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Carbohydrate intake and ketosis in self-sufficient multi-stage ultramarathon runners

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Abstract

Ultra-endurance athletes accumulate an energy deficit throughout their events and those competing in self-sufficient multi-stage races are particularly vulnerable due to load carriage considerations. Whilst urinary ketones have previously been noted in ultra-endurance exercise and attributed to insufficient carbohydrate (CHO) availability, not all studies have reported concomitant CHO intake. Our aim was to determine changes in blood glucose and β-hydroxybutyrate concentrations over five days (240 km) of a self-sufficient multi-stage ultramarathon in combination with quantification of energy and macronutrient intakes, estimated energy expenditure and evaluation of energy balance. Thirteen runners (8 male, 5 female, mean age 40 ± 8 years) participated in the study. Glucose and β-hydroxybutyrate were measured every day immediately post-running, and food diaries completed daily. CHO intakes of 301 ± 106 $g \cdot day^{-1}$ (4.3 ± 1.8 $g \cdot kg^{-1} \cdot day^{-1}$) were not sufficient to avoid ketosis (5-day mean β-hydroxybutyrate: 1.1 ± 0.6 $mmol.L^{-1}$). Furthermore, ketosis was not attenuated even when CHO intake was high (9 $g \cdot kg^{-1} \cdot day^{-1}$). This suggests that competing in a state of ketosis may be inevitable during multi-stage events where load reduction is prioritised over energy provisions. Attenuating negative impacts associated with such a metabolic shift in athletes unaccustomed to CHO and energy restriction requires further exploration.

Key words: ketones, running, ultra-endurance, carbohydrate, performance, nutrition, energy deficit

Introduction

Self-sufficient multi-stage ultramarathons are conducted over multiple days and athletes must carry all necessary clothing, equipment, and food required for the race in a backpack. Extreme environments, rough sleeping conditions and increased load carriage from backpacks result in long days of high physical, mental and emotional effort. It is recognised that many participants in ultra-endurance races compete in a state of negative energy balance and insufficient carbohydrate intake (Costa et al. 2013; Wardenaar et al. 2015). The reasons have been well explored elsewhere (Costa et al. 2016; Costa et al. 2017; Stuempfle and Hoffman 2015; Stuempfle et al. 2013) but some of the most common are gastrointestinal issues and the inability to consume enough calories to offset energy. In the case of self-sufficient events, these factors are compounded by a deliberate decision by athletes to compromise energy intake for reduced load to carry (Alcock et al. 2018; Lucas et al. 2016; McCubbin et al. 2016).

During periods of acute carbohydrate (CHO) insufficiency ketone bodies (acetoacetate (AcAc) and beta-hydroxybutyrate (β-HB)) are produced as an integral component of homeostasis (Cox and Clarke 2014). As blood glucose and insulin levels drop, free fatty acids are liberated from adipose tissue and partially oxidised in the liver producing ketones (Cahill 1981). The brain can then utilise these circulating ketone bodies for up to 60% of its energy requirements, the remainder coming from gluconeogenesis (Cox and Clarke 2014; Egan and D'Agostino 2016). Ketones also become an alternate energy source for the heart and skeletal muscle (Cox and Clarke 2014; Egan and D'Agostino 2016) reducing nitrogen depletion and allowing for the retention of lean muscle mass (Cahill 2006). This suggests that during a self-sufficient, multi-stage ultramarathon when limited by restricted exogenous energy and CHO sources, being in a state of ketosis may be beneficial to athletes.

In practice however, being in a state of ketosis during athletic endeavours is generally regarded as undesirable due to the initial 'adaptation period' characterised by lethargy and fatigue, as well as the potential for impaired performance due to glycogen depletion, inefficient utilization of muscle substrates, and reduced exercise economy (Burke et al. 2017; Phinney et al. 1983; Yeo et al. 2011). Although metabolic adaptations to low carbohydrate or calorie restricted diets demonstrably occur within five days (Goedecke et al. 1999) it has been suggested that athletes may require several weeks, if not months, to adapt fully (Volek et al. 2016). This may have negative implications for athletes who experience this transition period during multi-stage ultra-endurance events.

Early studies on starvation (Azar and Bloom 1963; Bloom and Azar 1963; Cahill 1981; Consolazio et al. 1968) demonstrated the link between CHO insufficiency and ketosis. Consolazio et al. (1968) similarly noted that when in a daily energy deficit of 11.7 MJ (2 800 kcal) induced through fasting and exercise, ketosis could be avoided by ingesting 1.8 MJ (420 kcal, ~100 g) of CHO per day, an amount equal to the carbohydrate requirements of the brain (Cahill 1981). Subsequent guidelines have thus recommended a daily CHO intake of >100 g combined with a fat intake of less than 160 g to prevent ketosis during periods of caloric restriction (Marriott 1995; Montain and Young 2003). There is scarce data on ketosis and CHO intake during ultra-endurance exercise. Although the presence of urinary ketones has been reported in athletes during ultra-endurance events and attributed to insufficient CHO (Costa et al. 2014; Costa et al. 2013), not all studies have reported concomitant CHO intake (Jablan et al. 2017; Weibel and Glonek 2007).

