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Original Article

Chronic carbohydrate restriction does not reduce endurance capacity in men and women

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Abstract: This study was designed to test whether adaptation to a CHO-restricted diet affects physical capacity during prolonged exercise. It is hypothesised that chronically reducing an individual's dietary carbohydrate intake during training will increase their ultra-endurance exercise capacity compared to a chronic high carbohydrate diet. Thirteen highly trained endurance athletes (eight males, VO_{2max} 66.0 ± 9.5 ml/kg/min, five females VO_{2max} 50.6 ± 8.4 ml/kg/min) consumed a moderate (>5 g CHO/kg/day) and a low (<2 g CHO/kg/day) carbohydrate training diet for four weeks in a randomized cross-over design. Performance was measured after a 24 h high carbohydrate "loading" regime, through a self-paced time trial to complete a fixed workload equivalent to five hours at a workload calculated to elicit 55% VO_{2max}. Although time to completion was not significantly different between diets, the average absolute (watts) and relative (W/kg) power outputs were significantly better on the carbohydrate restricted diet ($p = 0.03$ and 0.02 respectively). Both sexes responded similarly in terms of performance whilst only women significantly improved body composition when carbohydrate was restricted ($p = 0.02$). Results from this study highlight that when carbohydrate is restricted during training, trained endurance athletes do not suffer a reduction in ultra-endurance performance.

Keywords: beta-Oxidation; Low carbohydrate high fat; metabolic flexibility; ultra-endurance; carbohydrate loading.

1. Introduction

Of the many physiological, biochemical, and psychological changes that occur when an individual begins an endurance training program, some fundamental adaptations are considered most important in improving endurance exercise performance.

At the skeletal muscle level these include, but are not limited to, increases in capillarisation and oxygen (O_2) (Andersen, P., & Henriksson, J. 1977) transport, mitochondrial (volume) density (John O. Holloszy, 1967) and associated increased capacities for lipid oxidation (Molé, Oscai, & Holloszy, 1971) and other energy conversion pathways (Baldwin, Klinkerfuss, Terjung, Mole, & Holloszy, 1972), muscle fibre-type shifting (type IIx → type I) (Gollnick, Armstrong, Saubert, Piehl, & Saltin, 1972), and adrenergic stimulation (Butler, J., O'Brien, M., O'Malley, K., & Kelly, J. G, 1982)

Endurance training improves the capacity for fatty acid utilisation, enabling the continuation of work if muscle glycogen levels and circulating glucose levels decline.

The compensation for minimised glucose availability through increased fatty acid utilisation can be referred to as an increase in 'metabolic flexibility'. Kelly et al. 1999 are credited for coining, this term, describing how leg muscle of lean, healthy individuals can aptly switch from primarily fat oxidation in the fasted state to predominantly glucose oxidation with endogenous insulin stimulation. Since 'metabolic flexibility' fundamentally describes the capacity of an individual to alter fuel selection to best suit substrate availability, we can also apply this term to the physiological conditions where changes in fuel availability occur during both exercise and macronutrient-controlled diets.

Training-induced adaptations which increase the muscles' capacity for fat oxidation during exercise can be further enhanced by deliberately exercising with low CHO availability. Acute strategies such as exercising with low muscle glycogen content (Zderic, Davidson, Schenk, Byerley, & Coyle, 2004) or training after an overnight fast (Stannard et al., 2010) have been shown to upregulate the signalling pathways that promote mitochondrial biogenesis or increases in the abundance and activity of enzymes involved in fat oxidation (Bartlett, J. D., Hawley, J. A., & Morton, J. P., 2015). Chronic exposure to high fat, low CHO diets while undertaking endurance training leads to more profound retooling of the muscle to enhance fatty acid availability, transport/uptake, and oxidation (Skovbro, Boushel, Hansen, Helge, & Dela, 2011). Such changes occur in as little as five days of such exercise-nutrient manipulation and persist even when strategies to increase CHO availability are undertaken such as restoration of muscle glycogen stores, and achievement of high exogenous CHO availability via pre- and during- exercise CHO intake (Burke et al., 2000).

While these adaptations appear robust, the transfer to improvements in exercise capacity or sports performance has been less clear-cut mainly because of non-standard methodology and the complex nature of the performance-limiting factors (Burke et al., 2000). For example, limitations of the capacity for CHO oxidation appear to be a reciprocal outcome of fat adaptation protocols (Helge, 2000), and the latter thus impairs performance at higher intensities (Burke et al., 2017). This uncertainty suggests that fat adaptation strategies, with or without the acute restoration of CHO availability, are less suited to performance goals of endurance athletes who need to undertake the whole, or critical parts, of their event in such high-intensity domains. However, it is possible that an increased capacity for fat oxidation may be valuable for ultra-endurance, and this has been shown in the few low CHO studies completing exercise tests of three or more hours, citing equal or better performance when compared to a high CHO diet (Carey et al., 2001; Lize Havemann, Goedecke, Noakes, & Lambert, 2007; L. Havemann et al., 2006; Lambert et al., 2001; Phinney, Bistrian, Evans, Gervino, & Blackburn, 1983; Rowlands & Hopkins, 2002, Shaw, et al., 2019).

