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Keto-adaptation enhances exercise performance and body composition responses to training in endurance athletes

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ABSTRACT

Background. Low-carbohydrate diets have recently grown in popularity among endurance athletes, yet little is known about the long-term (>4 wk) performance implications of consuming a low-carbohydrate high fat ketogenic diet (LCKD) in well-trained athletes.

Methods. Twenty male endurance-trained athletes (age 33 ± 11 y, body mass 80 ± 11 kg; BMI 24.7 ± 3.1 kg/m^2) who habitually consumed a carbohydrate-based diet, self-selected into a high-carbohydrate (HC) group (n = 11, %carbohydrate:protein:fat = 65:14:20), or a LCKD group (n = 9, 6:17:77). Both groups performed the same training intervention (endurance, strength and high intensity interval training (HIIT)). Prior to and following successful completion of 12-weeks of diet and training, participants had their body composition assessed, and completed a 100 km time trial (TT), six second (SS) sprint, and a critical power test (CPT). During post-intervention testing the HC group consumed 30–60 g/h carbohydrate, whereas the LCKD group consumed water, and electrolytes.

Results. The LCKD group experienced a significantly greater decrease in body mass (HC −0.8 kg, LCKD −5.9 kg; P = 0.006, effect size (ES): 0.338) and percentage body fat percentage (HC −0.7%, LCKD −5.2%; P = 0.008, ES: 0.346). Fasting serum beta-hydroxybutyrate (βHB) significantly increased from 0.1 at baseline to 0.5 mmol/L in the LCKD group (P = 0.011, ES: 0.403) in week 12. There was no significant change in performance of the 100 km TT between groups (HC −1.13 min·s, LCKD −4.07 min·s, P = 0.057, ES: 0.196). SS sprint peak power increased by 0.8 watts per kilogram bodyweight (w/kg) in the LCKD group, versus a −0.1 w/kg reduction in the HC group (P = 0.025, ES: 0.263). CPT peak power decreased by −0.7 w/kg in the HC group, and increased by 1.4 w/kg in the LCKD group (P = 0.047, ES: 0.212). Fat oxidation in the LCKD group was significantly greater throughout the 100 km TT.

Conclusions. Compared to a HC comparison group, a 12-week period of keto-adaptation and exercise training, enhanced body composition, fat oxidation during exercise, and specific measures of performance relevant to competitive endurance athletes.

1. Introduction

Traditional sports nutrition guidelines recommend consumption of high-carbohydrate diets for endurance performance [1,2], yet a growing number of athletes have adopted a LCKD

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approach [3,4]. Endurance performance is limited when endogenous carbohydrates are the dominant fuel [5,6], necessitating provision of exogenous carbohydrate during exercise [7]. A LCKD increases oxidation of endogenous fat stores [8] partially relieving an athlete's dependency on glucose [4]. There is no universally agreed definition for a LCKD. The level of carbohydrate and protein restriction required to induce nutritional ketosis varies, however, some guidelines recommend consuming >75% of energy from fat, moderate protein (1.76–2.2 g per kg lean mass), and <50 g/d carbohydrate [5].

There is a scarcity of investigations examining the effects of a LCKD on performance [9–12] with a greater number of investigations examining low-carbohydrate high fat (LCHF) diets and performance [13–17]. A recent review [3] defined a LCHF diet to contain >60% energy from fat, with moderate levels of carbohydrate restriction (<25% energy). This definition of a LCHF diet is similar to a LCKD diet, both are higher in dietary fat than a traditional diet, and restrict carbohydrates. However, a LCHF diet may not optimise metabolic adaptations associated with accelerated fat oxidation and ketone-related metabolic and signaling effects [4,8]. LCHF diet investigations have focused on short (7–14 days) [13–15], to medium term adaptation periods (14–35 days) [16–17] in athletes. These investigations have reported consistent alterations in fuel utilization, and exercise metabolism in fasted, and carbohydrate depleted states, but fail to test the hypothesis surrounding long-term keto-adaptation and exercise performance [8]. When well-formulated ketogenic diets are implemented for a minimum of four weeks, enhanced fat oxidation rates are observed, with no decrement in aerobic capacity [9]. What happens to exercise performance beyond 4 weeks of keto-adaptation remains unclear, but empirically several endurance athletes using this approach remain highly competitive [8].

Changes in performance due to consumption of LCHF diets are mixed [13–17]. A recent cross sectional study examined the metabolic characteristics of keto-adapted ultra-endurance athletes who consumed a LCKD for 9–36 months [8]. Peak and sub-maximal fat oxidation rates during exercise in keto-adapted participants were more than two-fold higher compared to HC counterparts, and 50% higher than peak rates previously reported [18]. Two of the most notable differences between the LCKD investigation [4], and the current body of LCHF research are the level of carbohydrate restriction, and the length of the adaptation period.

LCKD research on performance has focused on short to medium term adaptation periods (21–30 days) [9–12], possibly due to challenges of long term dietary interventions. Two of these investigations should not be categorised as "ketogenic", since protein [11], and carbohydrate [10], were not sufficiently restricted. Nonetheless, strength, and time to exhaustion were not negatively affected [9–12], however two trials reported a decreased ability to perform at higher intensities [11], and decreased exercise economy [12]. Despite a lack of experimental scientific literature advocating clear performance benefits of adapting to a LCKD diet, interest in this dietary paradigm has continued to gather traction [3,4,19]. Keto-adaptation is believed to unlock a much larger fuel tank versus a carbohydrate-based diet [4,5]; hence reducing an athlete's need for carbohydrate supplementation during exercise. Thus, unlike previous long term cross-sectional LCKD investigations where keto-adaptation had already taken place [8,20] we designed an experimental study to investigate the long-term (12-week) performance implications of consuming a LCKD diet on performance relevant to competitive endurance athletes, and tested the hypothesis that a keto-adapted athlete can maintain/improve performance on a LCKD. This research also involved incorporation of training programme to enhance mitochondrial biogenesis and hence fuel utilization, an aspect not incorporated within previous research.

