liver cell damage. Monitoring changes in ALT and AST after intense exercise can help assess the degree of liver stress response and the state of liver cell damage. An increase in ALP (Alkaline Phosphatase) levels, normally ranging from 45 to 115 units per litre, may indicate stress or damage to the bile duct system. Prolonged intense exercise can lead to accelerated energy metabolism and extensive oxidation of fatty acids, potentially affecting bile duct function. Monitoring ALP levels can help identify stress and damage related to the bile duct system, especially potential issues like gallstones in athletes. An elevation in GGT (Gamma-Glutamyl Transferase), with normal levels typically between 9 and 48 units per litre, is usually associated with liver toxicity, alcohol intake, and the use of certain medications, making it particularly important for athletes who might be using supplements or specific medications to enhance performance. Monitoring GGT levels

can help identify liver stress or damage caused by drug abuse or excessive supplementation (92, 29).

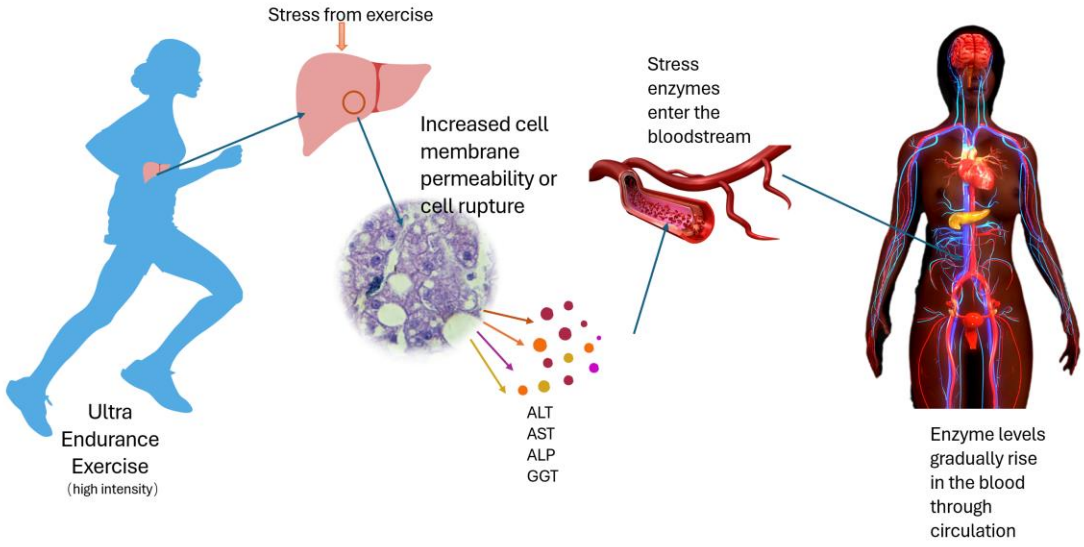

Figure 3. Mechanism of High-Intensity Ultra-Endurance Exercise on Liver Enzyme Levels

Total Bilirubin (TBIL) is a yellow-green bile pigment primarily derived from the breakdown of haemoglobin in damaged or aged red blood cells. Measuring total bilirubin is crucial for assessing liver function and diagnosing jaundice. It comprises direct bilirubin (conjugated bilirubin) and indirect bilirubin (unconjugated bilirubin), which are produced and metabolized through distinct biochemical processes. Total bilirubin serves as a key indicator of liver function, with its levels reflecting the liver's ability to handle extreme physical activities. Normal total bilirubin levels typically range from 0.1 to 1.2 mg/dL (82).

Factors like blood redistribution during ultra-endurance sports, energy metabolic stress, inflammatory responses, and oxidative stress can impact liver function, influencing bilirubin levels. For instance, studies have shown that endurance athletes may experience mild increases in total bilirubin due to the intense physical stress and increased breakdown of red blood cells commonly associated with long-duration

sports events. Such fluctuations, though generally within a safe range, can temporarily exceed normal levels, indicating heightened liver activity in response to physiological demands (30).

During prolonged exercise, the liver is required to break down more glycogen to supply energy and manage waste products from muscle breakdown, such as amino acids and lactic acid. This sustained high demand can lead to a temporary decline in liver function. Additionally, liver blood flow during ultra-endurance activities may be reduced due to the redistribution of blood towards exercising muscles, further affecting the liver's detoxification and metabolic functions. Furthermore, mechanical and chemical stresses induced by high-intensity exercise can increase the destruction of red blood cells, a process known as haemolysis, thereby increasing the production of unconjugated bilirubin. Unconjugated bilirubin is typically converted into soluble direct bilirubin in the liver and excreted through the bile (83, 84). However, in ultra-endurance activities, due to the temporary decline in liver function, this process may be impaired, leading to an accumulation of unconjugated bilirubin in the blood and, consequently, an increase in total bilirubin levels. Moreover, the liver's ability to detoxify may be limited while dealing with a large number of free radicals generated during exercise, further affecting its capacity to process bilirubin. Elevated levels of total bilirubin may be common in ultra-endurance athletes, reflecting the liver's stress response and functional changes under extreme physical activity (142). Although this increase in bilirubin levels is usually temporary, if levels remain abnormally high, particularly if accompanied by other abnormal liver function indicators such as significant increases in AST and ALT, it may indicate more severe liver damage, necessitating further medical evaluation and intervention. Therefore, monitoring total bilirubin levels after ultra-endurance activities can serve as a significant indicator for assessing the impact of exercise on liver health (143).

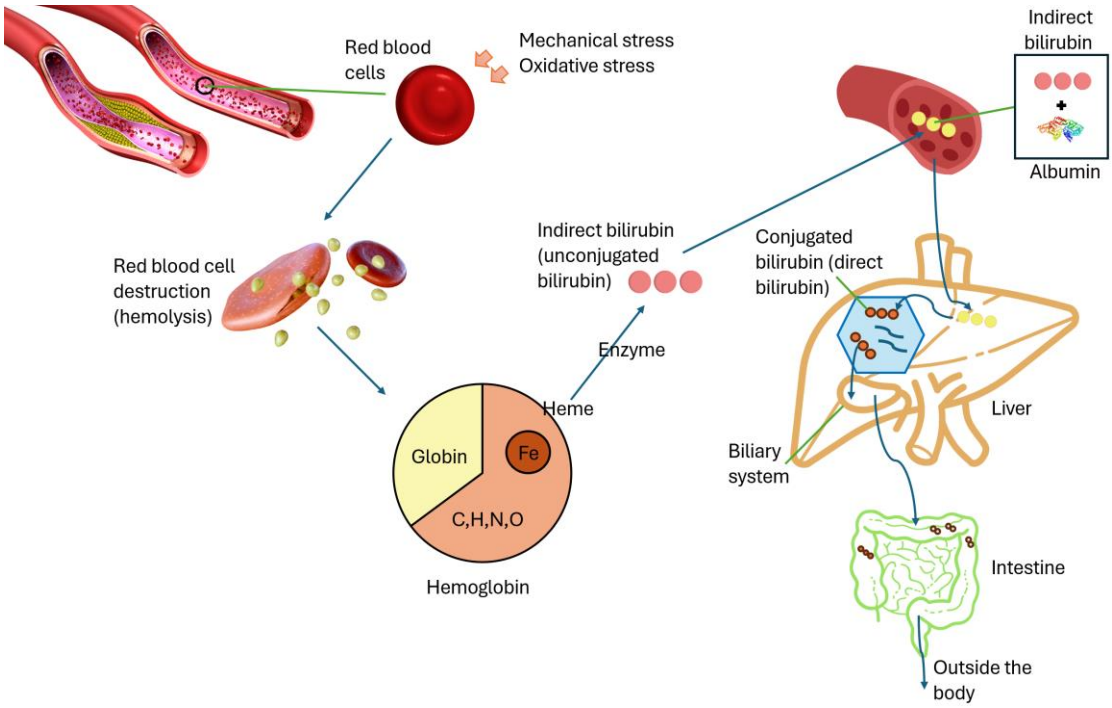

Figure 4. The process of bilirubin production. (During ultra-endurance exercise, due to temporary liver function decline, the liver's ability to process unconjugated bilirubin may be impaired, leading to the accumulation of unconjugated bilirubin in the blood.)

1.4.2 The Impact of Ultra-Endurance Sports on Liver Stress Enzyme Levels

Participation in ultra-marathons has been increasing over the past decades, and with it, understanding of the physiological changes induced by these physically demanding activities has grown through ongoing research. Several biomarkers, traditionally indicative of liver damage, tend to increase following endurance events but generally return to normal within a few days post-race. Historically, these increases have not been associated with any immediate or long-term adverse effects on athletes. In a high-altitude mountain ultra-marathon involving 669 starters, 352 (53%) successfully completed the race within the 30-hour limit. Observations revealed significant increases in the average levels of alanine aminotransferase (ALT) from 23 ± 10 U/L to 117 ± 106 U/L, aspartate aminotransferase (AST) from 23 ± 5 U/L to 485 ± 500 U/L, and bilirubin from 0.60 ± 0.29 mg/dL to 1.60 ± 0.61 mg/dL among finishers (all $P < 0.001$), while the mean alkaline phosphatase (ALP) levels remained unchanged ($P = 0.11$). Runners who completed the race exhibited elevated levels of ALT, AST, lactate

dehydrogenase (LDH), and bilirubin compared to non-finishers. Post-race biochemical analysis indicated no statistically significant correlations between the levels of ALT, AST, ALP, LDH, bilirubin, and total protein and the athletes' age, sex, body mass index, or finish time. However, significant positive linear correlations were observed between AST, ALT, and LDH with creatine kinase (CK) post-race (94). Another study tested white male athletes with an average age of 47, observing significant changes after completing a half-marathon. Notably, there was a marked decrease in body weight (-8.8%), slight increases in GGT (+6%) and AST (+10%), and more significant rises in CK (+38%), LDH (+23%), total bilirubin (+45%), and direct bilirubin (+50%). The activities of AST, LDH, total bilirubin, and direct bilirubin continued to increase up to 24 hours post-race, while GGT activity returned to pre-race levels after 6 hours. Among all observed changes, no athlete's ALT, ALP, or GGT exceeded the upper reference limit, whereas significant changes were observed in LDH, AST, CK, total bilirubin, and direct bilirubin. These results suggest that substantial aerobic exercise, like a half-marathon, can significantly alter the activities of traditional biomarkers of liver damage. Notably, increases in LDH, AST, CK, total bilirubin, and direct bilirubin were suggested to reflect common post-exercise muscle damage rather than direct liver injury. Increases in total and direct bilirubin may be partly due to intravascular haemolysis caused by foot strike, myocyte destruction due to osmotic stress, and membrane lipid peroxidation induced by free bilirubin (93).

1.4.3 The Impact of Ultra-Endurance Sports on Liver Stress and Athlete Health and Recovery

In ultra-endurance sports, the body's energy demands significantly increase, leading to a sharp rise in oxygen consumption. This process intensifies mitochondrial oxidative phosphorylation, resulting in the production of reactive oxygen species (ROS) and free radicals exceeding the clearance capacity of the antioxidant defence system. As the centre for metabolism and detoxification, the liver is required to manage these

increased free radicals, but excessive ROS can damage liver cell lipids, proteins, and DNA, causing cellular dysfunction and death. Additionally, micro-injuries to muscles and other tissues activate the immune system, triggering an inflammatory response. During this process, inflammatory mediators such as cytokines and chemokines are released into the bloodstream to promote the repair of damaged tissues. The liver plays a crucial role in this response, not only in producing some of these inflammatory mediators but also in clearing them. Prolonged or excessive inflammatory responses may exceed the liver's processing capacity, leading to liver inflammation and functional decline.

