Master's thesis

NTNU
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Effect of Carbohydrate Content in a Pre-Event Meal on Endurance Capacity, Maximal Oxygen Uptake, Lactate Threshold, and Work Economy: a randomized controlled crossover-trial

Master's thesis in Exercise Physiology

Supervisor: Øyvind B. Sandbakk, Arnt Erik Tjønna & Dionne Noordhof

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Norsk tittel: Effekten av karbohydratinnholdet i et pre-test-måltid på utholdenhetskapasitet, maksimalt oksygenopptak, laktatterskel og arbeidsøkonomi: En randomisert kontrollert overkrysningsstudie

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1 Abstract

Background: Pre-exercise exogenous carbohydrates (CHO) influence metabolism and lead to a shift in substrate utilization during subsequent exercise. This shift could influence energy delivery capacity, the energetic cost of exercise, and products of metabolism such as lactate. **Aim:** The primary aim was to investigate the effect of the relative CHO content in a pre-event meal on time to exhaustion (TTE), peak oxygen uptake (VO_{2peak}), lactate threshold (LT), and work economy (WE). A secondary aim was to compare these responses to pre-event CHO intake between endurance-trained and untrained individuals. **Material and methods:** 11 trained and 10 untrained men went through three trials in a randomized cross-over fashion. The three pre-test conditions were 1) a pre-event meal with a high CHO content (3 $g \cdot kg^{-1}$), 2) a pre-event meal with a low CHO content (0.5 $g \cdot kg^{-1}$), and 3) fasted state. The test consisted of five submaximal 5-minute constant-speed bouts of increasing intensities and a graded exercise test (GXT) to exhaustion. **Results:** TTE was 8.0% longer following high-CHO trial compared to the fasted trial ($p = 0.009$) and 7.2% longer compared to the low-CHO trial ($p = 0.010$), for both groups pooled. There was not a significant effect of the pre-test CHO conditions on VO_{2peak}, LT, onset of blood lactate accumulation (OBLA), or WE ($p > 0.087$). There was also no significant interaction effect between training status and the effect of pre-event CHO intake related to TTE or the three physiological variables ($p > 0.342$). **Conclusions:** High CHO content in the pre-event meal led to longer TTE measured during a GXT compared to a meal with a low CHO content and fasted state, both in trained and untrained participants. However, there was not an associated effect of the pre-test CHO conditions on the physiological performance-determining variables assessed here (VO_{2peak}, LT, or WE).

Keywords: Pre-exercise, pre-test meal, peak oxygen uptake, onset of blood lactate accumulation, oxygen cost, energy cost, incremental treadmill running, time to exhaustion, training status

Norsk abstrakt

Bakgrunn: Eksogen karbohydrat (CHO) før trening påvirker metabolismen og fører til en forskyvning i valg av drivstoff under trening. Denne forskyvningen kan påvirke utholdenhetskapasiteten, energikostnaden av arbeid og biprodukter av metabolisme, som laktat. **Formål**: Formålet med denne studien var å undersøke effekten av det relative CHO-innholdet i et pre-test-måltid på tid til utmattelse (TTE), maksimalt oksygenopptak (VO_{2peak}), laktatterskel (LT) og arbeidsøkonomi (WE). Et sekundært formål var å sammenligne responsen til CHO-inntak før trening mellom utholdenhetstrente og utrente individer. **Metode**: 11 trente og 10 utrente menn gjennomgikk tre forsøk i et randomisert overkrysningsdesign. Testforholdene var 1) et pre-test-måltid med et høyt CHO-innhold (3 $g \cdot kg^{-1}$), 2) et pre-test-måltid med et lavt CHO-innhold (0.5 $g \cdot kg^{-1}$), og 3) fastet tilstand. Testen besto av fem submaksimale 5-minutters drag med konstant hastighet, men av økende intensitet og avsluttet med en gradert treningstest (GXT) til utmattelse. **Resultat**: TTE var 8.0% lengre etter høy-CHO måltidet sammenlignet med fastet tilstand ($p = 0.009$) og 7.2% lengre sammenlignet med lav-CHO måltidet ($p = 0.010$), for begge gruppene samlet. Det var ingen signifikant effekt av pre-testforholdene på VO_{2peak}, LT, begynnelse av laktatakkumulering (OBLA) eller WE ($p > 0.087$). Det var heller ingen signifikant interaksjonseffekt mellom treningsstatus og effekten av CHO-inntak relatert til TTE eller de tre fysiologiske variablene ($p > 0.342$). **Konklusjon**: Høyt CHO-innhold i pre-test-måltidet førte til lengre TTE målt under en GXT sammenlignet med et måltid med lavt CHO-innhold og fastet tilstand, både hos trente og utrente deltakere. CHO-innholdet i pre-test-måltid hadde ingen assosiert effekt på de fysiologiske prestasjonsbestemmende faktorene vurdert i denne studien (VO_{2peak}, LT eller WE).

2 Abbreviations

ATP	Adenosine triphosphate
BG	Blood glucose
BM	Body mass
CHO	Carbohydrate
CPET	Cardiopulmonary exercise test
EC	Energy cost
FFA	Free fatty acids
$\dot{V}O_2$	Oxygen uptake
VO_{2max}	Maximal oxygen uptake
VO_{2peak}	Peak oxygen uptake
La^-	Blood lactate
$[La^-]b$	Blood lactate concentration
LT	Lactate threshold
OBLA	Onset of blood lactate accumulation
OC	Oxygen cost
RER	Respiratory exchange ratio
RPE	Rating of perceived exertion
TTE	Time to exhaustion
vLpeak	Peak running velocity
vLT	Velocity at lactate threshold
vOBLA	Velocity at OBLA

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3 Introduction

Nutrition is a key element for athletic performance, with carbohydrate (CHO) as a key source of fuel during high-intensity work (1-3). However, the endogenous CHO stores within the body are limited and can be rapidly depleted (2-4). Additionally, endogenous CHO availability can also be acutely manipulated by a single training session or by changes in daily intake (3, 5). Exogenous CHO ingestion has been shown to be a potent ergogenic aid for endurance performance and capacity (6-8), with the best effect being found when CHO is ingested both before and during exercise (7, 8). While the effect of CHO before exercise in isolation has yielded more contradictive results, this administration strategy does also lead to improved exercise capacity in most cases (7-11).

The potential mechanisms underlying improved endurance performance by pre-exercise CHO ingestion are increased endogenous CHO availability either by stimulating muscle glycogen synthesis (12), replenishing liver glycogen after an overnight sleep (12), or lastly by increasing other more temporary glucose stores in the body (i.e. circulating blood glucose (BG) or intestines) (13). This increase in availability leads to increased CHO oxidation before and during exercise (7, 8). Pre-exercise CHO ingestion also leads to elevation of BG and blood insulin concentrations (7, 8). CHO ingestion and the following hyperinsulinemia induce a temporary decrease in fat mobilization and utilization before and during exercise (7, 10, 11) with a concurrent increase in glucose uptake (14). Both the dosage and the timing of the pre-exercise CHO ingestion seems to be of importance for its subsequent effect on performance. The dosage must be of sufficient size to cover the energy deficit caused by the suppression of free fatty acids (FFA) oxidation and the excess amount of CHO oxidized for the exogenous CHO to have the best effect on endurance capacity and performance (7, 8, 15). Chryssanthopoulos et al. (8) found that a pre-event meal with a high CHO content (2.5 g · kg^{-1}) three hours before exercise led to longer time to exhaustion (TTE) compared to fasted exercise and Sherman et al. (15) found that both 1.1 and 2.2 g · kg^{-1} of CHO one hour before exercise led to improved time-trial performance despite elevated insulin concentration at onset of exercise and an initial drop in BG when compared to fasted state.

While the increased CHO availability and oxidation induced by pre-event CHO intake can lead to improved endurance performance and capacity, it is less clear if pre-event CHO intake could influence the measurements of physiological performance-determining factors and if this contributes to the ergogenic effect of CHO. Most of the studies are also carried out with either

trained or untrained participants. CHO uptake and utilization differ between trained and untrained individuals (16, 17). As these differences may affect the necessary pre-test precautions, further comparison of endurance-trained individuals and more sedentary individuals related to the effects of exogenous CHO is warranted.

3.1 Theoretical Background

A three-factor model has been proposed to account for the interindividual variance in aerobic endurance performance (18, 19). These three physiological factors are maximal oxygen uptake (VO_{2max}), lactate threshold (LT), and work economy (WE). This model is well accepted in the literature (20-22). These three factors are often measured during physiological testing when the goal is to assess fitness and potential aerobic endurance performance (3, 18, 19). Alteration of one of these three factors can be interpreted as a change in health or potential endurance performance. Therefore, it is of interest to investigate how the metabolic and physiological responses induced by pre-exercise CHO intake might influence the measurement of these three performance-determining physiological factors. An effect of pre-exercise CHO ingestion on one of the three mentioned physiological factors might contribute to the CHO-induced improvements of endurance performance and capacity, however; this has not been sufficiently investigated.

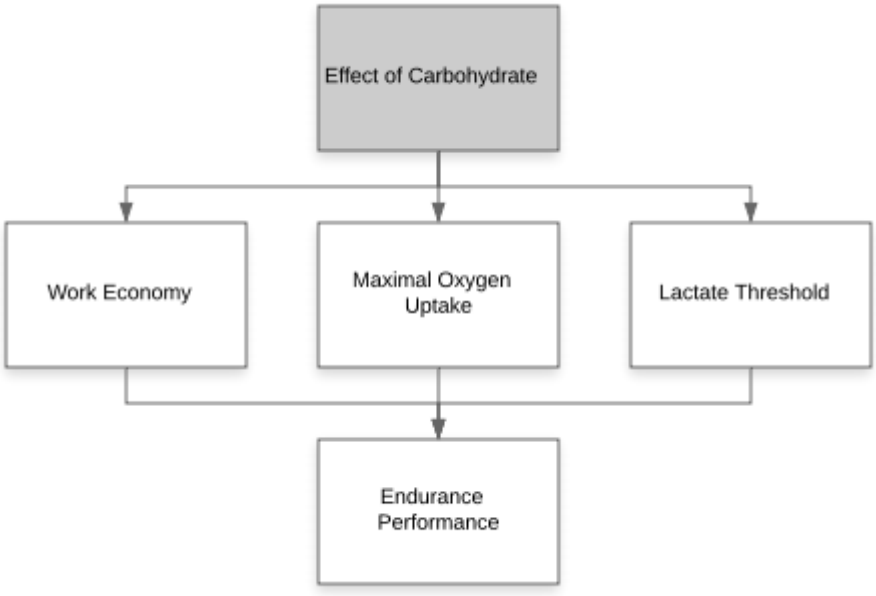

Figure 1. Illustration of the three main physiological performance-determining factors and the possible influence of CHO, adapted from Pate & Kriska (18) and Joyner & Coyle (19).

