

Compromised Energy and Macronutrient Intake of Ultra-endurance Runners During a Multi-stage Ultra-marathon Conducted in a Hot Ambient Environment

Ricardo J. S. Costa^{1,2,*}, Abigail J. M. Swancott², Samantha Gill², Joanne Hankey², Volker Scheer³, Andrew Murray⁴, Charles D. Thake²

¹Department of Health Professions, Coventry University, Priory Street, Coventry, CV1 5FB, United Kingdom

²Sport & Exercise Science Applied Research Group, Coventry University, Priory Street, Coventry, CV1 5FB, United Kingdom

³Sports Medicine Department, University of Heidelberg, Im Neunheimer Feld 710, 69120 Heidelberg, Germany

⁴Sports Scotland Institute of Sport, Airthrey Road, Stirling, FK9 5PH, United Kingdom

Abstract Energy and macronutrient intake of ultra-endurance runners (UER n=74; control (CON) n=12) during a 5-days 225km multi-stage ultra-marathon (MSUM) in the heat (T_{max} 32-40°C), were determined through dietary recall interview and analysed by dietary analysis software. Body mass (BM) and urinary ketones were determined pre- and post-stage. Recovery, appetite and gastrointestinal symptoms were monitored daily. Pre-stage BM, total daily energy (overall mean: 3348kcal/day), protein (1.5g/kgBM/day), carbohydrate (7.5g/kgBM/day) and fat (1.4g/kgBM/day) intakes did not differ between stages in UER. CON presented a daily macronutrient profile closer to benchmark recommendations than UER. Carbohydrate intake pre-stage (102g), during running (24g/h) and immediately post-stage (1.7g/kgBM), and protein intake post-stage (0.3g/kgBM) did not differ between stages, and were below benchmark recommendations in the majority of UER. Post-stage urinary ketones increased in UER as competition progressed (Stage 1: 16% vs. Stage 5: 32%). Gastrointestinal distresses and appetite suppression were reported by 85% and 72% of UER, respectively, along the MSUM. Correlations between subjective symptomology, energy and carbohydrate intakes were observed in UER ($P<0.05$). Sub-optimal macronutrient profile, carbohydrate intake, and recovery nutrition throughout the MSUM suggests energy quantity and quality may be compromised in ultra-runners along competition; indicating that specialised nutritional education may be beneficial in this population.

Keywords Heat, Running, Endurance, Gastrointestinal, Appetite, Carbohydrates

1. Introduction

Multi-stage ultra-marathon (MSUM) events have increased in popularity over the past decade, and are predicted for future growth within recreational endurance sports participation; especially amongst endurance enthusiasts that have successfully completed marathon and triathlon events (www.racingtheplanet.com). MSUM events are unique as they present additional challenges to ultra-runners. Not only are participants required to perform loaded (e.g. pack weight ranging from 5 to 15 kg) prolonged strenuous exercise, and sleep rough (e.g. outdoors, tents, and/or sports halls), on consecutive days (commonly ranging

from 5 to 8 days); but are also required to carry, prepare, and consume sufficient foods and fluids to maintain optimal exercise performance throughout competition. Associations between sub-optimal nutritional status and decrements in exercise performance have previously been well established, highlighting the importance of consistently meeting nutritional requirements on consecutive days of ultra-marathon competition[1]; especially during periods of greater endogenous energy solicitation[2,3].

Nutritional recommendations aimed at preventing fatigue, and subsequently attenuating decrements in endurance exercise performance have previously been developed to guide dietary strategies and aid endurance athletes meet their nutritional needs. For consecutive days of prolonged endurance exercise, achieving energy balance is recommended, alongside the provision of sufficient carbohydrate (CHO) to meet exercise load demands (up to 10 g CHO/kgBodyMass(BM)/day), and consumption of

* Corresponding author:

aa6914@coventry.ac.uk (Ricardo J.S. Costa)

Published online at <http://journal.sapub.org/sports>

Copyright © 2013 Scientific & Academic Publishing. All Rights Reserved

sufficient protein (PRO) to meet daily nitrogen balance (1.2 to 1.4 gPRO/kgBM/day)[1,4,5]. With regards to specific macronutrient intake and timing, >200 g of carbohydrate up to 2 h before prolonged strenuous exercise is recommended for consumption[6,7], and is thought to be particularly beneficial if carbohydrate intake during recovery fails to fully restore muscle glycogen storage, and additionally provides exogenous carbohydrate during the initial phase of exercise[8]. The consumption of 30 to 60 gCHO/h, in an individualised tolerable form, is recommended for endurance exercise lasting ≥ 2 h, with the aim of maintaining blood glucose concentration and sparing muscle glycogen stores; contributing towards the maintenance of exercise workload [9,10]. Immediately post-exercise the consumption of 1.0 to 1.5 gCHO/kgBM is recommended to assist muscle glycogen resynthesis, with some additional protein (up to 0.5 gPRO/kgBM) to aid repair and healing of exercise-induced tissue damage[5,11]. Moreover, carbohydrate and protein immediately after prolonged strenuous exercise has been shown to attenuate the exercise-induced depression in innate immune responses (e.g. neutrophil degranulation)[12,13] involved in tissue repair, wound healing, and prevention of illness/infection that commonly accompanies endurance exercise[14].

Anecdotal evidence suggests ultra-runners may not be following these recommendations during MSUM competition set in hot ambient conditions (2009 *Al Andalus Ultimate Trail*, Loja, Spain). This may be due to the lack of nutritional education, ultra-endurance sports cultural trends, development of unintentional symptoms (e.g. exercise and environmentally induced appetite suppression, taste fatigue, nausea, involuntary vomiting and other gastrointestinal distresses), and/or practical real-life factors (e.g. lack of food preparation facilities, equipment, location, time and/or motivation), associated with limiting total food and fluid intake during consecutive days of competition in extreme environmental conditions[15-18]. Interestingly, some observational evidence suggests faster ultra-runners generally tolerate greater food and fluid ingestion during ultra-marathon events compared with slower runners[19-21]. Moreover, the multitude of stressors including: strenuous exercise, phases of food and fluid rationing (acute under-nutrition and hypohydration), sleep disturbances, environmental extremes, accumulated fatigue and minor tissue injuries (e.g. blisters, abrasions, sunburn) that accompanies MSUM, individually and in combination have the potential to substantially increase nutritional requirements and/or exacerbate factors that would limit overall food and fluid ingestion[3,16,22,23].

Considering that most of the nutritional recommendations for endurance exercise are derived from controlled laboratory settings and generally amongst highly trained elite athletes; the majority of the ultra-marathon competitive population are recreational amateurs. To date, comprehensive research on the dietary practices of recreational ultra-runners during one-stage and multi-stage ultra-marathon running events is absent. It is therefore

plausible that current recommendations for endurance exercise may need adjusting to cater for the unintentional symptomology, real-life practical barriers, and specific race characteristics (e.g. degree of self sufficiency, environmental conditions and/or course topography) experienced by ultra-marathon competitors. With this in mind, the aims of the current observational study were to assess the adequacy of energy and macronutrient intake of ultra-runners during a semi self-sufficient MSUM conducted in a hot ambient environment.

2. Methods

2.1. Setting, Participants, and Experimental Design

The study was conducted during the 2010 and 2011 *Al Andalus Ultimate Trail*, held during the second week of July, in the region of Loja, Spain. The MSUM was conducted over five stages (5-days) totalling a distance of 225 km (Table 1), which was performed on a variety of terrains; predominantly off-road trails and paths, but also included steep and narrow mountain passes and occasional road. Sleeping arrangements from Stages 1 to 5 included a combination of outdoor tent and village sports hall accommodation.

