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Association between energy availability and menstrual function in elite Kenyan runners

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Abstract

Low energy availability (EA) has been recognized as an instigator of menstrual dysfunction and subsequent hypoestrogenism that leads to deterioration in bone health. Elite Kenyan male athletes have been reported to often function under low energy balance. Therefore, the purpose of this study was to determine EA and menstrual function (MF) among elite Kenyan female athletes; and to explore the association between EA and MF in the athletes. The data were collected from 25 elite Kenyan runners and 14 non-athletes. Energy intake (EI) minus exercise energy expenditure (EEE) normalized to fat free mass (FFM) determined EA. EI was determined through weight of all food and liquid consumed over three consecutive days. EEE was determined after isolating and deducting energy expended in exercise or physical activity above lifestyle level from the total energy expenditure output as measured by Actigraph GT3X+. FFM was assessed using DXA. A daily temperature-menstrual log kept for nine continuous months was used to establish menstrual function. Overall, EA below 45 kcal/kgFFM·d^{-1} was seen in 61.53% of the participants (athletes: 28.07 ±11.45 kcal/kgFFM·d^{-1}, non-athletes:56.97 ±21.38 kcal/kgFFM·d^{-1}). Results on menstrual dysfunction were as follows: oligomenorrhea (athletes: 40%; non-athletes: 14.3%) and amenorrhea (non-athletes: 14.3%). None of the athletes were amenorrheic. Results did not show any significant association between EA and MF, but the low to sub-optimal EA among elite Kenyan female athletes raises concern for their future menstrual and bone health. . Educating the athletes and coaches will enhance achievement of the specific dietary and nutritional needs appropriate to their competition events.

Keywords: Exercise energy expenditure, border-line energy availability, sub-optimal energy availability, negligible dietary restraint, eumenorrhea.

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Introduction

Optimal functioning of every cell is dependent on an uninterrupted energy supply of oxidizable substrate metabolized from food intake. Unfortunately, cellular functions associated with reproduction are considered non-essential to survival; and as such, during periods of energy deficiency, these energetically costly functions are suppressed (Wade & Jones, 2004). Normal pulsatile secretion of gonadotropin releasing hormone (GnRH) from the hypothalamus and subsequent pulsatile release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) by the pituitaral and are critical to optimal reproductive function (Loucks & Thuma, 2003). Optimal pulsatile secretion of GnRH, LH and FSH encourages oestrogen and progesterone production from the ovaries to ensure menstrual normalcy (Temme & Hoch, 2013). A chronically energy deficient environment, especially when accompanied by exercise (Loucks, 2003) poses grave metabolic challenge by denying the hypothalamus its optimum share of energy (Mircea, Lujan & Pierson, 2007). Acute sensitivity of the GnRH pulse generator to hypometabolic state triggers disruptive events in the hypothalamic-pituitary-ovarian (HPO) axis that eventually present as menstrual dysfunction (Gordon, 2010). Available evidence suggests that rather than depending on general EA, the brain's dependence on glucose availability, particularly from liver glycogen stores puts it in direct competition with skeletal muscle for available carbohydrate (Loucks & Thuma, 2003).

Energy availability is a reflection of the available energy that has been normalized to fat free mass after deducting energy expended in purposive exercise from energy consumed. It can be expressed as Energy Availability = (Energy Intake–Exercise Energy Expenditure)/Fat Free Mass or EA = (EI – EEE)/FFM (Manore, Kam & Loucks, 2007). While EA at ~ 45kcal/kgFFM$\cdot$d^{-1} (free fat mass/day) in healthy young women is considered energetically well balanced (West, Scheid & De Souza, 2009), abrupt disruption in menstrual function and bone formation begin when EA drops below the resting metabolic rate of 30kcal/kgFFM$\cdot$d^{-1} (Manore et al., 2007).

Increasing energy expenditure through exercise is one of the deliberate causative factors in low energy availability (Nattiv et al., 2007). Purposive restriction in dietary intake presenting as disordered eating (DE) has been recognized as the precursor to the more clinical eating disorder of bulimia nervosa (BN) that could degenerate into the extreme disorder of anorexia nervosa (AN) (Nazem & Ackerman, 2012). Athletes may also experience inadvertent energy deficiency through ignorance (Pantano, 2009), as well as inconvenient schedules that do not allow time for eating, and lack of food (Beals & Meyer, 2007). Regardless of the cause of energy deficiency, both, reproductive/menstrual function and skeletal health, experience dramatic hormonal and metabolic detrimental impact either directly or indirectly (West et al., 2009).