ALT and AST are two enzymes located within liver cells that normally have relatively low levels in the blood. The physical and chemical stresses caused by ultra-endurance sports can damage liver cells, causing these enzymes to leak into the bloodstream and increase blood levels. While elevated levels of ALT and AST are typically seen as indicators of liver damage, the increases observed following ultra-endurance sports are usually temporary and often return to normal within a few days. Although post-exercise increases in liver enzyme levels are generally temporary, if not properly managed, such as by continuing high-intensity training without adequate recovery, it may lead to long-term liver damage. Furthermore, a decline in liver function can impact its metabolic and detoxification capabilities during the recovery period after exercise, potentially leading to the accumulation of metabolic waste and delaying recovery processes. Previous case reports have described a rare instance of exercise-induced hepatitis in a healthy 48-year-old male marathon runner diagnosed after completing a 14-mile race. The patient sought medical attention for severe bilateral thigh and calf pain lasting over a week post-exercise, exhibiting jaundice and abnormal liver function indicators, including elevated levels of AST, ALT, ALP, and total bilirubin. Further examinations ruled out other common causes of liver injury, and a liver biopsy revealed severe ischemic injury characteristics such as parenchymal bile stasis and increased lipofuscin pigment, with no fat infiltration, inflammatory

changes, or structural abnormalities of the liver, confirming a diagnosis of exercise-induced hepatitis. This case illustrates that strenuous exercise can lead to severe ischemic liver injury, even in the absence of other risk factors. With aggressive hydration treatment and supportive measures, the patient's symptoms and liver function indicators gradually improved within one to two weeks (97). Heneghan et al. in 2014 described a young athlete participating in a 62-kilometre ultra-marathon who developed significant rhabdomyolysis and hypoxic hepatitis due to heat stroke, accompanied by multi-organ failure, including fulminant hepatic failure, resulting in intensive care and emergency hepatic resection followed by orthotopic liver transplantation (95). Carvalho et al. in 2016 reported a 25-year-old male who developed hyperthermia with neurological impairment during an ultra-marathon, progressing to acute liver failure necessitating close monitoring but ultimately recovering. These cases indicate that while increased liver injury biomarkers are usually reversible and serious liver damage is rare, severe liver function issues can still occur under specific circumstances (96).

The stress on the liver during ultra-endurance sports can significantly impact an athlete's recovery. The liver plays a pivotal role in processing metabolic waste, synthesizing proteins, regulating energy supply, and storing and metabolizing nutrients. When liver function is compromised, its ability to convert ammonia into urea may decrease, leading to an accumulation of ammonia. This can affect muscle recovery and the synthesis of new proteins. Moreover, the liver is crucial for the synthesis and breakdown of glycogen and maintaining glucose equilibrium. Liver stress may disrupt these processes, affecting the energy supply during an athlete's recovery period and thereby prolonging the time needed for recovery. The liver is also the primary site for the oxidation of fatty acids, which is essential for energy recovery; impaired function could slow the rate of fatty acid oxidation, reducing the efficiency of energy recovery. Additionally, the liver is responsible for synthesizing proteins critical for recovery, such as plasma proteins and clotting factors. Damage to

the liver may interfere with the synthesis of these proteins, affecting wound healing and muscle rebuilding. Furthermore, the liver stores vitamins such as A, D, E, and K, and minerals like iron and copper, which are crucial for maintaining normal immune function, bone health, and red blood cell production. Liver stress might interfere with the normal storage and utilization of these nutrients, impacting the nutritional status and recovery capabilities of athletes (98, 99, 100).

1.4.4 Dietary Impact on Liver Stress Enzymes

Malnutrition or excess can disrupt the balance between antioxidants and pro-oxidants, leading to liver disease. Studies show that specific micro and macronutrients are crucial for maintaining liver cell integrity, reducing fibrosis, inflammation, and fatty degeneration caused by oxidative stress (OS). The production and removal mechanisms of free radicals (FR) and other reactive substances in the liver are important for understanding the liver's capability to cope with physiological and pathological conditions. Proteins, carbohydrates, and lipids influence liver health and disease progression through various mechanisms. Proteins can directly scavenge ROS and inhibit the production of pro-inflammatory cytokines. High carbohydrate intake may promote liver steatosis and insulin resistance, while lipids, particularly saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA), impact liver health depending on their types and intake levels. Vitamins and minerals such as selenium, magnesium, manganese, copper, zinc, and iron are essential in combating OS and maintaining liver function. They act as cofactors in vital biochemical reactions that help maintain liver health (50). The enormous energy demand of ultra-endurance activities can quickly deplete the liver's glycogen reserves, impacting the energy supply chain and significantly reducing athletic performance. Long-term malnutrition can also impair basic metabolic functions of the liver, such as protein synthesis, detoxification, and lipid metabolism, not only delaying recovery post-exercise but also reducing immunity and potentially triggering liver-related diseases. Moreover, when the body lacks essential nutrients, particularly vital antioxidants like vitamins C,

E, and selenium, the liver's capacity to resist oxidative stress diminishes, making cells more susceptible to damage. Combined, these effects not only threaten the athlete's health and wellbeing but also severely impact their training and competitive performance. Nutritional excess, especially excessive intake of fats and simple carbohydrates, may pose several negative effects for ultra-endurance athletes. Excessive caloric intake can lead to liver fat accumulation, progressing to non-alcoholic fatty liver disease (NAFLD), a common liver condition that can further evolve into liver fibrosis and even cirrhosis. Additionally, excess nutritional intake, particularly of foods rich in unsaturated fats and high in added sugars, can increase levels of oxidative stress and inflammation in the body. These conditions not only damage liver cells but may also affect long-term liver health and function. Nutritional excess also imposes an additional metabolic burden on the liver; prolonged overload can lead to liver dysfunction, affecting the liver's normal metabolic and detoxification abilities. Therefore, ensuring adequate and balanced nutritional intake is crucial for maintaining the liver health and athletic performance of ultra-endurance athletes (101, 102, 103).

1.5 Aims, Objectives, Endpoint and Hypotheses

1.5.1 Aim

To investigate the effects of prolonged endurance exercise and consumption of carbohydrates with different GI values on liver enzyme and metabolic indicators in ultra-endurance athletes.

1.5.2 Objectives

- To assess the impact of consuming carbohydrates with different GI values (isomaltulose and maltodextrin) on liver function and recovery in ultra-endurance athletes following an acute fasted endurance run.

- To compare the effects on liver function and recovery of a low-GI diet (enriched with isomaltulose) versus a high-GI diet (enriched with maltodextrin) over a 28-day period of carbohydrate feeding.

1.5.3 Endpoint

Primary endpoint

- Changes in liver function markers: Including alterations in levels of liver enzymes such as AST, ALT, and GGT, which are direct biomarkers for assessing liver stress and damage.
- Metabolic recovery indicators: Primarily comprising the rapid restoration of post-exercise blood glucose and lactate levels, to evaluate the impact of carbohydrate type on the speed of metabolic recovery.

Secondary endpoints

- Changes in body composition: Such as body weight, body fat percentage, and lean body mass (LBM), which help to understand the impact of long-term carbohydrate intake on body shape.
- Variations in athletic performance: Assessing whether diets with different GI carbohydrates affect the performance of ultra-endurance athletes in standardized endurance tests.
- Energy intake and expenditure: Tracking and analysing the total energy intake and expenditure of ultra-endurance athletes during the study period, especially comparing different carbohydrate dietary backgrounds.

1.5.4 Hypothesis

Primary hypothesis: Consuming low-GI carbohydrates (such as isomaltulose) as opposed to high-GI carbohydrates (such as maltodextrin) will evoke less liver enzyme changes and faster metabolic recovery following ultra-endurance exercise.

Secondary hypothesis: A low-GI carbohydrate diet can improve long-term liver

function markers, reduce exercise-induced liver stress, and might have a positive impact on athletic performance.

CHAPTER 2–
Methods and Materials

2.1 Research Governance

This research protocol was approved by the Swansea University Ethics Review Committee. All participants provided written informed consent prior to the commencement of the study, having been fully informed of the research objectives, procedures, and potential risks involved.

2.2 Research Design

In the preliminary phase of this study, potential participants were initially assessed through screening visits. Selected participants were then randomly allocated to one of two different dietary intervention groups using a crossover design, with each dietary modification intervention lasting for 28 days. Additionally, within each phase, participants undertook two laboratory exercise tests, with an acute carbohydrate intake test conducted at the start and end of each 28-day period. The entire study lasted for 77 days, including one day of screening and a 14-day washout period.

2.3 Participants

A total of 10 participants were initially recruited for this experiment. All participants were from local running and triathlon clubs and groups that actively participated in ultra-endurance sporting events. During the study, one participant withdrew for personal reasons, leaving a final sample size of 9 participants (n=9). The group included 1 female and 8 males. Participants' ages ranged from 34 to 52 years, and their physical and body characteristics are listed in Table 1.

The participant group included 8 males and 1 female. While the inclusion of mixed genders enhances the generalisability of the findings, the predominantly male sample may limit the interpretation of gender-specific effects on liver enzyme responses.

Participant characteristics	Values (mean ± SD)
Males : Females	8:1
Age (years)	41 ± 7
Mass (kg)	79 ± 16
Height (cm)	176 ± 9
BMI (kg/m2)	26 ± 4
$\dot{V}O_{2peak}$ ($ml.kg^{-1}.min^{-1}$)	57 ± 4
HR_{Peak} (bpm)	182 ± 13
RPE_{Peak} (Borg)	19 ± 1
Run speed at $\dot{V}O_{2peak}$ ($km.hr^{-1}$)	16.0 ± 0.9

Table 1. Physical and cardiopulmonary screening test characteristics of participants (n=9).

2.4 Inclusion and exclusion criteria

Inclusion criteria

1) Informed consent was obtained
2) Male or female aged 18-65 years (both inclusive)
3) Otherwise healthy (as judged by premedical questionnaire) and participating in regular training volume in training (Self-declared >10 h training per week).

Participants were included if regularly training or participating in endurance events and/or $\dot{V}O2_{max}$ > 55 $ml.kg^{-1}.min^{-1}$.

Exclusion Criteria

1) Receipt of any investigational medicinal product within 1 month prior to screening for this trial.
2) Hemoglobin <8.0 mmol/L (male) or <7.0 mmol/L (female).
3) Use of systemic (oral or intravenous) corticosteroids, monoamine oxidase (MAO) inhibitors, non-selective beta-blockers, growth hormones, and non-routine vitamins and herbal products. Additionally, thyroid hormone use must have been stable for the 3 months prior to the start of the study.
4) Suffering from or having a history of a life-threatening disease or clinically severe condition that could directly affect the study results, as judged by the Investigator. This exclusion criterion did not apply to participants with basal cell carcinoma or squamous cell carcinoma of the skin, provided the cancer was in remission and deemed by the Investigator to have no impact on study outcomes or protocol compliance. Patients taking stable medications that affect metabolism (e.g., statins) or the cardio-respiratory system (e.g., asthma sprays) were not excluded, provided the therapy remained stable and was not adjusted during the trial.
5) Known cardiac issues were defined as decompensated heart failure (New York Heart Association (NYHA) class III and IV), angina pectoris within the last 12 months, or any occurrence of acute myocardial infarction.
6) Blood pressure at screening (after resting for 5 minutes in a supine position) was outside the range of 90-140 mmHg systolic or 50-90 mmHg diastolic (excluding white-coat hypertension; thus, if a repeated measurement on a second screening visit showed values within the range, the participant could be included in the trial). This exclusion criterion also applied to participants on antihypertensive medications.
7) History of clinically significant abnormal ECG findings, as judged by the Investigator.