2. Methods

2.1. *Experimental Approach*

This was a non-randomised control trial comparing long term performance implications of consuming a HC and LCKD, in male endurance trained athletes. A non-randomised approach was chosen due to the length of the adaptation period, and to promote dietary adherence. Participants were informed of the purpose, and any risks associated with taking part, prior to written consent being obtained. The investigation was approved by the research ethics committee at Waterford Institute of Technology, IE. At baseline participants completed a DXA scan, SS sprint, 100 km TT and CPT. Following baseline testing both groups began a 12-week dietary and training intervention (endurance, strength, and HIIT). Participants returned at the end of week 12 and repeated the testing protocol.

2.2. *Participants*

Forty-seven male endurance trained athletes (18–40 years) were enrolled. Twenty participants completed all requirements associated with the current study (Table 1). For this

Table 1 – Subject characteristics.

	HC diet (n = 11)		LCKD diet (n = 9)		t-Test
	Mean ± SD	Range	Mean ± SD	Range	P Value
Age, years	32.1 ± 6.4	20.0–38.0	33.8 ± 6.9	19.0–40.0	0.566
Height, cm	181.2 ± 4.9	177.0–192.1	183.1 ± 5.5	175.5–191.6	0.408
BMI, kg/m^2	23.9 ± 2.9	20.0–30.5	25.6 ± 3.0	22.2–31.2	0.090

investigation, an endurance athlete was an athlete who competed in endurance events, completed >7 h/week training with >2 years training experience. The reasons for dropout were: an injury or illness not related to the intervention (HC $n = 7$; LCKD $n = 9$), intervention too time consuming (HC $n = 1$; LCKD $n = 1$), dietary intervention too difficult to adhere to (LCKD $n = 5$), participants unable to complete post-intervention testing (LCKD $n = 2$), strength and HIIT training too difficult to incorporate into training week (HC $n = 1$), and technical difficulty at post-intervention testing (LCKD $n = 1$). Participants were recruited by contacting clubs, and via social media; Cycling Ireland, Triathlon Ireland, and Irish Triathlon. The following sports were represented: triathlon ($n = 6$), cycling ($n = 5$), Ironman ($n = 4$), marathon runners ($n = 3$), ultra-marathon runner ($n = 1$), and adventure racer ($n = 1$).

2.3. *Pre–Participation Screening*

Screening took place to ensure all participants were; male endurance athletes for >2 years, 18–40 years, and currently consumed a carbohydrate based diet (>50% kcal). Exclusion criteria included; diseases or conditions known to affect performance, use of pharmaceuticals that may affect any measurements of performance, and Illness or injury prior to start date.

2.4. *Pre-Intervention Testing*

Participants avoided racing or training 48 h prior to testing and maintained habitual carbohydrate based diet. Participants reported to the Human Performance Laboratory at 09:00 following 12 h fast (Fig. 1). Upon arrival, weight was recorded to the nearest 0.1 kg (SECA 711, Hamburg, Germany) and height was recorded to the nearest 0.1 cm (SECA 213, Hamburg, Germany). Body composition was measured by DXA (Norland XR-46) via whole-body scan set to a resolution of 4.5 × 9.0 mm and a scan speed 260 mm/s. Fasting blood samples were collected from an antecubital vein using a 21G BD Vacutainer blood collection set (BD Diagnostics). Blood samples were centrifuged and the resultant serum stored at –80 °C for later analysis.

This initial phase of testing was completed by 09:30 and participants were allowed 2 h to "fuel up" prior to the exercise trial. "Fueling up" included consumption of the individual's habitual breakfast, or pre-exercise nutrition (%carbohydrate:protein:fat HC = 52:20:28; LCKD = 64:16:20). Each group was allowed to self-select their pre exercise carbohydrate based meal, to ensure habitual dietary practices and performance measurements were obtained. Participants returned to the Human Performance Laboratory at 11:30, set up their bike position (Wattbike Ltd., Nottingham, UK), and began a 10 min warm up. The Wattbike is reported to sufficiently track performance changes in trained and untrained athletes with a reliability coefficient of 2.2% in a trained population and 6.7% in an untrained population [21]. After warm up participants completed a SS sprint on the Wattbike to determine peak and average power output. During the SS sprint a relative load of 0.5 of air resistance was applied for every 5kgs of body weight. Participants were then connected to the MOXUS Metabolic System (AEI Technologies, Chicago, IL) via mouthpiece (2700 series (large) 2 way T-shape non-rebreathing valve with saliva collector Hans Rudolph, Shawnee, KS) for the determination of energy expenditure, and began their 100 km TT. Participants were instructed to complete the 100 km TT as fast as possible. During the 100km TT, air resistance on the Wattbike was self selected. VO_2 and VCO_2 were averaged every 15 s for the first 5 min of every 20 km, and used to calculate oxygen uptake, minute ventilation, and carbohydrate and fat oxidation; which were presented as a respiratory exchange ratio (RER) value