Theoretically, menstrual function progresses sequentially along a health continuum that begins from optimal normal ovulatory cycle or eumenorrhea with intervals of 28±7 days (Temme & Hoch, 2013). Dysfunction descends to the tenuous asymptomatic sub-clinical luteal phase defect (LPD) manifest as shortened and more frequent menstrual cycles (Redman & Loucks, 2005). The more severe, longer than 35 days oligomenorrhoeic cycle could result from either energy deficiency or hyperandrogenism (Awdishu, Williams, Laredo & De Souza, 2009). Amenorrhea with cycles longer than 90 days is the most deleterious and common menstrual dysfunction in exercising women. Its association with extreme low-energy availability has earned it the special classification of functional hypothalamic amenorrhea (FHA) (Manore et al., 2007).

The close link between sufficiency of caloric intake relative to energy expended during exercise and menstrual function suggests a dose-response relationship between categories of menstrual dysfunction and energy availability (De Souza et al., 2007). Therefore, there appears to be no doubt about the direct detrimental impact of low energy availability on menstrual function, reproduction and fertility, and subsequently on bone health (Manore et al., 2007). It has been reported that Kenyan endurance runners often function under negative or borderline energy balance (Fudge et al., 2006). However, energy balance (EB) being a reflection of energy left over after use in all physiological functions of the day or total energy expenditure (TEE) could underestimate EA among those functioning in chronic dietary deficiency (Loucks, Kiens & Wright, 2011). Therefore, the objectives of this study were to (i) establish EA and (ii) explore the association between EA and MF in the elite Kenyan female long and middle distance runners. It was hypothesized that there would be differences in (i) the EI between the athletes (long and middle distance) and the non-athletes, (ii) EEE between the athletes and the non-athletes (iii) energy availability between the athletes and non-athletes and that (iv) EA would be associated with MF among the participants.

Methodology

Study design

This investigation was part of larger study that sought to establish the female athlete triad in elite Kenyan runners. Since no variable was manipulated and neither was there true random assignment of participants, the study was based on a quasi-experimental design. In view of the fact that the researchers had no control over the diet and training programmes of the participants; and that the results would be evaluated in retrospect, the study specifically used the ex post facto/causal-comparative design.

Ethical approval

Ethical approval, relevant permissions, and research permits were obtained from the Kenyatta University Ethics Review Committee (KU-ERC), Athletics Kenya (AK) and the National Council for Science and Technology (NCST) in Kenya respectively. Verbal and written information and the participant's expected role in the study was given to each participant, both, in *Swahili* (the Kenyan athlete's native language) and English, who subsequently signed the informed consent form.

Participants

Initially 16 long distance (LD) and 16 middle distance (MD) actively training elite Kenyan female runners, and 16 age-matched non-athletic (NA) controls, four each in 18-20, 21-23, 24-26, and 27-30 years age group clusters for parity were recruited for the study (N = 48). Non-athletes were certified as fit for participation in the study by the local Provincial Medical Officer. However, the 39 participants (LD=13, and MD=12, Non-athletes=14) who completed the requirements, provided data for the study.

Measurements

Energy availability (EA) was determined after deducting EEE from EI, and the remnant energy normalized for their fat free mass per day (FFF·d). Hence, EA = (EI-EEE)/FFM·d and was expressed as kcal/kgFFM·d^{-1}(Manore et al., 2007). Energy availability <45 kcal·kgFFM·d^{-1} was defined as low availability and EA >45 kcal·kgFFM·d^{-1} as healthy (Nattiv et al., 2007).