8) Severe retinopathy or maculopathy and/or severe neuropathy, particularly autonomic neuropathy, as judged by the Investigator.
9) Any chronic disorder or severe disease which, in the opinion of the Investigator, might jeopardize the participant's safety or compliance with the protocol.
10) History of allergies and/or intolerances to drugs or foods or a history of severe anaphylactic reaction e.g., fructose intolerance.
11) Significant history of alcoholism or drug/chemical abuse, as judged by the Investigator.
12) Smoker (defined as a participant who smokes more than 5 cigarettes or the equivalent per day).
13) Unable or unwilling to refrain from smoking or using nicotine substitute products during the monitoring period.
14) Participant with mental incapacity or language barriers preventing adequate understanding or cooperation, or who, in the opinion of their general practitioner or the Investigator, should not participate in the trial.
15) Potentially non-compliant or uncooperative during the trial, as judged by the Investigator.
16) Any condition that might interfere with trial participation or evaluation of results, as judged by the Investigator.
17) Any known history of diabetes mellitus, or the use of any anti-hyperglycaemic drug or insulin to treat diabetes and related conditions.

2.5 Experimental Procedure

The experiment lasted for 77 days in total. It was divided into two phases, each lasting four weeks. Additionally, there was a screening and mid-diet arm washout period (2 weeks), and a pre-experiment phase (1 week). An overview of the study schedule is shown in Figure 5. The laboratory trial protocol is depicted in Figure 9.

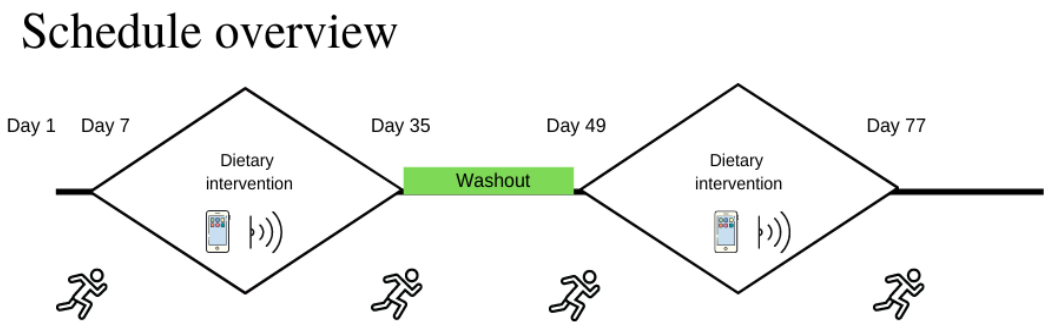

Figure 5. Overview of the Study Timeline

Figure 6. Sequence of Events on Familiarisation Day (DAY 1)

DAY 1: Screening and Cardio-Pulmonary Exercise Test

Participants underwent screening and a cardiopulmonary exercise test to initially familiarize themselves with the laboratory environment. The specific steps were as follows, in the order shown in Figure 6 above:

1) After trial numbers were assigned in ascending order, the research team collected participants' initial medical questionnaires (Appendix) and informed consent forms (Appendix) to ensure they met the study requirements.
2) Following the completion of informed consent, anthropometric characteristics including height (meters), weight (kilograms), Body Mass Index (BMI), bioelectrical impedance analysis (using Bodystat equipment), and blood pressure measurements were taken to confirm eligibility criteria.
3) Participants recorded their dietary intake throughout the trial using a diet (food

and liquid) management mobile application (Nutritics^{TM}) during the two 4-week dietary intervention periods. This process was regularly monitored by researchers to ensure adherence, with a compliance rate of 96.7% for the two diet groups.

4) Participants performed a continuous maximal incremental treadmill exercise, during which heart rate and respiratory data were collected. These data were later retrospectively analysed to determine participants' peak oxygen uptake ($\dot{V}O_{2peak}$) and submaximal exercise parameters. The peak heart rate (HR_{peak}) was used to set heart rate and speed standards for acute exercise intensity in laboratory tests.

   To determine maximum oxygen consumption ($\dot{V}O_{2max}$), participants completed a series of incremental ramp exercises on a treadmill (Pulsar 3P, Cosmos, Munich) until voluntary exhaustion. This testing protocol started with a 5-minute warm-up phase, followed by incremental step tests lasting 3 minutes each, with the speed increasing by 1 kilometre per minute, starting at a speed of 9 kilometres per hour. Heart rate and perceived exertion level (Borg scale) were measured throughout the testing process.

   Pulmonary gas exchange was continuously measured using a breath-by-breath analyser (Metamax 3B, Cortex, Leipzig). Data were continuously collected and retrospectively analysed to determine the highest oxygen uptake rate ($\dot{V}O_{2peak}$) and peak heart rate (HR_{peak}), as well as submaximal exercise parameters according to the guidelines of the British Association of Sport and Exercise Sciences (BASES) 2007. HR_{peak} was used to set submaximal heart rate targets (%HR_{peak}), and speed was set according to the speed obtained at $\dot{V}O_{2peak}$ (kilometres per hour) as a relative exercise intensity target for time-to-exhaustion tests on laboratory test days.

5) Participants then engaged in a home-based partial glycogen reduction protocol trial. This involved performing shorter duration exercises at the same intensity (approximately 70% of maximum heart rate) on the same course (approximately 1 lap), as in the acute trials.

6) Participants were randomly assigned to either low or high GI carbohydrate diet

groups. Participants followed their usual diets for 7 days, recording all food intake using the Nutritics^{TM} mobile app. Based on their regular dietary intake, they subsequently received written advice on appropriate substitutions of carbohydrate foods to ensure their chosen type of carbohydrate food had low or high GI variants.

Figure 7. Pulsar 3P, Cosmos, Munich

Figure 8. Metamax 3B, Cortex, Leipzig

2.6 Acute Exercise Test Performed at the start and end of each dietary arm

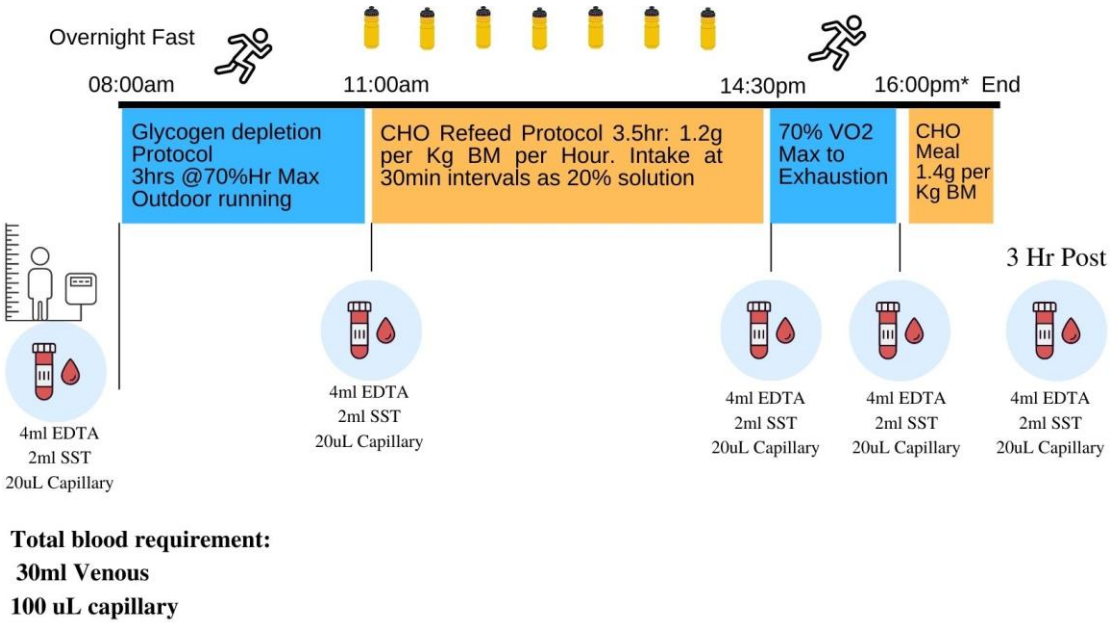

Figure 9. Laboratory Trial Procedures (Conducted at the Start and End of Both Dietary Groups)

1) Preparation Phase:
2) Participants arrived at the laboratory after fasting overnight (≥10 hours) and avoiding any physical activity for 24 hours before the trial day.
3) Laboratory Arrival and Baseline Data Collection:
    a) Anthropometric data of participants were collected in the laboratory.
    b) Venous blood samples were taken in a resting state: 4ml in EDTA tubes and 2ml in SST tubes (performed by a research team member trained in phlebotomy).
    c) Finger-prick capillary blood samples were collected: approximately 20 microlitres, for glucose and lactate analysis (specific sampling times and volumes are shown in Figure 9).
4) Glycogen Depletion Protocol (8:00 AM-11:00 AM):

5) Participants ran outdoors along a planned route for 3 hours, at an intensity corresponding to 70% $\dot{V}O_{2peak}$. Heart rate was maintained within ±5 bpm of this preset intensity, with running data collected by their own GPS watches, later downloaded by researchers from the TrainingPeaks^{TM} platform. They were required to consume at least 500 millilitres of water and 1 gram of electrolyte powder during this run, with additional water freely available.
6) Carbohydrate Supplementation Protocol (11:00 AM - 14:30 PM):
    a) Participants returned to the laboratory, where a catheter was inserted into the cubital vein for the collection of remaining blood samples (as shown in Figure 9).
    b) Consumption of a carbohydrate (CHO) drink containing 1 gram of electrolyte powder (0.75 grams/kg body weight per hour, 40% liquid solution), sourced from isomaltulose (BENEO, Mannheim, Germany) or maltodextrin (BENEO, Mannheim, Germany).
    c) Carbohydrate intake was carried out under resting conditions, lasting for 3.5 hours.
    d) After all carbohydrate supplements were consumed and before starting the run, blood samples were collected again (as shown in Figure 9).
7) Endurance Exercise to Exhaustion Protocol (14:30 PM - 16:00PM):
    a) Participants commenced running on a treadmill at a speed of approximately 74% $\dot{V}O_{2peak}$ (11.65 ± 0.60 km/h), until voluntary exhaustion.
8) Blood samples were collected immediately after the run and within 3 hours post-run.
9) Post-Run Supplementation and Recovery (16:00PM - End):
    a) After exercise, a CHO-rich meal was provided (Energy: 592 ± 16 kcal, Carbohydrates [70%]: 104 ± 3 grams, Fats [16%]: 11 ± 1 gram, and Proteins [14%]: 21 ± 1 gram).

After completing the first acute exercise trial, athletes resumed their regular training

schedules. The first set of trials ended on day 35 with the same acute exercise trial. Afterwards, athletes entered a two-week washout period during which they returned to their normal diets. After the washout period, athletes started the second set of dietary trials, lasting four weeks, until the completion of the entire study on day 77.