3.1.1 Maximal Oxygen Uptake

VO_{2max} reflects the body's ability to supply and utilize O_2 at maximal exercise intensity and is considered a strong predictor of aerobic endurance performance (20, 23). VO_{2max} is also considered the gold standard for assessment of an individual's cardiorespiratory fitness (24, 25) and is reached when oxygen uptake ($\dot{V}O_2$) per unit of time reaches its peak and remains constant despite increased workload (3, 19, 25). In healthy individuals, VO_{2max} is mainly limited by the supply of oxygen to the muscles (24, 26) and there does not seem to be a clear mechanism by which exogenous CHO intake would influence VO_{2max}. Mikulski et al. (27) had nine sedentary (VO_{2max}: 37.2 ml · kg^{-1} · min^{-1}) and 10 active men (VO_{2max}: 58.8 ml · kg^{-1} · min^{-1}) complete three tests following three different pre-test conditions, 1) following a high CHO content meal (4% protein, 1% fat and 95% CHO), 2) following a low CHO content meal (35% protein, 64% fat and 1% CHO), or 3) in a fasted state. Both meals contained approximately 1000 kcal and were given two hours before the tests. The preceding evenings subjects would complete 90-minute cycling at 70% VO_{2max} in order to deplete glycogen. They found no effect of the CHO content in the pre-event meals on VO_{2max} measured during an incremental ergometer cycle exercise to exhaustion where the intensity was increased 50 W every third minute, starting at 50 W. Yoshida (28) did also not find a significant effect on VO_{2max} when comparing a three-day high-CHO diet with a four-day low-CHO and a mixed diet. However, as pre-event CHO ingestion has repeatedly been shown to extend TTE (7, 8), pre-event CHO intake can likely influence peak running velocity (vLpeak) attained from a graded exercise test (GXT) to exhaustion. This is supported by Sabapathy et al. (29) who demonstrated significantly lower maximal power output during incremental ergometer cycle exercise to exhaustion with reduced muscle glycogen stores compared to normal levels.

3.1.2 Lactate Threshold

Lactate threshold (LT) is the exercise intensity or speed at which the blood lactate concentrations ($[La^-]b$) starts to increase exponentially during continuous exercise (3, 20). Accumulation of blood lactate (La^-) happens due to greater lactate production by anaerobic glycolysis compared to lactate utilization by the mitochondria (30). LT determines the relative intensity that can be sustained for a prolonged period of time (3, 31), as exercise above this threshold will lead to exponentially more rapid fatigue due to a non-linear increase in perceptual, metabolic and respiratory stress (32, 33), metabolic acidosis on the contractile functions (34), and accelerated depletion of muscle glycogen (35). LT has been determined both with fixed and non-fixed threshold values, in which this study uses the term onset of blood

lactate accumulation (OBLA) when referring to a fixed threshold variable (4 mmol · l^{-1}) and LT when referring to the non-fixed threshold variable.

Dietary interventions influence the metabolic response to prolonged submaximal exercise, such as [La^-]b (36, 37) and possibly also threshold variables calculated from [La^-]b (28, 36). Exogenous CHO either as pre-event intake or dietary intervention leads to a shift in metabolism from fat oxidation towards glycolytic domain (29, 38, 39). This has been shown to increase La^- production and in turn, mean and peak [La^-]b during exercise (8, 38, 40, 41). In a similar way that increased CHO availability increases the reliance on CHO oxidation, increased fat availability during exercise increases the reliance on fat oxidation, in turn, attenuating CHO oxidation and La^- production (42-44). The attenuation of La^- production can possibly be explained by FFA's oxidation inhibitory effect on glycolysis, as citrate (a product of FFA oxidation) inhibits the glycolytic enzymes phosphofructokinase and glycerol-3-phosphate dehydrogenase (42, 43).

Additionally, it has been demonstrated that the state of muscle glycogen stores has a clear influence on [La^-]b (29, 45-47). Depleted muscle glycogen stores lead to an attenuation of glycolysis and glycogenolysis during exercise (45-47). This attenuation leads to lower [La^-]b at the same work rates when compared to exercise done with normal glycogen levels (29, 41, 47) seen as a down- and rightward shift of the lactate workload curve (47, 48). There are also indications that glycogen loading can lead to an up- and leftward shift of the lactate workload curve (28, 40). Ivy et al. (41) investigated how acute alterations of substrate availability affected LT and OBLA measured during incremental ergometer cycle exercise to fatigue in nine male participants with three pre-test conditions; 1) 75 g of glucose 30 minutes prior to exercise, 2) a meal with a high-fat content 4- 4.5 hours before exercise (combined with heparin 30 minutes before), and 3) exercise done in a fasted state. Rotstein et al. (49) did a similar investigation with a lactate-minimum test in 15 male competitive runners using two pre-test conditions; 1) a 21 g CHO ingestion ingested 30 minutes prior to and one- time during exercise compared with 2) a calorie-free placebo. Neither Ivy et al. (41) nor Rotstein et al. (49) found an effect of CHO on LT or OBLA (41, 49). However, in respect to affecting LT, it can be argued that both studies utilized suboptimal CHO administration strategies. Compared to pre-event recommendations, both studies gave participants small CHO dosages very close to onset of exercise (50). The hypothetical effect of CHO on LT and OBLA seems dependent on the alteration of the muscle glycogen stores (28, 40, 49) as glycogen has been proposed to be the primary substrate for La^- production (51). It seems that dietary interventions can influence [La^-]b-related threshold

variables by alterations of muscle glycogen stores and substrate utilization (28, 40). A pre-event meal is capable of affecting both these mentioned prerequisites to a lesser degree (52) and thus, it is possible that a pre-event meal with a large CHO dosage given more than three hours before a test could affect [La^-]b during exercise and in turn OBLA. However, the effect of CHO in a real-life pre-event meal on [La^-]b and related threshold-variables has not been sufficiently investigated.

3.1.3 Work Economy

In a homogenous group with similar aerobic capacity like elite athletes, WE at a specific race pace is often better correlated to endurance performance than VO_{2max} (53). WE is a complex, systemic, multifactorial concept that is determined by biomechanical factors (18, 22, 54) and metabolic factors such as the efficiency of oxidative phosphorylation (18, 54, 55). WE is most often stated as the oxygen cost (OC), or steady-state $\dot{V}O_2$, of a given walking or running velocity (20, 21, 54). However, there are differences in the OC of metabolizing CHO and fat (56, 57), and alterations in substrate utilization during exercise can influence the reliability of OC (54). The OC of utilizing fat as an energy source is higher compared to CHO (58). Oxidation of CHO provides 5-11% more adenosine triphosphate (ATP) per litre of oxygen that is delivered to the mitochondria compared to fat oxidation (59, 60). CHO oxidation is thus preferred over fat oxidation when working at the higher range of intensities that can be supported by oxidative phosphorylation (5, 61). Energy cost (EC) has been used as an alternative term to state WE (61). EC has been proposed to be more comprehensive and valid compared to OC as it calculates WE while taking both OC and respiratory exchange ratio (RER) into account, and is thus, less influenced by alterations in substrate utilization (61, 62). As OC appears to be more influenced by dietary-induced changes in substrate utilization compared to EC, the term WE will refer to the EC of running throughout the thesis.

A low CHO diet leads to increased reliance on fat oxidation for energy production (37, 63, 64), which has been shown to lead to higher OC of race walking in elite race walkers (58). However, there are few studies that have investigated the effect of an acute CHO ingestion on WE. Brisswalter et al. (65) found that 8 ml · kg^{-1} of a 5.5% CHO-electrolyte solution given immediately before exercise and 2 ml · kg^{-1} of the same solution every 20-minute thereafter did not affect the WE measured before and after 80-minute running at ~80% VO_{2max} in 10 well-trained triathletes. Additionally, Sproule (66) found that 500 ml of a 6% CHO-electrolyte solution given immediately prior to 60-minute running at 80% of VO_{2max} did not have a significant effect on the deterioration of OC was measured after the 60-minute run at around 3

$m \cdot s^{-1}$ in 15 young untrained men. On the other side, Dumke et al. (67) found that pre-event CHO given immediately prior to exercise in combination with CHO during exercise attenuated the exercise-induced decrease in cycling efficiency. It was argued that this effect was likely due to better maintenance of metabolic efficiency, underlined as a significant correlation between BG concentrations and cycling efficiency and economy (67). There is currently a lack of investigations utilizing real-life meals in line with current recommendations (50) to assess the effect of pre-event CHO intake on WE, specifically WE expressed as EC measured during running.

3.1.4 Training Status

Endurance exercise leads to various physiological adaptations. Trained individuals have higher VO_{2max} regardless of exercise mode and specificity of training when compared to untrained individuals (16, 68). The higher VO_{2max} in endurance-trained individuals can be explained by various exercise-induced adaptations such as; increased cardiac output (mainly via increased stroke volume), increased blood volume, and increased capillary and mitochondrial density (3, 26). LT changes with the change in VO_{2max} and can also change as the percentage of VO_{2max} (18, 69). Trained endurance athletes have also been shown to have better WE compared to untrained individuals (55, 60, 70).

Jansson and Kajser (16) demonstrated that oxidative enzymes were ~100% more abundant in five trained cyclists (VO_{2max}: 72.9 $ml \cdot kg^{-1} \cdot min^{-1}$) compared to five untrained volunteers (VO_{2max}: 49.1 $ml \cdot kg^{-1} \cdot min^{-1}$) and in turn that ATP turnover from oxidative processes was 50% greater during ergometer cycling exercise at 65% of VO_{2max}. Additionally, respiratory exchange ratio (RER), leg lactate release, and accumulation, glycogen breakdown and utilization was lower during exercise in the trained participants compared to the untrained (16). The lower RER found in the trained subjects indicates that the contribution of fat to oxidative metabolism was 20% greater compared to the untrained subjects (~53% vs 33%) (16). However, as they found no difference in FFA the energy difference must have mainly been covered by intramuscular triglycerides (16).