There is a possibility that the increase in fat oxidation that occurs due to the training-induced adaptions previously outlined may occur to a lesser extent in females than males (Stannard, Buckley, Edge, & Thompson, 2010). Irrespective of diet females have demonstrated greater fatty acid utilisation and less CHO and protein metabolism than equally trained and nourished males, at submaximal intensities (Horton, Pagliassotti, Hobbs, & Hill, 1998; Knechtle et al., 2004; Tarnopolsky, MacDougall, Atkinson, Tarnopolsky, & Sutton, 1990). Therefore, the ability of females to increase their fat oxidation through a dietary stimulus may be reduced compared to males, indicating a potentially reduced metabolic flexibility.

The current study was designed to fill important gaps in the literature by providing a best-case scenario for testing the hypothesisthat chronic low CHO intake with CHO restoration improves exercise performance in men and women.

2. Materials and Methods

Experimental Design.

Thirteen highly trained endurance athletes consumed a moderate (>5 g CHO/kg/day) and a low (<2 g CHO/kg/day) carbohydrate training diet for four weeks in a randomized cross-over design with a five-week washout period. Performance was measured after a 24 h high carbohydrate "loading" regime, through a self-paced time trial to complete a fixed workload equivalent to five hours at a workload calculated to elicit 55% VO2max.

Participants. —Thirteen recreationally competitive endurance cyclists and triathletes, who trained ten to fifteen hours per week and had a minimum of five years racing experience (eight males, VO_{2max}: 66.0 ± 9.5 ml.kg.$^{-1}$min^{-1}; five females, VO_{2max}: 50.6 ± 8.4 ml/kg/min) participated in this study. The participants were recruited from local cycling and triathlon clubs. The participants were nationally competitive, and their characteristics are outlined in Table 1. All participants gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Massey University (14/09) in April 2014.

Table 1. Participant Overview

Sex	Body Mass (Kg)	VO_{2max} (ml/min/kg)	Maximum heart rate (bpm)	Body fat (%)	Age (yrs)
Female	63.9 ± 10.5	50.6 ± 7.7	180 ±6.2	21.7 ± 5.5	35 ± 10
Male	79.9 ± 13.7	66.0 ± 9.2	188 ± 6.8	14.2 ± 3.1	33 ± 9.4

Dietary Control —The composition of daily food intake was prescribed and monitored using "MyFitnessPal®" online and mobile software (MyFitnessPal®, Inc. 525 Brannan Street, San Francisco). MyFitnessPal® was selected due to the ease of use of the platform compared to more traditional, and potentially more reliable, diet diaries. Because MyFitnessPal® operates via a user-generated database, there is potential for the nutrient profile of foods to be recorded inaccurately. Thus, to avoid any misreporting, the researcher personally checked the participants' diaries every two-three day to ensure foods were being recorded accurately.

Both diets lasted four weeks, and protein intake was fixed at 15–20% of total energy intake. There were no caloric or other restrictions, to allow the diets to be as natural as possible. During the moderate CHO diet, a minimum of 50% of total energy came from CHO, and total CHO in grams was at least 5 g/kg/day; this was based off the common endurance athlete's diet (van Erp-Baart, Saris, Binkhorst, Vos, & Elvers, 1989). During the low CHO diet, CHO intake was restricted to <2 g/kg/day; this value selected because it has previously been shown to achieve a significant lowering of the participant's habitual CHO intake (J. W. Helge, 2002). The diet was not intended to induce ketosis.

At the beginning of both interventions, a food parcel and information pack were provided for the participants to help educate them in regard to what foods were high and/or low in CHO. Participants were under strict instructions to measure food volumes were possible.

The researcher had personal contact with all participants a minimum of three times per week and was available via phone and email to answer any queries that the participants had regarding the diet. In addition, the researcher was able to keep track of the participants' diets by regularly checking their data on MyFitnessPal®.

A minimum five-week washout period was implemented between diets, and diet recording started ten days prior to the start of each intervention to ensure habitual diets were similar.

VO_{2max} and submaximal exercise tests —Submaximal and VO_{2max} cycle exercise tests were completed before the beginning of the study phase. Participants cycled at four submaximal seven-minute workloads (men: 100, 150, 200, 250 W, women: 100, 125, 150 175 W), on an electronically braked cycle ergometer (Excalibur Sport, Lode BV, The Netherlands). During the last minute of each workload, ventilatory gases were collected into Douglas bags via a breathing apparatus. These were analysed for oxygen (O_2) and carbon dioxide (CO_2) concentrations, using a zirconia cell O_2 analyser (AEI Technologies Inc, Pittsburgh, USA), and an infra-red based CO_2 analyser, respectively (AEI Technologies Inc, Pittsburgh, USA). Volume measurement was made with a Harvard dry gas meter (Harvard, UK). Calculations of the rate of oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were made at Standard Temperature Pressure Dry (STPD).