After ethical approval from the Coventry University Ethics Committee that conforms with the 2008 Helsinki declaration for human research ethics, a convenience sampling observational cohort was studied, whereby 74 out of 134 ultra-endurance runners entered into the MSUM competition volunteered to participate in the study (mean: UER (Male $n=46$, Female $n=28$): age 41 ± 8 years, height 169 ± 14 cm, BM 70 ± 11 kg, body fat mass $17\pm 5\%$). Additionally, 12 age and anthropometrically matched individuals who accompanied the UER along the MSUM course, but did not compete (absence of exercise stress), volunteered to participate in the study as part of the control group (CON (Male $n=5$, Female $n=7$): age 35 ± 13 years, height 167 ± 9 cm, BM 70 ± 16 kg, body fat mass $21\pm 6\%$), for comparison only. For the purpose of data analysis, participants were divided into two groups. A slow group (SR), who completed the entire distance of the MSUM using a mixture of walking and running (overall mean speed < 8 km/h); and a fast group (FR), who completed the majority of the MSUM distance running (overall mean speed ≥ 8 km/h). This criterion was predetermined, and participants were grouped according to their overall race time, prior to data analysis.

Height was measured by a wall-mounted stadiometer. Baseline BM was determined using calibrated electronic scales (BF510, Omron Healthcare, Ukyo-ku, Kyoto, Japan) placed on a hard levelled surface. Waist and hip circumferences were measured using a standard clinical tape measure by trained researchers. BM and circumference measures were used in conducting multi-frequency bioelectrical impedance analysis (Quadscan 4000, Bodystat, Douglas, Isle of Man, UK), which was used to determine body composition.

The current MSUM was semi self-sufficient, whereby participants (including CON) planned and provided their own foods and fluids (except plain water) along the five days of competition. Participants' equipment and sustenance was transported to each stage section by the race organisation. Only plain water was provided by the race organisers *ad libitum* during the rest phase throughout competition. Additionally, aid stations along the running phase of competition were situated approximately 10 km apart, and only provided plain water, fruit (oranges and watermelon), and electrolyte supplementation (Elete electrolyte add-in, Mineral Resources International, South Ogden, Utah, US). UER and CON were advised to adhere to their programmed habitual dietary practices throughout the entire duration of the MSUM competition.

Each day, for five consecutive days, running stages commenced at either 08:00 h or 09:00 h. All participants consumed their breakfast 2 to 3 h before the start of each stage. Within the hour before the start of each running stage, pre-stage measurements were determined and samples collected. Participants were required to provide a mid-flow urine sample (2nd urine of the day upon waking) into 30 ml universal tubes (HR 120-EC, A & D instruments, Tokyo, Japan), before BM measurements. Immediately post-stage and before any foods or fluids could be consumed, BM was measured. Participants were then asked to provide a mid-flow urine sample at their earliest convenience. For consistency, the order of pre- and post-stage measurements and sampling were identical for all stages. Additionally, a final BM measurement was taken the morning following completion of the overall MSUM. To monitor carbohydrate adequacy[24], urine reagent strips (Multistix® 10SG Urinalysis strips, Siemens Healthcare Diagnostic, NY, USA) were used to identify urinary ketones (acetoacetic acid) from pre- and post-stage urine samples.

At the end of each competition day (20:00 to 22:00 h) on Stages 1 to 4, trained researchers conducted a standardised structured interview (dietary recall interview technique), on UER and CON, to ascertain total daily food and fluid ingestion. Due to practical and participant factors, it was not feasible to conduct the daily dietary assessment on Stage 5, since MSUM completion occurred within the duration of Stage 5, not completing a 24 h period. To avoid inter-observer variations, each researcher conducted the standardised structured interview on the same participant throughout the entire MSUM. Participants were educated and instructed to recall in detail all foods and fluids ingested along the competition day, which included specified food and beverage quantities (e.g. g, ml, litres, portions) and qualities (e.g. type of foods-beverages, brands of foods-beverages) ingested for breakfast (pre-stage), during the stage (during running), within the hour after stage completion (post-stage), and from the hour post-stage until sleep. Relevant nutritional information from all food-beverage packages was recorded by researchers. The

addition of carbohydrate, protein, and/or mixed macronutrient supplementations to foods and fluids was also recorded and combined with daily macronutrient intake. Additionally, gastrointestinal distress symptoms and subjective appetite sensation during running and rest periods were explored through a research generated symptomology tool on Stages 1 to 4. Participants also completed a general recovery log to determine subjective quality of recovery. The recovery log included a Likert Scale (-3 to +3, with 0 indicating a neutral response) which included: perceived recovery quality, anxiety levels, motivation, fatigue, and muscle soreness.

2.2. Dietary and Data Analysis

Total daily, pre-stage, during running, and post-stage energy and macronutrient intakes were calculated through Dietplan6 dietary analysis software (v6.60, Forestfield Software, Horsham, West Sussex, UK) by a trained dietetic researcher. To improve the validity of the dietary analysis, all the nutritional information gathered from food-beverage packages during the interview procedure was entered into the dietary analysis software program. In addition, to improve the reliability of the dietary analysis, all the completed dietary interview logs were blindly analysed by a 2nd trained dietetic researcher. The overall mean inter-observer coefficient of variation for energy and macronutrient variables analysed was 1.3% and 2.3%, respectively. In addition, estimated total daily energy expenditure was calculated through predictive equations[1] and verified through tri-axial accelerometry (SenseWear 7.0, BodyMedia Inc., Pittsburgh, PA, USA) in a sub-sample of participants, as previously used[25] to guide Sports Dietetic support during MSUM competition.

Data is presented as mean value \pm standard deviation (SD), otherwise specified. Descriptive statistics were used to explore urinary ketones, gastrointestinal symptomology, and subjective appetite sensation. Considering the potential influence of individual BM differences (especially in relation to gender and training status) on dietary intake variables, data analysis was performed on total values and corrected for BM, as previously reported[26]. A one-way ANOVA was applied to determine differences in variables between stages; while a two-way ANOVA was applied to determine differences between groups (UER vs. CON, and SR vs. FR), and between pre- and post-stage values within stages (SPSS v.17.0.2, Illinois, US). Assumptions of homogeneity were checked, with adjustments to the degrees of freedom and verification by non-parametric equivalents (Kruskal-Wallis and Friedman two-way, respectively) where appropriate. Significant main effects were analysed using post hoc Tukey's HSD test. Additionally, Spearman's correlation coefficient was used to determine relationships between variables. The acceptance level of significance was set at $P < 0.05$.

Table 1. Stage times and average speeds of ultra-endurance runners (UER) participating in a 225 km multi-stage ultra-marathon (MSUM) competition conducted in a hot ambient environment

	UER Running time (h:min) and speed (km/h)	SR Running time (h:min) and speed (km/h)	FR Running time (h:min) and speed (km/h)
Stage 1: 37 km 503 to 1443 m ASL T_{\max} : 32°C; RH_{\max} : 32%	4:54±0:51 7.6	5:29±0:35 6.8	4:10±0:28 8.9
Stage 2: 45 km 830 to 1338 m ASL T_{\max} : 34°C; RH_{\max} : 33%	6:37±1:20 6.8	7:32±1:06 6.0	5:38±0:46 8.0
Stage 3: 40 km 689 to 1302 m ASL T_{\max} : 38°C; RH_{\max} : 37%	4:59±0:53 8.0	5:37±0:39 7.1	4:15±0:27 9.4
Stage 4: 65 km 671 to 1152 m ASL T_{\max} : 40°C; RH_{\max} : 33%	7:51±1:25 8.3	8:52±1:01 7.3	6:48±0:54 9.6
Stage 5: 38 km 473 to 1065 m ASL T_{\max} : 40°C; RH_{\max} : 40%	4:16±1:05 8.9	4:49±1:05 7.9	3:35±0:35 10.6
Total: 225 km	28:03±4:34 8.0	31:52±2:51 7.1	24:22±2:20 9.2

Mean±SD: UER (n= 74); slow runners (SR: MSUM mean speed <8 km/h, n= 41); fast runners (FR: MSUM mean speed ≥8 km/h, n= 33). ASL (above sea level), T_{\max} (maximal ambient temperature), RH_{\max} (maximal relative humidity)

3. Results

No significant changes in pre- and post-stage BM were observed throughout the MSUM in UER and CON. Although MSUM participation tended to gradually reduce BM in UER (pre- to post-MSUM: UER -1.1% vs. CON 0.3%; SR -0.9% vs. FR -1.2%).