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There is a growing body of nutrition research in the field of ultra-endurance sports, but the focus is often on races where participants have access to exogenous food supplies through aid stations and/or crew assistance. Nutritional intake during a self-sufficient multi-stage race is restricted to what the participant is prepared to carry from the first day. This is, to our knowledge, the first study to quantify energy, CHO intake and β-HB during a fully self-sufficient multi-stage ultramarathon. The aim of this study was to determine changes in blood β-HB concentration during five days of a self-supported multi-day ultramarathon in combination with quantification of energy and macronutrient intakes.

Methods

Ethics statement

Ethical approval was granted by the University of Westminster FST Research Ethics Committee (Application VRE1516-0780). All work was performed in accordance with the principles of the Declaration of Helsinki and participants gave written, informed consent.

Participants

Participants were recruited from the pool of registered competitors via an email sent out by the race organisers. Details of the study were also posted on a social media platform with a request for volunteers.

Race conditions

The study was conducted during a 7-day self-sufficient, multi-stage ultramarathon in the Namibian Desert in May 2016. Race organisers provided shelter for sleeping (10 person canvas

tents) and plain water at overnight campsites and plain water only at aid stations positioned approximately 10 km apart on the course. Competitors were required to carry all other personal and mandatory equipment, including food, in a backpack for the duration of the race. Race regulations stipulated a minimum food requirement of 14 000 kcal for the entire race.

Course terrain was predominantly sandy (beach, dunes) with some vehicular dirt track, rocky sections and salt pans (hard packed mud and coral-like terrain). Recorded temperatures ranged between 16°C at night and 35°C during the day (mean daytime temperature 27°C ± 4°C). Humidity ranged from 25% to 51%.

The competitors took seven days to complete the race, which totalled 250 km. This investigation took place during the first five days of the race during which the participants completed a total of 240 km. Each day commenced at 08:00 and stage distances for the first four days were: 38 km, 42 km, 42 km and 41 km. The fifth day, known as 'the long stage', was 77 km and competitors were allowed 27 hours to complete the distance. This format is characteristic of this series of races, and in practice results in most competitors having a full 'rest' day on day six which is when the final measures were taken. Upon completion of the 10 km stage on day 7 (which was not included in the study) food and drink were provided by race organisers and then participants had a two hour bus ride back to the host town

Study design

Baseline measures were taken one day prior to the race (pre-race) and on the 'rest day' (day six), a minimum of 12 hours following the finish of the long stage. Blood and food diary data was collected on days one to five. A schematic of the study protocol is provided in Figure 1.

152 *** Figure 1 about here ****

153

154 ***Performance***

155 Although this study was not interventional, data collection took place during a competitive race
156 event. Therefore, finishing times were converted into average velocity ($km \cdot hr^{-1}$) and used as a
157 measure of performance. Race timings were recorded via timing chips carried by competitors
158 and official results were provided by the race organisers.

159

160 ***Anthropometry***

161 Anthropometric measures were taken with participants in their racing clothes comprised of shorts
162 (males) and shorts and bra tops (females) for both pre- and rest day measures. All participants
163 were sockless and shoeless during the measures. Height was measured pre-race to the nearest 0.1
164 cm (Seca 213 stadiometer, Seca, Birmingham, UK). Body mass was measured pre-race and on
165 the rest day to the nearest 100 g (Seca 877 flat scales, Seca, Birmingham, UK). Scales were
166 placed on a wooden board to provide a stable surface in the field.

167

168 The sum of skinfolds was determined pre-race and on the rest day using the four-site
169 Durnin/Womersley skinfold method (Durnin and Womersley 1974). Skinfold thicknesses were
170 measured on the right side of the body to the nearest 0.2 mm using Harpenden skinfold callipers
171 (Baty International, West Sussex, UK). All anthropometric measures were conducted by the
172 same investigator (technical error of measurement (TEM) of 3.5%)

173

174 ***Glucose and β-hydroxybutyrate***

175 Every day, immediately post-stage, blood glucose (GLUC) and β-hydroxybutyrate (KET) were
176 measured via capillary sampling from the fingertip using two CardioChek analysers (Polymer

177 Technology Systems, Indianapolis, USA) and PTS Panels single-analyte test strip. Each analyser
178 was specific either to GLUC or KET throughout the study. Limits of detection for GLUC and
179 KET were 1.11-33.3 $mmol \cdot L^{-1}$ and 0.19 - 6.72 $mmol \cdot L^{-1}$ respectively. Analyser testing using
180 check strips was performed daily on both analysers.
181
182 Blood samples were collected with participants in a standardised seated posture, immediately on
183 crossing the finish line of the stage. Ketosis was defined as a blood β-hydroxybutyrate
184 concentration of ≥ 0.5 $mmol \cdot L^{-1}$ (Volek et al. 2015).
185
186 Whilst the assessment of urinary ketones is a convenient and cost effective method in the field,
187 hyper- and hypohydration, both common issues in ultra-endurance events (Hoffman and
188 Stuempfle 2014; Hoffman et al. 2012), can result in false negatives and false positives
189 respectively (Brewster et al. 2017). Urine strip testing is subjective, semi-quantitative and cannot
190 control for how long urine has been sitting in the bladder. Blood analysis of ketones however
191 provides a quantifiable indication of current metabolic state through circulating β-HB.
192
193 ***Pack weights***
194 All competitors in the race had their packs weighed during check-in and results were provided
195 to the investigators by the race organisers.
196
197 ***Food diary and energy intake***
198 Participant food intake was restricted to what they chose to carry on day one, therefore similar
199 to the method employed in Stuempfle et al. (2013), individualised food diaries itemising every
200 food product carried on day one were prepared for each participant. The diary was provided to
201 participants at the end of each day for them to identify what and how much they had eaten, as