VO_{2max} —Five minutes after the final submaximal workload, VO_{2max} was measured using a 'ramp protocol' (men: 25 W/min, starting at 150 W; women: 20 W/min starting at 100W) until volitional fatigue. Respiratory gases were collected via repeated (~40 seconds) Douglas bags after the respiratory compensation point was identified. Douglas bags were analysed as above. Attainment of VO_{2max} was confirmed with an RER ≥1.1.

A linear relationship was drawn between steady-state O_2 consumption and workload as previously described (Figure 1) (Conway, Orr, & Stannard, 2003). After VO_{2max} was identified, a percentage of this figure was used in a linear equation (Figure 1) to calculate the power output (W) predicted to produce the desired % VO_{2max}.

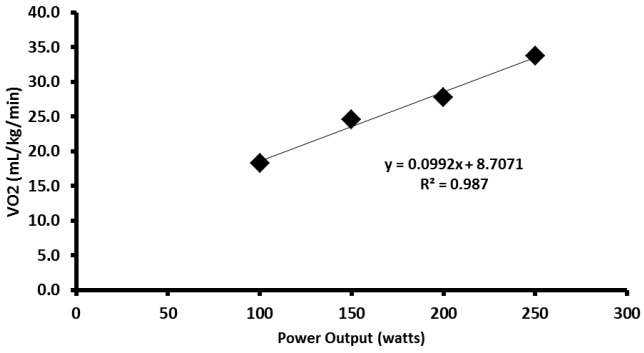

Figure 1. Linear relationship was drawn between steady-state O2 consumption and workload

Figure 1 Individual example of a linear relationship between relative VO_2 (mL/kg/min) and submaximal power output. Black diamonds represent data collection points.

Resting Metabolic Assessment — Resting metabolism was assessed by collecting respiratory gases, using the Douglas bag method, as outlined in the previous two sections. Prior to measurement, participants were seated for five minutes to allow resting homeostasis to be achieved before respiratory gases were collected (for a further five minutes).

Resting metabolic measurements were taken at two time points—on arrival to the laboratory before the beginning of any testing procedure or blood collection (to avoid stress-related influences), and again, post-exercise, at least ten minutes after the completion of physical testing and blood collection. Calculations of fat and CHO oxidation rates were determined using the Weir equation (Weir, 1949). Although this may not have been completely representative of resting metabolism, it did allow for an insight into the metabolic situation at rest, post-exercise.

Body Composition —Body composition was evaluated using a portable Bioelectrical Impedance Analyser (BIA, InBody 230, InBody®, USA). The InBody® 230 BIA was selected due to its practicality and low cost of implementation. Research reviewed before selection of the BIA suggested that its accuracy strongly correlated (r = 0.94–0.99) with that of traditionally-used, dual-energy X-ray absorptiometry (DXA) (Karelis, Chamberland, Aubertin-Leheudre, & Duval, 2013).

Body composition, body fat mass, and total body mass were measured at the following time points: after an overnight fast, at the

beginning, half-way, and end point of each four-week intervention. Participants were instructed to arrive hydrated and to wear the same cycling shorts and top, to ensure consistency between measurement points. However, beyond these instructions, no further protocols were put in place to ensure hydration status was similar between or within participants; thus, it is acknowledged that the participants' hydration statuses may have differed between measurement points.

Standardised Training —Training was standardised during dietary interventions to minimize variation in physical performance. Training sessions were prescribed so that each participant completed the same total workload per session. Training load was based on the participants' preliminary VO_{2max} data and consisted of four sessions per week—three interval sessions and one long aerobic session. The interval sessions were completed on participants own bikes using electronically-braked cycle ergometers (Wahoo kickr ®, Wahoo Fitness LLC, Atlanta, GA, USA) with resistance control by remote computer software (PerfPRO Studio, Vision Quest Virtual, LLC, USA). Heart rate (HR) was used to monitor intensity for the long aerobic ride, which was done outdoors. There was a slight variation in supplemental training for each athlete (e.g., running) which was replicated in the second intervention, to avoid within-subject variation of training loads.

Mood Questionnaire —A profile of mood state (POMS) test was used to assess the mood of each participant before and throughout each intervention. Each test gave a total mood disturbance score (TMD) which could be used for within and between subject comparisons. The test was completed weekly by the participants so that their changes in their TMD within each intervention could be examined.