Participants were supplemented with either high (HGI) or low (LGI) glycaemic index carbohydrate drinks, comprised of maltodextrin (MAL) or isomaltulose (ISO), respectively. The supplementation plan was displayed in Figure 6. Participants were encouraged to consume a bottle of carbohydrate solution (50 grams dissolved in 550 millilitres of water, forming a 9% solution) 1-2 hours before exercise. They were encouraged to consume more bottled drinks per hour during exercise. During the 90-minute recovery period after exercise, participants were encouraged to consume carbohydrates at 0.75 grams per kilogram of body weight, as a 40% solution.

Throughout the trial period, athletes used the MyFitnessPal^{TM} app to meticulously record their dietary intake. Depending on the group assigned, they replaced or adjusted food intake, choosing high GI or low GI food variants. For example, white potatoes might have been substituted with sweet potatoes. These suggestions were based on their regular dietary habits. To accurately record food intake, athletes were provided with a food scale.

Furthermore, athletes were required to use a smartwatch to log daily activities and training details. If athletes did not have an appropriate watch, the research team provided one during the initial screening. All data were synchronized with the TrainingPeaks^{TM} platform, allowing the research team direct access to this information without the need for athletes' personal accounts. Such measures ensured the accuracy of the data and the coherence of the study.

2.7 Data Collection

2.7.1 Capillary Blood Sample

Glucose samples obtained through capillary sampling were analysed for multiple indices, including the rate of change in glucose, peak concentration, mean concentration, and the area under the curve (AUC). In addition, blood lactate concentration (in millimoles per litre) was measured, and changes in lactate concentration throughout the trial period were illustrated in graphs.

2.7.2 Venous Blood

Venous blood samples were separated into EDTA plasma and serum and stored. Photometric testing analysis was conducted on the samples using commercially available ELISA kits and clinical chemistry analysers (such as those from Randox). These tests measured the concentrations of a range of liver stress enzymes, including alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), and total bilirubin levels.

2.8 Data Analysis

All statistical analyses of the raw data were conducted using IBM SPSS software and Excel (Microsoft Office). Data collected in all tests were presented as mean ± standard deviation (SD). Initially, each variable collected was subjected to descriptive statistics and a normality test (Shapiro-Wilk test) to determine the approach for further analysis. To understand if there were significant differences at various time points during the tests, before and after participants consumed ISO and MAL, repeated measures analysis of variance (ANOVA) was used, along with Bonferroni adjustments and the associated paired t-tests. Results with a p-value ≤ 0.05 were considered statistically significant.

2.9 Retrospective Statistical Power Analysis

A retrospective statistical power analysis evaluated the adequacy of the sample size in detecting overall differences in liver enzyme levels (ALT, AST, GGT and total bilirubin) between the low and high glycaemic index (GI) interventions. Using a combined effect size of |d|=0.29, derived from the weighted mean of absolute effect sizes, and a significance level (α\alphaα) of 0.05, the statistical power (1-β) was approximately 55%.

The analysis indicates that the sample size (n = 9 participants in a within-subject crossover design) provided moderate power for detecting small-to-moderate differences but was insufficient for smaller effects.

CHAPTER 3–
Results

3.1 Summary of Results

This study examined the effects of low and high glycaemic index (GI) carbohydrate diets on anthropometric measurements, endurance performance, liver enzyme responses, blood lactate, and glucose levels in ultra-endurance athletes. No significant changes in weight, BMI, body fat percentage, or lean body mass were observed between pre- and post-diet conditions or between the two dietary groups. During the 3-hour fasted outdoor run, running distance, heart rate, percentage of maximum heart rate, and running speed showed no significant differences between diets. Similarly, endurance performance metrics, including time to exhaustion and oxygen uptake, did not differ between the two diets. Significant post-diet increases in ALT and AST levels were observed in the isomaltulose group, with no changes in GGT, total bilirubin, blood lactate, or glucose levels across conditions.

3.2 Anthropometric data

	ISO $_{pre-diet}$	ISO $_{post-diet}$	MAL $_{pre-diet}$	MAL $_{post-diet}$	Overall p-value	LGI change	HGI change	Between dietary intervention p-value
Body mass (kg)	79.4±15.8	78.9±14.9	79.6±15.6	79.5±16.2	*p= 0.487*	-0.5±1.3	-0.2±1.4	*p=0.680*
BMI (kg/m2)	25.5±3.6	25.4±3.1	25.6±3.4	25.6±3.6	*p= 0.504*	-0.2±0.5	0.1±0.4	*p=0.641*
Body fat%	21±5	21±5	21±5	22±7	*p=0.598*	-1±2	0±2	*p=0.421*
Body fat mass	17.3±7.4	16.7±7.3	17.4±6.9	17.6±8.1	*p=0.509*	-0.6±1.4	0.3±2	*p=0.312*
LBM%	35.2±4.5	35.3±4.1	35±4.6	35±4.7	*p=0.678*	0.1±1.2	0.1±1	*p=0.927*
LBM (kg)	62.1±10.3	62.3±9.7	61.7±10.4	61.9±10.5	*P=0.847*	0.2±2.1	0.1±1.7	*p=0.920*

Table 2. Anthropometric measurements of participants on each trial day under conditions of consuming two different carbohydrates, isomaltulose or maltodextrin. This includes participant's weight, BMI, body fat, and LBM. Both body fat and LBM were recorded in percentages (percentage of body fat and lean body mass) and kilograms (fat mass and lean mass). Data are presented as mean ± SD (n=9).

Changes in body composition resulting from a 28-day dietary plan involving the intake of two carbohydrates with different glycemic indices, isomaltulose and maltodextrin:

There were no significant differences in anthropometric measurements between ISO $_{post-diet}$ and MAL $_{post-diet}$. In addition, there were no significant differences in anthropometric measurements between ISO $_{pre-diet}$ and ISO $_{post-diet}$, as well as between MAL $_{pre-diet}$ and MAL $_{post-diet}$.

3.3 Energy Intake

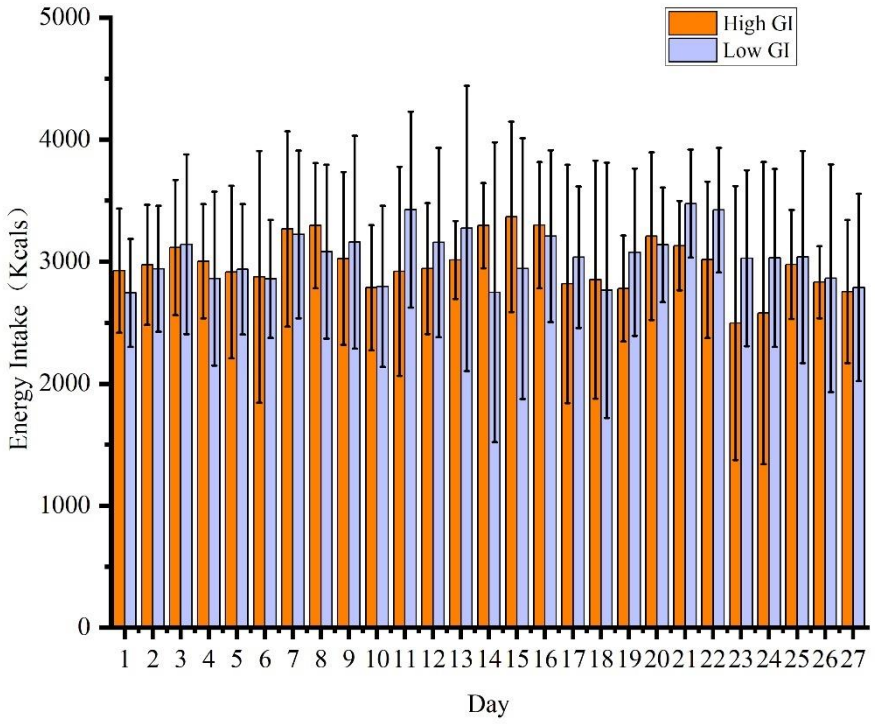

Figure 10. Mean energy intake (kcals) for each day of both dietary interventions. Data are displayed as mean ± SD (n=9).

	High GI	Low GI	P value
Energy (Kcals)	2980±219	3044±210	P=0.662
CHO (g)	448±46	429±70	P=0.349
PRO (g)	106±8	121±19	P=0.015
FAT (g)	83±12	92±17	P=0.203
CHO percentage of daily energy intake (%)	60±3	56±2	P=0.008
PRO percentage of daily energy intake (%)	14±1	16±2	P=0.002
FAT percentage of daily energy intake (%)	24±3	27±2	P=0.031
Supplemental CHO (g)	174±71	166±67	P=0.681

Table 3. Mean macronutrient intake of carbohydrates (CHO), fats (FAT), proteins

(PRO), and supplement powder. Data are presented as mean ± SD (n=9). A statistical difference is indicated if $p \leq 0.05$.

As shown in Figure 10, this represents the energy intake for each 28-day trial period. Table 3 displays the intake of macronutrients. In both carbohydrate diets, carbohydrate intake accounted for approximately 58% of daily energy intake. Under both dietary conditions, protein intake was significantly higher in the low GI diet than in the high GI diet, at 121±19 grams and 106±8 grams respectively (P=0.015). Fat intake was 92±17 grams in the low GI diet, higher than the 83±12 grams in the high GI diet, although this difference was not statistically significant (P=0.203). In terms of the percentage of total daily energy intake, the percentages of carbohydrate, protein, and fat intake in the low GI diet were 56±2%, 16±2%, and 27±2% respectively, compared to 60±3%, 14±1%, and 24±3% in the high GI diet. These differences in the percentage intake of protein and fat were significant (P=0.002 and P=0.031). When expressed as a percentage of overall daily energy intake, there were differences in the intake of all three macronutrients between each diet.

3.4 28-day physical activity data

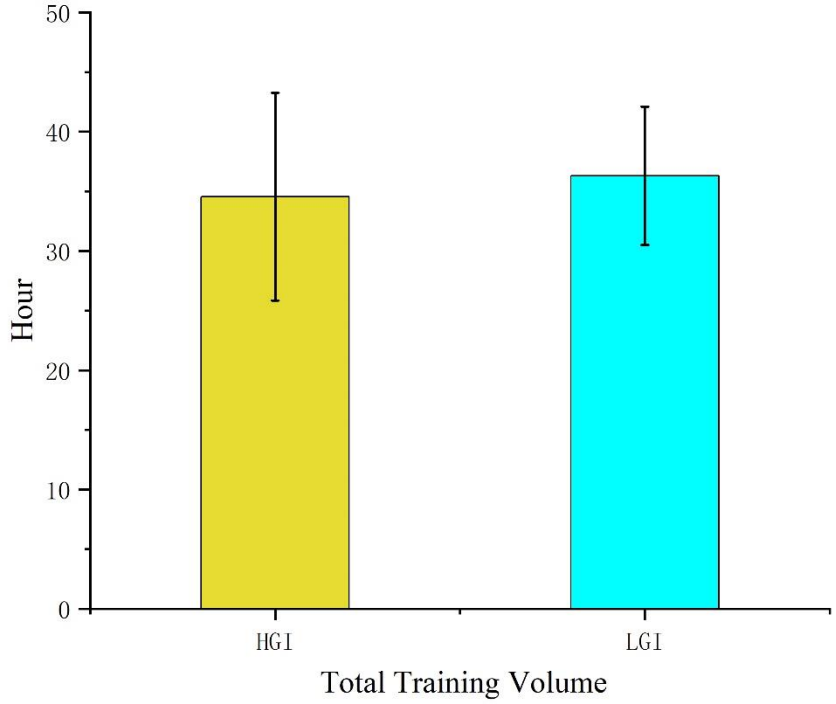

Figure 11. Average Training Volumes (Hours) During the Two Intervention Periods.