Energy and nutrient intake (EI) evaluations considered the lack of variety in a Kenyan athlete's diet throughout the week (Onywera et al., 2004), and the view that recording intake over three days could elicit adequate data (Thompson & Byers, 1994) to measure EI during three consecutive days. Initially, 48 trainee research assistants met with the principal investigator for training in food-weighing and questionnaire administration during the lunch hour every alternate working day for two weeks. Of these, only the 25 who showed proficiency and had attended at least 4 of the 6 training sessions were recruited as research assistants. Starting on the day of arrival at the centre, food weighing exercise was repeated at every dinner. Compliance at the participants' training centres was enhanced by random presence of the principal investigator during any food weighing session. A trained research assistant (RA) used a digital scale (Aston Meyers, model: 7766) to weigh all food and liquid consumed by her participant from 5.30 am until the last meal each evening during three consecutive days. At the end of a meal or a snack, weight of each item not consumed or remaining on the plate was deducted from the original amount to record the actual amount

consumed. Each participant was also instructed on how to note portions consumed after the departure of the RA each evening. These were matched for weight the next morning and recorded against the previous day's intake. Weight of actual amount of food and liquid consumed was entered into the Nutrisurvey for Windows 2007 (Erhardt, 2007) software programme which analysed the daily average EI and nutrients in kilocalories (kcals). In addition, the joint guidelines of the American Dietetic Association (American Dietetic Association (ADA), 2009), Dieticians of Canada, and the American College of Sports Medicine (2009) were used to establish whether participants' EI met minimum levels for their activity levels. When estimating energy requirements (EER), the physical activity coefficients used were 'very active' coefficient of 2.5 for the athletes and the 'moderate' 1.6 for the non-athletes (American Dietetic Association (ADA), 2009).

Exercise energy expenditure (EEE) was assessed using the Actigraph GT3X plus (GT3X+) tri-axial accelerometer (Actigraph, 2011). Except when bathing, the device was worn upright and flat on the right iliac crest continuously for 72 hours during the same period coinciding with the measurement of energy intake. Actilife, version 5.6 software (Actigraph LLC, Pensacola, FL, USA), was used to initialize, download and finally analyse data. Energy expenditure was determined using the Freedson work energy combination formula that incorporates participant weight (Freedson, Melanson & Sirard, 1998). Initialization of the device that was set at 1 s epoch length allowed the monitor to record even the shortest activity counts at various intensities and their durations during wear time. As required by the Freedson formula, each participant's weight nearest to 0.1 kilogrammes (kg) on a digital scale (A & D Precision Health Scale, Model: UC-322) was entered into the analysis template to determine her total energy expenditure. Actilife output considers activity counts higher than 1952 as corresponding to 3 to 6 Mets intensity, a level at which energy expenditure is higher than lifestyle energy expenditure. The daily living activities of walking to get anywhere, cultivating land, herding cattle, fetching water and firewood could account for substantial energy expenditure among the non-athletes. Therefore, for both, the athletes and non-athletes, all kcals corresponding to hourly activity counts above 1952 were deducted from total energy expenditure to account for their exercise energy expenditure.

Eating behaviour practices (EBP), thought to be contributory factors to EA, were explored using the Fairburn and Beglin's (2008) Eating Disorder Examination Questionnaire (EDE-Q). The EDE-Q has been validated for assessing eating psychopathologic behaviour comprising the subscales of dietary restraint, eating concern, shape concern and weight concern. Severity of each item in a subscale is based on a seven point (0 - 6) scoring scale. Whereas a score of 0 denotes absence of the subscale feature, every increase in a score is indicative of increase in severity in the subscale such that the score of 6 denotes

the most extreme presence of the feature. The questionnaire was completed in the presence of the principal investigator during the same period as when EI and EE were measured. Though the questionnaire was available in both, English and *Swahili*, the participant completed whichever she found more comprehensible, and where necessary, she asked for further clarification/explanation from the principal investigator, who is a native speaker of *Swahili.*

Fat free mass (FFM) was among several body composition assessments completed using dual energy x-ray absorptiometry (DXA) with Hologic®, Discovery (USA) at the Aga Khan University Hospital in Nairobi, Kenya. This was done under the direction and supervision of the head of the hospital's nuclear medicine. No scan was performed unless the system passed the daily quality control (QC) procedure using a phantom. In addition to providing whole/total body BMD measurements were taken at the lumbar spine (L1 – L4, CV= 1.0%) and left femoral neck (CV=1.0%). Initial evaluations were done according to the International Society of Clinical Densitometry (ISCD) (Lewiecki et al., 2008) guidelines, which require comparison of BMD against expected norms matched for age, sex and population/ethnicity match, and be expressed as *Z*-scores. In the ISCD guideline, a *Z*-score of less than -2.0 is considered the threshold below the expected range for age, sex and population. However, the American College of Sports Medicine (ACSM, 2007) cautions that since runners are expected to have better BMD than the general population, athletes with slightly better BMD values than the ISCD threshold could be at risk of fracture and/osteoporosis in the future. The daily-living physical activity level of the non-athletes also had the potential of affecting their BMD. Therefore, as recommended by the ACSM (2007), all participants with *Z*-scores below -1 were categorized further as having low BMD.