Nutritional Protocol for Exercise Test — In each intervention, all food consumed were standardised and strictly controlled from the completion of the fasted measures on the previous day to the commencement of the TT (Prior day: 5g/kg/day CHO, breakfast: 3g/kg CHO). Specifically, ready-made meals were provided for lunch and dinner following the submaximal test, and for breakfast on the morning of the TT, as well as snacks. The participants were required to eat all foods provided to them and were told not to eat any other foods besides this. On arrival into the laboratory for the TT, participants were asked whether they had complied with these requirements; however, no further checks were made to ensure that these dietary requirements had been followed. The food provided was selected to give the participants a CHO "load" to ensure a "like vs like" comparison for TT performance on each diet. It was assumed that this would mean participants started each TT with a similar glycogen status and thus conclusions drawn around performance could be related to the dietary intervention rather than pre-exercise glycogen status.

To ensure euglycemia was maintained during the TT, participants ingested 40 grams of CHO/hr during exercise via a commercially available sports drink (Gatorade, Frucor Beverages Ltd, New Zealand) for the first two hours. The quantity of 40g/hr CHO was selected to avoid gastrointestinal issues, which have been reported for CHO intakes ≥60 g /hr. After two hours, solid food was introduced in the form of a small chocolate bar (Snickers, Mars Inc.) which accounted for 10g of the 40g CHO/hr. Snickers were selected for practicality and the unanimous agreement of enjoyment. Once the solid food was introduced, the sports drink volume was decreased to maintain the glucose:H_2O concentration ratio. During the baseline TT, water was made available ad libitum, and the amount drunk by each participant was recorded and replicated for the remaining two TTs. In the last ten minutes of each hour during the TT, participants were warned that they only had a short period to consume the remaining sports drink and water that they had left for that hour. At the beginning of each hour, new

bottles of sports drink and water were provided.

Self-Paced Total Workload Time Trial — Before commencing the cycling TT, baseline respiratory gases, a venous blood sample (venepuncture) and HR (Polar RS800 HRM, Finland) measures were collected (approximately ten minutes before exercise started), following five minutes of rest in a seated position. The same measures were also taken ten minutes after the completion of the trial.

Following the pre-exercise resting measures, the participants mounted the electronically-braked cycle ergometer (Excalibur Sport, Lode BV, The Netherlands), which had previously been set up as closely as possible to the measurements of their own bike (this was replicated in each trial), and pedalled easily for ten minutes, to allow their legs to warm up. Subsequently, a linear factor was applied to the ergometer, which was designed so that a cadence of 90 rpm corresponded to a work rate which was predicted to elicit 55% of the participants' previously measured VO_{2max}. Thus, if the participant pedalled faster than 90 rpm, their power output and work rate would be greater than this, with the reverse occurring if they pedalled slower than 90rpm. This linear factor was chosen based on previous exercise tests from our laboratory, which have been conducted on athletes of similar fitness levels to the current study's participants. The total work requirement for the TT was based on an average power of 55% VO_{2max} for 5 hr expressed in kilojoules (1W = 3.6kJ). Participants were informed of their progress following the completion of each 5% segment of the TT; however, no other progress or performance feedback information was made available to the participants.

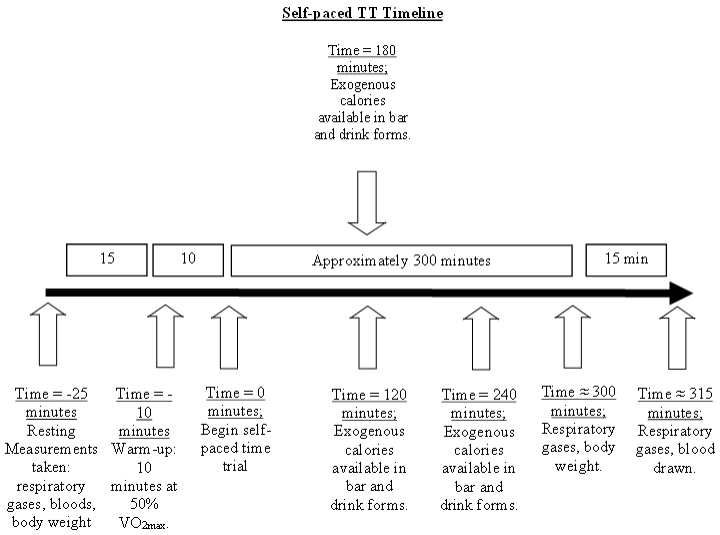

Figure 2. Timeline of events during the self paced time trial.