Figure 11 shows the comparison of average training volumes of participants over 28 days of consuming carbohydrates with different GI values (HGI:34.5±8.7 vs LGI:36.3±5.8h).

3.5 Acute carbohydrate supplementation trials

3-hour fixed intensity fasted outdoor run

	ISO $_{pre\text{-}diet}$	**ISO $_{post\text{-}diet}$**	**MAL $_{pre\text{-}diet}$**	**MAL $_{post\text{-}diet}$**	**Overall p-value**	**LGI change**	**HGI change**	**Between dietary intervention p-value**
Distance (km)	25.1±2.3	27±2.9	25.3±2.7	26.5±2.7	*p=0.272*	1.9±2.3	1.2±1.2	*p=0.469*
HR (bpm)	128±6	126±6	129±5	128±6	*p=0.333*	-2±4	-1±2	*p=0.633*
Percent of HR_{max} (%)	71±3	71±3	72±3	71±3	*p=0.333*	0±0	-1±0	*P=0.634*
Speed ($km.hr^{-1}$)	8.3±0.9	9±1	8.3±1	8.6±1.1	*p=0.382*	0.7±0.8	0.3±1	*P=0.443*

Table 4: All data for each participant during a 3-hour fasted morning run are presented as mean ± standard deviation (n=9). The table shows the data for distance, heart rate (HR), percentage of maximum heart rate, and running speed.

Running intensity was determined based on heart rate. Under the conditions of consuming two different carbohydrates, isomaltulose and maltodextrin, there were no significant differences in the data for the 3-hour runs between each trial (running distance, average heart rate, percentage of maximum heart rate, and average speed). Detailed data are shown in Table 4.

3.6 Analysis of Performance Testing

	ISO $_{pre-diet}$	**ISO $_{post-diet}$**	**MAL $_{pre-diet}$**	**MAL $_{post-diet}$**	**Overall p-value**	**LGI change**	**HGI change**	**Between dietary intervention p-value**
Run time (mins)	51±19	65±15	69±23	72±16	*p=0.264*	14±16	3±23	*p=0.328*
VO_2 ($ml.kg^{-1}.min^{-1}$)	42±4	42±3	41±3	42±5	*p=0.670*	0.4±2.8	0.7±5.2	*p=0.908*
VO_2max% (%)	82±6	83±6	81±8	82±6	*p=0.687*	0.9±5.7	0.9±11	*p=0.991*
VCO_2 ($ml.kg^{-1}.min^{-1}$)	40±4	40±4	39±4	39±4	*p=0.653*	0±2.4	-0.5±5.6	*p=0.857*
VCO_2max% (%)	73.7±6.8	73.7±7.1	72±8.5	70.9±6.9	*p=0.656*	0±4.6	-1.1±11.3	*p=0.822*
HR (bpm)	157±8	158±9	161±11	157±10	*p=0.350*	-2±5	-5±6	*p=0.115*
Percentage of HRmax (%)	88±3	88±5	90±6	87±6	*p=0.401*	-1±3	-3±3	*p=0.171*
RPE (Borg)	15±2	15±1	15±2	15±2	*p=0.700*	NA	NA	*NA*

Table 5. All data from the endurance performance tests are displayed as mean ± standard deviation (n=9). It shows the participants' test limit time, heart rate, oxygen intake, carbon dioxide output, percentages relative to limit gas exchange, and perceived exertion levels, as well as changes from ISO $_{pre-diet}$ to ISO $_{post-diet}$ and from MAL $_{pre-diet}$ to MAL $_{post-diet}$. P-values indicate statistical differences ($p \leq 0.05$).

Table 5 presents data from endurance performance tests. During the screening phase, participants underwent cardiopulmonary exercise testing on a treadmill, running until

subjective exhaustion. The intensity for the endurance performance tests was set at 74% of $\dot{V}O_{2peak}$ based on screening test results. The average speed during the test was 11.65 km/hr, with a standard deviation of 0.60 km/hr.

Acute carbohydrate supplementation response on exercise performance under pre-dietary conditions with 28 days of intake of two carbohydrates with different glycemic indices:

No significant differences were observed in the variables of the endurance performance tests between MAL $_{pre\text{-}diet}$ and ISO $_{pre\text{-}diet}$.

Response of acute carbohydrate supplementation on exercise performance under post-dietary conditions following 28 days of intake of two carbohydrates with different glycemic indices:

There were no differences in the variables of the endurance performance tests between MAL $_{post\text{-}diet}$ and ISO $_{post\text{-}diet}$.

Impact of acute carbohydrate supplementation on exercise response under conditions during a 28-day glycemic index carbohydrate diet:

There were no significant changes in the variables of the endurance performance tests from ISO $_{pre\text{-}diet}$ to ISO $_{post\text{-}diet}$, nor from MAL $_{pre\text{-}diet}$ to MAL $_{post\text{-}diet}$. Compared to the heart rate changes under conditions of maltodextrin intake over 28 days, participants using isomaltulose exhibited a smaller reduction in heart rate response during running performance.

3.7 Liver enzymes

Alanine aminotransferase (ALT) is displayed in Figure 12

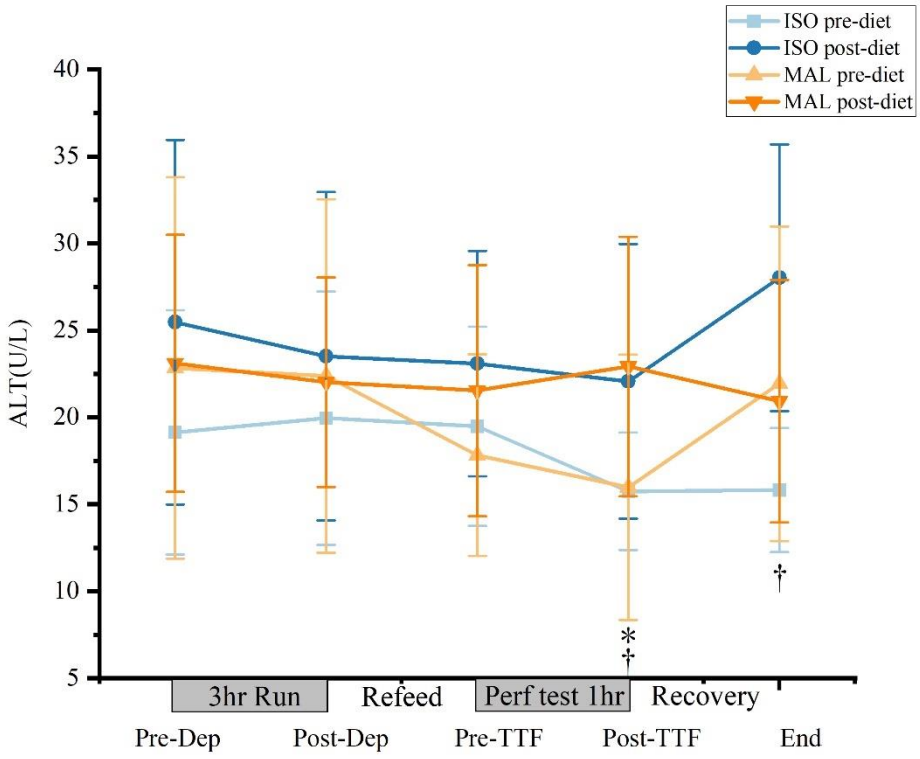

Figure 12. Plasma alanine aminotransferase (ALT) concentrations at each laboratory check. All data are presented as mean ± SD (n=9). † indicates a difference between pre- and post-ISO diet. * indicates a difference between pre- and post-MAL diet.

Before undergoing a 28-day long-term diet of two different types of carbohydrates, the comparison of acute carbohydrate intake (MAL and ISO) showed no difference in plasma alanine aminotransferase (ALT) concentrations between $MAL_{pre\text{-}diet}$ and $ISO_{pre\text{-}diet}$ participants.

After undergoing a 28-day long-term diet of two different types of carbohydrates, the comparison of acute carbohydrate intake (MAL and ISO) showed no difference in

plasma alanine aminotransferase (ALT) concentrations between $ISO_{post\text{-}diet}$ and $MAL_{post\text{-}diet}$ participants.

After undergoing a 28-day long-term diet of two different types of carbohydrates, the comparison of acute carbohydrate intake (MAL and ISO) showed a significant difference in plasma alanine aminotransferase (ALT) concentrations between $ISO_{pre\text{-}diet}$ and $ISO_{post\text{-}diet}$ participants (P=0.013). No difference was observed in plasma alanine aminotransferase (ALT) concentrations between $MAL_{pre\text{-}diet}$ and $MAL_{post\text{-}diet}$ participants.

Aspartate Aminotransferase (AST): Summary data are displayed in Figure 13.

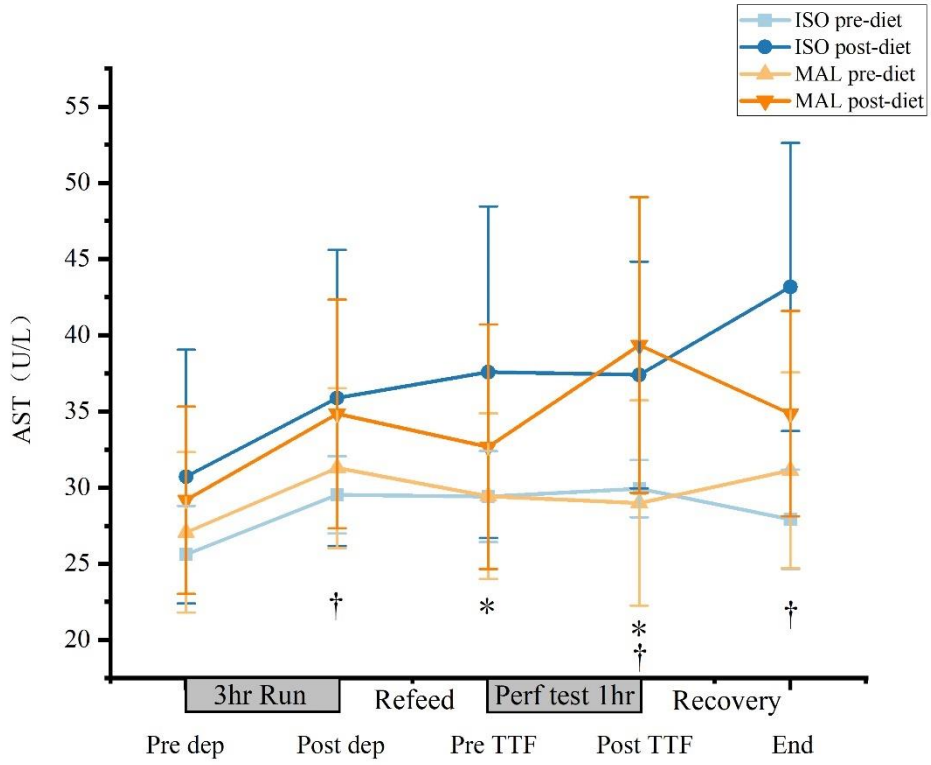

Figure 13. Plasma aspartate aminotransferase (AST) concentrations at each laboratory check. All data are presented as mean ± SD (n=9). † indicates a difference between pre- and post-ISO diet. * indicates a difference between pre- and post-MAL diet.

Before undergoing a 28-day long-term diet of two different types of carbohydrates, the comparison of acute carbohydrate intake (MAL and ISO) showed no difference in plasma aspartate aminotransferase (AST) concentrations between $MAL_{pre\text{-}diet}$ and $ISO_{pre\text{-}diet}$ participants.