Menstrual function (MF) was based on a nine-month daily temperature-menstruation log kept by the participant (US Department of Health and Human Services, 2006). Each participant was instructed on how to: (i) measure her temperature using an oral thermometer (Royal Flexible Waterproof Digital Thermometer, CEO197) (ii) record her temperature immediately upon waking every morning in the menstruation-temperature log, and (iii) complete the log every day for nine continuous months beginning on the morning after signing the informed consent. In this menstruation-temperature log, a complete cycle began on the day of first appearance of menstruation since start of the log and ended on the day previous to appearance of the next menstruation, which signalled start of a new cycle. Only complete cycles within the nine months of actual recording were considered for establishing menstrual functional status. The status was categorized as polymenorrheic (< 21 days) (Sloan, 2002), eumenorrheic (21–35 days), oligomenorrheic (35–90 days) and amenorrheic (>90days) (Nattiv et al., 2007).

In addition, the research assistant administered a standardized questionnaire that sought information about the participant's menstrual history since menarche and her daily living activities since childhood.

Statistical analysis was done using IBM SPSS Statistics Version 20. Means and standard deviations were computed to summarise values for of EI, EEE, menstrual status and EBPs. Independent samples *t*-test was used to examine whether significant differences existed in EI, EEE and EA between athletes and non-athletes. Further, analysis of variance (ANOVA) was used to test the significant differences in the means of EI, EEE and EA among the three groups of participants (MD, LD, NA). A significant *F* value warranted use of Tukey's post hoc to establish specific inter-group differences. The three categories of menstrual function which emerged were ranked from eumenorrheic/normal status that descended to the lesser oligomenorrheic dysfunction to the most serious dysfunction of amenorrhea. The two dysfunctional categories were combined and further subjected to Spearman's rank order correlation coefficient (rho) analysis to determine level of statistical significant association between energy availability and menstrual function. Pearson correlation coefficients (*r*) were computed to determine level of statistical significant contributory association between EBPs and EA. The probability level of ≤ 0.05 was set to determine statistical significance.

Results

Demographic information collected from 25 elite Kenyan female athletes and 14 non-athletes is summarized in Table 1.

Table 1: Demographic and anthropometric characteristics of all participants

Characteristics	**Athletes (*n*=25)**	**Non-Athletes (*n*=14)**	***p* value**
Age (years)	25 ± 3.21	24 ± 8.81	0.921
Age at menarche	15.8 ± 2.0	14.14 ± 1.02	0.005
Weight (kg)	49.8 ± 5.50	58.63 ± 5.83	0.001*
Height (m)	1.62 ± .05	1.60 ± .04	0.209
BMI (kg/m^2)	18.71 ± 1.28	22.76 ± 2.77	0.001*
Fat-free mass (kg)	38.63 ± 3.82	38.59 ± 2.57	0.971
Percentage body fat (%)	19.31 ± 3.21	30.51 ± 5.03	0.001

Notes: *p*-value computed using independent t-test, *$p<0.001$

Table 2 highlights the results of estimated energy requirements, energy and nutrient intake, exercise energy expenditure, energy availability and the global score in eating behaviour practises. Calculated energy requirements for the athletes and non-athetes were 4648.10kcal/kg·d^{-1} and 2409.1kcal/kg·d^{-1} , respectively.

Table 2: Estimated energy requirements, energy and nutrient intake characteristics among athletes and non-athletes.