Van Loon et al. (71) found that seven trained subjects (VO_{2max}: 71.7 $ml \cdot kg^{-1} \cdot min^{-1}$) had lower glycogen breakdown and utilization, lower contribution of CHO to energy expenditure and higher fat oxidation compared to eight untrained male subjects (VO_{2max}: 54.8 $ml \cdot kg^{-1} \cdot min^{-1}$) during 120-minute cycling at 50% of maximal workload (~55 % of VO_{2max}) (71). However, they did not find a significant difference in the oxidation of the exogenous CHO given during

exercise between the two groups (71). This is contradicted by Burelle et al. (17), who found using a slightly different CHO administration strategy (CHO given 30 minutes before and once during exercise) and exercise intensity (~68% of VO_{2max}, ~226 W for trained vs 140 W for untrained) that liver-, exogenous- and total CHO oxidation was greater in six well trained male participants (VO_{2max}: 66.8 ml · kg^{-1} · min^{-1}) compared to six sedentary male participants (VO_{2max}: 46.6 ml · kg^{-1} · min^{-1}) (17). As the difference in aerobic capacity between the trained and untrained groups in the two studies was comparable, the most important difference is arguably the inclusion of a pre-exercise CHO ingestion in Burelle et al. (17), where subjects were given 25 g of glucose 30 minutes before the onset of exercise, whereas subjects were only given CHO during exercise in Van Loon et al (71). Jeukendrup et al. (72) argued in their review that the different findings in these two studies' can be explained by glycogen stores being increased more in trained athletes postprandially. Trained individuals have greater insulin sensitivity and thus, greater CHO uptake compared to untrained (73-75). Due to the greater CHO uptake, glycogen accumulation and stores, and possible also greater utilization of exogenous CHO it is plausible that trained individuals might experience greater effect of the CHO content in a pre-event meal compared to untrained individuals; however, this has not yet been sufficiently investigated in relation to endurance capacity.

3.2 Aim and hypothesis

The primary aim of the present study was to investigate the effect of the relative CHO content in a pre-event meal on TTE, peak oxygen uptake (VO_{2peak}), LT, and WE. It was hypothesised that a pre-event meal with a high CHO content will extend TTE during a GXT when compared to the same test done in a fasted state. It was further hypothesised that there would be no effect of the pre-test CHO conditions on VO_{2max} or WE. However, it was hypothesised that high pre-test CHO availability would lead to higher $[La^-]b$ during exercise compared to low pre-test CHO availability, and as a result, OBLA would occur at a higher percentage of VO_{2max} following the pre-test CHO conditions with low CHO availability when compared to high. This hypothesis did not extend to LT based on an individualized $[La^-]b$ value. The secondary aim was to compare the response to pre-event CHO intake between endurance-trained individuals and untrained individuals. It was hypothesised that a pre-event meal with a high CHO content would have a stronger effect on TTE and $[La^-]b$ in the trained participants compared to the untrained.

4 Materials and Methods

4.1 Subjects

21 healthy, non-smoking men participated in this study. The participants were divided into a trained (N = 11) and untrained (N = 10) group (figure 2). All participants had to review and sign a consent form and go through an ASCM approved health-history questionnaire (American Council on Exercise, San Diego, CA, USA) (see appendix II) to establish low health risk and to reveal any possible food allergies before being allowed to participate in the study. Participants were recruited by means of posters dispersed over the different faculties at the local university, local gyms, social media, and oral presentations at various lectures at different classes belonging to various faculties of NTNU, in Trondheim.

The recruitment process was based on group-specific inclusion criteria. The trained participants had to be committed to an endurance training regimen of at least three sessions per week and must have been so for the last two years. Untrained participants could not be training endurance more than once per week and must have done so for the last two years. The participants that met the recruitment criteria went through a health- history form to establish that they were at low health risk and then a baseline test to determine if they met the fitness level-based inclusion criteria. In the untrained group a VO_{2max} of 53 ml · kg^{-1} · min^{-1} was set as the upper limit for aerobic capacity, in order to be below the average VO_{2max} in Norwegian men aged 21 to 29 years old (76). To get the desired group difference between the two groups, the trained participants were required to have a VO_{2max} above 60 ml · kg^{-1} · min^{-1}. All participants had to be between the age of 18 and 45 years old to participate in this study. The study was approved by The Institutional Review Board of NTNU and was carried out in accordance with the recommendation of the Norwegian Centre for Research Data (NSD) and the Declaration of Helsinki.

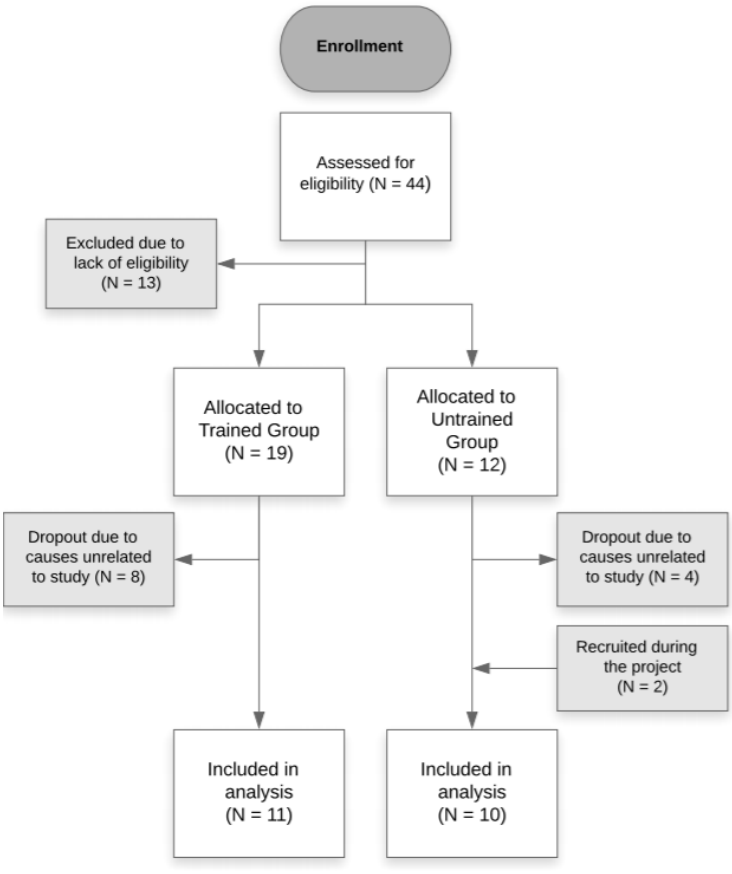

Figure 2: Flowchart of the study design

Sample size calculations were done using Stata 15.1 (Metrika consulting AB, Stockholm, Sweden). Using Chrysanthopoulos et al. (8) as reference, sample size calculation using TTE where the mean difference between the pre-event CHO trial and fasted trial was 9.0 with a mean SD 6.8, a power level of 80% and significance level at 5% showed that 11 participants per group (22 in total) would be needed.

4.2 Study Design

This study was a randomized controlled cross-over trial. All testing took place at the Next Move core facility at St. Olav's Hospital, NTNU – Norwegian University of Science and Technology, Trondheim.

4.2.1 Experimental Protocol

Participants completed a baseline test and subsequently three trials in randomized order. The trials were separated by a minimum of six days. The initial baseline test functioned as a familiarization session and consisted of a 10-minute warm-up with a pre-selected inclination of 5.3% and self-selected pace. After a three-minute break, the test finished off with a GXT to exhaustion in order to establish baseline VO_{2max}. The baseline GXT consisted of a 1 km · h^{-1} increase every minute until the test was terminated by the participants themselves. Starting speed for the baseline GXT was not standardized and was selected based on participants' self-selected pace during the warm-up. The GXT was used to establish VO_{2max} for the baseline test and VO_{2peak} for the three main trials. During the GXT participants were given strong verbal encouragement. Relevant measurements from the baseline GXTs were used to calculate the velocity of the submaximal stages for the following three main trials. The baseline test also allowed the participants to become familiar with the testing equipment and GXT to exhaustion.

The protocol of the three main trials consisted of three distinct steps. Between steps one and two and between steps two and three participants were allowed to drink water ad libitum. 1) A ten-minute warm-up at a velocity corresponding to ~40-50% VO_{2max} for the trained group and ~30-40% for the untrained group. Warm-up and all subsequent steps were performed at a 5.3 % inclination. 2) 5 · 5-minute exercise bouts with increasing intensities to establish LT and determine WE (54, 77). The speeds of the bouts were calculated to result in an intensity approximating 50, 60, 70, 80, and 90 % of participants' baseline VO_{2max}. There was a 90-second break between each exercise bout for sampling of capillary blood from the fingertip to determine $[La^-]b$ and BG. 3) After a three-minute break, participants completed a GXT to exhaustion to establish VO_{2peak}. The GXT of the three main trials started at a speed 2 km · h^{-1}

slower than the last submaximal exercise bout ended and otherwise followed the protocol as described above.

The pre-event meals were consumed at one of these three options according to what participants selected before their baseline test: 1) 07:00- 07:30, 2) 08.15-08.45, 3) 09.30-10.00. Tests were performed approximately three and a half hours later at; 1) 10.30-11.30, 2) 11.45-12.45, 3) 13.00-14.00. Prior to all test days, participants were told to refrain from any strenuous exercise 48 hours prior to the tests and to refrain from all exercise 24 hours prior. During the three days prior to the three main trials, participants were told to record and replicate their diet to minimize differences in glycogen stores between the test days. Participants recorded their diets using the app "dietist.net". During their first visit, participants were instructed on how to use the app and were told to try it out for a day or two prior to the first three- day period in order to minimize errors. Lastly, participants were told to refrain from the use of caffeine prior to all trials.

4.2.2 Experimental Trials

Participants arrived at the lab at their appointed time for the three trials following three different pre-test conditions: 1) A minimum of 12 hours of fasting before the test. 2) A standardized pre-event meal with a high CHO content (3 g · kg^{-1} CHO) given to the participants three and a half hours prior to the test. This meal consisted of white bread, jam, skimmed milk, oats, banana, and raisins. The dosage was chosen because it has been reported to increase both liver and muscle glycogen stores, is sufficient to cover the deficit in substrate supply, and has consistently been reported to lead to performance improvements (7, 8, 52, 78). 3) A standardized pre-event meal with a low CHO content (0.5 g · kg^{-1} CHO) given to participants three and a half hours prior to test. This meal consisted of yoghurt, almonds, and avocado. Option 2) and 3) were isoenergetic and the order of all three pre-test conditions was randomized. Having the pre-event meal three and a half hours prior to the tests was chosen because it takes more than three hours for blood insulin to reach basal levels following a high CHO ingestion (78, 79).

4.2.3 Anthropometrics and Allometric scaling

Prior to the onset of all tests body mass (BM) was measured with clothes but without shoes on (seca 877, Hamburg, Germany) to the nearest 0.1kg. Height was only measured before baseline testing and was measured without shoes to the nearest 1 cm. BMI was calculated as $\frac{weight\ (kg)}{height^2(m)}$ using the baseline height and weight.