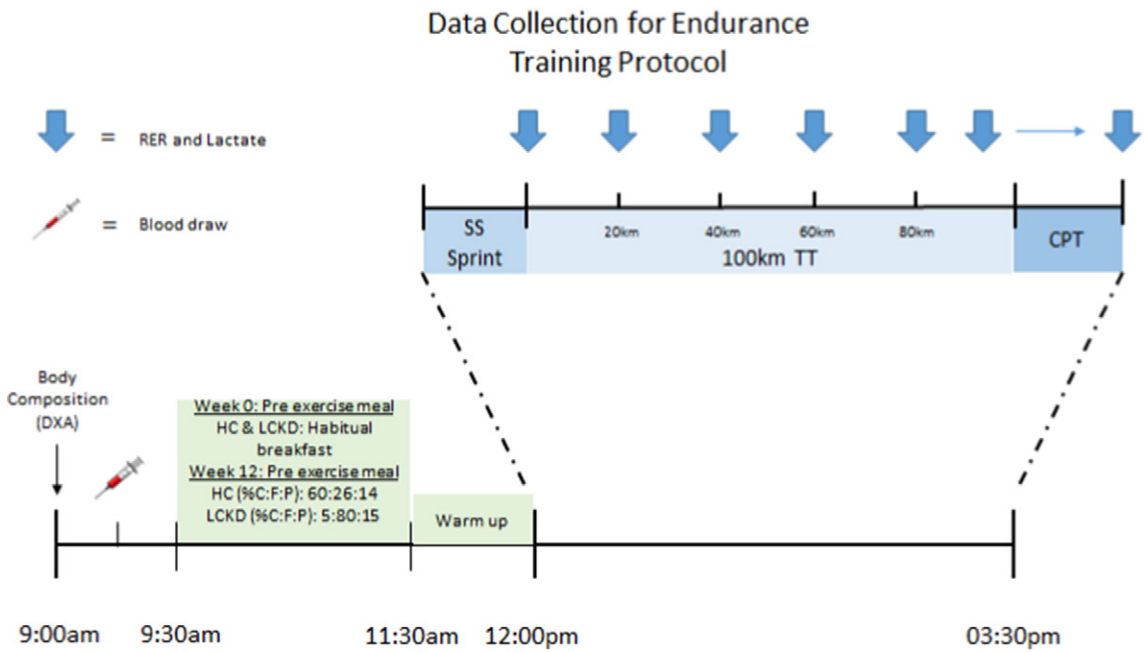

Fig. 1 – Experimental protocol implemented at pre and post-intervention testing.

(VCO_2/VO_2). During the final 30 s of these 5 min, a capillary blood sample was obtained and blood lactate concentrations analysed using a lactate analyser (Lactate Pro, Arkay, Shiga, Japan). Measurements were repeated at 20 km intervals up until 80 km (20 km, 40 km, 60 km & 80 km), the final measurement was the concluding 2500 m of the 100 km TT. Immediately after the 100 km TT, participants completed a CPT on a Wattbike, where peak VO_2, peak power (watts), and average power (watts) were recorded. Participants were instructed to maintain as high a power output as possible while remaining seated for 3-min, during which, the same resistance applied during the SS sprint, was applied. Absolute power measurements were converted to relative power (RP) by dividing watts by body mass in kg. Following completion of the CPT, the mouthpiece was removed and the final lactate measurement was recorded. Participants then completed a gradual self-selected cool down.

2.5. Intervention

2.5.1. Diet

Food diaries were obtained at baseline using a 3 day weighed food diary (2 week days and 1 weekend day). These were analysed using Nutritic's dietary analysis software (Nutritic's Professional v3.09, Nutritic's, Dublin, Ireland). The dietary and training intervention began the day after pre-intervention testing. The macronutrient goals were: HC 65% CHO, 20% fat and 14% protein, or LCKD >75% fat, 10–15% protein and <50 g/d CHO. HC participants were instructed to consume carbohydrates based on their daily energy requirements [7], whereas LCKD participants were instructed to adhere to carbohydrate and protein guidelines, and consume dietary fat ad libitum. 3 day food diaries were also obtained at week 12 and analysed.

2.5.2. Nutritional Counselling

Subsequent to pre-intervention testing each participant received a detailed handout, and nutritional counselling from the researcher. The researcher contacted each participant weekly to ensure dietary adherence, and a weighed food diary was submitted each week. The HC group's nutritional handout included guidelines on how to formulate a HC diet according to their daily energy requirements [7]. The LCKD group's handout included information on how to formulate a LCKD diet, a shopping list and example meal plans. To preclude orthostatic symptoms, LCKD participants were recommended to supplement salt to taste at meal times, consume electrolytes and water when exercising, and supplement 1–2 g/d of sodium from bouillon cubes, or homemade broth [5,9].

2.5.3. Training

Each group received the same training intervention, with endurance training (cycling and running), strength training and high intensity interval training (HIIT) to encourage mitochondrial biogenesis [22–23]. Each participant completed 7+ h a week endurance training (moderate intensity 56–68% VO_2max), 2 strength sessions; 6 sets of 8–10 reps on a leg press, or free squat (70–80% of participants 1RM), and 2 HIIT sessions/week (10 sets of 1 min bouts at 70% peak power with 1 min recovery). During endurance training the LCKD group minimised carbohydrate intake prior to training and limited food consumption during exercise.

2.6. Post-Intervention Testing

Post-intervention testing was similar to pre-intervention testing with the exception of fuelling prior to, and during the exercise trial. Following an overnight fast and preliminary tests, HC participants consumed a HC breakfast (%carbohydrate:fat:protein, 60:26:14) to meet dietary guidelines [2], and LCKD participants consumed a high fat breakfast (5:80:15) to maximize fat oxidation [24].

Participants were again allocated 2 h rest. Once exercise had commenced, participants in the HC group consumed 30–60+ g/h of carbohydrate (glucose, maltodextrin, sucrose and fructose), according to carbohydrate recommendations [2], whereas LCKD participants consumed water, and zero calorie electrolytes.