Average estimated total daily energy expenditure was calculated to range between 3831 to 4999 kcal/day in UER. Total daily energy intake did not vary significantly between stages in UER, SR and FR (Table 2). Total daily energy intake was greater in UER compared to CON on Stage 1 only; while total daily energy intake was greater in FR compared with SR on Stages 1, 3 and 4 ($P<0.001$). Although, when corrected for BM, no significant difference in total daily energy intake was observed between UER and CON (overall mean: 49±11 kcal/kgBM/day and 45±4 kcal/kgBM/day, respectively), nor between SR and FR (47±11 kcal/kgBM/day and 52±10 kcal/kgBM/day, respectively).

Total daily carbohydrate intake did not significantly vary between stages in UER, CON, SR and FR. However, a higher total daily carbohydrate intake was observed in UER compared to CON on Stage 1 only; while total daily carbohydrate intake was greater in FR compared to SR on Stages 3 and 4 ($P<0.001$; Table 2). Although when corrected for BM, no significant difference in total carbohydrate intake was observed between UER and CON (Figure 1A), nor between SR and FR (Figure 1B). Total daily protein intake did not significantly vary between stages in UER, CON, SR, and FR. Whereas, a higher total daily protein intake was observed in UER (50%) compared to CON, and in FR (23%) compared to SR, throughout the MSUM ($P<0.001$; Table 2). When corrected for BM, a significant difference in total daily

protein intake was only observed between UER and CON on Stages 1 and 3 (Figure 1A). A lower total daily fat intake was observed on Stage 3 compared with Stage 1 in UER only ($P=0.025$; Table 2). No other significant differences were observed for total daily fat intake. Total daily alcohol intake did not contribute significantly to overall energy intake in UER (53 kcal/day) and CON (0 kcal/day), and did not significantly differ between stages along the MSUM.

Total daily polysaccharide and oligo/di/monosaccharide (accounting for 39% and 61% in UER, and 33% and 67% in CON, of total daily carbohydrate intake, respectively), were similar between stages for UER and CON. Total daily fibre intake did not differ along the MSUM in UER (overall mean: 18±9 g/day), nor differ with CON (overall mean: 18±4 g/day). Total daily saturated, monounsaturated (MUFA) and polyunsaturated (PUFA) fat intakes accounted for 31%, 32% and 35% in UER, and 23%, 45% and 27% in CON, of total daily fat intake, respectively. Lower saturated fat intakes were observed on Stage 3 (23 g/day) compared with Stage 1 (36 g/day) in UER ($P<0.001$). A higher total saturated fat (29±13 g/day and 19±9 g/day, respectively) and $n6$ to $n3$ ratio (22:1±7:1 and 13:1±5:1, respectively) were observed in UER compared with CON along the MSUM ($P<0.001$).

No acetoacetic acid was identified in pre-stage urine samples throughout the MSUM in UER and CON. However, the presence of acetoacetic acid (range: 1.5 to 8.0 mmol/l) in post-stage urine samples was evident in 46% of UER at some point along the MSUM. The numbers of UER presenting post-stage urinary acetoacetic acid increased as the MSUM progressed (Stage 1: 16%, Stage 2: 22%, Stage 3: 27%, Stage 4: 30%, Stage 5: 32%). A weak but significant Spearman's correlation was observed between total daily intakes of energy ($r = -0.444$, $n = 92$, $P<0.001$) and carbohydrate ($r =$

-0.336, $n = 92$, $P = 0.001$) with presence of urinary acetoacetic acid in post-stage urine samples. No significant correlations were observed between intakes of energy and carbohydrate

(total and correct for BM) pre-stage and during running with presence of urinary acetoacetic acid in post-stage urine samples.

Table 2. Total daily energy and macronutrient intake (and overall macronutrient energy distribution (%)) of ultra-endurance runners (UER) participating in a 225 km multi-stage ultra-marathon (MSUM) competition conducted in a hot ambient environment

	Stage 1	Stage 2	Stage 3	Stage 4	Overall Mean
Energy (kcal/day)					
UER	3445±929 ^{bb}	3285±797	3252±934	3410±923	3348±750 ^b
CON	2491±338	3021±256 [†]	2886±399	3016±456 [†]	2858±159
SR	3315±971	3132±766	3023±900	3194±920	3166±734
FR	3626±850 ^c	3493±809	3601±896 ^c	3710±826 ^c	3608±701 ^c
Protein (g/day)					
UER	110±39 ^{bb}	101±33 ^{bb}	104±42 ^{bb}	105±39 ^{bb}	105±29 (13%) ^{bb}
CON	54±8	77±28	68±6	81±27	70±14 (10%)
SR	106±35	93±26	93±37	91±33	96±24 (12%)
FR	117±45 ^c	112±39 ^c	119±45 ^c	126±39 ^{cc}	118±31 (13%) ^{cc}
Carbohydrate (g/day)					
UER	515±146 ^b	511±136	529±149	527±137	520±116 (62%)
CON	400±58	487±37	469±47	484±45	460±19 (67%)
SR	491±131	483±113	494±139	496±126	491±100 (62%)
FR	551±162	552±156	581±151 ^c	572±142 ^c	564±127 (63%) ^c
Fat (g/day)					
UER	105±46	93±37	80±37 [†]	98±39	94±32 (25%)
CON	75±12	85±10	82±27	84±23	82±13 (23%)
SR	103±52	92±42	75±41	94±45	91±38 (26%)
FR	106±38	93±29	89±29	102±28	98±21 (24%)

Mean±SD: UER ($n = 54$); control (CON, $n = 12$); slow runners (SR; MSUM mean speed <8 km/h, $n = 32$); fast runners (FR; MSUM mean speed ≥8 km/h, $n = 22$). ^{††} $P < 0.01$ and [†] $P < 0.05$ vs. Stage 1; ^{bb} $P < 0.01$ and ^b $P < 0.05$ vs. CON; ^{cc} $P < 0.01$ and ^c $P < 0.05$ vs. SR