After undergoing a 28-day long-term diet of two different types of carbohydrates, the comparison of acute carbohydrate intake (MAL and ISO) showed no difference in plasma aspartate aminotransferase (AST) concentrations between $ISO_{post\text{-}diet}$ and $MAL_{post\text{-}diet}$ participants.

After undergoing a 28-day long-term diet of two different types of carbohydrates, the comparison of acute carbohydrate intake (MAL and ISO) showed a significant increase

in plasma aspartate aminotransferase (AST) concentrations from $ISO_{pre\text{-}diet}$ to $ISO_{post\text{-}diet}$ (P=0.006). Similarly, there was a significant increase in plasma aspartate aminotransferase (AST) concentrations from $MAL_{pre\text{-}diet}$ to $MAL_{post\text{-}diet}$ (P=0.028).

Gamma-Glutamyl Transferase (GGT): Summary data are displayed in Figure 14.

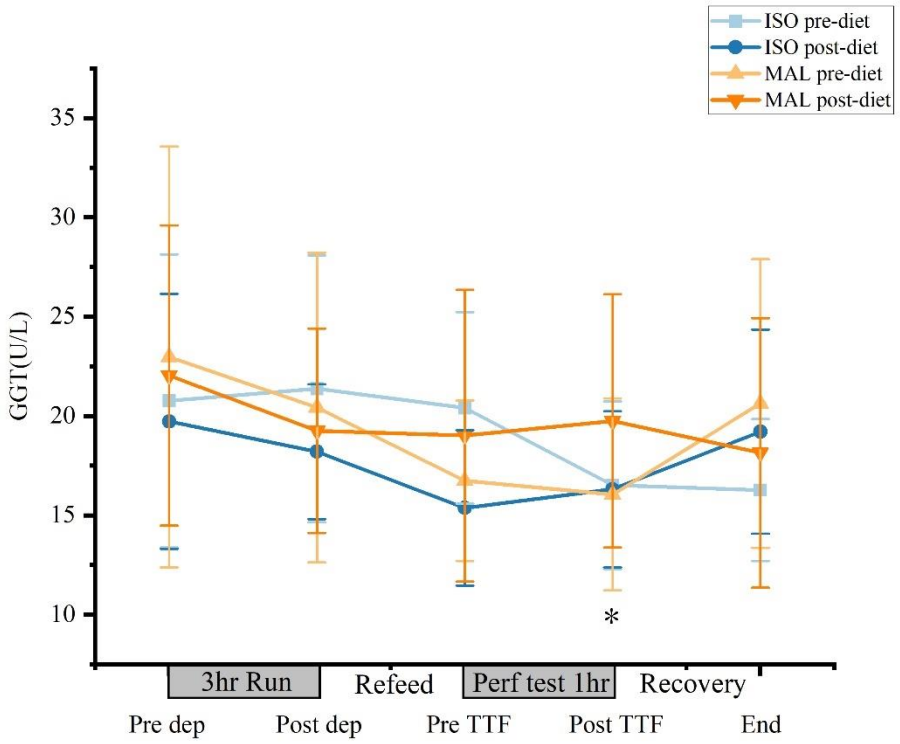

Figure 14. Plasma gamma-glutamyl transferase (GGT) concentrations at each laboratory check. All data are presented as mean ± SD (n=9). * indicates a difference between pre- and post-MAL diet.

Before undergoing a 28-day long-term diet of two different types of carbohydrates, the comparison of acute carbohydrate intake (MAL and ISO) showed no difference in plasma gamma-glutamyl transferase (GGT) concentrations between $MAL_{pre\text{-}diet}$ and $ISO_{pre\text{-}diet}$ participants.

After undergoing a 28-day long-term diet of two different types of carbohydrates, the comparison of acute carbohydrate intake (MAL and ISO) showed no difference in plasma gamma-glutamyl transferase (GGT) concentrations between $ISO_{post\text{-}diet}$ and $MAL_{post\text{-}diet}$ participants.

After undergoing a 28-day long-term diet of two different types of carbohydrates, the comparison of acute carbohydrate intake (MAL and ISO) showed no significant differences in plasma gamma-glutamyl transferase (GGT) concentrations between

$ISO_{pre\text{-}diet}$ and $ISO_{post\text{-}diet}$, as well as between $MAL_{pre\text{-}diet}$ and $MAL_{post\text{-}diet}$ participants.

Total Bilirubin: Summary data are displayed in Figure 15.

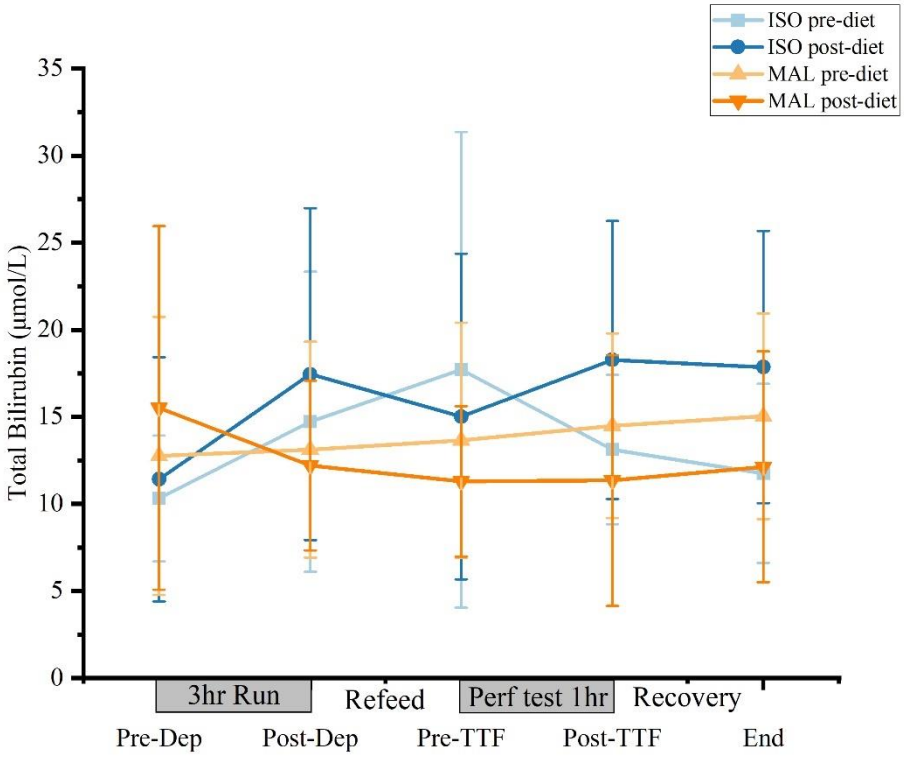

Figure 15. Plasma total bilirubin concentrations at each laboratory check. All data are presented as mean ± SD (n=9).

Before undergoing a 28-day long-term diet of two different types of carbohydrates, the comparison of acute carbohydrate intake (MAL and ISO) showed no difference in plasma total bilirubin concentrations between $MAL_{pre\text{-}diet}$ and $ISO_{pre\text{-}diet}$ participants.

After undergoing a 28-day long-term diet of two different types of carbohydrates, the comparison of acute carbohydrate intake (MAL and ISO) showed no difference in plasma total bilirubin concentrations between $ISO_{post\text{-}diet}$ and $MAL_{post\text{-}diet}$ participants.

After undergoing a 28-day long-term diet of two different types of carbohydrates, the comparison of acute carbohydrate intake (MAL and ISO) showed no significant differences in plasma total bilirubin concentrations between $ISO_{pre\text{-}diet}$ and $ISO_{post\text{-}diet}$, as well as between $MAL_{pre\text{-}diet}$ and $MAL_{post\text{-}diet}$ participants.

3.8 Blood glucose and blood lactate

Blood glucose: Summary data are displayed in Figure 16.

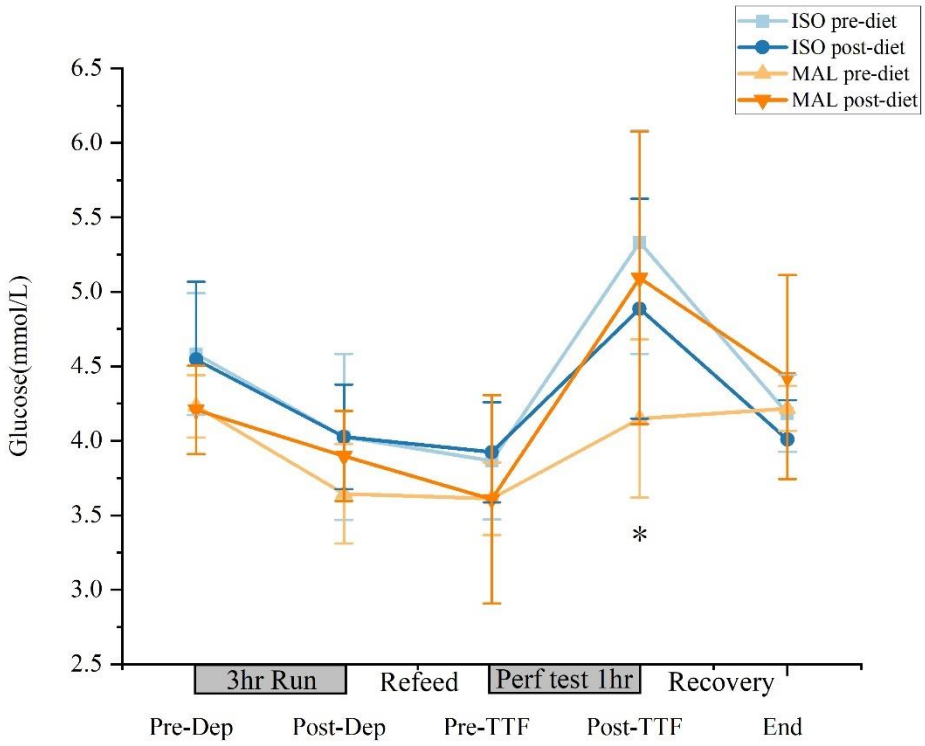

Figure 16. Blood glucose concentrations at each laboratory check. All data are presented as mean ± SD (n=9). * indicates a difference between pre- and post-MAL diet.

Before undergoing a 28-day long-term diet of two different types of carbohydrates, the comparison of acute carbohydrate intake (MAL and ISO) showed no difference in blood glucose concentrations between $MAL_{pre\text{-}diet}$ and $ISO_{pre\text{-}diet}$ participants.

After undergoing a 28-day long-term diet of two different types of carbohydrates, the comparison of acute carbohydrate intake (MAL and ISO) showed no difference in blood glucose concentrations between $ISO_{post\text{-}diet}$ and $MAL_{post\text{-}diet}$ participants.

After undergoing a 28-day long-term diet of two different types of carbohydrates, the comparison of acute carbohydrate intake (MAL and ISO) showed no significant differences in blood glucose concentrations between $ISO_{pre\text{-}diet}$ and $ISO_{post\text{-}diet}$, nor between $MAL_{pre\text{-}diet}$ and $MAL_{post\text{-}diet}$ participants.

Blood lactate: Summary data are displayed in Figure 17.