2.7. Blood Analysis

Fasting Beta-hydroxybutyrate (βHB) concentrations were determined at baseline and post-testing via colorimetric enzymatic assay (Sigma-Aldrich; St. Louis, MO). Intra-assay coefficient of variation was <10%. All samples were thawed one time prior to analysis.

2.8. Statistical Analyses

IBM Statistics SPSS 24 (Illinois, Chicago, USA) was used for statistical analysis. Data was tested for normality, with parametric tests used for normally distributed data or non-parametric, for data not normally distributed. Independent sample *t*-tests or Mann Whitney *U* test (if data was not normally distributed) were used to determine differences between HC and LCKD groups at baseline, with the alpha level for significance set at $P < 0.05$. Effects for each group were analysed using ANCOVA, with pre-intervention measures acting as a covariate. ANCOVA with baseline body fat (kg) as an additional covariate was carried out, due to a significant difference in body fat between HC and LCKD groups at baseline. As a measure of effect size, partial eta-squared (η_p^2) was used. Effect sizes were evaluated as: $\eta_p^2 = 0.01$ (small effect), $\eta_p^2 = 0.09$ (medium effect), and $\eta_p^2 = 0.25$ (large effect) [25]. Paired samples *t*-tests or Wilcoxon signed ranks test (if data was not normally distributed) examined changes over time within each group, if ANCOVA *P* value was <0.05.

3. Results

3.1. Baseline Subject Characteristics, Diet and Performance Measurements

Body fat (kg) ($P = 0.046$), and carbohydrate intake (g) ($P = 0.028$) were significantly different between the HC and LCKD group at baseline. All other physical characteristics (Table 1), dietary (Table 2), performance and anthropometric measurements (Table 3) were not statistically significant between groups ($P > 0.05$).

Table 2 – Daily energy intake, macronutrient distribution, and circulating ketones pre and post-intervention.

Dependent variables	HC diet (n = 11) [a]			LCKD diet (n = 9) [a]			ANCOVA		
	Pre	Post	Change	Pre	Post	Change			
	Mean ± SD	Mean ± SD	Mean	Mean ± SD	Mean ± SD	Mean	F – value	P value	ES [b]: η_p^2
Energy, kcal/d	2440.2 ± 773.8	2643.6 ± 358.0	+203.4	2843.8 ± 558.4	3022.3 ± 911.1	+178.5	(1,17) = 0.646	0.433	0.037
CHO, g/d	315.6 ± 107.5	400.3 ± 102.7 [d]	+84.7	454.8 ± 152.0 [c]	41.1 ± 13.3 [d]	−413.7	(1,17) = 77.71	0.000 *	0.821
CHO, g/kg BM	4.2 ± 1.6	5.3 ± 1.4	+1.1	5.2 ± 1.4	0.5 ± 0.2 [d]	−4.7	(1,17) = 95.93	0.000 *	0.849
Fat, g/d	77.7 ± 33.5	55.2 ± 10.7 [d]	−22.5	64.7 ± 39.1	259.3 ± 83.4 [d]	+194.6	(1,17) = 62.41	0.000 *	0.786
Fat, g/kg BM	1.0 ± 0.5	0.8 ± 0.5	−0.2	0.7 ± 0.1	3.2 ± 0.9 [d]	+2.5	(1,17) = 86.67	0.000 *	0.836
Protein, g/d	118.9 ± 31.8	90.9 ± 23.6	−28.0	110.3 ± 25.5	130.7 ± 35.8	+20.4	(1,17) = 8.270	0.010 *	0.327
Protein, g/kg BM	1.6 ± 0.5	1.2 ± 0.3	−0.4	1.2 ± 0.3	1.6 ± 0.4	+0.4	(1,17) = 5.306	0.034 *	0.238
βHB (mmol/L)	0.2 ± 0.3	0.1 ± 0.0	−0.1	0.1 ± 0.1	0.5 ± 0.4 [d]	+0.4	(1,17) = 8.780	0.011 *	0.403

Abbreviation: CHO, Carbohydrate.
[a] Original means and standard deviations, i.e. without adjustment for covariate (i.e. pre-treatment data).
[b] ES = effect size. η_p^2 = 0.01 (small effect), η_p^2 = 0.09 (medium effect), η_p^2 = 0.25 (large effect) [25].
[c] Significant difference between groups at baseline.
[d] Significant difference within group between pre and post-intervention.
* ANCOVA significant difference at $P < 0.05$.

3.2. *Diet and Exercise Adherence*

Mean duration of the intervention was 84 ± 2.8 days for the HC group, and 81.2 ± 4.9 days for the LCKD group. Energy intake remained unchanged in each group (Table 2). Mean carbohydrate intake significantly increased in the HC group (+85 g/d) and significantly decreased (−414 g/d) in the LCKD group (Table 2). Fat intake significantly decreased (−23 g/d) in the HC group, and significantly increased in the LCKD group (+195 g/d) (Table 2). Protein intake was significantly greater post intervention in LCKD group compared to the HC group (P = 0.010). There was no significant difference in HC and LCKD group's number of HITT (HC: 18.2 ± 2.0, versus LCKD: 19.7 ± 2.3), strength sessions (HC: 17.8 ± 2.1, versus LCKD: 18.3 ± 3.9) or hours endurance training (HC: 11.1 ± 1.7, versus LCKD: 13.0 ± 2.8) completed per week. βHB non-significantly decreased in the HC group (from 0.2 down to 0.1 mmol/L) and significantly increased in the LCKD group (from 0.1 up to 0.5 mmol/L) (P = 0.021).