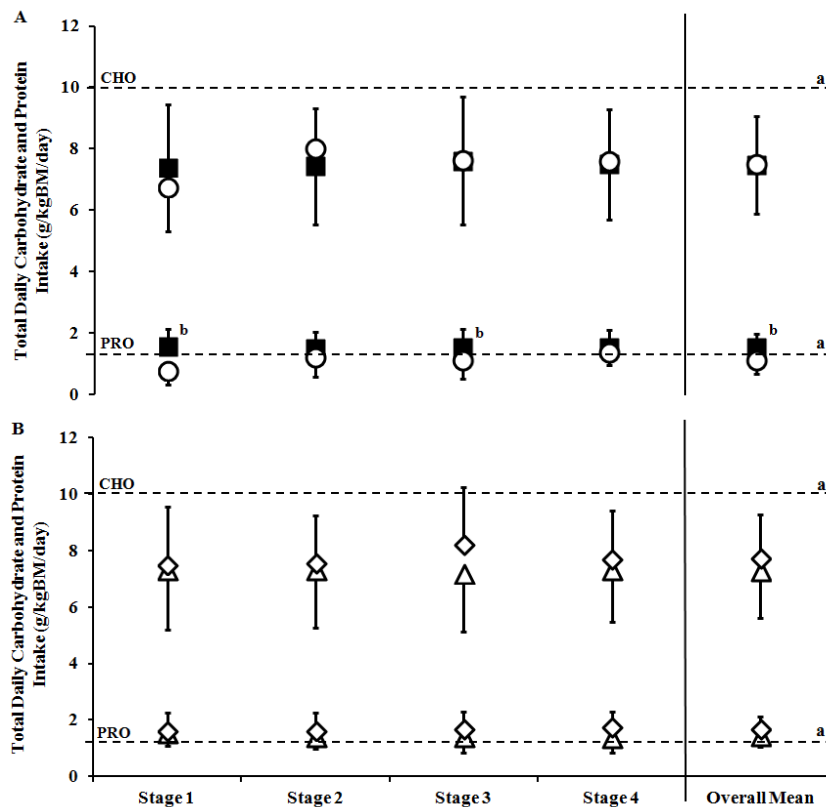


Figure 1. Total daily carbohydrate (CHO) and protein (PRO) intake (corrected for BM) of ultra-endurance runners (UER) participating in a 225 km multi-stage ultra-marathon (MSUM) competition conducted in a hot ambient environment. Mean±SD: (A) UER (■, $n = 54$) and control (CON ○, $n = 12$); (B) slow runners (SR △; MSUM mean speed <8 km/h, $n = 32$), and fast runners (FR ◇; MSUM mean speed ≥8 km/h, $n = 22$). ^a Benchmark recommendations [1,4]; ^b $P < 0.05$ vs. CON

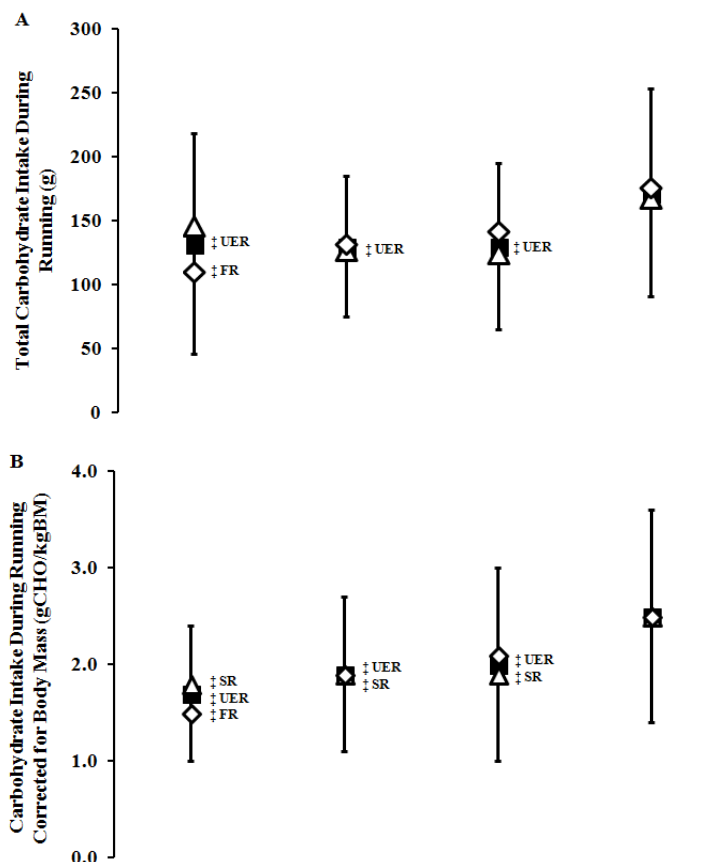
Lower pre-stage energy and protein intakes were observed on Stage 3 in UER ($P=0.05$ and $P=0.018$, respectively), and on Stages 3 and 4 in SR ($P=0.026$ and $P=0.014$, respectively), compared with Stage 1 (Table 3). Pre-stage carbohydrate intakes were also observed to be lower on Stages 3 and 4 in UER ($P=0.01$), and on Stage 4 only in SR ($P=0.015$),

compared with Stage 1. In FR, no significant differences in pre-stage energy and macronutrient intakes between stages were observed. Additionally, no significant difference was observed for pre-stage energy and macronutrient intakes between SR and FR within stages.

Table 3. Pre-stage total energy and macronutrient (and overall macronutrient energy distribution (%)) of ultra-endurance runners (UER) participating in a 225 km multi-stage ultra-marathon (MSUM) competition conducted in a hot ambient environment

	Stage 1	Stage 2	Stage 3	Stage 4	Overall Mean
Energy (kcal)					
UER	774±266	664±239	644±203†	644±256	680±166
SR	778±282	660±231	640±185†	595±242†	664±166
FR	757±246	671±254	647±230	718±269	696±169
Protein (g)					
UER	27±14	22±10	20±8††	22±11	23±8 (14%)
SR	28±14	22±10	20±8†	20±10†	22±7 (13%)
FR	25±15	23±11	20±8	24±11	23±9 (13%)
Carbohydrate (g)					
UER	117±39	99±34	96±37†	94±42††	102±28 (60%)
SR	117±40	98±37	95±36	86±40†	99±28 (60%)
FR	117±40	102±30	99±39	106±44	106±28 (61%)
Fat (g)					
UER	22±16	20±12	20±11	20±12	20±9 (26%)
SR	22±17	20±12	20±11	19±12	20±9 (27%)
FR	22±15	19±13	19±11	22±13	20±9 (26%)

Mean±SD: UER (n=54); slow runners (SR; MSUM mean speed <8 km/h, n=32); fast runners (FR; MSUM mean speed ≥8 km/h, n=22). †† $P<0.01$ and † $P<0.05$ vs. Stage 1



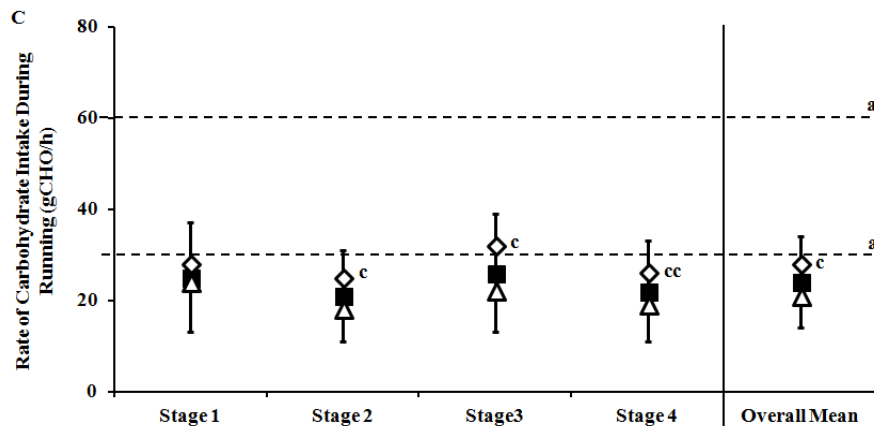


Figure 2. Total carbohydrate intake (A), carbohydrate intake corrected for BM (B), and rate of carbohydrate intake (C) during running, of ultra-endurance runners (UER) participating in a 225 km multi-stage ultra-marathon (MSUM) competition conducted in a hot ambient environment. Mean±SD: UER (■, n= 54), slow runners (SR △; MSUM mean speed <8 km/h, n= 32), and fast runners (FR ◇; MSUM mean speed ≥8 km/h, n= 22). ^a Benchmark recommendation [1,5]. ‡ P<0.05 vs. Stage 4; ^{cc} P<0.01 and ^c P<0.05 vs. SR.