Energy characteristics	Athletes (*n*=25)	Non-Athletes (*n*=14)	*p* value
Estimated energy requirements (kcals/d)	4648.10	2406.10	0.001*
Energy intake ($kcal/d^{-1}$)	1893.60 ±516	2258.82±799.1	0.091
Exercise energy expenditure ($kcal/d^{-1}$)	759.52±221.80	78.14±19.33	0.001*
Eating behaviour practice (Global score)	.62±.56	.57±.66	0.813

Notes: p-value computed using independent t-test, *p<0.001

Results of EI among the groups (Table 2) indicated that the athletes with a mean of 1893.60±516.kcal/kg.d^{-1} had lower intake than the non-athletic cohorts' (2270.01±766.38 kcal/kg.d^{-1}). However, *t*-test analysis did not reveal any statistically significant differences in energy intake between the athletes and non-athletes, nor did the ANOVA when participants were categorised by distance (LD and MD) and non-athletes. The independent *t*-test indicated significant difference (t=11.40; p<0.001) in EEE between the athletes (759.52±221.80) and non-athletes (78.14±19.33).

Table 3 details the macronutrient breakdown showing that 72.56% (±6.62) of the athletes' energy intake constituted carbohydrates (CHO) which translated to 12.91(±4.02) g/body weight in kg [BW], 10.72 %(±1.92) was protein (PRO) which is equivalent to 1.69 (±0.59) g/BW, and 16.96% (±1.29) was fat which reflected 1.43 (±0.70) g/BW. The non-athletes' intake showed that 74.64% (±5.74) was CHO providing 12.92 (±4.02) g/BW, 9.35% (±1.94) was PRO yielding 1.61 (±0.56) g/BW, and 16.07% (±5.13) was fat that amounted to 1.18 (±.32) g/BW. The independent *t*-tests revealed protein as the only micronutrient to show significant difference (t=2.11; p=0.041) between athletes (M=10.72±1.92) and non-athletes (M=9.35±1.94).

Table 3: Macronutrient intake among athletes and non-athletes

Category	CHO*	g/BW	PRO*	g/BW	Fat*	g/BW
Athletes	72.56±6.62	11.43±0.70	10.72±1.92	1.69±0.59	16.96±6.68	1.31±.91
Non-Athletes	74.64±5.74	12.92±4.02	9.35±1.94	1.61±0.56	16.07±5.13	1.18±.32

*Percentage of total energy intake; g/bw=grammes per body weight; CHO=Carbohydrates; PRO=Proteins

Table 4 summarises energy availability among the participants grouped as LD, MD runners and non-athletes.

Table 4: Energy availability by distance and non-athletic category

Participant category	*N*	Mean*	SD
MD	12	33.26	11.02
LD	13	23.27	9.94
NA	14	56.97	21.38

***** kcal/kgFFM.d-1

As is evident from Table 1, at 28.10 (± 11.45) kcal/kgFFM·d^{-1}, the athletes had lower EA than non-athletes who had a mean of 56.97 (±21.38) kcal/kgFFM.d^{-1}. The independent t-test revealed significant difference (p<0.001) between athletes (M=28.10±11.45kcal/kgFFM·d^{-1}) and non-athletes (M=56.97±21.38 kcal/kgFFM·d^{-1}). Analysis by distance and non-athletic category (Table 4) showed that MD athletes had a mean of 33.27 (±11.02) kcal/kgFFM·d^{-1}, LD athletes with a mean of 23.31 (±10.10) kcal/kgFFM·d^{-1} and a corresponding value of 56.97 (±21.38) for non-athletes. The ANOVA to compare EA means by distance category revealed a significant difference (F=17.251, df=36, p<0.001) among the MD, LD and NA groups. The Tukey-up post-hoc test showed that the source of the significant F ratio was the non-athletic group with the highest mean (56.97±21.38kcal/kgFFM·d^{-1}) compared to the MD (33.27 ± 11.02) and LD (23.31±10.10 kcal/kgFFM·d^{-1}) means. Distribution of participants along the EA health continuum showed that 36% of the 25 athletes (MD=8, LD=1) and 21% of the14 non-athletes had borderline sub-optimal EA of between 30 and 45 kcal/kgFFM·d^{-1}, while 56% of the athletes (MD=3, LD=11) and 7% of the non-athletic group (n=1) fell in the low EA category of ≤30 kcal/kgFFM·d^{-1}.

Results of the EDE-Q questionnaire that determined eating behavioural practices (EBP) (data not shown) revealed a global mean of 0.68 for the combined subscales of dietary restraint, eating concern, shape concern and weight concern. The Pearson correlation coefficient between EBPs and EA (r=0.142) was not significant (p=0.390), suggesting that eating psychopathological behaviour did not contribute towards the combined participant's low EA mean of 38.44 (±20.89) kcal/kgFFM·d^{-1}.