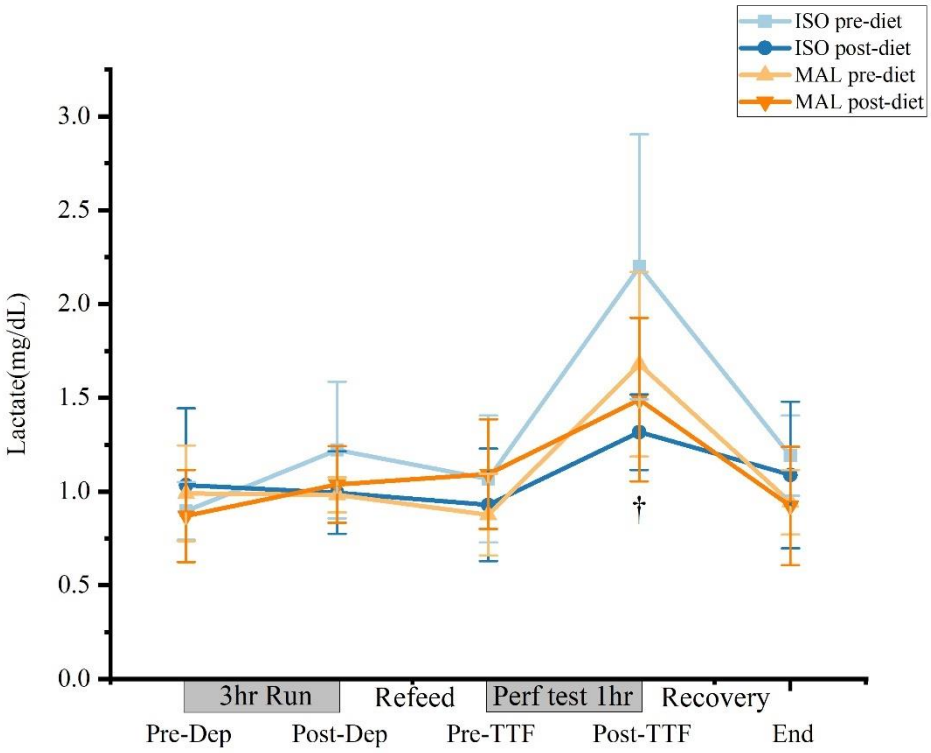

Figure 17. Plasma lactate concentrations at each laboratory check. All data are presented as mean ± SD (n=9). † indicates a difference between pre- and post-ISO diet.

Before undergoing a 28-day long-term diet of two different types of carbohydrates, the comparison of acute carbohydrate intake (MAL and ISO) showed no difference in blood lactate concentrations between $MAL_{pre\text{-}diet}$ and $ISO_{pre\text{-}diet}$ participants.

After undergoing a 28-day long-term diet of two different types of carbohydrates, the comparison of acute carbohydrate intake (MAL and ISO) showed no difference in blood lactate concentrations between $ISO_{post\text{-}diet}$ and $MAL_{post\text{-}diet}$ participants.

After undergoing a 28-day long-term diet of two different types of carbohydrates, the

comparison of acute carbohydrate intake (MAL and ISO) showed no significant differences in blood lactate concentrations between $ISO_{pre\text{-}diet}$ and $ISO_{post\text{-}diet}$, nor between $MAL_{pre\text{-}diet}$ and $MAL_{post\text{-}diet}$ participants.

CHAPTER 4–
Discussion

4.1 Aim

4.1.1

The purpose of this study was to assess the impact on liver function in ultra-endurance athletes after consuming carbohydrates with different GI values, following an acute endurance running performance.

4.1.2

To compare the effects on liver function and recovery of a low GI diet (with added isomaltulose) versus a high GI diet (with added maltodextrin) during a 28-day period of carbohydrate feeding with different GI values.

4.2 Main Research findings

4.2.1

The study found that after ultra-endurance athletes performed an acute endurance run followed by acute ingestion of isomaltulose and maltodextrin supplements and a subsequent performance test, there were no statistically significant changes in the concentrations of blood glucose, blood lactate, total bilirubin, ALT, or GGT in the participants' blood. There was also no noticeable difference in the running performance times between isomaltulose and maltodextrin.

4.2.2

The study found that during a 28-day endurance training period for ultra-endurance athletes, comparing the intake of a low glycaemic index diet (with added isomaltulose) with a high glycaemic index diet (with added maltodextrin) showed no

statistically significant differences in blood glucose, blood lactate, total bilirubin, ALT, or GGT after 28 days. Among these, the concentration of AST was higher in the $MAL_{post\text{-}diet}$ compared to $MAL_{pre\text{-}diet}$ (P=0.028) and higher in the $ISO_{post\text{-}diet}$ compared to $ISO_{pre\text{-}diet}$ (P=0.006) at the end of the 28-day glycaemic index diet.

4.3 The effect of a carbohydrate diet containing isomaltulose or maltodextrin for 28 days on anthropometric measurements in ultra-endurance athletes.

According to Table 2, after all participants completed 28 days on two different carbohydrate diets of isomaltulose or maltodextrin, all anthropometric characteristics remained unchanged. There were no significant differences in body weight, estimated LBM, and body fat after 28 days under the two dietary conditions. The study by McMillan-Price & Brand-Miller suggests that long-term intake of a low GI diet can enhance the rate of fat reduction and cardiovascular risk factors. Proposed mechanisms include increased satiety, higher metabolic rate, reduced postprandial blood glucose and/or insulinemia, and increased fat oxidation (118). However, similar changes were not observed in this study, possibly due to the shorter duration of the low GI diet. As shown in Table 3, the average daily energy intake on the low glycaemic index carbohydrate diet was approximately 80 kcal higher than that on the high glycaemic index carbohydrate diet, but the athletes maintained statistically similar isocaloric energy intakes, primarily due to an additional intake of 14 grams of protein per day with the low glycaemic index diet compared to the high glycaemic index carbohydrate diet. Additionally, as indicated in Figure 11, the volume of physical training during each 28-day training period was similar. With constant caloric intake and physical activity, the participants' anthropometric measurements were not affected by the type of carbohydrate consumed.

4.4 Metabolic effects of 28-day diet of high or low GI carbohydrates, on exercise

performance, recovery, and liver stress enzyme levels after prolonged submaximal exercise.

According to Table 3 and Figure 11, during the 28-day diet of either isomaltulose or maltodextrin carbohydrates, the energy intake in the diets of isomaltulose or maltodextrin remained essentially consistent, and the training volume carried out by participants on the two carbohydrate diets was also fundamentally consistent.

In this study, athletes fasted overnight and avoided physical activities 24 hours prior to visiting the laboratory. On an empty stomach in the morning, participants underwent resting venous blood sampling. The study compared the effects of two carbohydrate diets, isomaltulose (ISO) and maltodextrin (MAL), over 28 days on blood glucose, lactate, and liver enzymes (ALT, AST, GGT, total bilirubin). Results showed no significant differences in biochemical markers except for AST under both diets, indicating that short-term (28-day) changes in dietary carbohydrate GI did not significantly affect these markers in athletes. According to Figure 13, AST levels were higher in $MAL_{post\text{-}diet}$ compared to $MAL_{pre\text{-}diet}$ and higher in $ISO_{post\text{-}diet}$ compared to $ISO_{pre\text{-}diet}$, suggesting that both carbohydrate diets affected AST levels over 28 days. This is consistent with findings from Shlomit Chevion et al. (119) that intense exercise can raise AST levels, and from Purkins et al. (120) where high-calorie carbohydrate portions from sucrose also raised AST significantly compared to a regular high-calorie diet. However, the study design suggests multiple factors could have influenced the rise in AST levels. The participants in this study had an average carbohydrate intake representing 56±3% of total intake for the ISO group and 60±3% for the MAL group. A higher proportion of dietary carbohydrate intake could also be a potential factor in elevated AST levels. For ultra-endurance athletes, performance and recovery in endurance sports are also critical. Previous studies have shown that low-GI carbohydrates help preserve intramuscular carbohydrate reserves more effectively during long-term endurance activities by enhancing fat oxidation. This mechanism may be due to smaller blood glucose fluctuations caused by low-GI foods, reducing

the demand for insulin and enabling the mobilization and utilization of fatty acids (131). Another study on performance and recovery indicated that consuming low-GI meals before exercise could prolong endurance performance by stabilizing blood glucose levels, thereby preserving muscle glycogen reserves more effectively, and enhancing sustainable energy supply during exercise (132). Although there were no significant differences in other biochemical markers in this study, choosing a low-GI carbohydrate diet still holds potential benefits for ultra-endurance athletes' performance and recovery.

4.4.1 Before and after a 28-day diet of isomaltulose or maltodextrin carbohydrates, the effects of a 3-hour standardized submaximal outdoor run on participants' liver stress enzymes.

The study designed an outdoor mixed terrain running course where athletes engaged in loop running. Safety and route supervision were ensured, and the training environment was standardized. The experiment included four tests, each requiring the athletes to complete approximately 26 kilometers in three hours. Running speed was adjusted to closely match the target heart rate, with a permissible error of plus or minus 5 beats per minute. This control method effectively managed training intensity across the complex terrain, ensuring consistent physiological stress. The results showed an average heart rate of approximately 128 bpm (70% HR_{peak}) and an average speed of 8.5 km/h on mixed terrain. After three hours of running, blood samples were immediately collected in the lab.

Post-run, participants' blood glucose levels were similar to pre-run levels, showing no significant statistical change. Blood lactate levels were slightly elevated but not significantly different statistically and were similar between the ISO and MAL groups, before and after the 28-day diet of isomaltulose or maltodextrin. Post-run, there was an increase in the liver stress enzyme AST, significant only after the carbohydrate diet. According to Kłapcińska et al. (123), exercise stress can elevate AST levels, but changes in ALT were minor, suggesting that the damage may

primarily stem from muscle, which may be a major source of these enzymes. Another study suggests this could be linked to participants' long-term regular training (124) or possibly due to a high daily intake of carbohydrates (120).

4.4.2 During the recovery period after submaximal running to exhaustion, participants consumed either isomaltulose or maltodextrin and the impact on participants' liver stress enzymes was examined.

In this study, after a 3-hour run and refeeding with either isomaltulose or maltodextrin, participants generally exhibited hypoglycemia. The isomaltulose group showed similar blood glucose levels. This observation suggested slightly more stable values of isomaltulose conveyed a potential advantage in maintaining more stable blood glucose levels, which is crucial for recovery and stability after prolonged exercise.

During the trial, participants' blood lactate levels remained low and stable, indicating that neither low nor high GI carbohydrate intake significantly affected lactate metabolism. This suggests that post-exercise lactate production might be regulated by factors other than the type of carbohydrate consumed.

Additionally, there were no significant intra- or inter-trial differences in the levels of AST, ALT, GGT, or bilirubin during the refeeding period, indicating that liver function markers were not significantly affected by the type of carbohydrate consumed. This implies that liver function levels may remain stable and unaffected by carbohydrate GI after prolonged exercise.

4.4.3 Performance Testing

This study aimed to explore the impact of different carbohydrate intakes on exercise performance and heart rate. The results showed no significant difference in endurance performance across all carbohydrate trials under a set running intensity (equivalent to 74% of maximum oxygen uptake), as seen in Table 5. However, research indicates that the GI of carbohydrates may affect performance. Although heart rate changes

were not significant in this study, similar research suggests that low-GI carbohydrate intake can lead to more stable blood glucose levels and sustained energy release, potentially helping to stabilize heart rate during exercise (125). This aligns with the trend of reduced heart rate observed in the isomaltulose trial, which, although not statistically significant, warrants further exploration.