Total carbohydrate intake during running was lower on Stages 1 to 3 in UER compared with Stage 4 ($P=0.009$), and lower on Stage 1 only compared with Stage 4 in FR ($P=0.019$; Figure 2A). When corrected for BM, carbohydrate intake during running was lower on Stages 1 to 3 in UER ($P<0.001$) and SR ($P=0.007$) compared with Stage 4, and lower on Stage 1 only compared with Stage 4 in FR ($P=0.05$; Figure 2B). No difference in carbohydrate intake during running (total and corrected for BM) was observed between SR and FR within stages. A significant difference was however observed between SR and FR within stages when represented as rate of carbohydrate ingestion during running ($P<0.001$); whereby carbohydrate intake per hour of running was consistently greater in FR (33%) throughout the MSUM compared with SR (Figure 2C).

Total energy, protein, and fat intake immediately post-stage did not vary between stages for UER, SR and FR (Table 4). Whereas, total carbohydrate intake immediately after stage completion appeared to be generally higher in FR than SR ($P=0.038$). When corrected for BM (Figure 3), energy (UER: 9.3 kcal/kg BM), protein (UER: 0.3 g/kg BM), carbohydrate (UER: 1.6 g/kg BM), and fat (UER: 0.2 g/kg BM) intakes immediately post-stage did not differ between stages for UER, SR and FR; nor between SR and FR within stages.

Table 4. Post-stagetotal energy and macronutrient (and macronutrient energy distribution (%)) of ultra-endurance runners (UER) participating in a 225 km multi-stage ultra-marathon (MSUM) competition conducted in a hot ambient environment

	Stage 1	Stage 2	Stage 3	Stage 4	Overall Mean
Energy (kcal)					
UER	611±338	591±266	577±271	623±256	600±202
SR	538±299	588±279	581±281	633±276	584±185
FR	716±362	606±249	556±262	595±228	618±226
Protein (g·day)					
UER	17±17	15±13	14±13	14±12	15±10 (10%)
SR	17±16	16±14	14±13	14±11	15±9 (10%)
FR	18±18	14±12	13±13	14±13	15±11 (10%)
Carbohydrate (g)					
UER	111±55	108±52	110±52	108±45	109±36 (73%)
SR	95±47	104±60	111±56	106±47	104±35 (71%)
FR	134±58	115±37	108±48	110±42	117±37 (76%) ^c
Fat (g)					
UER	11±1	11±10	9±9	15±13	12±8 (17%)
SR	10±10	12±11	9±9	17±15	12±9 (19%)
FR	12±11	10±9	8±8	11±8	10±7 (14%)

Mean±SD: UER (n= 54); slow runners (SR; MSUM mean speed <8 km/h, n= 32); fast runners (FR; MSUM mean speed ≥8 km/h, n= 22). ^c P<0.05 vs. SR

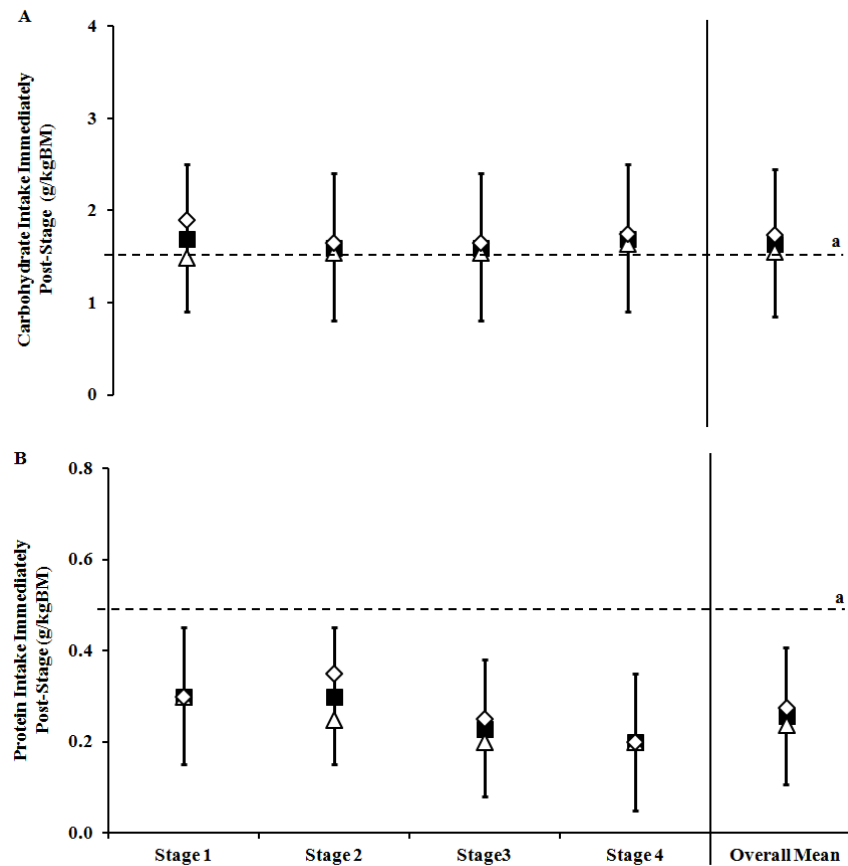


Figure 3. Carbohydrate (A) and protein (B) intake (corrected for BM) immediately post-stage of ultra-endurance runners (UER) participating in a 225 km multi-stage ultra-marathon (MSUM) competition conducted in a hot ambient environment. Mean \pm SD: UER (■, n= 54), slow runners (SR △; MSUM mean speed <8 km/h, n= 32), and fast runners (FR ◇; MSUM mean speed \geq 8 km/h, n= 22). ^a Benchmark recommendations[1,5,11]. No significant differences between stages, and between groups within stages

Gastrointestinal distress was a common feature, with 85% of UER presenting at least one severe gastrointestinal symptom along the MSUM, with rates of gastrointestinal distress being higher in SR (92%) than FR (76%) throughout competition. These included: nausea, gastrointestinal pain, vomiting, indigestion, and irritable bowel symptoms. No gastrointestinal symptoms were reported by CON throughout the MSUM. Weak but significant correlations between episodes of severe gastrointestinal distress, total daily energy ($r = -0.277$, $n = 54$, $P = 0.05$) and carbohydrate ($r = -0.348$, $n = 54$, $P = 0.013$) intakes were observed in UER.

Occurrence of appetite suppression was reported by 72% of UER along the course of the MSUM, with suppressed appetite constantly reported throughout the MSUM, but improved as stages progressed (Stage 1: 46%, Stage 2: 41%, Stage 3: 33%, Stage 4: 33%). Greater reports of suppressed appetite were observed in SR (78%) than FR (64%). Moreover, weak but significant correlations were observed between total daily energy ($r = 0.342$, $n = 54$, $P = 0.011$) and carbohydrate ($r = 0.337$, $n = 54$, $P = 0.013$) intake with appetite in UER.

Similarly, weak but significant correlations were observed between total daily energy ($r = 0.447$, $n = 54$, $P < 0.001$) and carbohydrate ($r = 0.376$, $n = 54$, $P = 0.005$) intakes with perceived recovery quality during the rest period between

stages in UER. While, weak but significant correlations were also observed between total post-stage energy ($r = 0.456$, $n = 54$, $P < 0.001$) and carbohydrate ($r = 0.429$, $n = 54$, $P < 0.001$) intakes with perceived recovery quality during the rest period between stages in UER.

4. Discussion

The current study was the first to comprehensively assess energy and macronutrient intake of ultra-runners during a MSUM conducted in hot ambient conditions. The results indicate that the majority of ultra-runners were not able to consistently meet estimated energy requirements along competition; possibly due to gastrointestinal distress, reduced appetite, and/or barriers to food/fluid preparation and consumption. Attempts to achieve energy balance, favoured the ingestion of fat dense foods, which was at the expense of carbohydrate energy substrate, with all ultra-runners failing to meet benchmark recommendations for carbohydrate intake on consecutive days of prolonged strenuous exercise[1,4,5]. Overall observations actually indicate that CON consumed a more appropriate macronutrient profile, for coping with exercise-stress on consecutive days compared with UER. Results also indicate

all ultra-runners failed to meet benchmark recommendations for carbohydrate intake during running and presented incomplete post-exercise recovery nutrition[1,5,11]. Of the total number of runners participating in the 2010 and 2011 Al Andalus Ultimate Trail, 55% volunteered to participate in the study. The strength of this sample size potentially gives a valid and reliable representation of current dietary habits of ultra-runners during MSUM conducted in hot ambient conditions.