Table 5 shows the distribution of eumenorrheic, oligomenorrheic and amenorrheic menstrual function among the participants in sub-groups of middle and long distance athletes and non-athletes.

Table 5: Distribution of menstrual function by sub-group categories

Menstrual Category	MD (n=12)	LD (n=13)	NA (n=14)
Eumenorrhea	67% (8)	7	71.4% (10)
Oligomenorrhea	33% (4)	6	14.3% (2)
Amenorrhea	0	0	14.3% (2)
Primary Amenorrhea	58% (7)	8	14.3% (2)

Consideration of MF in all 39 participants together showed that 5% were amenorrheic, 31% oligomenorrheic and 64% eumenorrheic. When considered in sub-groups as athletes and non-athletes, MF distribution among the 25 athletes showed 60% (MD=8, LD=7) were eumenorrheic and 40% (MD=4, LD=6) oligomenorrheic. Among the 14 non-athletes, 71.40% (*n*=10) were eumenorrheic, 14.30% (*n*=2) were oligomenorrheic and the remaining 14.30% (*n*=2) were amenorrhoeic. The presence of 44% (*n*=17) primary amenorrhea revealed that 88.24% (MD=7, LD=8) of these were athletes and 11.76% were non-athletes. Of these primary amenorrheics, 47% (MD=4, LD=4) were currently oligomenorrheic.

Results of Spearman's correlation analysis conducted to explore the association between EA and MF did not yield significant relationship (*rho*=.520; *p*=0.106), thus indicating that EA was poorly associated with MF among the Kenyan participants.

Discussion

At the initial point of EI, the Kenyan LD and MD runners, and non-athletic cohorts did not show statistically significant differences. However, the finding that EI is lower than the estimated energy required (EER) for all participants is indicative of energy deficiency and its spiralling impact on EA, subsequent menstrual dysfunction, impairment in bone health, risk of stress fractures, and endothelial dysfunction in athletic women (De Souza et al., 2010; Loucks et al., 2011). Intake of energy lower than the estimated energy requirement may be one of the reasons for the borderline sub-optimal to low EA among the elite Kenyan athletes and non-athletes. Though the results of the present study indicated that LD runners functioned with low EA and MD runners at a borderline sub-optimal EA, these differences were also not statistically significant between the groups of runners. EA among the non-athletes was in the above (45 kcal/kgFFM$\cdot$d^{-1}) healthy range.

Fudge et al. (2006) examined EA and utilisation in Kenyan runners during periods of intense training from the dimension of energy balance (EB) which reflects the energy remaining after use by all physiological functions through a day (Loucks et al., 2011). They confirmed earlier findings by Onywera et al. (2004) that Kenyan runners operated in borderline/negative energy balance. The current study investigated the energy issue from the context of exercise physiology which considers EA as the remnant energy put into the physiological systems after deducting only what is expended in purposive exercise (Loucks et al., 2011). Despite the contextual differences, the current study found that Kenyan runners still operate under borderline sub-optimal to very low EA.

The Hoch et al.'s (2009) investigation on prevalence of the female athlete triad in 13 to 18 year old high school athletes from a variety of sports has been one of the few studies so far to have used the concept of EA. With their low EA threshold set at ≤45 kcal/kg/LBM (lean body mass), 36% of the high school athletes had low EA. However, just 6% of these had EA ≤ 30 kcal/kg/LBM. Despite the differences in ages and sports involved, overall, 92% of current elite Kenyan athletes fell in the high school low EA threshold. Comparatively, at 36% more Kenyan athletes showed sub-optimal EA (30–45 kcal/kgFFM.d^{-1}) and at 56% more Kenyan athletes had EA below the threshold at which MF and BMD are expected to be disrupted (Loucks & Thuma, 2003). In the Hoch et al.'s (2009) study, of the 39% sedentary controls with low EA, only 4% were ≤30 kcal/kgFFM·d^{-1}. In contrast, 29% of the non-athletes presented EA≤30 kcal/kgFFM.d^{-1}.