3.3. *Body Composition*

Body mass significantly decreased in the LCKD group, with a loss of 5.9 kg compared to 0.8 kg in the HC group (Table 3). The significant change in body mass resulted from LCKD participants losing more body fat compared to the HC group (LCKD = −4.6 kg vs HC = −0.5 kg, P = 0.002). Despite significant loss in body mass, both groups maintained lean body mass (HC = +0.1 kg, LCKD = +0.3 kg). When baseline body fat was added as a covariate to body composition changes, similar levels of difference between HC and LCKD groups were obtained (body mass P = 0.009; lean mass P = 0.281).

Table 3 – Body composition and performance variables for pre and post-intervention.

Dependent variables	HC diet (n = 11) [a]			LCKD diet (n = 9) [a]			ANCOVA		
	Pre	Post	Change	Pre	Post	Change	P value		
	Mean ± SD	Mean ± SD	Mean	Mean ± SD	Mean ± SD	Mean	F – value	P value	ES [b]: η_p^2
Body mass, kg	76.5 ± 9.9	75.7 ± 8.7	−0.8	86.3 ± 14.3	80.4 ± 13.4 [d]	−5.9	(1,17) = 8.682	0.006 *	0.338
Lean mass, kg	63.6 ± 5.4	63.7 ± 5.0	+0.1	67.6 ± 9.0	67.9 ± 9.4	+0.3	(1,17) = 1.239	0.167	0.068
Body fat, kg	10.6 ± 6	10.1 ± 5.6	−0.5	15.8 ± 7.2 [c]	11.2 ± 5.0 [d]	−4.6	(1,17) = 13.05	0.002 *	0.434
Body fat, %	12.8 ± 5.1	12.1 ± 4.7	−0.7	17.5 ± 5.5	12.3 ± 4.7 [d]	−5.2	(1,17) = 8.998	0.008 *	0.346
Bone density (g/cm^2)	1.13 ± 0.12	1.12 ± 0.11	−0.01	1.16 ± 0.11	1.14 ± 0.11	−0.02	(1,17) = 0.001	0.978	0.000
VO_2max, ml/kg/min	52.6 ± 6.4	57.2 ± 6.1	+4.6	53.6 ± 6.8	57.3 ± 6.7	+3.7	(1,17) = 0.002	0.968	0.000
TT, min·s	169.57 ± 9.36	168.44 ± 9.14	−1.13	166.00 ± 12.38	161.53 ± 8.44	−4.07	(1,17) = 4.152	0.057	0.196
SS peak RP (w/kg)	13.9 ± 2.7	13.8 ± 2.2	−0.1	13.7 ± 1.4	14.5 ± 1.1 [d]	+0.8	(1,17) = 6.064	0.025 *	0.263
SS Av RP (w/kg)	12.2 ± 1.5	12.5 ± 1.7	+0.3	12.3 ± 1.3	12.8 ± 1.1	+0.5	(1,17) = 0.982	0.336	0.055
CPT peak RP (w/kg)	9.1 ± 2.6	8.4 ± 2.2	−0.7	8.3 ± 2.2	9.7 ± 2.3 [d]	+1.4	(1,17) = 4.574	0.047 *	0.212
CPT Av RP (w/kg)	4.5 ± 2.2	4.6 ± 2.2	+0.1	3.9 ± 0.7	4.0 ± 0.6	+0.1	(1,17) = 0.362	0.555	0.021

[a] Original means and standard deviations, i.e. without adjustment for covariate (i.e. pre-treatment data).
[b] ES = effect size. η_p^2 = 0.01 (small effect), η_p^2 = 0.09 (medium effect), η_p^2 = 0.25 (large effect) [25].
[c] Significant difference between groups at baseline.
[d] Significant difference within group between pre and post-intervention.
* ANCOVA significant difference at $P < 0.05$.

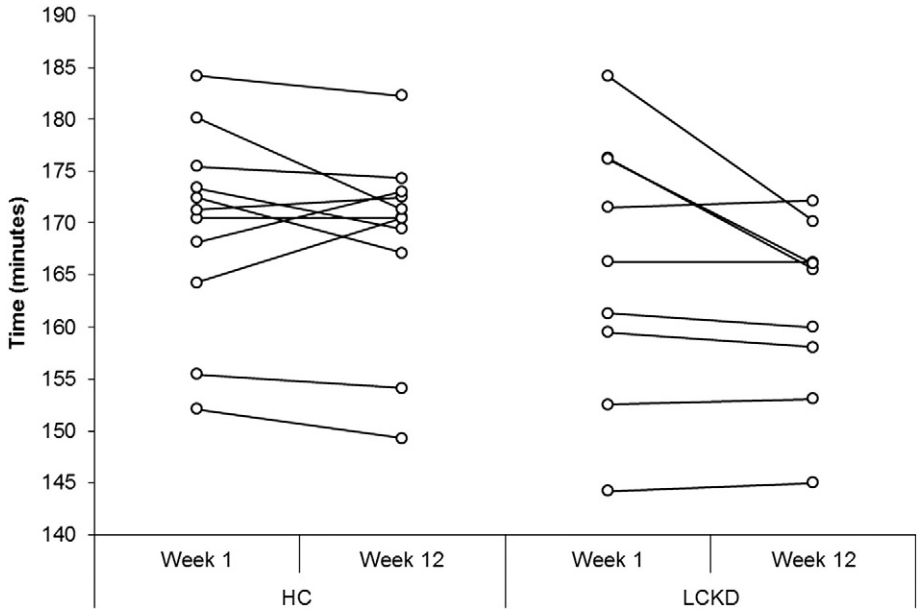

Fig. 2 – Individual 100 km TT times for HC, and LCKD groups at pre and post-intervention testing.