Statistical Analysis —All data were analysed using SPSS Statistics for Windows, Version 23.0. (IBM Corp, NY, USA). A one-way repeated measure analysis of variance (ANOVA) identified the differences in week four performance, measured as time to complete a fixed workload and average absolute (Watts) and relative power outputs (W/kg). A two-way repeated measures ANOVA tested diet x time (baseline, week one–four) interactions for changes in body mass, BF% and resting RER. Sex was included as a between-subjects factor. Mauchly's test of sphericity was used to test the sphericity of the ANOVA output, and unless otherwise specified, sphericity was assumed. Least squares difference post hoc tests were run to determine the locations of any significant ANOVA findings. To test the effect of order, the data was analysed, as trial one and trial two, with no reference of diet. A paired t-test was used to investigate the effect of order on the performance TT. Repeated measures two-way ANOVA tested the potential effect of order on changes in body mass and BF% within and between each intervention. Significance was set at $p \leq 0.05$. Confidence intervals were set at 95%. The effect sizes (ES) of significant interactions were calculated using Hedges's g (0.2 small; 0.5 medium; 0.8 large)

3. Results

There was no main effect of diet ($p = 0.06$, ES = 0.25) or sex on time to complete the time trial ($p = 0.31$, ES = 0.43) (Figure 5).

There was no interaction between the pacing profiles, represented as time to complete 5% segments, and condition (p = 0.99) (Figure 3).

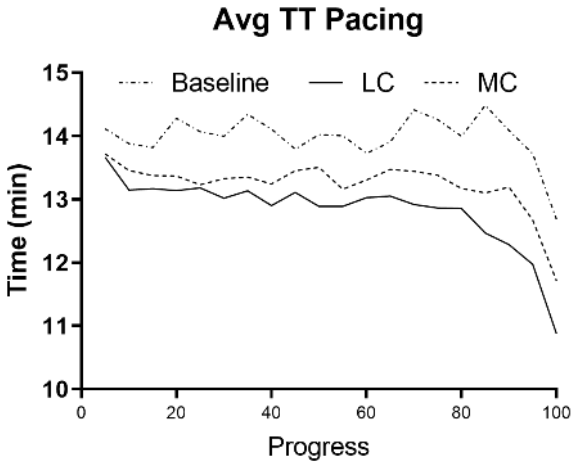

Figure 3 Pacing profile for average time to complete at baseline, post low carbohydrate (LC) and post moderate carbohydrate (MC)

Absolute Power Output

There was a positive main effect of low carbohydrate diet (p = 0.030) and negative main effect of sex (p = 0.030) on average power output produced during the time trial, and a near significant interaction between the two (p = 0.050) such that females produced more absolute power on a low CHO diet. Average power output was higher following the low CHO diet than at baseline and higher following the moderate CHO diet than at baseline (mean difference = 17.38 W, CI: 2.18 to 32.58: P = 0.03, ES = 0.34 & mean difference = 6.03 W, CI: -0.15 to 12.20: p = 0.06, ES = 0.25).

Relative Power Output

There was a main effect of diet (p = 0.023) on relative power output (W/Kg) such that a low CHO diet resulted in a higher relative power output, but there were no main effect or interactions of sex (p = 0.087). The participants held significantly more watts relative to body mass on the low CHO diet compared to baseline (mean difference = 0.261 W/kg, 95% CI: 0.064 to 0.458: p = 0.01, ES = 0.49) and compared to the moderate CHO diet (mean difference = 0.137 W/kg, CI: 0.047 to 0.227: p = 0.07, ES = 0.17) (Figure 6 2).

The order effect tested for any difference in means between trial one and two for time to complete the TT. No significant difference was detected (mean difference = 0.042 h, 95% CI: -0.172 to 0.257: p = 0.68, ES = 0.01).

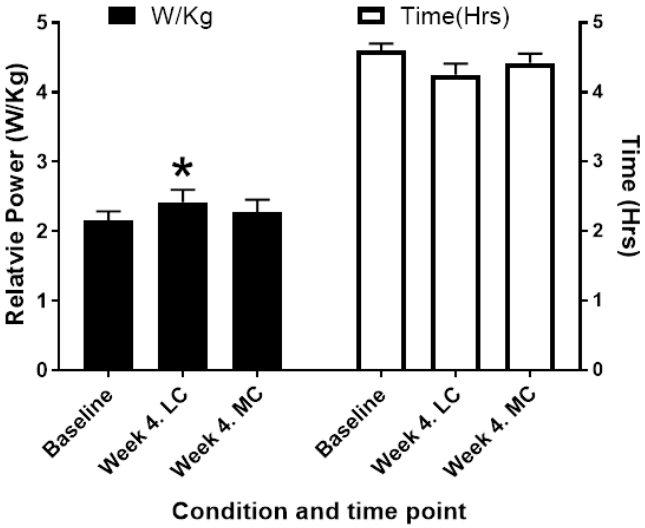

*Figure 4. Overall performance for baseline and week four self-paced cycle time trials, measured as watts per kg (black) and time to completion (white). * Denotes significant (p ≤ 0.05) difference between diets*.

Body composition

There was a significant main effect of diet on body mass (p = 0.01) but not sex (p = 0.09) such that a low CHO diet reduced participants body mass. Both time and the interaction of time and diet did not affect body mass (p = 0.14 and p = 0.11, respectively), nor was there any interaction between sex and the independent variables (diet, p = 0.49; and time, p = 0.45).