Disordered eating and ED, recognized as key contributory factors in energy deficiency, especially in sports favouring slimness or low body weight, have been used as surrogates for estimating EA (Nattiv et al., 2007). The results of EDE-Q administered to determine presence of DE and/ ED showed an almost negligible presence of eating psychopathological behaviour among the current participants. Similar low presence of eating psychopathological behaviour among elite Kenyan female runners was reported in a study that specifically compared eating disorders between elite British and Kenyan runners (Hulley, Currie, Njenga & Hill, 2007). Such low presence of eating psychopathological behaviour in both studies, and the non-significant association between EBPs and EA in the current athletes, suggests the very unlikely possibility of DE and ED being culprits in the low EA phenomenum found among the Kenyan athletes. As seen in the negative differences between EER and actual EI, inadvertent restriction or ignorance about maintaining balance between IE and energy expenditure (Pantano, 2009) may bear greater responsibility for the low EA than psychopathological constructs.

Numerous investigations and reports have dispelled any doubts about the direct detrimental impact of low EA on menstrual function, reproduction and fertility (Williams et al., 2001; Tietjen-Smith & Mercer, 2008; West et al., 2009; De Souza et al., 2010). It has also been widely reported that because of failure to adequately compensate for energy used in exercise, menstrual dysfunction is a lot more prevalent in exercising girls and women than in the general population (Beals & Meyer, 2007; Pantano, 2009; Nazem & Ackerman, 2012). Those participating in sports in which leanness or lower body weight could be more favourable, are more likely to experience low EA and are therefore more vulnerable to menstrual dysfunction (Manore et al., 2007). Consequently, the current study involving elite runners, for whom leanness and/ low body weight could be an advantage, reveals some intriguing findings, especially among the athletes. Whereas the mean EA among 56% of the athletes was definitely below

the threshold at which MF is supposedly disrupted, 60% of the athletes had eumenorrheic cycles. In such an apparent healthy menstrual functional environment, EA did not show any association with MF.

Cognizant of the distant female athletes' focus on low body weight and percentage body fat, Burke, Millet and Tarnopolsky (2007) advised LD female athletes that periodizing of CHO intake between 5 $g.kg^{-1}.d^{-1}$.and 10 $g.kg^{-1}.d^{-1}$through the season could satisfy, both, their weight and body composition concerns and training needs. At the same time, apart from emphasizing high capacity for fat oxidation in athletes during exercise, they do not provide any conclusive recommendation for fat intake by endurance athletes. The Stellingwerff, Boit and Res (2007) nutritional guidelines for MD runners do not distinguish between the sexes. They were quite emphatic that intake of CHO-rich foods ranging between 7 and 10 g/kg $BW^{-1}d^{-1}$ constitute majority of the daily energy requirements; and that during periods of hard training, PRO intake should range between 1.5 and 1.7 g/kg $BW^{-1}d^{-1}$. Stellingwerff et al. (2007) argue that MD runners should avoid increasing fat intake above normal intake. The negative effect of enhanced fat adaptation on CHO oxidation could prove counter–productive to performance for the MD runner. Interestingly, at 13 g/kg $BW^{-1}d^{-1}$, both groups of elite Kenyan runners showed a higher CHO intake than the ranges recommended (Burke et al., 2007; Stellingwerff et al., 2007).

Admittedly, though simultaneous logging of daily basal temperature and the exact date of start and finish of each cycle was useful in providing an overall picture of menstrual cyclic function, the method had its shortcomings. While it was possible to identify the obvious eumenorrheic, oligomenorrheic and amenorrheic cycles, without appropriate hormonal and/ovarian steroidal investigations, this study was unable to identify the presence of subtle sub-clinical conditions such as luteal phase deficiency (LDP) and anovulation. Using daily hormone measures, De Souza et al. (2010) confirmed the presence of LPD and anovulation in cycles that were apparently within normal eumenorrheic range. It has been reported that despite suppression in the pulsatile secretion of LH and FSH in an energetically challenged environment, oestrogen could be sufficient for uterine stimulation and menses without ovulation (De Souza & Williams, 2004). Loucks (2004) has raised the possibility of low CHO availability bearing greater responsibility for menstrual dysfunction than just general low EA. Perhaps the higher than recommended CHO intake was sufficient for the many Kenyan athletes who had eumenorrheic status.