well as noting if food had been lost, thrown away or exchanged with/obtained from, another competitor. Food diaries were then collected by the researcher following the last meal of the day prior to the participant retiring for the evening. Analysis of the energy content and macronutrient profile of foods was performed using Nutritics® dietary analysis software (v1.8, Nutritics Ltd, Dublin, Ireland). All packaged foods were analysed according to manufacturer provided data. Non-packaged foods were entered using equivalent foods existing in the database. Approximately 1 week postrace, participants were sent an email with their nutrition data and asked for clarifications and corrections.

Estimated energy expenditure

To provide a conservative estimate of total daily energy expenditure so as not to overestimate differences in energy balance, three components were calculated for each participant for each day of the study.

1. Sleeping: Predictive equations were used to estimate basal metabolic rate (BMR). The Cunningham (Cunningham 1980) and Harris Benedict (Harris and Benedict 1918) equations are recognised as being appropriate for athletic populations (Thomas et al. 2016), with the former more suitable for females and the latter for males (Jagim et al., 2017). It was assumed that participants slept for 8 hours per 24-hour period.
2. Racing: Metabolic Equivalent of Task (METs) (Ainsworth et al. 2011) were used to calculate the energy expenditure during racing each day based on average moving speed. Participant weight for day one was defined as pre-race weight plus starting backpack. Weight for subsequent days was calculated as pre-race weight plus starting pack weight minus average daily food weight (food eaten the previous day).

3. Rest: The remaining time (24 hours minus sleep and racing time) was defined as 'rest' and calculated at 1.3 METs (reclining, talking) (Ainsworth et al. 2011).

Two competitors took ~20 hours to complete the 'long stage' (day 5). In this instance, the assumptions were 20 hours racing, 1 hour 'rest' post-racing and 3 hours sleep for the 24 hour period. All other participants took less than 15 hours and were therefore estimated to have 8 hours of sleep and at least one hour of rest on this day.

Although the thermal effect of food accounts for approximately 10% of total daily energy expenditure, various factors such as body composition, macronutrient profile, meal timing, exercise and stress can all influence the metabolic response to feeding (Secor 2009). Therefore, rather than adding a blanket 10% to all estimates of energy expenditure this component has been excluded. The authors recognise this may result in underestimated energy expenditure and resultant calculated deficits.

Statistical analysis

Statistical analysis was completed using GraphPad Prism 7.4 for Windows (GraphPad Software, La Jolla California USA). All data was tested for normality using Shapiro-Wilk normality tests. Energy intake on day 5 did not pass the test for normality. Repeated measures ANOVA with Tukey's post-hoc analysis was used to determine differences in variables between stages (energy intake and blood measures). Pre-post measures were analysed using paired t-tests and Cohen's d was calculated for effect size.

Relationships between variables were determined using Pearson's correlation coefficient. In the case of non-parametric data (energy intake on day 5) Spearman's rank coefficient was utilised.

Relationship strength was classified for the absolute r-value using thresholds of 0.1, 0.3 and 0.5 for small, moderate and large respectively (Hopkins et al. 2009). Significance was set at $p < 0.05$. Data are presented as mean ± standard deviation (SD).

Results

Seventeen participants from a field of 219 entrants volunteered for the study, of which 13 were included in the final analysis (Table 1). Two withdrew from the race for reasons unrelated to the study (injuries sustained while running), one participant elected to withdraw from the study but continued with the race, and the nutritional data collected from one participant was incomplete and as such, their data was excluded from analysis. The 13 remaining participants represented 6.6% of the finishing field: eight males (5% of male finishers) and five females (11% of female finishers), All participants had trained for the event, had previous ultramarathon experience and none reported cardiovascular or metabolic disorders..

*** Table 1 about here ***

Estimated energy expenditure and energy and macronutrient intakes

Average total estimated energy expenditure for five days of racing was 113.6 ± 23.6 MJ which equates to 22.7 ± 6.6 $MJ \cdot day^{-1}$ and 2.3 ± 0.9 $MJ \cdot hour^{-1}$ during the racing periods of the day.

Compared to the race rules that stipulated competitors must start the race carrying food providing a minimum of 14 000 kcal (58.6 MJ), participants carried an average of 63.6 ± 10.5 MJ on day one (10.6 ± 1.7 $MJ \cdot day^{-1}$). Data from two participants were considered as outliers (>2 SD from

the mean) with one participant carrying 39.8 MJ and one carrying 88.5 MJ). The mean total energy content of the food carried by the 11 other participants was 63.5 ± 3.6 MJ.