There were significant main effects of both independent variables on BF% (diet p = 0.05, time p = 0.04) such that a low CHO diet and the duration of intervention were associated with lower BF%. However, there was no interaction (p = 0.16) between the two. Sex significantly interacted positively with time (p = 0.01) and the diet-time interaction (p = 0.03) but not diet (p = 0.99). Post-hoc inspection showed that when compared to

the start of the respective intervention, women lost BF% (mean difference = -0.82 %, CI, -1.49 to -0.15, p = 0.02, ES = 0.06) on the low CHO diet and trended toward gaining BF% (mean difference = 1.34 %, CI, -0.25 to 2.93, p = 0.09, ES = 0.01) on the moderate CHO diet, whereas men lost BF% on both low CHO (mean difference = -1.08 %, CI, -1.60 to -0.55, p = 0.001, ES = 0.18) and moderate CHO diets (mean difference = -1.64 %, CI, -2.90, -0.38, p = 0.015, ES = 0.26) (Figure 4).

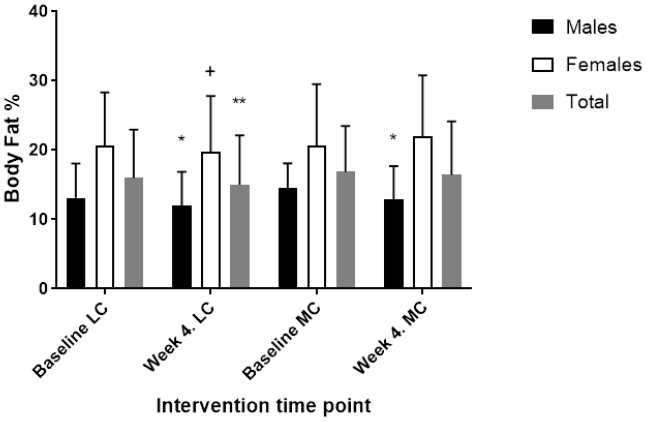

*Figure 5. Total mean and sex-specific percentage body fatness (BF%) measured before and after each four-week dietary intervention. * denotes significant (p ≤ 0.05) difference between baseline and week four measures across both interventions in males. ** denotes significantly lower BF% in week four of the low CHO diet compared to all other time points. + denotes a significant difference between baseline and week four of the low CHO diet in BF% for females.*

Resting metabolism

The results of the two-way repeated measures ANOVA including sex as a dichotomous variable are displayed in Figure 5 and show main effects of diet (p = 0.001) and time (p = 0.001) as well as a diet-time interaction (p = 0.04) on fasted and CHO-loaded resting RERs such that RER improve over time on a low CHO diet. Sex had no effect on either of the independent variables or their interaction. The post hoc analysis showed that fasted RER was significantly lower during the low CHO diet than both baseline (mean difference = - 0.108, CI, -0.160 to -0.056, p = 0.001, ES = 1.17) and the moderate CHO diet (mean difference = -0.072, CI, -0.105 to -0.038, p = 0.001, ES = 1.02). CHO loaded RER was higher at baseline than both fasted low CHO and moderate CHO diets (mean difference = 0.076, CI, .017 to 0.135, p = 0.02, ES = 0.62 and mean difference = 0.064, CI, .019 to .109, p = 0.01, ES = 0.68 respectively). There was a near significant difference between CHO-loaded low CHO and moderate CHO (p = 0.07).

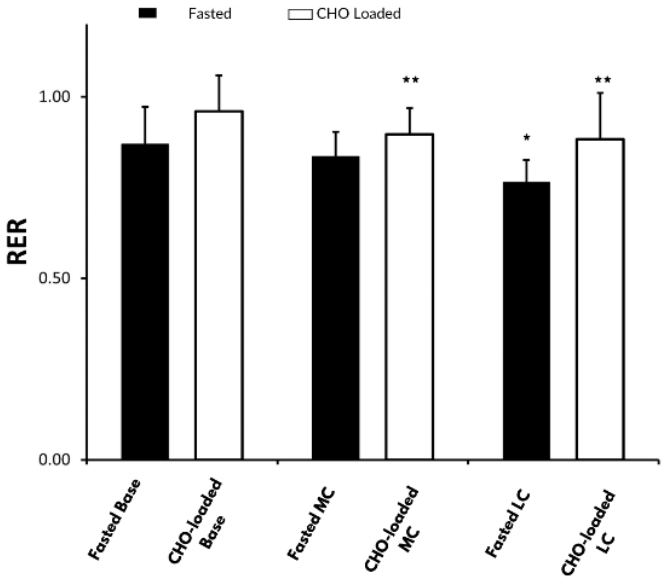

*Figure 6. Resting RER values in fasted (before 2 hr fixed intensity ride) and CHO loaded (before TT) states at baseline and weeks four of each diet. * denotes significant (p ≤ 0.05) difference between fasted low CHO and all other measures. ** denotes significant (p ≤ 0.05) difference between CHO-loaded RER and fasting RER.*

When testing for order, there were no significant effects of trial or time and no interaction between trial and time (p = 0.09) on body mass. Results were similar for BF%, with no effect of trial (p = 0.73), a significant effect of time (p =0.04), and no interaction between trial and time (p = 0.68).