3.4. *Performance and Fuel Utilization*

VO_2max changed similarly in HC (+8.7%) and LCKD (+6.9%) groups ($P = 0.968$). Time required to complete the 100 km TT was not significantly different between groups ($P = 0.057$, ES: 0.196) (Table 3), but change was numerically greater within the LCKD group (−4.07 min·s) compared to the HC group (−1.13 min·s) (Table 3). Participants individual 100 km TT times are shown in Fig. 2. Improvements in time were observed in 6 out of 9 LCKD participants, and 7 out of 11 HC participants. When baseline body fat was added as a covariate to time trial performance, performance differences between group remained insignificant ($P = 0.137$).

There was a significant difference in SS peak power between groups ($P = 0.025$) with a significant increase in the LCKD group (Table 3), but no changes to participants average power observed ($P = 0.336$). Similar to the SS Sprint, peak power in the CPT was significantly different between groups; decreasing in the HC group (−0.7 w/kg), and increasing in the LCKD group (1.4 w/kg) ($P = 0.047$) (Table 3), while average power during the CPT remained unchanged. Significant changes found in SS peak power ($P = 0.024$) and CPT peak power ($P = 0.045$) remained when baseline body fat was considered. Significant differences in RER were observed at 20 km ($P = 0.000$), 40 km ($P = 0.000$), 60 km ($P = 0.000$), 80 km ($P = 0.000$) and at 100 km ($P = 0.040$) (Fig. 3). These differences were present due to significant changes within the LCKD group. No changes were found in blood lactate responses to exercise for the HC and LCKD groups (Fig. 4). RER and blood lactate results remained similar when baseline fat was considered as a covariate.

4. Discussion

This study examined the effects of a consuming a 12 week LCKD diet versus a HC diet, while incorporating a training intervention, on exercise performance and body composition. We show compared to athletes consuming a HC diet, 12-weeks of keto-adaptation is associated with greater improvements in body composition, fat oxidation and peak power, with endurance performance being maintained in both groups. A 12-week period of keto-adaptation reduced total body mass and fat mass, while maintaining lean body mass and increased peak and average relative power during the SS sprint and CPT compared to HC participants. Performance of 100 km TT improved over the course of the intervention, but was not statistically different between groups.

Carbohydrate intake was greater within LCKD compared to HC participants at baseline (4.2 vs 5.2 g/kg). Increased fuel availability may have accounted for slightly better performance of LCKD participants at baseline, but this performance difference was not statistically significant. Due to high carbohydrate intake within LCKD participants at baseline, effect of carbohydrate reduction on fuel utilization was extreme, resultantly; LCKD participants reported fatigue and tiredness during initial adaptation period. The HC groups increase in carbohydrate intake to meet current carbohydrate recommendations [2] was moderate but enabled HC participant's equal LCKD baseline intake. Even though the HC participants consumed 5.3 g/kg of carbohydrate for 12 weeks, they did not equal the LCKD participant's mean time trial performance post intervention.

One of aims of this investigation was to determine if a HC athlete could improve/maintain endurance performance when keto-adapted. The findings here suggest endurance performance can be maintained, and in some cases improved when compared to a HC diet. This is evident in 3 LCKD participants, as 100 km TT performance improved, with maintenance observed in 6 LCKD participants. LCKD participants with the greatest improvements possessed the slowest baseline times. HC participants with similar baseline times did not experience similar improvements in performance, despite similar VO_2max gains. Strength training in cyclists alters muscle fibre type recruitment patterns, and improves exercise economy by increasing maximal strength of

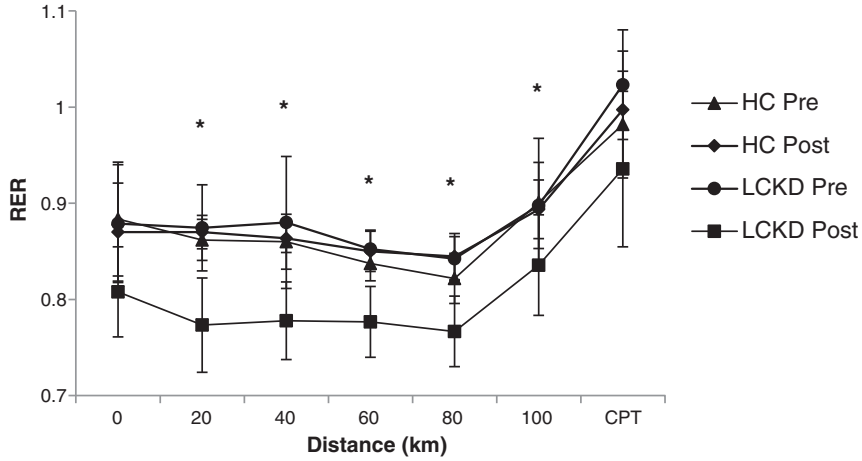

Fig. 3 – RER for HC and LCKD groups at 0 km, 20 km, 40 km, 60 km, 80 km, 100 km and CPT data points, at pre and post-intervention testing. *Indicates significant (P < 0.05) difference from ANCOVA, with changes within the LCKD group.

type 1 muscle fibres, and postponing activation of less economical type 2 muscle fibres [26]. Ability to maintain/increase performance may be due to favourable mitochondrial and oxidative enzyme adaptations occurring within muscle [27], due to diet and/or training effect within some LCKD participants. This challenges decades of conventional wisdom advocating a high-carbohydrate diet to optimise performance [3,28].

Two important factors in this study likely to contribute to the positive responses to training are diet composition and prolonged adaptation period.