Mean energy intake over five days of racing was 48.0 ± 10.0 MJ (9.6 ± 2.6 MJ·day^{-1}, range: 4.75 -11.5 MJ). All participants were in negative energy balance of 64.6 ± 22.2 MJ (12.9 ± 6.3 MJ·day^{-1}) after five days (range: -44.6 to -2.7 MJ Over the course of the race, no participant consumed all the food they carried from the first day.

Participants consumed 301 ± 106 g·day^{-1} CHO, contributing to 53% of total energy intake. Mean fat (FAT) intake was 85 ± 33 g·day^{-1} and protein (PRO) 85 ± 35 g·day^{-1} representing 32% and 15% of total energy intake respectively. Corrected for body mass, participants consumed 4.3 ± 1.8 g·kg^{-1}·day^{-1} CHO (range 1.6 to 9.1 g·kg^{-1}·day^{-1}), 1.2 ± 0.5 g·kg^{-1}·day^{-1} FAT (range: 0.6 to 2.0 g·kg^{-1}·day$^{-1)}$, and 1.2 ± 0.6 g·kg^{-1}·day^{-1} PRO (range: 0.4 to 2.6 g·kg^{-1}·day$^{-1)}$. Neither absolute nor corrected intakes of energy (Figure 2A) nor macronutrients (Figures 2B, 2C and 2D) differed between stages ($p > 0.5$).

*** Figure 2 about here ***

Blood glucose and β-hydroxybutyrate

Mean GLUC measured at the end of each stage of the race was 4.8 ± 1.0 mmol·L^{-1}. Mean daily concentrations are presented in Figure 3. Blood glucose did not differ between baseline (5.1 ± 0.97 mmol·L^{-1}) and race days ($p > 0.2$). GLUC indicating hypoglycaemia (< 3.2 mmol·L^{-1} (Mitrakou et al. 1991)) was reported once, in one participant on day five.

No participant registered blood β-HB > 0.5 mmol·L^{-1} pre-race. Ketosis was observed in four participants on day one and all participants on days two, three and five. There was a significant decrease in KET concentration on day four compared to days three and five in all participants (p < 0.001). On day four, three participants had KET < 0.5 mmol·L^{-1}. However, mean concentrations remained significantly higher than baseline (p < 0.002). Three participants (all different to those three with low KET on day four) did not meet the criteria for ketosis on the rest day at least 12 hours following the finish of the long stage. Mean KET during the race was 1.1 ± 0.6 mmol·L^{-1}.

*** Figure 3 about here ***

Performance

Participants took 39.9 ± 7.1 hours to complete the 240 km run during the study period at an average velocity of 6.35 ± 1.0 km·hr^{-1} including all stops at checkpoints along the course.

Pack weights

Mean pack weight for the full field of competitors that finished was 9.6 ± 1.9 kg (range 5.6 – 19 kg). Pack weight of the top 10 finishers was significantly lighter than those that finished in places 11 – 196 (7.66 ± 1.2 kg vs -9.7 ±1.9 kg, p = 0.006).

Correlations

Neither absolute nor relative intakes of CHO or FAT correlated with KET (CHO: absolute r = 0.03, p = 0.9, relative r = -0.004, p = 0.99; FAT: absolute r = -0.14, p = 0.65, relative r = -0.20, p = 0.50). There was a strong, negative relationship between both absolute PRO intake and KET (r = -0.61, p = 0.03) and relative PRO intake and KET (r = -0.54, p = 0.056).

Whilst there were no significant relationships between either daily energy intake or deficit ($MJ \cdot day^{-1}$) and GLUC (intake r = -0.19 p = 0.53, deficit r = -0.12 p = 0.71) or KET (intake r = -0.28, p = 0.35, deficit r = -0.27, p = 0.36), there was a moderate relationship between the cumulative energy deficit and KET (r = -0.44, p = 0.0002).

Performance was not correlated with mean KET (r = 0.22, p = 0.47) but had a strong relationship with total CHO intake (r = 0.62, p = 0.02).

As the first significant increase in KET occurred on day two, a Pearson correlation analysis was used to assess the relationship between overall performance and the magnitude of β-HB concentration increase from baseline to day two. There was a large positive relationship between the magnitude of β-HB increase and overall performance that approached significance (r = 0.54, p = 0.06, figure 4).

*** Figure 4 about here ***

Discussion

To the best of our knowledge this is the first study to investigate blood glucose and β-hydroxybutyrate (β-HB) concentrations throughout a fully self-sufficient multi-stage ultramarathon and the influence of nutritional intake on these substrates. The main finding is that all participants in this study entered a state of ketosis within two days of the race commencing, with ketosis not correlated with CHO intake.

Historically, an energy deficit *per se* has not been considered enough to induce ketosis (Consolazio et al. 1968): a concomitant reduction in CHO availability to less than 100g must occur. Here we show that despite a similar caloric deficit as that induced by Consolazio et al. (1968) ketosis still occurred in all participants despite a mean intake of 301 ± 106 $g \cdot day^{-1}$. Furthermore, ketosis was still evident in participants who consumed up to 600 $g \cdot day^{-1}$ of CHO. This lack of relationship between macronutrient intake and ketosis but large relationship between cumulative energy deficit and ketosis suggests that the magnitude of the ongoing energy deficit and the manner in which it is induced (i.e. exercise induced vs energy restriction) may play a greater role than previously appreciated.