Additionally, this study observed that after a 28-day carbohydrate feeding and acute carbohydrate supplementation, participants showed a significant improvement in endurance performance, evidenced by increased duration. This aligns with extensive literature supporting the critical role of carbohydrates in providing energy and delaying fatigue (126). Specifically, a systematic review found a strong association between continuous carbohydrate intake and improvements in exercise performance, especially in endurance sports (127). Thus, combining long-term and short-term carbohydrate intake could maximally enhance athletic performance, consistent with the increased durations observed in this study.

In the experiment, indirect calorimetry was used to collect values of oxygen and carbon dioxide in participants' exhaled gases, to assess their metabolic state under different carbohydrate intakes. Results indicated that exhaled gas values and the actual percentage of maximum oxygen uptake during the runs were nearly similar across all four trials, suggesting a relatively stable exercise intensity under different experimental conditions.

Despite the intended exercise intensity set at 74% $\dot{V}O_{2max}$, participants' actual running intensity during performance tests was equivalent to 82% of maximum oxygen uptake. This discrepancy might be attributed to fatigue caused by a three-hour outdoor run on an empty stomach in the morning.

Following the different carbohydrate trials, participants' blood glucose levels increased by an average of 0.96 mmol/L compared to pre-exercise, indicating some regulatory effect of carbohydrate intake on blood glucose levels. Notably, the increase

in blood glucose levels was only significant in the $MAL_{post\text{-}diet}$ trial, which may reflect a relatively higher increase in blood glucose levels after a 28-day period of carbohydrate feeding and acute supplementation.

This finding is consistent with previous research supporting the impact of carbohydrate intake on glucose metabolism. Earlier studies indicated that supplementing carbohydrates before or during exercise can increase blood glucose levels and delay fatigue to some extent (128). The significant increase in blood glucose levels observed in the $MAL_{post\text{-}diet}$ trial could be associated with the intake of high GI value carbohydrates (129).

After the exercise performance tests, participants showed a slight increase in blood lactate concentrations (0.4-0.8 mM), with statistically significant increases observed in the $MAL_{post\text{-}diet}$ and $ISO_{pre\text{-}diet}$ trials. This suggests that different carbohydrate intake regimes may impact lactate metabolism. Specifically, the increase in blood lactate concentration in the $MAL_{post\text{-}diet}$ trial could be associated with the intake of high-GI carbohydrates, as high-GI carbohydrate intake may lead to faster carbohydrate metabolism and lactate production (129).

Additionally, after a 28-day low-GI carbohydrate diet, an elevation in liver enzyme AST was observed, particularly in the maltodextrin group. However, ALT was unaffected and remained within the normal range across all trials. The elevation in AST within the maltodextrin group may reflect some degree of liver function alteration. AST, primarily found in the liver, heart, and muscle tissues, could elevate due to tissue damage or metabolic abnormalities (131). In this study, the increase in AST in the maltodextrin group may be related to the type of carbohydrates ingested and the dietary regimen. Maltodextrin, a high-GI carbohydrate, could cause rapid increases in blood sugar and insulin release, thereby affecting liver metabolic activity and physiological function. Additionally, exercise itself may impact AST levels. Studies have shown that intense or prolonged exercise can cause muscle damage, releasing muscle enzymes such as CK and LDH into the bloodstream, which are then

metabolized by the liver, leading to elevated AST levels (132). Thus, the post-exercise increases in AST levels observed in this study could partly be due to exercise-induced muscle damage. The concentration of total bilirubin remained unchanged and within the normal range in each trial, indicating that bilirubin metabolism was not significantly affected by different carbohydrate intakes.

Before the exercise performance test, although participants had some time to recover and refuel, the prolonged fasting outdoor run conducted on the morning of the test day could cause significant fatigue, thus impacting the subsequent performance tests. This effect may be due to the complexity of fatigue mechanisms and their impact on multiple physiological systems, including the depletion of energy reserves and changes in psychological state. Long-duration aerobic exercise, especially on an empty stomach, can increase reliance on muscle glycogen stores, leading to rapid depletion of these reserves and affecting both performance and recovery capabilities (133).

Despite some studies highlighting the specificity of isomaltulose oxidation rate which may positively influence fuel utilization efficiency in endurance sports, no significant advantages have been observed in overall exercise performance compared to conventional carbohydrates like glucose. This might relate to the complex mechanisms regulating energy supply during exercise, including but not limited to interactions involving insulin response, glycogen utilization, and fat oxidation (134).

In the experiment, a significant increase in AST was observed, although this might be linked to liver function damage, the absence of significant changes in ALT, GGT, and total bilirubin suggests there was no apparent liver damage. The rise in AST could be due to muscle damage or damage to other non-liver tissues, resulting from the release of enzymes during the damage and repair processes of muscle cells caused by prolonged, high-intensity exercise (135,123). Additionally, the combined factors of exercise and diet might also affect AST levels, such as oxidative stress and inflammatory responses following high-intensity exercise that could temporarily alter

serum enzyme activity (136).

After reaching their voluntary limit in an endurance run, participants underwent a three-hour recovery period. Initially during this recovery, all participants in their respective trial groups consumed a standardized isocaloric mixed meal to ensure consistency in dietary intervention. Achten and Jeukendrup (137) have noted that diet can significantly regulate fat oxidation, thus influencing post-exercise metabolic responses. Our study's subsequent blood sampling showed similar metabolic responses regarding blood glucose, lactate, and some liver enzymes (GGT, total bilirubin) across trial groups, indicating a consistent trend in metabolic recovery after short-term high-intensity exercise (138).

In the isomaltulose group, the concentration of the liver enzyme AST showed a significant increase post-diet compared to pre-diet and to the corresponding maltodextrin group (ALT). Bortolotti et al. (139) suggested that dietary components, such as protein intake, can significantly affect intrahepatic fat accumulation and liver enzyme activity, implying that changes in AST levels may be related to dietary components. Additionally, the increase in AST might also be related to muscle damage, as AST is expressed not only in the liver but also in muscle tissue. Thus, variations in AST levels should consider the potential for muscle damage and recovery.

4.5 Limitations of current work

One limitation of this study is the gender imbalance among participants, which is significant as Tarnopolsky's research (140) emphasizes the impact of gender differences on responses to exercise and nutrition. In this experiment, of the nine participants, eight were male and only one was female, suggesting that the 28-day carbohydrate intervention results might be more applicable to males. Future studies should ensure a balanced gender ratio or perform gender-stratified analyses to explore

the impact of gender on exercise and nutritional interventions. Additionally, the small sample size of nine limits the statistical power and increases the risk of results being due to chance. Larger-scale studies are recommended to improve statistical efficacy and provide more generalizable findings. Furthermore, the study only used running as a model for ultra-endurance testing, which might not apply to other forms like triathlons or cycling. Future research should include various exercise modes to assess the effects of carbohydrate intake across different forms of exercise or focus on a single mode. According to Jeukendrup (141), different sports may require different energy replenishment strategies, highlighting the need for diverse exercise models in research. Also, future experimental designs should clearly stratify ultra-endurance athletes at different life stages to systematically compare the physiological metabolic responses to different GI carbohydrates. This approach would offer deeper age-related insights into sports nutrition and might guide more personalized dietary strategies to optimize performance and health longevity.

4.6 Application of research findings

Research indicates that following various carbohydrate diets, particularly after isomaltulose intake, there is a significant increase in the liver enzyme AST. This finding has practical clinical applications, suggesting that even healthy ultra-endurance athletes may experience mild liver stress responses after specific diets. Medical professionals and nutritionists can use this information to provide more personalized nutritional advice to athletes, optimizing their diet to avoid potential liver strain. Routine health checks in sports medicine should include liver function monitoring, especially after intense training and specific dietary adjustments. Although no significant differences were observed in post-exercise metabolic recovery between carbohydrates with different GI values, this underscores the flexibility in choosing carbohydrate types in sports nutrition. This flexibility allows athletes and coaches to select carbohydrate sources based on individual digestive

adaptability and preference without overly concerning short-term metabolic differences. Additionally, the study's findings on the long-term and short-term effects of carbohydrate intake on liver enzyme levels offer guidance for clinical and nutritional planning, suggesting that the type and intake pattern of carbohydrates can have varying impacts on liver function.

4.7 Conclusion

This study conducted a systematic analysis of the physiological and metabolic responses of ultra-endurance athletes after consuming carbohydrates with different glycemic indices (GI). Over a 28-day experimental period, neither low-GI isomaltulose nor high-GI maltodextrin significantly impacted blood glucose, lactate, creatine kinase, or most liver function indicators (ALT, GGT, total bilirubin). However, the slight increase in AST provides important clues about liver and muscle stress responses, particularly in the comparison between low and high GI carbohydrate intake, possibly reflecting the different impacts of carbohydrate types on muscle cell integrity and their repair processes.

Although no significant liver damage was observed in this study, the elevation in AST levels may indicate post-exercise muscle micro-damage and its repair process, a common phenomenon in sports. The change in this biochemical marker not only relates to liver metabolic functions but is also closely associated with muscle tissue stress and recovery. Monitoring AST levels in the context of prolonged exercise and carbohydrate intake may be practically significant for understanding an athlete's recovery state and physiological stress response.

Additionally, considering the potential impact of exercise intensity and duration on liver and muscle metabolic activity, future research should further explore the long-term effects of different GI carbohydrates on liver enzymes, especially AST and ALT, under longer durations and higher exercise loads. Understanding these biochemical

parameter changes can help optimize training and recovery strategies for athletes, particularly when planning long-term or high-intensity training cycles.

In summary, although most liver function indicators in this study did not show significant changes, the slight increase in AST provides valuable insight into post-exercise muscle cell damage and repair mechanisms, which is of great importance for applications in sports medicine and sports nutrition. Future research, with more in-depth analysis, will help reveal the specific effects of different types of carbohydrates on post-exercise recovery and metabolic health.

5. Reference List

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6. Appendix

1. Physical Activity Readiness Questionnaire

Physical Activity Readiness Questionnaire (PAR-Q)

For most people physical activity should not pose any problem or hazard. PAR-Q has been designed to identify the small number of adults for whom physical activity might be inappropriate or those who should have medical advice before performing the type of exercise used in the current experiment.

Yes	No	1) Has a physician ever said you have a heart condition and you should only do physical activity recommended by a physician?
Yes	No	2) When you do physical activity, do you feel pain in your chest?
Yes	No	3) When you were not doing physical activity, have you had chest pain in the past month?
Yes	No	4) Do you ever lose consciousness or do you lose your balance because of dizziness?
Yes	No	5) Do you have a joint or bone problem that may be made worse by a change in your physical activity?
Yes	No	6) Is a physician currently prescribing medications for your blood pressure or heart condition?
Yes	No	7) Are you pregnant?
Yes	No	8) Do you have insulin dependent diabetes?
Yes	No	9) Do you know of any other reason you should not exercise?

If you answered YES to one or more questions:
If you have not recently done so, consult with your personal physician by telephone or in person before taking part in this exercise test.

If you answered NO to all questions:
If you answered PAR-Q accurately, you have reasonable assurance of your present suitability for this exercise test.

Name:
Date:
Signature:

2. Participant Consent Form

Please initial box

1. I confirm that I have read and understood the information sheet dated 15/07/20 (version number 1.1) for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that sections of any of data obtained may be looked at by responsible individuals from the Swansea University or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access these records. ☐

4. I understand that data I provide may be used in reports and academic publications in anonymous fashion ☐

5. I agree to take part in the above study. ☐

Name of Participant	Date	Signature
Name of Person taking consent	Date	Signature
Researcher	Date	Signature