Diet

There were main effects of diet (p = 0.01) and sex (p = 0.002) on total caloric intake. Post hoc comparisons showed that participants consumed more calories on the moderate CHO diet compared to their habitual diet (mean difference = 438.28 kcal, CI, 160.47 to 716.09, p = 0.006, ES = 64) and the low CHO diet (mean difference = 269.54 kcal, CI, -10.31 to 549.38, p = 0.057, ES = 0.23) and as expected, women consumed less calories than men (mean difference = -937.06 kcal, CI, -1429.36 to -444.36, p = 0.002, ES =1.92) (Table 2).

Table 2. Average daily macronutrient and caloric intake.

		Female			Male			Total		
		Mean (Kcal)	Std	%	Mean (Kcal)	Std	%	Mean (Kcal)	Std	%
Mod - CHO	CHO	1490	±320	66%	2013	±288	62%	1775	±396	62%
	Fat	432	±81	19%	790	±185	24%	627	±234	24%
	PRO	345	±83	15%	435	±124	14%	394	±112	14%
	Total	2267	±449		3237	±405		2796	±648	
Low - CHO	CHO	360	±39	19%	566	±75	19%	472	±123	19%
	Fat	1211	±247	62%	1889	±486	62%	1581	±517	62%
	PRO	382	±144	19%	558	±119	19%	478	±154	19%
	Total	1952	±353		3012	±595		2530	±730	
Habitual	CHO	857	±405	43%	1266	±225	48%	1080	±369	46%
	Fat	766	±230	41%	1027	±521	37%	908	±419	38%
	PRO	301	±58	16%	410	±138	15%	360	±119	15%
	Total	1923	±297		2704	±427		2349	±541	

Similarly, there were main effects of diet and sex on caloric intakes from CHO (p = 0.001 and 0.006) and fat (p = 0.001 and p = 0.02). CHO caloric intakes were lower during the low CHO diet relative to the participants' habitual diets (mean difference = 598.92 kcal, CI, 383.57, 814.27, p = 001, ES =2.12) and higher on the moderate CHO diet compared to habitual (mean difference = -689.45 kcal, CI, -939.47, -439.42, p = 0.001, ES =1.74). Fat caloric intakes were lower on the moderate CHO diet (mean difference = -285.55 kcal, CI, -539.69, -31.41, p = 0.032, ES = 0.84) and higher on the low CHO diet than the habitual diets (mean difference = 953.24 kcal, CI, 365.29, 941.18, p = 0.001, ES = 1.39). There was a strong tendency for diet and sex to affect protein intake (p = 0.06).

Total Mood Disturbance

Diet had no significant effect on total mood disturbance (TMD) (p = 0.20), whereas time showed a nearly significant effect (p = 0.07). The interaction between diet and time was significant (p = 0.03) and there was a trend towards significance (p = 0.09) for the diet x sex interaction.

Post hoc comparisons showed no significant difference in TMD across the moderate CHO intervention. However, within the low CHO diet, a significant negative (lower mood score) difference was found between baseline and week two (mean difference = -21.25, CI, -40.34, -2.16, p = 0.033, ES = 0.61) and a near significant positive (higher mood score) differences week two compared to week four (mean difference = 19.81, CI, -1.21, 40.84, p = 0.06, ES = 0.60).

4. Discussion

The primary purpose of this study was to investigate whether a four-week low CHO diet followed by CHO restoration could improve endurance performance in men and women when compared to a moderate CHO diet. Our findings demonstrate that, when assessed in the context of time to complete, there was no significant difference in performance between low carbohydrate and moderate carbohydrate diets. In absolute (W) and relative power outputs (W/kg) terms, ultra-endurance cycling performance improved on a low CHO diet when followed by CHO restoration. In part, due to a small but significant improvement in body composition.

While we do not present mechanistic data, our results very likely reflect contrasting changes in the oxidative capacity of the trained muscle on each diet. It can be seen from the fasted RER data that a moderate CHO diet results in a higher reliance on CHO as an energy source at rest (Figure 5) while the converse occurs during a period of low CHO intake. It is likely that the low CHO intervention provided an adaptive stimulus to metabolically active tissue other than skeletal muscle, specifically the liver (Webster et al., 2016), which has been shown to became more proficient at sparing CHO at rest (Nilsson & Hultman, 1973). Along these lines, it has previously been shown that fasted training or low CHO training diets in either sex produce a greater capacity to accumulate and store CHO as glycogen in the muscle (Stannard et al., 2010) and liver (Nilsson & Hultman, 1973; Saitoh, Shimomura, & Suzuki, 1993), which has been reported to assist an increase in endurance exercise capacity (Lambert et al., 2001). We did not control the timing of CHO ingestion post-exercise on either diet, a factor that could have impacted glycogen status and its associated metabolic effects (Zderic, Davidson, Schenk, Byerley, & Coyle, 2004, Jenjens & Juekenrup, 2003). Future studies in this area should consider controlling this variable to eliminate any potential effects on the results.