When comparing two highly heterogenic groups with variations in BM reporting VO_2- related measurements in absolute ($L \cdot min^{-1}$) or relative to BM ($mL \cdot kg^{-1} \cdot min^{-1}$) may be functionally imprecise (80, 81). As the OC and EC of movement do not scale linearly with BM, reporting measurements related to BM should be done with BM raised to the power of 0.75 (21, 81, 82). Allometric scaling has been reported to minimize the effect of BM, reveal smaller differences in WE and decrease SD of measurements affected by BM (21, 81-83). For these abovementioned reasons allometric scaling was used for certain key variables such as VO_{2max}- and $_{peak}$, as well as WE.

4.3 Assessment

4.3.1 Adherence & Dietary Habits

Upon arrival, participants filled out a form consisting of eight questions to assess their adherence to the pre-test protocol. Participants' self- reported nutritional data were analysed by a sports nutritionist to assess compliance with the pre-test dietary protocol. The dietary habits related to exercise were identified for the trained participants using two questions. They were asked to rate from 1 to 10 whether they usually exercised fasted or after a meal, 1 represented always exercising fasted and 10 represented always exercising with food ingested one to four hours prior to their sessions. For the second question participants were asked to rate from 1 to 10 whether their pre-training meal usually contained CHO or not. 1 represented that the pre-exercise meal consisted of no CHO and 10 represented that the pre-exercise meal predominately consisted of CHO rich foods. As the untrained participants did not have established dietary habits related to exercise they were not reported.

4.3.2 Heart Rate, Oxygen Uptake & Respiratory Exchange Ratio

The cardiopulmonary exercise test (CPET) has been shown to accurately estimate VO_{2max} (3, 84). All testing was performed on a treadmill (Woodway PPS 55, Waukesha, WI, USA). Participants were fitted with a facemask (7450 Series V2 CPET mask, Hans Rudolph, Shawnee, KS, USA). $\dot{V}O_2$, $\dot{V}CO_2$, and RER were measured by the use of an ergospirometry system with a mixing chamber (Metalyzer II, CORTEX Biophysik GmbH, Leipzig, Germany). Prior to all tests, the apparatus was calibrated in accordance with humidity conditions, ventilation (3L Calibration Syringe, Hans Rudolph, Lenexa, KS, USA) and ambient air. Prior to the first test of the day, the apparatus was calibrated in accordance with the barometric pressure using a weather station (Oregon Scientific, Tualatin, OR, USA) and with a reference gas mixture (5.00% CO_2 and 15.00% O_2, HiQ AGA, Norway). During baseline testing, when the number

of daily tests exceeded three, this last calibration step was repeated for every third test. $\dot{V}O_2$ measurements were taken at ten-seconds intervals. VO_{2max} was determined from the three continuous highest measurements and was accepted if the first and two of the remaining three criteria were met: 1) a plateau or a decrease in $\dot{V}O_2$ despite an increase in workload, 2) a RER ≥ 1.05, 3) $[La^-]b > 7$ mmol $\cdot$ l^{-1} and 4) a rate of perceived exertion (RPE) >18 on the Borg scale (25, 85-87). Due to the draining nature of the submaximal parts of the three main trials the criteria of the plateau was met for less than half the main trials. For this reason, all the highest $\dot{V}O_2$-measurements from the three main trials are defined as VO_{2peak} (85, 86).

A Polar H7 heart rate (HR) transmitter (Polar Electro, Kempele, Finland), compatible with the treadmill was used to measure HR. The highest HR measurement regardless of test was set as that participant's heart rate max (HR_{max}). The highest HR measurements from each of the three trials were set as HR_{peak} for the associated trial. HR was measured continuously and written down 30 seconds before the end of the warm-up, 30 seconds before the end of every exercise bout, and every 30-second during the GXT. HR measurements from minute seven to nine of the GXT was not reported due to too few participants lasting that long.

4.3.3 Perceived Exertion

Participants rated their self-perceived exertion using the Borg scale (88). Participants stated their RPE immediately after the warm-up, after each exercise bout and after the GXT. Before the baseline test and the first main trial was initiated, all participants were informed about the test procedure and instructed on how to use the Borg scale to most accurately state their RPE (88). They were also asked before the two remaining trials if they remembered the test procedure and how to use the Borg scale. Lastly, before the three main trials, participants were asked to terminate an exercise bout if their RPE exceeded 18 in order to not go into the GXT overly fatigued.

4.3.4 Endurance Capacity

TTE was measured during the GXT. The stopwatch was started at onset of the GXT for each trial and was stopped when participants jumped off, grabbed the railings or other parts of the treadmill. Participants' highest measured velocity during the GXT was reported as vLpeak.

4.3.5 Lactate Threshold, Blood Lactate, and Blood Glucose

For [La-]b, 20 µl capillary blood was sampled after a finger prick induced by a contact-activated safety lancet (Unistik Touch, Owen Mumford, Oxfordshire, United Kingdom) at rest before

onset of the test, after the warm-up, after each exercise bout, and after the GXT. The capillary blood sample was placed in a tube with haemolyzing liquid (EKF diagnostic, Barleben, Germany) and shaken lightly. [La-]b was established via analyse using a Biosen C-line lactate analyser (EKF-diagnostic GmbH, Leipzig, Germany). For BG concentration, 4 µl capillary blood was sampled using microcuvettes at the already mentioned time points as [La-]b, BG was established via analyse using a HemoCue Glucose 201 RT (HemoCue AB, Ängelholm, Sweden).

The five-minute length of the exercise bouts was chosen because it has been shown that 3-6 minutes is necessary to determine the desired metabolic inflection point (89, 90). The $[La^-]b$ of a given bout was included in the analysis if the bout lasted a minimum of three minutes. Weltman et al. (91) and others have reported that an exercise protocol based on a duration of three minutes will result in a valid determination of LT (77, 90, 91). LT was found when $[La^-]b$ was measured 2.3 mmol $\cdot$ l^{-1} above the individual warm-up $[La^-]b$ value, adapted from Helgerud et al. (87) to be appropriate for haemolyzed capillary blood samples (87, 92). OBLA was set at a $[La^-]b$ of 4 mmol $\cdot$ l^{-1} (77). The % of VO_{2max} where LT and OBLA occurred, and the velocity where lactate threshold (vLT) and OBLA (vOBLA) occurred was calculated using an exponential function in MatLab (R2019b, Natick, MA, USA). The equation used was $a + b \cdot \exp(c \cdot x)$, start points used for the fitting were [1 1.1 1].

4.3.6 Work Economy

$\dot{V}O_2$ measurements of a given bout were included in the analyses if the bout lasted at least three minutes. The minimum of three minutes was selected as it is approximately the time it takes to reach steady-state $\dot{V}O_2$ in these settings (3, 61). The mean $\dot{V}O_2$ of the last 60-seconds of a given exercise bout was used to establish the OC. $\dot{V}O_2$ and $\dot{V}CO_2$ during the same time period were used to calculate EC. An updated nonprotein respiratory quotient equation by Péronnet & Massicotte was used to calculate EC (56, 93). The equation by Péronnet & Massicotte (93) meticulously accounts for energy provided from glucose and fatty acids oxidation and is based on the latest chemical and physical data (62, 94). EC could only be calculated with this method if RER was < 1.00. The last bout for each participant where RER was < 1.00 was used for further analysis. The exercise bout selected for comparison for each participant had to be the same bout in all three trials. EC was reported as both SI units and non-SI units for better comparison with existing literature.

4.4 Statistical Analysis

The software program IBM SPSS, version 26.0 (Statistical Package for Social Science, Chicago, IL, USA) was used for the statistical analysis. All data were tested for normality using histograms, quantile-quantile (QQ) plots, and Shapiro-Wilk normality tests before proceeding to parametric tests. To assess the effect of the pre-test CHO conditions within-group and the interaction effect of group, two-way mixed factor ANOVA was used. This was followed up by a One-way repeated measure ANOVA for each of the two groups separately if the interaction effect was significant, and with both groups combined if the main effect of pre-test condition was significant, but the interaction effect was not. Whenever main or simple effects of pre-test condition were significant, pair-wise comparisons were made using Bonferroni adjustments. If the assumption of sphericity was violated for the two-way interaction, Mauchly's test of sphericity was displaced by a Greenhouse Geisser correction. Results are presented as mean (standard deviation (SD)), p-value, and confidence interval 95% (Cl 95%). To specifically describe either the main effect of group, main or simple effect of pre-test condition or the interaction effect; degrees of freedom (df) for two-way interaction term, df for the error term, F-value, p-value and the effect size using partial η^2 was used. The df for two-way interaction term, df for the error term, obtained F-values, p-values, and effect sizes not presented in text are presented in appendix III, along with bout specific EC and OC values, associated mean differences, p-values, and confidence intervals. In all cases, $P \leq 0.05$ was used as the level of significance.

5 Results

5.1 Participant characteristics, self-reported energy and carbohydrate intake, and self-reported dietary habits

Table 1. Participants characteristics

	Trained (n = 11)	**Untrained (n = 10)**
Age (years)	24.6 (2.3)	25.1 (3.5)
Height (cm)	183.1 (5.9)	179.4 (6.5)
BM (kg)	77.8 (9.2)*	92.3 (12.6)
BMI (kg · m^2)	23.2 (1.9)*	28.7 (4.4)
VO_{2max} (L · min^{-1})	5.60 (0.60)**	4.30 (0.69)
VO_{2max} (ml · kg^{-1} · min^{-1})	71.94 (5.08)**	46.85 (2.53)
VO_{2max} (ml · kg$^{-0,75}$ · min^{-1})	213.40 (13.70)**	144.84 (10.95)
HRmax (beats · min^{-1})	197.91 (6.88)	199.50 (5.04)

Data are presented as mean (SD), BM = body mass measured at baseline, BMI = body mass index, VO_{2max} = maximal oxygen uptake stated in absolute terms or relative and scaled to BM, HRmax = highest measured heart rate in the trained and the untrained group, respectively. * = significant differences between groups ($p \leq 0.05$), ** = significant differences between groups ($p < 0.001$).

There was not a significant difference in mean CHO intake ($_{Trained}$ ($F(2, 12) = 0.67$, $p = 0.532$, partial $\eta^2 = 0.01$), ($_{Untrained}$ ($F(2, 12) = 3.50$, $p = 0.064$, partial $\eta^2 = 0.37$) or energy intake ($_{Trained}$ ($F(2, 12) = 2.52$, $p = 0.122$, partial $\eta^2 = 0.30$), ($_{Untrained}$ ($F(2, 12) = 0.05$, $p = 0.948$, partial $\eta^2 = 0.01$) during the three days prior to each of the three trials.

The trained participants reported a mean score of 7.64 / 10 (1.63) on the question of whether they usually exercised with a meal prior to their training and a mean score of 7.91 / 10 (1.97) on the question whether their pre-exercise meal consisted predominately of CHO. Participants' self-reported CHO- and energy intake are presented in appendix III.