Prior studies have also noted the presence of urinary ketones in ultra-endurance athletes despite apparently adequate CHO intakes (Costa et al. 2014; Costa et al. 2013; Jablan et al. 2017; Weibel and Glonek 2007). Costa et al. (2013) noted that 46% of runners in a multi-day ultramarathon (5 days, 225 km) presented with urinary ketones indicative of ketosis at least once during the race despite CHO intakes of 520 $g \cdot day^{-1}$ (7.5 $g \cdot kg \cdot day^{-1}$). Likewise, in a 24 hour ultramarathon, urinary ketones were present in 90% of runners whose average CHO intake was 881 g (37 $g \cdot hr^{-1}$) (Costa et al. 2014). Weibel and Glonek (2007) found that in a six day race, 22 of 31 study participants produced urinary ketones, and although dietary intake was not recorded, they observed that some participants produced urinary ketones despite apparent high CHO intake. Likewise Jablan et al. (2017) reported a significant increase in urinary ketones in 81% of participants following a mountain ultra-marathon (mean race time 8.40 ± 1.28 hours) although CHO intake was not quantified. All these studies have attributed the presence of urinary ketones to insufficient CHO intake, but have not further explored the physiological implications of these findings. We suggest that rather than simply being an indication of participants not meeting CHO recommendations for performance, ketosis may be inevitable during certain ultra-endurance

events and that athletes will compete in a different physiological state than that which can be assumed for races where external supplies are available. This has practical implications for the self-sufficient, multi-stage athlete who must then manage the negative impacts of such an extreme dietary and metabolic shift whilst competing in an already physiologically stressful situation.

Although fat-oxidation rates have been shown to double following a ketogenic diet, this has not translated into performance improvements for elite endurance athletes (Burke et al. 2017; McSwiney et al. 2018). However, a benefit may exist for ultra-endurance athletes who are working at lower intensities for much longer periods of time with low CHO availability (Burke 2015). Recent evidence also suggests that long-term 'keto-adapted' athletes (those regularly in a ketogenic state for at least 6 months) not only have equivalent glycogen stores compared to athletes on high CHO diets, but are also able to replenish these stores in the absence of dietary CHO (Volek et al. 2016). This may be a crucial benefit for multi-stage athletes trying to recover for subsequent days of racing and suggests that incorporating periods of ketosis into training periods may better prepare athletes for these types of competitions. It is plausible that when confronted with such large energy deficits, athletes with greater metabolic flexibility, and specifically the ability to produce and utilise ketones more quickly would perform better. Our results show a trend in this direction and since to the best of our knowledge there are no studies quantifying the 'efficiency' of ketosis (how quickly people may become ketogenic without side effects), this deserves further exploration in future research. These results also raise the question of whether athletes might benefit from starting their events already in nutritional ketosis (without energy restriction) to avoid the adaptation period while racing.

In the present study we report a strong relationship between CHO intake and performance. Carbohydrate is undoubtedly ergogenic and we would expect to see increased CHO intake improve performance in ultra-endurance events (Mahon et al. 2014; Stellingwerff and Cox 2014). However, nutritional intakes in this study were constrained by food choices made by the participants prior to starting the race. While evening meals were of similar composition across the cohort (typically commercial freeze-dried meals) the faster competitors took a higher proportion of 'sports nutrition' products (gels, bars) for daytime consumption which were higher in CHO than 'real' foods taken by the slower competitors (meat jerky, nuts, seeds, cheese). It therefore cannot be ruled out that CHO intake may have been coincidental to better performance rather than causal. Since total weight of food is a key consideration, it is unclear as to whether CHO or total energy should be prioritised when optimising race nutrition for a self-supported event. The magnitude of the energy deficit induced by physical exercise in these events suggests that increasing energy content using energy dense high fat foods may improve performance, but the performance benefits of CHO are undeniable, as long as they are available.

There is little data on the relationship between increased pack weight through increased nutritional supplies (and therefore intake) and performance in multi-stage races. A recent case-study suggests the benefits of increased energy consumption outweigh the detriments associated with an increase in pack weight (Alcock et al. 2018), although carrying enough food to meet energy requirements resulted in a pack weight of 14 kg. This contrasts with the mean 7.6 kg carried by the top 10 competitors in this race and is more than 2.5 times the weight of the pack of the winning competitor. The current literature provides little incentive to increase weight carried if athletes are already performing well with much lighter packs and concomitant reduced energy consumption. In these circumstances, these findings should direct future research into

appropriate training and preparation to attenuate potential ketogenic adaptation issues during races and optimise performance.