Each sex responded similarly for performance but differently for body composition suggesting there are differing metabolic/mechanistic responses occurring between sexes. These deferring responses could be related to the inherent metabolic inflexibility of women, which may result in a blunted adaptation to a chronic moderate CHO intake. It could be said that men are more metabolically flexible than women and more able to respond to large changes in macronutrient availability, whereas women, although able to handle low CHO intakes, are far less metabolically able to tolerate large loads of CHO outside of exercise, resulting in a trend toward fat gain (Horton, Pagliassotti, Hobbs, & Hill, 1998; Knechtle et al., 2004; Tarnopolsky, MacDougall, Atkinson, Tarnopolsky, & Sutton, 1990). However, it is acknowledged that the overall small sample size may have impacted the sex comparison in the current study.

When CHO depletion is facilitating high rates of gluconeogenesis (Webster et al., 2016) and glycogen sparing (Sherman, Costill, Fink, & Miller, 1981), it provides a "best case scenario" for both sexes to exploit their full lipid utilising capacities during exercise, enabling better ultra-endurance performances when compared to the traditional athlete dietary recommendations (Academy of Nutrition and Dietetics, 2016; Louise M. Burke, Hawley, Wong, & Jeukendrup, 2011). During rest and submaximal exercise, high rates of lipid oxidation can be advantageous for reducing body mass and, more specifically, BF loss (St-Onge & Jones, 2003). Our data somewhat supported this thoery as women tended to gain BF on the moderate CHO diet ($p = 0.09$) and lose body fat on the low CHO diet ($p = 0.03$), whereas the men lost almost the same amount of BF on both diets (Figure 6 3). However, as with previous research (Volek et al., 2004), it was observed (albeit non-significantly) that women consumed less calories (approx. 300 kcal/day) on the low CHO diet, whereas men had near identical caloric intakes on both diets. This may reflect a level of discomfort in females about consuming fat-rich foods or a level of

pleasure in consuming CHO-rich foods that is independent of metabolic differences.

The POMs test results showed an initial reduction in TMD on the low CHO diet from baseline to week 2 ($p = 0.03$). This finding aligns with past research which showed a significant decline in TMD over the first eight weeks of a very low CHO diet (Brinkworth, Buckley, Noakes, Clifton, & Wilson, 2009). Since the baseline and week four TMD scores were not significantly different, the mood could be acting as a proxy for the body adapting to a chronic change in macronutrient intake. This would align with previously reported negative impacts of low CHO diets on endurance performance when the dietary intervention period was greater than seven days (Ref table L. M. Burke and Hawley (2002). Due to the nature of the diets, it was not possible to blind the athletes to either diet, so it is possible that prior perception of the diets may have affected the participants' behaviour. The authors, also acknowledge the lack of control for the menstrual cycle, which was done due to time constraints and to allow for easy inclusion of women in this study. In this regard, research is mixed, and multiple references have shown little effect of the menstrual cycle on endurance performance (Dombovy, Bonekat, Williams, & Staats, 1987; Nicklas, Hackney, & Sharp, 1989).

Based on the results observed in the current research, it is suggested that further research in the area of low CHO and endurance performance should be focused on periodisation of diet considering sex differences, as there are periods in a training and competition cycle where high CHO oxidation rates are more important than high rates of fatty acid oxidation and vice versa. The approaches taken to account for the changing demands of a competition cycle may differ between men and women. In a general sense, having high fatty acid oxidation levels during recovery and non-crucial periods of endurance events could be vital to preserving glycogen for high-intensity bouts that can occur within a race or on subsequent days of competition. Also, body composition is an important aspect of endurance sport where power-weight ratios can significantly influence performance and any means of improving body composition without sacrificing performance need to be considered.

5. Practical Applications.

A high fat low CHO diet should be considered an appropriate nutritional tool for both male and female endurance athletes. Although the incorporation of a low CHO diet does not appear to cause reductions in endurance performance, its application should be tested during non-crucial periods of competition (i.e., off-season).

Women appear to be less metabolically flexible than men. That is, women are not better at burning fat but rather, less able to burn CHO. As a result, we suggest that the appropriate level of CHO intake for endurance training is lower than the current recommendations.

6. Conclusions

A low CHO diet followed by CHO restoration did not result in improved ultra-endurance cycling performance in athletes of both sexes compared to the more traditional higher CHO dietary recommendations. However, in relation to power output, absolute (W) and relative (W/Kg), performance was enhanced on a carbohydrate restricted diet.

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