5.2 Blood Glucose and Lactate Concentrations

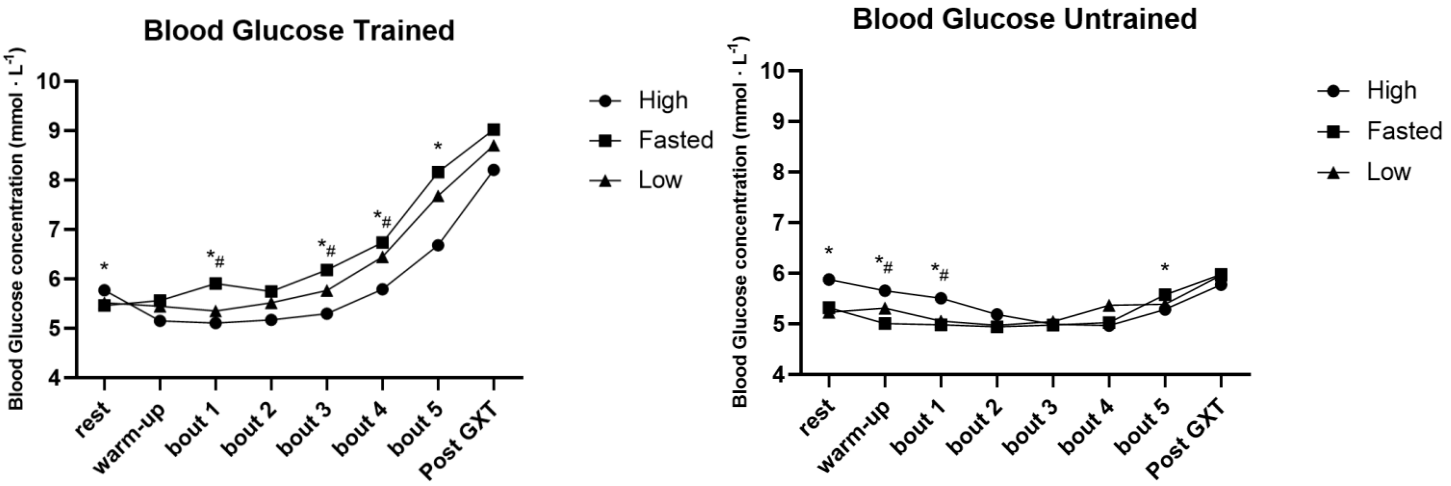

Figure 3 and 4. Mean blood glucose concentrations before and after warm-up, following all five exercise bouts, and after the graded exercise test (GXT) in the trained and the untrained group, respectively. * = significant effect of pre-test condition after a follow-up test with both groups included, *# = significant effect of pre-test condition after a follow-up test for the two groups separately. High = pre-event meal with high carbohydrate content, low = pre-event meal with low carbohydrate content, fasted = test in fasted state.

The effect of pre-test condition on mean BG was statistically significant in the trained group (F (2, 20) = 4.89, p = 0.019 partial η^2 = 0.33) but not the untrained group (F (2, 18) = 0.72, p = 0.718, partial η^2 = 0.07). The interaction effect between training status and the effect of pre-test condition was statistically significant for mean BG (F (2, 38) = 5.13, p = 0.011, partial η^2 = 0.21). In the trained group mean BG was 0.73 lower following the high-CHO trial compared to the fasted-trial (p = 0.042, Cl 95%: -1.44 to -0.02) and 0.43 lower compared to the low-CHO trial (p = 0.383, Cl 95%: -1.17 to 0.31). Ignoring pre-test condition, mean BG was significantly higher in the trained group compared to the untrained group (F (1, 19) = 26.49, p < 0.001, partial η^2 = 0.58).

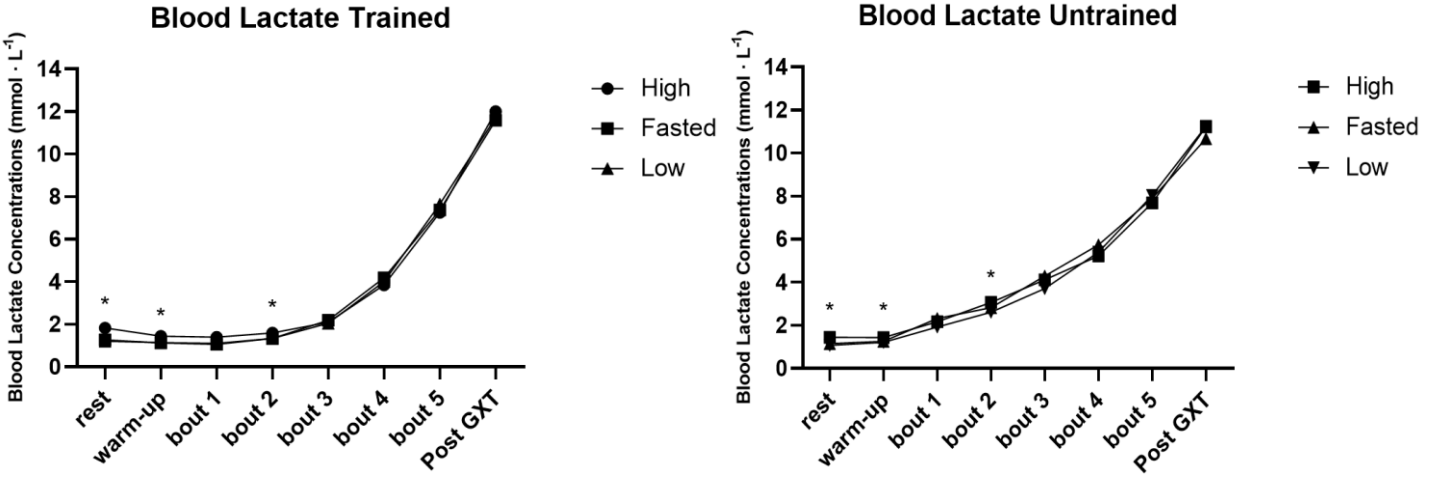

Figure 5 and 6. Mean blood lactate concentrations before and after warm-up, following all five exercise bouts and after the graded exercise test (GXT) in the trained and the untrained group, respectively. * = significant effect of pre-test condition after a follow-up test with both groups included, *# = significant effect of pre-test condition after a follow-up test for each group separately. High = pre-event meal with high carbohydrate content, low = pre-event meal with low carbohydrate content, fasted = test in fasted state.

The effect of pre-test condition on mean [La^-]b was not significant (F (2, 38) = 1.16, p = 0.326, partial η^2 = 0.06). There was no significant interaction effect between training status and the effect of pre-test condition. Ignoring pre-test condition, [La^-]b was significantly higher for the first three exercise bouts in the untrained group compared to the trained group (1^{st}: F (1, 19) = 12.43, p = 0.002, partial η^2 = 0.40), (2^{nd}: F (1, 17) = 18.16, p < 0.001, partial η^2 = 0.52), (3^{rd}: F (1, 19) = 15.74, p < 0.001, partial η^2 = 0.45).

5.3 Endurance Capacity

Table 2. Time to exhaustion and the peak velocity attained during the graded exercise test

	Trained (n = 11)			**Untrained (n = 10)**		
	High CHO	Low CHO	Fasted	High CHO	Low CHO	Fasted
TTE (min:ss.ss)	5:52.00 (1:31.45) *	5.30.30 (1:24.09)	5:22.50 (1:09.04)	5:45.21 (0:51.28)	5:17.30 (1:03.63)	5:18.90 (1:01.66)
vLpeak ($km \cdot h^{-1}$)	17.5 (1.5) *	16.9 (1.4)	16.9 (1.7)	11.3 (1.1)	10.8 (1.5)	10.9 (1.4)

Data are presented as mean (SD). High CHO = pre-event meal with high carbohydrate content, low CHO = pre-event meal with low carbohydrate content, fasted = test in fasted state, TTE = Time to exhaustion from the respective trial stated as minute : seconds, vLpeak = peak velocity attained during the graded exercise test in the respective trial in the trained and the untrained group, respectively. * = significant effect of pre-test condition after a follow-up test with both groups included.

There was a statistically significant effect of pre-test condition on TTE (F (2, 38) = 8.14, p = 0.001, partial η^2 = 0.30). TTE was significantly higher following the high-CHO trial compared to both the fasted trial (p = 0.009, Cl 95%: 6.49 to 49.31) and the low-CHO trial (p = 0.013, Cl 95%: 4.60 to 45.01). There was not a statistically significant interaction effect between training status and the effect of pre-test condition (F (2, 36) = 0.19, p = 0.828, partial η^2 = 0.01). Ignoring pre-test condition, TTE was not significantly different between groups (F (2, 18) = 0.07, p = 0.802, partial η^2 < 0.01).

5.4 Peak Oxygen Uptake

Table 3. Mean peak oxygen uptake stated as absolute, relative- and scaled to body mass

	Trained (n = 10)			**Untrained (n = 10)**		
	$L \cdot min^{-1}$	$ml \cdot kg^{-1} \cdot min^{-1}$	$ml \cdot kg^{-0,75} \cdot kg^{-1}$	$L \cdot min^{-1}$	$ml \cdot kg^{-1} \cdot min^{-1}$	$ml \cdot kg^{-0,75} \cdot kg^{-1}$
High CHO	5.21 (0.64)	67.98 (7.41)	200.90 (20.27)	4.17 (0.69)	45.08 (2.47)	139.60 (10.90)
Low CHO	5.15 (0.69)	67.14 (7.35)	198.51 (20.57)	4.00 (0.63)	43.39 (2.82)	134.22 (10.17)
Fasted	5.21 0.67)	67.91 (5.34)	200.73 (15.83)	4.07 (0.70)	44.28 (2.55)	136.91 (10.94)
BM (kg)	77.5 (8.7)	77.5 (9.2)	77.5 (9.0)	92.1 (11.3)	91.7 (12.1)	92.1 (12.5)

Data are presented as mean (SD). High CHO = pre-event meal with high carbohydrate content, low CHO = pre-event meal with low carbohydrate content, fasted = test in fasted state, Peak oxygen uptake stated in absolute, relative and scaled terms from the respective trial in the trained and the untrained group, respectively.

The effect of pre-test condition on VO_{2peak} was not significant ($_{absolute}$ F (2, 36) = 2.01, p = 0.149, partial η^2 = 0.10), ($_{relative}$ F (2, 36) = 1.78, p = 0.183, partial η^2 = 0.09), ($_{scaled}$ F (2, 36) = 1.85, p = 0.173, partial η^2 = 0.09). There was not a statistically significant interaction effect between training status and the effect of pre-test condition ($_{absolute}$ F (2, 36) = 0.53, p = 0.591, partial η^2 = 0.03), ($_{relative}$ F (2, 36) = 0.23, p = 0.794, partial η^2 = 0.01), ($_{scaled}$ F (2, 36) = 0.32, p = 0.729, partial η^2 = 0.02).