β-hydroxybutyrate levels were not different from baseline on day one, however, we observed a significant 5.4 fold increase from baseline on day two (0.25 ± 0.08 mmol·L^{-1} to 1.35 ± 0.60 mmol·L^{-1}, $p = 0.0003$), which rose to a 6.4 fold increase from baseline on day three (0.25 ± 0.08 mmol·L^{-1} to 1.6 ± 0.57 mmol·L^{-1}, $p < 0.0001$). All participants exhibited reduced KET on day four, albeit still significantly higher than baseline, and KET increased again on days five and six. The universal drop in KET on day four was unexpected and does not have a ready explanation given that it was unrelated to macronutrient intakes or changes in physical activity. Although speculative, the combined GLUC and KET pattern is indicative of the starvation response as identified by Cahill (1976), the initial stages of which have distinct adaptation phases. Previously consumed meals will provide fuel for up to eight hours. During the subsequent 24 - 48 hours, liver glycogen is used to maintain glucose homeostasis and ketone production increases. Thereafter, as liver glycogen is depleted, gluconeogenesis increases while ketone utilization reduces the demand for glucose from tissues. This results in a temporary increase in blood glucose and concomitant drop in ketone production. After four to five days, major adaptations to energy metabolism occur and ketone utilization increases with a concurrent reduction in gluconeogenesis (Cahill 1976). The starvation response has previously been identified in athletes participating in a 1 230 km ultra-endurance cycling event (Geesmann et al. 2017). During the 54 hour event, Geesmann et al. (2017) found that an energy deficit of 23.2 ± 19.1 MJ resulted in the suppression of testosterone, leptin and IGF-1. In some athletes these remained supressed for up to three days despite *ad-libitum* intake during recovery. Given the large energy deficits accumulated over five days by participants in this study, further research into the effects of starvation on the metabolic and hormonal health of ultra-endurance athletes is warranted. This is

447 of particular concern for athletes who train for, and compete in, several self-sufficient multi-
448 stage ultra-endurance events per year.
449
450 While a major strength of this study was its applied nature, contributing to understanding
451 metabolic shifts in athletes in a real-world, competitive environment, limitations must be
452 considered. .Energy expenditure was not directly quantified but the estimated energy
453 expenditure of 2.3 $MJ \cdot hr^{-1}$ whilst racing is in line with previous studies on ultra-endurance racing.
454 These include a 24 hr trail race (2.3 $MJ \cdot hr^{-1}$) (Costa et al. 2014), a 24 hr lab-simulated adventure
455 race (3.1 $MJ \cdot hr^{-1}$), and a 6 day adventure race (2.1 $MJ \cdot hr^{-1}$) (Enqvist et al. 2010). The average
456 daily energy expenditure in our study (22.7 $MJ \cdot day^{-1}$) was larger than previously reported for a
457 multi-day ultramarathon of a similar format (5 days, 225 km, 16.0 to 20.0 $MJ \cdot day^{-1}$) (Costa et al.
458 2013) although in the study of Costa et al. (2013) overall distance and running time was less than
459 our study. Additionally, participants in the study of Costa et al. (2013) had their food and
460 equipment transported each day meaning that they could take, and thus consume, more food
461 (intake in our study: 9.6 ± 2.6 $MJ \cdot day^{-1}$ compared to Costa et al. 2013: 14.0 ± 3.1$MJ \cdot day^{-1}$).
462 Furthermore, this daily transportation of provisions also means load carriage was reduced,
463 potentially resulting in a lower energy expenditure (Lucas et al. 2016), given that the cohort in
464 the present study had a mean starting pack weight of 8.6 kg. Therefore, we believe estimates of
465 energy expenditure and subsequent deficits in the present study are reasonable.
466
467 Due to logistical constraints in the field, morning pre-stage data collection of blood substrates
468 was not possible but would have added a greater understanding of changes over the course of the
469 race. Furthermore, although capillary testing is a well-recognised method of measuring ketones
470 (Brewster et al. 2017), venous blood samples could take into account changes in blood plasma
471 volume as well as identifying other biomarkers. It has been shown that blood plasma volume

increases over the course of a multi-stage ultramarathon (Alcock et al. 2018; Costa et al. 2014) suggesting that the participants' ketone levels may have been even higher than recorded. However, given the remote nature of this race the logistics of storing, transporting and analysing whole blood was beyond the scope of the study.

Conclusion

Health and performance related issues in ultra-endurance athletes have been well established and the focus is currently on solving these issues (e.g. gut-training for optimal CHO intake). Unlike races where optimal nutritional strategies may be applied through external access to food self-sufficient multi-stage ultra-marathons restrict intake to the load the athlete is prepared to carry from day one.. This is the first study to document changes in blood glucose and β-HB concentrations and concomitant nutritional intakes during a self-sufficient multi-stage ultra-marathon. We showed that all participants were ketogenic by day two. This suggests that rather than being a nutritional choice, competing in a state of ketosis may be unavoidable in multi-stage events where load carriage considerations encourage energy and CHO restriction. .Given the potential negative impacts associated with such an extreme metabolic shift in athletes unaccustomed to such restriction (fatigue, increased perceived effort and changes to the hormonal milieu), prior keto-adaptation could be a useful strategy to improve health and performance in these athletes, however further work is required to elucidate the benefits of such an approach.