According to ACSM guidelines[1] and tri-axial accelerometry verification[25], estimated lower and upper limits for daily energy expenditure during MSUM were calculated in UER to range between 3831 to 4999 kcal/day, respectively; while average energy intake for UER was 3348 kcal/day, indicating a negative energy status. Changes in resting BM have previously been used as a subjective tool in assessing energy balance, subsequently indicating changes in nutritional status[27]. Observed pre-stage BM values suggest energy needs for the majority of ultra-runners along the MSUM were generally met. However, using resting BM change as an indicator of energy balance throughout MSUM set in hot ambient conditions may be misleading; since progressive increases in resting total body water (Stage 1 to Stage 5 1.9 litres, supported by 20.4% increase in resting plasma volume from Stage 1 to Stage 5)[28], likely induced by heat acclimatisation[29,30], were observed as competition progressed. These progressive increases in body water likely contributed towards maintenance of pre-stage BM throughout MSUM, masking any negative energy balance induced by competition. It would be advised to consider measurements of body water when using BM to assess energy balance along MSUM in the heat.

Primarily due to food and fluid choices, and secondary to practical real-life factors and exercise-heat stress symptomology, all UER were not able to consistently meet benchmark recommendations for total daily carbohydrate intake (≥ 10 g/kgBM/day), previously suggested to support the replenishment of muscle glycogen stores during consecutive days of prolonged strenuous exercise[1,4-7]. If carbohydrate needs for exercise loads are not met, the predominance of fat as an energy substrate will become apparent through the production of ketones. Monitoring of urinary ketones at rest and after running may provide a useful non-invasive method to assess whether sufficient dietary carbohydrates are being consumed along MSUM[24]. Acetoacetic acid was identified in urine samples in UER throughout competition, but not in CON; and prevalence increasing as competition progressed. This provides some evidence of sub-optimal carbohydrate status in UER along the MSUM. Moreover, confounding factors known to increase urinary acetoacetic acid (e.g. severe dehydration, acute illness, high protein diet, and/or Diabetes Mellitus)[28,31] were not observed. These findings are not surprising taking into account that total daily carbohydrate intakes in UER (total running stress load over five days: $28:03 \pm 4:34$ h:min) did not significantly differ from CON (no running stress load) from Stage 2 onwards.

Carbohydrate consumption pre-exercise is thought to be particularly beneficial if carbohydrate intake during recovery fails to fully restore muscle glycogen storage, and additionally provides the working muscles with exogenous carbohydrate during the initial phase of exercise[8,9]. An overall average of 102 g of carbohydrates from awakening until race start (approximately 2 h) was observed in UER, with no participant achieving ≥ 200 g of carbohydrates during this period; despite 200 to 300 g of carbohydrate in a non-bulky form, up to 2 h before prolonged strenuous exercise, being recommended for consumption[6,7]. Predominant food-beverage types selected for consumption during this time period included: freeze-dried/dehydrated expedition meals (e.g. porridge), instant porridge, breakfast cereals, dried fruit and nuts, cereal bars, and fruit juices.

During the running phase of the MSUM, UER only managed to consume an overall average of 24 gCHO/h, far below the lower limit benchmark recommendations (30 to 60 gCHO/h)[1,5]; despite all UER reported carrying sufficient carbohydrate rich foods-beverages (e.g. isotonic drink powders, carbohydrate gels, energy bars, cereal bars, jelly sweets, dried fruit, soft drinks, and fruit juices) during each stage to meet recommendations. Moreover, FR were able to consistently consume higher rates of carbohydrate during running than SR; possibly since FR presenting less severe gastrointestinal distress and greater appetites along the MSUM. These results are similar to those observed during an Olympic course triathlon event and mountain marathon, whereby carbohydrate intake during competition also failed to meet benchmark recommendations in the majority of the studied population[19,26]. Nevertheless, appetite suppression, nausea, vomiting, and other gastrointestinal distresses are likely factors that prevented ultra-runners from consuming optimal quantities of carbohydrates during running, irrespective of carbohydrate type and/or running speed. Exercise-heat stress inducing splanchnic hypoperfusion, splanchnic ischemia, running impact on the gastrointestinal and splanchnic areas, potential increased gut permeability and subsequent endotoxin leakage, and/or an increased pro-inflammatory profile are all likely factors to explain the high rates of gastrointestinal distress and appetite suppression reported in both SR and FR along the MSUM[16-18,24,32-36]. Tailoring and interlinking training, heat acclimatisation, and dietary strategies to individual tolerance and symptomology may support ultra-runners in increasing their ability to consume more carbohydrates during exercise-heat stress[30,37]. Additionally, training the gastrointestinal tract to cope with food-drink ingestion during exercise-heat stress may also potentially increase the ability of ultra-runners to consume more carbohydrates during running throughout MSUM competition.

Previous reviews, based on laboratory controlled studies, have suggested the consumption of carbohydrate (1.0 to 1.5 gCHO/kgBM) and protein (up to 0.5 gPRO/kgBM) immediately after prolonged strenuous exercise improves muscle glycogen replenishment, and provides a nutrient base for tissue repair and healing during the recovery period[5,11].

The overall average carbohydrate and protein intake in UER immediately post-stage was 1.7 gCHO/kgBM and 0.3 gPRO/kgBM, respectively. Predominant food-beverage types selected for consumption during this time period included: soft drinks, fruit juices, fresh fruit (watermelon, oranges, and apples), salted crisps, salted pretzels, and cold meats. Moreover, <10% of UER reported consuming a formulated recovery drink during this time period, which provided sufficient carbohydrate and protein (variety of forms: whey, casein, skimmed milk powder, and soya) to meet benchmark recommendations. Although carbohydrate consumption was generally sufficient, ultra-runners would benefit from developing strategies that increases protein intake during this period (especially high biological value (HBV) protein that contains a reasonable leucine dose: cold meats, fish and seafood, eggs, milk and cheese, soya, pulses, and nuts). The consumption of HBV protein immediately after exercise, co-ingested with carbohydrate, has been linked with an increased amino acid blood pool and insulin response; subsequently suppressed muscle proteolytic activity, enhanced net amino acid muscle uptake, enhanced net intramuscular protein synthesis, and reductions in perceived muscle soreness after a period of rest[38-41]; responses which are likely to be advantageous to ultra-runners competing on consecutive days. In addition, carbohydrate and protein ingestion immediately after prolonged strenuous exercise appears to prevent the decline in neutrophil function (an important mechanism in tissue repair and healing) often observed after endurance exercise[12,13]. From a practical view point, race organisers simply providing a carbohydrate-protein enriched beverage (e.g. enriched milkshake or equivalent)[42-44] immediately after stage completion at the recovery feeding tent may be a positive initiative to improve overall general recovery of MSUM competitors, rather than just supplying plain water, soft drinks, and fruit juices.

Overall total daily protein intake of UER was above benchmark recommendations[1,4,45]. Even though overall total daily fat intake was generally within recommendations [4,6,7], fat quality appears unbalanced, with high intakes of saturated fat, and a *n*6 to *n*3 ratio of 22:1 being consumed by UER. Interestingly, on this occasion, CON presented a more appropriate fat profile compared with UER. The lack of nutritional education and ultra-endurance sports cultural trends encouraged UER, but not CON, to consume freeze-dried/dehydrated expedition meals (average of two meals per day; approximately 800 kcal/meal), which contains predominantly fat based ingredients (~53% average fat energy contribution), with accompanying low levels of carbohydrates (average~38% carbohydrate energy contribution). Taking into account the multiple stressors associated with MSUM, which have been associated with exacerbated pro-inflammatory cytokine responses[46,47], with or without clinical manifestation[24,48,49]; it would be favourable for ultra-runners during competition to select and consume foods that predominate with MUFA (e.g. olive oils) and marine based PUFA *n*3 (e.g. tinned oily fish), both of

which have been reported to present anti-inflammatory properties[51,52].