5.5 Lactate Threshold and Onset of Blood Lactate Accumulation

Table 4. Mean lactate threshold, onset of blood lactate accumulation and the velocity at lactate threshold and onset of blood lactate accumulation

	Trained (n = 11)			**Untrained (n = 10)**		
	HIGH CHO	LOW CHO	FASTED	HIGH CHO	LOW CHO	FASTED
LT % VO_{2max}	73.6 (5.8)	73.3 (7.2)	73.5 (3.9)	67.2 (8.4)	69.3 (9.4)	67.0 (10.0)
OBLA %VO_{2max}	75.0 (5.5)	76.1 (6.5)	76.8 (4.6)	70.9 (8.7)	73.0 (7.8)	70.3 (8.5)
$_{v}$LT ($km \cdot h^{-1}$)	12.4 (1.5)	12.2 (1.5)	12.1 (1.6)	6.5 (1.2)	6.5 (1.1)	6.3 (1.2)
$_{v}$OBLA ($km \cdot h^{-1}$)	12.8 (1.5)	12.6 (1.5)	12.6 (1.5)	6.7 (1.1)	6.8 (1.1)	6.6 (1.2)
$[La^-]b$	3.7 (0.4)*	3.3 (0.2)	3.4 (0.3)	3.7 (0.4)	3.4 (0.5)	3.5 (0.5)

Data are presented as mean (SD). High CHO = pre-event meal with high carbohydrate content, low CHO = pre-event meal with low carbohydrate content, fasted = test in fasted state, LT % and OBLA % = the percentage of maximal oxygen uptake where lactate threshold and onset of blood lactate accumulation occurred, $_{v}$LT and $_{v}$OBLA = velocity where lactate threshold and onset of blood lactate accumulation occurred, $[La^-]b$ = individual lactate concentration after warm-up + 2.3 in the trained and the untrained group respectively. * = significant effect of pre-test condition after a follow-up test with both groups included.

There was not a statistically significant effect of pre-test condition on LT or OBLA ($_{LT}$: F (2, 34) = 0.44, p = 0.645, partial η^2 = 0.03), ($_{OBLA}$: F (2, 34) = 1.04, p = 0.363, partial η^2 = 0.06). Neither was there a significant effect on vLT or vOBLA ($_{vLT}$: F (2, 34) = 2.48, p = 0.099, partial η^2 = 0.13), ($_{vOBLA}$: F (2, 34) = 0.84, p = 0.439, partial η^2 = 0.05). There was not a significant interaction effect between training status and the effect of pre-test condition regarding the [La^-]b-related threshold-variables (p > 0.342). Ignoring pre-test conditions, LT and OBLA was not significantly different between the trained and untrained group (p > 0.100). vLT and vOBLA was both significantly higher in the trained group compared to the untrained (p < 0.001). The effect pre-test condition on the individual [La^-]b threshold value was statistically significant (F (2, 38) = 6.04, p = 0.005, partial η^2 = 0.24).

5.6 Work Economy

Table 5. Work economy attained from the exercise bout with the highest accepted value

	Trained (n = 11)			**Untrained (n = 10)**		
Energy Cost	HIGH CHO	LOW CHO	FASTED	HIGH CHO	LOW CHO	FASTED
Absolute ($Kcal \cdot km^{-1}$)	101.65 (16.01)	103.35 (12.17)	104.32 (17.03)	142.47 (22.86)	149.46 (20.72)	146.56 (24.00)
Scaled ($Kcal \cdot kg^{-0.75} \cdot km^{-1}$)	3.88 (0.41)	3.97 (0.39)	3.99 (0.41)	4.80 (0.61)	5.06 (0.79)	4.96 (0.71)
SI-units ($J \cdot kg^{-1} \cdot m^{-1}$)	5.49 (0.55)	5.60 (0.59)	5.64 (0.52)	6.49 (0.88)	6.87 (1.26)	6.73 (1.07)
Oxygen cost **(**$ml \cdot kg^{-0.75} \cdot min^{-1}$)	148.01 (19.66)*	155.70 (22.37)	153.57 (21.18)	96.81 (17.98)*	101.88 (14.99)	100.41 (16.61)

Data are presented as mean (SD). High CHO = pre-event meal with high carbohydrate content, low CHO = pre-event meal with low carbohydrate content, fasted = test in fasted state, Energy cost = highest accepted energy cost value expressed as kcal per km, kcal per $kg^{-0.75}$ body mass per km and Joules per kg body mass per meter, Oxygen cost = scaled oxygen cost value equivalent to the energy cost value in the trained and the untrained group, respectively. * = significant effect of pre-test condition after a follow-up test with both groups included.

There was not a significant effect of pre-test condition on WE ($_{absolute\ EC}$ F (2, 38) = 2.07, p = 0.141, partial η^2 = 0.10) ($_{scaled\ EC}$ (F (2, 38) = 2.45, p = 0.099, partial η^2 = 0.11) ($_{SI\text{-}units}$ F (2, 38) = 2.60, p = 0.087, partial η^2 = 0.12). There was not a statistically significant interaction effect between training status and the effect of pre-test condition (p > 0.483). Ignoring pre-test condition, the EC was significantly lower in the trained group compared to the untrained group ($_{absolute\ EC}$ F (2, 19) = 29.62, p < 0.001, partial η^2 = 0.61), ($_{scaled\ EC}$ F (2, 19) = 18.96, p < 0.001, partial η^2 = 0.50), ($_{SI\text{-}units}$ F (2, 19) = 10.55, p = 0.004, partial η^2 = 0.36).

5.7 Respiratory Exchange Ratio

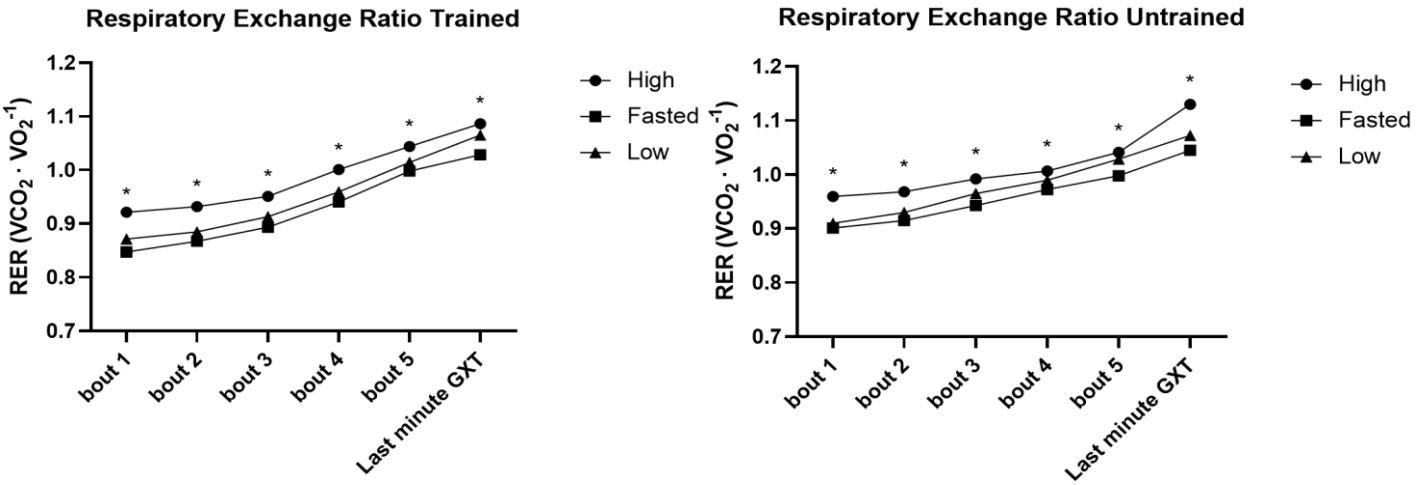

Figure 7 and 8: Mean respiratory exchange ratio (RER) during the final minute of all five exercise bouts and the graded exercise test (GXT) in the trained and untrained group, respectively. * = significant effect of pre-test condition after a follow-up test with both groups included. High = pre-event meal with high carbohydrate content, low = pre-event meal with low carbohydrate content, fasted = test in fasted state.

The effect of pre-test condition on mean RER was statistically significant ($F(2, 40) = 31.81$, $p < 0.001$, partial $\eta^2 = 0.61$). There was not a statistically significant interaction effect between training status and the effect of pre-test condition. Mean RER was 0.05 higher following the high-CHO trial compared to the fasted-trial ($p < 0.001$, Cl 95%: 0.03 to 0.08) and 0.04 higher compared to low-CHO trial ($p < 0.001$, Cl 95%: 0.02 to 0.05). Additionally, mean RER was significantly higher following the low-CHO trial compared to the fasted trial ($p = 0.048$, Cl 95%: -0.04 to -0.00). Ignoring pre-test condition, RER was significantly higher in the untrained group for the first three exercise bouts (1^{st} ($F(1, 18) = 16.48$, $p < 0.001$, partial $\eta^2 = 0.48$), (2^{nd} $F(2, 17) = 18.16$, $p < 0.001$, partial $\eta^2 = 0.52$), (3^{rd} $F(2, 19) = 15.26$, $p < 0.001$, partial $\eta^2 = 0.45$) compared to the trained group.