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Table 1: Participant characteristics

	Participant Characteristics (n= 13, 8 males, 5 females)		
	Pre-race	**Day 6**	**Cohen's *d***
Age (years)	40 ± 8	-	
Height (cm)	175.1 ± 8.1	-	
Body mass (kg)	73.1 ± 11.8	70.6 ± 11.6 *	0.1
Sum of 4 skinfolds (mm)	38.1 ± 12.2	32.0 ± 10.8 *	0.3
Starting pack weight (kg)	8.6 ± 1.3	-	

Note: Mean ± SD; * $p < 0.001$ vs pre-race;

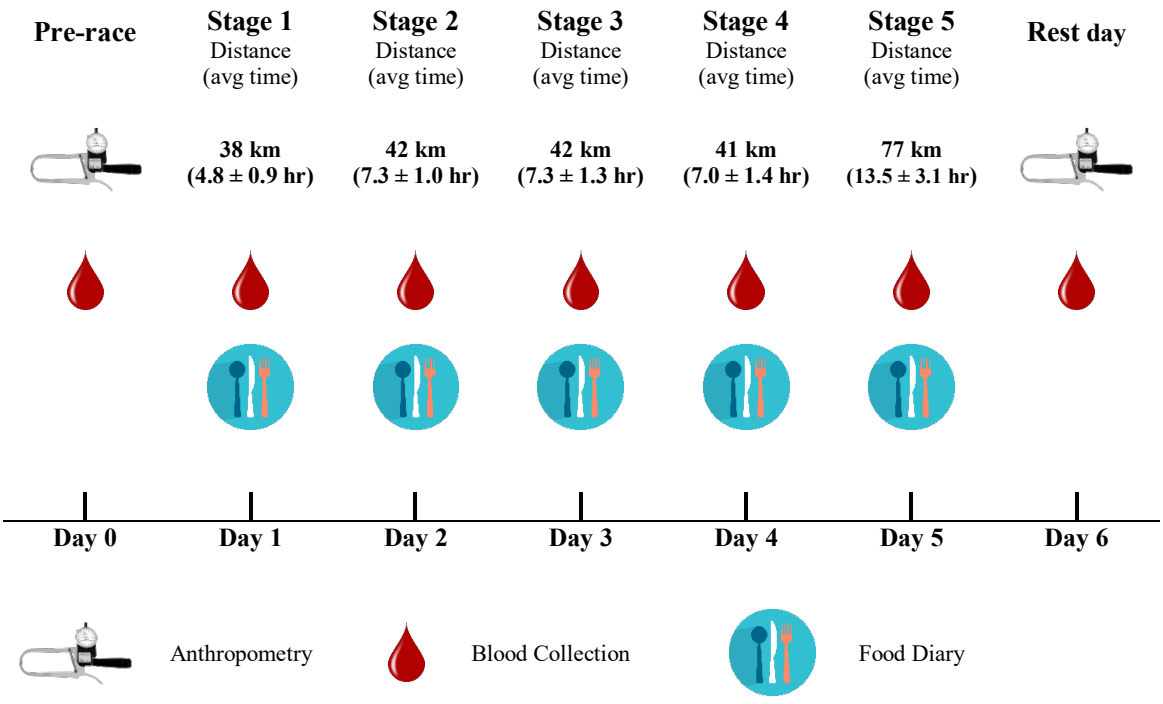

Figure 1. Schematic of study protocol. Blood samples were taken immediately post-stage every day at the finish line