5. Conclusions

Current food and fluid intakes of ultra-runners during a MSUM conducted in a hot ambient environment may not be sufficient to meet total energy requirements during consecutive days of competition; possibly due to a combination of exercise-heat stress induced gastrointestinal distress, suppressed appetite, and/or barriers to food/fluid preparation and consumption. Unbalanced total daily macronutrient intakes, primarily led by sub-optimal carbohydrate intake and food choices rich in unfavourable fats were observed consistently throughout the MSUM. Additionally, the amount of total daily energy and carbohydrate ingested (and post-stage energy and carbohydrate ingested) appears to contributed towards the degree of perceived recovery quality in-between stages. The findings from the current study indicate that nutritional education by qualified sport and exercise nutritional professionals (e.g. Sports Dietitians, Registered Sport & Exercise Nutritionists) focused at recreational ultra-runners is warranted.

Reflecting on results of the current study, nutritional education may include developing dietary strategies and promoting dietary changes aimed at: increasing total daily carbohydrate intake through meals, snacks, carbohydrate rich fluids, and carbohydrate supplementation (if required); introduce individualised tolerable carbohydrate intakes before, during, and after each stage completion; provide a diverse selection of HBV protein rich foods to meet daily nitrogen needs and supporting tissue repair and healing after running; introduce anti-inflammatory fats within dietary regimes throughout competition; modifying eating behaviours during periods of food-fluid disinterest, suppressed appetite, and gastrointestinal distress. A follow-up study should be conducted to evaluate the outcomes of such nutritional education on dietary practices of ultra-runners in proceeding MSUM events.

ACKNOWLEDGEMENTS

Firstly, the authors would like to thank all the ultra-endurance runners that volunteered to participate in this study. The authors acknowledge the *Al Andalus Ultimate Trail* (www.alandalus-ut.com) race directors Paul Bateson and Barbara Price; and Team Axarsport SL: Michelle Cutler and Eric Maroldo, for assisting and supporting various aspects of this study. The authors also acknowledge Jane Sheehy and Jagdeep Shergill from Coventry University for their technical support along the course of the study implementation; Lisa Hardy, Benjamin Lee, Vera Camões-Costa, Jessica Waterman, Emily Freeth, Edel Barrett, Slawomir Marczak, and Encarna Valero-Burgos for contributing towards various aspects of data and sample

collection. The study was funded by Coventry University Sport & Exercise Science Applied Research Group. All authors declare no conflicts of interest.

REFERENCES

- [1] American College of Sports Medicine, American Dietetic Association, Dietitians of Canada, 2009, American College of Sports Medicine position stand. Nutrition and athletic performance. *Med. Sci. Sports Exerc.*, 41, 709-731.
- [2] Febbraio, M.A., Snow, R.J., Hargreaves, M., Stathis, C.G., Martin, I.K., Carey, M.F., 1994, Muscle metabolism during exercise and heat stress in trained men: effect of acclimation. *J. Appl. Physiol.*, 76, 589-597.
- [3] Febbraio, M.A., Snow, R.J., Stathis, C.G., Hargreaves, M., Carey, M.F., 1994, Effect of heat stress on muscle energy metabolism during exercise. *J. Appl. Physiol.*, 77, 2827-2831.
- [4] Broad, E.M., and Cox, G.R., 2008, What is the optimal composition of an athlete's diet. *Eur. J. Sports Sci.*, 8, 57-65.
- [5] Kerksick, C., Harvey, T., Stout, J., Campbell, B., Wilborn, C., Kreider, R., Kalman, D., Ziegenfuss, T., Lopez, H., Landis, J., Ivy, J.L., Antonio, J., 2008, International Society of Sports Nutrition position stand: nutrient timing. *J. Int. Soc. Sports Nutr.*, 5: 17.
- [6] Burke, L.M., Kiens, B., Ivy, J.L., 2004, Carbohydrates and fat for training and recovery. *J. Sports Sci.*, 22(1), 15-30.
- [7] Burke, L.M., and Hawley, J.A., 2006, Fat and carbohydrate for exercise. *Curr. Opin. Clin. Nutr. Metab. Care*, 9(4), 476-481.
- [8] Jeukendrup, A.E., and Killer, S., 2010, The myths surrounding pre-exercise carbohydrate feeding. *Ann. Nutr. Metab.*, 57, 18-25.
- [9] Jeukendrup, A.E., 2008, Carbohydrate feeding during exercise. *Eur. J. Sports Sci.*, 8, 77-86.
- [10] Jeukendrup, A.E., 2010, Carbohydrate and exercise performance: the role of multiple transportable carbohydrates. *Curr. Opin. Clin. Nutr. Metab. Care*, 13, 447-451.
- [11] Beelen, M., Burke, L.M., Gibala, M.J., van Loon, L.J.C., 2010, Nutritional strategies to promote postexercise recovery. *Int. J. Sport Nutr. Exerc. Metab.*, 20, 515-532.
- [12] Costa, R.J., Oliver, S.J., Laing, S.J., Walters, R., Bilzon, J.L., Walsh, N.P., 2009, Influence of timing of postexercise carbohydrate-protein ingestion on selected immune indices. *Int. J. Sport Nutr. Exerc. Metab.*, 19, 366-384.
- [13] Costa, R.J.S., Walters, R., Bilzon, J.L.J., Walsh, N.P., 2011, Effects of immediate postexercise carbohydrate ingestion with and without protein on neutrophil degranulation. *Int. J. Sport Nutr. Exerc. Metab.*, 21, 205-213.
- [14] Walsh, N.P., Gleeson, M., Shephard, R.J., Gleeson, M., Woods, J.A., Bishop, N.C., Fleshner, M., Green, C., Pedersen, B.K., Hoffman-Goetz, L., Rogers, C.J., Northoff, H., Abbasi, A., Simon, P., 2011, Position statement. Part one: Immune function and exercise. *Exerc. Immunol. Rev.*, 17, 6-63.
- [15] Heaney, S., O'Connor, H., Michael, S., Gifford, J., Naughton, G., 2011, Nutrition knowledge in athletes: a systematic review. *Int. J. Sport Nutr. Exerc. Metab.*, 21, 248-261.
- [16] Rehrer, N.J., Brouns, F., Beckers, E.J., Frey, W.O., Villiger, B., Riddoch, C.J., Menheere, P.P., Saris, W.H., 1992, Physiological changes and gastro-intestinal symptoms as a result of ultra-endurance running. *Eur. J. Appl. Physiol. Occup. Physiol.*, 64, 1-8.
- [17] ter Steege, R.W.F., Geelkerken, R.H., Huisman, A.R., Kolkman, J.J., 2011, Abdominal symptom during physical exercise and the role of gastrointestinal ischemia: a study in 12 symptomatic athletes. *Br. J. Sport Med.*, Epub (Oct).
- [18] van Wijck, K., Lenaerts, K., Grootjans, J., Wijnands, K., Poeze, M., van Loon, L.J., Dejong, C.H., Buurman, W.A., 2012, Physiology and pathophysiology of splanchnic hypoperfusion and intestinal injury during exercise: strategies for evaluation and preventions. *Am. J. Physiol.*, Epub (April).
- [19] Kruseman, M., Bucher, S., Bovard, M., Kayser, B., Bovier, P.A., 2005, Nutrient intake and performance during a mountain marathon: an observational study. *Eur. J. Appl. Physiol.*, 94, 151-157.
- [20] Peters, E.M., and Goetzsche, J.M., 1997, Dietary practices of South African ultradistance runners. *Int. J. Sport Nutr.*, 7, 80-103.
- [21] Rehrer, N.J., 2001, Fluid and electrolyte balance in ultra-endurance sport. *Sports Med.*, 31, 701-715.
- [22] de Graaf, C., Blom, W.A., Smeets, P.A., Stafleu, A., Hendriks, H.F., 2004, Biomarkers of satiation and satiety. *Am. J. Clin. Nutr.*, 79, 946-961.
- [23] Park, A.J., and Bloom, S.R., 2005, Neuroendocrine control of food intake. *Curr. Opin. Gastroenterol.*, 21, 228-233.
- [24] Robson-Ansley, P.J., Gleeson, M., Ansley, L., 2009, Fatigue management in the preparation of Olympic athletes. *J. Sports Sci.*, 27, 1409-1420.
- [25] Britton, R., Dempster, S., Moore, J.P., Costa, R.J.S., 2011, The use of triaxial accelerometry to support dietary intervention during a multi-stage mountain ultra-marathon: A case study approach. *J. Sports Sci.*, 29, S132.
- [26] Cox, G.R., Snow, R.J., Burke, L.M., 2010, Race-day carbohydrate intake of elite triathletes contesting olympic-distance triathlon events. *Int. J. Sport Nutr. Exerc. Metab.*, 20, 299-306.
- [27] Seagle, H.M., Strain, G.W., Makris, A., Reeves, R.S., American Dietetic Association, 2009, Position of the American Dietetic Association: weight management. *J. Am. Diet. Assoc.*, 109, 330-346.
- [28] Costa, R.J.S., Teixeira, A., Rama, L., Swancott, A., Hardy, L., Lee, B., Camões-Costa, V., Gill, S., Waterman, J., Barrett, E., Freeth, E., Hankey, J., Marczak, S., Valero, E., Scheer, V., Murray, A., Thake, D., 2013, Water and sodium intake habits and status of ultra-endurance runners during a multi-stage ultra-marathon conducted in a hot ambient environment: an observational field based study. *Nutri. J.*, 12, 13.
- [29] Costa, R.J.S., Crockford, M.J., Moore, J.P., Walsh, N.P., 2012, Heat acclimation responses of an ultra-endurance running group preparing for hot desert based competition. *Euro. J. Sport Sci.*, Epub (March).