5.8 Rate of Perceived Exertion

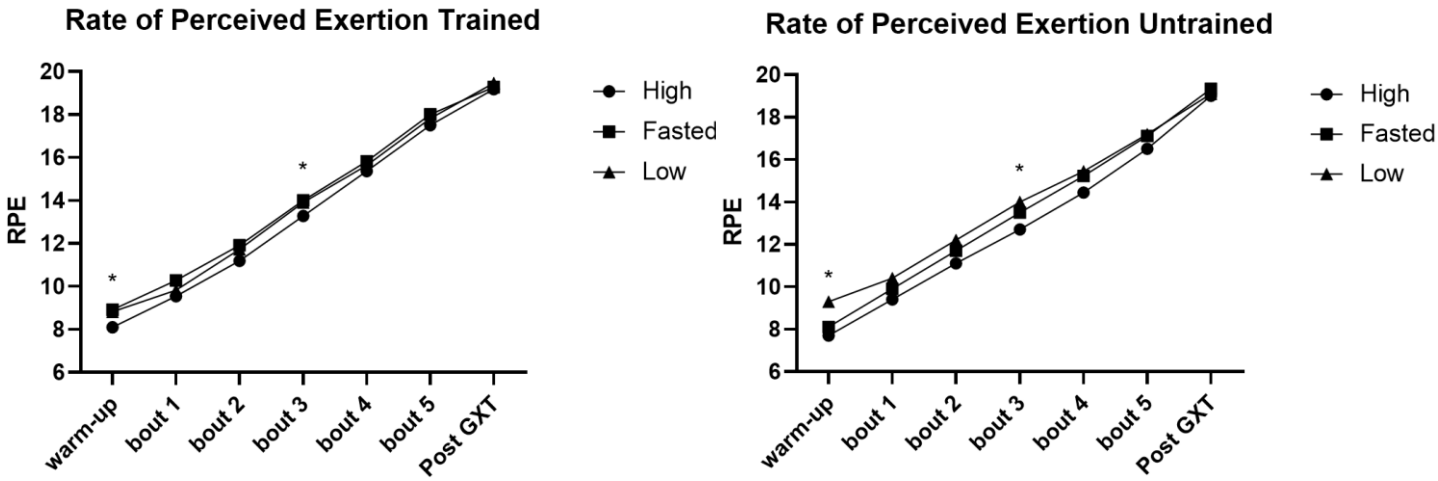

Figure 9 and 10: Mean rate of perceived exertion (RPE) after warm-up, following all five exercise bouts and after the graded exercise test (GXT) in the trained and untrained group, respectively. * = significant effect of pre-test condition after a follow-up test with both groups included. High = pre-event meal with high carbohydrate content, low = pre-event meal with low carbohydrate content, fasted = test in fasted state.

There was a significant effect of pre-test condition on mean RPE (F (2, 40) = 6.32, p = 0.004, partial η^2 = 0.24). Mean RPE was 0.63 lower following the high-CHO trial compared to the fasted trial (p = 0.084, Cl 95%: -1.33 to 0.07) and 0.77 lower compared to the low-CHO trial (p = 0.002, Cl 95%: -1.28 to -0.27). There was not a statistically significant interaction effect between training status and the effect of pre-test condition on mean RPE (F (2, 38) = 0.71, p = 0.500, partial η^2 = 0.04). Ignoring pre-test condition, mean RPE was not significantly different between the groups (F (1, 19) = 0.21, p = 0.653, partial η^2 = 0.01).

5.9 Heart Rate

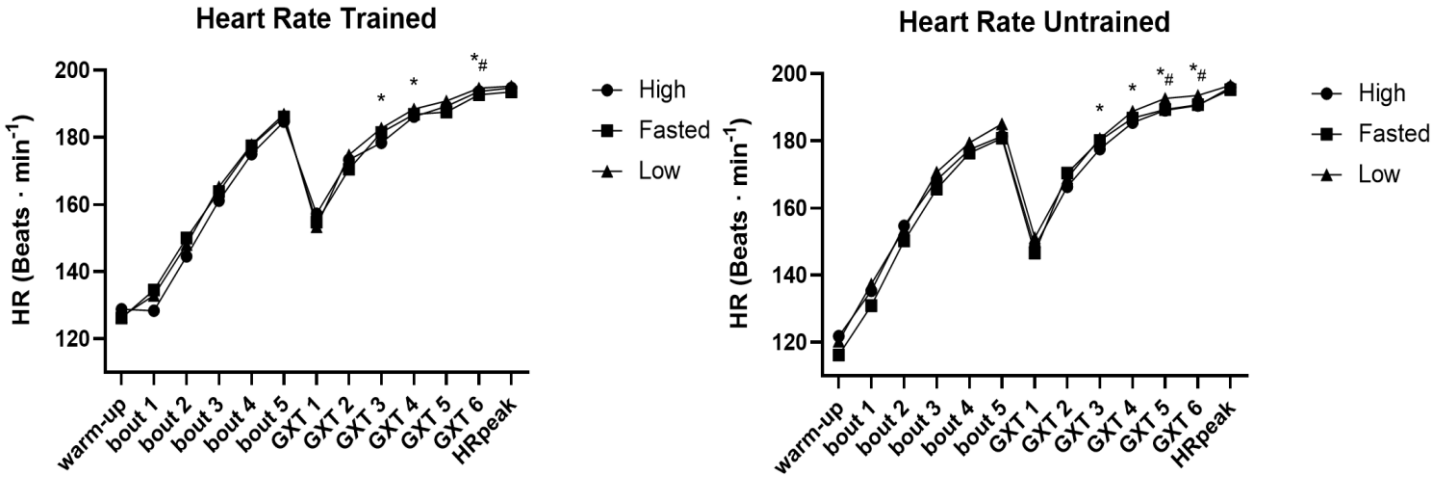

Figure 11 and 12: Mean heart rate (HR) during the final minute of the warm-up, all five exercise bouts, after each minute of the graded exercise test (GXT). peak heart rate (HRpeak) = highest HR measurement for the respective test in the trained and untrained group, respectively. * = significant effect of pre-test condition after a follow-up test with both groups included, *# = significant effect of pre-test condition after a follow-up test for each group separately. High = pre-event meal with high carbohydrate content, low = pre-event meal with low carbohydrate content, fasted = test in fasted state.

The effect of pre-test condition on mean HR was not statistically significant ($F(2, 34) = 1.33$, $p = 0.277$, partial $\eta^2 = 0.07$). Ignoring pre-test condition, mean HR was not significantly different between the groups ($F(1, 17) = 0.04$, $p = 0.846$, partial $\eta^2 = <0.01$).

6 Discussion

The aim of the present study was to investigate the effect of the relative CHO content in a pre-event meal on TTE, VO_{2peak}, LT, and WE, and to compare the response to pre-event CHO intake between endurance-trained and untrained participants. The main finding was that TTE was significantly longer following the high-CHO trial compared to both the fasted trial and the low-CHO trial in both trained individuals and the untrained individuals. There was no significant effect of the pre-test conditions or on VO_{2peak}, LT, OBLA, or WE in either group. Additionally, there was no significant effect of group on TTE or any of the three physiological performance-determining variables. However, greater mobilization of BG with increasing intensity and delayed reliance on anaerobic metabolism and CHO oxidation was found in the trained group compared to the untrained group.

6.1 Endurance capacity

In the trained group, TTE was 8.5% longer following the high-CHO trial compared to the fasted trial and 6.2% longer compared to the low-CHO trial. In the untrained group, TTE was 7.6% longer following the high-CHO trial compared to the fasted trial and 8.1% longer compared to the low-CHO trial (table 2). This is in accordance with our hypothesis and previous findings (8, 9, 29). However, comparisons with other studies are not straight forward, as TTE is often measured during constant- load exercise. Some comparisons for TTE and other variables have been done with studies that utilized constant-load running and others who used incremental exercise on ergometer cycle, and thus, should be interpreted with some care. Additionally, some studies such as Sabapathy et al. (29) have used glycogen depletion protocols by prolonged exercise on the night prior to the tests, followed by a fast until the next morning. This strategy was avoided in the current study due to it being less competition specific and as to not have residual fatigue caused by the depletion protocol affect the interpretation of the results.

Despite the relative short duration of the current study's protocol (~40 minutes of exercise in total), the percentage improvement of TTE is comparable with what other studies have reported using different administration strategies, testing protocols, and when comparing pre-exercise CHO ingestion to the same exercise done in a fasted state. Chryssanthopoulos (8) et al. reported 9% longer TTE measured during treadmill running at 70% VO_{2max} following a high CHO meal (2.5 g · kg^{-1}) given three hours before exercise in 10 trained male runners (VO_{2max}: 63.5 ml · kg^{-1} · min^{-1}). Davison et al. (9) found that a CHO and electrolyte solution given 15 minutes

prior to exercise improved TTE measured during a complex shuttle run test by 8% in 10 moderately trained men (VO_{2max}: 56.0 ml · kg^{-1} · min^{-1}). Lastly, Tokmakidis & Karamanolis (10) reported that 1 g · kg^{-1} of glucose given 15 minutes prior to exercise led to 12.8% longer TTE measured during an incremental running protocol consisting of five minutes at 60% VO_{2max}, 45 minutes at 70% VO_{2max} and 80% VO_{2max} until exhaustion in 10 male and 1 female recreational runners (VO_{2max}: 49.0 ml · kg^{-1} · min^{-1}). In the current study, the improved endurance capacity following the high-CHO trial in both groups is likely explained by the increased provision of additional CHO leading to greater CHO oxidation compared to the low-CHO and fasted trial, as shown by higher RER (figure 7 and 8). The greater CHO oxidation did not appear to be due to greater BG concentration, as it was consistently lowest following the high CHO trial in the trained group (figure 3 and 4). It is more likely that it is explained by greater glycogen stores before onset of exercise, as this was not measured it cannot be confirmed. Based on our findings, TTE measured during a GXT to exhaustion is influenced by relatively acute changes of pre-event CHO availability and the CHO content of a pre-event meal should be controlled as it is likely that TTE's reliability will suffer if it is not, regardless of the participants' training status.

6.2 Peak Oxygen Uptake

There was no effect of the pre-test CHO conditions on VO_{2peak} in either group (table 3). This indicates that VO_{2peak} did not contribute to the improved TTE during the GXT and that the participants reached a similar VO_{2peak} at a faster rate and at a lower velocity following the fasted- and the low-CHO trial compared to the high-CHO trial.

Our findings match those of previous investigations such as Mikulski et al. (27) who also found that the CHO content in a pre-event meal did not have a significant effect on VO_{2max} measured during an incremental ergometer cycle exercise. Additionally, both Yoshida (28) and Sabapathy et al. (29) did not find a significant difference in VO_{2max} when utilizing 3-4 days dietary interventions (28) or a glycogen depletion protocol respectively (29). These findings and ours suggest that the impact of CHO availability on TTE is not sufficient to influence the measurement of VO_{2max} or $_{peak}$ in either trained or untrained individuals as long as the testing protocol is sensible. Thus, when the goal is to measure VO_{2max} or $_{peak}$ during a GXT to exhaustion it does not seem to be necessary to take any specific precautions related to pre-event CHO intake.

6.3 Lactate Threshold and Onset of Blood Lactate Accumulation

There was not a significant effect of the pre-test conditions on LT, OBLA, vLT, or vOBLA in either group (table 4). This is in line with what Rotstein (49) and Ivy et al. (41) reported using more acute substrate manipulation and with what Quirion et al. (95) reported using two days dietary interventions, but in opposition to what Yoshida (28) reported using 3-4 days dietary interventions. Yoshida (28) found that a three-day high CHO diet consisting of 70% CHO, 20% fat and 10% protein led to OBLA occurring at a significantly lower percentage of VO_{2max} (66.3% of VO_{2max}) compared to a three day mixed diet consisting of 50% CHO, 30% fat and 20% protein (72.7% of VO_{2max}) and a four-day low CHO diet consisting of 30% CHO, 50% fat and 20% protein (75.8% of VO_{2max}) when measured during incremental ergometer cycle exercise.