4.1. *Diet Composition*

Nutritional counselling in this investigation focused on the fundamentals of a well-formulated LCKD diet with carbohydrate restricted to <50 g/day, protein consumed in moderation, and remaining energy derived from natural fat sources [5]. Dietary fat accounted for 81% of energy, protein 1.9 g/kg LBM, and carbohydrate 41 g/day. Macronutrient profile is similar to previous research [9,12], but inconsistent with others [10–11]. Studies where fat accounts for 55–70% of total energy [10,11], with higher protein intakes will fail to adequately induce nutritional ketosis, and may hinder adaptation [10,11]. Evidence of this derives from participant's haematology results [11]; although βHB significantly increased to 0.15 mmol/L, it was below the threshold of nutritional ketosis (>0.5–3.0 mmol) [5], failure of participants to reach nutritional ketosis may explain negative effects on performance, observed within the study by Zajac and colleagues. In contrast, βHB significantly increased from 0.1 at baseline to 0.5 mmol/L at week 12 in LCKD participants. Although nutritional ketosis was achieved [5], concentrations of βHB were less than previously observed in experimental trials (>1.0 mmol/L) [9,12], however, these trials were feeding trials, with βHB concentrations monitored throughout intervention periods. Greater control and accuracy of dietary prescription may contribute to greater βHB concentrations. A LCHF diet that does not achieve nutritional ketosis provides endurance athletes with a dilemma. When carbohydrates are restricted and protein consumed to a point that induces nutritional ketosis, ketones supply the brain with energy [5,29]. Consuming a high fat non-ketogenic diet may increase fat oxidation, but the brain is unable to use long-chain fatty acids for fuel. An interruption or inadequate supply of glucose

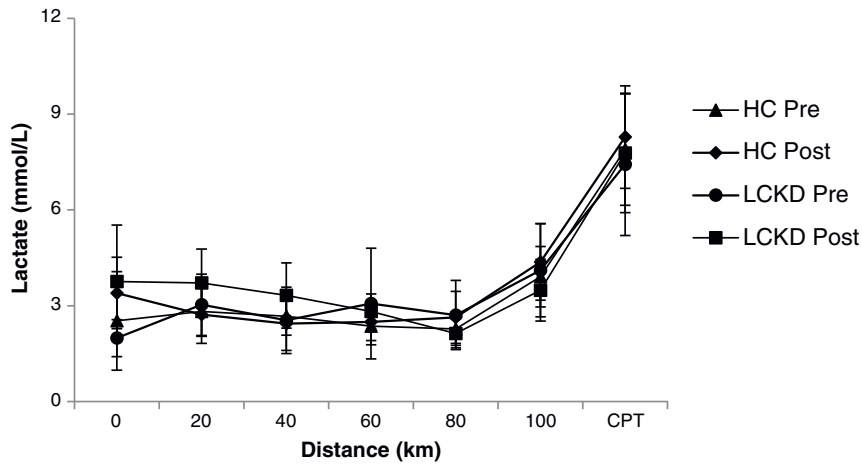

Fig. 4 – HC and LCKD groups lactate responses at 0km, 20km, 40km, 60km, 80km, 100km and CPT data points, at pre and post-intervention testing. P 0.05 at all time-points from ANCOVA.

to the brain in the absence of nutritional ketosis is the metabolic basis for reduced performance and bonking/hitting the wall. Phinney and colleagues [9] identified time to exhaustion was maintained following 28 days of LCKD diet with βHB concentrations >1.0 mmol/L, however, Burke et al. [12] recent investigation found performance adaptations were negated with βHB concentrations of >1.0 mmol/L following 21 days of a LCKD diet. Burke et al. [12] investigation did not assess endurance performance, as the 10 km race implemented was not sufficient to deplete muscle glycogen stores [2], with exercise times <46 min. It is important to highlight that the LCKD diet may not be suitable for everyone, 5 participants found the LCKD diet too difficult to adhere to, and two participants were unable to complete post-intervention testing. Participants unable to complete post-intervention testing were subsequently found to have βHB levels <0.2 mmol/L.

4.2. *Length of Keto-Adaptation*

Previous research incorporated 21–30 day adaptation periods [9–12], with one investigation in 'recreational endurance athletes' adopting a 10-week dietary protocol [30]. Previous LCKD investigations with 21–30 day adaptations noted decreased performance [31], decreases in exercise efficiency, and increases in rates of perceived exertion [12]. Decreased performance was also reported in a 10-week LCKD protocol [30]. However Zinn and colleagues [30] did not incorporate additional training into their study protocol, which the current trial possessed. A significant decrement in peak power was also reported [30] but study participants lost −4 ± 3.1 kg. The effect body mass loss had on power was not considered, decreased power reported by Zinn and colleagues [30] cannot be compared to current results, which are presented as relative power due to body mass loss. During this investigation, LCKD participants noted a drop in energy levels, and performance during the first 7–10, and a "lag" in performance for the first 4–6 weeks. The collective lag in performance, and that 5 LCKD participants dropped out because the dietary intervention was too difficult indicates a LCKD diet may not be for every athlete, should not be undertaken 4–6 weeks prior to an event, or without consideration of individual's dietary preferences.

Phinney et al. [31] first coined the term keto-adaptation to describe the physiological adaptations an individual goes through following consumption of a LCKD diet. They showed performance decreased 20% after one week of consuming a LCKD diet despite significant increases in fat oxidation, but time to exhaustion increased 155% at week 6. The temporal pattern and full scope of the keto-adapted phenotype have not been rigorously studied. The available evidence indicates at least 4–6 weeks is necessary to return to performance and additional time may be necessary to observe consistent increases in performance. Thus, we examined performance after 12-weeks of keto-adaption.