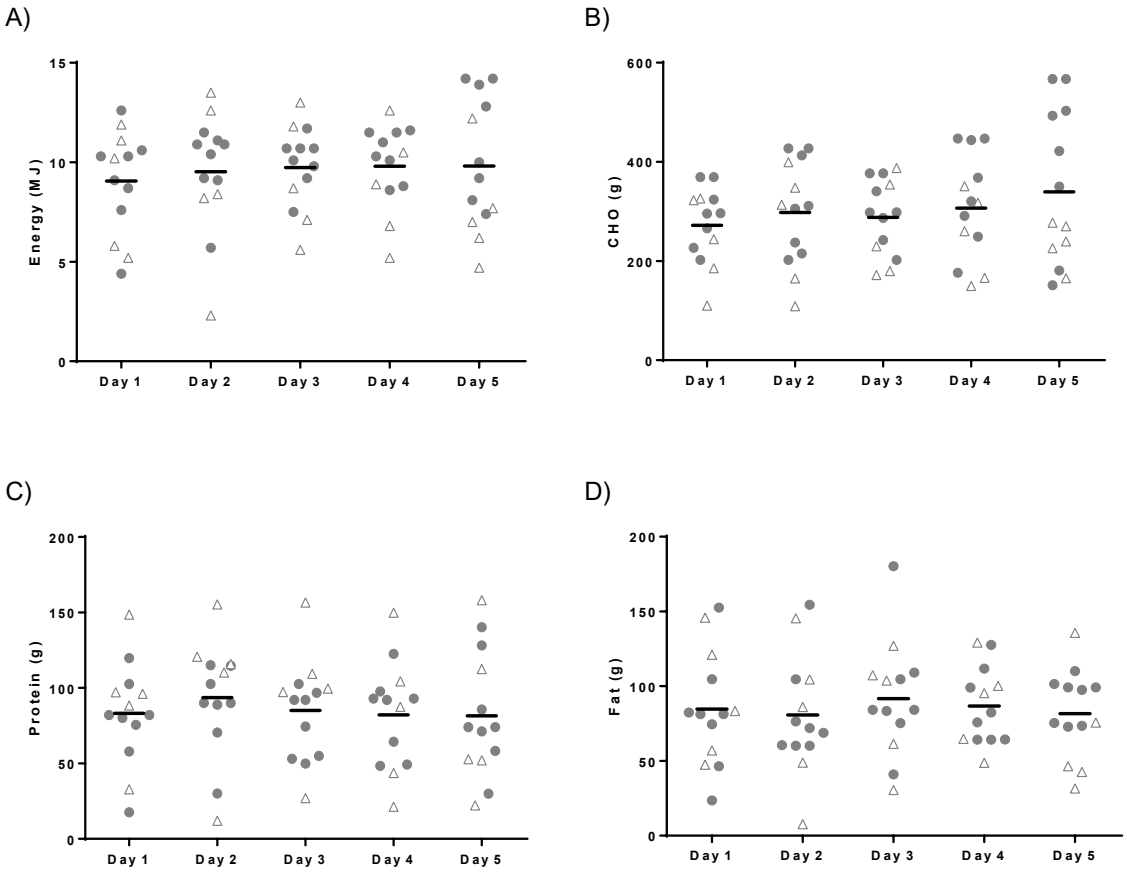

Figure 2: Mean daily intakes of (A) energy, (B) CHO, (C) PRO and (D) FAT. Δ Female; ● Male. Bars indicate mean intake

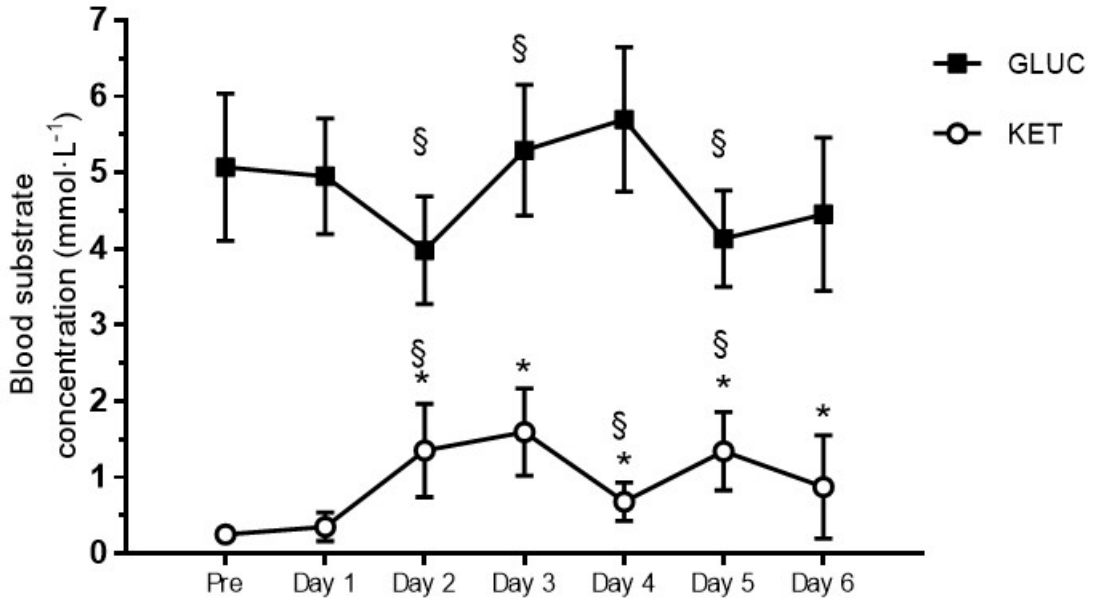

Figure 3. Blood GLUC and KET concentrations. * denotes a significant change from baseline. § denotes a significant change from the day before. Data are presented as mean ± SD.

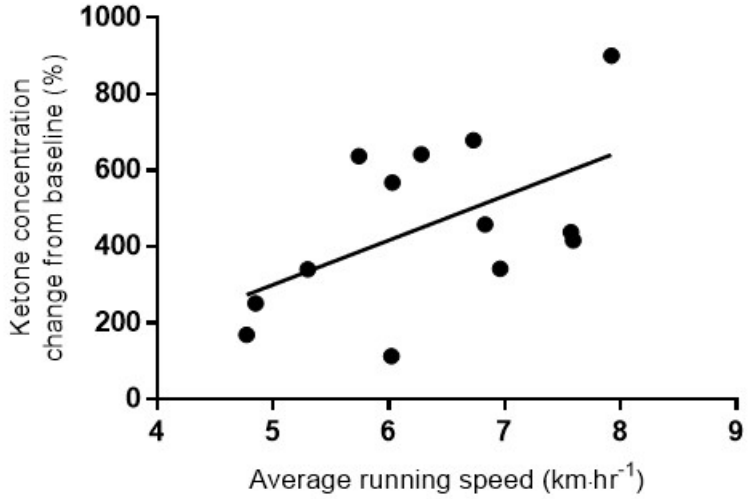

Figure 4. The relationship between the change in ketone concentration from baseline on day two and overall performance as defined by average speed throughout the race (r = 0.54, p = 0.06)