The effect of the pre-event meal on $[La^-]b$ and threshold variables was proposed to be dependent on the alteration of muscle glycogen stores and substrate utilization during exercise. Muscle glycogen was not measured but the timing and dosage of the high CHO meal should have been sufficient to increase the stores. There are two observations that suggest participants' endogenous CHO stores were likely increased following the high-CHO trial: 1) RER was elevated following the high-CHO trial and 2) resting $[La^-]b$ and BG were higher at rest following the high-CHO trial, both 1) and 2) indicative of greater utilization of CHO as fuel. Further support is provided by Chryssanthopoulos et al. (52), who found an 11% increase in muscle glycogen in the m. vastus lateralis using a highly comparable pre-test meal, with a slightly lower CHO dosage ($2.5\ g \cdot kg^{-1}$) given three hours before measurement.

It was speculated that the increase of muscle glycogen stores and CHO oxidation would lead to higher mean $[La^-]b$ following the high-CHO meal compared to the other two trials, and as a result that the pre-test conditions would have an effect on OBLA. This was not the case and there was no effect of the pre-test conditions on mean $[La^-]b$ (figure 5 and 6), and in turn, there was no effect on OBLA (table 4). This was unexpected as other studies with similar administration strategies have reported higher $[La^-]b$ during exercise following a pre-event CHO and when compared to the same exercise fasted (8, 38, 41) but not implausible as there are studies who also have reported no effect of pre-event CHO on $[La^-]b$ (27, 96, 97). As shown by RER (figure 7 and 8), CHO oxidation was greater following the high-CHO trial compared to both the fasted trial and the low-CHO trial, which is in line with previous findings (7, 27, 28). However, it can be speculated that the pre-event meal did not sufficiently alter muscle

glycogen and metabolism for there to be a measurable effect on LT and OBLA, which could explain the discrepancy between our findings and that of Yoshida (28) who found an effect on OBLA using a three-day dietary intervention and what was indicated in Busse et al. (40). Another possible explanation for the lack of effect could be that glycogen stores only affect OBLA or LT when elevated stores are compared with depleted stores, not when elevated stores are compared with normal or slightly reduced stores. The participants' self-reported dietary CHO intakes were moderate to high, see appendix III for specifics. This, combined with the abstinence from exercise 24-48 hours prior to the tests, likely led to participants' muscle glycogen stores being close to saturated before all three trials. The lack of a glycogen depleted state in any of the three trials could further explain the discrepancy between our findings and that of Yoshida (28) and Busse et al (40). Our findings further demonstrate that LT and OBLA are not influenced by acute (< 3.5 hours) changes in CHO availability (41, 49), and is not likely to vary in an individual following small and acute changes of muscle glycogen stores via changes in the pre-event meal.

6.4 Work Economy

There was no significant effect of the pre-test conditions on WE measured at ~69-73% of VO_{2max} in the trained group and ~66-70% of VO_{2max} in the untrained group. Changes in substrate availability before exercise alters substrate utilization during exercise (37, 63, 64). In the current study, the high CHO meal shifted participants' metabolism toward a greater reliance on CHO oxidation compared to the two other trials. However, the difference in substrate utilization induced by the pre-test conditions did not result in a significant effect on WE in either group. In line with our findings, Brisswalter et al. (65) did also not find a significant effect pre-exercise CHO ingestion on WE measured before and after 80-minute running at ~80% VO_{2max} in well-trained triathletes. Contrary, Dumke et al. (67) found that CHO ingestion immediately before and during exercise led to significantly greater cycling efficiency after 40 minutes cycling at ~75% VO_{2max} compared to placebo. The discrepancy between the findings of Dumke et al. (67) and that of the current study can largely be explained by the different exercise modalities used and that the testing protocols used by Dumke et al. (67) was longer in duration and more continuous in nature compared to the shorter, bout-based testing protocol used in the current study. Another difference worth mentioning is the administration strategy used, most specifically the addition of CHO ingestion during exercise by Dumke et al. (67), which was not included in the current study. Based on our findings it does not seem that

alteration of the CHO content of pre-event meals significantly affects WE measured during treadmill running.

6.5 Effect of Training Status

There were no significant differences in the responses to pre-event CHO intake between the trained and untrained participants related to TTE, VO_{2peak}, LT, OBLA, or WE. Therefore, based on our findings it does not seem to be necessary to take different precautions related to pre-event CHO intake based on training status when these abovementioned variables are measured. However, there were differences between the two groups in the measurements of RER, BG, and [La^-]b but not for all [La^-]-related threshold variables. Both vLT and vOBLA was significantly higher in the trained group compared to the untrained, and while both LT and OBLA was higher in the trained group compared to the untrained, this difference did not reach statistical significance. This is in line with what Helgerud and colleagues have previously reported when utilizing 8-week long training interventions (69, 98).

RER and [La^-]b were significantly higher in the first three exercise bouts in the untrained group compared to the trained (figure 5 to 8), indicative of an earlier reliance on CHO oxidation and anaerobic metabolism (27). This can be explained by the superior cardiorespiratory fitness in trained participants augmenting fat metabolism during both rest and exercise, thus, delaying the reliance on anaerobic glycolysis (99, 100). Additionally, trained individuals have been shown to have greater mitochondrial density which facilitates greater removal of La^- during low- and moderate-intensity exercise compared to the untrained individuals (30).

In the trained group of the current study there was a clear pattern of increasing BG concentration with increasing intensities, compared to a much flatter line in the untrained group (figure 3 and 4). This is likely explained by the fact that the trained participants were adapted to training with high CHO availability, and thus, through training had obtained a greater ability to mobilize glucose when facing increasing intensities compared to the untrained participants. The greater mobilization of BG in the trained participants probably derives from greater gluconeogenesis and hepatic glycogenolysis compared to the untrained participants (101). Although mean RER and RER for the first three bouts was significantly higher in the untrained group compared to the trained, both groups had an overall similar pattern of increasing RER with increasing intensities. Thus, it seems to be the case that the trained participants in the current study had a better ability to cover the increasing metabolic requirements of higher intensity by better

capability for mobilization of BG as an energy source while the untrained participants most likely had to rely on muscle glycogen to a greater extent, which is in line with what has been previously reported by Jansson and Kajser (16). The findings surrounding differences in BG mobilization expand upon what Suzuki et al. (102) reported with a smaller sample of one well trained female triathlete (VO_{2max}: 59.6 ml · kg^{-1} · min^{-1}) and one less aerobically trained female tennis player (VO_{2max}: 42.1 ml · kg^{-1} · min^{-1}). Based on the findings of the present study BG mobilization appears to be trainable and the differences related to training status are substantial and should be taken into considerations whenever this measurement is used.

6.6 Practical Implications of Findings

Based on the findings of the current study, pre-event CHO status does not seem to influence VO_{2peak}, LT, and WE in trained or untrained participants, thus, it is not necessary to change testing practice related to these three physiological variables. However, if TTE is a key variable, pre-test CHO availability should be controlled or adjusted for by standardizing pre-event meal and possibly also the pre-event diet. For both athletes and untrained recreational runners, the already established recommendation of having a pre-event meal with a higher CHO content (1-4 g · kg^{-1}) 1-4 hour before exercise still applies (50).

6.7 Strengths and Limitations

One strength of the current study is the large difference in aerobic capacity between the trained and the untrained group (table 1). The crossover design allows participants to act as their own controls and in turn, eliminate the effect of between-subject variability (103). However, the typical weakness of the crossover design was apparent in the current study as well. Beside the 21 participants that completed all three trials and were included in the analysis, 14 participants dropped out after completing one or two trials, making within-subject comparison impossible for these cases.

The present study did not include muscular biopsy or blood insulin measurements. The inclusion of these could have further explained the effect of pre-event CHO on TTE and the lack of effect of CHO on the three physiological performance- determining variables.

The use of real-life, relatively realistic meals in the current study increases the ecological validity of the findings. However, generalizability is reduced by the CHO dosage in the high CHO meal being equivalent to 90-100 % of normal daily CHO intake reported in well-trained

distance runners (104) and 95.1% of the self-reported CHO intake in the current study's trained participants. It is however worth mentioning that pre-event meals with 2.5 g · kg^{-1} CHO have been reported to not cause any discomfort during prolonged running at 70% VO_{2max} (13) and the dosage used in the current study is also in line with the general recommendations of 1-4 g · kg^{-1} CHO in the pre-event meal (50). The different food items included in the two pre-event meals also have other components besides their macronutrient compositions which could have affected the results. Additionally, the use of real-life meals removed the option of blinding. Based on the trained participants' self-reported dietary habits related to exercise, 10 out of 11 trained participants were adapted to having a pre-training meal consisting of CHO rich foods. This combined with the cultural aspect surrounding CHO in endurance sports might have resulted in an unconscious or conscious psychological effect related to the high CHO meal. This could have influenced both the TTE and OC measurements, as the participants might both have felt the best and expected themselves to perform the best on the test following a high CHO meal. This is supported by RPE, which was consistently the lowest at a given speed following the high-CHO trial (figure 11 and 12). Contrary to this, the same beneficial effect of CHO on TTE was found in the untrained group, where no dietary habits related to exercise were reported.

The method used to calculate the speed of the submaximal exercise bouts seemed to be affected by trained participants' WE and thus, it underestimated the velocity needed to elicit targeted intensity of 50-90% VO_{2max} compared to the untrained group where the calculation method brought them closer to the targeted intensity (see appendix III). This could have been discovered and been corrected with an additional familiarisation session where the five submaximal 5-minute exercise bouts were included.

In the current study, BM and BMI were significantly higher in the untrained group compared to the trained. This could have had an influence on metabolism and subsequently on the effect of the pre-event meal independent of the effect of training status (105, 106). This limitation was minimized by using appropriate allometric scaling on key variables, but an effect of BM and BMI on metabolism cannot be neglected.

7 Future Research

Future research should aim to investigate the effect of training status more isolated by having more similar BM and BMI in the two groups. There might also be relevant differences between male and female individuals, a similar study should also be done with female participants. Lastly, future research should include muscle biopsy and additional haematological measurements to better explain mechanisms of actions.

8 Conclusions

In conclusion, the pre-event meal with a high relative CHO content led to longer TTE measured during the GXT to exhaustion compared to fasted state and the pre-event meal with a low relative CHO content. This finding applies to both trained and untrained participants. In contrast to this performance-indicator, pre-event CHO intake did not have a significant effect on physiological performance-determining variables such as VO_{2peak}, LT, OBLA, or WE in trained nor untrained participants. However, untrained participants demonstrated an earlier reliance on anaerobic metabolism and inferior ability to mobilize BG for energy production with increasing exercise intensity and increasing CHO-demand compared to the trained participants.

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