4.3. *Body Composition*

LCKD participants had greater body fat (kg) pre-intervention which may have distorted fat loss findings. When differences in baseline data and body fat are considered, significance values remain similar. Despite LCKD participants losing 4.4 kg body fat, lean body mass increased 0.3 kg. Calorie intake in each group slightly increased during the trial, which contradicts how weight loss occurred. Weight loss is attributed to increased energy output, due to added training. Added weight loss within LCKD participants could be due to slightly greater volume of training undertaken each week. Loss of 4.4 kg body fat within LCKD participants and 0.7 kg body fat within HC participants resulted in both groups having very similar body fat post-intervention (HC 12.1%; LCKD 12.3%). Theses figure are similar to Ackland and colleagues finding that collegiate endurance athletes carry 5–11% body fat [32] and resulted in current study participants possessing the ideal body fat range for endurance athletes [33]. A LCKD diet is a useful tool for achieving weight loss in an untrained population [34], recreational athletes [30] and resistance trained males [35]. These findings suggest a LCKD diet could be a valuable aid to endurance athletes who struggle to maintain race weight, or athletes required to make competitive weight. Furthermore a recent cross-sectional study [36] positively correlated fat mass with increases in inflammatory biomarkers in male endurance trained athletes (17.1 ± 5.1% body fat), therefore reduced body fat is desirable.

4.4. *Performance*

The 100 km TT is short enough to encourage participants to work at high work rates, yet long enough to challenge fuel availability. During exercise there was a significant shift in fuel utilization in the LCKD group, with rates of fat oxidation significantly increasing throughout exercise. This pronounced shift in fuel utilization is a hallmark of keto-adaptation [3,8,9,37]. In attempts to simulate actual race conditions HC group athletes were allowed to fuel following standard dietary recommendations (30–60 g CHO/h). The observed shift in fuel utilization allowed LCKD athletes utilize a greater amount of endogenous lipid stores [4]. 100 km TT improved by 01:13 min·s (0.7%) in the HC group, and 04:07 min·s (2.5%) in the LCKD group (ES: 0.196). A 01.13 and 04:07 min·s increase in performance in 12-weeks in well-trained endurance athletes is practically significant, considering the difference between winning and losing the Tour de France may be seconds [38]. The training programme was designed to enhance mitochondrial biogenesis [22–23]. Although mitochondrial adaptations were not measured during this trial, it is possible that improvements in participant's mitochondrial density enhanced participant's abilities to utilize oxygen, attributing to increases in VO_2max, and performance. Prolonged keto-adaptation may result in an increased transcription and translation of lipid metabolism machinery. This may allow for an enhanced rate of lipolysis and subsequent energy production via oxidative pathways, and potentially explain the performance enhancement observed.

The SS sprint was performed prior to 100 km TT to determine participants' exercise capacity at higher intensities, in a non-fatigued state. Relative peak power increased in LCKD participants as did relative average power, with no changes observed in the HC group. SS sprint, due to the very

short duration, is primarily reliant on the phosphocreatine (PCr) energy system. Previous research indicates a carbohydrate restricted diet does not impair strength performance [10]. Our findings show 12-weeks keto-adaptation is associated with improvement in all-out short-duration exercise capacity, implying no detrimental effects on the phosphagen energy system.

The CPT was performed directly following the 100 km TT to mimic a sprint finish at the end of an endurance event. Previous research examining the effect of a non-ketogenic low carbohydrate diet for 2 weeks, demonstrated no effect on power [16], with other research indicating decreased performance at higher intensities [11]. This investigation incorporated a strength training programme and resulted in a significant increase in relative peak power, with no change in the HC group. Incorporating endurance training with strength training has resulted in increased maximal power output [26]. Improvements in power output observed in the LCKD group may be due to LCKD participants improved power to weight ratio. It is unlikely improvements in power output resulted from strength training, since HC participants had similar levels.

Increases in VO_2max were partially due to decreases in body weight (l/kg/min), and potentially due to improvements in participant's aerobic capacity from the new training stimuli and/or volume of aerobic training. There is little evidence to suggest strength training is an effective mode of improving an athlete's VO_2max [26], however investigations have positively correlated endurance training, and HIIT with improvements in aerobic parameters [39–41]. In a previous trial involving well trained keto-adapted athletes, muscle glycogen stores were not different to well-trained HC athletes after 180 min sub-maximal exercise [8]. Both LC and HC athletes demonstrated similar glycolytic and glycogen synthesis rates during and after exercise [8]. Although muscle glycogen was not assessed in this study, similar lactate responses between groups suggest keto-adaptation did not impair glycolysis during high-intensity exercise.

In summary, a LCKD diet may benefit some athletes, particularity those who struggle with maintaining competitive race weight. Adaptation to a LCKD diet for 12 weeks did not negate measures of performance relevant to endurance athletes, and caused more favourable adaptations to take place in some individuals. Thus, implementation or avoidance of this dietary protocol should be based on an individual's own dietary preference. Despite the concept of keto-adaption being over 30 years old [31], we are still in the early stages of understanding this dietary paradigm. The finding that 12-weeks of keto-adaptation improved aerobic and anaerobic exercise capacity, as well as body composition, in endurance athletes most certainly implies there is potential for using LCKD to improve performance and metabolism. In striving for a more individualistic approach to dietary prescription, keto-adaptation is one approach worth considering.

Contributions of Authors

The study was designed by Fionn McSwiney, Lorna Doyle, and Bruce Wardrop; data was collected by Fionn McSwiney, Lorna Doyle, and Bruce Wardrop; data interpretation and manuscript preparation were undertaken by Fionn McSwiney, Lorna Doyle, Bruce Wardrop, Parker Hyde, Richard LaFountain and Jeff Volek. All aforementioned authors approved the final version of the article.

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Conflicts of Interest

Dr. Volek receives royalties from books on nutrition and exercise.

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