It is very likely that despite regularity in cycles within normal range, the apparently healthy menstrual function in the current study underestimated the actual hormonal and/ ovarian steroidal status (De Souza et al., 2010). Bearing in mind the dose-response or energy conservation theory (De Souza et al., 2007) it is possible that elite Kenyan runners experienced fluctuations in hormonal

function ranging from healthy ovulatory cycles through non-obvious but subtle sub-clinical dysfunctions of LDP to anovulation corresponding to fluctuations in their EEE and EA (Awdishu et al., 2009). In the absence of early detection and management, such undetected subclinical dysfunctions could potentially cause as much harm to the athlete's health in the long-term as the deleterious amenorrhea (Gibbs, Williams & De Souza, 2013).

Closer scrutiny of the 40% athletes who had menstrual dysfunction revealed that 90% of them consumed 40% less energy than the EER and 30% expended more energy in exercise than the athletes' mean EEE. However, one athlete presenting oligomenorrhea and a history of primary amenorrhea showed healthy EA despite having the highest EEE among all athletes. Whereas energy deficiency is gaining increasing recognition as an instigator of menstrual dysfunction in athletic women, it may not always be the culprit in oligomenorrhea. As speculated, hyperandrogenism may bear greater responsibility for oligomenorrhea (Awdishu et al., 2009). The robust energy status of the oligomenorrheic athlete warrants further clinical investigation to determine what factors, other than EA, are probably more involved in her oligomenorrheic status. While signs and symptoms may give rise to speculation, treatment and management of menstrual disorders among these athletes will ultimately depend on the underlying causes. Correct diagnosis of menstrual dysfunction by process of elimination should include clinical and biochemical investigations in the athletes to rule out factors such as hyperandrogenism (Awdishu et al., 2009). Use of hormonal contraceptives (HC) was reported by just 30% (3) of the athletes. In addition to all of them presenting with oligomenorrhea, two had EA well below the 30 $kcal/kgFFM \cdot d^{-1}$ threshold, and the third athlete's EA laid midway in the borderline suboptimal category. Apart from the need to manipulate cycles for competition convenience and avoid pregnancy, no other reason was given for HC use. It is most likely that the athletes' oligomenorrhea is the combined outcome of this manipulation and low to borderline suboptimal EA, exposing them to greater risk of low bone mineral density (Manore et al., 2007).

Strengths and Limitations

The combination of direct food weighing/EI by a trained, individually assigned RA during all waking hours throughout the day, use of objective accelerometry to measure EEE, and use of the gold standard DXA for assessing FFM in this study, avoided much of the reported error and or bias associated with methods used for assessing EA (Gibbs et al., 2013). This assessment technique enhanced the strength of the study. Apart from those who did not complete the requirements of the study, high compliance among those remaining was enhanced through direct monthly contact at the training centres between participants and the principal investigator; and bi-weekly mobile phone communication between participants and their assigned RAs.

However, it must be recognized that estimation of energy expenditure from activity counts measured using acclerometry could introduce errors in analysis. While participant enthusiasm and compliance allowed accurate determination of start and finish of each menstrual cycle, mistakes made in reading temperatures may confound accurate estimation of ovulatory cycles. Unfortunately, this failure combined with the lack of either direct hormonal and steroidal assessments or their biomarkers, highlights a weakness of the study. In the absence of these assessments and consequent inability to establish the presence of subtle sub-clinical menstrual dysfunctions, the seemingly healthy menstrual profile may be an overestimation of the status.

In conclusion, the hypothesized expected differences in EI between the athletes and non-athletes did not materialize. Significant differences were found between the athletes and non-athletes in EEE and EA. The envisaged significant association between EA and MF was not evident in elite Kenyan athletes nor was it significant in the non-athletes. Despite the low to sub-optimal EA, elite Kenyan athletes showed a relatively healthy eumenorrheic menstrual status. In view of the adequate CHO intake that could replenish glycogen stores and maintain health (Burke et al., 2007; Stellingwerff et al., 2007), the apparent eumenorrheic menstrual condition among the elite Kenyan athletes should not be a surprising revelation. However, since such chronic low to sub-optimal EA could lead to more serious health consequences, the over-all low to sub-optimal EA is cause for concern and further investigation. Though the signs of psychopathological EBPs were negligent, it may be necessary for Kenyan athletes to increase IE by force rather than depend on the appetite (Loucks, 2004). In addition to regular updates and education of athletes and their coaches about dietary and nutritional requirements of endurance training, there is need for regular health screening that will identify pathologies in need of intervention.

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