- [30] Wendt, D., van Loon, L.J., Lichtenbelt, W.D., 2007, Thermoregulation during exercise in the heat: strategies for maintaining health and performance. *Sports Med.*, 37, 669-682.
- [31] Fischback, F.T., Dunning, M.B., 2004, A manual of laboratory and diagnostic tests 7th edition. Lippincott Williams and Wilkins, Philadelphia, PA, pp. 199-201.
- [32] French, S.J., Cecil, J.E., 2001, Oral, gastric and intestinal influences on human feeding. *Physiol. Behav.*, 74, 729-734.
- [33] Lambert, G.P., 2009, Stress-induced gastrointestinal barrier dysfunction and its inflammatory effects. *J. Anim. Sci.*, 87, E101-E108.
- [34] Otte, J.A., Oostveen, E., Geelkerken, R.H., Groeneveld, A.B., Kolkman, J.J., 2001, Exercise induces gastric ischemia in healthy volunteers: a tonometry study. *J. Appl. Physiol.*, 91, 866-871.
- [35] Rehrer, N.J., Meijer, G.A., 1991, Biomechanical vibration of the abdominal region during running and bicycling. *J. Sports Med. Phys. Fitness.*, 31, 231-234.
- [36] Wright, H., Collins, M., Schwellnus, M.P., 2009, Gastrointestinal (GIT) symptoms in athletes: A review of risk factors associated with the development of GIT symptoms during exercise. *Int. Sports Med. J.*, 10(3), 116-123.
- [37] Cox, G.R., Clark, S.A., Cox, A.J., Halson, S.L., Hargreaves, M., Hawley, J.A., Jeacocke, N., Snow, R.J., Yeo, W.K., Burke, L.M., 2010, Daily training with high carbohydrate availability increases exogenous carbohydrate oxidation during endurance cycling. *J. Appl. Physiol.*, 109(1), 126-134.
- [38] Churchward-Venne, T.A., Burd, N.A., Mitchell, C.J., West, D.W., Philp, A., Marcotte, G.R., Barker, S.K., Baar, K. and Phillips, S.M., 2012, Supplementation of a suboptimal protein dose with leucine or EAA: Effects on myofibrillar protein synthesis at rest and following resistance exercise in men. *J. Physiol.*, Epub (March).
- [39] Rennie, M.J., and Tipton, K.D., 2000, Protein and amino acid metabolism during and after exercise and the effects of nutrition. *Annu. Rev. Nutr.*, 20, 457-483.
- [40] Saunders, M.J., 2007, Coingestion of carbohydrate-protein during endurance exercise: influence on performance and recovery. *Int. J. Sport Nutr. Exerc. Metab.*, 17, S87-S103.
- [41] Tipton, K.D., and Witard, O.C., 2007, Protein requirements and recommendations for athletes: relevance of ivory tower arguments for practical recommendations. *Clin. Sports Med.* 26(1), 17-36.
- [42] Pritchett, K., Bishop, P., Pritchett, R., Green, M., Katica C., 2009, Acute effects of chocolate milk and a commercial recovery beverage on postexercise recovery indices and endurance cycling performance. *Appl. Physiol. Nutr. Metab.*, 34(6), 1017-1022.
- [43] Roy, BD, 2008, Milk: the new sports drink? A Review. *J. Int. Soc. Sports Nutr.*, 5, 15.
- [44] Shirreffs, S.M., Watson, P., Maughan, R.J., 2007, Milk as an effective post-exercise rehydration drink. *Br. J. Nutr.*, 98, 173-180.
- [45] Tipton, K.D., and Wolfe, R.R., 2004, Protein and amino acids for athletes. *J. Sports Sci.*, 22(1), 65-79.
- [46] Suzuki, K., Nakaji, S., Yamada, M., Totsuka, M., Sato, K., Sugawara, K., 2002, Systemic inflammatory response to exhaustive exercise. *Cytokine kinetics. Exerc. Immunol. Rev.*, 8, 6-48.
- [47] Walsh, N.P., and Whitham, M., 2006, Exercising in environmental extremes- A greater threat to immune function? *Sports Med.*, 36, 941-976.
- [48] Lambert, G.P., 2004, Role of gastrointestinal permeability in exertional heatstroke. *Exerc. Sport Sci. Rev.*, 32(4), 185-190.
- [49] Lim, C.L., and Mackinnon, L.T., 2006, The roles of exercise-induced immune system disturbances in the pathology of heat stroke : the dual pathway model of heat stroke. *Sports Med.*, 36(1), 39-64.
- [50] Papageorgiou, N., Tousoulis, D., Psaltopoulou, T., Giolis, A., Antoniadis, C., Tsiamis, E., Miliou, A., Toutouzias, K., Siasos, G., Stefanadis C., 2011, Divergent anti-inflammatory effects of different oil acute consumption on healthy individuals. *Eur. J. Clin. Nutr.*, 65(4), 514-519.
- [51] Tartibian B., Maleki B.H., Abbasi A., 2011, Omega-3 fatty acids supplementation attenuates inflammatory markers after eccentric exercise in untrained men. *Clin. J. Sport Med.*, 21(2), 131-137.
- [52] Wardhana, Surachmanto, E.S., Datau, E.A., 2011, The role of omega-3 fatty acids contained in olive oil on chronic inflammation. *Acta Med. Indones.*, 43(2), 138-143.